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African Journal of Agricultural Research

Table of Contents: Volume 11 Number 35, 1 September, 2016

ARTICLES

African Journal of Agricultural Research

Table of Contents: Volume 11 Number 35, 1 September, 2016

ARTICLES

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Review

Logistics and storage of soybean in Brazil

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Soybean is one of the most important cultures harvested in the world, and Brazil is ranked the second major producer and the top exporter of this oilseed. Brazil presents good edaphoclimatic conditions for soybean cropping, it has a strong research structure and has capacity to enhance its cropping area, and however, the faulty infrastructure in its own terrain hinders logistic operations for soybean flow and exportation. Brazil has a huge deficit in grain storing, forcing producers to flow their production right after harvesting. Brazilian transportation is centered on a road system, and it is not viable once road conditions are precarious and also there are long distances to run. Soybean should then be transported by water or railway, but these means of transportation are not enough in the country due to low exploitation of existing waterways and the short railway system. Besides, Brazilian ports are inefficient, when the soybean harvest flow is at its peak for exportation; there are long lines of trucks and ships. This series of internal barriers increase the costs of Brazilian soybean and reduces its competition in the foreign market. Thus, it is of pivotal importance that the Brazilian government performs investments in building warehouses. It is also vital to invest in the improvement of the road network, extend railways and waterways and enhance ports, so that the costs with soybean logistics drop and producers should be able to increase their profits and competitiveness in the overseas market.

Key words: *Glycine max* L., transport, packaging.

INTRODUCTION

The soybean (*Glycine max* (L.) Mer.) is a legume and an oilseed harvested as a source of food for humans and animals and it is an essential culture in global economy. Its grains have been used more intensively by chemical and pharmaceutical industries, as well as by agribusiness, in the production of soy oil and bran. Into this perspective, there are various factors that have contributed for the growth of the soy population in the last years, with its high levels of proteins (more than 40%) and oil (around 20%), its used as animal feed, there are even scientific findings that proof of grain efficiency in the production of cosmetics and medicine (Freitas, 2011).

While soy production has advanced through the interior of the country, the investments on flow alternatives of

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production were not sufficient. Currently the roads are precarious, mainly in the bordering regions of agriculture, there is a lack of connections among transportation modes, with the predominance of road transportation and infrastructure problems, making the flow harder and resulting in losses and reducing competitiveness for Brazilian soybeans (Zemolin, 2013).

Lack of investments is historical in this sector. In the 1980's and 1990's, government contribution to transportation infrastructure was Lilliputian: Around 0.2% of annual PIB (NGP), whereas in countries such as China, the average is of 3.5%. The absence of resource contributions in this period took a heavy toll on national logistics, which yielded around R$ 350 billion in 2012, twice as much as ten years ago (PBLOG, 2013).

According to Correa and Ramos (2010), to reduce logistics inefficiency, projects that motivate transportation interconnection are imperative, along with the widening of the capacity of water and railway modals, besides the widening of ports' capacity and the development of seafaring/cabotage in the country.

This way, this work aims to make a literary review about the logistics and soybean storage in Brazil.

THE IMPORTANCE OF SOYBEAN CULTURE

The soybean is the most important oilseed harvested in the world, standing out as 56% of grain production with oil content (US Soybean Export Council, 2008). The main producers are the U.S (32% of worldwide production), followed closely by Brazil (30%) and Argentina (19%), and in the harvest of 2013/14 the worldwide production was 285.3 million tons (USDA, 2014).

Soybean is the activity which presented a higher expansion among agricultural crops in 1970/71 and 2010/11, with a production increase of 526%. The greatest soy production increase may be attributed to various factors, among which five are highlighted: (1) The soybean presents a high levels of excellent quality protein (around 40%), either as animal or human feed; (2) The oilseed contains a considerable oil level (approximately 20%), that can be used for different purposes, specially related to human feed and biofuel production; (3) The soybean is a standard and uniform *commodity*, therefore, owning the characteristic of being produced and commercialized by producers in many countries; (4) The oilseed presents high levels of liquidity and demand and; (5) withal in the last decades, there has been an expressive raise in technological production offering, which allowed to enhance substantially the area and productivity of the oilseed (Hirakuri and Lazzarotto, 2011).

SOYBEAN CULTURE IN BRAZIL

Brazilian soybean production in the harvest in 2013/2014 was of 86.121 million tons, being the main producers Mato Grosso (30.7% of national production), followed by Paraná (17%) and Rio Grande do Sul (15%). The Center-South accounts for 88.4% of the national production and the region North/Northeast for 11.6% (CONAB, 2014).

Although soybean had been introduced in Brazil at the end of the 19th century, it has been a declassed culture for several decades. However, in the early 1970's, the oilseed broke the boundaries form Rio Grande do Sul state and the traditional production system, to become one of the most important crops in the Brazilian agriculture. From 1970 to 1985, soybean expansion happened through the opening and consolidation of new agricultural areas in the South and Mid-West regions (Hirakuri and Lazzarotto, 2011).

Brazilian soybean production presented a great expansion, boosted not only by the increase of the production areas, but mainly by the enhancement of productivity. Considering the period between the harvests of 1976/77 to 2013/14, on the one hand, the area presented a growth rate of 334.2%, going from 6.95 to 30.17 million of hectares, on the other hand, production reached a growth rate of 609%, going from 12.14 to 86.12 millions of tons. In this same period, productivity went from 1.748 to 2.854 kg ha^{-1} which represents an increase on productivity of 63% (CONAB, 2014).

To Brazil, the soybean complex has an expressive economic importance. Besides involving a great number of agents and organizations linked to the various economic sectors, it plays an important role for the national gross product (NGP), as well as generation of foreign currency. From 1995 to 2009 the soybean economic performance presented an expressive growth. While NGP grew under annual taxes of 2.86%, the gross value of soy production (derived from the sales prices times the quantity of product yielded) grew at an average rate close to 7.75% a year. The Brazilian soybean complex is accounted for job generation (Hirakuri and Lazzarotto, 2011). According to surveys conducted by Roessing and Lazzarotto (2004) this complex is responsible for generating approximately 5.0 million job openings. From this figure, it can be highlighted that for each hectare cultivated in Brazil, an average of 0.24-job would be generated by the whole complex.

The jobs generated by soybean's productive chain are related to agriculture activity and with several sectors that draw up this chain. According to Cavalett and Ortega (2007) soybean chain has several stages: Agricultural production, transportation up to grain crushing and processing industries, where the extraction of the main derivatives, bran and oil take place, also redirecting to refineries and other derivatives, and afterwards, market distribution through wholesale and retail.

THE IMPORTANCE OF LOGISTICS

Logistics is unexpendable to any company in the world, because more and more the transportation of goods, storing, inventory management, personalized customer service are basic premises for the success of any enterprise, and these are within the logistics role. Among the various logistic functions the one that stands out is transportation, for all sectors in economy, one way or the other, depend on transportation, either to carry their products or services or for purchasing raw material to produce them (Resende et al., 2007).

Logistics mission is to plan and coordinate all activities to reach quality levels at the lowest cost possible (Christopher, 2011). For Soares and Caixeta-Filho (1997) the productive systems have advanced significantly, especially regarding development and the spread of new production techniques, however, the final competitiveness of the products is impaired by the bottlenecks throughout a specific chain, being logistics and transportation fundamental in this context. Transportation is one the main logistics functions, because it accounts for the majority of the costs in most organizations.

The best transportation choice to haul a certain good is the greatest challenge for logistics operators, to reach a decision they take into consideration: Agility, flexibility, security, costs, transportation capacity and kind of cargo (Resende et al., 2007). Inside agribusiness logistics, the productive chain is divided into three segments: 'Anti gate' – includes inputs, machinery, equipment, chemicals, fertilizers, credit, rural insurance and whatever necessary for agricultural production; the segment 'in gate' from planting to harvesting; and 'post gate' – including all channels of product distribution up to the final destination (storing, industrialization and distribution) (Callado, 2008).

Agroindustrial products feature low relative value per weight unit or volume. Still, the productive and consumer regions are most of the times, far from each other. Altogether, these factors input considerable costs to product distribution. Its price fluctuation, due to seasonal characteristics of harvesting, is another aspect that has to be considered. During harvesting, there is a focus on offering, followed by a reduction in relative prices and a higher need for production flow. This context explains the increase on the demand for transportation services, which makes the prices skyrocket. Thus, there has the combination of low cargo price with high transportation price. This is the main characteristic of agribusiness logistics (Gameiro and Caixeta-Filho, 2010).

THE IMPORTANCE OF STORING

Commercialization includes a series of activities, through which goods and services are allotted from productive sector to final consumers. These activities that compose the commercialization process have to do with processing, transportation and storing, wherein each of these phases add up value to the product (Barros, 2004).

Amongst the activities involved in the commercialization process, storing stands as a relevant contribution, because agricultural production presents seasonal production, and can be transferred within time, assuring the availability of the product for consuming. When storing is performed in a competitive market environment, where individuals search for the maximization of gain, the decision of storing part of the production for the next period is made under future price expectation. Thus, storing will only be economically viable if the difference between the future and the current prices are higher than the costs for storing the product (Ferrari, 2006).

From a technological point of view, storing is an essential activity to reduce agricultural loss and for grain conservation. It is also considered to be a fundamental backup activity for the transportation and commerce phases, for the presence of storing units close to production areas, markets, ports and agribusiness makes it feasible rationalization of transportation costs, the strategical apportioning of inventory and still, favors inter-regional commerce (Biagi et al., 2002). Warehouses are also important, because during the commercialization process, the grains need to go through warehouses for cleaning up and for reduction of humidity, for product conservation and optimization of the right transportation modal to be deployed (Ferrari, 2006).

CHARACTERIZATION OF THE STORING UNITS

Warehouses in general, can be grouped according to local features of the region where they will be set. Thereby, there are units installed in the farm, collection units, subterminal and terminal units. There is even other one known as intermediate unit, which is simply the grouping that involves collection and subterminal units (Ferrari, 2006).

Units installed in agricultural properties are generally for private usage. The existence of these units is fundamental to guarantee the producer's autonomy, making it possible to choose the right moment to perform the product sale and production flow (Frederico, 2008). Besides autonomy, the units in the farm hold a series of other advantages: They avoid the overload that the transportation system suffers at harvesting periods, increasing freight costs; they prevent the spread of humidity and impurities; they allow for cleanability, drying and storage at low cost; the producer keeps organic residues; they lower total cost per ton produced; among others (Weber, 2005).

The collection units, normally used by groups of producers, are located in the vicinage of the production area. The cooperatives and warehouse complexes, mainly set in the Brazilian Mid-West, can be fit into this category. The subterminal units are those installed close to the main road systems (railways and waterways included) and are operationally capable of receiving the incoming product from collection units and those set in the farms, in addition to perform staggering of products in port terminals. Now the terminal units are those located in the consumer centers, ports and agribusiness (Ferrari, 2006).

According to Puzzi (2000) amongst these different units that form the storage net, it is noticeable that it is not necessary to follow the sequence for the categories presented, for specific cases, such as subterminal units, might not be needed to stagger flow. The different kinds of storing units should be set in such a way that they allow for a fast and continuous flow of goods and should be dimensioned so that none of the units get overloaded (Frederico, 2008).

In 2011 the static capacity regarding storage unit location was: 13% of static capacity was in farms, 35% was in the countryside, 45% in urban areas and 6% in waterfronts (Maia et al., 2013).

STORING CAPACITY OF GRAINS IN BRAZIL

In accordance to Nogueira Junior and Tsunechiro (2011) there are many obstacles found in storing structure that have become chronic, because of the rhythm of crop harvesting growth in Brazil: (1) The mismatch between offer and demand of grains in years with high yields. In specific years there is a worsening in the situation resulting in logistics and harvest transportation problems and even jams in the ports due to the high flow of goods; (2) A direct effect from the inadequacy of the harvest flow system (transportation and storage) reducing product prices due to the necessity of readiness for commercialization right after harvesting. Because of the high offering in the Market, grain prices lower and producers do not avail the best moment to collect profits; (3) The growth in production for different goods (transgenic, organic, new grains, such as canola, millet and triticale) which require specific cell sorting; (4) Lack and inadequacy of storing units, either regarding quality or geographical location. Due to crop migration, various regions as the Mid-West of the country, where lots of important grain producing areas still do not have a satisfactory storing structure, forming the so called logistic void. However, in other regions like the state of São Paulo, because of the decrease of coffee culture, a lot of warehouses and silos are located in areas that do not produce coffee anymore, resulting in surpluses; (5) Profiles created by CONAB (2014) point out a great number of storing units with obstructions. Although, apparently, there is no veto, the working conditions of these units are not in accordance to servicing and for loss reduction after harvesting; (6) Substantial part of agriculturists still have outstanding bills, and a high sum of resources required for the construction of silos hardens the expansion and modernization for the sector. For small producers hiring financing is difficult, for a compensatory production volume is demanded; (7) Low storage capacity in rural properties do not reach 20%, resulting an unfavorable position for Brazil in relation to major worldwide grain producers, who have in field the greatest parcel of units to store their products. Moreover, this condition compels the producer to commercialize his/her crops readily when prices are low. Besides, this causes logistics problems such as jams in intermediate and terminal storage networks; (8) High volumes of sugar and fertilizers contend storing space with grains. These products have not been taken into account in the statistics of storing demand, which masks economics analysis and; (9) Transportation headquarters focused on trucks result in jams and delay in unloading cargo in warehouses/silos and ports, also increasing transportation costs. Because of its extensive territory, the ideal stage for Brazil would be the use of railways and waterways for crop transportation, which would enhance partially competitiveness of products after harvesting.

In Table 1 the relation between soybean production and the statistical storing capacity is at display (CONAB, 2014) divided into states and into Brazilian regions. It is noticeable that only the Southeast region do not present shortage in storage, this is because of its low production of soybean, and the existence of several warehouses which were built for coffee storing and are not functioning (Nogueira Junior and Tsunechiro, 2011). The South region, which is the second major soybean producer, also has a deficit in storing, however, due to the fact that the producing regions are close to ports such as Paranaguá and Rio Grande, that makes production flow easier and this deficit is not as strong as it is in the Mid-West region.

In the Mid-West region, the major soybean producer and second grain producer, this shortage becomes more severe, for this region is far away from ports, and when producers perform harvesting, they are obliged to flow their production by paying high freight prices, due to the high demand of this service. If we give special attention to the state of Mato Grosso, the greatest Brazilian soybean and grain producer, this storage deficit is higher than 37%. The states forming what is called MATOPIBA (Maranhão, Tocantins, Piauí and Bahia) show a pretty high storage deficit, and the state of Piauí, for instance, presents a deficit above 65%. This high index is because these states presented and increase in soybean production for the past years (CONAB, 2014) and still have not built adequate and sufficient storing infra-structure. The macro regions North/Northeast and Mid-South show a deficit of 44.36 and 11.77%, respectively. However, the deficit in the Mid-South, in absolute values, is higher than in the region North/Northeast, because these regions present production of 172.4 and 23.1 million tons, respectively. Brazil presents a deficit of 15.62% which accounts for roughly 30 million tons.

SOYBEAN TRANSPORTATION MODALS

Transportation is the main compound in the logistics

Table 1. Static storage capacity (thousand tons), grain production (thousand tons) and percentage of deficit or surplus in storage, by region and states of Brazil.

Region/Federation unity	Static storage capacity (thousand tons.)	Grain production (thousand tons.)	Deficit/surplus (%)
NORTH	3.652.88	6.309.80	-42.11
RR	147.48	142.20	3.71
RO	733.55	1.223.70	-40.05
AC	29.28	123.80	-76.35
AM	347.60	42.00	727.62
AP	6.28	5.60	12.07
PA	856.13	1.416.50	-39.56
TO	1.532.57	3.356.00	-54.33
NORTHEAST	9.199.20	16.788.10	-45.20
MA	1.997.01	4.300.60	-53.56
PI	963.78	2.777.40	-65.30
CE	384.58	573.00	-32.88
RN	63.50	38.30	65.79
PB	99.84	58.20	71.54
PE	977.74	187.80	420.63
AL	550.72	67.20	719.52
SE	3.20	1.123.50	-99.72
BA	4.158.84	7.662.10	-45.72
MID-WEST	67.707.83	81.636.50	-17.06
MT	29.626.23	47.702.60	-37.89
MS	8.274.12	14.470.50	-42.82
GO	29.326.66	18.333.40	59.96
DF	480.82	1.130.00	-57.45
SOUTHEAST	22.194.62	17.887.70	24.08
MG	9.048.69	11.655.30	-22.36
ES	1.489.14	74.10	1909.63
RJ	184.50	15.90	1060.36
SP	11.472.29	6.142.40	86.77
SUL	62.177.66	72.845.00	-14.64
PR	28.316.26	35.840.60	-20.99
SC	5.201.62	6.572.20	-20.85
RS	28.659.79	30.432.20	-5.82
NORTH / EAST	12.852.09	23.097.90	-44.36
SOUTH CENTER	152.080.11	172.369.20	-11.77
BRAZIL	164.932.19	195.467.10	-15.62

Source: CONAB (2014).

system, and represents in average, 60% of total logistics resources employed in exportation operations. Besides, it is responsible for having a crucial role in the quality of logistics services, because it impacts directly on delivery times, on buyer’s trust and product security (Vasques, 2009). A modal choice should be based not only on the time to be spent in transportation, but also with its cost and product to be used (Caixeta Filho, 2010).

Being a product of low aggregated value and which can be transacted in high volumes, the soybean needs a transportation modal with high capacity and at low unitary cost, even though other attributes might not be considered, such as frequency and production delivery deadlines (Fleury, 2005).

The road modal turns to be the most adequate when it comes to transportation to short distances, that is, for trips up to 186 miles (300 km). This modal would be applied in the so called edges – from the original place (in this case the producing farms) to warehouses or railway or waterway terminals, which then, would be responsible for transportation to long distances, due to a greater cargo capacity and the possibility of cost and loss reduction

(Hijjar, 2004).

The Brazilian soybean, even when coming from regions that are more distant from the ports, is transported preferably through roads. This makes the transportation of the soybean produced in the Mid-West region to be inefficient, just because of the choice of road modal as a unimodal means – connecting the cargo origin to its final destination – instead of using it as a multimodal connection (road-waterway or road-railway) (Correa and Ramos, 2010).

According to Correa and Ramos (2010) although other means for the transportation of the produced soybean in the Mid-West exist, such as the waterways in Madeira and Tietê, the road modal is still predominant, and it accounts for 67% of existing modals, waterways only 5% and railways 28%.

Road modal

It is the most expressive when it comes to cargo transportation in Brazil, practically reaching all ends of the national territory, because since the 50's, with the implementation of automobile industry and road paving, this modal expanded in such a way that today it is the most used one (Rodrigues, 2007).

Even with a low quality road system, it is responsible for 60% of everything that is transported in Brazil. It is a high index, even when compared to other countries with a huge continental extension: In the U.S. road participation accounts for only 26% of transported cargo and in Australia 24%. This scenario shows Brazil's dependence on this modal, and opens a spot in the need for actions that result in a balance in the national transportation matrix, giving other modals – railway, waterway and airway, more participation (PBLOG, 2013).

The usage of road modals, even for products and distances where it is not the most competitive, comes from the lack of options in the use of others modals for short, medium and long distances (Petraglia, 2009). This overdependence on road modal gets even worse when Brazilian great territorial extent is taken into account, as well as its precarious and insufficient infrastructure to cope with demand (Pontes et al., 2009).

According to the Department of Transportation (2014), Brazil has 1.7 million kilometers (1,056.331 miles) of roads, which 79.5% are not paved roads. In general, the preservation conditions of these roads is terrible and the bad pavement quality leads to higher maintenance costs, to breaks wear and tear, and also to more expenses with tires, which wear off faster once pavement quality is so low. These conditions cause an increase in operational costs. A study conducted by the extinct Departamento Nacional de Estradas e Rodagem (DNER) shows that when pavement is in great conditions, there is no increase in operational costs, when it is in good conditions there is an increase of 18.8%. Now roads under bad conditions add 91.5% to operational costs. Thus, a transporter who drives on a road with great conditions would have operational costs of R$ 100.00 in specific kilometers, whereas if an individual drove the same distance under bad conditions, one would have R$ 191.50 in operational costs (CNT, 2014).

When one analyses the quality of the pavement in regional terms in Brazil, the scenario is even more critical for regions North and Mid-West, which present an increase to average cost between 37.6 and 27.5%, respectively. The regions South and Southeast show the best pavement conditions, which reflects on the estimate for cost increase for the regions that are below the national average of 26.0% (CNT, 2014). This increase in the operational costs pushes to freight price, and this way, the producers in Mid-West region, for instance, have their profits cut short compared to the ones in the South region. Because, besides having to transport the soybeans for a longer distance up to ports, they face the worst road conditions which make the price of freight more expensive.

Linked to the bad road conditions, soybean transportation suffers another problem. According to Agência Nacional de Transportes Terrestres (ANTT) the fleet responsible for cargo transportation is 13.1 years old in average (ANTT, 2014), that is, many of the vehicles used in soybean transportation do not meet adequate conditions, which results on quantitative and qualitative loss during the trips.

Although it presents a great number of disadvantages related to the sector such as: A lesser relative capacity of cargo, its more fragile security, because it is bound to theft and damage and its higher operational cost compared to railway modal, the advantages of this modal are centered in its door-to-door service capacity, in the frequency and availability of its access ways, a lesser time for loading and unloading cargo, and its facility of replacement in case of accidents or technical problems with the vehicles (Vasques, 2009).

Railway modal

The first Brazilian railway started its operations in 1845 by Barão de Mauá, connecting Praia da Estrela to Petrópolis. From 1873 to 1930, it played a decisive role in coffee exportation (Rodrigues, 2007). Since 1922 the changes to Brazilian railways were incipient, which still counts on the same unbelievable 29 thousand kilometers (18.019 miles) of rail tracks. That is, the construction of railways in Brazil is still very incipient compared to other countries with similar territorial proportions, like the United States, which hold the world's longest railway system-225.000 km (139.808 miles). Russia registers 87.000 km (54.059 miles), followed by China 86.000 km (53.437 miles), India 64.000 km (39.767miles) and Canada 46.000 km (28.583 miles). In this *ranking*, Brazil holds the 122nd place, behind Cuba and Ukraine (PBLOG, 2013).

The railway transportation sector is an economic transformation fact. There are several profit possibilities derived from a greater use of the railway modal for cargo transportation. Its convenience presents advantages to Brazil, which even more, consolidates its worldwide position as a major agricultural and mineral exporter. The physical-volumetric features of these goods make it feasible the use of railway transportation, generating economy to producers and competitiveness in the foreign market (CNT, 2014).

The railway modal is the most appropriate when it comes to the transportation of great volumes and which involves goods of the lowest aggregate value, which is the case of soybeans and grains in general. It is an extremely efficient and competitive means of transportation in trips of medium and long duration that do not need transshipment, because of its high cargo capacity added to its low freight cost. Moreover, the operational cost for freight and maintenance and backup structure is low (Faro and Faro, 2010).

For Brazil presents continental dimensions, the railway modal stands as a huge opportunity for cost reduction on terrestrial freight paid by soybean exporters in the flow of grains to the ports. However, this modal has been, in practice, left aside due to the high investments needed to amplify its operational capacity (Pontes et al., 2009).

According to ANTT (2014) in 2013, 20.5 million tons of soybeans and bran were hauled through railway modal, which is a very high figure. However, it is of pivotal importance that governments invest in this sector so that railway systems are to expand and reach the regions that produce soybeans, where this modal is yet not used as a means of transportation.

In accordance to PBLOG (2013), the federal government has as goal, the expansion of the railway system to 40 thousand kilometers until 2020, investing R$ 200 billion. The new railway matrix that is being designed for the following years, will give the economy of the regions South, Southeast and Mid-West a new strength, allowing their production to reach the European, American, Caribbean and Asian markets through the regions North and Northeast. Besides that, this configuration will promote, concomitantly, inner integration, contributing to commerce dynamization between the North and the rest of Brazil. The federal government has presented five expansion proposals for the railway system, to interconnect the national territory as follows: (1) Construction of the railroad EF-354 – Transcontinental. This railroad will link the state of Rio de Janeiro to Acre, going through Minas Gerais, Goiás, Mato Grosso and Rondônia; (2) Railroad EF-170 - Cuiabá-Santarém. This railroad will be of great importance for production flow in the Mid-West – from 15 to 20 million tons of grains (soy and corn); (3) Conclusion of North-South railroad, Anápolis and Rio Grande (RS) (EF-151). This railroad will link the state of Goiás to Rio Grande do Sul, going through São Paulo, Paraná and Santa Catarina; (4) Conclusion of EF-334 - West-East Figueirópolis (TO) to Ilhéus (BA). This railroad will open a new exportation corridor through the Atlantic Ocean, with benefits to Regions Mid-West, South and North and an important part of the Northeast and; (5) Integration Axis Maracaju and Guaíra (MS) to São Francisco port (SC) or to Paranaguá port (PR). It will also be a major route for grain flow.

To demonstrate the importance of railway modal for transportation of agricultural products, mainly soybeans, a Confederação Nacional do Transporte (CNT) has conducted a case study comparing possible routes of flow for soybeans in Mato Grosso, having as a distribution center the city of Lucas do Rio Verde. The route that presented the highest cost was the one with final destination at the Paranaguá port, using the road modal (R$ 232.74 per ton) and the route with the lowest cost was the one with final destination in the Itaqui port, using the railway modal (R$ 148.58 per ton). This route depends on the construction of the Mid-West Integration railroad (CNT, 2013). This study shows the loss of competitiveness for agribusiness when road modal is used for long distances, with an existing need for alternate routes for soybean flow with exportation ends.

Waterway modal

Fluvial and lacustrine transportation are composed by waterways in interior navigation, performed in rivers and lakes, respectively. Due to its reduced operational cost, the waterway transportation allows the hauling of great quantities of cargo through long distances, which is ideal for *commodities* as soybeans. The waterway transportation is quite used in some regions of the world, like in Europe, whereas lacustrine transportation is practically narrowed down to the borderline between the U.S and Canada, in the region of the Greats Lakes (Faro and Faro, 2010).

According to Alfredini and Arasaki (2009) the waterway scenario is associated to an increase in the international competitiveness. Introducing this means would ensure a planned and omnibus development, connecting regions and promoting the shifting of inputs, products and people. The possibility of navigation creates a transportation alternative of low cost for shifting great cargo volumes at a low unit cost, less energetic expenses, not taking into account arising environmental costs, compared to direct competitive modes.

Brazil presents an immense potential for the use of fluvial navigation, with 63 thousand kilometers (39.146 miles) of rivers and lakes/ponds, distributed all over the national territory. Of this total, more than 40 thousand kilometers (24.854 miles) are potentially navigable. All the same, commercial navigation occurs in less than 13 thousand kilometers (8.077 miles), with relevant concentration in the Amazon, where rives do not need greater investments and the population cannot afford having terrestrial modal options (Ministério dos Transportes, 2010).

The full potential of waterways is still little exploited. Only 13.6% the cargo amount transported in Brail are performed though waterways – fluvial, seafaring and long haul transportation – despite of the comparative advantages with other models, due to the lowest operational costs, lowest environment impact, besides offering security and cargo concentration. The Agência Nacional de Transportes Aquaviários (Antaq) shows an idea of its comparative advantages between road and railroad modals: 1.5 thousand tons of cargo can be hauled in a single flatboat, whereas the same quantity would need 15 *Jumbo Hoppers* cars with capacity of 100 tons each, or 60 trucks with capacity of 25 tons each (PBLOG, 2013). For Afonso (2006), the use of waterway modal for cargo hauling would represent a cost reduction of 44% in relation to railroads, and 84% relative to road system.

According to Antaq data, Brazil has experienced a light, but consistent growth in cargo transportation through waterway modal. In 2011, 60.855 million of TKU (Tons per Useful Kilometers) were hauled, against 57.880 million of TKU, a growth of 5.1%. Being the main products transported through this modal in 2011: bauxite (36.71%); containers (15%); soybeans (9.80%); fuel and mineral oil and products (8.94%); altogether contributed with 70.80% of all hauled cargo in that year (PBLOG, 2013).

For the expansion of the waterway modal, the introduction of transshipment terminals and road/rail access to the waterways is vital Thus, in spite of presenting environmental and economic advantages over the other modals, noticeably when it comes to shifting great quantities of cargo through long distances, this alternative depends heavily on the tentacles of the multimodal net to assure cargo access in the loading points, and to enable distribution in delivery points. In Brazil, the multimodal transportation resents on the difficulty of cargo transfer from one modal to another, for the quantity of terminals set to multimodality is yet to grow (Ministério dos Transportes, 2010).

The importance and advantages of waterway transportation, allied with the existing national hydrous potential, has stimulated Brazilian governments to make efforts to increase the participation of waterway modal in cargo transportation matrix. The Federal Government goal is to double the participation for waterway transportation (waterways and seafaring navigation), with a leap of 13% to 25% up to 2020. The waterway system will count on R$ 2.7 billion for 48 projects which embrace the construction of 34 terminals and seven corridors. This will supply the country, up to 2020, with around 40 thousand kilometers (24.854 miles) of navigable ways. For the expansion of this modal, it will be necessary some investments as the construction of locks to gain over of gaps caused mainly by the construction of damns, and also, it is essential that some dragging work is performed, to increase the depth of rivers (PBLOG, 2013). According to Alfredini and Arasaki (2009) the main fluvial networks in Brazil are the ones from Maderia-Amazonas, Araguaia-Tocantins, São Francisco, Paraguai-Paraná and Tietê-Paraná rivers.

THE PORT SYSTEM AND SOYBEAN EXPORTATION

According to Goularti Filho (2007) in the Brazilian port system offering has always been dragged by demand, that is, investments applied in the ports (upgrading, re-equipping and modernization) have always been insufficient to cope with the increasing volume of Brazilian external commerce. Investments matured rapidly going through strangling, demanding more and new investments, yet, more complex and expensive than the previous one.

A grand problem faced by ports, to be highlighted, is the one encountered by the users of the Brazilian port system, in relation to the average of waiting time for mooring. For ships with containers, this term has been reduced between 2006 and 2007 from 13.5 to 9 h per ship. In terms of grains, waiting is much longer: In 2007, the national average for the several solid grains was of 54 hours/ship. the situation reaches the extremes in Paranaguá port, for instance, where waiting times for transshipping comes to 389 h/ship (approximately 16 days waiting) (Neto et al., 2009). This problem with delays for boarding increases the "Brazil Cost" (a set of structural, economic and bureaucratic difficulties which enhance investments), because the costs with demurrage (A charge required as compensation for the delay of a ship or freight car or other cargo beyond its scheduled time of departure) is of US$ 50 thousand, a day, in average (Hijjar, 2004).

An inefficient and expensive port system implies in significant additional costs for a series of productive endeavors, engendering, as a direct consequence, a less propitious environment for the growth at the economic activity level and the appeal for new investments (Uderman et al., 2012).

The port sector presents strategical importance for the country's economy, due to its expressive participation in the total shift of goods. In 2013, the sector shifted in tons, 98.3% for exportation and 90.4% for imports, it also has shifted, a total of 931 million tons – according to data from Ministério do Desenvolvimento, Indústria e Comércio Exterior e da Antaq. The Brazilian port system is formed by 37 public ports – located in 16 Federative Units, being 34 salt-water and 3 fluvial – and 130 terminals for private use. Among public ports, 14 are empowered, granted or managed by state or district

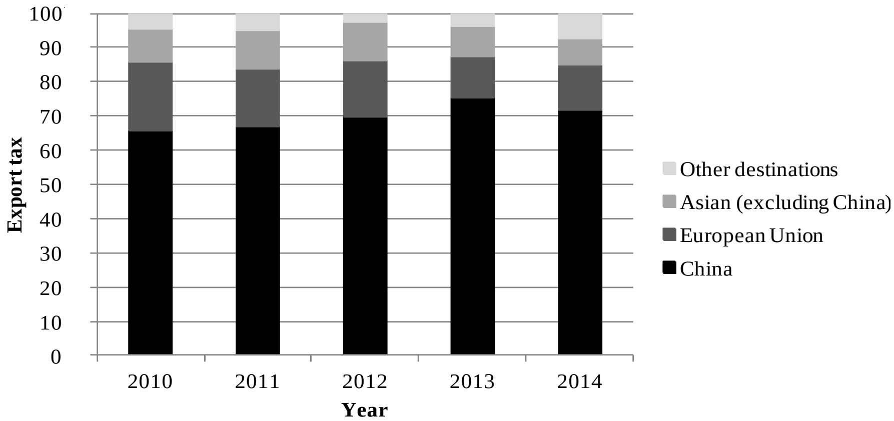

Figure 1. Brazilian soybean exports in grain (%) according to the destinations, between the years 2010 and 2014. Source: ABIOVE (2014).

Table 2. Quantity exported and the participation of ports used in soybean exports in the years 2013 and 2014 of Brazil.

Port	Unit federation	2013		2014	
		Tons	Participation (%)	Tons	Participation (%)
Santos	SP	12.892.151	30	12.719.177	28
Rio Grande	RS	8.206.122	19	8.078.602	18
Paranaguá	PR	7.723.033	18	7.526.407	17
São Francisco do Sul	SC	4.032.264	9	4.910.909	11
Vitória	ES	2.823.224	7	3.148.465	7
São Luís	MA	2.974.624	7	3.116.084	7
Salvador	BA	1.778.558	4	2.015.194	4
Manaus	AM	1.251.487	3	1.411.104	3
Barcarena	PA	0	0	1.110.852	2
Others	-	1.073.197	3	1.516.626	3
Grand total		42.754.659	100	45.553.419	100

Source: ABIOVE (2014).

governments, whereas the remaining 23 are managed by Companhias Docas, a mix economic society, whose majority shareholder is the federal government. In 2011, from private terminals, 73 presented marine activity. The fluvial and lacustrine ports, however, are under Ministério dos Transportes management (CNT, 2014).

According to a research conducted by USDA (2014) Brazilian soybean exportation hit the charts of 46.83 millions of tons in 2014, although, according to Associação Brasileira das Indústrias de Óleos Vegetais (ABIOVE). Brazil exported 45.6 millions of tons (ABIOVE, 2014). In both researches, Brazilian soybean exportation was superior to the North-American, that exported 44.82 millions of tons in 2014 (USDA, 2014), Therefore, in that year Brazil was the worldwide top exporter of soybeans.

From the Brazilian soybean exportations in 2014, 72% went to China, 13% to European Union, 8% to Asia (except China) and 7% to other countries. Figure 1 depict the exportation destinations for Brazilian soybean from 2010 to 2014.

Table 2 show the amount exported and the participation of ports used in the soybean exportations in 2013 and 2014.

The ports in the regions South and Southeast in 2014 were responsible for 81% of the exportations, whereas the remaining ones represent only 19% of the exported volume, and only three ports (Santos, Rio Grande and Paranaguá) are responsible for 63% of soybean exportations. This scenario shows the inefficiency of the Brazilian logistics structure, because ports like Santarém and Itacoatiara that could be used for soybean exportation, are not even shown in the statistics.

PERSPECTIVES FOR SOYBEAN LOGISTICS INFRASTRUCTURE

From the moment when the economic functions of transportation infrastructure and its importance became crystal clear for development, the internal affairs of a

Figure 2. Structural Axis proposed by Projeto de Integração Nacional from the federal government, Brazil. Source: CNT (2014).

country are more debated, with the aim to boost its insertion into globalization. Therefore, the establishment of a set of national objectives that would make the country more competitive in the global scenario is of extreme importance for the success in international trade (Caixeta Filho, 2010).

For Roessing et al. (2007) the issue on logistics optimization for Brazilian soybean is related to the need for the enhancement of competitiveness in the national production compared to external ones, on this account, among the segments that infer in the efficiency of several sectors of an economy, transportation is the most significant one. This way, the Brazilian soybean producer suffers an average loss of 25% of his/her income with transportation costs, whereas for a North-American producer this average expense is less than 10% of the total income.

The increase in competitiveness of the country's productive structure and, therefore, the soybean production in the Mid-West region, for instance, depends on investments in the expansion of transportation infrastructure, both in the development of alternative modals (rail and waterway) and revitalization of the road system (Correa and Ramos, 2010).

The expansion for soybean production for bordering areas has demanded more efficient modal flow means, besides alternatives to routes used by traditional regions. An alternative route for the flow of soybeans produced in the Northern Mato Grosso and in the states of the North region is that one destined to the ports of the North region of the country, like the port of Itacoatiara (AM). Because they are close to grain producing regions and even closer to external consumer markets, such as Europe, these exportation spots provide more competitive advantages, if compared to traditional ones. Besides the shorter distance, these ports offer a better structure, taking in bigger ships, that among other factors provide smaller operational costs and, this way, lower marine freights (Timossi, 2003).

According to Almeida et al. (2013) an alternative route for soybean harvest flow China bound would be the Transoceânica road. This road connects Brasil to Peru and to Chile, coming from the city of Porto Velho (RO) up to the ports of Ilo and Matarani in Peru and up to the port of Arica in Chile. This alternative might be used by the states of Rondônia and Acre which have their agricultural productions increased even more. The Northern Mato Grosso can also benefit from this route.

The federal government, through Projetos de Integração Nacional, Brazil, intends to enhance the logistics infrastructure of the country, by the creation of Structural Axis, which consist of adequacy and construction of aero/port, terminal, rail, inner navigation, ports and road infrastructure. Nine Structural Axes were defined altogether, being the majority multimodal (Figure 2); there are seven axes with infrastructure with at least two modals and two with only one modal. The axes are described as follows: (1) Northeast-South Axis: Composed by road and railway modals; (2) Coastal Axis: Comprised of roads only; (3) North-South Axis: Constitutes water and road ways; (4) Amazon Axis: Containing only waterway mode; (5) Central-North Axis: Constituted water and road ways; (6) North-Southeast Axis: Formed by roads, railways and waterways; (7) East-West Axis: Comprised of roads and waterways; (8) Northeast-Southeast Axis: Composed of roads, railroads and waterways and; (9) Cabotage Axis: Connecting the main Brazilian marine ports through possible operational navigation cabotage routes, from Macapá (AP) to Rio Grande (RS) (CNT, 2014).

FINAL CONSIDERATIONS

Each year, Brazil has been increasing its soybean production and the perspectives are that briefly, it will go past the U.S. and will become the major worldwide producer, due to favorable weather conditions, to innovation in production technology, to the possibility of increasing the cropping area, among others. In the harvest of 2013/14, Brazil achieved the title of the world's greatest soybean exporter, which is of great importance regarding foreign exchange to the country. However, Brazilian soybean competitiveness stumbles on difficulties found in a crippled logistics infrastructure regarding storing, transportation and ports in the country. Overall, railroads and waterways are not enough, making road modal the main way of production flow, encumbering costs due to the high freight price. Because of this scenario, the Brazilian producer suffers an average income loss of 25% with flow expenses, whereas for a North-American producer this expense is less than 10%.

It is vital that Federal Government invest in revitalization of Brazilian roads, in the expansion of rail and waterway systems, in the construction of warehouses and improvement of ports infrastructure. Furthermore, release of lines of credit for producers to build warehouses in their properties, so that they are able to store their production, avoiding the hindrance of having to flow their soybeans right after harvest, improving their income.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Abiove (2014). Associação Brasileira das Indústrias de Óleos Vegetais. Relatório de Exportações do complexo soja. Novembro/2014. Disponível em: <http://www.abiove.com.br/site/_FILES/Portugues/11122014-135245-exp_201411.pdf>.

Afonso HCAG (2006). Análise dos custos de transporte da soja brasileira. 2006. 138f. Tese de Mestrado (Engenharia de Transportes) – Instituto Militar de Engenharia, Rio de Janeiro. [In Portuguese].

Alfredini P, Arasaki E (2009). Obras e Gestão de Portos e Costas. 2ª edição, Editora Blucher, 2009. [In Portuguese].

Almeida CA, Seleme R, Neto JC (2013). Rodovia transoceânica: uma alternativa logística para o escoamento das exportações da soja brasileira com destino à China. Rev. Econ. Sociol. Rural Piracicaba SP. 51(2):351-368.

Antt (2014). Agência Nacional De Transportes Terrrestres. Idade média dos veículos, por tipo de veículo e categoria do transportador. Disponível em: <http://anuario.antt.gov.br/suroc-i.php>. [In Portuguese].

Barros GSAC (2004). Economia da comercialização. Piracicaba: FEALQ, 227p.

Biagi JD, Bertol R, Carneiro MC (2002). Armazéns em unidades centrais de armazenamento. In: LORINI I, MIIKE LH, SCUSSEL VM (Ed.). Armazenagem de grãos. Campinas: Instituto Bio Geneziz, cap. [In Portuguese]. 3:157-174.

Caixeta Filho JV (2010). Logística para a agricultura brasileira. Revista Brasileira de Comércio Exterior. São Paulo, [In Portuguese]. 24(103):18-30.

Callado AAC (2008). Agronegócio. 2. ed. São Paulo: Atlas. [In Portuguese].

Cavalett O, Ortega E (2007). Emergy and fair trade assessment of soybean production and process in Brazil. Manag. Environ. Qual. Int. J. 18(6):657-668.

Christopher M (2011). Logística e gerenciamento da cadeia de suprimentos: criando redes que agregam valor. 2ª Ed. São Paulo: Cengage Learning. [In Portuguese].

Cnt (2013). CONFEDERAÇÃO NACIONAL DO TRANSPORTE. O sistema ferroviário brasileiro. Brasília: CNT, 58p.

Cnt (2014). CONFEDERAÇÃO NACIONAL DO TRANSPORTE. Plano CNT de transporte e logística, 2014. Disponível em: <http://www.cnt.org.br/Paginas/index.aspx>. [In Portuguese].

Conab (2014). Companhia Nacional de Abastecimento. Acompanhamento da safra brasileira: Grãos, v.1 – Safra 2013/14, n. 12 - décimo segundo levantamento, setembro. [In Portuguese].

Correa VHC, Ramos P (2010). A precariedade do transporte rodoviário para o escoamento da produção de soja do Centro-Oeste: situação e perspectivas. Rev. Econ. Soc. Rural, Piracicaba, SP, [In Portuguese]. 48(02):447-472.

Faro R, Faro F (2010). Competitividade no Comércio Internacional: acesso das empresas brasileiras aos mercados globais. 1. ed. São Paulo: Atlas, [In Portuguese] pp.15-36.

Ferrari RC (2006). Utilização de modelo matemático de otimização para identificação de locais para instalação de unidades armazenadoras de soja no estado do Mato Grosso. 2006, 185f. Dissertação (Mestrado) – ESALQ/USP (Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo). Piracicaba. [In Portuguese].

Fleury F (2005). A infra-estrutura e os desafios logísticos das exportações brasileiras. Centro de Estudos em Logísticas (CEL), Instituto COPPEAD de Administração, Universidade Federal do Rio de Janeiro (UFRJ). [In Portuguese].

Frederico S (2008). O novo tempo do cerrado: expansão das fronteiras agrícolas e controle do sistema de armazenamento de grãos. 2008, 273f. Tese (Doutorado) – Universidade de São Paulo (Faculdade de Filosofia, Letras e Ciências Humanas/Departamento de Geografia).

São Paulo. [In Portuguese].
Freitas MCM (2011). A Cultura da Soja no Brasil: O crescimento da produção brasileira e o surgimento de uma nova fronteira agrícola. Enciclopédia Biosfera – Centro Científico Conhecer, Goiânia, [In Portuguese] 7(12):1-12.
Gameiro AH, Caixeta-Filho JV (2010). Índices de preços para o transporte de cargas: o caso da soja. Nova Economia, Belo Horizonte,. [In Portuguese] 20(1):121-163.
Goularti FA (2007). Melhoramentos, reaparelhamentos e modernização dos portos brasileiros: a longa e constante espera. Economia e Sociedade, Campinas, [In Portuguese] 16(3)455-489.
Hijjar MF (2004). Logística, soja e comércio internacional. Rev. Tecnol. Centro Estudo Log. [In Portuguese] 9:70-75.
Hirakuri MH, Lazzarotto JJ (2011). Evolução e perspectiva de desempenho econômico associados com a produção de soja nos contextos mundial e brasileiro. 3.ed. Londrina: Embrapa Soja, 67 p. (Documentos / Embrapa Soja, ISSN 2176-2937; P. 319).
Maia GBS, Pinto AR, Marques CYT, Lyra DD, Roitman FB (2013). Panorama da armazenagem de produtos agrícolas no Brasil. Rev. do BNDES, [In Portuguese] 40:161-194.
Ministério dos Transportes (2010). Secretaria de Política Nacional de Transportes. Diretrizes da Politíca Nacional de Transporte Hidroviário. Brasília, DF. [In Portuguese].
Ministério dos Transportes (2014). Transporte rodoviário. Disponível em: <http://www.transportes.gov.br/transporte-rodoviario-relevancia.html>. [In Portuguese].
Neto CASC, Filho BP, Romminger AE, Ferreira IM, Vasconcelos LFS (2009). Gargalos e Demandas da Infraestrutura Portuária e os Investimentos do PAC: Mapeamento IPEA de Obras Portuárias. Primeira Edição, Inst. Pesqui. Econ. Aplicada: Bras. [In Portuguese]. http://www.ipea.gov.br/portal/index.php?option=com_content&view=article&id=4979
Nogueira JS, Tsunechiro A (2011). Pontos críticos da armazenagem de grãos no Brasil. Análise e Indicadores do Agronegócio. Inst. Econ. Agríc. [In Portuguese]. 6(4):5.
Pblog (2013). Plano Brasil De Infraestrutura Logística: uma abordagem sistémica. Sistema CFA/CRAs, Brasília, DF, [In Portuguese] 124 p.
Petraglia J (2009). Infraestrutura logística sob o prisma da exportação de etanol brasileiro. Rev. Gestão Reg. Santos [In Portuguese] 25(74):107-116.
Pontes H, Carmo B, Porto A (2009). Problemas logísticos na exportação brasileira da soja em grão. Rev. Eletr. Sistemas & Gestão, [In Portuguese] 4(2):155-181.
Puzzi D (2000). Abastecimento e armazenamento de grãos. Campinas: Instituto Campineiro de Estudos Agrícolas, [In Portuguese] 660 p.
Resende ES, Bonjour SCM, Figueiredo AMR (2007). Análise de rotas alternativas de transporte: o caso da soja de Mato Grosso. Revista de Estudos Sociais, ano 9, n. 1 e 2. [In Portuguese].
Rodrigues PRA (2007). Introdução aos sistemas de transporte no Brasil e à logística internacional. 4 ed. rev. e apl. São Paulo: Aduaneiras. [In Portuguese].
Roessing AC, Lazzarotto JJ (2004). Criação de empregos pelo complexo agroindustrial da soja. Londrina: Embrapa Soja, (Embrapa Soja. Documentos, 233). [In Portuguese] 50 p.
Roessing AC, Telles TS, Guimarães MF (2007). Perfil da infraestrutura de transportes para o escoamento da soja no Brasil. In: XLV Congresso da Sociedade Brasileira de Economia, Administração e Sociologia Rural SOBER, 45, Londrina, Paraná: Universidade Estadual de Londrina, 22 a 25 de jul. [In Portuguese].
Soares MG, Caixeta-Filho JV (1997). Caracterização do mercado de fretes rodoviários para produtos agrícolas. Gestão & Produção, [In Portuguese] 4(2):186-203.
Timossi AJ (2003). Grandes mudanças no escoamento de grãos. FNP Consultoria & Agroinformativos. [In Portuguese] pp. 427-478.
Uderman S, Rocha CH, Cavalcante LR (2012). Modernização do sistema portuário no Brasil: uma proposta metodológica. J. Transport Literature, [In Portuguese] 6(1):221-240.
US Soybean Export Council (2008). How the global oilseed and grain trade works. Soyatech, LLC, Southwest Harbor, Maine, US.
Usda (2014). United States Departament of Agrculture. World Agricultural Supply and Demand Estimates. Dez. 2014. Disponível em: <http://www.usda.gov/oce/commodity/wasde/latest.pdf>.
Vasques EF, Eidelchtein C (2009). Logística Internacional. In: SEGRE, German (Org.). Manual prático de comércio exterior. 2. ed. São Paulo: Atlas, [In Portuguese] pp. 121-151.
Weber AE (2005). Excelência em beneficiamento e armazenagem de grãos. Canoas, RS: Sales Editora. [In Portuguese].
Zemolin EM (2013). Análise da evolução da competitividade e da inserção externa do complexo soja brasileiro. 2013, 111f. Dissertação (Mestrado) – Universidade Federal do Rio Grande do Sul, Faculdade de Ciências Agronômicas. Porto Alegre. [In Portuguese].

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Full Length Research Paper

Physiological quality of second crop soybean seeds after drying and storage

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This work was carried out with the goal of determining the immediate and latent effects of different temperatures of drying air on the physiological quality of soybean seeds produced during the second crop. The seeds, which were collected close to their physiological maturity and had a moisture content of approximately 23% (w.b.), were exposed to different drying temperatures (40, 50, 60, 70, and 80°C) until their moisture content reached 12.5 ± 0.7% (w.b.). These were then stored in an environment with no temperature or humidity control for 180 days. The physiological quality was evaluated every 45 days thereafter by germination, first count, accelerated aging, modified cold, electrical conductivity, and tetrazolium chloride-based tests. From the results obtained, we conclude that a) an increase in the temperature of drying air influences the physiological quality of the soybean seeds produced during the second crop, and this effect is enhanced by the duration of storage; b) the viability and vigor are inversely related to both of these factors; and c) an air temperature of 40 °C can be recommended for drying second crop soybean seeds.

Key words: *Glycine max* L., viability, seed vigor, post-harvest.

INTRODUCTION

The attainment and maintenance of soybean seed quality has always been one of the main challenges of the production system (Marcos Filho, 2013). In this context the use of good quality seeds enables access to genetic advances, with quality assurance and localization technologies in different regions, ensuring higher productivity. Therefore, the soybean crop establishment with high quality seeds is of key importance (Pádua et al., 2014). However, given the rational use of new agricultural frontiers there is an increase demand for some changes towards the soybean seeds production systems.

The implementation of crops oriented toward the production of soybean seeds, once commonly limited to main crops, started to occur also in second crops (Albrecht et al., 2009). Thus, because the temperature and humidity levels vary through different stages of physiological maturity, as well as in the post-harvest phase, some questions arise. Namely, issues arose

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concerning the procedures used in harvesting, drying,and storage, given the likelihood that frosts can force an early harvest, and therefore essentially force the artificial drying of the crop. However, other scenarios also came up concerning the environmental conditions commonly found in these periods; as, for example, the possibility of storing seeds in an uncontrolled environment, although with temperatures below 25°C. This would reduce refrigeration costs while guaranteeing the quality of the seeds.

Drying essentially reduces the biological activity of the seeds, thus hindering the possibility of chemical and physical changes that could occur in the product during storage (Barrozo et al., 2014). This in turn aims to enable the storing of the product in a safe and effective manner until its commercialization. However, both operations could result in a reduction of the quality of the seeds, if not carried out properly. Drying can have an immediate damaging effect on the harvested product and storage might amplify such damages (Afonso Júnior and Corrêa, 2000; Deliberali et al., 2010; Schuh et al., 2011).

Regarding the damage that can result from these factors, we mainly highlight the detrimental effect on the seeds' physiological characteristics. In particular, seeds exposed to improper drying and storage show a reduction in germination and vigor (Resende et al., 2012; Faria et al., 2014; Rathinavel, 2014; Paraginski et al., 2015). Because of its high cost/benefit, even a single soy seed is of high value within a soybean production system (Sediyama et al., 2013). Therefore, it is important to understand the relationship between the quality of seeds and the factors that affect their performance - such as drying and storage. Thus, the goal of this work was to evaluate the immediate and latent effects of different drying air temperatures on the physiological quality of soybean seeds harvested from second crops.

MATERIALS AND METHODS

This study consisted of two phases. The first phase dealt with seeds production, occurring between January and May of 2014, at the São Lourenço's farm, located at 22°11′58.06″ S, 54°53′24.32″ W, and at 452 m of altitude, in the municipality of Dourados, MS. The second phase covered the drying, storage, and evaluation of the physiological qualities of the seeds, carried out at the Laboratory for Pre-processing and Storage of Agricultural Products, and the Laboratory of Physical Properties of Agricultural Products, both of which belong to the School of Agricultural Sciences of the Federal University of Grande Dourados (UFGD), also located in the municipality of Dourados, MS.

The soybean cultivar used to produce seeds was SYN 1059 RR (V-TOP), which was manually harvested at full physiological maturity, according to the specifications by Fehr and Caviness (1977), with moisture content of approximately 23 ± 0.5% (w.b.). After this phase, the seeds were subjected to drying process at temperatures of 40, 50, 60, 70, and 80 °C. The reduction in the mass of the product was monitored by a gravimetric method until a final moisture content of 12.5 ± 0.7% (w.b.) was achieved. In this process, the times required for each drying temperature to achieve the predetermined moisture content were 230, 160, 112, 75, and 57 minutes, respectively.

The drying took place in an experimental fixed-bed dryer, with a drying chamber of 0.80 m diameter and 1.0 m height. The experimental dryer used a series of electric resistors as a heating source, had a total power of 12 kW, and associated to this, an IBRAM centrifugal fan (model VSI-160) with a power of 0.75 kW. Temperature control was achieved using a universal Proportional-Integral-Derivative (PID) process controller (model N1200, Novus). The air flow rate used was 0.2 $m^3 s^{-1} m^{-2}$, selected by a frequency inverter connected to the fan motor. The water reduction rate (WRR) of soybean seeds was determined by monitoring the drying process for each selected temperature used. This was determined using Equation 1, according to Corrêa et al. (2001), who defined WRR as the amount of water that a certain product loses per dry matter unit per unit of time:

$$\mathrm{WRR} = \frac{\mathrm{Mw_0} - \mathrm{Mw_i}}{\mathrm{MS}\,(\mathrm{t_i} - \mathrm{t_0})} \quad (1)$$

where, WRR: water reduction rate, in kg $kg^{-1} h^{-1}$; Mw_0: previous total water mass, in kg; Mw_i: current total water mass, in kg; MS: dry matter mass, in kg; t_0: total time for previous drying, in h; and t_i: total time for current drying, in h.

After drying, soybean seeds were conditioned through a drying process in non-hermetic metal containers. These, when closed, were kept in an environment with no temperature or relative humidity control for a period of 180 days. The physiological qualities of the seeds and their moisture content were evaluated and measured immediately after drying and every 45 days thereafter. The temperature and relative humidity of environmental air were recorded during the storage period by using two thermo-hygrometers installed next to the containers.

The quality of the seeds was determined by means of germination, first count, accelerated aging, modified cold, electrical conductivity, and tetrazolium chloride-based tests. A gravimetric method was employed to determine the moisture content levels. The test was performed in two repetitions, using an oven kept at 105 ± 3 °C for 24 h (Brasil, 2009). The germination and first count tests were performed concurrently, using four sub-samples of 50 seeds for each treatment, distributed on paper towels moistened with distilled water, in a volume equal to 2.5 times the mass of the dried paper used and then kept at 25 °C in a Mangelsdorf-type germinator. The analyses were carried out on the fifth and eighth days after the test. The percentage of normal seedlings on the fifth day was used for first count, while that on the eighth day was used for the germination test, according to the criteria established in Rules for Seeds Analyses (Brasil, 2009).

The accelerated aging test was carried out according to the methodology described by Marcos Filho (1999), for which 300 seeds for each treatment were distributed in a single uniform layer on stainless steel screens. These were then suspended inside a Gerbox type plastic box, containing 40 ml of distilled water at the bottom. The boxes were then closed and kept in a BOD chamber for 48 h at a controlled temperature of 41 °C. After this period, the moisture content of the seeds was determined, and a germination test (Brasil, 2009) was also performed, with the percentage of normal seedlings being computed on the fifth day after starting the test.

The cold modified test was performed according to methodology described by Barros et al. (1999). Four subsamples of 50 seeds from each treatment were used. The seeds were distributed on paper towels moistened with distilled water, in a volume equal to 3 times the dry paper. These rolls were kept in a BOD chamber for 5 days at a temperature of 10 °C. They were then transferred to a Mangelsdorf-type germinator, with a controlled temperature of 25 °C, where they stayed for an additional four days, after which the percentage of normal seedlings was computed.

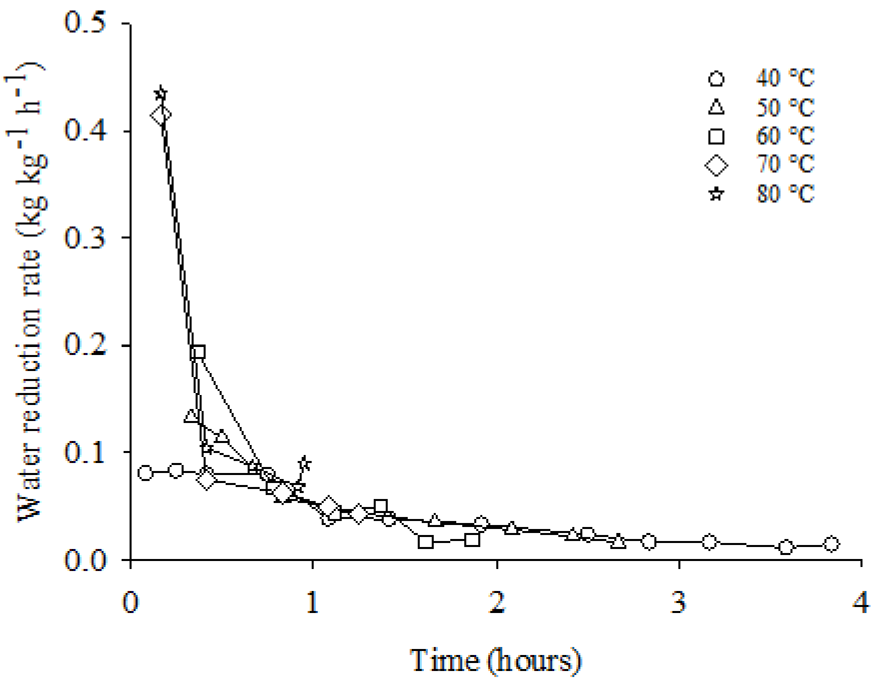


Figure 1. Water reduction rate for soybean seeds during the drying process at different temperatures.

In the electrical conductivity evaluation, four subsamples of 50 seeds for each treatment were weighed and left to soak in 75 ml distilled and deionized water for 24 h at 25°C, in 200 ml plastic cups. After this period, the electrical conductivity of the soaking solution was measured using the CG 1800 conductivity meter made by Gehaka. The results obtained from the readings, in µS cm^{-1}, were divided by the respective mass, with the final result being related to the test expressed in µS cm^{-1} g^{-1} (Vieira and Krzyzanowski, 1999).

The tetrazolium test, performed the following method described by Delouche (1962) and adapted by França Neto et al. (1999), made use of two subsamples of 50 seeds for each treatment. First the seeds were preconditioned for 16 h at 25°C in a wet towel moistened with distilled water, the volume of which corresponded to 2.5 times the mass of the dry paper. Then the seeds were taken through a coloring phase, where they were submerged in a tetrazolium chloride solution (0.075%) for 180 min, at a temperature of 40°C. At the end of that period, seeds were classified from 1 to 5 on their viability and from 1 to 3 on their vigor.

The experiment was carried out in 5 × 5 sub-divided plot arrangements, with five air-drying temperatures in each plot, and 5 different storage periods in each sub-plot, in a completely randomized design. A polynomial regression analysis was carried out to evaluate the latent effect of drying temperatures. The models were selected taking into consideration the magnitude of the coefficient of determination (R^2), the significance of the regression, by the F-test, and the phenomenon under study.

RESULTS AND DISCUSSION

Curves of water reduction rate obtained during the soybean seeds drying process showed that, according to the use of higher temperatures, such as 60, 70, and 80°C, this variable was clearly pronounced at the beginning of the process (Figure 1). However, over time this behavior became more homogenous between the drying treatments, since the water on the surface of the product was gradually substituted by a front of evaporation that moved into the interior of the same. Furthermore, due to the involvement of more complex mechanisms in water displacement to the interior or exterior of the seed, such as liquid diffusion and capillary action, the speed of the process decreased. Thus, the similarity in WRR among the drying treatments applied becomes evident 50 min after the start of the drying.

The variations in temperature and relative humidity throughout the experiment can be seen in Figure 2. Then, under these circumstances, the mean temperature observed during the storage period was of 21.4°C, where the maximum and minimum temperatures registered were 28.4 and 14.5°C, respectively. As for the relative humidity, the mean value observed was of 57.9%, the maximum was 83.4%, and the minimum was of 47.3%

It can be seen in Table 1 that, due to seed hygroscopicity, the moisture content might vary during storage, mainly due to the uncontrolled temperature and relative humidity conditions favoring phenomena such as sorption and desorption, as already observed by Tiecker Junior et al. (2014) and Bessa et al. (2015). Moreover, over the 180 days of storage, we also found that all of the batches analyzed showed increased water levels in evaluations carried out at 45 and 180 days of storage. This is likely because those reading dates followed

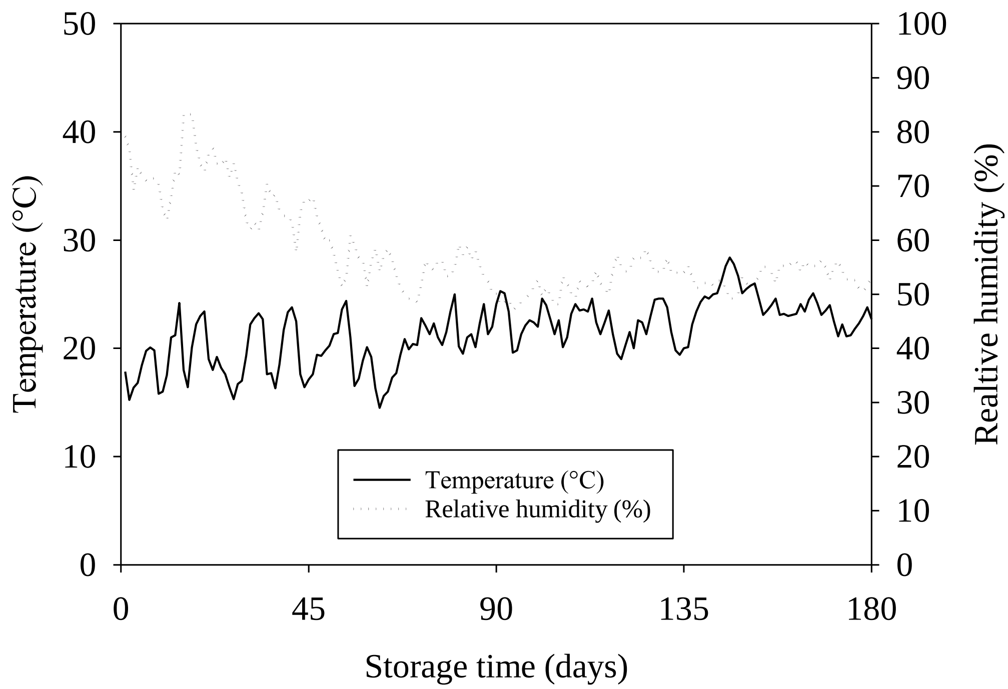


Figure 2. Daily mean of air relative humidity and temperature, during 180 days of storage of soybean seeds in a non-controlled environment.

Table 1. Mean moisture content (% w.b.) of soybean seeds after submitted to drying temperature and storage time.

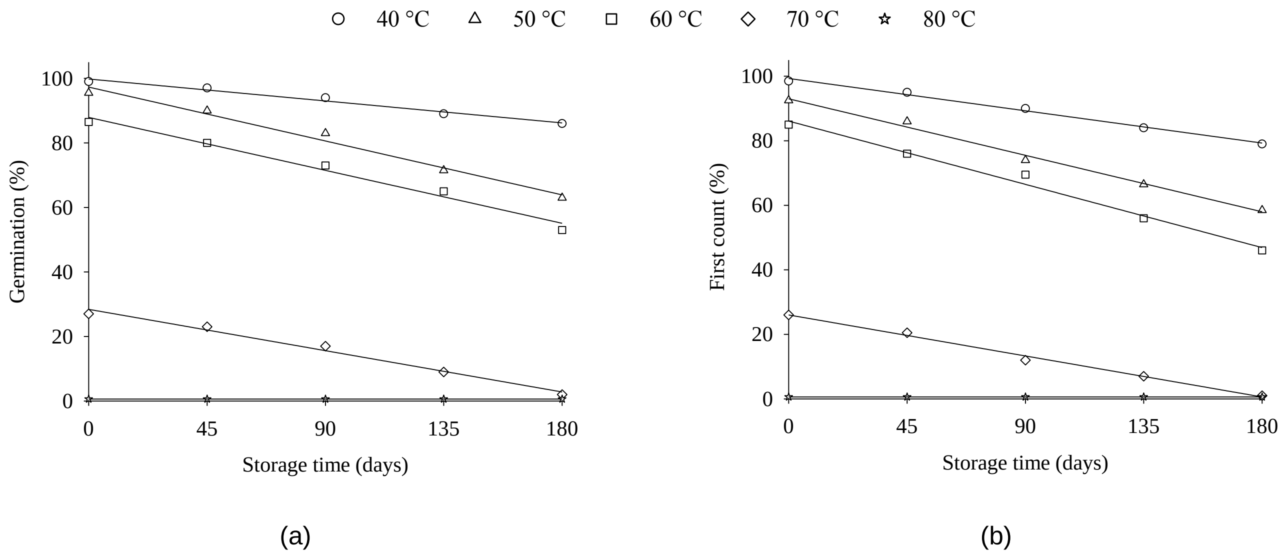
Storage time (days)	Drying temperature (°C)				
	40	50	60	70	80
0	13.2	12.7	12.4	12.5	12.0
45	13.4	12.8	12.4	12.6	12.3
90	12.6	12.1	12.0	11.8	11.4
135	11.1	10.6	10.6	10.5	10.2
180	11.3	11.0	10.8	10.8	10.4

periods of higher relative humidity, which would favor sorption.

Nevertheless, in general there was a reduction in moisture content in every batch evaluated (Table 1). This is probably due to the reduction in the values referring to the relative humidity or the atmosphere according to the weather (Figure 2) and also the centesimal composition of seeds, which for being aleuro-oleaginous tend to retain less water inside, since oily substances are less hydrophilic. Considering the germination and first count behavior against the tested factors, it can be observed that both variables had a lower percentage of normal seedlings. This negative effect was noticed immediately during the drying process and was caused by the increase in air temperature being linearly enhanced throughout the storage period (Figure 3).

The germination percentages were registered immediately after drying, for temperatures of 40, 50, 60, 70, and 80°C, which were of 100, 97, 88, 28, and 1%, respectively. It is worth noting the small difference between drying treatments at 40 and 50°C (Figure 3a). However, due to the latent behavior previously mentioned to occur, already in the evaluation carried out at 45 days the difference between the two treatments started to be clearer. The daily reduction in the percentage of normal seedlings caused at 50°C was equal to twice that favored by a temperature of 40°C, as assessed for temperatures of 60, 70, and 80°C.

In view of this behavior, at the end of the experiment the germination values registered for temperatures 40, 50, 60, 70, and 80°C were of 86, 64, 55, 3, and 1% (Figure 3a). Then, if adopted the parameters established in the standard guidelines number 45 instituted by Brasil (2013), which defines as lower limit for commercialization of soybean seeds 80% germination, only the batch of seeds dried at 40°C could be commercialized by the end



$G_{40\,°C} = 99.8000 - 0.0756\ ST \quad (R^2 = 0.9729)$
$G_{50\,°C} = 97.3000 - 0.1856\ ST \quad (R^2 = 0.9784)$
$G_{60\,°C} = 87.9000 - 0.1822\ ST \quad (R^2 = 0.9774)$
$G_{70\,°C} = 28.4000 - 0.1422\ ST \quad (R^2 = 0.9820)$
$G_{80\,°C} = 0.6$

$FC_{40\,°C} = 99.3000 - 0.1111\ ST \quad (R^2 = 0.9905)$
$FC_{50\,°C} = 93.0000 - 0.1944\ ST \quad (R^2 = 0.9898)$
$FC_{60\,°C} = 86.1000 - 0.2178\ ST \quad (R^2 = 0.9841)$
$FC_{70\,°C} = 26.0000 - 0.1411\ ST \quad (R^2 = 0.9915)$
$FC_{80\,°C} = 0.6$

Figure 3. Results of the percentage of normal seedlings for germination test (a) and first count (b) of soybean seeds, as a function of drying temperature and storage time.

of the experiment. The product dried with a temperature of 50 and 60°C loses this germination level approximately 150 and 101 days after drying, while the others lose this level as early as during the drying process itself.

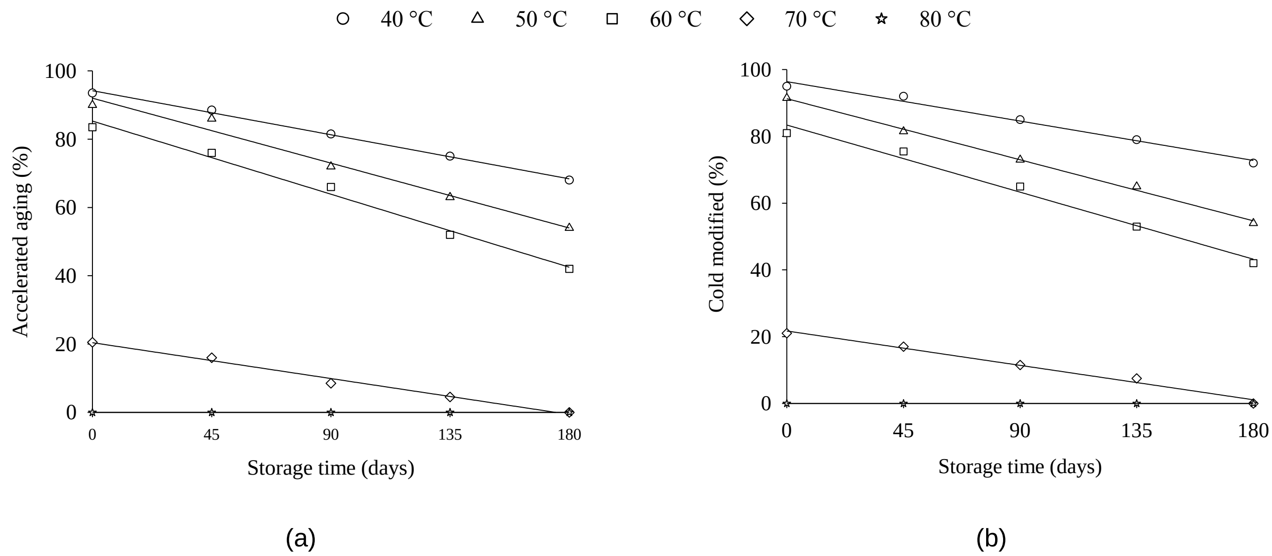
The first count test showed a behavior analogous to that of the germination test, but also showed other parameters beside the presence of normal seedlings at the fifth day. It also indirectly indicates the speed of the germination process, as already documented by Tillmann and Menezes (2012). Thus, since the results obtained are reduced when the air temperature and the duration of the storage period increases, it is possible to infer that germination speed was immediately reduced while latent. This is indicative of a decrease in seeds vigor and their respective storage potential (Figure 3b).

Ullmann et al. (2015), evaluating the effect of different drying temperatures on the physiological quality of sweet sorghum seeds, found similar results to those of this study. The authors found that the increase in drying air temperature results in a decrease of both germination and germination speed, expressed indirectly by the first count. As suggested by Afrakhteh et al. (2013), and also shown in this work, this fact results from the increase in the rate at which water is drained from the product, the WRR, which increased along with the drying temperature. This great temperature gradient between the inside and the periphery leads to the creation of ridges in the soft tissue and micro fissures in the cotyledon, and negatively affects the quality of the seed. In addition, as clarified by Mbofung et al. (2013) and observed in this study, this kind of situation can increase the susceptibility of the material to latent damages, or even increase its deterioration, thus reducing the longevity and physiological quality of the product itself.

The accelerated aging tests and the modified cold test also indicate an immediate and long-term reduction in the vigor of seeds with an increase in both of the factors (Figure 4). They also indicate a possible progressive increase of material's susceptibility to adverse environmental conditions imposed by the tests, since all results assessed by these tests were lower than those assessed by germination and first count tests, which in turn were conducted under conditions theoretically ideal for the crop (Figure 3).

Immediately after drying, for example, the accelerated aging test revealed normal seedling levels of 94, 92, 85, and 20% for drying processes of 40, 50, 60, and 70°C (Figure 4a). As for the modified cold test, the results for these same temperatures tested were 96, 91, 83, and 22% of normal seedlings (Figure 4b), indicating the deleterious effects of temperatures above 40°C on seed vigor.

Although the results obtained by Menezes et al. (2012) refer to rice culture, a species belonging to another family and with different physical and chemical structures from soybean, they corroborate the results of this study. The



$AA_{40\,°C} = 94.2000 - 0.1433\ ST \quad (R^2 = 0.9959)$
$AA_{50\,°C} = 92.0000 - 0.2111\ ST \quad (R^2 = 0.9746)$
$AA_{60\,°C} = 85.3000 - 0.2378\ ST \quad (R^2 = 0.9870)$
$AA_{70\,°C} = 20.4000 - 0.1167\ ST \quad (R^2 = 0.9853)$
$AA_{80\,°C} = 0$

$CM_{40\,°C} = 96.4000 - 0.1311\ ST \quad (R^2 = 0.9807)$
$CM_{50\,°C} = 91.3000 - 0.2033\ ST \quad (R^2 = 0.9964)$
$CM_{60\,°C} = 83.4000 - 0.2233\ ST \quad (R^2 = 0.9808)$
$CM_{70\,°C} = 21.7000 - 0.1144\ ST \quad (R^2 = 0.9828)$
$CM_{80\,°C} = 0$

Figure 4. Results of the percentage of normal seedlings for tests of accelerated aging (a) and modified cold (b) of soybean seeds, according to the drying temperature and storage time.

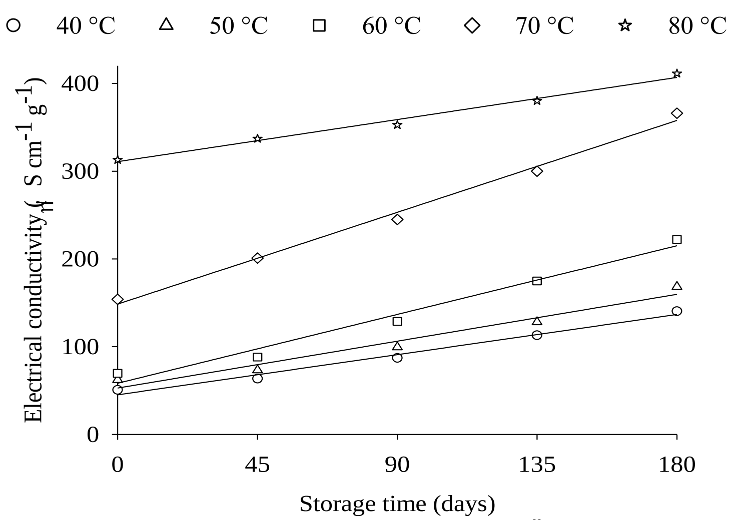
authors also found that the susceptibility of seeds to adverse environmental conditions, such as high temperatures, relative humidity, and cold, is greater in seeds subjected to higher drying temperatures. This fact is explained by the authors as the result of an increased number of cracks found in seeds dried at higher temperatures.

The electrical conductivity test confirms that the increased drying temperature is a determining factor in the reduction of the physiological quality of the seeds, as well in the worsening of conditions in the course of the storage. The results obtained by this test immediately following drying increased and, over time, continued to show a linear growth over time for every drying treatment (Figure 5).

In addition, this test provides information about the integrity of the material, especially concerning its cellular membranes (Marcos Filho, 2015). The results obtained allow hypothesizing that the increase in both factors, besides causing a physical disorder in the tegumentary tissue of the seeds in the surface of the cotyledons, might have resulted in a sort of metabolic disorder that might have led to severe leaching of a series of nutrients essential for germination and the development of vigorous seedlings. According to Menezes et al. (2012), the electrical conductivity test is very effective in identifying damage resulting from drying, since the values obtained by the test typically correlate directly with the number of cracks on the seeds and the temperatures used in drying. Increasing the drying temperature to impair tegumentary and cellular integrity favors solutes leaching, since cell membranes do not efficiently perform their function as selective barriers in first stages of the soaking process. This eventually increases the conductivity values of the solution (Resende et al., 2012).

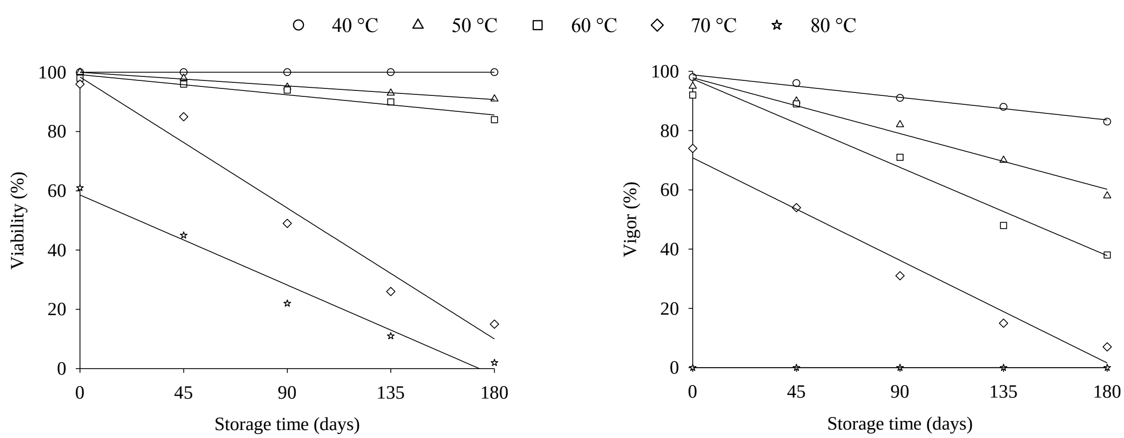
A series of results similar to those found in the electrical conductivity test were found using other crops sensitive to temperatures higher than 40 °C, as bean seeds (Faroni et al., 2006), jatropha (Ullmann et al., 2010), sweet sorghum (Ullmann et al., 2015) and for adzuki bean seeds (Resende et al., 2012). The tetrazolium test also demonstrated a negative effect caused by the increase in air temperature and, subsequently, the amplification of this effect during storage. However, only during storage was it possible to check the difference between the drying treatment, since for viability only the batch of seeds dried at 80°C was immediately inferior to the others, and for the vigor the batches dried at 70 and 80°C showed similar behavior (Figure 6).

According to the results obtained, there was a growing negative tendency correlated with an increase in drying temperature. This shows how much the interference in the initial quality of a seed batch might negatively affect its storage potential and latent maintenance necessary for its physiological quality (Figure 6).



$EC_{40\ °C} = 45,1447 + 0,5082\ ST \quad (R^2 = 0,9799)$
$EC_{50\ °C} = 52,8178 + 0,5932\ ST \quad (R^2 = 0,9506)$
$EC_{60\ °C} = 58,3232 + 0,8707\ ST \quad (R^2 = 0,9719)$
$EC_{70\ °C} = 148,5115 + 1,1625\ ST \quad (R^2 = 0,9906)$
$EC_{80\ °C} = 310,8511 + 0,5329\ ST \quad (R^2 = 0,9833)$

Figure 5. Results of electrical conductivity of the soaking solution of soybean seeds, according to drying temperature and storage time.



(a) (b)

$VIA_{40\ °C} = 100.0000$
$VIA_{50\ °C} = 100.0000 - 0.0511\ ST \quad (R^2 = 0.9925)$
$VIA_{60\ °C} = 99.2000 - 0.0756\ ST \quad (R^2 = 0.9177)$
$VIA_{70\ °C} = 98.4000 - 0.4911\ ST \quad (R^2 = 0.9550)$
$VIA_{80\ °C} = 58.6000 - 0.3378\ ST \quad (R^2 = 0.9617)$

$VIG_{40\ °C} = 98.8000 - 0.0844\ ST \quad (R^2 = 0.9782)$
$VIG_{50\ °C} = 97.8000 - 0.2089\ ST \quad (R^2 = 0.9642)$
$VIG_{60\ °C} = 97.4000 - 0.3311\ ST \quad (R^2 = 0.9397)$
$VIG_{70\ °C} = 70.8000 - 0.3844\ ST \quad (R^2 = 0.9645)$
$VIG_{80\ °C} = 0$

Figure 6. Results of the percentages of viable seeds (a) and vigor (b) in the context of the tetrazolium chloride test, according to the drying temperature and storage time.

Afonso Júnior and Corrêa (2000) obtained similar results as those found in this study when they evaluatedthe physiological quality of bean seeds. The authors verified that when they imposed a drying temperature of 40°C, both immediately drying and after 180 days of storage, the viability and vigor of seeds harvested with approximately 25% moisture content was mostly unaltered. However, when the imposed temperatures were higher than this, for example 50°C, especially during storage, the same parameters were reduced since the latent effect resulting from the increased drying temperature was characterized by favoring the action of degenerating problems and, subsequently, having a negative impact on the physiological quality of the harvested product.

Conclusion

The increase in the drying temperature influences the physiological quality of soybean seeds produced by a second crop, and this effect is enhanced by the storage period. In the range from 40 to 80°C, an air drying temperature of 40°C should be recommended to dry soybean seeds produced in a second crop. In this way, the germination, viability, and vigor are all diminished with the increase in drying temperature, as well as with storage time of up to 180 days.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENT

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REFERENCES

Afonso JPC, Corrêa PC (2000). Immediate and latent drying effects of bean seeds harvested with different moisture levels. Ciênc. Agrotechnol. 24(Special edition): pp. 33-40.

Afrakhteh S, Frahmandfar E, Hamidi A, Ramandi HD (2013). Evaluation of Growth Characteristics and Seedling Vigor in Two Cultivars of Soybean dried under different Temperature and Fluidized bed dryer. Intl. J. Agric. Crop Sci. 5(21):2537-2544.

Albrecht LP, Braccini AL, Ávila MR, Scapim CA, Barbosa MC, Stülp M (2009). Soybean seeds produced in out season in west of Paraná state. Acta Sci. Agron. 31(1):121-127.

Barros ASR, Dias MCLL, Cicero SM, Krzyzanowski FC (1999). Testes de frio. In Vigor de sementes: conceitos e testes, (Eds. Krzyzanowski FC, Vieira RD, França NJB) Abrates, Londrina-PR. pp. 5(1):5-15.

Barrozo MAS, Mujumdar A, Freire JT (2014). Air-Drying of seeds: A review. Dry Technol. 32(10):1127-1141.

Bessa JFV, Donadon JR, Resende O, Alves RMV, Sales JF, Costa LM (2015). Storage of crambe seeds in different containers and environments: Part I - Physiological quality. Rev. Bras. Eng. Agrí. Ambient. 19(3):224-230.

Brasil (2013). Ministério da Agricultura, Pecuária e Abastecimento, Instrução Normativa n° 45, de 17 de setembro de 2013. Padrões de Identidade e Qualidade para a produção e a comercialização de sementes. Diário Oficial da União da República Federativa do Brasil de 20/09/2013. Brasília. Seção 1.

Brasil (2009). Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes. Secretaria de Defesa Agrícola. Brasília: MAPA/ACS 399 p.

Corrêa PC, Machado PF, Andrade ET (2001). Kinetics of drying and the quality of popcorn. Ciênc. Agrotec. 25(1):134-142.

Deliberali J, Oliveira M, Durigon A, Dias ARG, Gutkoski LC, Elias MC (2010). Effects of drying process and storage time on technological quality of wheat. Ciênc. Agrotec. 34(5):1285-1292.

Faria RQ, Teixeira IR, Cunha DA, Honorato JM, Devilla A (2014). Physiological quality of crambe seeds submitted to drying. Rev. Ciênc. Agron. 45(3):453-460.

Faroni LRA, Cordeiro IC, Alencar ER, Rozado AF, Alves WM (2006). Influence of the harvest moisture contente and of the drying temperaure upon the bean quality. Rev. Bras. Eng. Agríc. Ambient. 10(1):148-154.

Fehr WR, Caviness CE (1977). Stages of soybean development. Special Report, no. 80, Ames: Iowa State University of Sci. Technol. 11 p.

França NJB, Krzyzanowski FC, Costa NP (1999). Metodologia do teste de tetrazólio em sementes de soja. In Vigor de sementes: conceitos e testes, (eds. Krzyzanowski FC, Vieira RD, França NJB). Abrates, Londrina-PR 8(5):1-28.

Marcos FJ (2015). Fisiologia de sementes de plantas cultivadas. 2.ed. Londrina, PR: Abrates 660 p.

Marcos Filho J (2013). Importance of the physiological potential of soybean seed. Informativo Abrates 23(1):21-24.

Marcos Filho J (1999). Teste de envelhecimento acelerado. In Vigor de sementes: conceitos e testes (eds. Krzyzanowski FC, Vieira RD, França Neto JB). Abrates, Londrina-PR. 3(1):3-24.

Mbofung GCY, Goggi AS, Leandro LFS, Mullen RE (2013). Effects of storage temperature and relative humidity on viability and vigor of treated soybean seeds. Crop Sci. 53(3):1086-1095.

Menezes NL, Pasqualli LL, Barbieri APP, Vidal MD, Conceição GM (2012). Drying temperatures on physical integrity, physiological quality and chemical composition of rice seeds. Pesqui. Agropec. Trop. 42(4):430-436.

Pádua GP, França NJB, Rossi RF, Cândido HG (2014). Agroclimatic zoning of the state of Minas Gerais for the production of high quality soybean seeds. J. Seed Sci. 36(4):413-418.

Paraginski RT, Rockenbach BA, Santos RF, Elias MC, Oliveira M (2015). Quality of maize grains stored at different temperatures. Rev. Bras. Eng. Agríc. Ambient. 19(4):358-363.

Rathinavel K (2014). Influence of storage temperature and seed treatments on viability of cotton seed (*Gossypium hirsutum* L.). Cotton Res. J. 6(1):01-06.

Resende O, Almeida DP, Costa LM, Mendes UC, Sales JF (2012). Adzuki beans (Vigna angularis) seed quality under several drying conditions. Food Sci. Technol. 32(1):151-155.

Schuh G, Gottardi R, Ferrari FE, Antunes LEG, Dionello RG (2011). Effects of two methods of drying on quality physical and chemical grain winter corn – RS, stored for 6 months. Semina: Cienc. Agrár. 32(1):235-244.

Sediyama T, Oliveira RCT, Nogueira APO (2013). Importância econômica da semente. In Tecnologia de produção de sementes de soja (ed. Sediyama T) pp.11-14, Mecenas, Londrina-PR.

Tiecker Junior A, Guimarães LE, Ferrari FE, Castro B, Del Ponte EM, Dionello RG (2014). Physicochemical quality of maize grain stored with different moisture contents in airtight and non-airtight environments. Rev. Bras. Milho Sorgo 13(2):174-186.

Tillmann MAA, Menezes NL (2012). Análise de sementes. In Sementes: fundamentos científicos e tecnológicos (eds. Peske ST, Villela FA, Meneghello GE). Editora Universitária da UFPel, Pelotas-RS. 3.Ed. pp.161-272,

Ullmann R, Resende O, Chaves TH, Oliveira DEC, Costa LM (2015). Physiological quality of sweet sorghum seeds dried under different conditions of air. Rev. Bras. Eng. Agríc. Ambient. 19(1):64-69.

Ullmann R, Resende O, Sales JF, Chaves TH (2010). Seed quality of jatropha under different drying air conditions. Rev. Ciênc. Agron. 41(3):442-447.

Vieira RD, Krzyzanowski FC (1999). Teste de condutividade elétrica. In Vigor de sementes: conceitos e testes. (eds. Krzyzanowski FC, Vieira RD, França NJB) Abrates. Londrina-PR. pp. 4(1):26.

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Full Length Research Paper

Response of Chinese cabbage to source and rate of N topdressing application in tropical soil in Brazil

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The aim of this study was to evaluate the response of Chinese cabbage (hybrid Deneko) to source and rate of N topdressing application. The experimental design was a randomized complete block, consisting of nine treatments (4x2+1 factorial design), with five replications, that is, four rates of N (54, 108, 162 and 216 kgha^{-1}); two sources of N (urea and ammonium sulphate); and a control (without N topdressing). The experiment was conducted from July to October, 2013. Forty days after transplanting, SPAD index (relative chlorophyll density) was evaluated. After harvesting, fresh matter; leaf number; head diameter and head height were evaluated. It was observed that sources of N did not influence any evaluated traits. For the SPAD index, head height and head diameter linearly increased by improving the rate of N, with values which range from 27.0 to 41.1 SPAD units; 25.9 to 29.3 cm; and 8.9 to 13.4 cm, respectively. The effect of N was quadratic for head fresh matter, with maximum value estimated at1217 g for162 kg N ha^{-1}.

Key words: *Brassica pekinensis*, urea, ammonium sulphate.

INTRODUCTION

Chinese cabbage (*Brassica pekinensis*) is a *brassicaceae* and it is characterized by its bright green leaves; a prominent white midrib; a short thick stalk; and a large compact globular head. These cultivars are good for growing areas with mild temperatures (Filgueira, 2008).
Chinese cabbage is rich in vitamins A, B, C, calcium, potassium, and fibre, which stimulates intestinal activity. It also provides vitamin B3 (niacin), which helps in gastrointestinal problems and nervous system disorders (Gordin et al., 2010). The state of São Paulo is the largest vegetable producer in Brazil and also excels in the production of Chinese cabbage with a 12,588 t of productivity per year (Filho and Camargo, 2015).

Brassicaceae extracts large amounts of nutrients from

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agricultural soil; accumulating high N levels, but they also have a high demand for S (Rathke et al., 2006; Berry et al., 2010). Additionally, the development of a sustainable and productive agriculture has to supplement fertilization management that involves appropriate nutrient management practice; right fertilizer source to each species and the best application time (Fageria and Baligar, 2005). In the literature, it is common to find studies showing that sources and rates of N can have a significant effect on the plants' nutrition; therefore, influencing growth, development and production of plants (Neeteson and Carton, 2001; Rahn, 2002; Sady et al., 2008). Nitrogen is a constituent of cellular components, proteins, nitrogenous bases, triptophane amino acid and chlorophyll (Fagan et al., 2016). Adequate rates of N in vegetative phase augment vegetative growth augmenting a capitation of light, ameliorating the photosynthesis resulting in higher yield.

When seedlings were transplanted to the groove in low and mid fertility soils, Filgueira (2008) recommended for tropical soils, an application of 40; 150-300; and 100-150 kg ha^{-1} of N, P_2O_5 and K_2O, respectively. However, Raij et al. (1997) recommended an application of 60; 400-600; and 180-240 kg ha^{-1} of N, P_2O_5 and K_2O, respectively. In addition, Raij et al. (1997) also suggested topdressing fertilization, such as 15-200 kg N ha^{-1} and 60-120 kg K_2O ha^{-1}. Tropical regions are characterized by weathered soils, greater intensity of rain and acidic soil which facilitates leaching and lack both N and S. In addition, tropical soils are characterized as presenting small amounts of organic matter because of the high temperatures and high microorganisms activity, reducing the availability of N and S over most cultures cycle, requiring replacement of nutrients by organic or chemical fertilizer.

Furthermore, sulphur is also present in many agricultural regions in the world and this nutrient has become a limiting factor for crop production, as an adequate S availability for plants stimulates higher growth, yield responses and quality (Abbey et al., 2002; Eriksen et al., 2004). In Brazil, the S is not considered a very important nutrient. In addition, it uses many formulations of nitrogen, phosphorus and sulfur and not potassium, which causes a reduction in the availability of sulfur in the soil for crops.

Plants have different abilities to absorb, translocate and use sulfur and therefore require different amounts of available ground SO_4^{-2}. Some plants, such as those of the family of legumes, brassicas and Liliaceae only express their genetic potential in terms of productivity and quality when the availability of this nutrient is high, and then established a critical level of 10 mg dm^{-3}, while for the remaining species, this low value is 5 mg dm^{-3}. Considering these reference values for the surface layer of soil, about 50% of the total area of tropical soils and sub-tropical South America can be considered deficient in sulfur. The availability of organic sulfur to plants depends on the processing of the inorganic forms almost exclusively in the form of sulfate ($SO4^{-2}$). In tropical soil conditions due to increased precipitation and temperature, there is a rapid depletion of organic matter and consequently low S content available to plants. In addition, both the total amount of sulfur as the adsorption capacity of SO_4^{-2} are lower in soils with low clay content and retention is further reduced by the application of lime and phosphate, these are highly practiced in Brazil because of the present acidic soils. Thus, there is a shift of this ion to the deeper layers, where it can be adsorbed because of higher clay content and lower levels of organic matter and pH (Rheinheimer et al., 2005). This same behavior is observed for N, and leaching losses due to high precipitation.

Schonhof et al. (2007) observed an increase in the broccoli head matter by applying sulphur. The sulfur is a constituent of diverse enzymes when cysteine and methionine amino acids are used in protein synthesis. The cysteine is the first stable organic compound synthesized in the sulfate assimilation pathway. They are used for the synthesis of a variety of metabolites and other important compounds, such as methionine, SAM, S-methylmethionine, [Fe/S] clusters, hormones, proteins, vitamins and enzyme cofactors. Methionine is sulfur containing essential amino acid, which is an important methyl group donor and a precursor of several metabolites such as ethylene, polyamines, and dimethyl sulfoniopropionate. It is also an important constituent of several peptides and proteins (Buchanan et al., 2015). Besides, the interaction between nitrogen and sulphur enables important growth parameters, such as biomass and yield, as S is an essential constituent of enzymes when associated with nitrogen metabolism, that is, nitrate and nitrite reductase (Salvagiotti and Miralles, 2008; Mendel, 1997; Campbell, 1999; Swamy et al., 2005; Takahashi and Saito, 1996; Koprivova et al., 2000; Hesse et al., 2004; Carfagna et al, 2011).

Taken all these considerations into account, this study aimed to evaluate the response of Chinese cabbage to source (ammonium sulphate and urea) and rate of N topdressing application.

MATERIALS AND METHODS

The experiment was conducted in the Sao Manuel Experimental Farm, Botucatu School of Agronomy, UNESP, Brazil (22°46'28"S, 48°34'37"W; 740 mm altitude). According to the Köppen classification, the climate in the region is mesothermic, *Cwa*, in other words, humid and subtropical; dry winter; rainy season from November to April. The mean annual rainfall of São Manuel is 1445 mm; the mean annual temperature of the warmest month is 22°C; and the mean temperature of the coldest month is 18°C (Cunha

Table 1. SPAD index (SPAD units), fresh matter, head diameter and head height of Chinese cabbage according to different sources of nitrogen. Agronomic Science School, São Manuel-SP, 2013.

Source of N	SPAD index (units)	Fresh matter (g plant^{-1})	Head diameter (cm)	Head heigth (cm)
Urea	35.8[a]	1082[a]	12.0[a]	28.1[a]
Ammonium sulphate	36.4[a]	1125[a]	12.1[a]	28.6[a]
C.V.	9.8%	10.1 %	8.2%	4.7%

Means followed by different letters differ by Tukey test at 5% probability.

and Martins, 2009).

The soil is classified as Dystrophic Red Latosol (Oxisoil). Soil samples were collected in agricultural area characterized per sandy soil. This soil were collected in 20 point in experimental area at a depth of 0-20 cm to determine their chemical properties: pH in $CaCl_2$, 6.4; Organic matter, 11 g dm^{-3}; phosphorus$_{resin}$, 13 mg dm^{-3}; H+Al, 12 mmol$_c$ dm^{-3}; Potassium, 1.9 mmol$_c$ dm^{-3}; calcium, 20 mmol$_c$ dm^{-3}; magnesium, 10 mmol$_c$dm^{-3}; base sum, 32 mmol$_c$ dm^{-3}; cation exchange capacity, 44 mmol$_c$ dm^{-3}; base saturation 82%.

The experimental design was a randomized complete block, with nine treatments (4x2+1 factorial design), five replications; 15 plants per plots, but only the central five were evaluated. Therefore, treatments consisted of five rates of N topdressing (0, 54, 108, 162 and 216 kg ha^{-1}); two sources of N (urea and ammonium sulphate) and a control (without N topdressing). According to the methodology described by Raij et al. (1997), 60 kg N ha^{-1}; 400 kg P_2O_5 ha^{-1}; 180 kg K_2O ha^{-1}; 40 t ha^{-1} of organic compost trade mark Provaso® (moisture content of 37.9%) was applied, which was evenly distributed over the whole bed surface (1.2 m width and 0.3 m height). S was not utilized in fertilizer application.

Hybrid Deneko® of enterprise Bejo was used. On July 10, 2013, sowing was performed in polypropylene trays of 200 cells, filled with coconut fibre substrate. On August 13, 2013, seedlings were transplanted separately into microplots of size 0.5 x 0.4 m. Weed control was done in hand form and sprinkler was used for irrigation.

Regarding the crop, the included doses exceeded the recommendation of Raij et al. (1997), that is, from 15 to 200 kg N ha^{-1}. At 15, 30 and 45 days after transplanting, topdressing was performed by adding 1/3 of the dose in each date. In addition, 90 kg ha^{-1} of potassium chloride was applied to the commercial source which is cheap according to the methodology described by Raij et al. (1997).

When the head started to close (40 days after transplanting), it was determined the SPAD index (relative chlorophyll density). Therefore, chlorophyll content was indirectly measured by using the Clorofilog® device. The measurement was performed in fully developed young leaves (40 days after transplanting) of three plants per plot.

On October 10, 2013 (three month after sowing), plants were harvested. the fresh matter, head height and head diameter were evaluated. Thus, all outer leaves were removed to evaluate the head. For fresh matter, a semi-analytical balance with a precision of 0.1 g was used; for head diameter, a digital calliper was used; and for height, a graduated ruler expressed in centimetres (cm) was used.

Data were subjected to analysis of variance and regression for rates of N. For the source of N, data were compared by Tukey test ($p < 0.05$) by the Sisvar software (Ferreira, 2010).

RESULTS AND DISCUSSION

With regards to the source of N, there were no differences for all the characteristics evaluated (Table 1), since the fertilizer was applied at the recommended rates (30-60 kg S ha^{-1}) as proposed by Raij et al. (1997) and Filgueira (2008) for brassica in tropical soils. This recommendation is similar to that of other brassicas like cabbage and cauliflower. A few studies have been conducted in order to evaluate the response of Chinese cabbage to this nutrient. However, Sanderson and Ivany (1996) presented an increase in the head cabbage matter, when S is applied. The sulfur is constituent of diverse enzymes when cysteine and methionine is the sulfur-containing amino acids used in protein synthesis. The cysteine is the first stable organic compound synthesized in the sulfate assimilation pathway. It is used for the synthesis of a variety of metabolites and other important compounds, such as methionine, SAM, S-methylmethionine, [Fe/S] clusters, hormones, proteins, vitamins and enzyme cofactors. Methionine is sulfur containing essential amino acid, which is an important methyl group donor and a precursor for several metabolites such as ethylene, polyamines and dimethyl sulfoniopropionate. It is also an important constituent of several peptides and proteins (Buchanan et al., 2015). Corrêa et al. (2013) evaluated rates of potassium in cabbage, with and without sulphur topdressing fertilization; but also presented no differences between sources for all the evaluated traits. Losák et al. (2008), when evaluating kohlrabi (another *brassicaceae*), also did not obtain any difference in the production when sulphur was added, which reduced the content of inorganic nitrate (NO_3^-, an anion) in the leaves; this anion could be harmful to human health if consumed in higher levels. High dietary intake due to the high nitrate content of certain vegetables has generated concern about the possible health effects. The toxicity of nitrate per se is low, but in humans, 5 to 10% of the ingested nitrate is converted to the more toxic nitrite by salivary or gastrointestinal reduction. Although, earlier reports linking nitrate with the occurrence of cancer are largely un-substantiated, other nitrate-induced syndromes, such as methaemoglobinaemia in infants (blue baby syndrome) have been confirmed (Elwam and El-Hamed, 2011).

However, Sanderson (2003), Schonhof et al. (2007) and Salvagiotti et al. (2009) observed an increase in broccoli productivity by applying sulphur. It is as a result of diverse enzymes used for the synthesis of hormones, proteins, vitamins and enzyme cofactors and a precursor for several metabolites such as ethylene and polyamines (Buchanan et al., 2015).

The lack of effect of the sources, that is, no difference by applying doses of S topdressing, perhaps indicates that the application thereof is not necessary. Considering that all the plots received 40 t ha^{-1} of organic compost (37.9% of moisture content), therefore, it may have met the nutrient demands for the plants throughout the cycle, that is, 0.19% of S in the compost dry matter, released up to 288 kg S ha^{-1}, which is higher than the maximum applied dose of ammonium sulphate (110 kg S ha^{-1}). For most vegetables, an application of large amounts of organic compost, which may be sufficient to meet the plants demand for S is recommended. Therefore, sulphur fertilizer is probably unnecessary when using organic fertilizer.

Regarding the rates of nitrogen, all evaluated characteristics presented some differences, independent of different N source. Therefore, a linear increase was obtained for the SPAD index (Figure 1). Pôrto et al. (2014) reported that the SPAD index has a positive correlation with chlorophyll and N in the leaf of cucumber; also, stating that SPAD index can be an easy and fast alternative to diagnose N nutritional status of plant. These significant correlations between have been found by many authors (Chapman and Barreto, 1997), as most of the N from the leaves take part in the structure of chlorophyll molecules because N is part of tetrapirrolic ring of chlorophyll molecule. So, greater concentration of N results in greater availability of this nutrient from formation of chlorophyll. SPAD index has a positive correlation with chlorophyll and so, one form indirect to determine of N. The higher the N rate, the greater the averages of head diameter and head height (Figures 1B and C). For the head fresh matter, a quadratic effect with maximum value of 1216.6 g was obtained by applying 162 kg N ha^{-1} (Figure 1D). The nitrogen is a constituent of cellular components, proteins, nitrogenous bases, triptofane amino acid and chlorophyll (Fagan et al., 2016). Adequate rates of N in vegetative phase augment vegetative growth capitation of light, ameliorating the photosynthesis resulting in higher head diameter, head height and head fresh matter.

Seabra Junior et al. (2013) observed in broccoli, a quadratic effect on productivity when N and K fertilizers were applied, obtaining a maximum estimated production of 306 g $plant^{-1}$ (588 kg K_2O ha^{-1} and 150 kg N ha^{-1}); 370 g $plant^{-1}$ (541 kg K_2O ha^{-1} and 300 kg N ha^{-1}); and 303 g $plant^{-1}$ (751 kg K_2O ha^{-1} and 450kg N ha^{-1}), broccoli production was expressed in inflorescence per plant. At higher rates of N, there was an abortion of flowers due excessive growth of vegetation, bringing out the unbalance of carbohydrates between leaves and flowers. Furthermore, they found that higher doses of K topdressing reduced the severity of black rot in broccoli (*Xanthomonas campestris pv. campestris*) as compared to equilibrate the absorption of nitrogenous. Nitrogen, which is held largely in leaves and plays an important role in mineral nutrition and physiology of plants and is responsible for important physiological processes such as photosynthesis, respiration, development and activity of roots, ionic absorption of other nutrients, growth and cell differentiation. The effect of nitrogen in disease resistance is totally dependent on dose, because in appropriate doses contributes to the synthesis of phenols and alkaloids, but in high concentrations, it reduces the production of phenolics, lignans, due to the carbon demand in photosynthetic pathway cycle Krebs, affecting the synthesis of secondary metabolites via the shikimic acid, and contribute to the production of young tissues extending the vegetative stage and creating favorable conditions for the pathogen attack. The K assists in disease resistance by balancing the nitrogen fertilization and makes them more fibrous tissue, increasing fungal and bacterial resistance. This favorable effect is a direct action, making the establishment and development of the pathogen in the host, in addition to acting indirectly, promoting wound healing and hindering the penetration of pathogens. Potassium plays a role in the conversion of simple sugars and nitrogen compounds of high molecular weight compounds such as cellulose, starch and protein, amino acids and reducing sugars that are food for pests and diseases.

According to Huett and Dettmann (1991), N stimulates growth and development of plants, that is, having direct effect on the drain source relationship; raising the production of assimilates (carbohydrates). Adequate rates of N in vegetative phase augment vegetative growth capitation of light, ameliorating the photosynthesis resulting in higher head diameter and head height and head fresh matter. The higher the rates of N (till dose-limiting extent), the larger the leaf area; for that reason, affecting photosynthesis and so, production of carbo-hydrates, plant growth and crop production.

Despite the N and organic compost fertilization, all plants need regular supply of nitrogen, which confirms the necessity of multiple split application, as recommended by Raij et al. (1997). Considering the fresh matter, the necessary rate of N to obtain the maximum value was 162 kg ha^{-1}, which is slightly below the maximum recommended by Raij et al. (1997), that is, 200

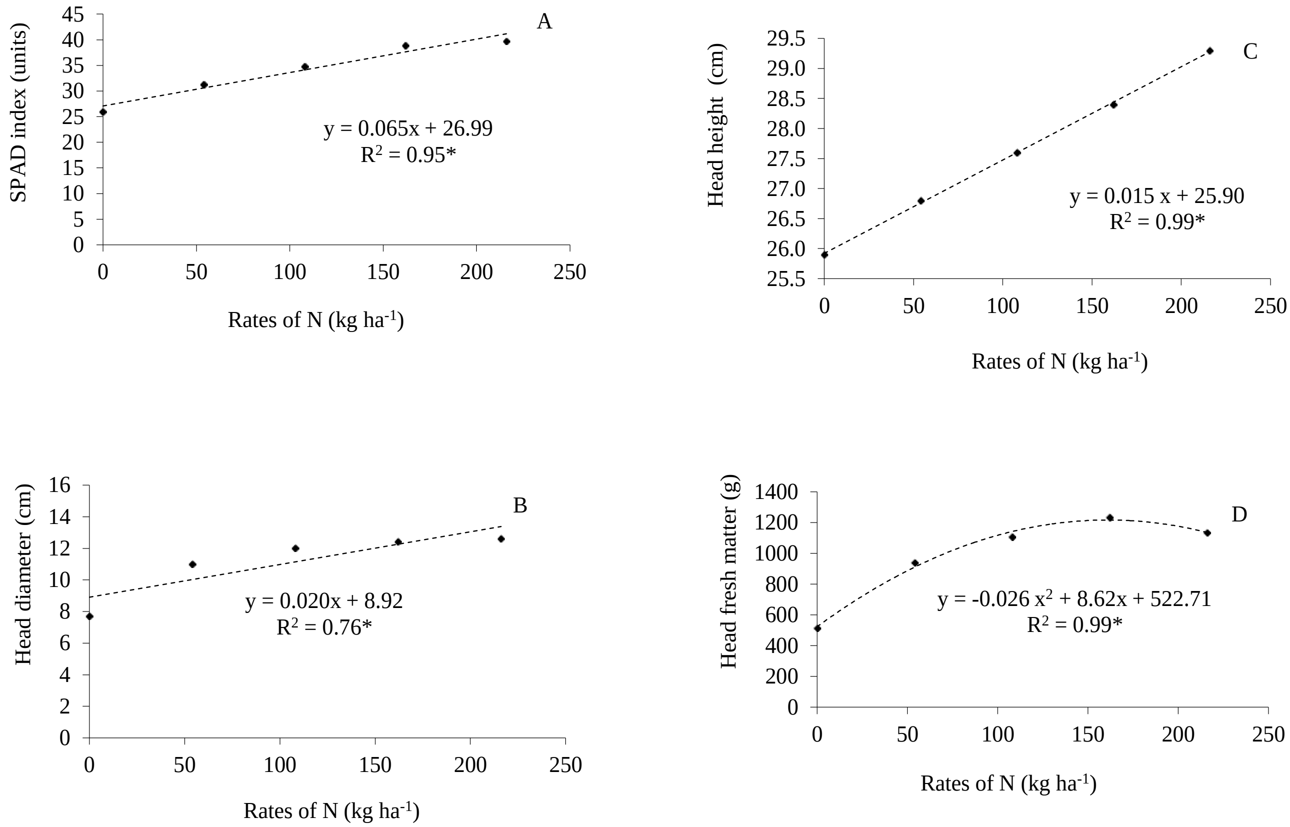

Figure 1. SPAD index, fresh matter, head diameter and head height of Chinese cabbage according to different rates of nitrogen. Agronomic Science School, São Manuel-SP, 2013. *Differ by Tukey test at 5% probability.

kg N ha^{-1}. In this study, the soil was sandy (about 87%sand), thereby, a greater potential to lose N from leaching and, consequently, split applications were effective.

There are some studies that confirm the real increase in production per increase head height and fresh matter by applying N topdressing on *brassicaceae*, such as cabbage (Aquino et al., 2009; Moreira e Vidigal, 2011) and broccoli (Elwan et al., 2011).

Although, studies also confirm the need for split application of total N rate, there are little information on source and rate of sulphur application; despite the importance of this nutrient for brassica, the results are not yet sufficient to recommend the use of S to improve productivity.

Conclusion

The evaluated characteristics were not affected by the sources of N in this study. The largest head fresh matter was estimated to be about 162 kg N ha^{-1}. By improving the nitrogen dose, the SPAD index, head height and head diameter linearly increased.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Abbey L, Jouce DC, Aked J, Smith B (2002). Genotype, sulfur nutrition and soil type effects on growth and dry matter production of spring onion. J. Hortic. Sci. Biotechnol. 77(3):340-345.
Aquino LA, Puiatti M, Lélis MM; Pereira PRG, Pereira FHF (2009). Produção de biomassa, teor e exportação de macronutrientes em

plantas de repolho em função de doses de nitrogênio e de espaçamentos. Ciênc. Agrotecnol. 33 (5):1295-1300.
Berry PM, Spink J, Foulkes MJ, White PJ (2010). The physiological basis ofgenotypic differences in nitrogen use efficiency in oilseed rape (*Brassica napus* L). Field Crops Res. (119):365-373.
Buchanan BB, Gruissem W, Jones RL (2015). Biochemistry & Molecular biology Of plants. American Society of plant Biologists. Second edition. USA, Oxford 1283 p.
Camargo Filho WP, Camargo FP (2015). Acomodação da produção olerícola no Brasil e em São Paulo, 1990- 2010 – Análise Prospectiva e Tendências 2015. Instituto de Economia Agrícola. São Paulo 24 p.
Campbell WH (1999). Nitrate reductase structure, function and regulation: bridging the gap between biochemistry and physiology. Annu. Rev. Plant Physiol. Plant. Mol. Biol. (50):277-303.
Carfagna S, Vona V, Di Martino V, Esposito S, Rigano C (2011). Nitrogen assimilation and cysteine biosynthesis in barley: evidence for root sulphur assimilation upon recovery from N deprivation. Environ. Exp. Bot. 71(1):18-24.
Chapman SC, Barreto HJ (1997). Using a chlorophyll meter to estimate specific leaf nitrogen of tropical maize during vegetative growth. Agr. J. 89(4):557-592.
Corrêa CV, Cardoso AII, Cláudio MTR (2013). Produção de repolho em função de doses e fontes de potássio em cobertura. Semina 34(5):2129-2138.
Cunha AR, Martins D (2009). Classificação climática para os municípios de Botucatu e São Manuel, SP. Irriga 14:1-11.
Elwan MWM, Abd El-Hamed KE (2011). Influence of nitrogen form, growing season and sulfur fertilization on yield and the content of nitrate and vitamin C of broccoli. Sci. Hortic. 127(3):181-187.
Eriksen J, Thorup-Kristensen K, Askegard M (2004). Plant availability of catch cropsulfur following spring incorporation. J. Plant Nutr. Soil Sci. 167(5):609-615.
Fagan EB, Ono EO, Rodrigues JD, Soares LH, Dourado Neto D (2016). Fisiologia vegetal: Metabolismo e Nutrição Mineral. Editora Andrei, São Paulo, Brasil 306p.
Fageria NK, Baligar VC (2005). Enhancing nitrogen use efficiency in crop plants. Adv. Agron. (88):97-185.
Ferreira DF (2010). Sistema Sisvar para análises estatísticas. Lavras: UFLA. Disponível em: http://www.dex.ufla.br. Acesso em: 20 nov. 2013.
Filgueira FAR (2008). Novo manual de olericultura: agrotecnologia moderna na produção e comercialização de hortaliças. Viçosa: UFV P 421.
Gordin CRB, Biscaro GA, Santos AM, Pagliarini MK, Peixoto PPP (2010). Níveis de fertirrigação nas características morfofisiológicas de mudas de couve chinesa. Rev. Agrar. 3(10):253-260.
Hesse H, Nikiforova V, Gakiere B, Hoefgen R (2004). Molecular analysis and control of cysteine biosynthesis, integration of nitrogen and sulphur metabolism. J. Exp. Bot. 55(401):1283-1292.
Huett DO, Dettmann EB (1991). Nitrogen response surface models of zucchini squash, head lettuce and potato. Plant Soil 134 (2):243-254.
Koprivova A, Suter M, Op den Camp R, Brunold C, Kopriva S (2000). Regulation of sulfate assimilation by nitrogen in Arabidopsis. Plant Physiol. (122):737-746.
Losák T, Hlusek J, Kráemar S, Varga L (2008). The effect of nitrogen and sulphur fertilization on yield and quality of kohlrabi (*Brassica oleracea* L.). Revista Brasileira de Ciência do Solo. 32(2): 697-703.
Mendel R (1997). Molybdenum cofactor of higher plants: biosynthesis and molecular biology. Planta 203(4):399-405.
Moreira MA, Vidigal SM (2011). Evolução das características da planta associadas à nutrição nitrogenada de repolho. Rev. Ceres. 58(2):243-248.
Neeteson JJ, Carton OT (2001). The environmental impact of nitrogen in field vegetable production. Acta Hort. (563):21-28.
Pôrto MLA, Puiatti M, Fontes PCR, Cecon PR, Alves JC (2014). Índice SPAD para o diagnóstico do estado de nitrogênio na cultura do pepino japonês em ambiente protegido. Hortic. Bras. 32(3):292-296.
Rahn CR (2002). Management strategies to reduce nutrient losses from vegetable crops. Acta Hort. 571:19-29.
Raij BV, Cantarella H, Quaggio JÁ, Furlani AMC (1997) Recomendações de adubação e calagem para o estado de São Paulo. Campinas: Instituto Agronômico & Fundação IAC P 285.
Rathke GW, Behrens T, Diepenbrock W (2006).Integrated nitrogen managementstrategies to improve seed yield, oil content and nitrogen efficiency of winter oilseed rape (*Brassica napus* L.): a review. Agric. Ecosyst. Environ. (117):80-108.
Rheinheimer DS, Alvarez JW, Osorio Filho BD, Silva LS, Bortoluzzi EC (2005). Resposta de culturas à aplicação de enxofre e a teores de sulfato num solo de textura arenosa sob plantio direto. Ciênc. Rur. Santa Maria 35(3):562-569.
Sady W, Rozek S, Domagala-Swiatkiewicz I, Wojciechowska R, Kolton A (2008). Effect of nitrogen fertilization on yield, NH_4^+ and NO_3^-content of white cabbage. Acta Sci. Pol. Hortorum Cultus 7(2):41-51.
Salvagiotti F, Miralles DJ (2008). Radiation interception, biomass production and grain yield as affected by the interaction of nitrogen and sulfur fertilization in wheat. Euro J. Agron. 28(3):282-290.
Salvagiotti F, Castellarin JM, Miralles DJ, Pedrol HM (2009). Sulfur fertilization improves nitrogen use efficiency in wheat by increasing nitrogen uptake. Field Crop Res. 113(2):170-177.
Sanderson KR (2003). Broccoli and cauliflower response to supplemental soil sulphur and calcium. Acta Hort. 1(627):171-179.
Sanderson KR, Ivany JA (1996). Supplemental soil sulphur increases cabbage yield. Canadian J. Plant Sci. (76):857-859.
Schonhof I, Blankenburg D, Mueller S, Krumbein A (2007). Sulfur and nitrogen supply influence growth, product appearance, and glucosinolate concentration of broccoli. J. Plant Nutr. Soil Sci. 170(1):1-8.
Seabra Junior S, Lalla JG, Goto R, Maringoni AC, Villas Boas RL, Rouws JRC, Oriani EE (2013). Suscetibilidade à podridão negra e produtividade de brócolis em função de doses de nitrogênio e potássio. Hortic. Bras. 31(3):426-431.
Swamy U, Wang M, Tripathy JN, Kim SK, Hirasawa M, Knaff DB, Allen JP(2005). Structure of spinach nitrite reductase: implications for multi-electronreactions by the iron–sulfur: siroheme cofactor. Biochemistry 44(49):16054-16063.
Takahashi H, Saito K (1996). Sub cellular localization of spinach cysteine synthase isoforms and regulation of their gene expression by nitrogen and sulphur. Plant Physiol. 112(1):273-280.

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Full Length Research Paper

Response of broccoli to sulphur application at topdressing in the presence or absence of organic compost at planting

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The literature has confirmed the efficacy of sulphur (S) when growing *Brassicaceae*. However, there are no studies with this nutrient on broccoli. Therefore, the aim of this study was to evaluate the response of broccoli (hybrid Avenger) to sulphur rates applied at top dressing in the presence or absence of organic compost at planting. The experimental design was a randomized complete block, with ten treatments (5 x 2 factorial design) and four repetitions. Treatments consisted of five S (ammonium sulphate) rates (0, 31, 62, 93 and 124 kg ha^{-1}); with organic compost (100 t ha^{-1}) in planting or without organic compost in planting. After harvesting, head fresh matter, head diameter and number of leaves were evaluated. In general, head diameter, head height and fresh matter increased in all treatments by adding organic compost. A quadratic effect was observed for head fresh matter and head height by applying organic compost (100 tha^{-1}), as the maximum values were 620.6 g and 17.2cm at the rate of 66.9 and 49.2 kg S ha^{-1}, respectively.

Key words: *Brassica oleracea* var. italica, fertilization, sulphate.

INTRODUCTION

Broccoli (*Brassica oleracea* var. *italica*) belongs to the B*rassicaceae* family. It is well known for its nutritional value, as it provides vitamins and fibre, preventing against some types of cancers and heart diseases; easily produced; and has already spread its popularity on global market (Keck, 2004; Baenas et al., 2016; Bachiega et al., 2016; Ciancaleoni et al., 2016). In Brazil, there are two types of broccoli cultivars, that is, “ramoso” and “single-head” (Filgueira, 2008).

“Ramoso” is characterized by a small main head; coarse-grained; a considered number of side shoots; multiple crops; and sold by the bunch (stems). Whilst,

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"single-head" type features one large central head and reaches an average of about 400 g. Furthermore, "single-head" is more compact and presents fewer shoots than "ramoso". But, both consist of tightly closely buds; dark green colour and tender stems.

Among the crops, brassica has a good response to organic fertilization. Therefore, this type of fertilization benefits the soil, which supports plant growth by improving water retention and, consequently, penetration capacity; improving the structure; aeration; drainage; influencing microbial community and, hence, eliminating the undesirable ones; and increasing plant nutrient availability (Filgueira, 2008).

By using a large rate of organic compost (25 tha^{-1}), Diniz et al. (2008) showed the highest production of broccoli and large amount of dry matter in the experiment. The amounts of soil nutrients extracted by brassica are large, for example, sulphur (s) and nitrogen (Rathke et al., 2006; Berry et al., 2010). S is an essential nutrient for plant growth, as it forms amino acids; vitamins; cofactors; and secondary products, such as glucosinolates (Marschener, 2011). There are a few studies on the effects of S fertilizer with brassica under tropical conditions. There are some studies on cabbage, but no production differences were found due to application of S fertilizer (Correa et al., 2013).

Plants have different abilities to absorb, translocate and use sulfur and therefore require different amounts of available ground SO_4^{-2}. Some plants, such as those of the family of legumes, brassicas and Liliaceae only express their genetic potential in terms of productivity and quality when the availability of this nutrient is high, and then established a critical level of 10 mg dm^{-3}, while for the remaining species this low value to 5 mg dm^{-3}.

Considering these reference values for the surface layer of soil, about 50% of the total area of tropical soils and sub-tropical South America can be considered deficient in sulfur. The availability of organic sulfur to plants depends from the processing of the inorganic forms almost exclusively in the form of sulfate ($SO4^{-2}$). In tropical soil conditions due to increased precipitation and temperature there is a rapid depletion of organic matter and consequently low S content available to plants. In addition, both the total amount of sulfur as the adsorption capacity of SO_4^{-2} are lower in soils with low clay content and retention is further reduced by the application of lime and phosphate, practices these highly practiced in Brazil because present acidic soils. Thus, there is a shift of this ion to the deeper layers, where it can be adsorbed because of higher clay content and lower levels of organic matter and pH (Rheinheimer et al., 2005).

In current days, agriculture aims to develop a sustainable and productive manner, such as fertilizer best management practices to achieve a better production (Fageria and Baligar, 2005). Given all that has been earlier mentioned, this current study aimed to evaluate the response of broccoli to S application at top dressing, in the presence or absence of organic compost at planting.

MATERIALS AND METHODS

The experiment was conducted in the Sao Manuel Experimental Farm, Botucatu School of Agronomy, UNESP (22°46'28"S, 48°34'37"W; 740 m altitude), Brazil. According to the Köppen classification, the climate in the region is mesothermic, *Cwa*, that is, humid and subtropical, dry in the winter with a rainy season between November and April. The mean annual rainfall of São Manuel is 1445 mm; the mean annual temperature of the warmest month is 22°C; and the mean temperature of the coldest month is 17.5°C (Cunha and Martins, 2009).

The soil is classified as Dystrophic Red Latosol (Oxisoil) of texture sandy. Soil samples were collected for analysis at several sets from depths of 0 to 0,20m to determine their chemical attributes: pH in $CaCl_2$, 6.0; M.O., 10 g dm^{-3}; P_{resin},90 mg dm^{-3}; H+Al, 14 $mmol_c$ dm^{-3}; K, 3.2 $mmol_c$ dm^{-3}; Ca, 24 $mmol_c$ dm^{-3}; Mg, 8 $mmol_c$ dm^{-3}; SB, 35 $mmol_c$ dm^{-3}; CTC, 49 $mmol_c$ dm^{-3}; V%, 72; and S, 4.0 $mmol_c$ dm^{-3}.

The experimental design was a randomized complete block, with ten treatments (5 x 2 factorial schemes) and four replications; three rows, 11 plants per row, but only the central line were evaluated. It was applied five S rates (0, 31, 62, 93 and 124 $kgha^{-1}$) at planting in the presence (100 tha^{-1}) and without organic compost in planting. For the treatments receiving S topdressing fertilization, the highest rate of ammonium sulphate (23% S and 20% N) recommended by Raij et al. (1997) (108 kg N ha^{-1}) was used as reference. This rate is recommended from the state of São Paulo, Brazil.

With regards to the soil analysis, based on the recommendations of Raij et al. (1997), it was applied 60 kg N ha^{-1}; 200 kg P_2O_5 ha^{-1}; 120 kg K_2O ha^{-1}; in formulation 4-14-8 (NPK) and organic compost at planting by walking tractor. The organic compost Provaso® was chosen and its chemical analysis showed values of pH, 8.2; M.O, 13.3; N, 0.43; P_2O_5, 0.62; K_2O, 0.48; Ca, 1.61; Mg, 0.17; and S, 0.20, all expressed in g $kg^{-1}$of dry matter. The ratio of C/N was 19/1; and the moisture content of the compost was 38%.

In the topdressing fertilization, S was applied into three times, every 15 days after transplanting. According to the methodology described by Raij et al. (1997), it was applied N (108 $kgha^{-1}$) and K_2O (90 kg ha^{-1}) too, 1/3 of the respective doses in the same dates of the topdressing. The source of N and S was ammonium sulphate (23% S and 20% N). Additionally, source of N was completed with urea (45% N); and source of K was accomplished with potassium chloride (60% K_2O).

The hybrid Avenger® of Sakata was used. On March 6, 2014, sowing was performed in polypropylene trays of 200 cells, containing coconut fibre substrate for vegetable seedling production. On March 26, 2014, seedlings were transplanted separately into a microplot of size 0.5 x 0.4m. Sprinkler irrigation and weed hand control were used from culture tracts.

From 9 to 23rd of July, 2014, (90 the 110 days after sowing) plants were collected to evaluate the following characteristics: fresh matter; head height; head diameter; and number of green leaves. For matter, it was used a semi-analytical balance with a precision of 0.1g (expressed in grams per plant); for diameter, caliper was used (expressed in centimetres); and for height, a graduated ruler (expressed in cm). The harvesting of the clumps cutting was performed manually with the aid of a knife approximately 5 cm below the insertion of the inflorescence. three harvests were made

Table 1. Fresh matter; head diameter, head height (cm) in the presence (100 t ha^{-1}) or absence of organic compost with different rates of S topdressing at planting. FCA/UNESP, São Manuel-SP, 2014.

Compost (t ha^{-1})	Sulphur rates (kg ha^{-1})				
	0	31	62	93	124
Head fresh matter (g $plant^{-1}$)					
Without	305.3^b	432.5^b	435.8^b	466.0^b	341.3^b
With	351.0^a	590.8^a	631.0^a	526.3^a	467.5^a
CV (%)			6.08		
Head diameter (cm)					
Without	15.7^b	18.1^b	19.0^b	19.2^a	18.3^b
With	19.4^a	20.7^a	23.3^a	19.4^a	20.7^a
CV (%)			6.35		
Head height (cm)					
Without	11.9^b	12.4^b	12.3^b	15.4^a	13.0^a
With	15.4^a	17.2^a	18.3^a	14.3^b	14.4^a
CV (%)			6.76		

Means followed by different letters differ by Tukey test at 5% probability.

because the inflorescences did not show up formed at the same time, demonstrating the non-uniformity of the hybrid employed.

Data were subjected to analysis of variance (ANOVA) and regression for S rates. For the S topdressing fertilization in the presence or absence of organic compost, means were compared by Tukey test ($p < 0.05$) by the Sisvar software (Ferreira, 2010).

RESULTS AND DISCUSSION

For all the traits, the interaction between the presence or absence of organic compost and S topdressing fertilization were significant, except of number of leaves per plant that had no differences with or without organic compost, as both presented an average of 24 leaves per plant. These results corroborates with the one found by Kano et al. (2008) and Ferreira et al. (2013), 23.5 and 24.0 per plant, respectively.

In general, all treatments with organic compost (100 t ha^{-1}) at planting have increased fresh matter, head diameter and head height (Table 1). Studies have revealed that organic fertilizers release nutrients (Cardoso et al, 2011), which are absorbed by plants, consequently, increasing the production (Dinis et al., 2008; Ferreira et al., 2013).

The recommended rates of organic compost for *Brassicaceae* lie generally between 10 to100 t ha^{-1}, but higher levels are not uncommon. However, the amount of organic compost may depend on the crop; quality assurance of the materials; soil properties; time management and environmental conditions (Villas et al., 2004; Ferris et al., 2012).

Additionally, in this experiment the soil was very sandy (more than 70% of sand), which requires more organic fertilizers than recommended. Furthermore, organic compost also improves the physical and biological properties of the soil when adequate macro and micronutrients are supplied (Reeve et al 2016). The organic compost benefits the soil, which supports plant growth by improving water retention and, consequently, penetration capacity; improving the structure; aeration; drainage; influencing microbial community. With regards to sulphur topdressing fertilization, a quadratic effect was obtained for head fresh matter according to the rates in the presence or absence of organic compost at planting (Figure 1).

The maximum values were estimated at 465.4g $plant^{-1}$ (without organic compost) and 620.6 g $plant^{-1}$(with organic compost) at the rate of 66.9 kg S ha^{-1}. For the control treatment (zero dose), it was obtained an increase in more than 50% for the head fresh matter, i.e., 158.5g $plant^{-1}$ (without organic compost) and 247.0 g $plant^{-1}$ (with organic compost). This result demonstrates the importance of applying sulphur to plant growth and development of plants (Khan et al., 2015; Asgher et al., 2014). Schonhof et al. (2007) observed an increase in the broccoli head matter by applying adequate rates of sulphur. Therefore, these results confirmed findings of previous studies on broccoli (Schonhof et al., 2007;

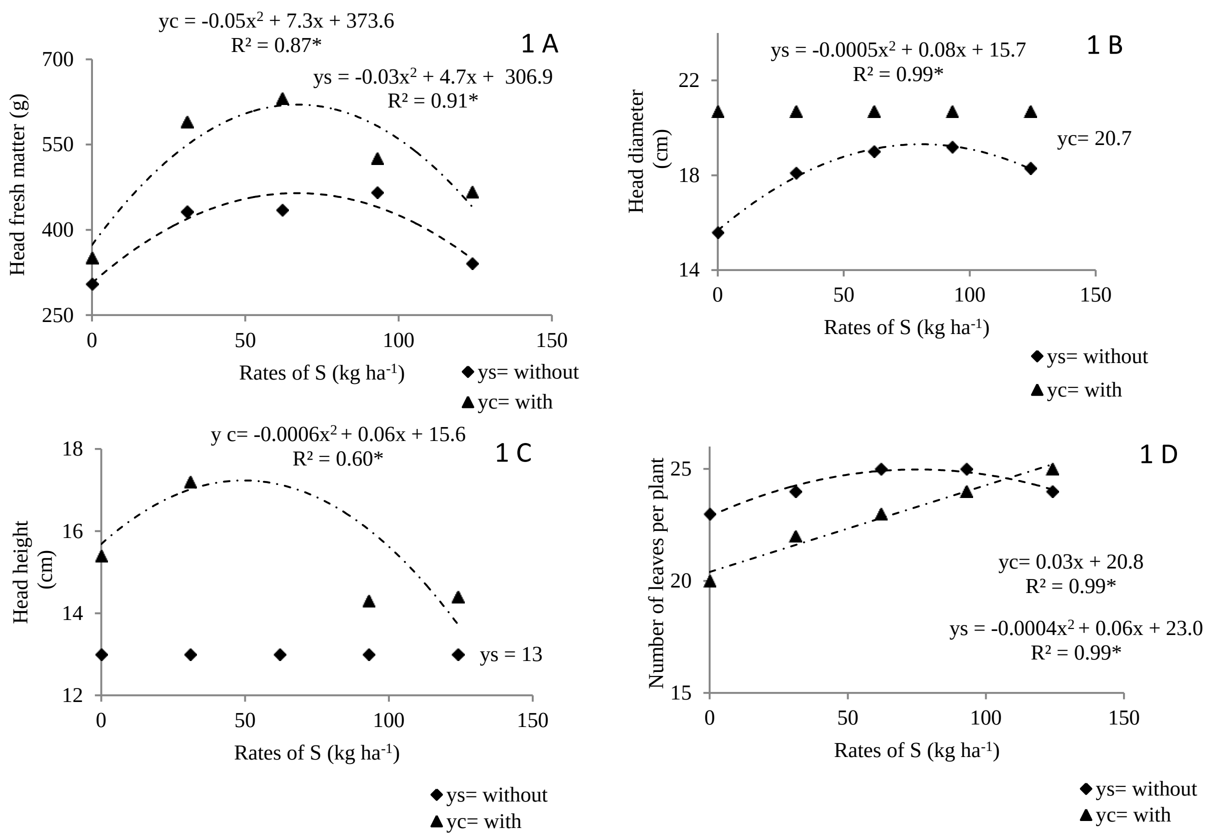

Figure 1. Fresh matter, head diameter, head height and number of leaves per plant by applying different rates of S topdressing in with (y_c) or without (y_s) of organic compost at planting. FCA/UNESP, São Manuel-SP, 2014.

Elwan et al., 2010).

For the treatments in the absence of fertilizer, the minimum value was 306.9 g plant^{-1}. However, with organic compost, the maximum value was 620.6 g plant^{-1} by applying 66.9 kg ha^{-1}of S in topdressing. Therefore, the results were close to those found by Diniz et al. (2008), Lalla et al. (2010) and Freitas et al. (2011), who obtained maximum value which ranged from 405 to 600 g plant^{-1} in broccolis. Although, these results were lower than those reported by Kano et al. (2008), who obtained a maximum value of 963.2 g plant^{-1}. Bearing in mind the different management and environmental conditions, the results were compatible.

For the head diameter, a quadratic effect was obtained, reaching the maximum value of 19.3 cm by applying 81.1 kg ha^{-1} of S in topdressing without organic compost (Figure 1 B). However, it was not observed any difference between the analysis of variance and regression to the sulphur rates when organic compost was added, with an average of 20.7cm. Therefore, the application of organic compost within the studied range may dispense any further application of S in topdressing. These results corroborates with the one found by Pizetta et al. (2005) and Kano et al. (2008), 20.4 and 20.5 cm in broccolis, respectively. However, these values were higher than those reported by Lalla et al. (2010) and Ferreira et al. (2013), that is, 15.5 cm and 13.0 cm, respectively. For the head height, it presented an average of 13.0 cm without organic compost at planting. However, it was observed a quadratic effect with organic compost, reaching a maximum value of 17.2 cm at a rate of49.2 kg S ha^{-1} (Figure 1 C).

The organic matter is a source of all the macro and micronutrients necessary for the development of vegetables. In the presence of organic matter is observed larger heads precisely for allowing nutrient supply, and improved soil characteristics such as increased water retention, increased aeration. It can be seen that for the characteristic head diameter, S have little influence in the presence of organic matter, and absence of organic matter influence its diameter at low doses. Excessive doses of S do not contribute to increase the size of broccoli head. Excessive doses can be lost by leaching very common in sandy soils with low organic matter as used in this research. The application of organic matter allows improvements as greater availability of nutrients, and lower losses by leaching. From the figures in general, there is a great deal of sulfur (66.9 kg ha^{-1}) and organic compound (100 t ha^{-1}) for best broccoli yields, showing the interaction between chemical and organic fertilizers.

The number of leaves per plant presented a linear effect to the equation in the presence of organic compost at planting. However, a quadratic effect was observed without organic compost, reaching a maximum average of 25.3 per plant by applying 75 kg Sha^{-1}in topdressing (Figure 1 D). These values are close to those reported by Ferreira et al. (2013), who obtained a higher number of leaves per plant (that is, 24 leaves) with bokashi compost (10 t ha^{-1}) in the broccoli production.

In general, the application of S in topdressing influenced all the evaluated traits of the head. S is constituent of diverse enzymes used for the synthesis of hormones, proteins, vitamins, and enzyme cofactors and a precursor for several metabolites such as ethylene, polyamines, plays a role in photosynthesis; nitrogen fixation; chlorophyll biosynthesis; and micro and macronutrients uptake (Salvagiotti et al., 2009). Thus, it is an essential element for the plant growth and production (Leustek, 2002).

Despite all these functions, S has been neglected by most of the researchers. In brassica, there are reports that this nutrient are accumulated to more than twice the P levels (Yamada et al., 2007); it is also the second most accumulated nutrient in cauliflower seeds (Cardoso et al., 2016). Although, the effect of S on the head characteristics is often quadratic, indicating that in excess can be harmful. Taking into account, the highest rate of this study (124 kg S ha^{-1}), it was just obtained by using the highest rate of N recommended by Raij et al. (1997), but this amount of N, as ammonium sulphate, can be detrimental. Considering all the treatments with organic compost (100 t ha^{-1}) (Figure 1), it reached 373.6 g for head matter (0 dose); maximum value of 620.6g (at a rate of 66.9 kg S ha^{-1}); and 510.0 g (at a rate of 124 kg S ha^{-1}).

Therefore, within this study conditions; and to be recommended as a topdressing fertilizer, only half of N should be applied, as ammonium sulphate; and the other half as another source, which should not contain any sulphur.

Conclusion

At planting, organic compost increased broccoli production. Moreover, there was the largest head matter by applying up to 66.9 kg ha^{-1} of S in topdressing and 100 t ha^{-1} of organic compost.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Asgher M, Khan NA, Khan MIR, Fatma M, Masood A (2014). Ethylene production is associated with alleviation of cadmium-induced oxidative stress by sulfur in mustard types differing in ethylene sensitivity. Ecotoxicol. Environ. Safety 106:54-61.

Baenas N, Villaño D, García-Viguera C, Moreno DA (2016). Optimizing elicitation and seed priming to enrich broccoli and radish sprouts in glucosinolates. Food Chem. 204:314-319.

Bachiega P, Salgado JM, Carvalho JE, Ruiz ALTG, Schwarz K, Tezotto T, Morzelle MC (2016). Antioxidant and antiproliferative activities in different maturation stages of broccoli (*Brassica oleracea Italica*) biofortified with selenium. Food Chem. 190:771-776.

Berry PM, Spink J, Foulkes MJ, White PJ (2010). The physiological basis ofgenotypic differences in nitrogen use efficiency in oilseed rape (*Brassica napus* L). Field Crops Res. 119:365-373.

Ciancaleoni S, Onofri A, Torricelli R, Negri V (2016). Broccoli yield response to environmental factors in sustainable agriculture. Eur. J. Agron. 72:1-9.

Correa CV, Cardoso AII, Claúdio MTR (2013) Cabbage production in function of doses and sources of potassium coverage. Semina 34(5):2129-2138.

Diniz ER, Santos RHS, Urquiaga SS, Peternelli LA, Barrella TP, Freitas (2008) Growth and broccoli production organic compound doses system function. Cienc. Agrotec. 32 (5):1428-1434.

Elwan MWM, Abd El-Hamed KE (2011). Influence of nitrogen form, growing season and sulfur fertilization on yield and the content of nitrate and vitamin C of broccoli. Sci. Hortic. 127(3):181-187.

Fageria NK, Baligar VC (2005). Enhancing nitrogen use efficiency in crop plants.Adv.Agron. (88):97-185.

Ferreira S, Souza RJ, Gomes LAA (2013). Summer broccoli productivity with different doses of Bokashi. Rev. Agrogeoambiental 5(2):31-38.

Ferris H, Sánchez-Moreno S, Brennan EB (2012). Structure, functions and interguild relationships of the soil nematode assemblage in organic vegetable production. Appl. Soil Ecol. 61:16-25.

Ferreira DF (2010). Sisvar system for statistical analysis. Lavras: UFLA. Available in: <http://www.dex.ufla.br>. Acesso em: 20 nov. 2014.

Filgueira FAR (2008). New manual of horticulture: modern agro-technology in the production and marketing of vegetable. Viçosa: UFV P 421.

Keck AS, Finley JW (2004). Cruciferous vegetables: cancer protective mechanisms of glucosinolate hydrolysis products and selenium. Integr. Cancer Ther. 3(1):5-12.

Kano C, Godoy AR, Higuti ARO, Castro MM, Cardoso AII (2008). Broccoli production in function of tray type and seedling age. Cienc. Agrotecn. 32 (1):110-114.

Khan MIR, Nazir F, Asgher M, Per TS, Khan NA (2015).Selenium and sulfur influence ethylene,formation and alleviate cadmium-induced oxidative stress by improving proline and glutathione production in wheat. J. Plant Physiol. (173):9-18.

Lalla JG, Laura VA, Rodrigues APDC, Seabra Júnior S, Silveira DS, Zago VH, Dornas MF (2010). Competition broccoli cultivars single head type in Campo Grande. Hortic. Bras. 28 (3):360-363.

Leustek T (2002). Sulfate metabolism. In: Somerville CA, Meyerowitz EM (Eds.), The Arabidopsis Book. American Society of PlantBiologists, Rockville, MD pp. 1-17.

Marschener H (2011). Mineral Nutrition of Higher Plants. Academic Press, 3 edition, New York P 672.

Pizetta LC, Ferreira ME, Cruz MCP, Barbosa JC (2005). Broccoli response, cauliflower and cabbage to fertilization with boron in sandy soil. Hortic. Bras. 23(1):51-56.

Raij BV, Cantarella H, Quaggio JÁ, Furlani AMC (1997). Recommendations fertilization and liming for the state of São Paulo. Campinas: Instituto Agronômico&Fundação IAC P 285.

Rathke GW, Behrens T, Diepenbrock W (2006).Integrated nitrogen management strategies to improve seed yield, oil content and nitrogen efficiency of winter oilseed rape (*Brassica napus* L.): A review. Agric. Ecosyst. Environ. (117):80-108.

Reeve JR, Hoagland LA, Villalba JJ, Carr PM, Atucha A, Cambardella C, Davis DR, Delate K (2016). Organic Farming, Soil Health, and Food Quality: Considering Possible Links. Adv. Agron. pp. 1-49.

Rheinheimer DS, Alvarez JW, Osorio Filho BD, Silva LS, Bortoluzzi EC (2005). Response cultures for sulfur application and sulfate levels in a sandy soil under no-tillage. Ciênc. Rur. Santa Maria 35(3):562-569.

Salvagiotti F, Castellarin JM, Miralles DJ, Pedrol HM (2009). Sulfur fertilization improves nitrogen use efficiency in wheat by increasing nitrogen uptake. Field Crop Res. (113):170-177.

Schonhof I, Blankenburg D, Mueller S, Krumbein A (2007). Sulfur and nitrogensupply influence growth, product appearance, and glucosinolate concentration of broccoli. J. Plant Nutr. Soil Sci. 170(1):1-8.

Villas BRL, Passos JC, Maximino D, Büll LT, Cezar VRS, Goto R (2004). Effect of doses and types of organic compounds in the production of lettuce in two soils under protected environment. Hortic. Bras. 22(1):28-34.

Yamada T, Abdala SRS, Vitti GC (2007). Symposium on nitrogen and sulphur in brazilian agriculture. (Piracicaba, SP), Anals... IPNI, Brasil P 722.

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Full Length Research Paper

Nitrogen accumulation and productivity of green corn in function of ways and seasons of top-dressing nitrogen fertilizer application

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Nitrogen is one of the nutrients required in large amounts by plants and mostly influences corn yield. With the objective of evaluating the best timing and methods of N fertilizer application in topdressing on corn, this experiment was carried out in Ipameri County, State of Goiás, Brazil, on a Red Yellow Latossol (Ferralsol). The treatments were distributed in a randomized complete block design with four replications. The applied treatments were: control: 100% N applied on the surface after planting; 100% N applied on the surface in V4 phase; 50% N applied on the surface at V4 and 50% N applied on the surface at V6 phases; 100% N applied on the surface in V6 phase; 100% N applied in continuous fillet at V4 phase; 100% N applied in continuous fillet at V6 phase; 50% N in continuous fillet applied at V4 and 50% N in continuous fillet applied at V6 phases. The data were subjected to variance analysis by test 'F' and the means were compared by Tukey test ($P < 0.05$). The nitrogen fertilization increased the length of ears with and without husk, the diameter of ear without husk, ears green yield with and without husk, number of grains per row and grain yield. The N application mode in continuous fillet provided greater averages for the characteristics evaluated. The N application time influences the absorption and accumulation of N in the shoots of corn.

Key words: Absorption, nitrogen fertilization, application methods, nutrition.

INTRODUCTION

The culture of green maize has become an alternative of great economic value to the producer because of good market price and of the demand for the product in nature (Matos, 2007), mainly, for subsistence agriculture. It is intended for consumption in the green stage of maturation, both in the form of cooked or roasted ear, juice, cooked and canned grains, among others, differentiating its characteristics from common corn. In Brazil, the production of green maize is intended mainly for canning (Sawazaki et al., 1990; Oliveira Junior et al., 2006). To meet requirements of the consumer, the ears should be large, cylindrical and with husk (Freire et al., 2010).

One of the aspects of great importance in the

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production of green maize is the management of nutrition, mainly in terms of nitrogen (N), nutrient required in great quantity. The management of nitrogen fertilization in the production of green maize is crucial, because it is directly related to the increased productivity and improved quality, with numerous relevant functions in its physiological activities, as photosynthesis, respiration, protein synthesis and ionic absorption. The recommendations of the nitrogen fertilization are 80 and 120 kg.ha^{-1} of N, and m is based on the fact that culture requires about 20 kg.ha^{-1} of N for each ton of grain produced, and for green maize, is based on information relating to grains production (Borin et al., 2010). In Brazil, the regional recommendations of N, presented variations, with doses that vary from 20 to 180 kg ha^{-1} (Almeida and Sanches, 2012). Higher doses are associated with larger productivity, but the energy efficiency has great interference in the results. According to Matos (2007), 70 to 90% of the assays with maize in Brazil respond to N.

The availability of N, in many production systems, is almost always a limiting factor, along the crop cycle, influencing the growth of the plant more than any other nutrient (Carmo et al., 2012). In addition, N presents high mobility in the soil and can be easily lost, mainly, by the immobilization, denitrification, leaching and volatilization. For this reason, it is important to minimize the losses, maximize the absorption and metabolism of N in the interior of the plants (Almeida and Sanches, 2012).

The phenological stage of application can interfere with the efficiency of the use of nitrogen fertilizers by crops. On one hand, early applications can favor losses by leaching, due to longer exposure to rain and lower rate of nutrient uptake by the crop from sowing to the first days after emergence. Late fertilization may not make plant to produce more due to plant productive potential in the early stages, depending on the availability of nutrients. Better grain productivity was obtained by Silva et al. (2005a), with the incorporation of N during planting or 15 days after the emergency. However, Rambo et al. (2004) obtained an increase in productivity of grains by applying N in the emergency of ear stage. According to Silva et al. (2005b), response to late fertilizing depends on the cultivar. For this reason, it is of fundamental importance to search for more precise information on the requirements for the production of green maize, as well as determine the application modes and phenological stage, in which the fertilizer is more required, according to the absorption and the accumulation of nutrient in different stages of plant development (Borin et al., 2010).

Thus, the objective of this work was to evaluate the accumulation of N and the productivity of green maize in function of modes and phenological stages of application of N.

MATERIALS AND METHODS

The work was conducted in the experimental field of State University of Goiás, Campus Ipameri, Goiás (Lat. 17° 43' 19'' S, Long. 48° 9' 35'' W, Alt. 773 m) in the agricultural year of 2014/2015. According to the classification of Köppen, the climate of the region is tropical humid (AW) with annual rainfall of 1,447 mm, average temperature of 21.9°C, relative humidity ranging from 58 to 81%. In Figure 1, the climate data corresponding to the period and the year of development of field work is shown.

The soil of the experimental area is classified as Red Yellow Latossol (Ferralsol) (EMBRAPA, 2013), whose physical-chemical attributes are presented in Table 1. The experimental design was randomized blocks, with 8 treatments and four repetitions. The treatments were composed of: control (without application of N in coverage); 100% N applied to the surface after sowing; 100% N applied to the surface in V4 phase; 50% N applied to the surface at V4 and 50% N applied to the surface at V6 phases; 100% N applied to the surface in V6 phase; 100% N applied in continuous fillet at V4 phase; 100% N applied in continuous fillet at V6 phase; 50% N in continuous fillet applied at V4 and 50% N in continuous fillet applied at V6 phases.

The sowing of maize for "*in natura*" consumption, Agroceres 1051, was performed on 12/17/2014 under no-tillage, with the aid of a seeder with tractor traction, with eight rows spaced 0.80 m, by distributing five seeds per linear meter. The useful area of the experimental plot constituted of four central rows, with 3.0 m length, removing a meter at each end. The fertilizing was based on the chemical analysis of the soil and in the demands of culture, with the application of 27.5 kg ha^{-1} of N, 137.5 of kg ha^{-1} of P_2O_5 and 82.5 kg ha^{-1} of K_2O, using the fertilizer with formulated NPK 05-25-15. The nitrogen fertilization application was 120 kg ha^{-1} using urea as source.

The nitrogenous fertilization was performed with the soil at field capacity, according to the treatment mentioned, using urea as source of nitrogen (N). The weed control was performed by means of the applications of atrazine in pre-emergence and Tembrotiona® in post-emergence. The control of pests was performed with application of insecticide provided and ingestion of Premio® with concentration of 125 mL ha^{-1}, plus the growth regulator insecticide, Intrepid 240SC with 180 mL ha^{-1} volume of 200 L ha^{-1}syrup.

During the reproductive phase in stage R_3 (grain pasty), 10 plants were evaluated in the useful area of each plot: I- height of insertion of the first ear (IFE), measured from the stem base to the point of insertion of the first ear; then, the plants were harvested manually following the evaluations of: II- husked ear length (HEL) and unhusked (CED) and were obtained by measuring the distance between the base and the apex of the ear in cm; III- husked ear diameter (DED), a digital pachymeter with precision to 0.1 cm, measured at the point corresponding to the center of ear was used; IV - Productivity of husked (PEE) and unhusked cob (PED) in Mg.ha^{-1} obtained with the mass of the ears harvested and extrapolated to 1 ha; V - row number per ear (relation); VI - Number of grain per row (NGF); VII - grain yield (PG), the grains were separated with cut close to the surface of cob, these were weighed and the quantity obtained was extrapolated to 1 ha (Mg ha^{-1}).

For the determination of N content, they were collected in the aerial part of four plants per plot, in stages V_{12} and R_3. Subsequently, the plants were washed and weighed to determine the wet mass, then stored in paper bags and placed to dry in an oven with forced air circulation and the temperature was kept in the range of 65 to 70°C, until the samples reached constant weight. Subsequently, the samples were milled and subjected to chemical analyzes, for the determination of concentrations of N, according to the procedures described by Malavolta et al. (1997). In these results, the cumulative quantities of this nutrient per plant and the quantity obtained extrapolated to 1 ha (kg ha^{-1}) were estimated.

The data were subjected to analysis of variance by test 'F' at the level of 0.05 of probability and the averages were compared by the Tukey test with the aid of the Statistical Program System for Analysis of Variance - SISVAR®.

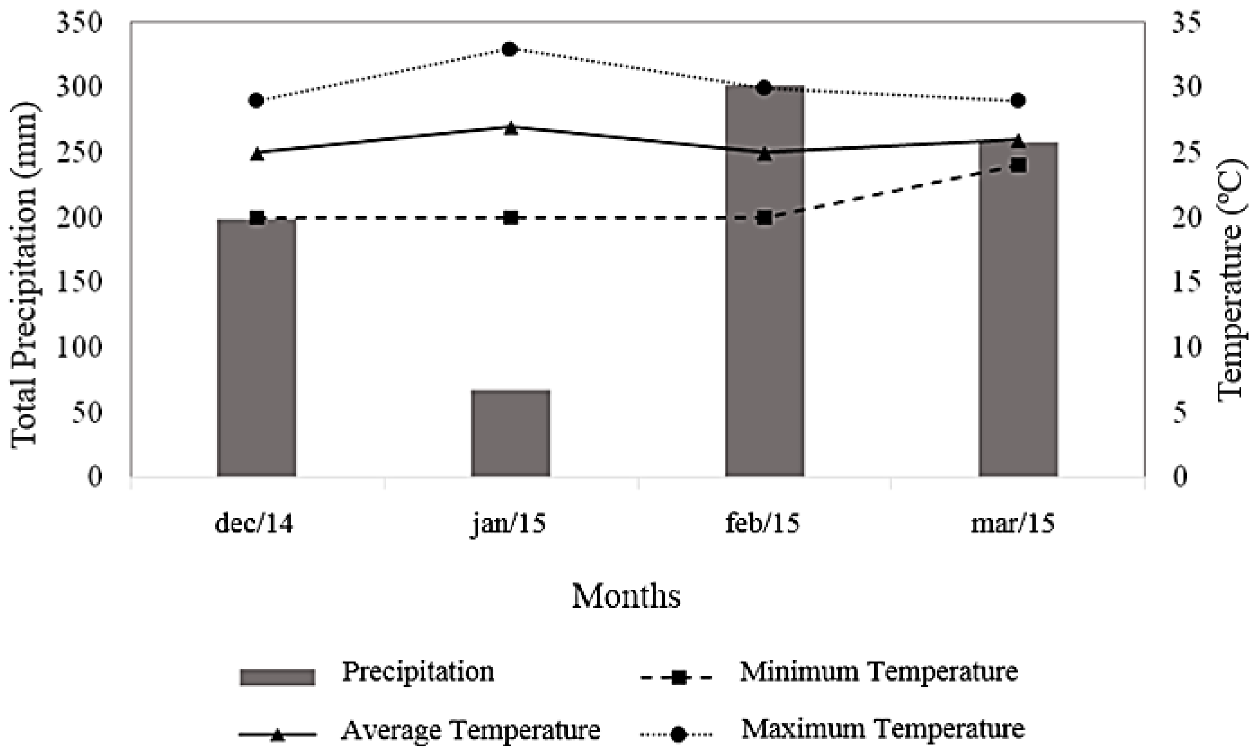

Figure 1. Pluviometric data of the meteorological station of Ipameri-GO, 2014/15, relating to the precipitation and temperature of the air during the months of development of the crop of sweet maize.

Table 1. The chemical characteristics of the soil, in a depth of 00 to 05 cm and 05 to 20 cm, sampled before the installation of the experiment.

Depth	pH	O.M.	P-Mehlich1	Macronutrients ($cmol\ dm^{-3}$)							Micronutrients ($mg\ dm^{-3}$)					V (%)
(cm)	$CaCl_2$	($g\ dm^{-3}$)	($mg\ dm^{-3}$)	K	Ca	Mg	Al	H+Al	SB	CTC	B	Fe	Mn	Zn	Cu	
00 to 05	5.2	3.5	12.6	0.1	1.8	0.6	0.0	2.8	1.7	5.29	0.61	24	3.4	3.0	0.5	47
05 to 20	4.8	2.9	5.6	0.1	1.2	0.4	0.1	3.8	1.7	5.53	0.34	43	2.3	1.4	0.5	31

O.M. = Organic matter; V% = saturation of bases; SB = sum of bases.

RESULTS AND DISCUSSION

The modes and phenological stages tested with nitrogen fertilization in green maize differed statistically ($P<0.05$), for all the variables, except for the row number per ear (relation), which shows the variability in the treatments in the study. According to the classification proposed by Scarpin et al. (1995), it may be inferred that the experiment had experimental accuracy within the normality standards, with coefficients of variation oscillating between 3.19%, for the husked ear diameter (DED) and 19.46% for N accumulated in the area (NPA) of sweet corn in stage V_{12} (Tables 2, 3 and 4).

The maximum height of insertion of the first ear (IFE) was observed in treatment with 50% N with total area in the stages V_4 to V_6, with an average of 1.20 m, differing statistically from the control and treatment with 100% N after planting, presenting a mean of 1.05 and 1.07 m, respectively (Table 2). The IFE is an important characteristic, because plants with higher values present advantages during harvest. The mode and phenological stage also influenced the height of insertion of the first ear in the work of Silva et al. (2005a), and the highest values were obtained in the treatments in which N was applied 15 days after emergence (DAE), the whole N at sowing and standard system of experimental farm. These results differ from those obtained by Neumann et al. (2005), who found no significant responses on plant height with regards to N fertilization in the maize for silage.

The length of husked cob (HEL) showed significant difference, and all the treatments were higher than the

Table 2. Average values of insertion of the first ear (IFE), husked ear length (HEL), unhusked ear length (CED), husked ear diameter (DED), husked ear productivity (PEE) and productivity of unhusked ear (PED).

Mode and season of application of N in coverage	IFE (m)	HEL (cm)	CED (cm)	DED (cm)	PEE (Mg ha^{-1})	PED (Mg ha^{-1})
Witness	1.05^{b}	18.9c	16.6c	4.1^{b}	22.9^{b}	12.3^{b}
100% N to launch after planting	1.07^{b}	20.2^{b}c	17.7^{b}c	4.4^{a}	28.3^{ab}	17.3^{ab}
100% N to launch total area V_4	1.11^{ab}	22.3^{ab}	19.6^{ab}	4.5^{a}	27.3^{ab}	17.4^{a}
50% N to launch total area V_4 and V_6	1.20^{a}	22.2^{ab}	19.4^{ab}	4.6^{a}	30.3^{a}	17.2^{ab}
100% N to launch total area V_6	1.11^{ab}	21.7^{ab}	19.1^{ab}	4.5^{a}	28.6^{a}	17.4^{a}
100% N continuous thread V_4	1.15^{ab}	22.8^{a}	19.9^{a}	4.6^{a}	32.7^{a}	19.5^{a}
100% N continuous thread V_6	1.14^{ab}	21.7^{ab}	19.1^{ab}	4.6^{a}	29.6^{a}	17.7^{a}
50% N continuous thread V_4 and V_6	1.12^{ab}	22.4^{ab}	19.6^{ab}	4.6^{a}	29.6^{a}	17.9^{a}
CV (%)	4.38	5.12	5.04	3.19	8.25	12.26

Averages followed by the same letter in the column do not differ by Tukey test ($P<0.05$).

Table 3. Average values of number of rows per ear (relation, NFE), number of grains per row (NGF) and grain yield (PG) evaluated in the culture of sweet corn.

Mode and season of application of N in coverage	NFE	NGF	PG (Mg ha^{-1})
Witness	16.18^{a}	28.87^{b}	5.48^{b}
100% N to launch after planting	16.87^{a}	36.72^{a}	8.73^{ab}
100% N to launch total area V_4	17.18^{a}	37.17^{a}	9.25^{a}
50% N to launch total area V_4 and V_6	17.12^{a}	36.86^{a}	8.94^{ab}
100% N to launch total areaV_6	16.50^{a}	36.39^{a}	9.20^{a}
100% N continuous thread V_4	16.62^{a}	38.14^{a}	10.57^{a}
100% N continuous thread V_6	16.81^{a}	36.15^{a}	9.38^{a}
50% N continuous thread V_4 and V_6	16.25^{a}	36.59^{a}	9.72^{a}
CV (%)	6.87	5.08	17.15

Averages followed by the same letter in the column do not differ by Tukey test ($P<0.05$) significant at 5% level of significance.

control, except the treatment with 100% N at the launching after planting. This is a very important characteristic in selling of green maize, when it is to be used for free fairs and greengrocery. However, the highest average HEL was with application of 100% nitrogen (N) in continuous thread in stage V_4, with 22.85 cm (Table 2). Similar results were obtained by Cardoso et al. (2011), when the performance of sweet maize cultivars in the municipality of Teresina, Piauí was evaluated, classifying unhusked green ears with lengths of 26.4 cm, as appropriate for marketing *in natura*.

The phenological stages and modes of application of nitrogen (N), influenced the unhusked ear length (CED), and the diameter of the husked ear (DED), presenting averages of 18.87 cm and 4.5 cm, respectively (Table 2). Almost all the treatments presented values of CED of preference by industry which is for ears that have length of about 20 cm. Only the control presented lower mean, with 16.62 cm, not differing statistically from treatment with 100% N, applied to launching after planting. For DED, there was no statistical difference between the control and all other treatments; however, all of them presented values higher than 4 cm, while the minimum value for trade is 3 cm. Significant results were also found by Oktem et al. (2010), when the authors evaluated the effects of different doses of N from 0 to 360 kg ha^{-1} applied at sowing and stage V_6, showing higher values of CED and DED, with 21.8 and 5.15 cm, respectively.

The productivity of husked cob (PEE) showed significant difference and five treatments were higher than the control (Table 2). The treatment with 100% N in continuous thread, in stage V_4, was higher with greater PEE, 32.7 Mg ha^{-1}, which is 30% greater than the control. These yields were higher than those of Freire et al. (2010), with the supply of urea, obtaining maximum productivity of 14.8 Mg ha^{-1} of green ears with straw. PEE is an important parameter considered in the marketing of green corn, due to the greater conservation of grain by husk protection.

Another important characteristic, directly related with

Table 4. Cumulative quantity of N in the aerial part at the stage V_{12} (NPA), in the aerial part without the grains in stage R_3 (NPASG), on grain (NG) and in the aerial part total (NPAT) of plants of sweet maize, depending on the mode and season of application of N in coverage.

Mode and season of application of N in coverage	N accumulated			
	NPA (V_{12}) ($kg\ ha^{-1}$)	NPASG (R_3) ($kg\ ha^{-1}$)	NG (R_3) ($kg\ ha^{-1}$)	NPAT (R_3) ($kg\ ha^{-1}$)
Witness	74.27^{b}	136.45^{b}	35.54^{b}	171.99^{b}
100% N to launch after planting	133.49^{a}	266.38^{a}	68.53^{a}	334.91^{a}
100% N to launch total area V_4	124.59^{ab}	258.48^{a}	66.65^{a}	325.13^{a}
50% N to launch total area V_4 and V_6	143.66^{a}	245.74^{a}	64.15^{a}	309.89^{a}
100% N to launch total area V_6	115.82^{ab}	286.59^{a}	66.27^{a}	352.86^{a}
100% N continuous thread V_4	129.75^{a}	254.32^{a}	79.77^{a}	334.09^{a}
100% N continuous thread V_6	120.37^{ab}	301.89^{a}	68.81^{a}	370.70^{a}
50% N continuous thread V_4 and V_6	118.59^{ab}	274.62^{a}	70.62^{a}	345.24^{a}
CV (%)	19.46	17.85	18.46	16.02

Averages followed by the same letter in the column do not differ by Tukey test ($P<0.05$).

the quality of the ear, is the productivity of unhusked ear (PED), considered as a commercial factor crucial both for the fresh market and for industry. For these characteristics, five treatments were higher than the witness, not differing statistically from the other two treatments with nitrogen fertilization. The treatment with 100% N in continuous thread, in stage V_4, stood out with greater PED, 19.47 Mg ha^{-1}, which is 37% greater than the control (Table 2).

The number of rows of grains per ear (relation, NFE) showed no statistical difference between the treatments, with lower mean of 17.18 rows (Table 3). Now, for the number of grains per row (NGF) and grain yield (PG), all the treatments were higher than the control; however, for PG treatment with 100% N applied after planting, and with 50% N applied in total area in the stages V_4 and V_6, significantly differ from the witness (Table 3). Souza et al. (2013) found similar results, when studying the yield components of sweet maize in different population densities, and the NFE also had no statistical differences; however, for the NGF, there was no statistical differences differentiating the results found in this work.

The three treatments that stood out for grain yield were the same as those that stood out in the productivity of unhusked corn ears, with 100% N applied in continuous thread in phase V_4, 50% N applied in continuous thread in stages V_4 and V_6 and 100% N applied in continuous thread in phase V_6, showing PG of 10.57, 9.72 and 9.38 Mg ha^{-1}, PED 19.5, 17.9 and 17.7 Mg ha^{-1}, respectively.

When the N was divided and applied in continuous thread, this provided greater productivity as compared to application in total area, probably, in function of minor losses by leaching due to fragmentation, less volatilization by less contact with the ground and, consequently, with the enzyme urease, as well as greater efficiency of absorption, when applied next to plants.

For the variable accumulation of N in the area of green maize in the stage V_{12}, it is observed in Table 4 that there was significant difference and three treatments were higher than the control. The treatments with: 50% N launching in stages V_4 and V_6; 100% N launching after planting; and 100% N in continuous thread in the stage V_4, highlighted and exhibited greater averages of N accumulated with 143.66, 133.49 and 129.75 kg ha^{-1}, respectively. In this way, the period of application of N soon after the planting and the V 4 stage V_4 promoted the highest absorption and accumulation of N in the stage V_{12}, probably, in function of this nutrient which is available to the plants for a longer period of time.

The accumulation of N in the stage R_3 for the various parts of plants of sweet maize (vegetative parts, grains and total plant) were significant and all treatments differed statistically from witness, as shown in Table 4. The largest mean values of N, accumulated in the aerial part without the grain and in the total aerial, were observed in the treatments with: 100% N in continuous thread and 100% N in total area in the stage V_6, with an average of 1.89 and 286.59 kg ha^{-1}, for N accumulated in the aerial part without the grain, and 370.70 and 352.86 kg ha^{-1}, for the aerial part total, respectively. The application of N in the V $stage_6$ was crucial and provided the largest accumulation of N in stage R_3, both for the air without grains as compared to the aerial part total, in culture of sweet maize.

The results of this study do not correspond with the results obtained by Borin et al. (2010), when absorption and accumulation of N was assessed in the culture of sweet-maize grown in field conditions, in which total extraction of N by aerial part was of 123,05 kg ha^{-1}.

The treatments with: 100% N in continuous thread in the stage V_4; 50% nitrogen (N) in continuous thread in $stageV_4$ and V_6; and 100% N in continuous thread in stage $V_{6,}$ showed the highest average values of N accumulated in the grain with 79.77, 68.81 and 70.62 kg ha^{-1}, respectively. When the N was divided and applied in continuous thread, this provided greater accumulations of N in grains in relation to application in total area.

Conclusions

The mode (in-line application, in total area) and season (after planting, V_4 and V_6 stage) of application influenced the quality of ears and the productivity of corn maize. The application of nitrogen (N) in continuous thread offered the highest averages for the characteristics evaluated.

The season of application of nitrogen (N) influences the absorption and the accumulation of nitrogen (N) in the aerial part of green maize. The mode of application in continuous thread allows greater accumulation of nitrogen (N) in grains as compared to application in total area.

Conflict of interest

The authors have not declared any conflict of interest.

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REFERENCES

Almeida RF, Sanches BC (2012). Fertizilantes with nitrogen slow release and stabilized in agriculture. Green J. Mossoró RN 7(5):31-35.

Borin ALDC, Lana RMQ, Pereira HS (2010). Absorption, accumulation and export of macronutrients in sweet corn cultivated under field conditions. Ciênc. Agrotecnol. Lavras 34:1591-1597.

Cardoso MJ, Ribeiro VQ, Melo FB (2011). Performance of green corn cultivars in the city of Teresina, Piauí. Embrapa Mid-North. Tech. Notice 227:1-4.

Carmo MS, Cruz SCS, Souza EJ, Campos LFC, Machado CG (2012). Sources and doses of nitrogen en the desenvelopment of culture end productivity of sweet corn (*Zea mays* convar. *Saccharata* var. *rugosa*). Biosci. J. Uberlândia 28:223-231.

EMBRAPA (2013). Brasilian Company of Agriculture and Livestock research. *Brazilian system of soil classification.* Brasília, DF: Embrapa, 3ª ed. rev. ampl. 353 p.

Freire FM, Viana MCM, Mascarenhas MHT, Pedrosa MW, Coelho AM, Andrade CLT (2010). Economic yield and production components of green maize ears influenced by nitrogen fertilization. Braz. J. Maize Sorghum 9(3):213-222.

Malavolta E, Vitti GC, Oliveira SA (1997). *Avaliação do estado nutricional das plantas:* princípios e aplicações. 2ª ed. Piracicaba: Potafós.

Matos EHSF (2007). Plantação do Milho Verde. In: MATOS, E. H. S. F. Cultivo do Milho Verde – Dossiê Técnico. CTD/UnB.

Neumann M, Sandini IE, Lustosa SBC, Romano MA, Falbo MK, Pansera R (2005). Yield and production components of corn (Zea mays L.) for silage as a result of nitrogen fertilization. Ver. Bras.de Milho e Sorgo 4(3):418-427.

Oktem A, Oktem AG, Emeklier HY (2010). Effect of nitrogen on yield and some quality parameters of sweet Corn. Communications in Soil Science and Plant Analysis. Lincoln 41:832-847.

Oliveira Junior LFG, Deliza R, Bressan-Smith R, Pereira MG, Chiquiere TB (2006). Selection of corn on the cob genotypes more appropriate to the in natura consumption. Food Technol. Sci. Campinas 26(1):159-165.

Rambo L, Silva PRF, Argenta G, Bayer C (2004). Soil nitrate tests as complementary indices for management of corn nitrogen application. Ciênc. Rural 34(4):1279-1287.

Sawazaki E, Ishimura I, Rosseto CJ, Maeda JA, Saes LA (1990). Green corn: evaluation of earworm resistance, pericarp thickness and other agronomic characters. Bragantia Campinas 49(2):241-251.

Scarpin CA, Carvalho CGP, Cruz CD (1995). Proposal to categorize coefficients of variation for yield and plant height in soybean. Braz. Agric. Res. Bras. 30(5):683-686.

Silva EC, Ferreira SM, Silva GP, Assis RL, Guimarães GL (2005a). Timing and methods of nitrogen application to no-tillage corn on cerrado soil. Braz. J. Soil Sci. 29:725-733.

Silva PRF, Strieder ML, Cose RPS, Rambo L, Sangoi L, Argenta G, Forsthofer EL, Silva AA (2005b). Grain yield and kernel crude protein content increases of maize hybrids with late nitrogen side-dressing. Sci. Agríc. 62(5):487-492.

Souza RS, Vidigal FPS, Scapim CA, Marques OJ, Queiroz DC, Okumura RS, José JV, Tavore RV (2013). Yield elements of sweet corn in different population densities. Comun. Sci. 4(3):285-292.

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Full Length Research Paper

Selectivity and efficiency of herbicides in warm season turfgrass varieties

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Weed infestations and the few post-emergent control alternatives available are some of the main problems in the management of ornamental and sports lawns. Given the exposed, the study aims to evaluate the selectivity of five herbicides to four grass varieties: *Paspalum notatum* (bahia grass), *Cynodon dactylon* (bahama grass- ITG6), *Zoysia japonica* (japanese lawn grass) and *Zoysia japonica* (imperial – ITG5), as well as the control efficiency of those upon nutsedge (*Cyperus rotundus*) and sudan grass (*Urochloa decumbens*). The following herbicides were applied in the recommended doses: sulfentrazone + diuron, isoxaflutole, triclopyr, 2,4-D and halosulfuron. Visual phytotoxicity symptoms in turfgrass and weed control were performed at 7, 14, 21, 28, 35 and 42 days after herbicide application and, at the end of the evaluations, was quantified the dry weight. The herbicides sulfentrazone + diuron and isoxaflutole were not selective to any turfgrass, although sulfentrazone + diuron controlled *C. rotundus* and *U. decumbens* species and isoxaflutole controlled only *U. decumbens.* However, halosulfuron and 2,4-D herbicides were selective to all of grasses and promoted weed species control. The herbicide triclopyr was selective to *P. notatum*, *Z. japonica* and *Z. japonica*– ITG5, and it can be used to control *C. rotundus* and *U. decumbens.*

Key words: *Urochloa decumbens, Cynodon dactylon*–ITG6, *Cyperus rotundus, Paspalum notatum, Zoysia japonica*.

INTRODUCTION

Warm-season (C4) grass species are the most adaptable to Brazil's environmental conditions. The main turfgrasses used in tropical regions are *Cynodon* spp., *Zoysia* spp., *Penicillium notatum, Axonopus compressus* (lawn grass), *Stenotaphrum secundatum* (st Augustine grass) *Pennisetum clandestinum* (kikuyu grass) (Gurgel, 2003).

During the grass field formation, the presence of weeds is one factor that interfere the most to diminish its aesthetic quality and its usage, depreciating the garden's value. The aesthetic value is defined by its beauty and the value the garden adds to the scenery. In addition, the usability is the durability of a given field to sports or reduction in the erosion effects (McElroy and Martins, 2013), due its unique characteristics such as fast growth, deep and penetrating root system, and high tolerance to adverse conditions (Xiao et al., 2011).

Among the weeds that infest gardens are sudan grass (*Urochloa decumbens*) and nutsedge (*Cyperus rotundus*). *C. rotundus* is a weed that occurs during the entire cycle of several commercial crops and has a difficult management (Silveira et al., 2010) due to its competition capabilities and its aggressiveness, as well as its control and eradication difficulty. *Urochloa* species are weeds of perennial cycle and slower growth (Bianco et al., 2005) and also of hard control due factors inherent to the species such as morphological characteristics and the large amount of seed produced (Santos et al., 2012).

In turfgrasses, besides the difficulty of controlling weed, the current shortage of post-emergent products registered for the crop is another problem too. The availability of different mechanisms of action is an essential factor for crop management to reduce selection pressure and to create alternatives of control. Post emergence control of grass weeds is a great challenge, since few selective herbicides to grasses actually may affect these weed species to death. As so, too little alternatives may be used in post-emergence when control is necessary (Unruh et al., 2013). Selectivity refers to the capacity of a particular herbicide to eliminate weeds found in a crop, without affecting yield (Velini et al., 2000).

Regarding said information, the experiment was conducted aiming to evaluate the selectivity of herbicides on bahia grass (*Paspalum notatum*), bermuda grass (*Cynodon dactylon* – ITG6), japanese lawn grass (*Zoysia japonica*) and imperial grass (*Zoysia japonica* – ITG5) associated to sudan grass and nutsedge control.

MATERIALS AND METHODS

The experiment was conducted in Jaboticabal, São Paulo, located at 21°14'05" S latitude and 48°17'09" W longitude, at a 615 m altitude, from January to August 2015, using 120 plastic boxes with 5.44 liter capacity (35.5 x 25.5 x 6 cm) filled with soil collected from the arable part of an oxisol. The soil chemical characteristics are described in Table 1, according to Raij et al. (2001).

Turfgrass varieties evaluated in the experiment were: *P. notatum* (bahia grass), *C. dactylon* (bermuda grass – ITG6), *Zostera Japonica* (japanese lawn grass) and *Z. Japonica* ITG5 (ITG5 - imperial), which were planted as pads in 96 boxes, using 24 boxes for each variety. On the 24 remaining boxes were sown *U. decumbens* (sudam grass) and planted tubercles of *C. rotundus* (nutsedge). For Sudam grass sowing, 30 seeds m^{-2} were deposited on the length direction of the boxes and, parallel to it, 50 nutsedge tubercles m^{-2} were planted. Fertilizer was applied at an amount of 400 kg ha^{-1} NPK fertilizer 4-20-20 and soil's capacity was maintained at 100%.

Applied herbicides upon the grasses and weeds were: sulfentrazone (600 g $a.i.ha^{-1}$) + diuron (3840 g $a.i.ha^{-1}$), isoxaflutole (190 g $a.i.ha^{-1}$), triclopyr (1.19 g $a.i.ha^{-1}$), 2,4-D (1612 g $a.i.ha^{-1}$) and halosulfuron (112,5 g $a.i.ha^{-1}$), with a control plot in which no herbicides were applied. The herbicides were applied 35 days after planting/sowing (DAS) on the boxes, when the weeds showed 4 to 5 leaves and the garden grasses were visually rooted, with buds and covered the entire box. A CO_2 constant pressure backpack sprayer was used equipped with a XR 11002 nozzle. The equipment was adjusted at 2.2 bar pressure to spray the equivalent of 200 $l.ha^{-1}$ herbicide solution. The herbicides were applied at closed environment, with humid substratum, 27°C temperature and air humidity of 74%.

Phytotoxicity symptoms on the grasses were evaluated visually, adopting a visual scale of one to nine, in which 1 equals the absence of toxicity and 9 equals the death of the plants (Ewrc, 1964). Such evaluation was performed every 7 days after herbicide application and repeated until 42 days after application (DAA). Visual evaluations of the weed control were performed at 40 DAA, in which they were attributed control grades ranging from 0 to 100% depending on the symptoms' intensity (ALAM, 1974), according to Table 2.

At 42 DAA, the plants were collected in order to analyze the dry matter (to estimate growth). For the garden grasses, a 10x10 cm pad was collected from each box, taking only the live part (which was not affected by the herbicide), from which were separated the leaves and roots/rhizomes, that after being washed, were placed in forced air circulation stoves at 70°C to dry for 72 h.

The experimental design used for each grass and weed was the complete randomized design, with five treatments (herbicides) and four replications. The statistical analysis was performed separately for each species. Data found was submitted to the variance analysis through F-test and means compared through Tukey test at 95% probability, using the AgroEstat software (Barbosa and Maldonado, 2010).

RESULTS AND DISCUSSION

Bahia grass

It may be observed that, for bahia grass, among the evaluated treatments, sulfentrazone + diuron and isoxaflutole herbicides were the most phytotoxic treatments, because they induced initial symptoms of foliar chlorosis and yellowed leaves with white spots,

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Table 1. Results of collected soil chemical analysis.

pH	M.O.	P	K	Ca	Mg	H+Al	SB	T	V
$CaCl_2$	$g.dm^3$	resin $mg.dm^3$			------------------------------$mmol_c.dm^{-3}$-----------------------------				Percentage (%)
5.6	18	125	4.6	42	15	28	61.6	89.6	69

Analysis Laboratory of Soil and Plant, Department of Soils and Fertilizers, Faculdade de Ciências Agrárias e Veterinárias/UNESP - Jaboticabal-SP.

Table 2. ALAN grade scale used to evaluate the effectiveness of weed control.

Percentage	Grau de controle
0-40	None or poor
41-60	Regular
61-70	Enough
71-80	Good
81-90	Very good
91-100	Excelent

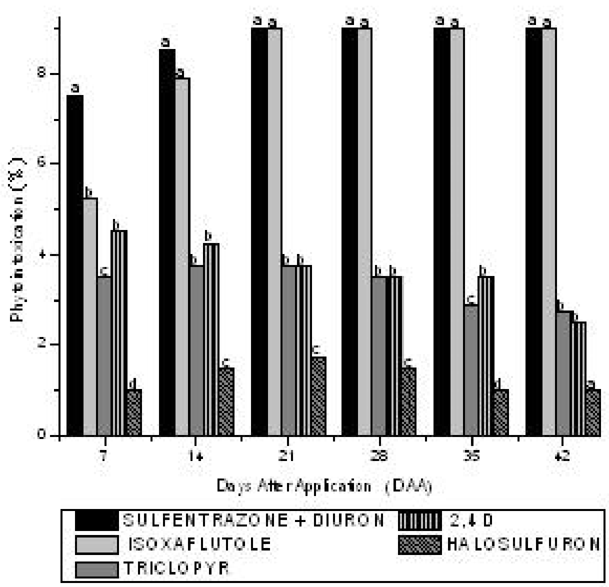

Figure 1. Phytointoxication of *P. notatum* submitted to herbicide application after 7, 14, 21, 28, 35 and 42 days.

respectively, at 14 DAA, causing the death of the specimens at 42 DAA (Figure 1). The application of triclopyr gave a brand level of toxicity (3.5) at 7 DAA, with foliar chlorosis. This symptom progressed slowly (3.75) until the 21 DAA, and after this, the species were recovered and, at 42 DAA, the symptoms were very brand (2.75).

Freitas et al. (2003) verified that the usage of triclopyr showed a phytotoxicity of 4 (0.96 kg $a.i.ha^{-1}$) and 10% (1.20 kg $a.i.ha^{-1}$) on bahia grass, at 10 DAA, and that at 20 DAA there was no phytotoxic effect of this treatment. In the 2,4-D treatment, the herbicide caused a foliar chlorosis effect (7DAA) with toxicity level of 4.5, but the plants recovered from this initial damage at the 35 DAA, and at the 42 DAA, the toxicity level was 2.3 (Figure 1). Akanda et al. (1997), studying the effects of 2,4-D upon the chemical control of *P. notatum* observed that the herbicide lead to brand symptoms (≤2.0%) until the 35 DAA evaluation, and after this period, the symptoms did not evolve and the plants recovered. Machado et al. (2010), evaluating the effect upon other species (white clover), showed it promoted a toxicity level of 60% at 21 DAA and, at 84 DAA, the symptoms were reduced to 22%. The authors also show that moderate symptoms induced only a little reduction in the productivity. It may be observed that these symptons of 2,4-D upon bahia grass had a reduction on its growth too (around 50% in aerial part).

Halosulfuron caused the least phytotoxical symptoms when compared to the other treatments. The highest toxicity level occured at 21 DAA, being considered as very brand (1.75). At the end of the evaluations, the foliar chlorosis symptoms reduced, being zero at 42 DAA (Figure 1). These results showed an elevated selectivity degree of this herbicide upon the bahia grass. Costa et al. (2010) observed that the halosulfuron application at 112.5 g a.i. ha^{-1} induced severe phytotoxicity levels at 7 DAA. However, at 14 DAA, the symptoms degraded to brand levels, disapearing at the 26 DAA.

Phytotoxicitysymptoms occurring in the species regarding the herbicide application are related to the lesser production of aerial parts dry matter. As for the roots/rhizomes dry matter production, the herbicides triclopyr, 2,4-D and halosulfuron did not differ from the control plot (Table 3).

Bermuda grass

For bermuda grass, it was observed that sulfentrazone + diuron and triclopyr were the most phytotoxic herbicides, providing severer phytotoxicity symptoms – 7.3 and 8,

Table 3. Herbicide effect upon dry matter of leaves and roots/rhizomes of *P. notatum* evaluated after 42 days.

Treatment	Aerial parts (g)	Roots/Rhizomes (g)
Sulfentrazone + Diuron	0.00[c]	0.00[b]
Isoxaflutole	0.00[c]	0.00[b]
Triclopyr	2.76[ab]	13.13[a]
2,4-D	2.01[b]	16.03[a]
Halosulfuron	2.40[ab]	16.91[a]
Control	4.62[a]	15.66[a]
CV(%)	46.94	37.20
MSD	2.07	8.60
F	14.65**	17.77**

Means followed by same letter in a column do not differ statistically through Tukey test (p<0.05); CV (%) = coefficient of variation; MSD = minimal significant difference.

Table 4. Herbicide effect upon dry matter of leaves and roots/rhizomes of *C. dactylon* ITG6 evaluated after 42 days.

Treatment	Aerial parts (g)	Roots/Rhizomes (g)
Sulfentrazone + Diuron	0.00[b]	0.00[c]
Isoxaflutole	1.98[a]	7.84[b]
Triclopyr	0.00[b]	0.00[c]
2,4-D	2.16[a]	9.20b
Halosulfuron	2.30[a]	12.57[ab]
Control	3.36[a]	17.11a
CV(%)	41.75	30.98
MSD	1.53	5.42
F	15.75**	32.04**

Means followed by same letter in a column do not differ statistically through Tukey test (p<0.05); CV (%) = coefficient of variation; MSD = minimal significant difference.

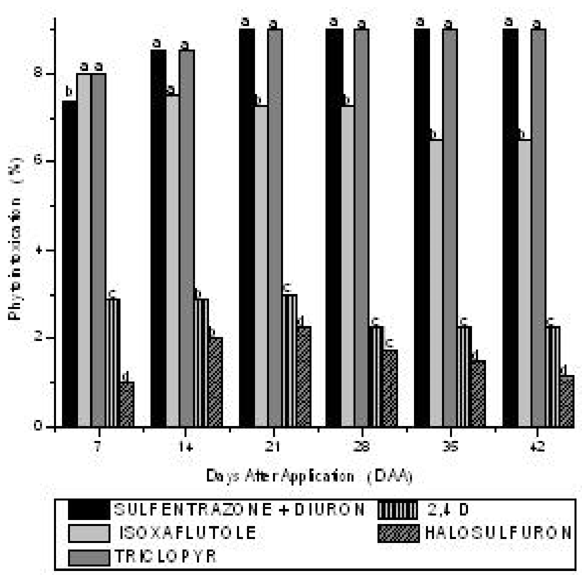

Figure 2. Phytointoxication of *C. dactylon* – ITG6 submitted to herbicide application after 7, 14, 21, 28, 35 and 42 days.

respectively – at 7 DAA. These symptoms are characterized by the yellowing and foliar discoloration, respectively. The symptoms evolved during the length of the experiments evaluations, causing all the plants to be dead at the 42 DAA evaluation (Figure 2).

Some papers show triclopyr toxicity effect upon *Cynodon* species. McElroy and Breeden (2006) evaluating its effect at 1.23 kg a.e.ha^{-1}, attained a 47% suppressing effect upon this species. McElroy et al. (2005) observed that the application of triclopyr + clopyralid reducing soil cover about 56%, at 21 DAA and, these injuries were associated mainly by the herbicide triclopyr. Johnson and Duncan (2001) found that applications of triclopyr cause injuries around to 30% at 2 DAA. 2,4-D and halosulfuron provoked the least phytotoxicity upon the bermuda grass, such as with bahia grass. Symptoms at 21 DAA evaluation were classified as brand (3.5) and very brand (2.3), respectively, attaining a toxicity level near 2 (2,4-D) and 0 (halosulfuron) at 42 DAA (Figure 2).

It is noticed that dry matter on 2,4-D treated plants, for aerial parts and roots, was slightly inferior than the obtained for halosulfuron, but no significant difference was shown for aerial parts through the statistical analysis (Table 4). Data obtained from the halosulfuron application shows this herbicide was selective for bermuda grass.

Isoxaflutole caused severe symptoms at 7 DAA and 14 DAA, causing the death of 85% of the grass pad. In the remaining evaluations, the initial symptoms were reduced and the plant growth restarted indicating, at 42 DAA, a moderate recovery with almost strong toxicity level (6.5). These symptoms caused roots/rhizomes dry mass loss by about 54%, when it was compared to control, however no differences occurred to aerial parts dry mass (Table 4).

Japanese lawn grass

Herbicides sulfentrazone + diuron and isoxaflutole were the most phytotoxical, causing initial symptoms of foliar chlorosis and yellowed leaves, causing death of the plants at 42 DAA (Figure 3). Triclopyr application caused moderate phytotoxicity symptoms (4) at 7 and 14 DAA,

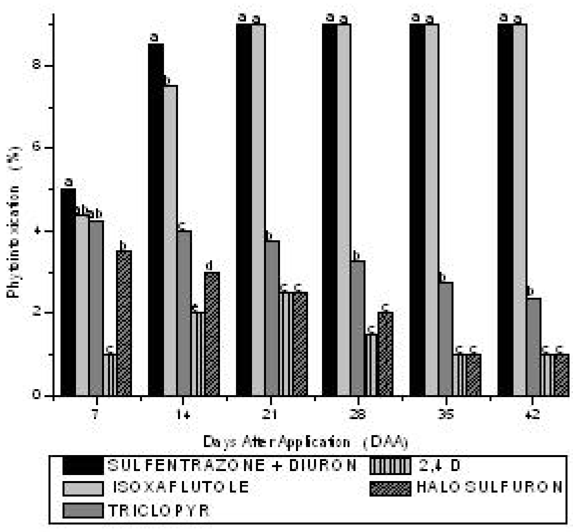

Figure 3. Phytointoxication of *Z. japonica* submitted to herbicide application after 7, 14, 21, 28, 35 and 42 days.

Table 5. Herbicide effect upon dry matter of leaves and roots/rhizomes of *Z. japonica* evaluated after 42 days.

Treatment	Aerial parts (g)	Roots/Rhizomes (g)
Sulfentrazone + Diuron	0.00[b]	0.00[d]
Isoxaflutole	0.00[b]	0.00[d]
Triclopyr	3.89[a]	10.15[bc]
2,4-D	6.16[a]	21.52[a]
Halosulfuron	6.11[a]	17.87[ab]
Control	3.88[a]	13.12[b]
CV(%)	36.76	31.40
MSD	3.01	12.76
F	12.33*	8.13**

Means followed by same letter in a column do not differ statistically through Tukey test ($p<0.05$); CV (%) = coefficient of variation; MSD = minimal significant difference.

being characterized as foliar chlorosis and tissue necrosis. However, during the evaluations, the symptoms reduced and, at the 42 DAA, the phytotoxicity level was very brand (2.4). During the evaluations, it was noticed that triclopyr caused a reduction of 20% in the grass pads, having a slight growth recovery toward the end of evaluations , which may be noticed by the lower roots dry matter value when compared to 2,4-D and halosulfuron (Table 5). About the herbicide selectivity of 2,4-D and halosulfuron, the toxicity level was equal to 0 and 3.5 (brand), respectively, at 7 DAA. From 21 DAA, both 2,4-D and halosulfuron had their symptoms reduced along the evaluations, disappearing at the 42 DAA. Analyzing data from dry matter of aerial parts and roots, it may be observed that the allocation was similar to control.

ITG5 – Imperial grass

Regarding results obtained for Imperial grass, suulfentrazone + diuron proportioned an elevated toxicity level (4.5) already at 7 DAA. Symptoms developed along the evaluations until 21 DAA, reaching a toxicity level next to 7, considered strong. However, during the following evaluations, the initial symptoms decreased and plant growth continued, indicating a slight recovery at 42 DAA (Figure 4). As for the dry matter, a lower mass allocation regarding aerial parts of plants treated with sulfentrazone + diuron was observed, when compared to the other treatments and the control plot, and roots dry matter showed no difference from the control plot (Table 6).

Isoxaflutole applied to imperial grass at 7 DAA proportioned brand toxicity symptoms (2.5). However, the symptoms advanced and, at 21 DAA, the herbicide provoked the death of the plants (Figure 4). It is noticed that this herbicide also shows low selectivity to bahia grass, bermuda grass and japanese lawn grass. Injury symptoms were present due to the application of triclopyr being more intense at 21 DAA, reaching toxicity level of 4 (moderate symptoms). On the following evaluations, there was no evolution of symptoms, which consisted of bleached leaves, and plants continued to grow and, at 42 DAA, no more signs of the initial symptoms were observed. McElroy and Breeden (2006) after applying triclopyr (1.23 kg a.e.ha^{-1}) show the injuries were of 10% or bellow. Similar results were obtained by Lewis et al. (2012). Dry matter accumulation effects due to application were similar to the control, with slight reductions of aerial parts and roots dry matter (Table 6).

Herbicides 2,4-D and halosulfuron have the lowest toxicity levels for imperial grass. The most elevated levels were at 21 DAA, considered as brand (3) and very brand (2). On the remaining evaluations, the symtoms lessened significantly and, at 42 DAA, no injury symptom was observed (Figure 4). Data about dry matter of aerial parts and roots of these herbicides show similar matter aggregation to control (Table 6). Similar to bahia grass and bermuda grass, data from halosulfuron application shows imperial grass is also very tolerant to this herbicide.

Differences on the toxicity symptoms found between the species and cultivars may be due to their own distinctions about herbicide tolerance, given the difference

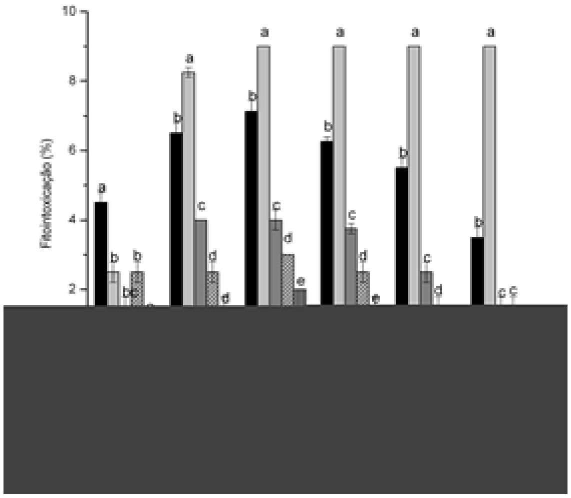

Figure 4. Phytotoxicity of *Z. japonica* – ITG5 submitted to herbicide application after 7, 14, 21, 28, 35 and 42 days.

Table 6. Herbicide effect upon dry matter of leaves and roots/rhizomes of *Z. japonica* – ITG5 evaluated after 42 days.

Treatment	Aerial parts (g)	Roots/Rhizomes (g)
Sulfentrazone + Diuron	2.40b	12.56a
Isoxaflutole	0.00c	0.00b
Triclopyr	3.17ab	10.48ab
2,4-D	4.50ab	13.27a
Halosulfuron	5.29a	17.50a
Control	4.01ab	14.36a
CV(%)	29.36	41.65
MSD	2.13	10.62
F	15.64**	6.41**

Means followed by same letter in a column do not differ statistically through Tukey test ($p<0.05$); CV (%) = coefficient of variation; MSD = minimal significant difference.

from plant to plant to metabolize a given herbicide, its low penetration or limited translocation along the plant (Kelley and Riechers, 2007).

Control of weeds

Regarding the control of nutsedge, sulfentrazone + diuron caused yellowing and necrosis symptoms, with a control level of 60% at 7 DAA. Along the evaluations, these symptoms evolved, causing the death of the weed at 35 DAA (Figure 5). Some papers show the efficiency of sulfentrazone on *C. rotundus* control, (Martins et al., 2009; Silva et al., 2014), however, some experiments like those of Gannon et al. (2012), applying 0.035 and 0.07 kg ha^{-1}, show unacceptable control levels of this species. For diuron, it is known that it is used for pre-emergence control on grass gardens for annual and broad-leaf weeds (McElroy and Martins, 2013).

Triclopyr shows a similar result to sulfentrazone + diuron. However, the symptoms were more severe after the 14 DAA, when it caused 60% control. Along the evaluations, the symptoms became more intense and, at 35 DAA, this herbicide caused the death of the weeds. Symptoms observed were growth paralyzation, foliar epinasty, chlorosis (Kelley et al, 2005; Grossmann, 2010), becoming yellowed and, eventually, death. Isoxaflutole was not efficient on *C. rotundus* control. Between 7 and 21 DAA, control levels were considered very low, about 20%. From the following evaluations, the initial symptoms were reduced and the plant growth restarted, indicating a recovery from the symptoms (Figure 5).

Halosulfuron and 2,4-D treatments had similar responses about the evolution of the symptoms, which increased during the evaluations. At the end of the 42 DAA evaluation, these herbicides caused a 80 and 72% control, respectively, which are considered good control levels. Durigan et al. (2005) evaluating the application of halosulfuron (0.0937, 0.1125 and 0.1312 kg ha^{-1}) and 2,4-D (2.01 kg ha^{-1}), relate halosulfuron had an 80% control and that it reduced the tubercle percentage above 92%, and the control efficacy and tubercles reduction from 2,4-D application was considered low and inconstant. Similar results were found by Oliveira et al. (2010) regarding the best performance of halosulfuron (85% control) than 2,4-D (60%) on the control and epigeal manifestations of *C. rotundus*. Brecke et al. (2005) show halosulfuron reduced the number and viability of nutseged tubercles in a mean value of 55%. About *Urochloa decumbens* control, sulfentrazone + diuron, isoxaflutole and triclopyr treatments show better results than the remaining treatments. At 7 DAA, the control was already considered good - about 80% - and at 35 DAA, these treatments caused the death of the weeds (Figure 5).

Carbonari et al. (2010) evaluating isoxaflutole application in dry and wet ways (150 and 225 g a.i. ha^{-1}) in post-emergence control of *U. decumbens* stated a mean control of 90% - for solid way at 75 and 110 DAA – 90 and 45% (wet way) at 75 and 110 DAA, respectively. It is observed that 2,4-D and halosulfuron treatments, unlike *C. rotundus* response, were not effective to control Sudan grass. Both herbicides, at the final evaluation (42 DAA) caused only a 30% mean weed control, considered insatisfactory.

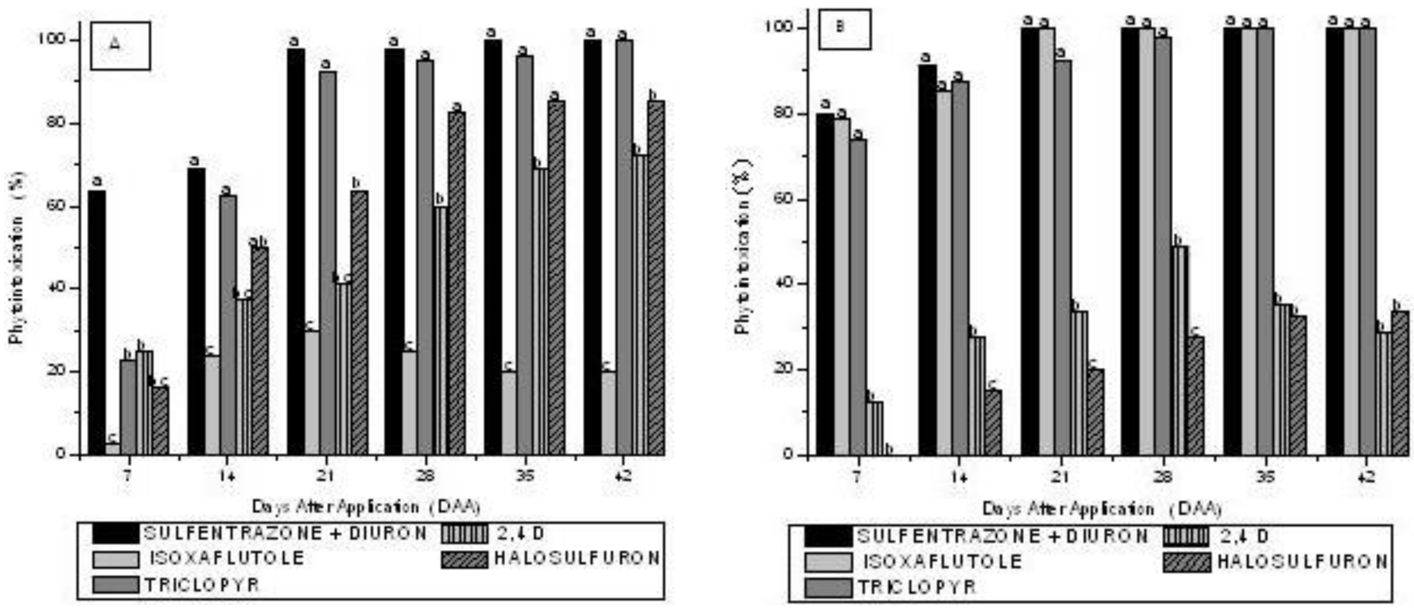

Figure 5. Percentage of control of sulfentrazone + diuron, isoxaflutole, triclopyr, 2,4-D and halosulfuron herbicides applied upon *C. rotundus* (A) *and U. decumbens* (B).

About auxin mimic herbicides, those are highly used on commercial gardens, golf courses and crops for its selectivity to grasses and its control upon dicotyledonous weeds (Song, 2014). Herbicides that have this action mechanism are mainly active on broadleaf species (McElroy and Martins, 2013). However, as it has been cited, species tolerance is dependent of many factors such as development stage, species, herbicide, among others, which may explain the control difference of grass species by auxinic herbicides.

Conclusions

The herbicides sulfentrazone + diuron and isoxaflutole were not selective to *P. notatum*, *C. dactylon* – ITG6, *Z. Japonica* and *Z. Japonica*– ITG5 grasses due to its phytotoxicity, however, sulfentrazone + diuron controlled *C. rotundus* and *U. decumbens* weeds and isoxaflutole controlled *U. decumbens.* Halosulfuron and 2,4-D were selective for all four grasses and both controlled just *C. rotundus.* The herbicide triclopyr was selective for *P. notatum*, *Z. japonica* and *Z. japonica* – ITG5, and may be used to control *C. rotundus* and *U. decumbens.*

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Akanda RU (1997). Influence of postemergence herbicides on tropical soda apple (*Solanum viarum*) and bahia grass (*Paspalum notatum*). Weed Technol. 11(2):656-661.

Barbosa JC, Maldonado Jr W (2010). *AgroEstat*: sistema para análises estatísticas de ensaios agronômicos. Versão 1.0. Jaboticabal: Unesp.

Bianco S, Tonhão MAR, Pitelli RA (2005). Growth and Mineral Nutrition of Surinam Grass*.* Planta Daninha 23(3):423-428.

Brecke, BJ, Stephenson, DO, Unruh, JB (2005). Control of purple nutsedge (cyperus rotundus) with herbicides and mowing. Weed Technol. 19(4):809-814.

Carbonari CA, Velini ED, Silva JRM, Bentivenha SRP, Takahashi EM (2010). Efficacy of the aerial application of the herbicides sulfentrazone and isoxaflutole using clay granules in eucalyptus area. Planta Daninha 28(1):207-212.

Costa NV, Martins D, Rodrigues ACP, Cardoso LA (2010). Selectivity of herbicides applied on bahia grass and broadleaf carpetgrass turfs. Planta Daninha 28(2):365-374.

Durigan JC, Timossi PC, Leite GJ (2004). Chemical control of *Cyperus rotundus*, with and without sugar cane straw cover. Planta Daninha 22(1):127-135.

European weed research council (EWRC) (1964). Report of 3rd and 4rd meetings of EWRC. Committee of methods in weed research. Weed Res. 4(1):88.

Freitas FCL, Ferreira LR, Silva AA, Barbosa JG, Miranda GV, Machado AFL (2003). Triclopyr efficiency in controlling weeds in the lawn (*Paspalum notatum*). Planta Daninha 21(1):159-164.

Gannon TWG, Yelverton FH, Tredway LP (2012). Purple nutsedge (*Cyperus rotundus*) and false-green kyllinga (*Kyllinga gracillima*) control in bermudagrass turf. Weed Technol. 26(1):61-70.

Grossmann K (2010). Auxin herbicides: current status of mechanism and mode of action. Pest Manage. Sci. 66:113-120.

Gurgel RGA (2003). Principais espécies e variedades de grama. *In:* I SIGRA – Simpósio Sobre Gramados – “Produção, Implantação e

Manutenção", 1, 2003, Botucatu. *Anais*... Botucatu: FCA/UNESP. 1.CD-ROM.

Johnson BJ, Duncan RR (2001). Effects of herbicide treatments on suppression of seashore paspalum (*Paspalum vaginatum*) in bermudagrass (*Cynodon* spp). Weed Technol. 15(1):163–169.

Kelley KB, Riechers DE (2007). Recent developments in auxin biology and new opportunities for auxinic herbicide research. Pesticide Biochem. Physiol. 89:1-11.

Kelley KB, Wax LM, Hager AG, Riechers DE (2005). Soybean response to plant growth regulator herbicides is affected by other postemergence herbicides. Weed Sci. 53(1):101-112.

Lewis DF, McElroy S, Breeden GK (2012). Control of summer annual grasses during seeded zoysiagrass establishment with various timings and rates of fluazifop, triclopyr, and fluazifop plus triclopyr. Weed Technol. 26(2):267-271.

Machado D, Lustosa SBC, Baldisserai TC, Turok JDNT, Machado M, Watzlawick LF, Mendonça CG, Pelissari A (2013). Herbicide selectivity on white clover in phenological stage of first trifoliate expanding. Ciência Rural 43(12):2132-2138.

Martins D, Tomazela MS, David DV, Martins CC (2009). Purple nutsedge control with sulfentrazone and diclosulam and tubers viability in different soil depths. Bragantia 68(2):357-366.

McElroy JS, Breeden GK (2006). Triclopyr safens the use of fluazifop and fenoxaprop on zoysiagrass while maintaining bermudagrass suppression. Applied Turfgrass Science 3(1):0-0.

Mcelroy JS, Breeden GK, Yelverton FH, Gannon TW, Askew SD, Derr JF (2005). Seeded bermudagrass response to herbicides during establishment. Golf Course Manage. 73:154-158.

McElroy JS, Martins D (2013). Use of herbicides on turfgrass. Planta Daninha 31(1):455-467.

Oliveira AR, Freitas SP, Freitas ILJ (2010). Control of *Cyperus rotundus* L. with trifloxysulfuron sodium in sugar-cane. Rev. Ceres 57(6):736-741.

Raij BV, Andrade JC, Cantarella H, Quaggio JÁ (2001). Análise química para avaliação da fertilidade de solos tropicais. Campinas Inst. Agronô. 284p.

Santos MV, Ferreira FA, Freitas FCL, Fonseca DMF, Carvalho AJ, Braz TGS (2012). Brachiaria brizantha control by using fluazifop-p-butil on Tifton 85 pasture formation. Rev. Bras. Zootec. 41(2):281-285.

Silva BP, Almeida ROA, Salgado TP, Alves PLCA (2014). Efficacy of imazapic, halosulfuron and sulfentrazone for *Cyperus rotundus* L. control in response to weed tuber density. Afr. J. Agric. Res. 9(47):3458-3464.

Silveira HRO, Ferraz EO, Matos CC, Alvarenga ICA, Guilherme DO, Tuffi Santos LD, Martins ER (2010). Allelopathy and Homeopathy in the Management of Nutsedge (*Cyperus rotundus*). Planta Daninha 28(3):499-506.

Song Y (2014). Insight into the mode of action of 2,4-dichlorophenoxyacetic acid (2,4-D) as an herbicide. J. Integr. Plant Biol. 56(2):106 -113.

Unruh JB, Leon RG Brecke BJ, Trenholm LE (2013). Weed Management Guide for Florida Lawns. ENH884. University of Florida (IFAS), Gainesville, FL. Disponível em: http://edis.ifas.ufl.edu/ep141<Acessed in 24 Feb. 2015.

Velini ED, Martins D, Manoel LA, Matsuoka S, Travain JC, Carvalho JC (2000). Avaliação da seletividade da mistura de oxyfluorfen e ametryne, aplicada em pré ou pósemergência, a dez variedades de cana-de-açúcar (cana planta). Planta Daninha 18(1):123-134.

Xiao B, Wang QH, Wang HF, Dai QH, Wu JY (2011).The effects of narrow grass hedges on soil and water loss on sloping lands with alfalfa (*Medicago sativa* L.) in Northern China. Geoderma pp. 167-168:91-102.

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Full Length Research Paper

Effect of humic substances and nitrogen fertilization on yellow passion fruit cultivation in the Brazilian semiarid region

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Fruit production of yellow passion fruit (*Passiflora edulis* Sims. f. *flavicarpa* Deg.) is affected by several factors, such as climate, soil and agricultural practices, including fertilization and irrigation, which are essential for high crop yield. Thus, an experiment was carried on to evaluate the effect of humic substances and different nitrogen doses supplied through fertigation on fruit production of yellow passion fruit in Brazilian semiarid. The experiment was carried on from December 2012 to December 2013 on Curaçá irrigation area, Juazeiro County, Brazil. The experimental design was randomized complete block in a split plot arrangement, considering humic substances (absence and presence) as main plots and N doses (180, 200, 260, 330 and 350 kg ha^{-1} yr^{-1} of N) as subplots with five replications and six plants in each parcel. The interaction of nitrogen doses and humic substances affected foliar nitrogen concentrations, stem diameter, production per plant and yield. No isolated effect of humic substances on any variable studied was significant. The higher yellow passion fruit yields are recorded at 290 and 350 kg ha^{-1} N doses without and with humic substances, respectively.

Key words: Passionflower, plant nutrition, organic acids.

INTRODUCTION

Brazil is the center of origin for yellow passion fruit (*Passiflora edulis Sims* f. *flavicarpa* Deg.), where 823,284 tons of passion fruit were produced in 2014, characterizing the country as the world's largest producer

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(FAOSTAT, 2015). Nearly 72.59% of Brazilian yellow passion fruit has been produced in Northeast region, and Bahia is the largest producer state (IBGE, 2015).

Yellow passion fruit production is affected by several factors, among which climate, soil and crop management practices, including fertilization and irrigation are predominant (Silva et al., 2015). The nutrients should be supplied at compatible levels according to the plant's requirements, preferentially using a fertigation technique, that's been currently proven effective (Borges et al., 2006), especially for yellow passion fruit crop which, according to Haag et al. (1973), demands large amounts of nutrient during development and budding phases; and nitrogen (N) is the most absorbed nutrient by this crop, observing an extraction of 205.5 kg ha^{-1} $year^{-1}$ and an N exportation through fruit harvest of 44.55 kg ha^{-1} for a fruit yield of 16.3 t ha^{-1}.

Fertigation allows applying, beyond soluble fertilizers, different inputs to plants such as herbicides, insecticides, bio-fertilizers and humic substances (HS). HS are formed by the transformation of biomolecules during the humification process of plants and animals residues in the environment (Silva and Mendonça, 2007).

Due to the beneficial effects of HS to soils and plants, it is possible to find in the scientific literature, some studies aiming to increase the efficiency of nutrients absorption, particularly for N, through the association of N fertilizing with HS for important commercial fruit crops such as grape (Ferrara and Brunetti, 2008), pineapple (Baldotto et al., 2009), custard apple (Cavalcante et al., 2012, 2014) and guava (Nunes et al., 2014), although for yellow passion fruit information are still scarce. A study on the effect of HS on fruit quality of yellow passion fruit was made by Silva et al. (2015). The study was justified by largely recognizing the influence of the HS on chemical, physical and biological soil properties and consequently in root growth, higher nutrient availability and chlorophyll biosynthesis (Ferrara and Brunetti, 2008).

Hence, the present study aimed to evaluate the effect of humic substances and different nitrogen doses supplied through fertigation on fruit production of yellow passion fruit in Brazilian semiarid.

METHODS AND MATERIALS

The experiment was carried out from December/2012 to December/2013 in Curaçá irrigated area, Juazeiro County, Bahia State, Brazil at the geographic coordinates of latitude 09° 07' S, and longitude 40° 04' W with altitude of 376 m. The climate of the region is classified, according to Köppen (1918), as hot and dry semiarid (Bswh).

During the execution of the experiment, the climatic data were collected by a meteorological station installed near the experimental farm (Figure 1), while physical and chemical characteristics of the soil from samples taken before executing the experiment are in Table 1. The soil is a Ultisols Ustult (American classification Soil Taxonomy).

For the propagation of seedlings polyethylene bags were used as containers with dimensions of 22 x 5.5 cm in height and width, respectively. The substrate used in the production of seedlings was composed of soil : sieved sand : bovine manure at a 1:1:2 ratio. The planting holes were opened in the dimension 60 x 60 x 60 cm, received part of phosphate fertilizer in foundation and transplanting of seedlings was performed 60 days after seeding. The seedlings were transplanted in February 2013, at 3 x 3 m spacing distance, and conducted on vertical cordon, with a smooth wire no. 14, 1.8 m from the ground. Plants were drip-irrigated daily with three emitters per plant installed every 0.30 m for a flow of 1.6 L h^{-1} each one, following the potential evapotranspiration (ETo) and yellow passion fruit's Kc coefficient defined by Souza et al. (2009).

The nitrogen source used was urea (45% N), fertigated once a week, beginning at 30 days after transplanting (DAT), according to Borges and Coelho (2009) recommendation. Phosphorus and potassium fertilizations were performed using monoammonium phosphate MAP (50% of P_2O_5; 11% of N) and potassium chloride (60% K_2O), respectively. The phosphate fertilization, 120 kg ha^{-1} yr^{-1} of P_2O_5, was applied at 90 and 210 DAT, while potassium (140 kg ha^{-1} yr^{-1} of K_2O) was weekly parceled from 90 DAT until the end of the experiment. The nitrogen, potassium and phosphate fertilizers were supplied through fertigation system (Viqua® venturi injector of 1" at 10 bar operating pressure), according to soil analysis. Foliar fertilization with micronutrients was performed every 15 days from 90 DAT following plant demand.

The source of humic substances used in the experiment was the commercial product KS 100 (Omnia®) from leonardite, with composition of K_2O (15%), total organic carbon (45%), humic acids (70%) fulvic acids (8%), electrical conductivity (0.37 mS/cm) salt index (24), pH (10) and solubility (140 g L^{-1}). The fertigation with HS were performed once every 30 days, following the manufacturer recommendations (5 kg ha^{-1} of the product throughout the crop cycle) and the amount of K_2O discounted when the potassium fertigation was performed with the humic substances. All management practices for pruning, control of weeds, pests and diseases were performed following the instructions of Lima et al. (2002).

The experimental design was randomized complete block in a split plot arrangement, considering of humic substances (absence and presence) as main plots and N doses (180, 200, 260, 330 and 350 kg ha^{-1} yr^{-1} of N) as subplots with five replications and six plants in each parcel. The N doses were defined according to Borges and Coelho (2009) recommendations. Choice for N levels with differences ranging from 20 to 60 is not clear.

The following variables were evaluated: i) stem diameter (mm) at 270 days after transplanting (DAT), at 10 cm of height from the soil using a digital paquimeter (0.01 - 300 mm, Digimess®); ii) at the beginning of flowering, the leaf chlorophyll readings (chlorophyll *a*, *b* and total) were measured using a chlorophyll meter (Falker®, Brazil) in three leaves within the canopy of each replication (plant) between 0900 and 1000 H, following the methodology described by El-Hendawy et al. (2005). Readings were taken in the middle of the canopy, avoiding necrotic areas by the attack of pests and diseases; iii) the same leaves were collected immediately after performing leaf chlorophyll readings, and chemically analyzed. After washing and rinsing with distilled water, the leaves were dried at 70°C for 48 h. Total N concentrations were analyzed using the Kjeldahl method, following the methodology properly described by Malavolta et al. (1997); and iv) HS and N doses treatments were also evaluated by determining the cumulative fruit production (kg $plant^{-1}$) and fruit yield (ton ha^{-1}), when yellow passion fruits were harvested twice a week placed in plastic boxes and weighted using a Filizola® CF15 brand precision scale (0.5 grams of precision). After the registration of fruit production per plant, the fruit yield (ton ha^{-1}) was calculated in each treatment.

Statistical analyses included analysis of variance, mean separation of HS using Tukey's test, and simple regression to separation of N fertilizing doses. All the calculations were performed using the SAS Statistical Program (SAS, 2011), considering

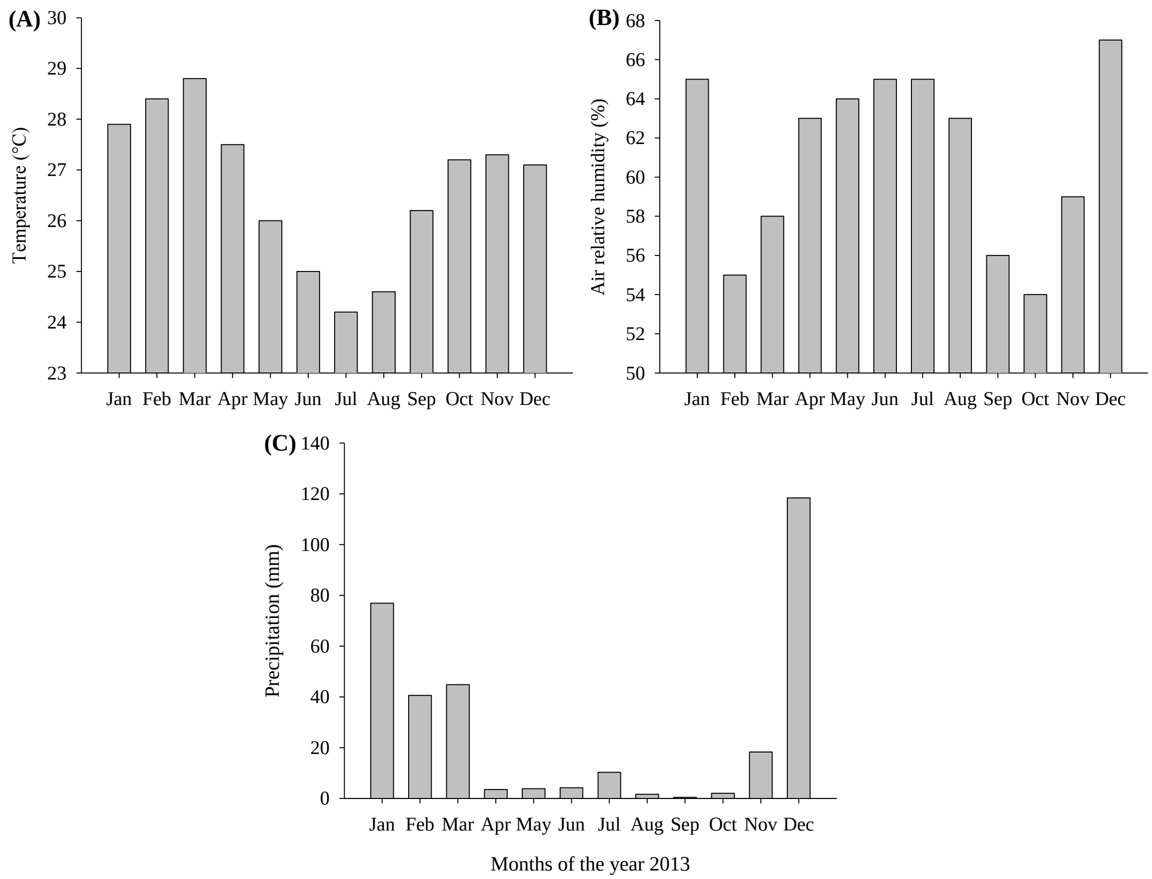

Figure 1. Monthly air temperature (A), air humidity (B) and precipitation (C) during the experiment.

Table 1. Chemical and physical characteristics of the soil (0-30 cm soil depth) where the experiment was carried out.

Chemical atributes		**Physical atributes**	
pH (water - 1:2,5)	5.6	Sand (g kg^{-1})	658
P (mg dm^{-3})	10.0	Silt(g kg^{-1})	277
K^+ ($cmol_c$ dm^{-3})	0.28	Clay (g kg^{-1})	65
Ca^{2+} ($cmol_c$ dm^{-3})	3.50	SD (kg dm^{-3})	1.52
Mg^{2+} ($cmol_c$ dm^{-3})	1.50	DP (kg dm^{-3})	2.51
Na^+ ($cmol_c$ dm^{-3})	0.29	Total porosity (%)	39.61
SB ($cmol_c$ dm^{-3})	5.57	Textural classification	Franco sandy
Al^{3+} ($cmol_c$ dm^{-3})	0.00		
$H^+ + Al^{3+}$ ($cmol_c$ dm^{-3})	1.60	Carbon stocks in soil	
CEC ($cmol_c$ dm^{-3})	7.17	Fractions	Initial condition (t ha^{-1})
V (%)	78	TOC	6.54
O.M. (g dm^{-3})	12.1	CHF	2.41
C (g dm^{-3})	7.0	CHAF	1.11
E.C. (dS m^{-1})	2.0	CFAF	0.64

P, K^+ e Na^+ Mehlich-1 extractor (HCl^+ H_2SO_4); Al3$^+$, Ca^{2+}, Mg^{2+} extractor KCl 1M; OM= organic matter; SB= base saturation; V= base saturation; CEC= cation exchange capacity; SD= soil density and DP= density of particles; E.C.= electric conductivity; TOC= total organic carbon; CHF; CHAF e CFAF= carbon in the humin fraction, humic and fulvic acids, respectively.

Table 2. Stem diameter (SD), leaf nitrogen concentration (N), leaf chlorophyll index (*a*, *b* and total), fruit production (FP) and fruit yield (FY) of yellow passion fruit as a function of nitrogen fertilizing and humic substances grown in Brazilian semiarid.

Source of variation	SD	N	Chl *a*	Chl *b*	Total Chl	FP	FY
	mm	g kg^{-1}	CI			kg plant^{-1}	t ha^{-1}
HS (Value "F")	0.36^{ns}	0.01^{ns}	4.91^{ns}	0.38^{ns}	2.09^{ns}	0.98^{ns}	1.06^{ns}
Without HS	27.36^{a}	53.22^{a}	33.54^{a}	10.04^{a}	43.59^{a}	12.28^{a}	13.60^{a}
With HS	28.22^{a}	53.46^{a}	34.84^{a}	10.47^{a}	45.32^{a}	13.64^{a}	15.20^{a}
LSD	3.31	8.41	1.35	1.58	2.74	3.16	3.48
N doses(Value "F")	2.17^{ns}	1.00^{ns}	0.40^{ns}	1.88^{ns}	0.85^{ns}	4.11**	3.23*
180	26.99	50.08	33.75	9.31	43.05	12.53	12.56
200	26.80	61.25	34.47	10.04	44.52	10.71	13.37
260	28.55	50.54	34.81	11.62	46.43	13.31	14.78
330	28.94	52.91	33.62	10.11	43.74	13.98	15.54
350	27.69	51.95	34.33	10.22	44.55	14.27	15.85
HS X N (Value "F")	2.88*	1.19**	0.28^{ns}	0.95^{ns}	0.25^{ns}	3.28*	3.95*
CV%	7.24	26.93	7.27	18.85	9.72	17.19	17.21

HS = Humic substances; LSD = least significant difference; CI = chlorophyll index; ns = no significant; ** = significant at the 0.01 level of probability; * = significant at the 0.05 level of probability; means followed by the same letter in the columns do not differ statistically by Tukey test at 0.05 and 0.01 probability.

significant at $P \leq 0.01$.

RESULTS AND DISCUSSION

The interaction between the nitrogen (N) doses and humic substances (HS) affected the stem diameter ($p < 0.05$), leaf nitrogen concentration ($p < 0.01$), fruit production and yield ($p < 0.05$), a result that shows interdependence between the studied factors for these variables, although any effect on chlorophyll index has been registered. It is also observed that humic substances and N single doses did not significantly affect any of the studied variables (Table 2).

For treatments with HS, the stem diameter (SD) presented a better significant data adjustment to the quadratic regression model as a functions of N levels, characterized by the SD increase followed by decrease with the increasing of N doses applied through fertigation (Figure 2A and B); while for treatments without HS, SD increased with N doses enhancement, increasing 4.1 mm from 180 kg ha^{-1} to the maximum estimated dose of 281.33 kg ha^{-1} N (Figure 2A). This result is in agreement with Rebequi et al. (2011) who registered an increase of 4.9 mm on SD of yellow passion fruit from the lowest to the highest N dose evaluated, but it disagrees with Santos et al. (2011) that did not find significant difference for SD of yellow passion fruit, as a function of nitrogen fertilization doses.

Treatments with HS presents the maximum estimated dose of 260 kg ha^{-1} N reaching a peak a 28.9 mm, which corresponds to the reduction of 30.16% of N fertilizer applied as compared to the plants grown without HS (Figure 2B), that could occurred because HS increases nutrient absorption, among them, N (Primo et al. 2011). Thus, the plants treated with HS had a better N use, reaching the maximum stem diameter with a lower dose of N. Nunes et al. (2014) evaluated the growth and leaf nutritional status of guava grown with HS and soil mulching, and registered no significant effect on stem diameter. On the other hand, Baldotto et al. (2009) observed significant increases on growth and development of pineapple seedlings propagated by tissue culture as a function of humic acids isolated from vermicomposting during the acclimatization period. These facts may have occurred due to the benefits provided to the plants by the humic acids addition, which are formed by heterogeneous molecular aggregates and stabilized by hydrogen connections and hydrophobic interactions, favoring root system development (Zandonadi et al., 2007) and nutrients accumulation (Chen et al., 2004).

As shown in Figure 2C, plants grown without HS at 180 kg ha^{-1} of N presented nearly 56.5 g kg^{-1} of N, with exponential decrease of N concentration with N doses increasing, a result in disagreement with Venancio et al. (2013) who verified linear increase of N leaf concentrations with higher N doses applied, with higher N concentration of 40.49 g kg^{-1} of N corresponding to the higher N dose applied (210 kg ha^{-1} of N). According to adequate range of supply defined by Prado and Natale (2006), (43-55 g kg^{-1}), the plants were N adequately supplied, and there was no visual toxic symptoms of N excess, although the recorded values are strongly higher than those reported by Silva Júnior et al. (2013) in a study on the bio-fertilizers use as a HS source for yellow passion fruit plants.

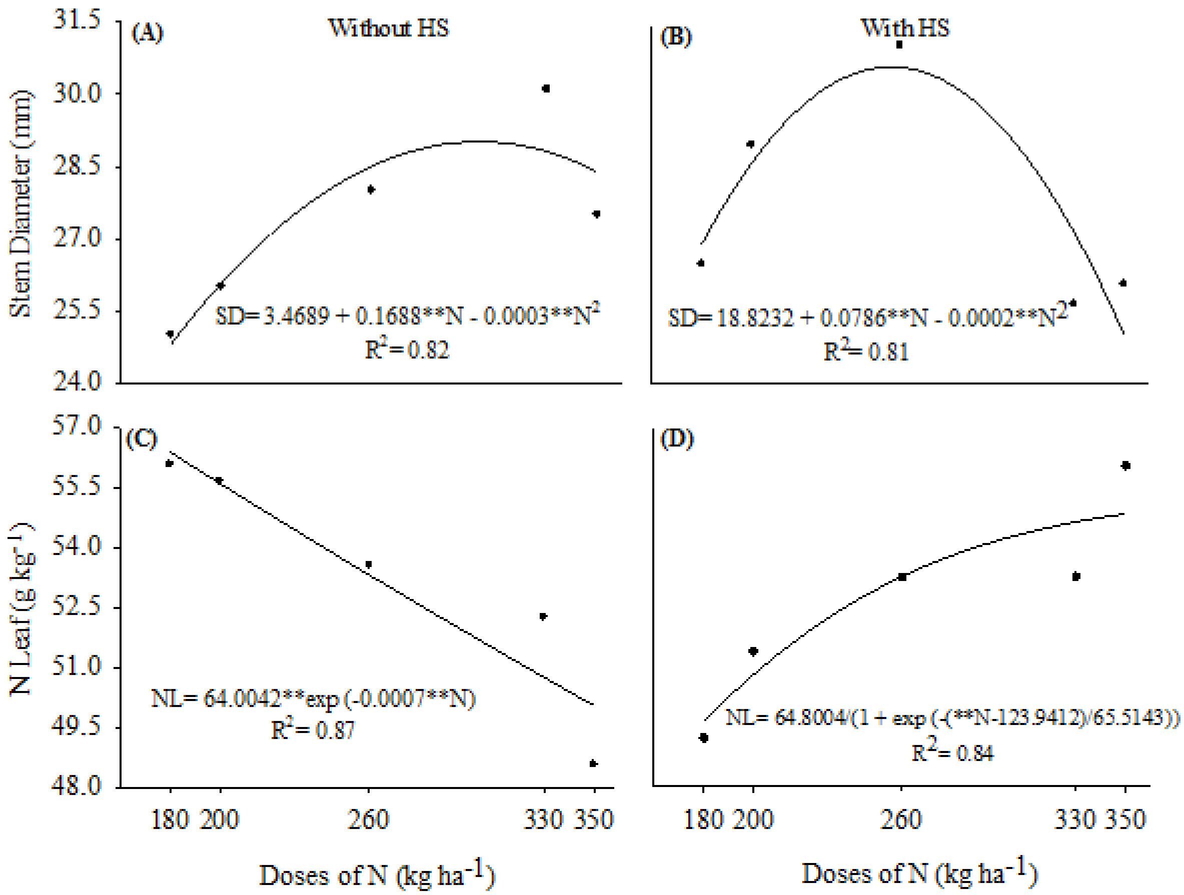

Figure 2. Stem diameter (A and B) and leaf nitrogen concentration(C and D) of yellow passion fruit as a function of N doses and humic substances.

The N leaf concentrations of yellow passion fruit presented exponential growth with N increase using HS (Figure 2D), with the highest dose (64 g kg^{-1} of N) recorded at the maximum leaf N level (350 kg ha^{-1} of N). This N leaf concentration increase can be explained by the positive effect provided by HS to nitrogen root absorption as ammonium nitrate (Keeling et al., 2003). In addition, according to Figure 2D, it is possible to infer that there was a "luxury consumption" in plants that received HS because those plants presented an adequate N leaf concentration for a satisfactory yield when fertilized with 260 kg ha^{-1} N (Prado and Natale, 2006).

The leaf chlorophyll indexes *a*, *b* and total of yellow passion fruit were not affected by N doses, independently of HS use (Table 2) that is congruent with the results of Cavalcante et al. (2014) for custard apple crop. On the other hand, increases on leaf chlorophyll as a function of foliar spray of humic acids (5 and 20 mg L^{-1}) of grape were reported by Ferrara and Brunetti (2008). Primo et al. (2011) and Baldotto et al. (2009) found that humic acids provided higher photosynthetic pigments levels and significant increase of chlorophyll *a* and *b* ratio, as compared to the control.

Yellow passion fruit plants grown without HS produced more than 14.4 kg $plant^{-1}$ when fertilized with 290 kg ha^{-1} $year^{-1}$ N (Figure 3A), a result lower than that quoted by Cavalcante et al. (2012b), who obtained a fruit production of 17.81 kg $plant^{-1}$ with NPK soil fertilizing. However, these results exceed the values recorded by Cavalcante et al. (2005) who obtained a fruit production of 7.1 kg $plant^{-1}$ and 8.4 kg $plant^{-1}$ presented by Cavalcante et al. (2007) in soil fertilized with liquid bovine bio-fertilizer. The positive response to N doses can be attributed to the low soil organic matter content, even for a semiarid region (Table 1), as Bayer and Mielniczuk (2008) stated that organic matter is the main soil N source.

The plants fertigated with HS increased fruit production from 12.0 to 15.2 kg $plant^{-1}$ (26.70%) from the lowest to the highest N dose applied (Figure 3B), which is congruent to the results of Cunha et al. (2015) who observed significant interactions between N doses and HS on custard apple (*Annona squamosa* L.) production and yield, also in a semiarid climate. This increment can be explained by the effect of HS on soil chemical and biological properties (Pimenta et al., 2009) with direct effect on nutrient uptake (Primo et al., 2011) and

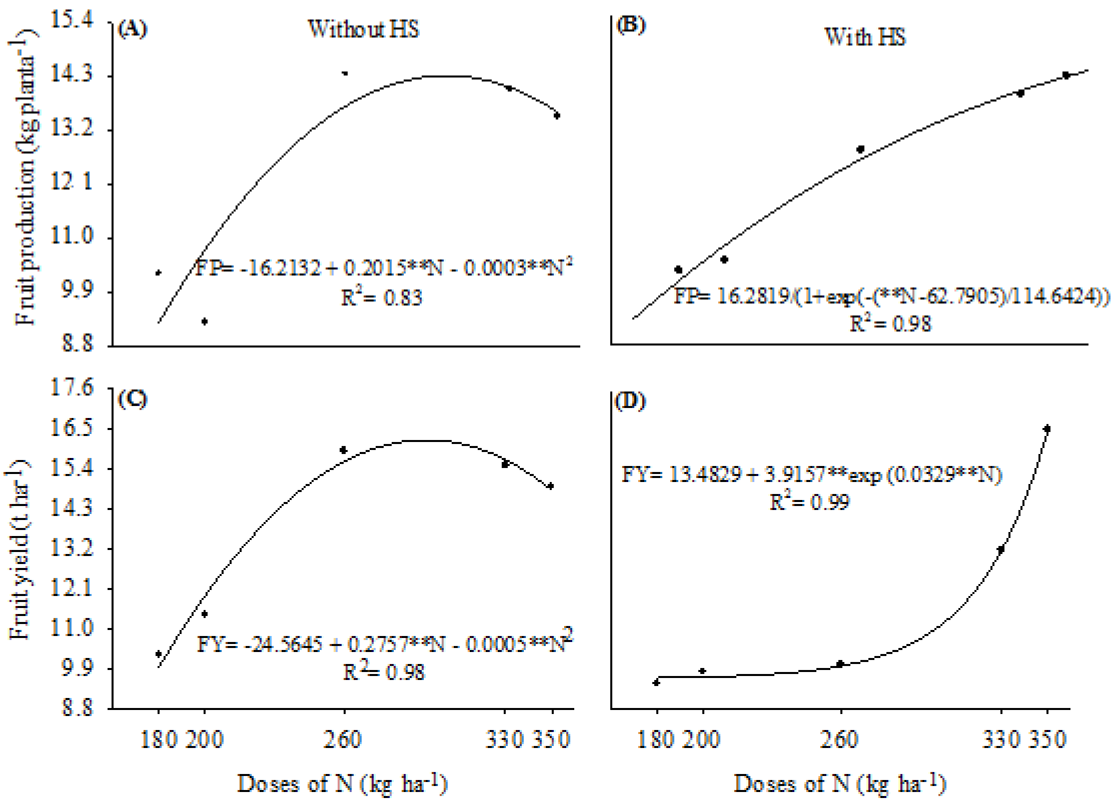

Figure 3. Fruit production (A and B) and fruit yield (C and D) of yellow passion fruit of yellow passion fruit as a function of N doses and humic substances.

consequently to the plants. According to Eyheraguibel et al. (2008), the HS effects promote significantly higher water consumption, and consequently better plant growth and production. Additionally, much of the humic acid's biostimulants effects have been credited to the HS activity, which is similar to the plant hormones of auxin class, that is, they can promote plant growth in relatively small concentrations (Baldotto et al., 2009).

The average fruit yield of the fertigated yellow passion fruit presented different data distributions to the HS effects (Figures 3C and D). In plants without HS, the maximum estimated fruit yield was 16.5 t ha^{-1} for 290 kg ha^{-1} N dose, and, therefore, below the 17.5 t ha^{-1} of the treatments with the N dose of 350 kg ha^{-1}, in plants with HS. These results are above the Brazilian average fruit yield of 13.42 t ha^{-1} $year^{-1}$ (Agrianual, 2014), but, however, they are lower than those recorded by Venancio et al. (2013) who evaluated the production, fruit quality and leaf nitrogen content in yellow passion fruit under nitrogen fertilization, and obtained an average yield of 18.5 t ha^{-1} yr^{-1} under the conditions of Aquidauana - MS.

Conclusions

The stem diameter, leaf N concentrations and fruit production of yellow passion fruit are affected interdependently by nitrogen doses and humic substances. The leaf indexes of chlorophyll *a*, *b* and total are not affected by the nitrogen fertigationor by humic substances. The higher yellow passion fruit yields are recorded at 290 and 350 kg ha^{-1}N doses without and with humic substances, respectively.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Agrianual (2014). Anuário estatístico da agricultura brasileira. FNP, São Paulo 136 p.

Baldotto LEB, Baldotto MA, Giro VB, Canellas LP, Olivares FL, Bressan-Smith R (2009). Desempenho do abacaxizeiro 'vitória' em resposta à aplicação de ácidos húmicos durante a aclimatação. Rev. Bras. Cienc. Solo 33(4):979-990.

Bayer C, Mielniczuk J (2008). Dinâmica e função da matéria orgânica. In: Santos GA, Silva LS, Canella LP, Camargo FAO (2008). Fundamentos da matéria orgânica do solo: ecossistemas tropicais e subtropicais. (2ª edição). Metrópole, Porto Alegre pp. 7-25.

Borges AL, Caldas RC, Lima AA (2006). Doses e fontes de nitrogênio em fertirrigação no cultivo do maracujá amarelo. Rev. Bras. Frutic. 28(2):301-304.

Borges AL, Coelho EF (2009). Fertirrigação em frutíferas tropicais.

Embrapa Mandioca e Fruticultura Tropical, Cruz das Almas 180 p.
Cavalcante IHL, Cunha MS, Beckmann-Cavalcante MZ, Osajima JA, Souza JSN (2012). Relationship between chlorophyll meter readings and leaf nitrogen concentration in custard apple. Philipp. J. Crop. Sci. 37(3):7-11.
Cavalcante ÍHL, Cunha MS, Rocha LF, Santos EM, Silva Júnior GB (2014). Physiological indexes of custard apple as a function of nitrogen fertilization and humic substances. Rev. Cienc. Agrar. 57(1):85-89.
Cavalcante LF, Cavalcante ÍHL, Rodolfo Júnior F, Beckmann-Cavalcante MZ, Santos GP (2012b). Leaf-macronutrient status and fruit yield of biofertilized yellow passion fruit plants. J. Plant Nutr. 35(2):176-191.
Cavalcante LF, Costa JRM, Oliveira FKD, Cavalcante ÍHL, Araújo FAR (2005). Produção do maracujazeiro amarelo irrigado com água salina em covas protegidas contras perdas hídricas. Irrigation 10(3):229-240.
Cavalcante LF, Santos GD, Oliveira FA, Cavalcante ÍHL, Gondim SC, Cavalcante MZB (2007).Crescimento e produção do maracujazeiro amarelo em solo de baixa fertilidade tratado com biofertilizantes líquidos. Rev. Bras. Cienc. Agrar. 2(1):15-19.
Chen CR, Xu ZH, Mathers NJ (2004). Soil carbon pools in adjacent natural and plantation forests of subtropical. Soil Sci. Soc. Am. J. 68(1):282-291.
Cunha MS, Cavalcante ÍHL, Mancin AC, Albano FG, Marques AS (2015). Impact of humic substances and nitrogen fertilising on the fruit quality and yield of custard apple. Acta Sci. Agron. 37(2):211-218.
El-Hendawy S, Hu Y, Schimidhalter U (2005). Growth, ion content, gas exchange, and water relations of wheat genotypes differing in salt tolerances. Aust. J. Agric. 56(2):123-134.
Eyheraguibel B, Silvestre J, Morand P (2008). Effects of humic substances derived from organic waste enhancement on the growth and mineral nutrition of maize. Bioresour. Technol. 99(10):4206-4212.
Ferrara G, Brunetti G (2008). Influence of foliar applications of humic acids on yield and fruit quality of table grape cv. Itália. J. Inter. Sci.Vigne Vin. 42(2):79-87.
Food and Agriculture Organization of the United Nations- FAOSTAT (2015). Data base of agricultural production. Disponível em: http://apps.fao.org
Haag HP, Oliveira GD, Borducchi AS, Sarruge JR (1973). Absorção de nutrientes por duas variedades de maracujá. Anais da ESALQ 30:267-279.
Instituto Brasileiro de Geografia e Estatística-IBGE (2015). Produção Agrícola Municipal, 2015 [Online]. Disponível em: http://www.sidra.ibge.govbr
Keeling AA, Mccallum KR, Beckwith CP (2003). Matune Green waste compost enchances growth and nitrogen uptake in wheat (*Triticum aestivum* L.) and oilseed (*Brassica napus* L,) through the action of water-extractable factors. Bioresour. Technol. 90(2):127-132.
Köppen W (1918). Klassification der klimate nach temperatura, niederschlag und jahreslauf. Petermanns Geographische Mitteilungen. Gotha 64:193-203.
Lima AA, Junqueira NTV, Veras MCM, Cunha MAP (2002).Tratos culturais. In: Lima AA (Ed.) Embrapa. Maracujá Produção: Aspectos Técnicos. Informação Tecnol. Bras. pp. 41-47.
Malavolta E, Vitti GC, Oliveira AS (1997). Avaliação do estado nutricional das plantas: princípios e aplicações. POTAFOS, Piracicaba. 281p.
Nunes JC, Cavalcante LF, Lima Neto AJ, Silva JA, Souto AGL, Rocha LF (2014). Humitec® e cobertura morta do solo no crescimento inicial da goiabeira cv. 'Paluma' no campo. Rev. Agro@mb. On-line 8(1):89-96.
Pimenta AS, Santana JAS, Anjos RM, Benites VM, Araújo SO (2009). Caracterização de ácidos húmicos produzidos a partir de carvão vegetal de duas espécies florestais do semiárido: jurema preta (Mimosa tenuiflora) e pereiro (Aspidosperma pyrifolium). Rev. Verde Agroecol. Desenvolvimento Sustentável 4(4):01-11.
Prado RM, Natale W (2006). Nutrição e adubação do maracujazeiro no Brasil. EDUFU, Uberlândia. 192 p.
Primo DC, Menezes RSC, Silva TO (2011). Substâncias húmicas da matéria orgânica do solo: uma revisão de técnicas analíticas e estudos no nordeste brasileiro. Sci. Plena 7(5):1-13.
Rebequi AM, Cavalcante LF, Diniz AA, Nunes JC, Brehm MAS, Oliveira FA (2011). Crescimento e produção de maracujazeiro amarelo sob diferentes níveis e combinações de adubações nitrogenada e potássica no solo e foliar nas plantas. Magistra 23(1):45-52.
Santos PC, Lopes LC, Freitas SJ, Sousa LB, Carvalho AJC (2011). Crescimento inicial e teor nutricional do maracujazeiro amarelo submetido à adubação com diferentes fontes nitrogenadas. Rev. Bras. Frutic. pp. 722-728.
Silva IR, Mendonça ES (2007). Matéria orgânica do solo. In: Novais RF, Alvarez VVH, Barros NF, Fontes RLF, Cantarutti RB, Neves JCL (2007). Fertilidade do solo. 1ª edição. Soc. Bras. Ciênc. Solo Viçosa pp. 374-470.
Silva Júnior GB, Cavalcante ÍHL, Albano FG, Osajima JA (2013). Estado nutricional e clorofila foliar do maracujazeiro amarelo em função de biofertilizantes, calagem e adubação com N e K. Rev. Cienc. Agrar. (Lisboa) 36(2):163-173.
Silva RL, Cavalcante ÍHL, Sousa KSM, Galhardo CX, Santana EA, Lima DD (2015). Qualidade do maracujá amarelo fertirrigado com nitrogênio e substâncias húmicas. Comun. Sci. 6(4):479-487.
Souza MSM, Bezerra FML, Viana TVA, Teofilo EM, Cavalcante ÍHL (2009). Evapotranspiração do maracujá nas condições do vale do curu. Rev. Caatinga 22(2):11-16.
Statistical Analisys System-SAS (2011). Institute Inc. SAS. User's guide: Statistics. 5th edition. SAS Inst., Inc., Cary, NC.
Venancio JB, Cavalcante ET, Silveira MV, Araújo WF, Chagas EA, Castro AM (2013). Produção, qualidade dos frutos e teores de nitrogênio foliar em maracujazeiro amarelo sob adubação nitrogenada. Científica 41(1):11-20.
Zandonadi DB, Canellas LP, Façanha AR (2007). Indolacetic and humic acids induce lateral root development through a concerted plasmalemma and tonoplast H^+ pumps activation. Planta 225(6):1583-1595.

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Full Length Research Paper

Soybean seed treatment with micronutrients, hormones and amino acids on physiological characteristics of plants

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The study is aimed at contributing to the research on physiological effects of seed treatment with micronutrients, hormones and amino acids. Two experiments were conducted, first in a greenhouse (partially controlled conditions) and second in the field at Patos de Minas, MG, Brazil. Four seed treatments were arranged in a randomized block design (control; micronutrients Zn, Mn, B, Mo, Ni and Co; hormones: indole butyric acid, gibberellic acid and kinetin, and amino acids: glutamic acid, arginine, glycine, methionine and cysteine) with six and six replications for each treatment in the greenhouse and in the field, respectively. Results showed that seed treatment with micronutrients increased the activity of nitrate reductase (NR) by 51%, the net photosynthesis (NP) by 50%, the chlorophyll content (SPAD value - Soil Plant Analysis Development) by 52%, and the plant growth rate (GR) by 28%, all compared to the control. On the other hand, the use of amino acids and hormones reduces the level of stress of the plants during the initial period of growth and increases the mass of dry matter production. Finally, the seed treatment with micronutrients, hormones and amino acids represented an increase in productivity as compared to the control.

Key words: Biostimulants, *Glycine max* (L.) Merrill, soybean productivity.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is the most widely cultivated species around the world, with approximately 80% of its production concentrated in three countries: The United States, Brazil and Argentina (United States Department of Agriculture - USDA, 2013). In these countries the average productivity does not exceed 4,000 kg ha^{-1}, far from the genetic potential of the plant, which can reach over 20,000 kg ha^{-1} (Navarro Junior and Costa,, 2002).

The productive potential of a crop is governed by the genetic characteristics of seeds. However, due to the environmental conditions of cultivation such as

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temperature, water regime, photoperiod, chemical and physical soil quality, competition with weeds, among other factors, a productivity depletion occurs reaching mean values currently obtained.

Soybean studies show productivity records like 10,760 kg ha^{-1}, indicating that there is space for the possibility of better exploring the genetic characteristics of the crop culture (Haegele and Below, 2013). The potentiation of the early growth of plants by treating seeds with biostimulants substances has been one of the adopted alternatives. Often these promote physiological changes that stimulate growth.

Among the biostimulants, algae extracts, synthetic hormones and fermentation by-products stand out in the last years. Seaweed extracts contain various compounds that promote the growth of plants, including amino acids and hormones (Khan et al., 2009; Craigie, 2011). Synthetic hormones that regulate growth perform important functions during plant development (Werner and Schumülling, 2009; Zhao et al., 2010), and many substances among which amino acids, are present in fermentation byproducts. However, little is known about the exact composition of these products and why they promote the growth of plants. On the other hand, some studies show that micronutrients when used in seeds treatments act as stimulants of the initial growth of the plants (Soares, 2013).

On the other hand, some studies show that micro-nutrients when used in seeds treatments act an stimulants of the initial growth of the plants (Soares, 2013).

Thus, the objective of this study was to understand the effects of micronutrients, amino acids and hormones applied to soybean seeds on plant growth.

MATERIALS AND METHODS

Two experiments were conducted at the University Center of 'Patos de Minas' (UNIPAM), 'Patos de Minas', 'Minas Gerais' State, Brazil, (18° 34 '39 " S, 46° 29 '15 "W and 890 m above sea level), one under greenhouse conditions and the other in the field. Both experiments consisted of the same treatments as shown in Table 1. The treatments with amino acids and micronutrients consisted of the rates also shown in Table 1, applied as syrup on the seeds (1 mL per kg of seeds).

Physiological aspects of the crop under partially controlled conditions

Ten seeds of the soybean cultivar NA-7255-RR were sown in pots with 10 dm^3 of washed sand. The experimental design of randomized blocks with four treatments (Table 1) and six replicates was chosen, considering each pot as a sample unit. After emergence and plant establishment the number of plants was thinned to four plants per pot. Irrigation was performed daily in order to maintain the vessel at about 80% of the laboratory determined field capacity, and fertilization was performed weekly using as irrigation water the nutrient solution proposed by Johnson et al. (1957).

Evaluations consisted of % emergency (E) at 10 days after sowing (DAS) during thinning, activity of nitrate reductase (NR) (Cataldo et al., 1975), lipid peroxidation (LP) (Heath and Packer, 1968), net photosynthesis (NP) and SPAD value at 26 days after emergency (DAE), and root growth rate (RGR), stem growth rate (SGR) and leaf growth rate (LGR), total dry matter (DM) and leaf area (LA) at 50 DAE. NP was determined using a portable open system of gas exchange, IRGA (Infra Red Gas Analyzer), LI-6200 (Li-Cor®), always for the leaves of the upper plant extract, fully expanded and fully exposed to solar radiation. To determine the SPAD index the Chlorophyll Soil Plant Analyzes Development (SPAD, Minolta brand, SPAD-502® model) was used, allowing instantaneous readings of relative chlorophyll content in the leaf without destroying the sample. The dry matter mass determinations (DM_1 and DM_2) for root, stem, leaf and all were performed using one plant for each replicate, at DAE_1 = 26 and DAE_2 = 50. These data were used to estimate the growth rate of organ i according to GR = (DM_2-DM_1) / (DAE_2 - DAE_1). The leaf area was determined by disc method, removing randomly 10 discs from 20 leaves of four plants, totaling 40 discs, with the aid of a punch with an area AD = 0.9672 cm^2. LA was calculated according to $LA = (8AD.DM_l)/DM_d$, in which DM_l represents the dry matter mass of the leaves of the five plants (g), and DM_d the sum of the dry matter of the 40 discs, resulting cm^2 $plant^{-1}$.

Effect on emergency and productivity of the soybean in the field

Cultivar NS-8490-RR was grown in the field. The soil was classified as an Oxisol (Soil Survey, 1999). Before sowing time the chemical characteristics of the 0-20 cm top soil layer were: Organic matter 29.6 g kg^{-1}; pH (H_2O) 5.7; Ca^{2+}, Mg^{2+} and Al^{3+} (KCl 1 mol L^{-1}) 2.45; 0.42 and 0.03 $cmol_c$ dm^{-3}, respectively; K^+ and P (Mehlich-1) 43.87 and 13.17 mg dm^{-3}, respectively; H + Al (Ca (OAc) 2 0.5 mol L^{-1}) 4.7 $cmol_c$ dm^{-3}; CECpH 7.0 7.68 $cmol_c$ dm^{-3}; base saturation 38.8%; O (500 mg L^{-1} to P 2 in HOAc mol L^{-1}) 2.13 mg dm^{-3}; Cu, Mn, Zn and Fe (Mehlich-1) 0.5, 10, 0.6 and 50 mg dm^{-3}, respectively and B (hot water) 0.32 mg dm^{-3}.

Based on these values a maintenance fertilization of 430 kg ha^{-1} formulation 8:28:16 (NPK) + 1.01% Ca + 3.42% S + 0.2% B + 0.2% Zn was used. The seeds were inoculated with Gelfix 5® (2 mL kg^{-1} seed), treated with Standak Top® [Fipronil (250 g ai L^{-1}) + Pyraclostrobin (25 g ai L^{-1}) + Thiophanate Methyl (225 g ai L^{-1})] at a dose of 2 ml kg^{-1} . Seeds were planted mechanically spaced 0.5 m between rows and 22 seeds m^{-1}. After the emergency the crop was thinned to 10 plants m^{-1}, with a final population of 200,000 plants ha^{-1}. For weed control Roundup Ultra® (Glyphosate - 650 g a.i. U^{-1}) was used at a rate of 2.2 kg b.w. ha^{-1}, applied at 32 and 80 DAS. For insect control, Lanatte® was used (Methomyl - 215 g a.i. ha^{-1}) at a dose of 1.5 U b.w. ha^{-1} at 80 and 106 DAS, and Metafós® (Methamidophos - 600 g a.i. L^{-1}) at a rate of 1.0 L p.c. ha^{-1} at 90 DAS. Disease control was promoted by application of Opera® [Pyraclostrobin (133 g a.i. ha^{-1}) + Epoxiconazole (50 g a.i. ha^{-1})] at a dose of 0.6 L p.c. ha^{-1} and Bendazol® (Carbendazim - 663 g a.i. U^{-1}) at a dose of 0.6 U b.w. ha^{-1} at 90 and 106 DAS.

The experimental design was also in randomized blocks with four treatments (Table 1) and six replicates, with each experimental unit consisting of four lines seven meters long each, occupying an area of 14 m^2.

The emergence (10 DAE) and productivity were evaluated. The emergence was determined counting the number of emerged seedlings in two linear meters and the results expressed as a percentage (%). To determine the yield, plants were harvested manually considering the two central rows neglecting 0.5 m at each end. Water content of the grain was determined to calculate the

Table 1. Description of the treatments used in the management of soybean seeds.

Treatment	Composition	Source	Rate (g kg^{-1} of seeds)
Control (C)	-	-	-
Micronutrients (M)	Zinc (Zn)	Zn EDTA 14% (Librel Zn)	0.875
	Manganese (Mn)	Mn EDTA 13% (Librel Mn)	0.187
	Boron (B)	B-MEA-Monoetanolamine 10% (Vitta Boro®) -	0.062
	Molybdenum (Mo)	Ammonium molybdate	0.400
	Cobalt (Co)	Co EDTA 13.7% (Librel Co)	0.044
	Nickel (Ni)	Ni EDTA 13.7%	0.013
Hormones (H)	4-(indol-3-yl)butyric acid (IBA) + kinetin (CK) + gibberellic acid (GA_3)	Stimulate® (IBA - 0.05 g i.a L^{-1} + CK - 0.09 g i.a L^{-1} + GA_3- 0.05 g i.a L^{-1})	5.0 *
Amino acids (Aa)	Glutamic acid	Glutamic acid (Sigma®)	0.00314
	Glycine	Glycine (Sigma®)	0.00300
	Arginine	Arginine (Sigma®)	0.00343
	Methionine	Methionine (Sigma®)	0.00420
	Cysteine	Cysteine (Sigma®)	0.00370

*mL kg^{-1} of seeds.

productivity adjusting to 13% (0.13 g g^{-1}).

Statistical analysis

Statistical analysis was performed with the aid of the SAS 9.0 software. The normality of ANOVA residues and the homogeneity of variances were tested by the Shapiro-Wilk and Levene tests, respectively, both at the 1% significance level. After words the analysis of variance was performed, and comparisons were made between means using the Tukey test at 5% significance (SAS Institute, 2004).

RESULTS

Physiological aspects of the crop under partially controlled conditions

Seed treatment with micronutrients reduced percentage of seedlings (Table 2), but this treatment did not differ from that which received amino acids. However, the use of micronutrients increased the activity of NR in 51.6% compared to control. The treatment that received amino acids presented low activity of nitrate reductase (NR).

The SPAD value data are shown in Table 2. A significant difference is noticed between the control in relation to other treatments. On average, the soybean plants that received seed treatment with micronutrients, hormones and amino acids presented an increase of 58% in all measured variables compared to control. The increase in the SPAD index reflected in higher net photosynthesis of plants, as all treatments showed an increase compared to untreated seeds (control), and this was 53% on average.

Seed treatment with hormones and micronutrients provided higher RGR. The average increase was 21% in relation to the control, and 28% in relation to the treatment with amino acids. The increase in RGR provided by micronutrient application reflected in higher SGR (Table 2).

Treatment with micronutrients leads to greater LGR. The increases were 37, 70 and 28% compared to treatment with hormones, amino acids and control, respectively (Table 2). This behavior was responsible for a greater accumulation of DM because all seed treatments presented higher values compared to the control (Table 2).

Effect of micronutrientes, amino acids and hormones in the field

Seed treatment with hormones promoted higher seed emergency. The other treatments did not differ in this aspect (Table 3). Seed treatment with micronutrients led to higher productivity, however not different from the amino acids treatment. The increase was 46 and 19% compared to the treatment with hormones and control.

DISCUSSION

Although Castro and Vieira (2001) have not evaluated seed emergency, they observed an increase in the

Table 2. Emergency (E, %) nitrate reductase activity (NR, µg N-NO_2 g^{-1} de green mass h^{-1}), lipid peroxidase (LP, nmol TBARS g^{-1} MF), SPAD, net photosynthesis (NP, µmol CO_2 m^{-2} s^{-1}), root growth rate (RGR, g plant day^{-1}), stem (SGR, g plant day^{-1}) and leaf (LGR, g plant day^{-1}), total dry mass (DMt, g $plant^{-1}$) and leaf area (LA, cm^2 plant a^{-1}) of soybean plants treated (T) with -micronutrients (M); –hormones (H); amino acids (Aa), and control (C).

T	E	NR	LLP	SPAD	NP	RGR	SGR	LGR	TDM	LA
C	80^{a}*	27.3^{b}	54.0^{a}	21.0^{b}	12.8^{b}	0.0106^{ab}	0.0101^{ab}	0.0149^{b}	0.108^{b}	152^{c}
M	58^{b}	41.4^{a}	54.8^{a}	31.9^{a}	19.3^{a}	0.0129^{a}	0.0122^{a}	0.0191^{a}	0.140^{a}	201^{a}
H	83^{a}	24.9^{b}	48.5^{b}	34.3^{a}	20.8^{a}	0.0131^{a}	0.0080^{b}	0.0139^{bc}	0.138^{a}	181^{ab}
Aa	72^{ab}	18.3^{b}	48.0^{b}	33^{a}	18.4^{a}	0.0100^{b}	0.0105^{ab}	0.0112^{c}	0.130^{a}	159^{bc}
cv(%)	16.1	29	4.1	5.3	10.5	15.4	15.3	13.8	5.2	7.9
DMS	20.4	13.5	4.7	26.6	31.3	0.0021	0.0026	0.0034	0.11	23

*Averages followed by the same letters in a column do not differ between them by the Tukey test at 5% probability.

Table 3. Emergency (E, %) and productivity (P, kg ha^{-1}) of soybean crops with seeds treatments.

T	E	P
C	54.5^{b}*	1321^{c}
M	63.0^{b}	1939^{a}
H	76.0^{a}	1623^{b}
Aa	58.7^{b}	1847^{ab}
cv (%)	9.71	7.23
DMS	12.86	255.61

*Averages followed by the same letters in a column do not differ between them by the Tukey test at 5% probability. C, Control; M, micronutrients; H, hormones; Aa, amino acids.

number of normal plants when the seeds were treated with hormones. On the other hand, the lowest percentage of emergence was observed in treatments with micronutrients, possibly due to the salt effect. One of the problems related to the treatment of seeds with micronutrients is the toxicity, the boundary between the ideal level to promote germination and the toxic level, which is very narrow, and the seeds do not have efficient physiological mechanisms to control their entry into the seed (Pessoa et al., 2000). This information allows us to infer that the application of micronutrients reduces seed germination under controlled conditions, although in the field the damaging effects could be mitigated by adsorption mechanisms. Therefore, under field conditions, benefits of application of micronutrients can be observed. According to Werner and Witte (2011) the micronutrients can act on seeds reserve degradation, which induced an increase in the initial growth of plants. Besides that, the application of micronutrients assisted in the mitigation of oxidative stresses (Gill and Tuteja, 2010).

The results found highlight the role of micronutrients in the nitrate reductase activity. Among the micronutrients used, molybdenum plays a key role. NR is an enzyme dimer with three prosthetic groups of electron transfer, the flavin (FAD), heme and cofactor molybdenum (MoCo). The MoCo is one Mo atom covalently bonded to two sulfur atoms of pterin molecule. Mo in MoCo is connected to a third sulfur atom of a cysteine residue (Gonzalez-Guerrero et al., 2014). Another nutrient that plays a key role in the nitrate reductase activity is Mn, although indirectly. NR is a highly energy-dependent enzyme, thereby Mn deficient plants which have a reduced photosynthetic rate have less capacity to supply the energy required for the reduction of NO^{3-}. Additionally, the lower growth rate due to Mn deficiency reduces the nitrogen demand by inhibiting the activity of NR (Marschner, 2012; Fischer et al., 2015).

Among the micronutrient used in this study, only the Mn performs direct function in the production of chlorophyll. Mn is involved in the activation of enzymes present in the biosynthetic pathway of chlorophyll (Fischer et al., 2015). However, Mo and Co have a fundamental role in nitrogen metabolism and may have contributed to the increase in the SPAD value compared to the control (Gonzalez-Guerrero et al., 2014; Marschner, 2012).

The application B increased the assimilation of N, due to the stimulus in the nitrate reductase activity, as observed by Camacho-Cristóbal and González-Fontes (2007), and also promoted an increase in yield.

Ni and Co have an indirect role in maintaining the chlorophylls because they reduce the synthesis of ethylene via inhibition of the enzyme acid 1-carboxylic acid-1-amino cyclopropane oxidase (ACC oxidase) (Alarcón et al., 2009; Taiz and Zeiger, 2013).

The good results obtained in both experiments of the use of amino acids as seed treatment, might be related to their important role in various physiological activities of the plant, for example, chlorophyll synthesis. Two of the chlorophyll biosynthetic pathways have been described. One uses glutamic acid as a starting substance, while the other is based on glycine. The representatives of these two pathways is not known, however it is established that both are important for plants (Taiz and Zeiger, 2013; Marschner, 2012), it is however possible that the amino acids may have contributed to the increase of chlorophyll over the control.

The micronutrients, amino acids and hormones used here, play a key role in the metabolism of the plant. Manganese is a constituent of the water photolysis in the photosystem II complex. The change of the manganese atom in the oxidation state (S0-S4) by the incidence of light flashes makes manganese able to release electrons for photochemical reactions. The S4 state oxidizes water, releasing O_2, and retrieves the donated electrons (Yano and Yachandra, 2014; Fischer et al., 2015).

In relation to the amino acids, glutamic acid and glycine are known as precursors of chlorophyll synthesis, which enhance the photosynthetic activity (Taiz and Zeiger, 2013; Marschner, 2012). However, amino acids may also have been used to form other photosynthetic structures, since they all are considered parts of proteins (Hildebrandt et al., 2015).

Another function of the amino acids is related to their role in plant defense against stresses. Cysteine is an important amino acid, as well as being a source of sulfur, it is involved in the phytochelatin production process and, together with glycine operates in the synthesis of glutathione, an important molecule in the defense system of plants (Foyer and Noctor, 2005; Gill and Tuteja, 2010). On the other hand, glycine, besides participating in the glutathione and phytochelatin biosynthesis, is involved in the glycine betaine formation process, a compound normally accumulated in plants that are under water or salt stress, cold, heat and freezing, and helps to keep the integrity of cell membranes while maintaining photosynthetic efficiency (Ashraf and Foolad, 2007). Another important amino acid used in seed treatment is the glutamic acid that can be considered a key amino acid in plants; it can be used in several biosynthetic pathways and serves as the basis for the formation of various amino acids such as arginine, proline, glutamine, and aspartate (El-Ghamry et al., 2009; Taiz and Zeiger, 2013). Furthermore, this amino acid is the precursor of the chlorophyll molecule, through the formation of δ-aminolevulinic acid. Thus, one of the benefits of the application of amino acids can be observed in this experiment, since the application of this compound reduced the lipid peroxidation in relation to the other treatments, which means that these plants are under less stress level. Furthermore, it was observed that the plants subjected to amino acids treatments lead to SPAD values which are directly connected to the chlorophylls, and higher photosynthetic activity

Among the micronutrients used in this study, Zn, B and Co play a key role in the formation of roots because they are involved with auxins. Zn operates in the biosynthesis of tryptophan which is a precursor of auxins, while the B regulates AIA-oxidase activity and thus the concentration of auxins in tissues (Marschner, 2012). Recently the function of Co was described in the formation of lateral roots. This activates some heme oxygenases that mediate auxinic response, inducing the formation of lateral roots (Hsu et al., 2013).

Regarding the effect of hormones in plants, auxin directly influences root growth. In Arabidopsis it is known that auxins accumulate in cells adjacent to the pericycle cells of the xylem in order to initiate the formation of lateral roots in these cells. Moreover, they are also involved in the growth and organization of root primordia and the emergence from the main root (Hodge et al., 2009).

In the case of soybeans, the roots are the points of infection of bacteria that fix atmospheric nitrogen. High availability of roots early in the seedling growth maximizes infection by bacteria and enhances the biological nitrogen fixation. This is because normally the infecting bacteria preferably act in the stretching regions of the roots and in root hairs. Furthermore, higher root formation during the initial growth period makes these plants more tolerant to possible stresses due to increased soil volume that they explore (Câmara, 2014). Under field conditions, at the amendments rates used, the micronutrients provide benefits that were not seen in the greenhouse. Micronutrients did not reduce the plant stand, therefore, probably portions of the micronutrients were adsorbed by soil colloids and did not cause adverse effects (Marques et al., 2004). All benefits observed by the use of micronutrients, described above, under partially controlled conditions may have led to increased productivity in the field. The increase in nitrogen assimilation, coming from the higher activity of nitrate reductase, facilitated by the increase in leaf area, provides greater assimilation of CO_2 by photosynthesis (Romano, 2005). This caused an increase in the net photosynthesis and, consequently, increased production of sugars which were subsequently translocated for organ growth, in this case the grain filling.

Conclusions

1. Soybean seed treatment with micronutrients potentiates nitrogen assimilation and net photosynthesis, increases the chlorophyll content (SPAD value) and plant growth, which lead to 46% increase in productivity;
2. The use of amino acids or hormones applied to soybean seeds reduced the level of physiologic stress of the plants during the initial period of growth and increased the mass of dry matter production;
3. All seed treatments (application of micronutrients, amino acids and hormones) increased soybean productivity.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Alarcón MV, Lloret-Salamanca A, Lloret PG, Iglesias DJ, Talón M, Salguero J (2009). Effects of antagonists and inhibitors of ethylene biosynthesis on maize root elongation. Plant Signal Behav. 4(12):1154-1156.

Ashraf M, Foolad MR (2007). Roles of glycine betaine and proline in improving plant abiotic stress tolerance. Environ. Exp. Bot. 59:206-216.

Camacho-Cristobal JJ, González-Fontes A (2007). Boron deficiency decreases plasmalemma H^+-ATPase expression and nitrate uptake, and promotes ammonium assimilation into asparagine in tobacco roots. Planta 226:443-451.

Câmara GMS (2014). Fixação biológica do nitrogênio em soja. Inform. Agron. 147:1-9. Available at: http://www.ipni.net/publication/ia-brasil.nsf/0/B7FB85D4FAD745CF83257D660046A90D/$FILE/Page1-9-147.pdfCastro Castro PRC, Vieira EL (2001). Aplicação de reguladores vegetais na agricultura tropical. Guaíba: Agropecuária.

Cataldo DA, Haroon LE, Schrader LE, Youngs VL (1975). Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun. Soil Sci. Plant Anal. 6:71-80.

Craigie JS (2011). Seaweed extract stimuli in plant science and agriculture. J. Appl. Phycol. 23:371-393.

El-Ghamry AM, Abd KM, Ghoneem K (2009). Amino and humic acids promote growth, yield and disease resistance of faba bean cultivated in clayey soil. Aust. J. Basic Appl. Sci. 3(2):731-739. Available at: http://ajbasweb.com/old/ajbas/2009/731-739.pdf

Fischer WW, Hemp J, Johnson JE (2015). Manganese and the Evolution of Photosynthesis. Orig. Life Evol. Biosph. 45(3):351-357.

Foyer CH, Noctor G (2005). Redox homeostasis and antioxidante signaling: A metabolic interface between stress perception and physiological responses. Plant Cell 1:1866-1875.

Gill SS, Tuteja N (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48:909-930.

Gonzalez-Guerrero M, Matthiadis A, Sáez A, Long TA (2014). Fixating on metals: new insights into the role of metals in nodulation and symbiotic nitrogen fixation. Front. Plant Sci. 5:1-6.

Haegele JW, Below FE (2013). The six secrets of soybean success: improving management practices for high yield soybean production. Available at: http://cropphysiology.cropsci.illinois.edu/documents/2012%20Six%20Secrets%20of%20Soybean%20Success%20report.pdf.

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplast: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125:189-198.

Hildebrandt TM, Nunes AN, Araújo WL, Braun HP (2015). Amino Acid Catabolism in Plants. Mol. Plant 8(11):1563-1579.

Hodge A, Berta G, Doussan C, Merchan F, Crespi M (2009). Plant root growth, architecture and function. Plant Soil 321:153-187.

Hsu YY, Chao YY, Kao CH (2013). Methyl jasmonate-induced lateral root formation in rice: the role of heme oxygenase and calcium. J. Plant Physiol. 170:63-69.

Johnson CM, Stout PR, Boyer TC, Carlton AB (1957). Comparative chlorine requirements of different plants species. Plant Soil 8:337-353.

Khan W, Rayirath UP, Subramanian S, Jithesh MN, Rayorath P, Hodges DM, Critchley AT, Craigie JS, Norrie J, Prithiviraj B (2009). Seaweed extracts as biostimulants of plant growth and development. J. Plant Growth Regul. 28:386-399.

Marques JJ, Schulze DG, Curi N, Mertzman SA (2004). Trace element geochemistry in Brazilian Cerrado soils. Geoderma 121:31-43.

Marschner P (2012). Mineral nutrition of higher plants. Amsterdam: Elsevier.

Navarro Júnior HM, Costa AC (2002). Contribuição relativa dos componentes do crescimento para produção de grãos de soja. Pesqui. Agropecu. Bras. 37(2):269-274. Available at: http://www.alice.cnptia.embrapa.br/bitstream/doc/106777/1/pab20377.pdf

Pessoa ACS, Luchese EB, Luchese AV (2000). Germinação e desenvolvimento inicial de plantas de milho, em resposta ao tratamento de sementes com boro. R. Bras. Ci. Solo 24:939-945.

Romano MR (2005). Desempenho fisiológico da cultura de milho com plantas de arquitetura constante: parâmetros para modelos de crescimento. 100 p. Tese. USP-ESALQ. Piracicaba. Available at: http://www.teses.usp.br/teses/disponiveis/11/11136/tde-08062005-161952/pt-br.php

SAS Institute (2004). SAS/STAT Statistical Analysis System Manual (V. 9.0). SAS Institute, Cary, NC.

Soares LH (2014). Manejo fisiológico com base em tratamento de sementes e aplicação de organominerais via foliar para sistemas de alto potencial produtivo de soja. Dissertação (Mestrado em Ciências) – Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo, Piracicaba. Available at: www.teses.usp.br/teses/disponiveis/11/11136/tde.../Luis_Henrique_Soares.pdf

Soil Survey Staff (1999). Soil Taxonomy: A basic system of soil classification for making and interpreting soil surveys. Second ed. USDA-SCS Agric. Handb. 436. US Gov. Print. Office, Washington, DC.

Taiz L, Zeiger E (2013). Plant Physiology**.** 5. ed, Sunderland: Sinauer Associates. 782 p.

United States Department of Agriculture (2013). Available at: http://www.fas.usda.gov/psdonline/psdQuery.aspx

Werner AK, Witte CP (2011). The biochemistry of nitrogen remobilization: purines rings catabolism. Trends Plant Sci. 16:381-387.

Werner T, Schumülling T (2009). Cytokinin action in plant development. Plant Biol. 12:1-12.

Yano J, Yachandra V (2014) Mn_4Ca Cluster in Photosynthesis: Where and How Water is Oxidized to Dioxygen. Chem. Rev. 114:4175−4205.

Zhao Z, Andersen SU, Ljung K, Dolezal K, Miotk A, Schultheiss SJ, Lohmann JU (2010). Hormonal control of the shoot stem-cell niche. Nature 465(7301):1089-1092.

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Full Length Research Paper

Fermented milk drink flavored with Murici pulp added of passion fruit bark flour

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This study aimed to evaluate the physicochemical parameters, antioxidant activity, color and sensory profile of fermented milk drink with murici and passion fruit bark flour (FCM). Four milk drink formulations were processed: 0% FCM + 5% murici pulp (Treatment 1 - control); 0.5% FCM + 5% murici pulp (Treatment 2); 1.0% FCM + 5% murici pulp (Treatment 3) and 1.5% FCM + 5% murici pulp (Treatment 4). The scanning electron microscopy (SEM) of FCM presented quite irregular particles and the presence of starch of circular shape and fibrous compounds connected to fragmented walls. After the analyses of milk drinks, a slight decrease in the mean pH values and an increase in the antioxidant activity according to the increase in FCM content in milk drinks were observed. The color of milk drinks tended to yellow and red in samples added of FCM due to the presence of carotenoids. In the sensory profile, the highest mean value was for the texture of yogurt without addition of FCM, and the lowest mean value was for taste of yogurt with 1.50% FCM.

Key words: *Byrsonima crassifolia* (L.) Rich.), *Passiflora edulis* Sims, functional drinks.

INTRODUCTION

Murici (*Byrsonima ssp.*) is a fruit from the Brazilian cerrado usually consumed fresh. When mature, the fruit is yellowish and smells like rancid cheese (Rezende et al., 2003). The pulp is soft and fleshy (Alves et al., 2003) and can be used to develop new products such as fermented drinks.

Functional foods provide additional benefits to consumers, reduce the risk of diseases and improve diet with the intake of substances whose beneficial effect is not obtained through usual diet (Palanca et al., 2006). Due to the benefits in the digestive process, recommendations for the intake of dietary fiber have increased in recent years; however, there are few studies on the application of these fibers, especially when they come from agro-industrial wastes (Miranda et al., 2013).

Many agro-industrial wastes that may have bioactive characteristics, due to their low popularity and little knowledge on commercial applications, have not received much attention as a source of natural antioxidants. Nevertheless, these still unexplored sources result in

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many benefits to human health, where wastes can be turned into new products (Dimitrios, 2006). Among these wastes, passion fruit bark reduces cholesterol levels and blood glucose and improves the performance of the gastrointestinal system, besides being a source of protein and fiber (Cordova et al., 2005).

The aim of this study was to develop milk drink formulations added of murici pulp and different passion fruit bark flour concentrations in order to add value to this waste and improve the functional qualities of the milk drink by increasing the fiber content. The study also assessed the best formulation and product acceptance by sensory analysis, as well as the physicochemical characteristics and antioxidant activity of the proposed formulations.

MATERIAL AND METHODS

Murici pulp

Fruits (Figure 1) were collected at Fazenda Gameleira, municipality of Montes Claros de Goiás, Brazil, located at 16°07 'S and 51 18' W, altitude of 592 m and transported to the Laboratory of Fruits and Vegetables, Federal Institute of Goiás, Rio Verde Campus. Fruits were washed, sanitized and selected according to the degree of maturation homogeneity. Subsequently, fruits were manually pulped to obtain whole pulp, packed in polyethylene bags, identified by labeling and stored at -18°C for later use. The experiment received positive opinion for execution under protocol number 020/2013.

Passion fruit bark flour

For drying of passion fruit bark, 1 kg of previously ground bark was used. Drying was carried out by positioning the tray with dimensions of 800 mm x 600 mm at the central region of the drying oven with air circulating and renewal model MA 035 Marconi® at temperature of 60°C and air flow of 7.728 kg / m^2.s. After drying, passion fruit bark flour was obtained by three millings in Diogomaq® multi-purpose grinder and conditioned at room temperature in low density polyethylene bags for later use.

Fermented milk drinks

Two and a half liters of milk base were used for each formulation (30% skimmed milk serum and 70% whole milk) with addition of 10% sucrose, followed by submitting the mixture to pasteurization heat treatment at temperature of 90°C for 3 min, followed by lowering the temperature to 42°C, with subsequent addition of starter cultures composed of *Streptococcus Thermophilus, Lactobacillus Acidophilus* and *Bifidobacterium Lactis*.

Milk drink samples were incubated in an oven (BOD Quimis® Model Q-315d) at temperature of 42°C until pH reached 4.5. After coagulation, the oven temperature was readjusted to 20°C and clot was broken using a glass rod in a circular motion for 1 min. After temperature stabilization, thawed murici pulp pasteurized at 75°C for 15 s at concentration of 5% was added.

Four formulations of milk drink flavored with murici and added of passion fruit bark flour (FCM) were developed according to the following treatments: Treatment 1 - Milk drink flavored with murici without the addition of passion fruit bark flour (control); Treatment 2 - Milk drink flavored with murici plus 0.50% passion fruit bark flour; Treatment 3 - Milk drink flavored with murici plus 1% passion fruit bark flour and Tr7eatment 4 - Milk drink flavored with murici plus 1.50% passion fruit bark flour.

After addition the FCM, milk drink samples were filled into aseptic and identified polypropylene packages exposed in a laminar flow hood under ultraviolet light (UV) for 30 to 45 min followed by filling. Immediately, samples were stored under refrigeration at 4°C ± 1°C.

Analyses

The analyses of mineral macro and micronutrients of passion fruit bark flour were carried out at the Solocria Agricultural Laboratory located in Goiânia - Goiás, Brazil. Nitrogen was analyzed by distillation and complexation with boric acid with mixed indicator (bromocresol green + methyl red) and subsequent titration with 0.025 mol / L H_2SO_4 (sulfuric acid).

Phosphorus was analyzed by digestion of sample (FCM) with nitropercloric mixture and determination by colorimetry with Ammonium Molybdate and Ascorbic Acid. Potassium was analyzed by digestion of sample (FCM) with nitropercloric mixture and determination by flame photometry. Calcium, magnesium and micronutrients (Cu, Fe, Mn, Zn, Co, Mo) were analyzed by digestion of sample with nitropercloric mixture and elements were determined by atomic absorption. Finally, for boron determination, the sample was incinerated at 550°C for two hours in a muffle furnace. The residue was dissolved with 1: 3 HCl and the element was determined by colorimetry with Azomethine-H.

For physical assessment of murici pulp, dehydration was performed with sample previously frozen in freeze dryer - model enterprise (Terroni®). Lyophilized murici pulp and passion fruit bark flour were analyzed in a scanning electron microscope, Jeol, JSM-6610, equipped with EDS, ThermoScientific NSS SpectralImaging. The antioxidant activity of murici pulp and four types of treatments of milk drink flavored with murici and added of FCM was determined by the capacity to scavenge the DPPH free radical, according to methodology described by Rufino et al. (2007).

pH was measured using digital bench potentiometer - model LUCA® - 210P. The electrode was inserted in murici pulp samples and in milk drinks after homogenization without touching the bottom or sides of the package and thus reading was carried out. Three points in each pot were analyzed, corresponding to three replicates of each treatment. For titration of murici pulp, 10 g of sample were added to 100 ml of distilled water, the mixture was then filtered. Shortly after, four drops of 1% phenolphthalein solution was added to the filtrate and the mixture was titrated with 0.1 N sodium hydroxide solution up to the appearance of persistent pink color for about 30 seconds (Brazil, 2006). Acidity was determined in triplicate according to the following equation, and expressed as lactic acid percentage: lactic acid (%) = ((V x f x 0.9)/m), where: V = volume of 0.1 N sodium hydroxide solution used in the titration, in ml, f = correction factor of the 0.1 N sodium hydroxide solution; m = sample weight in grams.

The determination of total soluble solids of murici pulp was performed by direct reading in manual refractometer (ATAGO®) and expressed in °Brix. The results represent the average of three readings. The dry matter of passion fruit bark flour was determined by the difference between 100 and the moisture that was calculated according to AOAC method No. 925.09 (2000) up to obtaining constant weight. The analysis of the ether extract of passion fruit bark flour was performed according to AOAC method No. 925.38 (2000). The water activity (Aw) of passion fruit bark flour was determined in AqcuaLab device, CX-2, Washington, USA, at constant temperature (24±1°C).

The moisture contents in murici pulp, passion fruit bark flour and milk drinks were determined using an oven with forced air circulation. About 5 g of sample were weighted in previously dried and tared crucibles. Drying was carried out according to

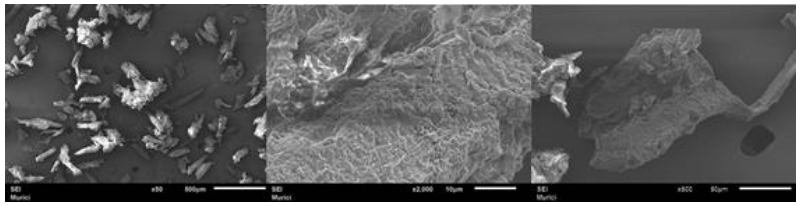

Figure 1. Scanning electron microscopy of lyophilized Murici pulp used in the processing of fermented milk drinks flavored with Murici (*Byrsonimacrassifolia* (L.) Rich.) added of passion fruit bark flour.

methodology proposed by AOAC (1995) for a period of 24 h at 105°C. The dry matter content of passion fruit bark flour was calculated by the difference between 100 and the moisture content. The samples used for analysis of moisture in murici pulp, passion fruit bark flour and the different milk drink treatments were also used for the analysis of ashes. Ashes were determined by the total carbonization of the organic matter in a muffle furnace (Bravac, M2) at 550°C for about 10 h or until clear ashes are obtained as described in paragraph of AOAC 923.03 official method (1995).

For the crude protein analysis, total nitrogen was determined by micro-Kjeldahl method for murici pulp, passion fruit bark flour and milk drink treatments according to the AOAC International official method No. 960.52 (1995). Total nitrogen was converted into crude protein using factor 6.25 for the murici pulp and passion fruit bark flour. For milk drinks, factor 6.38 was used.

Fat was assessed using the Gerber method by placing 10 ml of sulfuric acid (density 1.820), 11 ml of milk drink and 1 ml of amyl alcohol in a butyrometer. The butyrometer was sealed with appropriate stopper. The mixture was placed in a Gerber centrifuge for five minutes at 1200 rpm. After this time, the butyrometer was transferred to a water bath at 65°C for 5 to 7 min with stopper down, and then, holding the stopper down, the fat layer was allocated within the butyrometer scale and the reading was taken in the inner part of the meniscus. The reading results directly indicated the fat percentage, and the analysis was performed in triplicate. The carbohydrate content of milk drinks was estimated by difference, subtracting values obtained for moisture, ash, protein and fat from one hundred.

Instrumental color parameters (L*, a* and b*) of milk drink and passion fruit bark flour samples were analyzed in HunterLab colorimeter model Color Flex EZ at the Laboratory of Postharvest of Plant Products, Federal Institute of Goiás, Rio Verde *Campus*, GO, Brazil. These parameters were used to determine the color indices: Hue angle, which is indicative of hue and chroma and define color intensity. Nine results were analyzed per treatment.

Texture was evaluated in Brookfield texturometer model CT3 Texture Analyzer for the following parameters: compression test, probe tip TA4 / 100, distance 50%, trigger load of 5 g, speed of 1mm / s. All milk drink treatments were analyzed in triplicate, with readings at 1, 8, 15, 22 and 29 days of storage. Samples were removed from the refrigerator moments before the test so that there was no change in results. Data were collected through the Texture Expert software for Windows software - version 1.20 (Stable Micro Systems). Hardness and adhesiveness parameters were used for assessing the results, which represent the average of three readings for each formulation.

The evaluation of the sensory characteristics was conducted in order to quantify the consumer preference for different types of milk drinks flavored with murici pulp and added of FCM, as well as the purchase intent. Sensory analyses were performed with students, teachers and servers of the Federal Institute of Goiás, Rio Verde Campus, GO, Brazil. To perform the sensory analysis, 50 untrained panelists composed the evaluation team. Four milk drink formulations added of murici were sensorially evaluated: without FCM (control) and with the addition of 0.5%, 1% and 1.5% passion fruit bark flour.

The model adopted for sensory analysis was the acceptance test for the comparison of milk drinks with different passion fruit bark flour concentrations. Sensory evaluation was based on scores given by judges through a 9-point hedonic scale, where value one (1) represents" disliked extremely" and nine (9) "liked extremely", in which overall impression (color), flavor, aroma and texture were judged. Along with the global aspect of products, the purchase intent of panelists on each of the samples was analyzed with a 5-point hedonic scale, where one (1) represented "certainly would not buy" and five (5) represented "I would definitely buy" (Ial, 2005).

Sensory analysis was performed in individual booths at the Laboratory of Sensory Analysis, Federal Institute of Goiás, Rio Verde Campus, GO, Brazil. The four samples were coded with three-digit numbers and delivered under white light in 50 ml white cups to each of the panelists. The amount of samples served was the same, about 20 mL, aiming not to influence their opinion. Milk drink samples were presented to panelists at temperature of approximately 6°C in a balanced and randomized form.

Statistical analysis

Graphs have been presented through the Microsoft Office Excel software version 2007. The SISVAR software (Ferreira, 2010) was used for the mean comparison tests.

RESULTS AND DISCUSSION

The quantitative analysis of macro and micronutrients of passion fruit bark flour is shown in Table 1. The mineral detected in greatest amount was potassium, with 2.80 g / 100 g. This mineral is attributed antihypertensive effect by inducing loss of sodium and water by the body, increasing the secretion of prostaglandins and reducing vascular resistance (Tomazoni and Siviero, 2009).

Santos et al. (2008) characterized macro and micronutrients in passion fruit bark (*Passiflora nitida*) and observed similar values for sodium and manganese, with average values of 103 mg / kg and 15.80 mg / kg respectively; however, the macronutrient contents (Ca = 2.35 g / Kg, P = 0.83 g / kg, N = 16.92 g / kg, K = 23.98 g / kg Mg = 1.01 g / kg, and S = 5.29 g / kg) were higher than those of passion fruit variety of this study (Table 1). SEM aims to analyze the sizes and shapes of crystalline and amorphous, inorganic and biological structures of a sample. Figures 2 and 3 show the scanning electron

Table 1. Values of macro and micronutrients present in passion fruit bark flour used in the processing of fermented milk drinks flavored with murici and passion fruit bark flour (*Byrsonimacrassifolia* (L.) Rich.).

Parameters	Value	Unit
Calcium	0.34	g/kg
Phosphorus	0.18	
Nitrogen	1.50	
Potassium	2.80	
Magnesium	0.10	
Sulfur	0.15	
Sodium	102.00	mg/kg
Copper	1.00	
Iron	210.00	
Manganese	19.00	
Zinc	43.00	
Cobalt	0.16	
Molybdenum	0.60	
Boron	91.00	

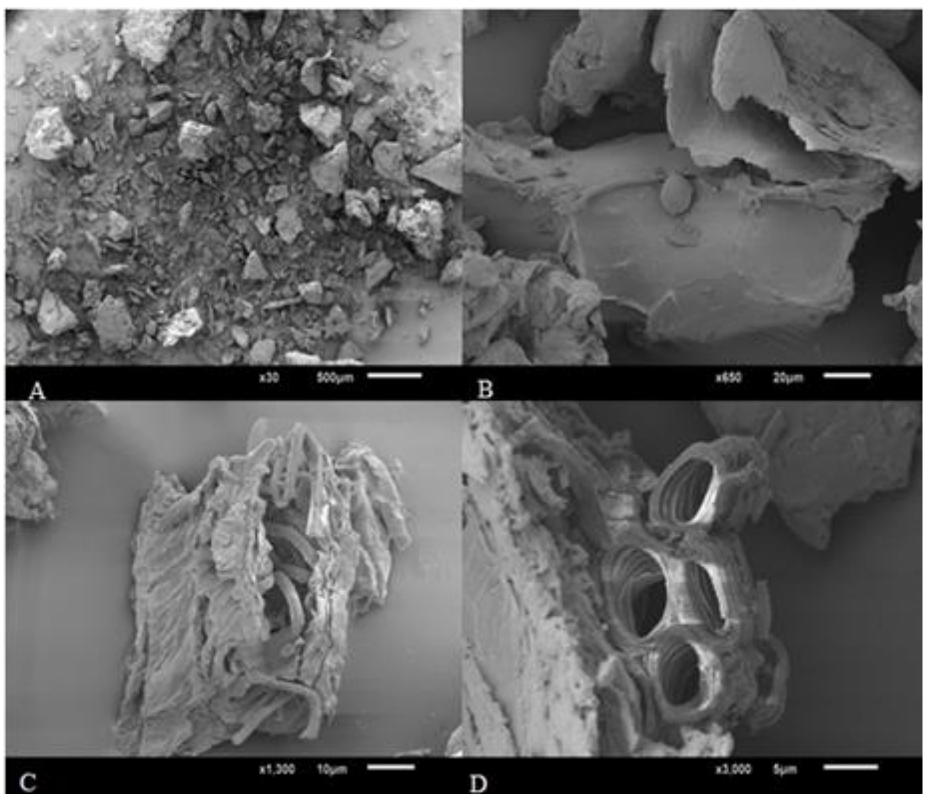

Figure 2. Scanning electron microscopy of passion fruit bark flour used in the processing of fermented milk drinks flavored with Murici (*Byrsonimacrassifolia* (L.) Rich.) added of passion fruit bark flour.

microscopy (SEM) of murici pulp and passion fruit bark flour, respectively.

When using magnification of 50 times, murici pulp (Figure 2) showed various particles predominantly of irregular shapes. Using magnification of 500 times, the composition is asymmetrical, showing the presence of a

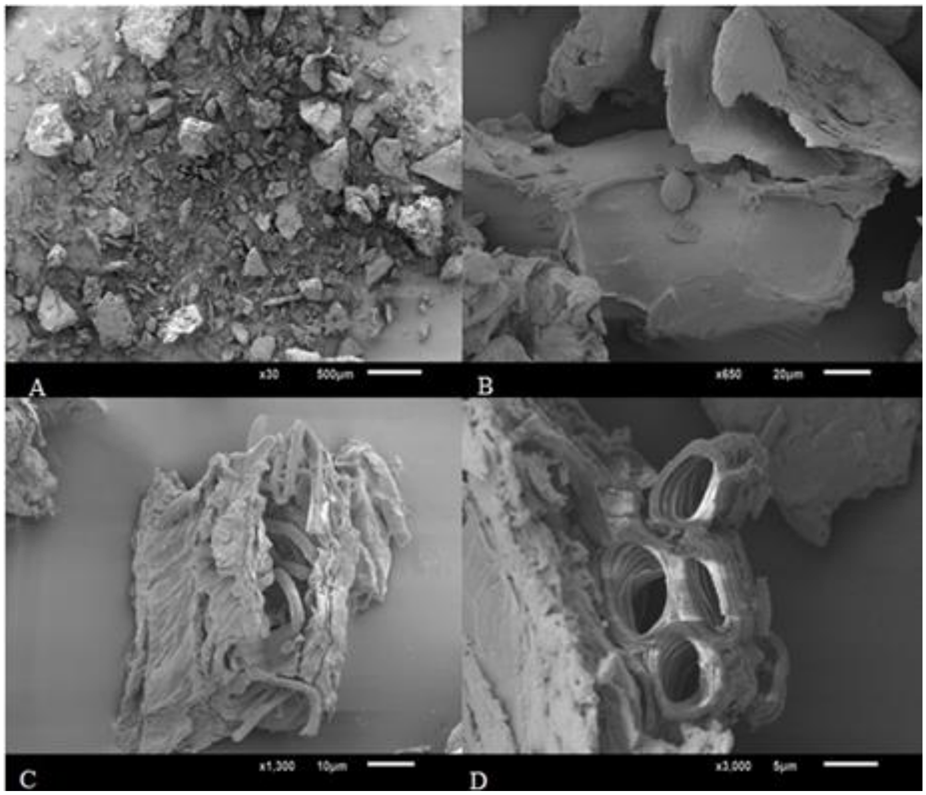

Figure 3. Scanning electron microscopy of passion fruit bark flour used in the processing of fermented milk drinks flavored with Murici (*Byrsonimacrassifolia* (L.) Rich.) added of passion fruit bark flour.

few fibrous filaments and dense and compact surface. Using magnification of 2,000 times, micrographs showed greater roughness and porosity, being possible to observe the disposition of some fibrous membranes.

Particles of different sizes in flour characterizes the degradation of the molecular matrix, granular structures and in the shape of lentils, represents the starch fraction and protein fraction, respectively (Roman-Gutierrez et al., 2002). In Figure 3, with magnification of 30 times, particles of different sizes and irregular surface structures with flat portions and some holes were observed. Figure 3B, with magnification of 650 times, shows a compact and amorphous mass, where it is possible to distinguish some starch granules indicated by the arrow from non-starchy material.

With magnification of 1,300 times (Figure 3C), the arrangement of fiber filamentous was observed. Unlike starch, fibers have no rounded structures, but rather more geometric shapes and some gaps that are responsible for the high incidence of permeable pores in fibers, which leads to high water absorption, characterizing fiber as a highly hygroscopic structure (Fiorda et al., 2013). It is known that FCM has considerable protein content, and Figure 3D shows particles of different sizes and shapes and structures with circular and lentil shape, suggesting characterization of polysaccharides and protein matrix.

The antioxidant activity of murici pulp extract and four milk drink treatments was assessed at three concentrations: 200%, 100% and 50% and their respective absorbance values were determined using equation with better fit and respective correlation coefficient R^2. The results were expressed as EC_{50} (extract concentration in g / L capable of reacting with 50% of the radical present in the DPPH solution). Therefore, the lower the EC_{50} value, the higher the antioxidant activity of the extract analyzed (Vieira et al., 2011). The standard curve constructed with the absorbance parameters in UV and concentration was determined by the percentage of scavenged DPPH with EC_{50} value = 182.07 g / L, with linearity and good correlation with the correlation determination coefficient R^2 = 0.953.

Roesler et al. (2007) reported lower antioxidant activity by DPPH of typical cerrado fruits such as cagaita pulp (EC_{50} = 387.5), pequi pulp (EC_{50} = 298.7), and higher antioxidant activity in araticum pulp (EC_{50} = 148.8) and lobeira pulp (EC_{50} = 163.0). Siguemoto (2013) also reported lower antioxidant activity in murici fruits collected in the city of Marabá - PA, Brazil, in which EC_{50} = 330.5 was obtained. This can be explained by the fact that fruits

Table 2. Proximate composition and instrumental color parameters (L *, a * and b *) of passion fruit bark flour.

Parameter	FCM
Water activity (Aw)	0.323
Dry matter (g/100 g)	90.83
Moisture (g/100 g)	9.17
Crude protein (g/100 g)	13.86
Ash (g/100 g)	9.85
Ether extract (g/100 g)	0.43
L*	70.78
a*	5.32
b*	21.30

were collected in different regions, thus obtaining different antioxidant activity values.

The antioxidant activity of milk drink flavored with murici and added of passion fruit bark flour was determined for all four treatments (0, 0.5, 1.0 and 1.5% FCM). A slight increase in the antioxidant activity of milk drinks with increasing passion fruit bark flour concentration was observed, and milk drink with 0% flour showed EC_{50} = 238.244 g / L with R^2 = 0.983 and milk drink with 1.5 % showed EC_{50} = 224.302 g / L, with R^2 = 0.997. This is because passion fruit bark exhibits high antioxidant capacity. Zeraik (2010) observed EC_{50} value = 35 g / L in passion fruit peel.

This study revealed that the higher the passion fruit bark flour concentration added to milk drinks flavored with murici, the greater their antioxidant capacity. pH, titratable acidity and total soluble solids (TSS) of murici pulp were 3.63; 0.77 g of lactic acid / 100 ml and 10.9 °Brix, respectively. Similar results were reported by GUIMARÃES & SILVA (2008), who analyzed murici pulp and obtained pH 3.42 and TSS of 10.67 °Brix.

The moisture content, ash and protein values of murici pulp analyzed in the present study were 73.29 g / 100 g; 0.97 g / 100g 1.72 g / 100g respectively, which were similar to values found by ROESLER et al. (2007), who studied the proximate composition of cerrado fruit pulps and found in araticum pulp (*Annonacrassiflora*) 67.85 g / 100 g of moisture; 0.77 g / 100 g of ashes and 1.80 g / 100 g of protein. Table 2 shows the proximate composition and instrumental color parameters (L *, a * and b *) of passion fruit bark flour.

The flour obtained by drying and grinding passion fruit bark showed low moisture content and aW, which protects flour against microbiological changes, since bacterial growth occurs at aW between 0.6 and 0.9 (LABUZA & ALTUNAKAR, 2008). Similar water activity and moisture values were found by Cazarin et al. (2014), who studied this flour and obtained aW of 0.43 and moisture of 9.48 g / 100 g.

Crude protein value of 13.86g / 100g was observed, which is greater than that reported by Souza et al. (2008), who found value of 11.76g / 100g for passion fruit bark flour and Pena et al. (2008), who analyzed passion fruit flour obtained by drying at 70°C and reported 11.3g / 100 g of protein. This allows classifying the passion fruit bark flour of the present study as source in protein, being an alternative for use in diets that require protein increment.

In the analysis of ash, relatively high result was obtained (9.85g / 100 g), when compared to result obtained by Souza et al. (2008), who found value of 8.13g / 100g for passion fruit bark flour and Cazarin et al. (2014), who reported 6.88g / 100 g of ash content for the same waste. The values of color instrumental parameters indicated that passion fruit bark flour can be clear, as it presented brightness close to white (70.78), because the closer to 100, the clearer the flour. Values observed by Vilhalva et al. (2011) for cassava bark flour were close to the FCM, whose L * and a * values were 64.54 and 4.51, respectively.

As for chromaticity coordinates a * and b *, the flour has color tending to red, with positive a * value (5.32) and to yellow, with positive b * value and away from zero (21, 30). The tendency of colors to red and yellow confirmed the presence of carotenoids, which are natural pigments present in passion fruits (Silva and Mercadante, 2002). Table 3 shows the average results of moisture, ash, protein, fat and carbohydrates of fermented milk drinks flavored with Murici (*Byrsonima crassifolia* (L.) Rich.) added of different passion fruit bark flour concentrations.

Regarding the proximate composition of milk drinks flavored with murici and enriched with FCM, Table 3 shows that the moisture content of samples decreased with increasing FCM concentration in milk drinks, and drink with 1.5% flour significantly differed ($p < 0.05$) from the others. According to Gambelli et al. (1999), yogurt moisture content is about 87 / 100 g; however, this value depends on the type of milk and available soluble solids, because in this study, in addition to milk, milk whey, murici pulp and FCM were also used and thus the results corroborate those by Toledo (2013), who produced yogurt with passion fruit pulp and flour, in which yogurt without flour showed moisture of 78.73g / 100 g and for yogurt with the highest flour concentration (8 g / 100g), the moisture content was 73.13g / 100g.

There was a significant difference ($p < 0.05$) in the ash content of milk drinks without flour and sample with the highest FCM concentration. This parameter concomitantly increased the FCM concentration, a result that was expected since FCM presented ash content of 9.85 g / 100 g, and its addition is a factor that significantly contributed to increase the ash content of milk drinks.

As can be seen in Table 3, the protein content of milk drinks increased with the addition of flour. This fact can be explained by the high protein content of passion fruit bark flour (11.76g / 100g) observed by Souza (2008) and the protein content of 13.86g / 100 g of FCM used in this

Table 3. Average moisture (g / 100 g), ash (g / 100g), protein (g / 100 g), fat (g / 100 g) and carbohydrate results of fermented dairy drinks flavored with Murici (Byrsonimacrassifolia (L .) Rich.) added of passion fruit bark flour.

Parameters	Passion fruit bark flour concentrations (%)				VC (%)
	0.0	0.5	1.0	1.5	
Moisture	82.12±0.08a	81.73±0.32a	81.65±0.32a	80.77±0.33b	0.35
Ash	0.54±0.14ª	0.67±0.02ab	0.69±0.03ab	0.78±0.01b	10.71
Protein	1.97±0.04ª	2.01±0.07ab	2.10±0.05b	2.26±0.02c	2.32
Fat	2.00±0.20ª	1.73±0.64ª	1.23±0.31a	0.93±0.92a	40.11
Carbohydrate	13.37±0.25a	13.84±0.76ab	14.31±0.55ab	15.25±0.97b	4.84

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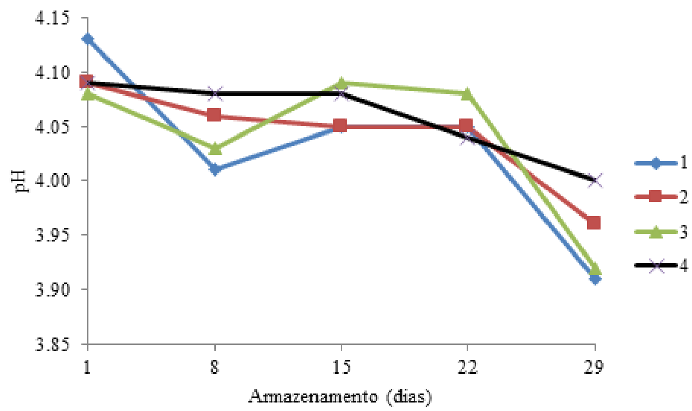

Figure 4. pH of fermented milk drinks flavored with Murici (*Byrsonimacrassifolia* (L) Rich.) added of passion fruit bark flour.

study. The four milk drink formulations showed content above legal standards (1.7%) defined by Brazilian legislation (Brazil, 2005).

The protein percentage of milk drinks with passion fruit bark flour was similar to those described by Thamer and Penna (2006), who prepared milk drinks added of probiotic and prebiotic bacteria and found protein content ranging from 1.93 g / 100 g to 2.46 g / 100 g.

The fat content showed no significant different (p> 0.05) among the four milk drink formulations. Cunha et al. (2009) found fat content ranging from 1.43 g / 100 g to 2.01 g / 100 g, in a study on the influence of the use of milk whey and probiotic bacteria in the properties of fermented milk drinks. Finco et al. (2011) observed higher lipid content (3.36 g / 100 g) in natural yogurt enriched with sesame flour.

In relation to the carbohydrate contents, there was a significant difference (p <0.05) between sample without the addition of flour and those with the highest FCM concentration. It was observed that with increased flour concentration, a slight increase in the carbohydrate content was observed. Similar results were reported by Pagamunici (2009), with average carbohydrate content of 13.78 g / 100 g for yogurt added of jaracatiá bran. The pH results of milk drinks flavored with murici and with 0% FCM - Control; 0.5% FCM (Treatment 2); 1.0% FCM (Treatment 3) and 1.5% FCM (Treatment 4) are shown in Figure 4.

The analysis of data allowed observing that during the storage period of milk drinks, pH showed a considerable reduction, since the initial pH ranged from 4.13 (Treatment 1) to 4.09 (Treatment 4), reaching 3.91 (Treatment 1) and 4.00 (Treatment 4) on day 29 of storage. Similar pH results were reported by Sivieri and Oliveira (2002), who analyzed milk drinks prepared with "fatreplacers" during 28 days of storage. The pH values at baseline ranged from 4.0 to 4.6, and after 28 days of storage, it ranged from 3.9 and 4.5.

According to Ellis (1996), microorganisms present in yogurt remain viable and even keeping the product at temperature of 5°C, they continue reproducing and acidifying the medium. Therefore, to establish whether yogurt is fit for consumption, pH and titratable acidity should be checked. The low pH from day 22 of storage on can be related to the action of *Lactobacilus acidophilus*, a microorganism known for its high capacity to produce acid in the fermentation medium (Macedo, 1997). According to Souza (1990), the yogurt acidity is quite variable and largely influences consumption. Thus, pH values from 4.6 to 3.7 are common. However, values

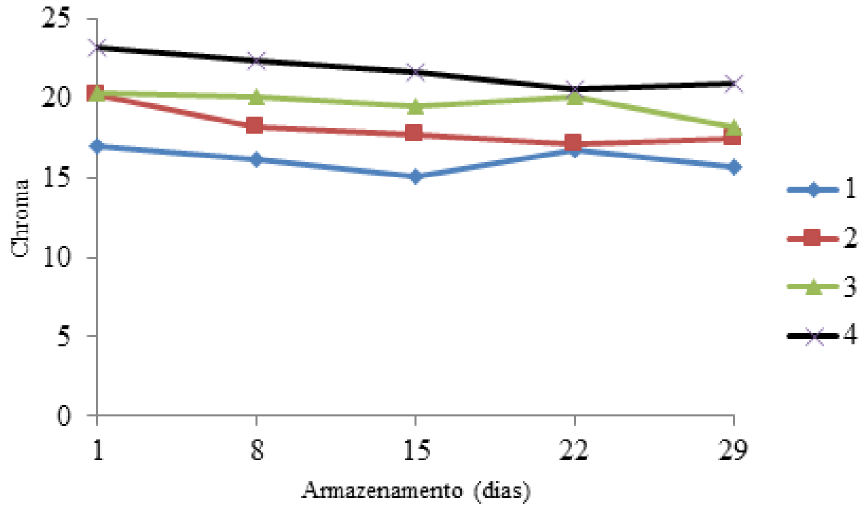

Figure 5. Chroma of fermented milk drinks flavored with Murici (*Byrsonima crassifolia* (L.) Rich.) added of passion fruit bark flour.

similar to those considered as ideal were found in milk drinks even after 29 days of storage.

Regarding the color of milk drinks, it was observed that the addition of FCM had no effect on chroma (C *) and Hue value (H *). The results are shown on Figures 5 and 6. The first feature observed in a food is its color, and this preview determines taste and quality expectations (Henry, 1996). The quality of a color is obtained by hue (h *) and saturation (C *). Hue is a feature that characterizes the quality of color such as red, green and blue, for example, enabling them to be differentiated. The hue angle (h *) ranges from 0° to 360°: 0° is the angle corresponding to the red color, 90° to yellow color, 180° to green color and 270 ° to blue color (Abreu et al., 2011). Saturation, also called purity, describes the intensity or quantity of a color, indicating the proportion in which it is mixed with black, white or gray, allowing differentiating strong from weak colors and when this variable has values near 0, there is predominance of neutral colors (gray) and when it has values close to 60, there is predominance of vivid colors (Ramos and Gomide, 2007).

Regarding chromaticity (C *), samples added of passion fruit bark flour showed higher values (20.37 to 23.35) compared to treatment without the addition of flour. Lower C * values correspond to a weaker color pattern and higher C * values correspond to a stronger color pattern. Thus, the addition of passion fruit bark flour resulted in milk drinks with more vivid colors. The analysis of data allowed observing that during the storage period, C * showed significant decreases since the initial data ranged from 16.98 (Treatment 1) to 23.18 (Treatment 4), reaching 15.63 (Treatment 1) and 20.91 (Treatment 4) on day 29 of storage, as can be seen in Figure 5.

Similar results were found by Abreu et al. (2011), who developed mixed drinks with mango, passion fruit and cashew pulp added of different concentrations of prebiotics and observed C * values between 13.04 and 13.45 for drinks with the lowest and highest amounts of prebiotic, respectively. However, the opposite was observed by Toledo (2013), who produced yogurt with passion fruit flour and pulp and observed that samples added of 2 g / 100 g to 8 g / 100 g of passion fruit flour showed significantly lower C *values compared to treatment without flour (yogurt with 0% flour), concluding that the addition of the pulp and flour resulted in yogurt with more consistent color ("weak") due to the effect of the dark color of flour on the product.

The average Hue angle values (Figure 6) ranged from 85.30 (Treatment 1) to 80.13 (Treatment 4), indicating that milk drinks added of pulp and flour were within the yellow hue. It was found that as the concentration of passion fruit bark flour increased, the samples distanced from yellowish color. Thus, samples containing 0% and 1.5% passion fruit bark flour showed, respectively, the highest and lowest proportions of yellow color.

Toledo (2013) produced yogurt with passion fruit pulp and flour, and obtained similar results for the Hue angle, observing that as the concentration of passion fruit bark flour increased, the h * values decreased to 96.68 for yogurt without flour and 89.95 for yogurt with 8g / 100g of flour. The results of the texture analyses (hardness and adhesiveness) are shown as a function of the days of storage in Figures 7 and 8. It was observed that hardness increased for all formulations over the days, with minor variations in results.

Noronha et al. (2007) reported that the increase in hardness may be due to the decreased hydration of the protein network, resulting in lower plasticizing effect. According to Sodini et al. (2002), the main factors influencing yogurt hardness are protein content and type.

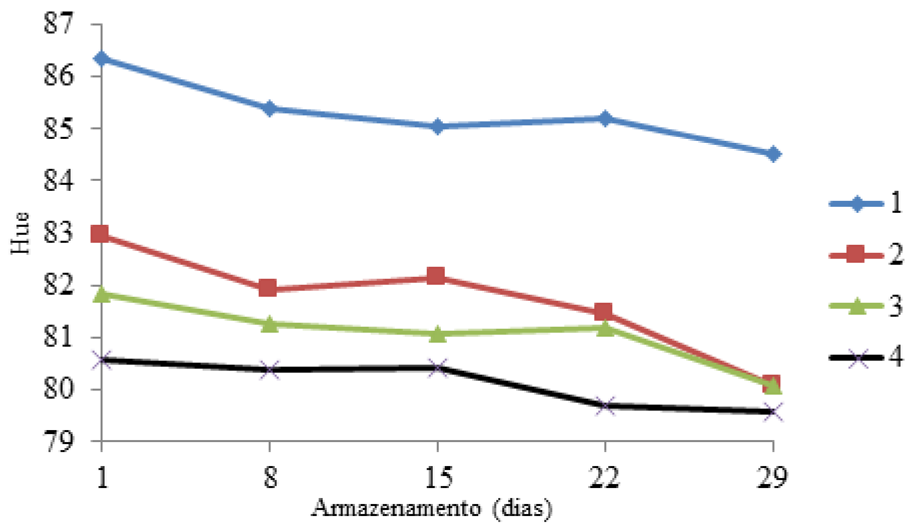

Figure 6. Hue angle of fermented milk drinks flavored with Murici (*Byrsonimacrassifolia* (L.) Rich.) added of passion fruit bark flour.

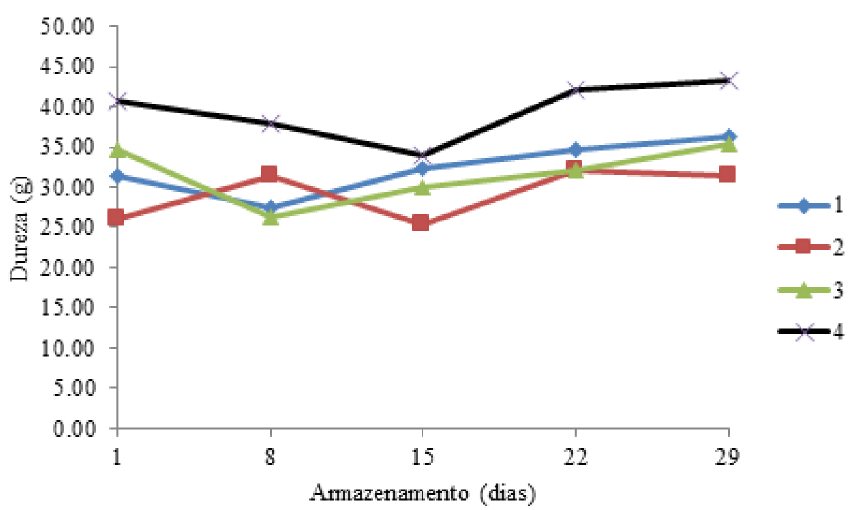

Figure 7. Hardness of fermented milk drinks flavored with Murici (*Byrsonimacrassifolia* (L.) Rich.) added of passion fruit bark flour.

A possible explanation for a slight variation during the storage period for each treatment would be the amount of FCM added to milk drinks. The higher hardness presented by drink with 1.5% FCM can be related to the higher total solids content (FCM). Cardarelli et al. (2003) observed a significant increase in hardness over 28 days of storage at 4°C for two symbiotic petit-suisse cheese formulations in which xanthan gum was added as a stabilizer and Castro (2007) observed increased hardness in milk drinks added of oligofructose compared to milk drinks without oligofructose. Stability or small variation in hardness during the storage period is desired, since, in this way, it is found that after a few weeks of storage, the product has characteristics similar to a newly manufactured product. Stability is highly desirable to keep physical, chemical and sensory characteristics throughout the product shelf-life (Maruyama et al., 2006). Similarly to what was observed for hardness, it was found that the adhesiveness values were higher for milk drinks with higher FCM concentration (Figure 8). Higher mean adhesiveness values were observed for Treatment 4 (average of 0.78) compared to Treatment 1 with lower FCM concentration (average of 0.44). According to Fox et al. (2000), adhesiveness refers to the force required to remove the food that is stuck in the mouth (generally on the palate) during the normal chewing process.

During the storage period, there was an increase in adhesiveness values for all milk drink formulations, which

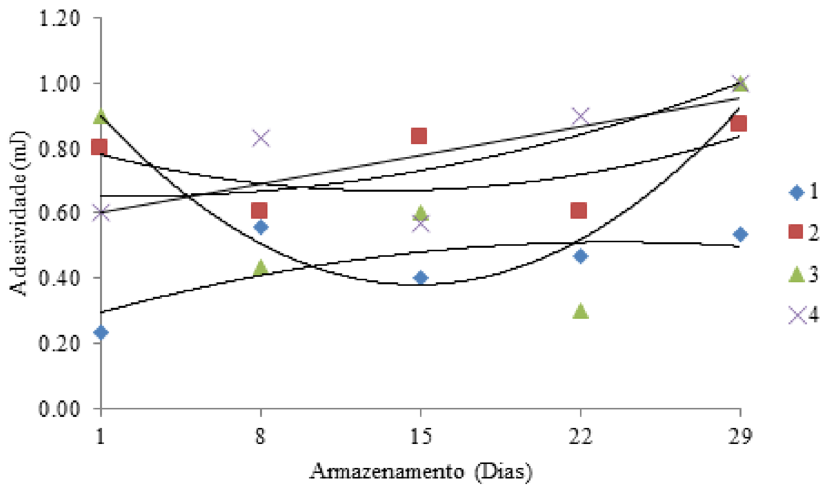

Figure 8. Adhesiveness of fermented milk drinks flavored with Murici (*Byrsonimacrassifolia* (L.) Rich.) added of passion fruit bark flour.

Table 4. Mean values and standard deviation of the sensory analysis of parameters color, flavor, aroma, texture and purchase intent (IC) of fermented milk drinks flavored with Murici (*Byrsonima crassifolia* (L.) Rich.) added of passion fruit bark flour.

Parameters	FCM concentration (%)				CV (%)
	0.0	0.5	1.0	1.5	
Color	7.18±1.77ª	6.60±1.63a	5.36±2.18b	4.78±2.34b	33.48
Flavor	6.86±1.85ª	5.38±1.93b	4.18±2.04c	3.60±2.14c	39.79
Aroma	6.02±2.19ª	5.26±2.20ab	4.46±2.27b	4.40±2.42b	45.15
Texture	6.30±2.08ª	5.22±1.76ab	4.74±2.05b	4.18±2.40b	40.80
IC	3.68±1.06ª	2.80±1.05b	2.32±0.98bc	1.82±0.87c	37.39

Different letters on the line significantly differ by the Tukey test at 5% probability.

is similar to results found by Buriti et al. (2010), who observed an increase of adhesiveness values for guava mousse added of inulin during refrigerated storage. Table 4 presents the average values of sensory parameters color, flavor, aroma, texture and purchase intent for the four types of milk drink flavored with murici and added of passion fruit bark flour at eight days of storage. The sensory evaluation of the color of milk drinks flavored with murici with different FCM concentrations (0%, 0.5, 1.0 and 1.5%) showed that panelists gave higher score to drink without FCM. The color of milk drink with 1.5% flour was less attractive for panelists and drinks containing 0% and 0.5% significantly differed (p <0.05) from drinks containing 1.0 and 1.5% of flour. As seen in Table 4, the higher the flour concentration present in the sample, the greater the rejection by panelists. Thus, it was found that samples containing 0 and 1.5% were those obtaining the highest and lowest scores, respectively.

Lupatini et al. (2011) evaluated biscuits enriched with passion fruit bark flour and okara and also obtained lower acceptance for treatment with the highest flour percentage, this fact is due to the bitter aftertaste in the product added of passion fruit bark flour. The result for flavor and texture of milk drinks flavored with murici and with different FCM concentrations showed that panelists gave the highest score to drink with 0% flour. The aroma and texture of drinks with 1.5% flour were less attractive; however, milk drink with 0.5% murici pulp did not differ significantly (p> 0.05) from drinks with 0 and 1.0% of flour.

Regarding purchase intent, significant difference (p <0.05) was observed among treatments 1, 2 and 4, and the highest score was given to treatment 1 (3.68%), followed by treatments 2, 3 and 4. Despite the higher acceptance and purchase intent of drink with 0% FCM, many panelists declared they would consume sample with 0.5% flour by associating it to a more healthy and nutritious product due to the presence of fibers and proteins from flour.

These results indicated that although panelists not have preferred samples with high FCM concentration, some panelists declared they would consume drink with0.5% FCM, which can be a food product of great interest, since the addition of passion fruit bark flour greatly contributes to enhance nutritional properties.

CONCLUSION

The addition of passion fruit bark flour in milk drinks flavored with murici intensified the color, resulting in milk drinks with more vivid colors, tending to yellow and red. Regarding the physicochemical aspects, it was concluded that the addition of passion fruit bark flour had a positive influence on the texture of milk drinks, which increased with increasing FCM concentration, as well as the protein content and antioxidant activity of milk drinks.

Regarding the sensory evaluation, higher acceptance was obtained for drinks without the addition of flour. However, milk purchase intent, showing that the addition of pulp and flour influenced the sensory profile of samples, and treatments with 0 and 0.5% FCM showed the highest scores for most desirable attributes for milk drink flavored with murici added of passion fruit bark flour.

Further studies should be carried out in order to establish the best levels of FCM addition to improve acceptance, sensory characteristics and nutritional and technological qualities of milk drinks, being an alternative to reduce the disposal of this food industry by-product.

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Conflict of interests

The authors have not declared any conflict of interests.

REFERENCES

Abreu DA, Silva LMR, Lima ADS, Maia GA, Figueiredo RWD, Sousa PHM (2011). Desenvolvimento De Bebidas Mistas À Base De Manga, Maracujá E Caju Adicionadas De Prebióticos Development of mixed beverages of mango, passion fruit and cashew apple added with prebiotics. Alimentos e Nutrição Araraquara 22(2):197-203.

Alves GL, Franco MRB (2003). Headspace gas chromatography–mass spectrometry of volatile compounds in murici (*Byrsonima crassifolia L. Rich*). J. Chromatogr. A 985(1):297-301.

Association of Official Agricultural Chemists (1995). Official methods of the Association of the Agricultural Chemists. 16. ed. Washington, DC, 2:1094.

Association of Official Agricultural Chemists (2000). Official methods of the Association of the Agricultural Chemists. 17. ed. Washington, DC, 2:1175.

Brazil (2005). Ministério da Agricultura, Pecuária e Abastecimento. Padrões de Identidade Qualidade de Bebidas Lácteas. Instrução Normativa nº 16, de 23 de agosto de 2005. Regulamento técnico de identificação e qualidade de bebidas lácteas. Publicada no Diário Oficial da República Federativa do Brasil. Seção I, 23 ago.

Buriti FC, Castro IA, Saad SM (2010). Viability of Lactobacillus acidophilus in synbiotic guava mousses and its survival under in vitro simulated gastrointestinal conditions. Int. J. Food Microbiol. 137(2):121-129.

Cardarelli H, Febbo C, Saad S (2003). Monitoramento de parâmetros de textura instrumental em queijo petit-suisse probiótico ao longo de sua vida de prateleira. Rev. Bras. Cienc. Farm 39(supl 3):147-149.

Castro FPD (2007). Influência de diferentes proporções de soro de queijo e oligofrutose sobre as propriedades de bebidas lácteas fermentadas simbióticas.

Cazarin CBB, da Silva JK, Colomeu TC, de Lima ZR, Junior MRM (2014). Capacidade antioxidante e composição química da casca de maracujá ("Passiflora edulis"). Cienc. Rural 44(9):1699-1704.

Cordova KRV, Gama TMMTB, Winter CMG, Kaskantzis Neto G, Freitas RD (2005). Características físico-químicas da casca do maracujá amarelo (Passiflora edulis Flavicarpa Degener) obtida por secagem. B. CEPPA 23(2):221-230.

Dimitrios B (2006). Sources of natural phenolic antioxidants.Trends Food Sci. Technol., 17(9):505-512.

Ellis MJ (1996). Shelf life evaluation of foods. London: Black Acad. Prof. 321 p.

Finco AMO, Garmus TT, Bezerra JRMV, Córdova KRV (2011). Elaboração de iogurte com adição de farinha de gergelim. Ambiência Guarapuava (PR) 7(2):217-227.

Fiorda FA, Júnior MSS, da Silva FA, Souto LRF, Grossmann MVE (2013). Farinha de bagaço de mandioca: aproveitamento de subproduto e comparação com fécula de mandioca. Pesqui. Agropecu. Trop. (Agricultural Research in the Tropics), 43(4):10-1590.

Fox PF, Guinee TP, Cogan TM, Mcsweeney PLH (2000) Fundamentals of cheese science. Gaithersburg: Aspen, 587p.

Gambelli L, Belloni P, Ingrao G, Pizzoferrato L, Santaroni GP (1999) Minerals and trace elements in some italian dairy products. J. Food Compost. Anal. San Diego 12:27-35.

Guimarães MM, SILVA MS (2008). Valor nutricional e características químicas e físicas de frutos de murici-passa (Byrsonima verbascifolia). Ciênc. Tecnol. Alimentos 28(4):817-821.

Henry BS (1996). Natural food colours. In: Hendry GAF, Houghton JD. Natural Food Colorants, 2. Ed. Great Britain: Chapman e Hall pp. 40-79.

Instituto adolfo lutz (Ial) (2005). Normas analíticas do Instituto Adolfo Lutz: métodos químicos e físicos para análise de alimentos. 4 ed. São Paulo: IMES

Labuza TP, Altunakar L (2008). Water activity prediction and moisture sorption isotherms. In: Barbosa-Cánovas GV. et al (Eds.). Water activity in foods: Fundamentals and Applications. Local: Oxford. Blackwell Publishing pp. 109-154.

Lupatini AL, Fudo RM, Mesomo MC, Conceição WAS, Coutinho MR (2011). Desenvolvimento de Biscoitos com Farinha de Casca de Maracujá-amarelo e Okara. Rev. Ciênc. Exatas Nat. 13(3):317-329.

Lutz IA (1985). Normas analíticas, métodos químicos e físicos para análise de alimentos. São Paulo: Instituto Adolfo Lutz P. 1.

Macedo REF (1997). Desenvolvimento de bebida láctea fermentada a base de extrato hidrossolúvel de soja e soro de leite de búfala por cultura mista de Lactobacillus casei Shirota e Bifi dobacterium adolescentis. 112f. Dissertação (*Mestrado em Tecnologia Química*) – Universidade Federal do Paraná, Curitiba.

Maruyama LY, Cardarelli HR, Buriti FCA, Saad SMI (2006). Textura instrumental de queijo petit-suisse potencialmente probiótico: influência de diferentes combinações de gomas. Ciência e Tecnologia de Alimentos, 26(2):386-393.

Miranda AA, Caixeta ACA, Flávio EF, Pinho L (2013). Fibras da farinha da casca do maracujá. Alim. Nutr. = Braz. J. Food Nutr., Araraquara, 24(2):225-232.

Noronha N, O'riordan ED, O'sullivan M (2007). Replacement of fat with functional fibre in imitation cheese. Int. Dairy J. 17:1073-1082.

Pagamunici LM (2009). Utilização de fibra de jaracatiá no enriquecimento de iogurte. Dissertação (Mestrado em Ciências de

Alimentos) –Universidade Estadual de Londrina, Londrina.
Palanca V, Rodríguez E, Señoráns J, Reglero G (2006). Bases científicas para el desarrollo de productos cárnicos funcionales com actividad biológica combinada. Alimentos funcionales: Nutr. Hosp. 21(2):199-202.
Pena RS, Silva DMS, Mendonça NB, Almeida MDC (2008). estudo da secagem da fibra residual do maracujá. Rev. Bras. Tecnol. Agroind. 2(1):1-13.
Ramos EM, Gomide LAM (2007). Avaliação da qualidade de carnes: fundamentos e metodologias. Cap 7. Ed. UFV, Viçosa-MG, pp. 287-370.
Rezende CM, Fraga SR (2003). Chemical and aroma determination of the pulp and seeds of murici (*Byrsonima crassifolia L.*). J. Braz. Chem. Soc. 14(3):425-428.
Roesler R, Malta LG, Carrasco LC, Holanda RB, Sousa CAS, Pastore GM (2007). Atividade antioxidante de frutas do cerrado. Ciênc. Tecnol. Alimentos 27(1):53-60.
Roman-gutierrez AD, Guilbert S, Cuq B (2002). Microstructural changes in wheat flour and flour components during hydration by using environmental scanning electron microscopy. Lebensmittel Wissenschaft Technol. (35):730-740.
Rufino MSM, Alves RE, Brito ES, Morais SM, Sampaio CG, Pérez-Jiménez J, Saura-Calixto FD (2007). Metodologia Científica: Determinação da Atividade Antioxidante Total em Frutas pela Captura do Radical Livre DPPH. Comunicado Técnico 127 – EMBRAPA.
Santos ALB, Faleiro FG, Costa AM, Campos AVS (2008). TUPINAMBÁ DD, SILVA KN. Caracterização de macro e micro nutrientes em cascas de frutos de maracujá-suspiro (*Passiflora nitida* KUNTH.). II Simpósio Int. Savanas Trop. Bras.
Siguemoto SE (2013). Composição nutricional e propriedades funcionais do murici *(Byrsonima crassifolia)* e da moringa (*Moringa Oleifera*). [Dissertação de mestrado]. São Paulo: Faculdade de Saúde Pública. Universidade de São Paulo.
Silva DB, Silva JA, Junqueira NTV, Andrade LRM (2001). Frutas do cerrado. Bras. Embrapa Inf. Tecnol. 179 p.
Silva SR, Mercadante AZ (2002). Composição de carotenóides de maracujá-amarelo (*Passiflora edulis flavicarpa*) in natura. Ciênc. Tecnol. Aliment., Campinas 22(3):254-258.
Sivieri K, Oliveira MN (2002). Avaliação da vida-de-prateleira de bebidas lácteas preparadas com "fat replacers" (*Litesse e Dairy-lo*). Ciênc. Tecnol. Alimentos, 22(1):24-31.
Sodini I, Lucas A, Oliveira MN, Remeuf F, Corrieu G (2002). Effect of milk base and starter culture on acidification, texture, and probiotic cell counts in fermented milk processing. J. Dairy Sci. 85(10):2479-2488.
Souza ACG, Sandi D (2001). Industrialização. In: *Maracujá:* tecnologia de produção, pós-colheita, agroindústria, mercado. Porto Alegre: Cinco continentes, pp. 305-343.
Souza G (1990). Fatores de qualidade do iogurte. Colet. Inst. Tecnol. Alim., Campinas 21(1):20-27.
Souza MWS, Ferreira TBO, Vieira IFR (2008). Composição centesimal e propriedades funcionais tecnológicas da farinha da casca do maracujá. Alimentos e Nutrição, Araraquara 19(1):33-36.
Thamer KG, Penna ALB (2006). Caracterização de bebidas lácteas funcionais fermentadas por probióticos e acrescidas de prebiótico. Ciênc. Tecnol. Alimentos 26(3):589-595.
Toledo NMV (2013). Aproveitamento de subprodutos da industrialização do maracujá para elaboração de iogurte. 2013. 129f. Dissertação (Mestrado) – Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba.
Tomazoni T, Siviero J (2009). Consumo de potássio de idosos hipertensos participantes do Programa Hiperdia do município de Caxias do Sul, RS. Rev. Bras. Hipertensão 16(4):246-250.
Vieira LM, Sousa MSB, Mancini-Filho J, Lima A (2011). Fenólicos totais e capacidade antioxidante in vitro de polpas de frutos tropicais. Rev. Bras. Frutic. 33(3):888-897.
Vilhalva DAA, Junior MSS, Moura CMA, Caliari M, Souza TAC, Silva FA (2011). Aproveitamento da farinha de casca de mandioca na elaboração de pão de forma. Rev. Inst. Adolfo Lutz 70(4):514-521.
Zeraik ML (2010). Estudo analítico dos flavonóides dos frutos do maracujá (*Passiflora edulis* Sims f. *flavicarpa* Degener). Tese (Doutorado em Ciências, área Química Analítica) Universidade de São Paulo, São Carlos.

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Full Length Research Paper

Decomposition rate of crambe phytomass on Haplortox under different soil management practices

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The aim of this study was to assess the decomposition rate of crambe straw under different soil management practices based on no-tillage system. The experiment was carried out at the experimental area of the Agronomic Institute of Paraná - IAPAR, regional center of Santa Tereza do Oeste, Paraná, Brazil. Treatments consisted of four soil management practices: Traditional No-tillage System - TNTS – Chisel Plowing Tillage System - CPTS – No-tillage System with Application of Gypsum - NTSG - and Quality No-tillage System - QNTS. The treatments were distributed in randomized order in parts subdivided on time with fifteen macro-plots of 20 m x 25 m. The assessments took place on 0 (zero), 7, 15, 30, 60, 90, and 120 days after crambe crop harvest. Decomposition was determined quantitatively through the analysis of the decomposition rate of crop residues using litter bags. Average loss of crambe phytomass was significantly different ($p>0.05$), mainly for CPTS and NTSG, around 0. 63% day^{-1} and 0. 71% day^{-1}, respectively. The longest half-life period (66 days) was observed on QNTS with bristle oat and white lupin consortium, and the shortest half-life period (45 days) was observed on CPTS.

Key words: *Crambe abyssinica*, litter bags, half-life period.

INTRODUCTION

The alterations on physical, chemical and biological properties of a soil that is intensively used for agricultural purposes can, and normally will, result in negative impacts on the natural balance of its ecosystem. The consequence is disequilibrium, which affects the decomposition of organic matter and nutrient cycling in the soil. In addition to the chosen method of soil management, the type of crop residue also has great influence on the rate of decomposition of organic matter. For Teixeira et al. (2009), the leguminous plants have a higher rate of decomposition compared cultures of grasses. Among the crops, crambe has great advantages

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such as low production cost, ruggedness, easy adaptability to low soil fertility and high tolerance to drought, does not require new machinery and equipment for cultivation (Neves et al., 2007). The crambe culture can still be an option for the "safrinha" after the summer crop harvest, to be more suited to the autumn and winter seasons. It is a precocious culture, with full cycle varying from 80 to 100 days, it depends on variety, sowing time and climate conditions (Onorevoli, 2012).

The degree of impact on the soil ecosystem is closely related to management practices adopted in agricultural production. Therefore, choosing the proper method for preparing the soil is a major step in maintaining and structuring its ecosystem. There are innumerous studies on different types of soil that point to the organic matter as the essential element for soil conservation. For instance, organic matter improves different factors such as soil aggregation, physical attributes, permeability and porosity. In addition, it increases cationic exchange, water retention, nutrient cycling, fertility, and total organic carbon stocks in the soil (Kong et al., 2005; Amado et al., 2006; Bayer et al., 2006; Calegari et al., 2008; Sá and Lal, 2009; Chioderoli, et al., 2010). For structuring the soil, it is highly important that soil organic matter (SOM) is conserved (Calegari et al., 2006). For better understanding of the process of conservation of soil quality, the knowledge of the dynamics of decomposition of crop residue is fundamental (Kliemannet al., 2006). SOM protection must be grounded on a proper set of processes that favors its preservation and its contribution to the soil. Among those processes, cultivation method is the one that should receive greater attention.

From that perspective, choosing quality agricultural management and practices involves adopting the usage of cover crops. When the objective is to conserve the agro ecosystem soil through decomposition and cycling of nutrients of crop phytomass, the adoption of crop rotation with crops that produce large amounts of biomass, above 6 Mg ha^{-1} dry mass, is recommended (Nunes et al., 2006).

In order to carry out management practices that contribute to the maximum protection of the soil, to carbon accumulation and use of recycled nutrients by the succession of cover crops, outlining crop systems involves selecting the best succession of cover crops. The effectiveness of the process of preparing the soil is also related to the knowledge of the process of decomposition and cycling of nutrients from crop residue (Esther et al., 2013). Early studies with crambe culture (Crambe abyssinica Hoechst) were performed in the first decades of the twentieth century.

The motivation of the production of crambe was its high concentration of erucic acid. The fatty acid can be used for the production of a wide range of products and byproducts, including lubricants, insulating paints, resins, surfactants (Gonzales and Cihacek, 1991). Thus, numerous studies began to be developed, however, in the 1960s, the oil crambe weakened front to products and petroleum by-products that have become economic again.

Currently, the new perspective, especially environmental, for new energy sources such as biofuels, the crambe back to be back to be studied due to its large oil production potential with good commercial characteristics, especially in Brazil. Among the oleaginous plants the crambe shown as a non-food annual crops most promising composing the commercial crops framework in the agricultural sector. This research was design on the premise that different species of crop present different rates of decomposition according to soil management practices and contribute in distinct ways to the increase of carbon in the soil and quality of SOM. The aim is to understand the dynamics of *crambe abyssinica* phytomass decomposition, as well as the influence of the management system by determining dry phytomass of this crop, and by doing so, providing data that may be valuable to farmers, technicians and other professionals when making decision on the best management practice to be taken into account.

MATERIALS AND METHODS

Experiment placement and description of environment

This study is part of a long duration experiment in soil management systems. The experiment was carried out in the Experimental area of the Agronomic Institute of Paraná - IAPAR, regional center of Santa Tereza do Oeste, Paraná, Brazil, coordinates 25º08' (S) latitude and 53º58' (W) longitude, average altitude 750 m above sea level. The climate characteristic of the region according to Köppen classification is Mesothermal-Humid Subtropical, Cfa, with averages superior to 22°C in the hottest month and inferior to 18°C in the coldest month. The region has no dry season defined, hot summers and occasional frost. Rainfall is abundant and well distributed along the year, annual average between 1,800 to 2,000 mm. Relative humidity around 75 to 80% (IAPAR, 2000). The landscape of the experimental area has a slightly undulated relief with average declivity of 3 to 8%. The soil is classified as dystropherric red Latossol (LVdf) of clayey texture (Donagema et al., 2011).

Background of the area

The assessment took place in experimental area in the institute formerly used for agricultural practices under non-tillage system for at least 18 years. The last liming record was of 3.0 Mg ha^{-1} of dolomitic limestone applied on the area in 2011. Table 1 shows the chemical analysis for soil fertility for each experimental stand prior to the installation of the experiment. Samples were collected in triplicates in layers of 0.00 to 0.20 m. Analysis based on methodology proposed by Pava et al. (1992).

Climatological review

For local climatological review during the period of experiment (March/2015 to December/2015), medium, maximum and minimal temperature and precipitation were measured on a daily basis. A

Table 1. Result of chemical analysis for each experimental stand in the beginning of the assessment for the present study on layers from 0.00 to 0.20 m.

Experimental stand (treatment)	pH	C	P	K	Ca	Mg	Al	H+Al	*S	*T	*V	*Al
		g dm^{-3}	mg dm^{-3}	----------------			cmol$_c$ dm^{-3}	----------------				%
1 (QNTS-1)	4.68	25.26	20.80	0.38	4.72	2.30	0.74	9.46	7.40	16.86	43.80	16.52
2 (QNTS-2)	4.70	21.29	12.61	0.41	4.04	2.18	0.40	8.27	6.63	14.89	43.30	9.90
3 (CPTS)	4.77	21.30	12.99	0.38	4.29	1.96	0.12	7.69	6.63	14.33	44.15	2.90
4 (QNTS-3)	4.50	19.28	14.97	0.30	2.83	1.43	0.33	8.86	4.55	13.62	32.44	7.40
5 (QNTS-4)	4.94	18.96	3.60	0.26	4.15	1.89	0.13	6.35	6.30	12.65	48.11	4.15
6 (NTSG)	4.63	18.05	8.95	0.27	3.20	1.53	0.31	7.26	4.99	12.54	37.74	10.00
7 (QNTS-5)	4.54	26.75	19.09	0.34	4.27	2.39	0.44	9.99	7.00	16.99	40.80	8.05
8 (SPDQ-6)	4.57	22.40	10.43	0.29	3.56	1.62	0.46	9.21	5.48	14.68	35.88	13.48
9 (TNTS)	4.27	22.01	11.53	0.22	2.40	1.02	0.83	21.47	3.64	14.27	24.62	24.60

C – Organic carbon - P – Available phosphorus - K – Exchangeable potassium - Ca – Calcium exchangeable - Mg – Magnesium exchangeable - Al – Exchangeable aluminum H+Al - Potential acidity - S* - Base sum - T* - Cations exchange capacity - V* - Base saturation - Al* - Aluminum saturation.

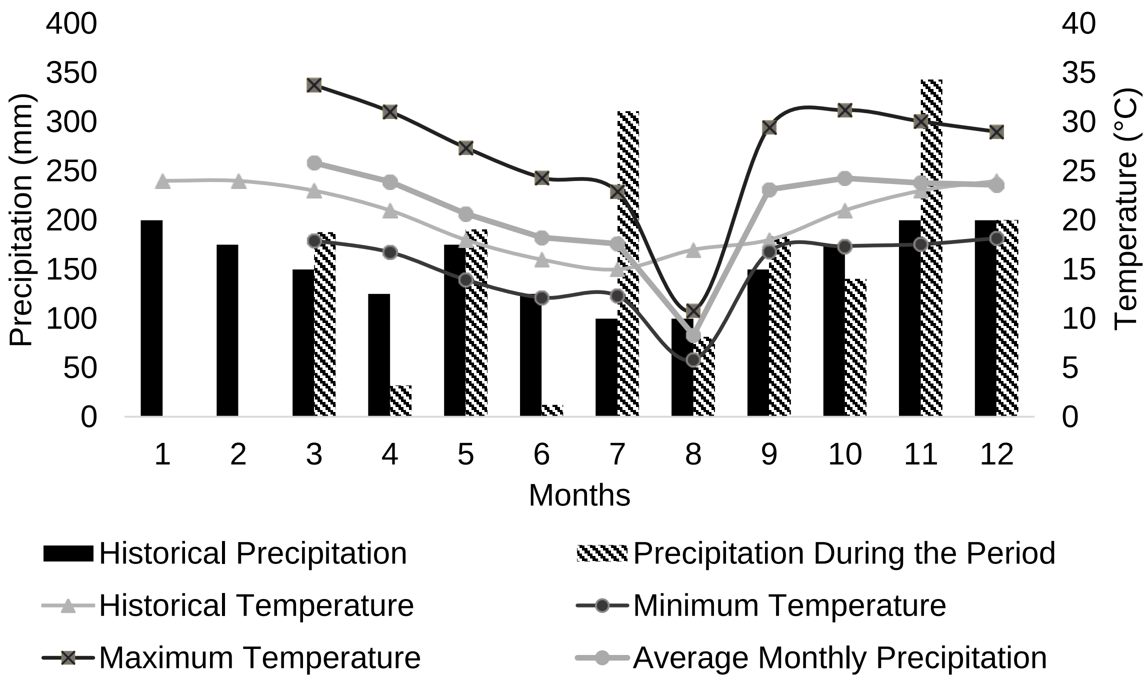

Figure 1. Average monthly precipitation, temperature for the period of 26 years (historical average) of Santa Tereza do Oeste – PR. Source: IAPAR 2000. Maximum, minimum and average temperature from March/2015 to December/2015.

dry bulb thermometer was used for recording temperature and a field pluviometer for precipitation. The results were compared to the historical data of the region according to IAPAR weather charts (2000). Figure 1 shows historical data for average precipitation and temperature in Santa Tereza do Oeste – PR for a period of 26 years (1972-1998), and climate data, including duration of research, average monthly precipitation, maximum, minimum and average temperature for the experimental period. The results of precipitation in the region on July, November and December exceed the averages on the historical data. According to Technical Bulletin of Paraná Meteorological System, for the year 2015, the pluvial average for November exceeded historical precipitation for the last ten years, reaching the 343 mm accumulated in that season (SIMEPAR, 2015). In addition, average temperature throughout the experiment was also above historical data.

Experimental design and description of treatments

The experiment was set in completely randomized design, composed of four soil management practices: Traditional No-tillage System - TNTS – Chisel Plowing Tillage System - CPTS – No-tillage System with Application of Gypsum - NTSG - and Quality No-tillage System - QNTS. Treatments were distributed in parts subdivided on time with nine macro-plots of 20 m X 25 m, among which six compose the QNTS treatment. The six treatments QNTS are composed of winter cover crops during off-season of commercial crops. Namely: QNTS-1 – with common oat (*Avena sativa L.*); QNTS-2 – with bristle oat (*Avena strigosa Schieb*); QNTS-3 – with rye IPR89 (*Secale cereale* L.); QNTS-4 – with bristle oat (*Avena strigosa Schieb*) and radish (*Raphanus sativus* L.) consortium; QNTS-5 – with bristle oat (*Avena strigosa Schieb*) and white lupin (*Lupinus albus*) consortium; and QNTS-6 – with bristle oat (*Avena strigosa Schieb*) and field pea (*Pisum sativum subsp. Arvense L*.) consortium. For TNTS treatment and witness treatment, soil management practice was the same as the adopted by local farmers, who use fallow system farming after summer season. For the CPTS, land was plowed at a depth of 0.30 m. For NTSG treatment, 3.0Mg ha^{-1} agricultural gypsum was applied a month following the beginning of the experiment.

Before setting the experiment, soybean had been cultivated at the location. After succession of crops and treatments management was as follows: a) QNTS treatments: winter cover crops/ soy/ crambe; b) CPTS, NTSG and TNTS: fallow/ soy/ crambe. Winter cover crop management, rolling and desiccation processes occurred at flowering stage. For commercial crops, however, the analytical determination occured only after a full cycle. Crambe seeds were sown on March 31, 2015, and analysis of phytomass production occurred at flowering stage on 15 July, 2015. The crop was harvested on 17 August, 2015, and the analysis for the present study began at the same day.

Evaluation and analytical determination

Decomposition was determined quantitatively through the analysis of decomposition rate of crop residue in seven periods, with five repetitions per period during decomposition. Four samples in 1 m^2 were collected for each treatment, totaling 4 m^2 per treatment. After collecting the material, it was sent to a laboratory where it was washed in running water, then in distilled water, dried in forced air stove at 65°C until reaching constant weight. The material was then weighed to determine organic matter. The decomposition of crambe crop residue was assessed through method proposed by Thomas and Asakawa (1993) using litterbags of 2 mm mesh and opening of 0.15 m x 0.15 m. Thirty bags containing the respective crop residue from each portion were distributed for each treatment. The quantity of crop residue in grams was proportional to the biomass of the dry matter produced in the treatment stand. The litterbags were randomly disposed on the soil surface of each experimental stand and collected in periods of 0 (zero), 7, 15, 30, 60, 90 and 120 day from the harvest of crambe.

The samples were sent to the laboratory where the exceeding dirtiness (soil and other vegetation that were not relevant for the analysis) was removed. Later, the remaining phytomass in the litterbags was dried. Analytical analysis was carried out for each period quantifying the remaining dry biomass of the species.

In order to describe crop residue decomposition rate, the exponential model $X = X_0 e^{-kt}$, decribed by Wieder and Lang (1982), adjusted by Thomas and Asakawa (1993) was applied. Where X is the amount of dry mass (kg ha^{-1}) in time (days) t; X_0 is the fraction of dry mass potentially decomposable, and *k* is the constant of decomposition of the residue (g g^{-1} day^{-1}). According to the model, it is possible to do the regression analysis of the residue decomposition as well as determine the constant of decomposition (*k*) applying napierian logarithm (in) in: $k = \ln (X / Xo) / t$. With the value obtained from k, the half-life period (T(½)) of the dry mass and the nutrients in the remaining crop residue is calculated, that is, the necessary time for 50% of dry mass to be decomposed and nutrients released. To calculate the decomposed biomass half-life, the mathematical formula proposed by Paul and Clark (1989) was used, where $T(½) = \ln(2)/k$. Where T (½) is the half-life time for the decomposition of the biomass or liberation of the nutrients and *k* is the constant of decomposition of biomass.

Statistical analysis

The results of the present study were submitted to analysis of variance (ANOVA), applying the F test to identify the differences between the averages of treatments. For significant results, averages were compared through the test of Turkey at 5% probability ($p < 0.05$). Regression analysis related to decomposition of dry mass was carried out based on the formulations by Thomas and Asakawa (1993).

RESULTS AND DISCUSSION

Dry phytomass production and dynamics of crambe crop residue

Table 2 presents the values of the production of crambe dry mass, as well as its decomposition rate. There is statistical difference between the treatments studied for the production of dry phytomass. The highest amount was observed in treatment TNTS, with 3.716.46 kg ha^{-1}, this is the management practice commonly adopted by local farmers. Following are treatments QNTS-6, with average production estimated in 3.657.45 kg ha^{-1} using bristle oat and field pea consortium, and QNTS-4, estimated in 3.635,16 kg ha^{-1} using bristle oat as a cover crop. The treatments with lower amounts were treatments QNTS-1 and CPTS, with production estimated in 2.763.29 kg ha^{-1} and 2.831.15 kg ha^{-1}, respectively.

The production of dry matter in all the experimental stands was superior to what has been observed in the literature. Pitol et al. (2010), Heinz et al. (2011) and Mauad et al. (2013) showed values of dried phytomass of 1.742 kg ha^{-1}, 2.688 kg ha^{-1} and 2.837 kg ha^{-1}, respectively. The difference between the studies may be attributed to climate conditions, soil type, species, chemical condition of the experimental unit, seeding season, and stage and type of management practice. Decomposition rates of crambe crop residue varied according to treatment used. This result is possibly associated with the amount of residue generated, the topographic localization of the experimental stand, and management practice adopted. The highest decomposition rate is observed in treatment CPTS, with 0.71% day^{-1}. The same treatment however showed one of the lowest averages of mass loss per day because of initial low phytomass content, 20.18 kg ha^{-1} day^{-1}. With reduced volume of straw, daily loss was low when compared, for example, to treatment QNTS-6, which reduced its phytomass to approximately 25.51 kg ha^{-1} day^{-1} but reached a rate of 0.70 % day^{-1}. Similar to treatment CPTS, mass loss in treatment QNTS-2 was approximately

Table 2. Total production of dry phytomass of crambe crop; decomposed phytomass and remainings after 120 days, and decomposition rate.

Treatment	Phytomass			Decomposition rate	
	Produced†	Decomposed (%)	Remaining	$kg\ ha^{-1}\ day^{-1}$	$\%\ day^{-1}$†
	---------------------- $kg\ ha^{-1}$	----------------------			
QNTS-1	2.763.29$^{c\ (\pm272)}$	2.155.92 (78)	607.36^{ab}	17.97^{e}	0.65$^{\pm0,01}$
QNTS-2	3.635.16$^{a\ (\pm351)}$	3.039.84 (84)	595.33^{ab}	25.33^{b}	0.70$^{\pm0,01}$
QNTS-3	3.517.26$^{a\ (\pm85)}$	2.862.78 (81)	654.48^{ab}	23.86^{b}	0.68$^{\pm0,02}$
QNTS-4	3.497.59$^{a\ (\pm409)}$	2.837.23 (81)	660.36^{ab}	23.64^{b}	0.68$^{\pm0,01}$
QNTS-5	3.300.42$^{abc\ (\pm340)}$	2.606.90 (79)	596.06^{ab}	20.72^{d}	0.66$^{\pm0,01}$
QNTS-6	3.657.45$^{a\ (\pm370)}$	3.061.39 (84)	596.06^{ab}	25.51^{a}	0.70$^{\pm0,00}$
TNTS	3.719.46$^{a\ (\pm180)}$	2.902.28 (78)	817.19^{a}	24.19^{b}	0.65$^{\pm0,02}$
CPTS	2.831.15$^{bc\ (\pm80)}$	2.422.16 (86)	408.99^{b}	20.18^{d}	0.71$^{\pm0,01}$
NTSG	3.446,89$^{ab\ (\pm172)}$	2.588.77 (75)	858.12^{a}	21.57^{c}	0.63$^{\pm0,02}$

† - Average standard deviation (±). Averages of treatments followed by the same lowercase letter show no significant difference on Turkey test at 5% of probability.

25.33 kg ha^{-1} day^{-1}, representing a decomposition rate of 0.70 % day^{-1} of the dry mass produced. By the end of the 120 days of experiment, treatment CPTS decomposed 86% of the dry mass produced. This treatment presented the highest percentage rate of decomposition. What may explain the low productivity and the accelerated process of degradation of straw as a cover crop is that in the present treatment chisel plowing management system was adopted. As a result, crop residue is in direct contact with the soil due to low protection and to the fact that the soil had already decomposed the organic matter contained in it. Hence, the material in it is more susceptible to microbial attack even more exposed to decomposition agents.

Lowest decomposition rates were observed in treatments NTSG, QNTS-1 and TNTS, respectively. Besides management practice adopted, the succession of crops may possibly have contributed in different ways to the local soil ecosystem, reducing or increasing the decomposition rate of the succeeding crops. For treatment NTSG, mass loss was approximately of 21.57 kg ha^{-1} day^{-1}, which represents a decomposition rate close to 0.63% dia^{-1}. After 120 days, treatment NTSG reduced its initial mass of cover straw in approximately 75%. For treatments QNTS-1 and TNTS, with common oat and fallow, mass loss was of 17.97 and 24.19 kg ha^{-1} day^{-1}, respectively. Those were the lowest losses observed during the period studied. Decomposition rate for treatments QNTS-1 and TNTS were of 0.65% day^{-1}, decomposing by the end of the process 78% of the initial volume produced (Table 2).

The results from the present research support those of Mielniczuk and Martin-Neto (2000), who observed in their study the influence of management practices on organic matter decomposition rate. They noticed that decomposition rate on soils not revolved mechanically was lower (0.029 ano^{-1}). The usage of single crops (*Pisum sativum* subesp. arvense) show faster decomposition (0.0752 dia^{-1}) due to the chemical composition of the residues and of the relation C/N (Doneda et al., 2012). In addition, the organic matter decomposition reaction is modified according to the barriers and protection of the SOM (Marín et al., 2011). They contribute to exemplify the high rates of decomposition due to higher reactive oxidation by macro and micro biota of the soil for treatment CPTS in opposition to treatment QNTS-6, for example. It is worth pointing that for the period of study there were atypical climatological events. With the high amount of rainfall, the material was left at constant humidity possibly favoring the high rates of decomposition. The results also show that different management practices, specially the use of chisel plowing, have a direct impact on the duration of soil protection.

Half-life of crambe crop residue

Table 3 shows the coefficient of equation proposed by Wieder and Lang (1982), adjusted by Thomas and Asakawa (1339), as well as the coefficient of determination of exponential regression and the half-life of the crop residue. The kinetics of crop residue decomposition showed initially a decaying behavior followed by a more constant phase and a slight decay at the end (T120). Half-life of the material (T (½)) started reducing again by the end of November and beginning of December due to high precipitation. The high humidity content on the soil promoted decomposition and consequent reduction of T (½). Differences for T (½) were highly observed on treatments CPTS and QNTS-5. CPTS, with soil revolving, resulted in reduction of approximately 20 days in T (½). As has been noted, soils that undergo management practices that include revolving tend to decompose faster crop residue (Bayer et al.,

Table 3. Coefficient of regression of crop residue decomposition $X = X_0 \exp^{(-kt)}$ (Wieder e Lang, 1982), coefficient of determination (R^2) and half-life of the material T (½) = 0,693/k of crambe crop residue.

Treatment		QNTS-1	QNTS-2	QNTS-3	QNTS-4	QNTS-5	QNTS-6	TNTS	CPTS	NTSG
X_0		2.190.40	2.920.10	2.954.10	2.752.50	2.920.80	3.128.70	3.275.10	2.309.40	3.026.70
R^2		0.93	0.94	0.98	0.94	0.97	0.97	0.98	0.96	0.97
T7	*k*	0.042^{a}	0.039^{abc}	0.035^{abc}	0.042^{a}	0.030^{c}	0.039^{abc}	0.030^{c}	0.042^{ab}	0.032^{bc}
	T(½)	16	18	20	17	23	18	23	17	22
T15	*k*	0.035^{a}	0.032^{abc}	0.031^{abc}	0.034^{ab}	0.026^{c}	0.034^{ab}	0.028^{abc}	0.030^{abc}	0.027^{bc}
	T(½)	20	21	23	20	27	21	25	23	25
T30	*k*	0.021^{ab}	0.020^{ab}	0.020^{b}	0.022^{ab}	0.017^{b}	0.022^{ab}	0.018^{b}	0.028^{a}	0.018^{b}
	T(½)	33	34	34	31	41	32	38	25	40
T60	*k*	0.017^{ab}	0.018^{a}	0.016^{ab}	0.017^{a}	0.012^{c}	0.018^{a}	0.014^{bc}	0.018^{a}	0.014^{bc}
	T(½)	42	38	43	40	58	38	51	38	51
T90	*k*	0.012^{cde}	0.013^{bcde}	0.014^{abc}	0.013^{bcd}	0.011^{e}	0.014^{ab}	0.013^{bcde}	0.015^{a}	0.011^{de}
	T(½)	59	55	50	53	66	49	54	45	62
T120	*k*	0.013^{cde}	0.013^{cd}	0.014^{bc}	0.014^{bc}	0.011^{e}	0.015^{ab}	0.013^{cde}	0.016^{a}	0.012^{de}
	T(½)	55	52	49	50	62	46	55	43	60

Lowercase letters in the lines do not show significant difference by Turkey test at 5% probability. T7 – 7 days after the beginning of the experiment; T15 – 15 days after; T30 – 30 days after; T60 – 60 days after; T90 – 90 days after; T120 – 120 days after; *k* – coefficient of decomposition (g $g^{-1}day^{-1}$); T(½) – half-life period (day); X_0 – initial decomposable mass (kg ha^{-1}).

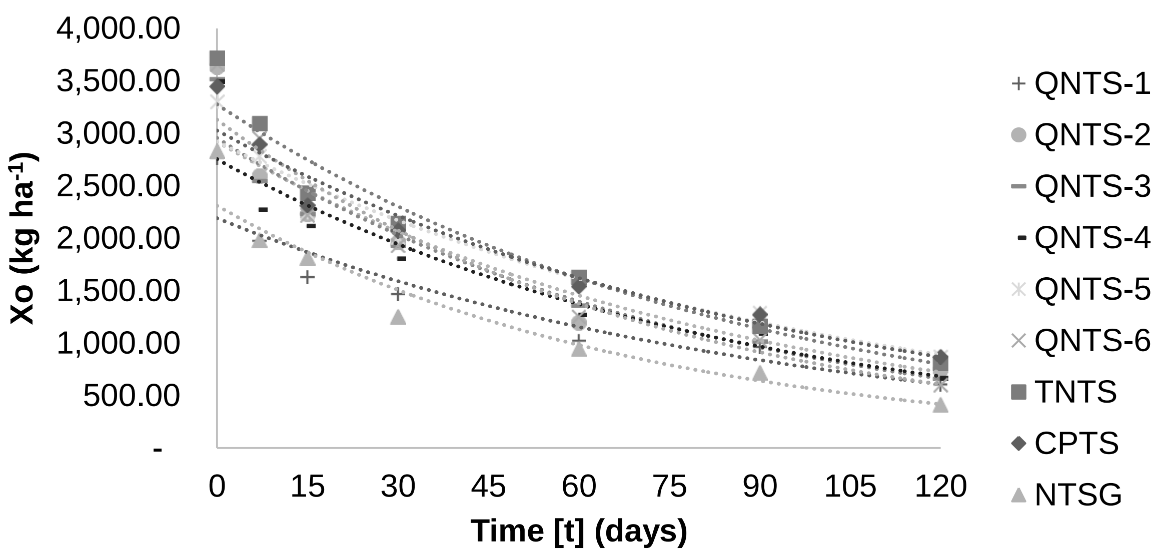

Figure 2. Exponential tendencies of crambe crop residue decomposition after 120 days of experiment.

2000). When management system is more intense with mechanical revolving, the soil foster higher rates of oxidation, and that is one negative result for it which reduces the amount of organic matter in the soil (Barros et al., 2015).

After 60 days of experiment, the values of decomposition reached approximately 51 to 67%. The highest rates of decomposition were seen on the first 15 days, when about 37% of the residue had already been decomposed. The low amount of cover straw disposed on the field little protected the soil. Thus, the exposure of the residue to the decomposition agent was higher, what consequently favored the rapid decomposition of crambe crop residue. In essence, soil microbiologic activity, and consequently, decomposition, suffers great influence from the type of crop or management practice applied to the soil (Meena et al., 2014). Figure 2 shows tendency lines for each treatment from the 120th day after implementing the experiment. The residue tends to decompose faster soon after crop residue management.

Data show that mass loss tends to balance after 45 to 60 days after the implementation of decomposition tests. Such an occurrence can be explained by the strong influence of carbon and nitrogen ratio (C/N) of the species. At the start of the decomposition nutrients potassium, phosphorus, nitrogen, calcium and magnesium are released rapidly, leaving a higher concentration of carbon, which causes the material to become recalcitrant and therefore pass to resist longer decomposing the remaining waste. According to the researchers Rheinheimer et al. (1998), the C / N ratio, the chemical composition of plant residues also changes the decomposition process. Santos et al. (2009) also affirm in their studies that the higher the C/N ratio, cellulose content, hemicellulose, lignin and polyphenol is a slower decomposition of biomass.

Conclusion

Different cover crop species contribute with different decomposition rates according to management practice adopted for the soil, especially soil revolving practice, which leads to accelerated decomposition. The dynamics of crambe crop decomposition presented different results according to treatment management. Decomposition rates of crop residue remained in the range of 0.63% day^{-1} to 0.71% day^{-1} of decomposable mass, and T(½) suffered a reduction of about 20 days with CPTS. Decomposition rate was also affected by the amount of residue generated by the crop. The highest the amount, the highest the decomposition rate of the residue.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Amado TJC, Bayer C, Conceição PC, Spagnollo E, De Campos BHC, Da Veiga M (2006). Potential of carbon accumulation in no-till soils with intensive use and cover crops in southern Brazil. J. Environ. Qual. 35(4):1599-1607.

Barros JDS, Chaves LG, Pereira WE (2015). Carbon and nitrogen stocks under different management systems in the Paraiban Serto. African J. Agric. Res. 10(3):130-136.

Bayer C, Martin-Neto L, Mielniczuk J, Pavinato A, Dieckow J (2006). Carbon sequestration in two Brazilian Cerrado soils under no-till. Soil Till. Res. 86(2):237-245.

Bayer C, Mielniczuk J, Martin-Neto L (2000). Efeito de sistemas de preparo e de cultura na dinâmica da matéria orgânica e na mitigação das emissões de CO2. Rev. Bras. Ciênc. Solo 24(3):599-607.

Calegari A, de Castro Filho C, Tavares Filho J, Ralisch R, de Fátima Guimarães M (2006). Melhoria da agregação do solo através do sistema plantio direto. Semina: Ciênc. Agrár. 27(2):147-158.

Calegari A, Hargrove WL, Rheinheimer DDS, Ralisch R, Tessier D, de Tourdonnet S, de Fatima Guimarães M (2008). Impact of long-term no-tillage and cropping system management on soil organic carbon in an Oxisol: a model for sustainability. Agron. J. 100(4):1013-1019.

Chioderoli CA, de Mello LM, Grigolli PJ, Silva JODR, Cesarin AL (2010). Consorciação de braquiárias com milho outonal em plantio direto sob pivô central. Engenharia Agríc. pp. 1101-1109.

Donagema GK, Campos DD, Calderano SB, Teixeira WG, Viana JHM (2011). Manual de métodos de análise de solos. rev. Rio de Janeiro: Embrapa Solos 230.

Doneda A, Aita C, Giacomini SJ, Miola ECC, Giacomini DA, Schirmann J, Gonzatto R (2012). Fitomassa e decomposição de resíduos de plantas de cobertura puras e consorciadas. Rev. Bras. Ciênc. Solo 36(6):1714-1723.

Esther OJ, Hong TX, Hui GC (2013). Influence of straw degrading microbial compound on wheat straw decomposition and soil biological properties. Afr. J. Microbiol. Res. 7(28):3597-3604.

Gonzales PB, Cihacek LJ (1991). Crambe fertilization: a literature review. North Dakota. Farm Res. 49:17-19.

Heinz R, Garbiate MV, Neto ALV, Mota LDS, Correia AMP, Vitorino ACT (2011). Decomposição e liberação de nutrientes de resíduos culturais de crambe e nabo forrageiro. Ciênc. Rural 41(9):1549-1555.

Kliemann HJ, Braz AJPB, Silveira PM (2007). Taxas de decomposição de resíduos de espécies de cobertura em Latossolo Vermelho distroférrico. Pesqu. Agropec. Trop. 36(1):21-28.

Kong AY, Six J, Bryant DC, Denison RF, Van Kessel C (2005). The relationship between carbon input, aggregation, and soil organic carbon stabilization in sustainable cropping systems. Soil Sci. Soc. Am. J. 69(4):1078-1085.

Marín SMA, Spínola AG, Cano AF, Ortíz RS, López ÁL, Mendoza DG (2011). Decomposition rates of plant residues under different land uses. Afr. J. Agric. Res. 6(12):2856-2860.

Mauad M, Garcia RA, Vitorino ACT, Silva RMMF, Garbiate MV, Coelho LCF (2013). Matéria seca e acúmulo de macronutrientes na parte aérea das plantas de Crambe. Ciênc. Rural 43(5):771-778.

Meena VS, Maurya BR, Meena RS, Meena SK, Singh NP, Malik VK, Kumar V, Jat LK (2014). Microbial dynamics as influenced by concentrate manure and inorganic fertilizer in alluvium soil of Varanasi, India. Afr. J. Microbiol. Res. 8(3):257-263.

Neves MB, Trzeciak MB, Vinholes OS, Tillmann CAC, Villela FA (2007). Qualidade fisiológica em cultura de crambe produzidas em Mato Grosso do Sul. EMBRAPA. Disponível em: <http://www.cpact.embrapa.br/publicacoes/download/ livro/Agroenergia_2007/Agroener/trabalhos/Outras%20culturas.../Neves_1.pdf>.

Nunes UR, Andrade Júnior VC, SILVA EDB, Santos NF, Costa HAO, Ferreira CA (2006). Produção de palhada de plantas de cobertura e rendimento do feijão em plantio direto. Pesqu. Agropec. Bras. 41(6):943-948.

Onorevoli B (2012). Estudo do *Crambe abyssinica* como fonte de matérias primas oleaginosas: óleo vegetal, ésteres metílicos e bio-óleo. 132 f. Dissertação (Mestrado em Ciência de Materiais), Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul.

Pavan MA, Fatima BM, Costa ZH (1992). Manual de análise química de solo e controle de qualidade. Londrina: IAPAR.

Pitol C, Broch DL, Roscoe R (2010). Tecnologia e produção: Crambe 2010. Maracaju, Fundação MS. 60p.

Rheinheimer DS, Kaminski J, Lupatini GC, Santos EJS (1998). Modificações em atributos químicos de solo arenoso sob sistema de plantio direto. Rev. Bras. Ciênc. Solo Campinas 22:713-723.

Sa JCM, Lal R (2009). Stratification ratio of soil organic matter pools as an indicator of carbon sequestration in a tillage chronosequence on a Brazilian Oxisol. Soil Till. Res. 103(1):46-56.

Santos HP, Fontaneli RS, Spera ST, Tomm GO (2009). Efeito de sistemas de produção integração lavoura-pecuária (ILP) sobre a fertilidade do solo. Acta Scient. Agron. 31(4):719-727.

SIMEPAR (Sistema Meteorológico do Paraná) (2015). SETI - Secretaria de Ciência, Tecnologia e Ensino Superior do Estado do Paraná. Análise climática para o período 18 a 30 de novembro de 2015 para o Estado do Paraná. (2015). Disponível em: <www.simepar.br>. Acesso em: 15 jan. 2016.

Teixeira CM, Carvalho GJ, Andrade MJB, Silva CA, Pereira JM (2009). Decomposição e liberação de nutrientes das palhadas de milheto e milheto + crotalária no plantio direto do feijoeiro. Acta Scient. 31(4):647-653.

Thomas RJ, Asakawa NM (1993). Decomposition of leaf litter from

tropical forage grasses and legumes. Soil Biol. Biochem. 25(10):1351-1361.
Wieder RK, Lang GE (1982). A critique of the analytical methods used in examining decomposition data obtained from litter bags. Ecology 63(6):1636-1642.

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Genetic variability and correlation analysis of rice (*Oryza sativa* L.) inbred lines based on agro-morphological traits

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In order to evaluate genetic variability of agro-morphological traits and also determine the correlation between grain yield with its components in rice lines, 17 recombinants inbred lines, their parents and a check variety were grown in research station of Africa rice center in Benin republic during two consecutive years 2013 and 2014. The experiments were laid out in a randomized complete block design with four replications. Phenotypic coefficients of variance were higher than genotypic coefficients of variance in all the characters across the two years. High heritability in broad sense (H^2) estimates were obtained for biomass (68.77%), date of 50% flowering (98.11%), plant height (81.94%), leaf area (82.90%), number of panicles (64.40%), leaf dry weight (72.91%), root weight (67.43%) and yield/plant (62.23%) suggesting that the traits were primarily under genetic control. A joint consideration of broad sense heritability (H^2) and genetic advance as per cent mean expected (GAM) revealed that leaves dries weight and roots weight combined high heritability and high GAM. Furthermore, high (H^2) and high GAM recorded in these characters could be explained by additive gene action. However, high estimates (H^2) combined with moderate GAM recorded for biomass, day to 50% flowering, leaf area, number of panicle and yield/plant could be due to non-additive gene effect. Grain yield/plant recorded positive and significant correlation with stem weight (r=0.5262) and biomass (r=0.9291). This result indicates that selection based on these two characters will be highly effective for yield improvement in rice.

Key words: Agro-morphological traits, correlation, genetic variability, heritability, rice.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. It is a staple food crop for more than half of the world's human population. Rice grain contains 75 to 80% starch, 12% water and 7% protein (Oko et al., 2012; Hossain et al., 2015). Minerals like calcium, magnesium and phosphorus are present along with some traces of iron, copper, zinc and manganese. In addition, rice is a good source of niacin, thiamine and riboflavin (Yousaf, 1992; Oko et al., 2012).

Rice is grown in 117 countries across all habitable continents covering a total area of about 163 mha with a global production of about 740 mt and an average yield of about 4,539 kg / ha (FAOSTAT, 2014). The Asian continent ranks first with over 90.1% of world production, followed by the American continent (5.1%), African continent (4.2%), Europe (0.5%) and Oceania (0.1%). The major producing countries are China (206.5 million tons), India (157.2 million tons), Indonesia (70.8 million tons), Bangladesh (52.2 million tons) and Viet Nam with 44.9 million tons (FAOSTAT, 2014). In Africa, rice is grown and consumed in more than 40 countries. Its production has increased significantly from year 2000 to 2013. More than 20 million farmers in Africa are engaged on rice production in these countries and about 100 million people are dependent on it directly for their livelihood (Nwanze et al., 2006). For example in Burkina Faso, rice production has grown from 103,087 tons on year 2000 to 305,382 tons on 2013. It is no longer a luxury food but has become the cereal that constitutes a major source of calories for almost urban and rural people of Africa (Seck et al., 2013).

In a report published by United Nations the world population is going to cross the 8 billion mark by 2030 and 9.6 billion by the year 2050 and rice production must be increased by 50% in order to meet the growing demand. This demand in Sub-Saharan Africa is expected to grown substantially as the population is currently growing at the rate of 3 to 4% per annum (Ogunbayo et al., 2014). To meet the future demand resulting from population growth, development of news high yielding rice genotypes is therefore a necessity. Thus, to meet this demand and attend rice self-sufficiency, plant breeders have to develop high yielding cultivars with desirable agronomic traits for diverse ecosystems.

The development of new genotypes requires some knowledge about the genetic variability presents in the germplasm of the crop to build efficient breeding programme. The knowledge about genetic variability can help to know if these variations are heritable or non-heritable. The magnitude of variation due to heritable component is very important because, it would be a guide for selection of parents for crop improvement (Dutta et al., 2013). Therefore, selection for high yield requires knowledge about genetic variability and good understanding of correlation between yield and yield components regarding to the genetic material that is on hand. Genetic variability for agronomic traits is the key component of breeding programme for broadening the gene pool of rice (Dutta et al., 2013).

Heritability estimates provide authentic information about a particular genetic attribute which will be transmitted to the successive generations and constitute an efficient guide for breeders in the choice of parents for crop improvement programmes (Rafi and Nath, 2004). However, heritability in broad sense alone may not be helpful for selection based on phenotype, because it's influenced by environment. Thus, estimate heritability along with genetic advance conjointly are reliable helpful in predicting the gain under selection than heritability alone (Ogunbayo et al., 2014). Moosavi et al. (2015) reported that grain yield is a complex trait, quantitative in nature and a combined function of a number of constituent traits. Consequently, selection for yield may not be satisfying without taking into consideration yield component traits. Thus, positives correlated between yield and yield components are requires for effective yield component breeding increasing grain yield in rice (Ogunbayo et al., 2014). So, it is important for plant breeders to understand the degree of correlation between yield and its components.

Therefore, the objective of the present study was to assess and evaluate genetic variability of rice recombinant inbred lines based on agro-morphological traits and analyse the relationships between these traits.

MATERIALS AND METHODS

The experiment was conducted in the greenhouse of Africa rice center research station in Cotonou (Benin republic) from March to July 2013 and from February to June 2014. The site is located between 6°25.415N and 2°19.684E at an altitude of 21 m above sea level. Experiments were conducted in greenhouse using a randomized complete block design with four replications. Individual plant of each genotype was grown in 2 L pots containing natural field soil. Management practices such as irrigation and fertilization were performed by following the standard procedures (IRRI, 2002).

A total of 20 genotypes consisting of 17 F_5 inbred lines from the *indica*'s cross included their two parents and the *indica* variety APO were used in this study. The inbred lines were obtained from the *indica*'s cross IR64 X B6144F-MR-6-0-0.The parent, B6144F-MR-6-0-0, a drought resistant landrace was crossed with the variety IR64, a variety which possesses many agronomical superior traits. The variety APO, a popular variety known for its long term adaptation in drought prone ecosystem was used as a control.

Observations were recorded on one plant of each pot. Thus, morphological and agronomical data were collected for 11 quantitative characters at appropriate growth stage of rice plant following the standard evaluation system indicated by IRRI (IRRI, 2002). The characters that were evaluated included days to 50% flowering (50%DF, day), plant height (PH, cm), leaf area (LA, cm^2), number of tillers (NT), number of panicles (NP), number of fertile spikelets (NFS), leaf dry weight (LDW, g), roots weight (RW, g), panicles weight (PW, g), stem weight (SW, g), 1000 grains weight (1000GW, g), biomass (biom, g) and grain yield per plant (yield/plant, g/plant).

The data recorded on 12 morphological and agronomical traits from the genotypes used, were subjected to statistical analysis. Analysis of variance (ANOVA) was carried out to access the genotype effect and their interaction using R program package. The correlation analysis was performed using the same software to

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Table 1. Mean square of combined analysis of variance for all the characters studies of 20 rice genotypes.

Source	df	Biom	D50F	PH	LA	NT	NP	NFS	LDW	RW	PW	SW	1000GW	Yield/plant
Rep	3	31.6	4.126	49.06	95.51	1.22	48.35	75.88	8.32	15.47	3.26	20.73	9.68	24.95
Geno	19	89.1**	337.24**	66.37**	2992.44**	6.20**	11.66**	234.93**	4.72**	31.20**	1.03**	8.89**	11.35**	80.94**
Year	1	278.44**	120.16**	1863.36**	14.5ns	46.71**	30.11**	1956.69**	27.23**	38.43**	3.48**	1.52ns	47.91**	222.34**
G x Y	19	6ns	2.23ns	4.80ns	333.7**	0.22ns	0.20ns	53.25ns	0.49ns	2.59ns	0.21ns	2.40ns	6.21**	14.44ns
Error	117	10.58	1.4	2.923	71.56	1.26	2.04	65.6	0.36	3.74	0.33	2.38	2.57	8.64

1000 GW: 1000 grains weight; D50F: day to 50% flowering; LA: leaf area; LDW: leaf dry weight; NP: number of panicle; NFS: number of fertile spikelets; NT: number of tiller; PH: plant height; PW: panicle weight; RW: root weight; SW: stem weight; Yield/p: yield per plant; biom: biomass; ns: no significant; * : significant at 5% level probability; **: significant at 1% level probability.

determine the degree of correlation between yield and its components. In order to assess and quantify the genetic variability among the genotypes for the characters under study the variance components and values of heritability and genetic advance were estimated following the formula given by Burton and De Vane (1953); and Johnson et al. (1955) and applied by Tuhina-Khatun et al. (2015).

Phenotypic and genotypic variances were estimated using the following formula:

$$Vg = (MS_g – MS_{gxy}) / ry$$

$$Vgxy = (MS_{gxy} - MS_e) / r$$

$$Ve = MS_e$$

$$Vp = Vg + Vgxy / y + Ve / ry$$

Where, Vg = genotypic variance; Vgxy = genotype x year variance; Ve = environment variance; Vp= phenotypic variance; MS_g = mean square of genotypes, MS_{gxy}= mean square of genotype x year; MS_e= mean square of error, r = number of replications and y = number of year.

Both genotypic and phenotypic coefficients of variability were estimated using the formula below:

$$GCV = \frac{\sqrt{Vg}}{X} 100$$

$$PCV = \frac{\sqrt{Vp}}{X} 100$$

Where GCV = genotypic coefficient of variability; PCV = Phenotypic coefficient of variability; $\sqrt{Vg}$ = genotypic standard deviation; $\sqrt{Vp}$ = phenotypic standard deviation and X= general mean of the character.

Heritability in broad sense (H^2) was computed as the ratio of genetic (Vg) variance to the total phenotypic variance (Vp).

$$H^2 = \frac{Vg}{Vp} 100$$

The genetic advance (GA) and genetic advance as per cent of mean (GAM) were estimate using the formula given below:

$$GA = H^2 k\sqrt{Vp}$$

$$GAM = \frac{GA}{X} 100$$

Where H^2= heritability in broad sense; k = Selection differential which is equal to 2.06 at 5% intensity of selection; X= general mean of the character.

RESULTS

Analysis of variance

The results of combined analysis of variance for the all characters are shown in Table 1. Significant effects of genotype were observed for all the characters under study. High significant effect of year for all the characters except the leaf area (LA) and stem weight (SW) were shown from this analysis. The genotype x year was highly significant only for the characters leaf area (LA) and 1000 grain weight (1000 GW). Environmental conditions were not similar in two years, meaning that climate changes were observed during the study suggesting that the differences among genotypes were not stable across years.

Estimate of genetic parameters

Estimates of genotypic (Vg) and phenotypic variances (Vp), genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), broad sense heritability (H^2), genetic advance (GA) and genetic advance as percentage of the mean (GAM) are shown in Table 2.

High genotypic and phenotypic variances were recorded with leaf area, 332.34 and 400.89, respectively. High genotypic and phenotypic variances were equally observed for 50% flowering day (41.88 and 42.68) and the number of fertile spikelets 22.71 and 53.97, respectively. The low values of genotypic and phenotypic variances were observed with the characters panicle weight (0.10 and 0.26), stem weight (0.81 and 2.01) leaf dries weight (0.53 and 0.73), number

Table 2. Genotypic (Vp) and phenotypic variance (Vp), genotypic coefficient (GCV) and phenotypic coefficient of variance (PCV), broad sense heritability (H^2) , genetic advance (GA) and genetic advance as per cent of mean (GAM) for all the traits.

Characters	Min	Means	Max	Vg	Vp	GCV	PCV	H2 (%)	GA	GAM
Biomass (g)	39.79	50.34	62.85	10.39	15.11	6.40	7.72	68.77	5.51	10.94
Day to 50% flowering	81.60	92.67	118.30	41.88	42.68	6.98	7.05	98.11	13.20	14.25
Plant height (cm)	79.00	87.24	100.30	7.70	9.39	3.18	3.51	81.94	5.17	5.93
Leaf area (cm^2)	175.20	213.60	264.70	332.34	400.89	8.53	9.37	82.90	34.19	16.01
Number of tillers	10.00	13.43	16.89	0.75	1.25	6.44	8.33	59.79	1.38	10.26
Number of panicles	6.50	10.29	15.07	1.43	2.23	11.63	14.50	64.40	1.98	19.23
Number of fertile spikelets	8.81	86.39	101.30	22.71	53.97	5.52	8.50	42.08	6.37	7.37
Leaf dry weight (g)	3.02	5.09	9.35	0.53	0.73	14.31	16.75	72.91	1.28	25.16
Roots weight (g)	3.05	9.44	16.86	3.58	5.30	20.03	24.39	67.43	3.20	33.87
Panicle weight (g)	1.17	2.17	8.73	0.10	0.26	14.78	23.30	40.25	0.42	19.32
Stem weight (g)	3.48	8.00	13.78	0.81	2.01	11.27	17.71	40.49	1.18	14.77
1000 grains weight (g)	12.85	23.49	29.28	0.64	2.39	3.41	6.58	26.95	0.86	3.65
Yield/plant (g/plant)	22.79	36.04	45.79	8.31	13.36	8.00	10.14	62.23	4.69	13.00

number of tiller (0.75 and 1.25) and 1000 grains weight (0.64 and 2.39), respectively. In general, the phenotypic variances were higher than genotypic variances for all the characters.

Genotypic coefficients of variance (GCV) were ranged from 3.18% for plant height to 20.03% for root weight, whereas phenotypic coefficients of variance (PCV) were ranged from 3.51% for plant height to 24.39% for root weight. According to Sivasubramanian and Menon (1973) PCV and GCV values more than 20% are regarded as high, whereas values less than 10% are considered to be low and values between 10 and 20% to be moderate. Based on this delineation, GCV and PCV values were low for biomass, day to 50% flowering, plant height and 1000 grains weight; medium for number of panicle, leaf dry weight and stem weight; high for root weight. The panicle weight and plant height were recorded with low GCV and moderate PCV. In addition, PCV values were higher than theirs corresponding GCV values for all the characters considered. However, this difference was low for all the characters except the panicle weight and stem weight.

Heritability analyses estimate the relative contributions of differences in genetic and non-genetic factors to the total phenotypic variance in a population. It is an important concept in quantitative genetics, particularly in selective breeding. The heritability in broad sense (H^2) estimate varied from 26.95% to 98.11%, respectively for 1000 grains weight and day to 50% flowering. All the characters studies had high heritability (>60%) except the numbers of fertile spikelets, panicle weight, stem weight and 1000 grains weight. This result indicates that these characters could be easily improved by selection.

Genetic advance (GA) under selection refers to the improvement of characters in genotypic value for the new population compared with the base population under one cycle of selection at a given selection intensity (Wolie et al., 2013). The high value of GA was recorded with leaves area (34.19) and the low (0.42) with panicle weight. Estimates of GA for yield/plant was 4.69 g/plant indicating that whenever we select the best, 5% high yielding genotypes as parents, average grain yield/plant of progenies could be improved by 4.69 g/plant.

Genetic advance as per cent mean expected (GAM) had a general range between 3.65% for 1000 grains weight and 33.87% for roots weight. Among the characters high values of GAM (>20%) were recorded only for roots weight and leaf dry weight (25.16%). It was moderate (10 to 20%) for biomass, day to 50% flowering, leaf area, number of tillers, number of panicle, panicle weight, stem weight and yield/plant; low (<10%) for plant height, number of fertile spikelets and 1000 grains weight. Leaf dry weight and roots weight had high heritability and high GAM, whereas biomass, day to 50% flowering, leaf area, number of panicle and yield/plant had high heritability but moderate GAM. Panicle weight and stem weight had both moderates heritability and GAM.

Correlation

The degree of correlation between the traits is important in plant breeding. It can be used as tool for indirect selection. Correlation studies help the plant breeder during selection and provide the understanding of yield components. The results of correlation analysis showed in Table 3 reveals that there was positive and highly significant correlation between day to 50% flowering (50%DF) with leaf dry weight (r=0.6223), number of panicle (r=0.7091) and stem weight (r=0.5566). The characters which had positive relationship with grain yield/ plant were day to 50% flowering (r=0.3997), leaf area (r = 0.0382), leaf dry weight (r=0.101), number of

Table 3. Correlation coefficients among agronomical and morphological characters in twenty recombinants inbreed rice lines.

Correlation	100GW	D50F	LA	LDW	NP	NFS	NT	PH	PW	RW	SW	Yield/p
100GW	-											
D50F	-0.0842^{ns}	-										
LA	0.119^{ns}	-0.3095^{ns}	-									
LDW	0.2171^{ns}	0.6223**	-0.08^{ns}	-								
NP	-0.2698^{ns}	0.7091**	-0.5265*	0.4235^{ns}	-							
NFS	0.3861^{ns}	-0.2657^{ns}	-0.1247^{ns}	0.1743^{ns}	-0.1863^{ns}	-						
NT	-0.0061^{ns}	0.4241^{ns}	-0.0837^{ns}	0.1964^{ns}	0.5345*	-0.3501^{ns}	-					
PH	0.0526^{ns}	0.4329^{ns}	-0.1598^{ns}	0.0653^{ns}	0.2572^{ns}	-0.4627*	0.1598^{ns}	-				
PW	-0.207^{ns}	0.3297^{ns}	-0.4161^{ns}	0.039^{ns}	0.5134*	-0.3552^{ns}	0.1554^{ns}	0.2856^{ns}	-			
RW	0.1602^{ns}	-0.0833^{ns}	0.0456^{ns}	0.1133^{ns}	0.0952^{ns}	-0.0023^{ns}	0.0906^{ns}	-0.064^{ns}	0.2736^{ns}	-		
SW	0.037^{ns}	0.5566*	0.0116^{ns}	0.4164^{ns}	0.2989^{ns}	-0.3386^{ns}	0.1536^{ns}	0.255^{ns}	0.1883^{ns}	0.1161^{ns}	-	
Yield/p	-0.0241^{ns}	0.3997^{ns}	0.0382^{ns}	0.101^{ns}	0.1286^{ns}	-0.3007^{ns}	-0.1425^{ns}	0.2794^{ns}	0.112^{ns}	-0.1489^{ns}	0.5262*	-
Biom	-0.0366^{ns}	0.3658^{ns}	0.1106^{ns}	0.0596^{ns}	0.1783^{ns}	-0.3813^{ns}	-0.1343^{ns}	0.2902^{ns}	0.1895^{ns}	-0.0332^{ns}	0.5556*	0.9291**

1000GW: 1000 grains weight; D50F: day to 50% flowering; LA: leaf area; LDW: leaf dry weight; NP: number of panicle; NFS: number of fertile spikelets; NT: number of tiller; PH: plant height; PW: panicle weight; RW: root weight; SW: stem weight; Yield/p: yield per plant; biom: biomass; ns: no significant; * : significant at 5% level probability; **: significant at 1% level probability.

panicle (0.1286), plant height (r=0.2794), panicle weight (r=0.112), stem weight (r=0.5262) and biomass (r=0.9291). Among these characters only stem weight and biomass showed significant positive correlation with grain yield per plant. On the contrary 1000 grains weight (r=-0.024), number of fertile spikelets (r=-0.3007), number of tiller (r=-0.1425) and root weight (r=-0.1489) were inversely but no significant correlated with grain yield per plant. Plant height had positive correlation with all the characters except leaves area, number of fertile spikelets and root weight. In the same time, number of fertile spikelets had negative correlation with almost the characters except 1000 grains weight and leaf dry weight. Positive and significant correlation were shown for biomass with root weigh (r=0.5556) and for number of panicle with number of tillers and panicle weight (r=0.5345 and r=0.5134, respectively). However, plant height and number of panicle showed significant negative correlation with number of fertile spikelets (r=-0.4627) and leaf (r=0.5265), respectively.

DISCUSSION

Genetic diversity in breeding is very important. It is the key of crop improvement. More variability is observed in the basic population more is the chance of improvement. In the present study, results from ANOVA showed highly significant difference among the genotypes for all the characters, indicating huge genetic variability existing among the genotypes. So, the parents used for crossing were genetically different. Significant year effects were observed for all the characters except leaf area and stem weight, meaning that climate change were observed during the study. Environmental conditions were not similar during the two years. The results are in accordance to those found by Ogunbayo et al., (2014). All the characters, except leaf area and 1000 grains weight, exhibited stability across the seasons since the significance of genotype × year interaction was not detected and the differences among genotypes were clear. This appears to show that further improvement through selection for these characters may be effective. On the other hand, the significant effect of genotype x year interaction for leaf area and 1000 grains weight, indicating that genotypic difference in these characters was greatly influenced by the environment.

The current study suggests that phenotypic variance (Vp) and phenotypic coefficient variance (PCV) were higher than theirs corresponding genotypic variance (Vg) and genotypic coefficient of variance (GCV) respectively for all the characters studies, indicating that the expression of these characters was influenced by environment. Similar results were reported by Dutta et al., (2013); Singh et al., (2014) and Tuhina-Khatun et al., (2015) in rice. It is interesting to note that this difference was low for all the characters except panicle weight and stem weight, indicating that these characters were less influenced by environment. It also suggests that selection based on these characters would be effective for future crossing. Similar result was also found by Prajapati et al*.,* (2011) and Singh et al., (2014) for these traits. However the other traits like panicle weight and stem weight which showed a higher difference between PCV and GCV indicated that environmental effect on the expression of those traits is higher.

The most important function of the heritability in the genetic study of quantitative characters is its predictive role to indicate the reliability of the phenotypic value as a guide to breeding value (Falconer and Mackay, 1996; Al-Tabbal et al., 2012). High heritability estimates for yield/plant, plant height, day to 50% flowering, biomass and number of tillers, indicated a high response to selection in these traits. Similar results were also reported by Al-Tabbal et al., 2012; Dutta et al., 2013; Rafii et al., 2014, which support the present findings. Heritability in broad sense and the genetic advance are also important selection parameters. It is more useful as a selection tool when considered jointly with heritability. The estimates of genetic advance can help to understand the type of gene action of various polygenic characters. Johnson et al. (1955) suggested that high heritability estimates along with the high genetic advance is more helpful in predicting gain under selection than heritability estimates alone. Thus, the heritability estimates will be reliable if accompanied by high genetic advance. The present study revealed high heritability accompanied with high genetic advance as percent of the mean for leaf dry weight and root weight; high heritability and moderate genetic advance as percent of the mean for biomass, day to 50% flowering, leaf area, number of panicle and yield/plant. These results could be explained by additive gene action and their selection may be done in early generations. Similar findings have been reported by Wolie et al. (2013), Ogunbayo et al. (2014) on rice and Reza et al. (2015) on wheat. However, panicle weight and stem weight had moderate heritability coupled with low to moderate genetic advance as percent of the mean indicates non-additive gene effects; suggesting that these characters could be improved by developing varieties through recurrent selection method (Ogunbayo et al., 2014).

Selection of traits contributing simultaneously to a character will improve it in subsequent segregation population (Nor et al., 2013). Hence, the correlation analysis is therefore necessary to determine the direction of selection and the numbers of characteristics need to be considered in improving any character such as grain yield. The present study showed that there was a highly significant correlation between grain yield per plant with biomass at the 1% level and stem weight at the 5% level indicating that simultaneous selection for these characters would result in improvement of yield. Similar findings were earlier reported by Gulzar and Subhasl (2012), Azarpour (2013) and Moosavi et al. (2015). Also, day to 50% flowering exhibits a significant positive correlation with leaf area, number of panicle and stem weight. The observed positive correlation of date to 50% flowering was supported by earlier researchers such as Zhou et al. (2010) and Khan et al. (2014) for number of panicle. Plant height has not significant correlated with yield per plant. This result is in accordance with those of Golam et al. (2011) and Nor et al. (2013). However, this is in contrast with the previous study of Khan et al. (2014) and Moosavi et al. (2015) that presented the negative correlation between plant height and yield per plant. Positive and significant correlation was shown between plant height and number of fertile spikelets. Similar results were early reported by Aris et al. (2010) and Kohnaki et al. (2013). Azarpour (2013) also reported that plant height in rice had significant and positive correlated with spikelet fertility per plant.

Conclusion

The present study highlighted the existence of diversity among the 17 rice recombinant inbred lines, their parents and the check variety. High heritability in broad sense recorded for biomass, day to 50% flowering, plant height, leaf area, number of panicles, leaf dry weight, roots weight and grain yield per plant demonstrates that these characters could be successfully transferred to offsprings if their selection is performed in hybridization programme. The correlation analysis revealed that 8 agronomical traits such as day to 50% flowering, leaf area, leaf dry weight, number of panicle, plant height, panicle weight, stem weight and biomass have the positive contribution to grain yield. Among these characters, biomass and stem weight showed significant correlation with grain yield. So, these two traits may be considered as the selection criteria for the improvement of grain yield in rice.

Conflict of Interests

The authors have not declared any conflict of interest.

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REFERENCES

Al-Tabbal JA, Al-Fraihat AH (2012). Genetic variation, heritability, phenotypic and genotypic correlation studies for yield and yield components in promising barley genotypes. J. Agric. Sci. 4:3.

Aris H, Bambang K, Supartopo S (2010). Correlation analysis of agronomic characters and grain yield of rice for tidal swamp areas. Indonesian J. Agric. Sci. 11(1):11-15

Azarpour E (2013). Path coefficient analysis for the yield components of rice cultivars in Iran under different nitrogen levels. J.Biodivers. Environ. Sci. (JBES) 3(10):24-30.

Burton G.W, De Van EH, (1953). Estimating heritability in Tall Fescue (*Fescuta arundinacea*) from replicated clonal material. Agron. J. 45:481-487.

Dutta P, Dutta P.N. and Borua P. K (2013). Morphological Traits as Selection Indices in Rice: A Statistical View. Univ. J. Agric. Res. 1(3):85-96.

Falconer DS, Mackay TFC (1996). Introduction to quantitative genetics. 4th Edn.,Benjamin Cummings, England, ISBN-10:0582243025.

FAOSTAT (2014). Statistical data base. Food and Agriculture Organizations of the United Nations. Rome, Italia.

Golam F, Yin YH, Masitah A, Afnierna N, Majid NA, Khalid N, Osman M (2011). Analysis of aroma and yield components of aromatic rice in Malaysian tropical environment. AJCS. 5(11):1318-1325.

Gulzar SS, Subhash KC (2012). Genetic Parameters and Selection Indicesin F3 Progenies of Hill Rice Genotypes. Not. Sci. Biol. 4(4):110-114.

Hossain S, Maksudu HMD, Jamilur RJ (2015). Genetic Variability, Correlation and Path Coefficient Analysis of Morphological Traits in some Extinct Local Aman Rice (Oryza sativa L). J. Rice Res. 3:158.

IRRI (2002). Standard evaluation system for rice. International Rice Research Institute, Manila.

Johnson HW, Robinson HF, Comstock RE (1955). Estimation of genetic and environmental variability in soybeans. Agron. J. 47:314-318.

Khan MSK, Iqbal J, Saeed M (2014). Comparative study of agronomic traits of different rice varieties grown under saline and normal conditions. J. Anim. Plant Sci. 24(2):634-642.

Kohnaki ME, Kiani G, Nematzadeh G (2013). Relationship between morphological traits in rice restorer lines at F3 generation using multivariate analysis. Int. J. Adv. Biol. Biomed. Res. 1(6):2013:572-577

Moosavi M, Ranjbar G, Zarrini HN, Gilani A (2015). Correlation between morphological and physiological traits and path analysis of grain yield in rice genotypes under Khuzestan. Biol. Forum Int. J. 7(1):43-47.

Nor AH, Abdul RH, Mohd RY, Norain MN, NurIzzah J (2013). Correlation analysis on agronomic aharacters in F1 population derived from a cross of PongsuSeribu 2 and MR 264. ScFund MOSTI (06-03-01-SF0110) and RAGS (600RMI/RAGS 5/3).

Nwanze KF, Mohapatra S, Kormawa P, Keya S, Bruce-Olivier S (2006). Rice developmentt in sub-saharanAfrica. J. Sci. Food Agric. 86:675-677;

Ogunbayo SA, Sié M, Ojo DK, Sanni KA, Akinwale MG, Toulou B, Shittu A, Idehen EO, Popoola AR, Daniel IO, Gregoria GB (2014). Genetic variation and heritability of yield and related traits in promising rice genotypes (*Oryza sativa* L.). J. Plant Breed. Crop Sci. 6(11):153-159.

Oko AO, Ubi BE, Efisue AA, Dambaba N (2012). Comparative analysis of the chemical nutrient composition of selected local and newly introduced rice varieties grown in Ebonyi State of Nigeria. Int. J. Agric. For. 2(2):16-23.

Prajapati MK, Singh CM, Suresh BG, Roopa LG, Jadhav P (2011). Genetic parameters for grain yield and its component characters in rice.Electron. J. Plant Breed. 2(2):235-238.

Rafi SA, Nath UK (2004). Variability, Heritability, Genetic advance and relationship of yield and yield contributing characters in dry Bean (*Phaselous vulgaris* L.). J. BioI. Sci. 4(2):157-159.

Rafii MY, Zakiah MZ, Asfaliza R, Iffah HMD, Latif MA, Malek MA (2014). Grain quality performance and heritability estimation in selected f_1 rice genotypes. Sains Malaysiana 43(1):1–7

Reza M, Pour F (2015). Investigation of genetic variation for agronomic traits among the recombinant inbred lines of wheat from the norstar × zagross cross under water stress condition. Int. J. Biol. Biomol. Agric. Food Biotechnol. Eng. 9:9.

Seck PA, Touré AA, Coulibaly JY, Diagne A, Wopereisv MCS (2013). Africa's rice economy before and after 2008 rice crisis. Africa rice center Cotonou, Benin and CABI International UK 480 p.

Singh A, Singh AK, Singh V, Singh N, Singh VN, Shamin M, Vikram P, Singh S (2014). Genetic variability among traits associated with grain yield of rice (*Oryza sativa* L.) exposed to drought at flowering stage. Afr. J. Res. 9(16):1252.

Sivasubramanian S, Menon M (1973). Heterosis and inbreeding depression in rice. Madras Agric. J. 60:1139.

Tuhina-Khatun M, Hanafi MM, Yusop MR, Wong MY, Salleh FM, Ferdous J (2015). Genetic variation, heritability, and diversity analysis of upland rice (*Oryza sativa* L.) genotypes based on quantitative traits. BioMed. Res. Inter. ID 290861.

Wolie A, Dessalegn T, Belete K (2013). Heritability, variance components and genetic advance of some yield and yield related traits in Ethiopian collections of finger millet (*Eleusinecoracana* (L.) Gaertn.) genotypes. Afr. J. Biotechnol. 12(36):5529-5534.

Yousaf M (1992). Study on some Physico-chemical characteristics affecting cooking and eating qualities of some Pakistani Rice Varieties, M.Sc. Thesis Department of Food Technology, University of Agriculture Faisalabad, Pakistan.

Zhou HK, Hayat Y, Fang LJ, Guo RF, He JM, Xu HM (2010). Analysis of genetic and genotype X environment interaction effects for agronomic traits of rice (*Oryza sativa* L.) in salt tolerance. Pak. J. Bot. 42:3239-3246.

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Full Length Research Paper

Inorganic solute accumulation in noni (*Morinda citrifolia* Linn) under salt stress during initial growth

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Noni (*Morinda citrifolia*) is a fruit species which is well adapted to different soil and climate conditions, and may be a good option for farmers in arid and semi-arid regions. To evaluate the tolerance of noni plant in the initial phase to salinity and the accumulation of inorganic solutes in its organs, an experiment was carried out in a greenhouse under hydroponic system. The experimental design was completely randomized, with five levels of NaCl (0, 25, 50, 75 or 100 mM) in the nutrient solution, and four replicates. Plant height, stem diameter, number of leaves, dry matter of leaves, stems and roots, leaf area, leaf succulence, sclerophylly index and the contents of Na^+, Cl^- and K^+ in different plant organs were evaluated after 40 days of stress. Salinity reduced the growth of all plant organs in the initial phase, in contrast, leaf succulence and sclerophylly index increased by 32% at the highest saline level, compared with control plants. The contents of Na^+ and Cl^- in leaves, stems and roots increased, while K^+ contents decreased with the increment in salinity. The highest K^+ contents were observed in the roots and the highest Na^+ and Cl^- contents were observed in the leaves. Salinity induced disturbances in the ionic homeostasis of noni plants, which can explain, at least in part, the salt-induced growth reduction.

Key words: Salinity, sodium chloride, toxic ion.

INTRODUCTION

The cultivation of salt-tolerant species has called the attention of researchers and farmers in order to achieve an economically viable production in salt-affected areas. The saline soils are located mainly in arid and semi-arid regions. In these areas, the soil and climatic conditions and inadequate irrigation management favor the salt accumulation in soil and water. In Brazil, the semi-arid region occupies an area of 980,133.079 km², located

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mainly in the Northeast of the country, with about 53 million inhabitants (IBGE, 2011; Santos et al., 2014). Therefore, the introduction of salt-tolerant plants is essential to maintain the crop production in these areas. The noni plant acclimates to the most diverse environmental conditions, including high levels of salts in the soil (Nelson and Elevitch, 2006). Under favorable conditions, the plant produces fruits nine to twelve months after planting (Chan-Blanco et al., 2006), which turns the noni (*Morinda citrifolia*) as an interesting crop to be introduced in salt-affected areas.

Noni is an exotic plant, belonging to the Rubiaceae family, and a fruit crop with medicinal and nutritional value that has been cultivated in several states of Brazil (Correia, 2010). This plant has been used for thousands of years by the Polynesians in the combat of various diseases. Noni seeds, peel and pulp have significant amounts of carbohydrates, proteins, vitamin C, carotenoids and phenolic compounds, which have antioxidant activity *in vitro* (Costa et al., 2013).

Salinity affects plant development inducing osmotic and ionic stress (Munns and Tester, 2008). However, the plant can develop mechanisms to survive to these conditions. Na^+ and Cl^- are the main ions found in saline soils. These salts, when absorbed and accumulated by plants, can contribute to osmotic adjustment or be toxic; however, the amounts of salts and the capacity of the plant to cope with salt stress will determine the crop production. Therefore, understanding the mechanisms of plant tolerance to salinity is fundamental for the introduction of this crop in salt-affected areas. Thus, the study aimed to evaluate the effect of salinity on the initial growth and accumulation of inorganic solutes in the different parts of noni plants in order to know its tolerance level to salt stress.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse of the Center of Soil and Water Engineering, at the Federal University of Recôncavo of Bahia, in Cruz das Almas-BA, Brazil (12º40'19"S, 39º 6'23"W). The mean values of temperature, relative air humidity and photosynthetically active radiation (at noon) during the experimental period were 25° C, 81% and 1200 μmol m^{-2} s^{-1}, respectively.

Mature noni fruits were selected for collection of seeds, prioritizing the characteristics of plant sanitary conditions and size. After the removal of seeds, dormancy breaking was performed by immersing the seeds for 48 h in water (Leite et al., 2012). After 50 days of sowing, the seedlings with two cotyledonary leaves were transferred to 500-mL plastic cups, containing a mixture of Red-Yellow Latosol and humus (1:1), irrigated daily with well water and weekly with 50 mL of nutrient solution (Furlani, 1998).

Noni seedlings three months old with four pairs of leaves were transferred to containers with 12 L of Furlani nutrient solution, in a floating hydroponic system, where they remained for four days for acclimation. After this period, the seedlings were subjected to five treatments, which consisted in the addition of 0, 25, 50, 75 or 100 mM of NaCl in the nutrient solution. The NaCl was gradually added (25 mM day^{-1}), in order to avoid the osmotic shock. The volume of the solutions was completed daily with water and the renewal was performed weekly. The pH was maintained at 6.0 ± 0.2 by adding NaOH or HCl. The system was maintained under intermittent aeration of 15 min every hour, using an air compressor coupled to a timer.

At the end of the experimental period (40 days), the plants from all treatments were carefully removed from the nutrient solution, the roots were washed with distilled water and the plants were divided into different organs for fresh mass (FM), dry mass (DM), and leaf area (LA) determinations. LA was measured with the WinDIAS image analysis system, model W-C110-PC (Delta-T Devices Ltd, Cambridge, UK). The leaf (LDM), stem (SDM), and root (RDM) dry masses were determined after drying of plant parts in an oven at 65°C, for 72 h. These data were used to calculate the shoot to root dry mass ratio (S/R). The values of leaf fresh mass (LFM), LDM and LA were used to calculate leaf succulence and sclerophylly index (SI), according to the equations proposed by Benincasa (2003):

Succulence = (LFM-LDM)/LA
SI = LDM (mg)/LA (cm^2).

For determination of Na^+, K^+ and Cl^- contents, the extracts were prepared as described by Jones (2001), with minor modifications. 100 mg of leaf, stem or root ground material were mixed with 10 mL of deionized water in test tubes. The tubes were maintained for 1 h at 80°C in water bath, with shaking every 15 min. After this period, the tubes were centrifuged at 5,000 × g for 15 min, at room temperature. The Na^+ and K^+ contents were determined by flame photometry (Faithfull, 2002) and the Cl^- contents by spectrophotometry (Jones, 2001).

The experiment was conducted in a completely randomized design, with five salinity levels (0, 25, 50, 75 or 100 mM of NaCl) and four replicates. The data were subjected to analysis of variance and regression using SISVAR 4.6 statistical software (Ferreira, 2011). The models were selected based on significance level by F test and determination coefficient (R^2).

RESULTS AND DISCUSSION

The increase of NaCl in the nutrient solution linearly decreased the plant height, stem diameter and number of leaves, with reductions of 0.225 cm, 0.041 mm and 0.255, respectively, per mM NaCl in solution (Figure 1). Thus, among biometric variables, the reduction in 100 mM NaCl was higher in the plant height (62%), number of leaves (62%) and lower in the stem diameter (35%), as compared to the control. Similar results were also reported by Souto et al. (2013), in noni plants grown in soil and irrigated with saline water. The reduction in plant height, stem diameter and number of leaves may affect biomass production. Studies on noni have demonstrated that salt-induced changes in growth variables directly affect crop yield (Souto et al., 2015, 2016).

Figure 2 shows that LDM, SDM and TDM presented decreasing linear response, with reductions of 0.146, 0.074 and 0.329 g per mM NaCl, hence reductions of 72, 49 and 68% at the highest NaCl level, respectively. However, for the S/R, succulence and SI, there were increases of 0.0319, 0.0823 mg H_2O cm^{-2} and 0.0202 mg of DM cm^{-2} for each increment in salinity, that is, increases of 117, 32 and 32% at 100 mM of NaCl, in

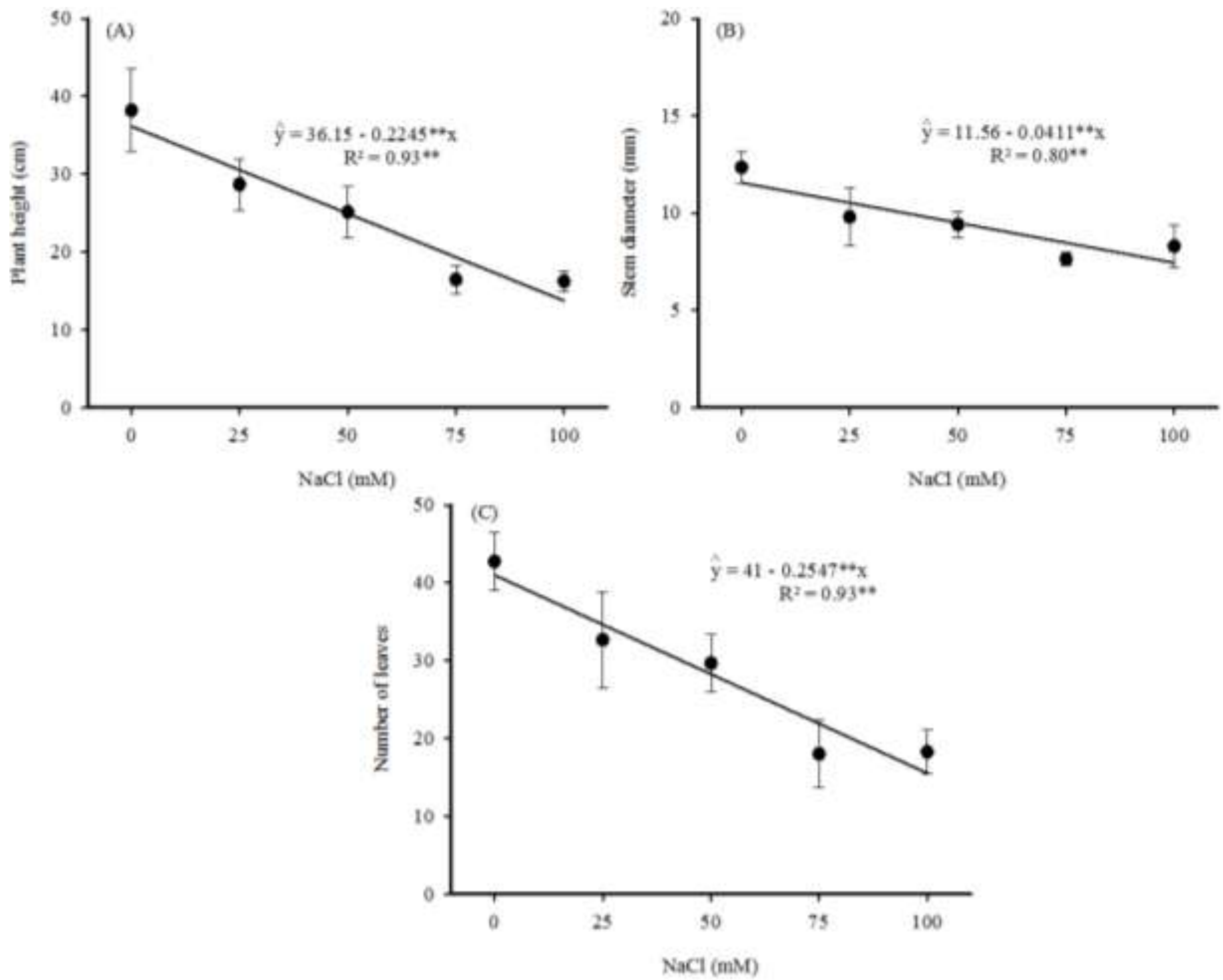

Figure 1. Plant height (A), stem diameter (B) and number of leaves (C) of noni plants 40 days after treatments in hydroponic system as a function of NaCl doses in the nutrient solution.

relation to control treatment.

RDM and LA followed a quadratic polynomial model, with more pronounced reductions (35 and 31%) at 25 mM NaCl in comparison with control, and reductions of 77 and 74%, respectively in 100 mM NaCl. The reduction in noni LA may decrease photosynthesis, absorption of water and nutrients, and consequently, affect growth and biomass production (Souto et al., 2013).

Comparing the different plant organs, the roots showed the greatest reduction in dry mass induced by the saline stress suggesting that the root is the most sensitive organ to the deleterious effects of salinity in noni plants. Rodrigues et al. (2014) observed a reduction of 52% in *Ricinus communis* roots, which was the most affected organ at 150 mM NaCl in the nutrient solution, compared with the control plants. Although Abreu et al. (2008) suggested that roots have greater capacity for osmotic adjustment and better protection against oxidative stress under saline stress conditions, this growth reduction may occur because the root is the first organ directly exposed to the salt stress (Azevedo Neto et al., 2006).

The higher sensitivity of roots to salt stress, in comparison to the shoots, led to a linear increase in the S/R, which varied from 2.72 in the control to 5.91 in the treatment of 100 mM NaCl. According to Marana et al. (2008), in coffee seedlings, a plant of the same family of noni, values between 4.7 and 7.0 are considered as satisfactory for plant development.

Plants, when subjected to abiotic stress, including salinity, can develop mechanisms to survive to environmental conditions, such as changes in leaf succulence and SI. The increment in succulence observed in the present study indicates an increase in the amount of H_2O per unit of LA, which may be important for saving water through transpiration, water storage, and dilution of the toxic ions Na^+ and Cl^-. In turn, increased SI indicates that

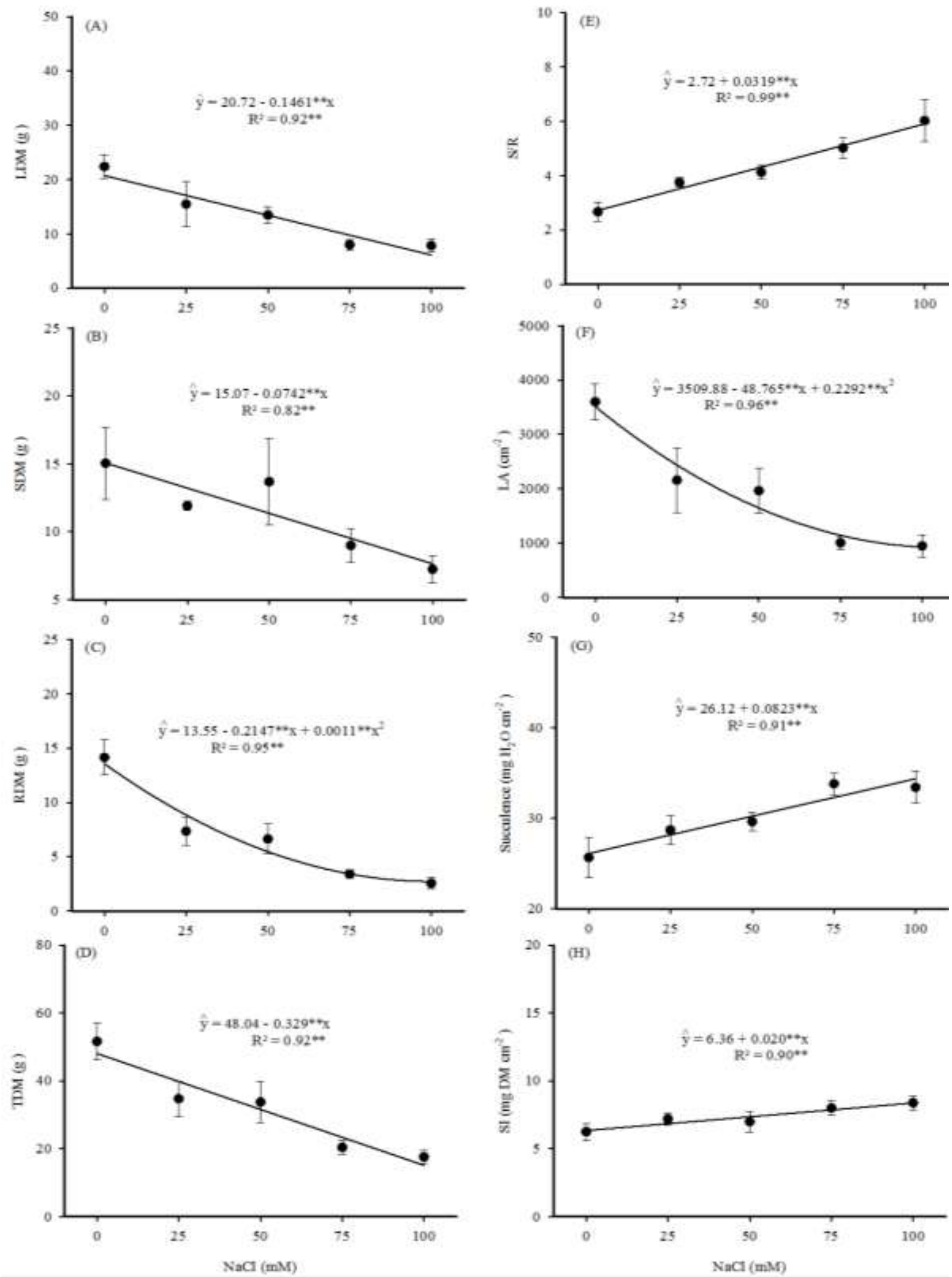

Figure 2. Leaf dry matter - LDM (A), stem dry matter - SDM (B), root dry matter - RDM (C) and total dry matter - TDM (D), shoot/root ratio - S/R (E), leaf area - LA (F), leaf succulence (G) and sclerophylly index - SI (H) in noni plants 40 days after treatments in hydroponic system as a function of NaCl doses in the nutrient solution.

the leaves are thickened, with more strata of photosynthetic tissues, which may be important to reduce the deleterious effects of salinity on plant growth (Oliveira et al., 2012).

Reductions in growth variables of noni irrigated with saline water have recently been reported in the literature (Souto et al., 2013; Souza et al., 2014; Souto et al., 2015). These authors attributed the results to the increase in soil salinity, which affects water absorption, and to the accumulation of toxic ions and reduction of photosynthetic pigments. The accumulation of the toxic ions Na^+ and Cl^- in the different plant organs, the nutritional imbalance and/or the water deficit induced by salinity can affect the plant growth (Munns et al., 2006; Munns and Tester, 2008; Bosco et al., 2009).

The Na^+ and Cl^- contents in leaves, stems and roots of noni plants, as a function of NaCl concentrations in the nutrient solution are shown in Figure 3. It can be observed that the Na^+ and Cl^- contents increased quadratically, except for the Cl^- contents in the roots, which followed a linear model. In relation to the control, the contents of Na^+ and Cl^- in 100 mM of NaCl increased, respectively, 15 and 6 times in the leaves, 31 and 4 times in the stem and 6 and 5 times in the roots. It can also be observed in Figure 3 that, at 100 mM of NaCl, the estimated values for Na^+ and Cl^- were 1.81 and 1.17 mmol g^{-1} DM in the leaves, 0.80 and 0.75 mmol g^{-1} DM in roots, respectively.

The high content of Na^+ in the leaf tissue can affect physiological and biochemical processes, such as stomatal opening, respiration and synthesis of proteins, consequently affecting photosynthesis, growth and yield of the crops (Apse and Blumwald, 2007; Tavakkoli et al., 2010; Furtado et al., 2013). Thus, among the characteristics of salt-tolerant plants, the capacity to restrict the transport and accumulation of toxic ions in the leaves has been considered as the most important (Munns and Tester, 2008).

Chloride in high concentrations can reduce photosynthetic capacity and quantum yield, due to chlorophyll degradation (Tavakkoli et al., 2010). On the other hand, Rodrigues et al. (2014) reported that the accumulation of Na^+ and Cl^- in leaves of *R. communis* played an important role in the osmotic adjustment.

Considering that the highest Na^+ and Cl^- accumulations were observed in the leaves, the results obtained in this study indicate that noni plants have no mechanisms of exclusion of these ions in the organs with smaller metabolic activity, such as stem and roots.

According to Taiz and Zeiger (2013), values above 100 mM of Na^+ and Cl^- become cytotoxic, causing protein denaturation and destabilization of membranes. Considering the data of LFM and LDM, the estimated concentrations of Na^+ and Cl^- in the leaf were, respectively, 188 and 134 mM at the lowest salinity level (25 mM NaCl). These values are similar to those reported by Azevedo Neto and Tabosa (2000) in the leaves of maize plants grown in nutrient solution with 100 mM NaCl. These authors considered that the ionic compartmentalization and cytoplasmic accumulation of compatible solutes was necessary for the reduction of ionic toxicity. Thus, the growth reduction observed in noni can, at least in part, be related to the toxic effects of Na^+ and Cl^- accumulation. However, since the NaCl levels were not lethal and did not cause early senescence of the leaves, the data of the present study also suggest that noni plant utilizes mechanism of Na^+ and Cl^- compartmentalization.

The K^+ content and the K^+/Na^+ ratio in leaves, stems and roots of noni plants as a function of NaCl doses applied in the nutrient solution are shown in Figure 4. The K^+ contents in the plant organs decreased with the increment of NaCl in the nutrient solution and fitted to an exponential regression model in the leaves, quadratic in the stem and linear in the roots, which represented reductions of 65, 59 and 41%, respectively, in 100 mM NaCl.

According to Bosco et al. (2009), high Na^+ concentrations can reduce K^+ absorption by plants. Various factors can affect the absorption of this ion under saline conditions, especially the antagonism between K^+ and Na^+ for absorption sites in the plasmalemma and disorders in the membrane integrity (Apse and Blumwald, 2007). These results corroborate with those of Rodrigues et al. (2014), in castor bean subjected to increasing levels of NaCl in the nutrient solution, leading to a reduction of photosynthesis and dry mass yield and increase of S/R ratio.

The K^+/Na^+ ratio has been considered as an important variable in studies on the plant nutrition under salt stress (Bosco et al., 2009). In the present study, this variable decreased exponentially in all plant organs. Thus, the K^+/Na^+ ratio in leaf, stem and root decreased by 96, 97 and 93%, respectively, at 100 mM NaCl. The abrupt reduction of the K^+/Na^+ ratio in noni plants was due to the increase in Na^+ uptake in conjunction with the decrease in K^+ uptake. Many studies have shown that, under saline conditions, several crops alter the K^+/Na^+ ratio, causing nutritional imbalance, due to the antagonism between these elements (Silva et al., 2008; Bosco et al., 2009). According to Greenway and Munns (1980), the K^+/Na^+ ratio in glycophytes must be higher than 1.0 for the maintenance of ionic homeostasis and an optimal metabolic efficiency. Based on the regression equation of K^+/Na^+ ratio in the leaves, the maximum dose would be equal to 15 mM NaCl for the maintenance of a ratio adequate for the metabolism in noni.

Conclusions

1. Salinity negatively affects biomass production in noni

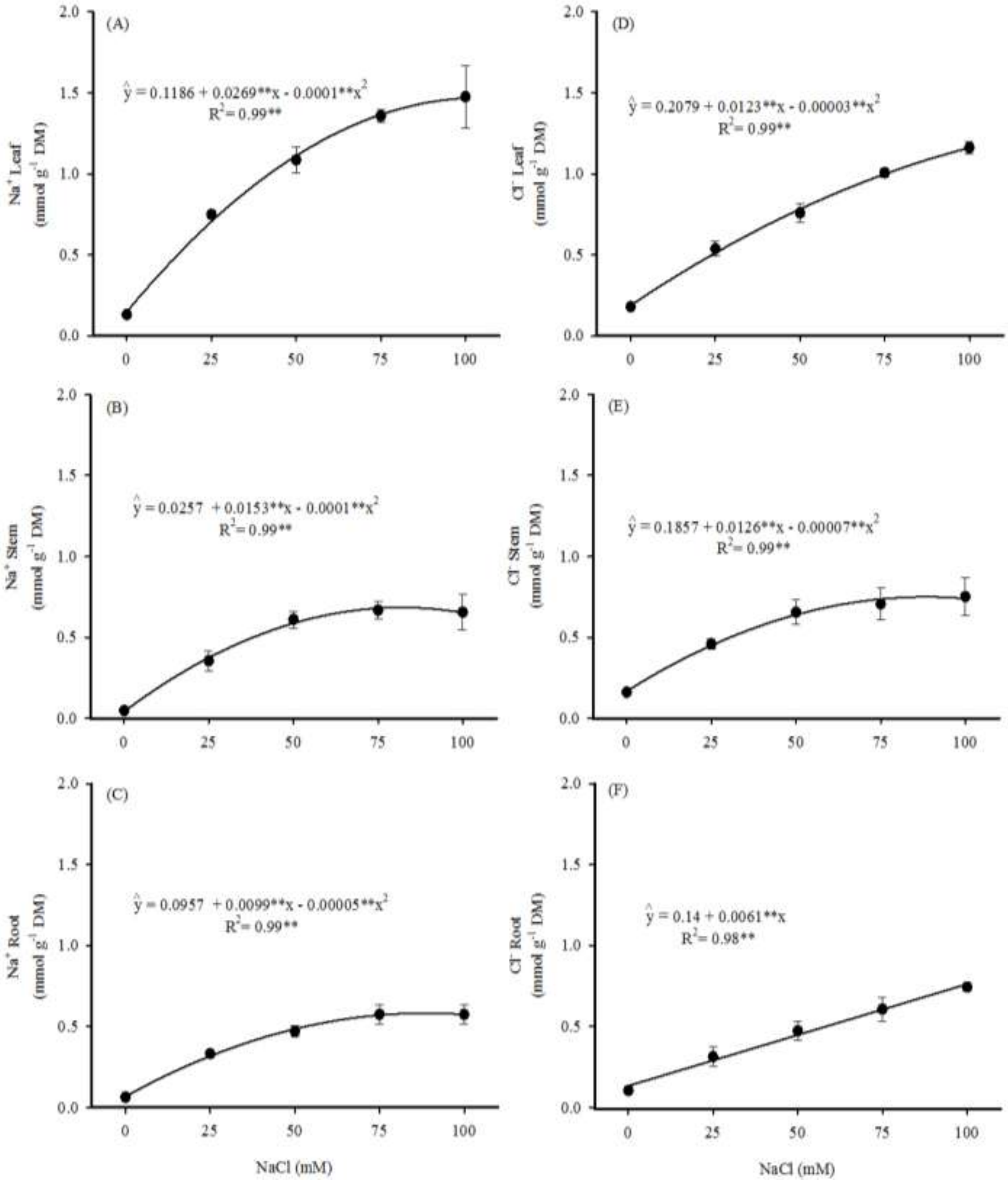

Figure 3. Contents of Na^+ (A, B, C) and Cl^- (D, E, F) in leaves, stem and roots of noni 40 days after treatment in hydroponic system as a function of NaCl doses in the nutrient solution.

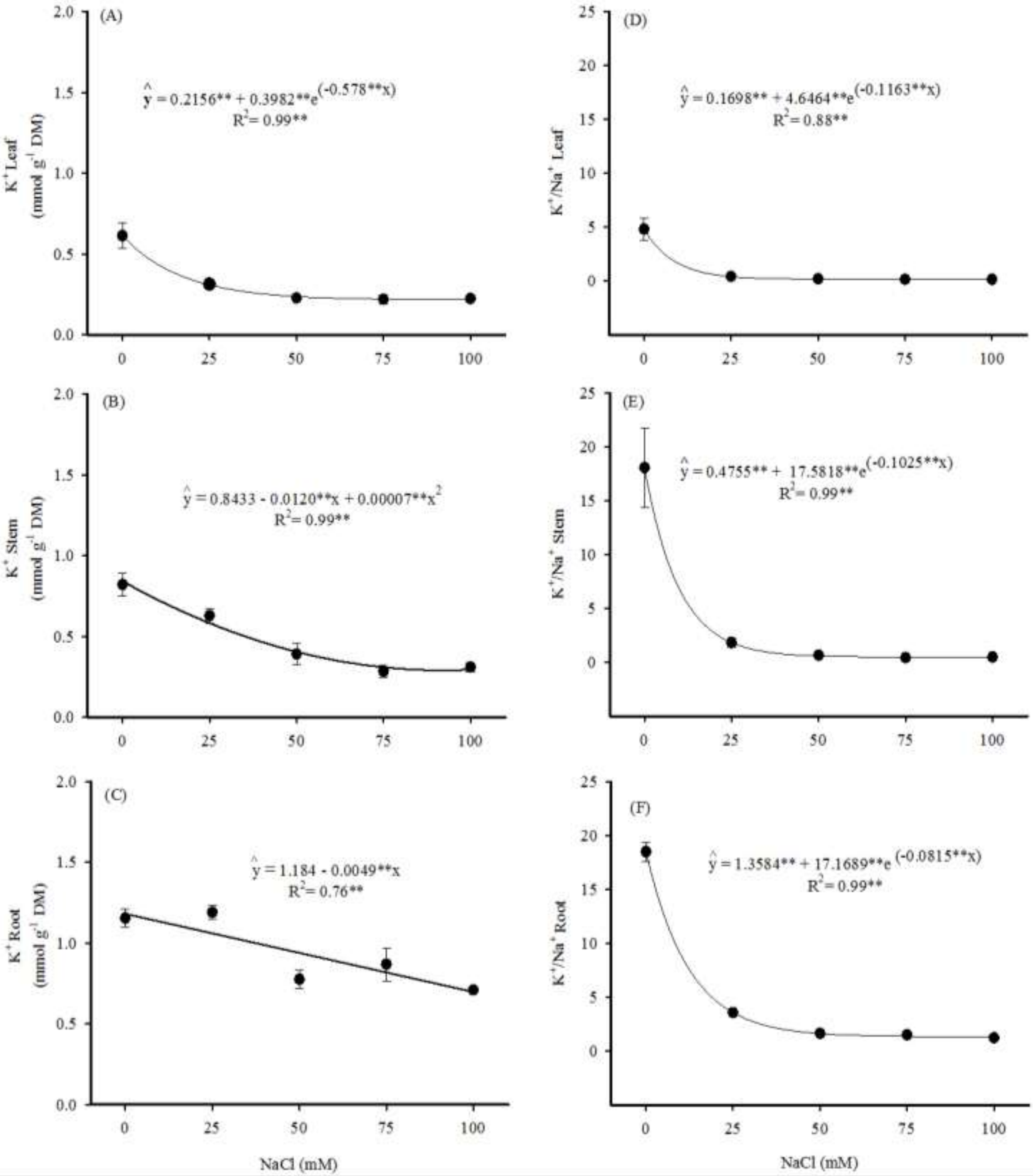

Figure 4. Contents of K^+(A, B, C) and K^+/Na^+ ratio (D, E, F)in leaves, stems and roots of noni plants 40 days after treatment in hydroponic system as a function of NaCl doses in the nutrient solution.

plant and the root is the main organ affected by the stress in the initial phase;

2. Salinity reduces leaf area and increases succulence and sclerophylly index as a mechanism of tolerance to salinity in noni plant;

3. Noni plant in the initial phase does not have mechanisms of restriction to the transport of Na^+ and Cl^- to the leaves, which show higher contents of these ions, while having the compartmentalization of Na^+ and Cl^- as a mechanism of tolerance to salinity;

4. The increment in salinity until the dose of 100 mM causes alterations in the ionic homeostasis of noni plants, which can, at least partially, explain the salt-induced growth reduction.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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REFERENCES

Abreu CEB, Prisco JT, Nogueira ARC, Bezerra MA, Lacerda, CF, Gomes-Filho, E (2008). Physiological and biochemical changes occurring in dwarf-cashew seedlings subjected to salt stress. Braz. J. Plant Physiol. 20(2):105-118.

Apse MP, Blumwald E (2007). Na+ transport in plants. FEBS Letters. 581(1):2247-2254.

Azevedo Neto AD, Prisco JT, Enéas-Filho J, Abreu CEB, Gomes-Filho E (2006). Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environ. Exp. Bot. 56(1):87-94.

Azevedo Neto AD, Tabosa JN (2000). Salt stress in maize seedlings: Part II Distribution of cationic macronutrients and its relation with sodium. Rev. Bras. Eng. Agric. Ambient. 4(2):165-171.

Benincasa MMP (2003). Análise de crescimento de plantas: noções básicas. 2.ed. Jaboticabal: Funep. Brazil 41 p.

Bosco MRO, Oliveira AB, Hernandez FFF, Lacerda CL (2009). Influence of salt stress on the mineral composition of eggplant seedlings. Rev. Ciênc. Agron. 40(2):157-164.

Chan-Blanco Y, Vaillant F, Perez AM, Reynes M, Brillouet J-M, Brat P (2006). The noni fruit (*Morinda citrifolia* L.): A review of agricultural research, nutritional and therapeutic properties. J. Food Compos. Anal. 19(1):645-654.

Correia AAS (2010). Maceração enzimática da polpa de noni (*Morinda citrifolia* L.), Universidade Federal do Ceará, Fortaleza, Brazil 106 p.

Costa AB, Oliveira AMC, Silva AMO, Macini-Filho J, Lima A (2013). Atividade antioxidante da polpa, casca e sementes do noni (*Morinda citrifolia* Linn). Rev. Bras. Frutic. 35(2):345-354.

Faithfull NT (2002). Methods in agricultural chemical analysis: A practical handbook. Wallingford: CABI Publishing 266 p.

Ferreira DF (2011). Sisvar: A computer statistical analysis system. Ciênc. Agrotec. 35(6):1039-1042.

Furlani PR (1998). Instrução para o cultivo de hortaliça de folha pela técnica de hidroponia - NFT. Campinas: Instituto Agronômico, (Boletim Técnico, 168).

Furtado GF, Soares LAA, Sousa JRM, Andrade EMG, Guerra HOC (2013). Physiological changes in cowpea under saline water and nitrogen fertilization. Rev. Verde Agroecologia Desenvol. Sustent. 8(3):175-181.

Greenway H, Munns R (1989). Mechanisms of salt tolerance in non-halophytes. Annu. Rev. Plant. Physiol. 31(1):149-190.

Instituto Brasileiro de Geografia e Estatística – IBGE (2011). Censo Demográfico. 2010. Rio de Janeiro: IBGE.

Jones JB (2001). Laboratory guide for conducting soil tests and plant analysis. Boca Raton: CRC. Press pp. 205-206.

Leite GA, Cunha PSCF, Mendonça LFM, Medeiroa PVQ, Mendonça V (2012). Superação de dormência de sementes de noni. Rev. Verde Agroecologia e Desenvolv. Sustent. 7(4):120-128.

Marana JP, Miglioranza E, Fonseca EP, Kainuma HK (2008). Seedling quality in coffee grown in containers. Cienc. Rural. 38(1):39-45.

Munns R, James RA, Lauchli A (2006). Approaches to increasing the salt tolerance of wheat and other cereals. J. Exp. Bot. 57(5):1025-1043.

Munns R, Tester M (2008). Mecanisms of salinity tolerance. Annu. Rev. Plant. Biol. 59(1):651-681.

Nelson SC, Elevitch CR. (2006) Workshop manual to supplement Noni: The complete guide for consumers and growers for noni processing, marketing and field training Workshop for YAP. Permanent Agriculture Resources, Holualoa, Hawaii 18 p.

Oliveira FA, Medeiros JF, Oliveira FRA, Oliveira MKT, Freire AG (2012). Sensibility of cotton crops to mepiquat chloride under saline conditions. Rev. Ciênc. Agron. 43(3):484-492.

Rodrigues CRF, Silva EN, Moura RM, Anjos DC, Hernandez FFF, Viégas RA (2014). Physiological adjustment to salt stress in *R. communis* seedlings is associated with a probable mechanism of osmotic adjustment and a reduction in water lost by transpiration. Ind. Crops Prod. 54(1):233-239.

Santos APS, Perez-Marin AM, Forero LFU, Moreira JM, Medeiros AML, Lima RCSA, Bezerra HA, Bezerra BG, Silva LL (2014). O Semiárido brasileiro riquezas, diversidades e saberes. Instituto Nacional do Semiárido, Campina Grande, Brazil http://www.insa.gov.br/wp-content/uploads/2014/08/Cartilha-semi%C3%A1rido-final.pdf

Silva EC, Nogueira RJMC, Araújo FP, Melo NF, Azevedo Neto AD (2008). Physiological responses to salt stress in young umbu plants. Environ. Exp. Bot. 63(1):147-157.

Souto AGL, Cavalcante LF, Lima Neto AJ, Mesquita FO, Santos JB (2016). Biometrics in noni plants under irrigation with saline water and the leaching of salts from the soil. Rev. Ciênc. Agon. 47(2):316-324.

Souto AGL, Cavalcante LF, Diniz BLMT, Mesquita FO, Nascimento JAM, Lima Neto AJ (2015). Saline water and bovine biofertilizer on yield and biomass allocation in noni plant (*Morinda citrifolia* L.). Rev. Bras. Pl. Med. 17(2):340-348.

Souto AGL, Cavalcante LF, Nascimento JAM, Mesquita FO, Lima Neto AJ (2013). Behavior of noni plants (*Morinda citrifolia* L.) to salinity of irrigation water in soil with bovine biofertilizer. Rev. Irriga. 18(3):442-453.

Souza MCMR, Lacerda CF, Amorim, AV, Menezes, AS (2014). Influence of organic matter and the environment in growth and in solute accumulation in *Morinda citrifolia* submitted to salinity. Rev. Bras. Frutic. 36(3):704-712

Tavakkoli E, Rengasamy P, Mcdonald GK (2010). High concentrations of Na+ and Cl- ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. J. Exp. Bot. 61(15):4449-4459.

Taiz L, Zeiger E (2013). Fisiologia vegetal. Artmed, Porto Alegre, BR 918 p.

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Full Length Research Paper

Use of *Bacillus* spp. as growth promoter in carrot crop

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Growth promoting rhizobacteria may increase the yield of some crops. Several microbial products that stimulate plant growth have been marketed. Therefore, the objective of this study was to evaluate the effect of bacteria from Bacillus genera on the production of commercial carrot roots (*Daucus carota* L.) in field conditions. The following isolates: SF 264 (*Bacillus* spp.), SF 268 (*Bacillus* spp.) and commercial formulations based on SF 202 (Rizos®, *B. subtilis*), SF 266 (Quartz®, *B. methylotrophicus*) and SF 267 (Onix®, *B. methylotrophicus*) were evaluated in four experiments conducted in commercial fields of carrot production in the municipality of Rio Paranaíba, Minas Gerais - Brazil. Each plot was 6 m long and 1.75 m wide (10.5 m^2) including four double rows. An additional treatment containing only water was the control treatment. The experiment was designed as randomized blocks with five replications. The products Rizos®, Quartz® and Onix® containing *Bacillus* spp. increased the production of commercial carrots roots of all cultivars and sites. The SF 268 and SF 264 isolates were efficient only in two and three experiments, respectively.

Key words: *Daucus carota*, rhizobacteria, plant growth, *Bacillus.*

INTRODUCTION

In Brazil, carrot is among the five main garden crops grown and 80 % of the total production supplies Brazilian domestic market. The Southeast, Northeast, and South regions are the largest producers of this root crop. The State of Minas Gerais stands out in the production of this vegetable crop and the Alto Paranaiba region is one of the main producers. Yield potential of the crop is from 100 to 120 t ha^{-1}; however, the Brazilian average is much lower around 33 tons ha^{-1} (Embrapa, 2010).

Free-living bacteria are found in the rhizosphere of plants and parts of them are known as Plant Growth Promoting Rhizobacteria - PGPR (Alves, 2007). *Pseudomonas, Bacillus, Azospirillum, Agrobacterium* and *Azotobacte* have been reported as PGPR, being *Pseudomonas* and *Bacillus* the PGPR widely reported.

The mechanisms that PGPR, including *Bacillus* spp., increase the plant growth may vary from production or changes on phytohormones concentration, ethylene synthesis inhibition, siderophore and antibiotics production, nitrogen fixation, phosphate solubilization and systemic resistance induction to pathogens (Singh et al., 2011; Vafadar et al., 2014). The beneficial effect of

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rhizobacteria in commercially cultivated crops is world-wide known in onion (Harthmann et al., 2010), potato (Sottero et al., 2006) and tomato Mena-Violante and Olalde-Portugal, 2007). However, this potential is not yet known in several crops, such as carrot (Konusny-Andreani et al., 2014).

Carrot marketing depends on the roots pattern that considers the length, diameter and the defects absence (Ceasaminas, 2015). Thus, the application of PGPR may increase the production of commercial roots, resulting in lower waste and consequently higher profitability to farmers. *Bacillus* spp. in special is easily grown in liquid culture media with low cost, which make easier its mass production in industrial fermenters. Furthermore, they produce resistant endospores, which increase the shelf life of products and integrated use with chemical products (Lanna Filho, 2010).

Therefore, the aim of this study was to evaluate the efficiency of five *Bacillus* spp. isolates in increasing the production of commercial roots of carrot under field conditions.

MATERIALS AND METHODS

PGPR Isolates

The following isolates: SF 264 (*Bacillus* spp.), SF 268 (*Bacillus* spp.), SF 202 (*Bacillus subtilis* - commercial product Rizos®), SF 266 (*B. methylotrophicus* - commercial product Quartz®) and SF 267 (*Bacillus methylotrophicus* - commercial product Onix®). They were tested in 4 fields with commercial carrot production in the municipality of Rio Paranaíba - MG (site 1 – S.: 19°19´09´´; W.: 46°14´04.2''; site 2 – S.: 19°14´14.7´´; W.: 46°08´47.5.2''; site 3 – S.: 19°25´45.8´´; W.: 46°14´10.2''; site 4 – S.: 19°13´04´´; W.: 46°14´20''). Isolates and commercial products belong to Farroupilha`s group from Patos de Minas, Minas Gerais. The experiments were carried out during February and March 2012.

Carrot cultivars

The cultivar Brasilia, developed by EMBRAPA, was used in two sites. In the two other sites the cultivars were Suprema Max® (Isla Sementes Ltda, Porto Alegre, Brasil) and Juliana® (Seminis Vegetable Seeds, St. Louis, EUA), respectively.

Soil and sites characterization

The chemical and physical characteristics of the soils are presented in the Table 1.

All isolates were prepared in liquid culture media and formulated as a protocol used in the company. The concentration of the isolates SF 264, SF 266, SF 267 and SF 268 was 1 x 10^9 colony forming units (cfu) per mL, while the concentration of SF 202 was 5 x 109 cfu mL^{-1}.

At 15 days after planting, the isolate suspensions were applied on the plants using a CO_2 -pressurized backpack sprayer furnished with three 110-02 nozzles working in constant pressure of 2.2 bar, spaced at 0.5 m. The dose and volume of the spray solution were 4 L ha^{-1} and 200 L ha^{-1}, respectively. Immediately after the application of the bacterial isolates, each experimental area was irrigated at 5 mm water depth in order to facilitate the bacteria propagules percolation in the soil profile. The sprays were done after 3 pm.

Each plot was 6 m long and 1.75 m wide (10.5 m^2), including four double rows. An additional treatment containing only water composed the control treatment. The experiment was designed as randomized blocks. Crop management was carried out following the pattern adopted by the growers, including irrigation, topdressing fertilizations, pest management and rough-hewing.

Yield evaluation

The harvest was carried out at 105 days after planting. The fresh weight of commercial roots of carrot was evaluated in 1 m^2, located at the center of each plot (useful plot). All roots in the useful plots were classified according to the carrot standard classification of a Brazilian program for commercial standards and horticultural packaging improving (Ceasaminas, 2015). It was considered only the roots with a length between 14 and 26 cm without defects. Later the selected roots were weighed using a digital scale.

Statistical analysis

The data were submitted to variance analysis (F test, P = 0.05) and the means compared by Scott-Knott test (P = 0.05) using the software SISVAR 5.1 Build 72 (Ferreira, 2007).

RESULTS AND DISCUSSION

The bacteria isolates increased the production of commercial roots of the cultivar Juliana in both sites (Table 2). For the cultivar Suprema Max, the SF 267, SF 266, SF 264 and SF 202, even in different groups, increased the carrot production. For the cultivar Juliana, yields with SF 202, SF 266 and SF 267 application were 10.40; 12.48 and 23.32 higher than the control.

The SF 267, SF 266 and SF 202 isolates of *B. methylotrophicus* are soil nitrifying bacteria, which means, they may convert ammonia to nitrite and later nitrite to nitrate which is a readily assimilable nitrogen form by plant roots (Zhang et al., 2012). This characteristic may explain the outstanding performance observed for SF 266 and SF 267 isolates to increase the production of commercial roots of carrot. Furthermore, Yan et al. (2011) and Dev Sharma et al. (2013) reported in several studies that *B. methylotrophicus* isolates produced antagonistic metabolites against several pathogenic bacteria and fungi.

Andreani et al. (2014) evaluated the effect of rhizobacteria isolates from *Crotalaria spectabilis* on the development of carrot (cultivar Nantes), in greenhouse conditions, through seed microbiolization with bacterial suspensions. In this study the bacterial isolates could not be identified. They were merely coded as UCCBj-CE's. The UCCBj-CE 04, UCCBj-CE 11, UCCBj-CE 17 and UCCBj-CE 18 isolates induced higher productivity in carrot crop.

According to Lanna Filho (2010), the growth-promotion induced by *B. subtilis* may be a consequence of increasing nitrogen fixation, nutrients solubilization, hormone synthesis and soil conditions improvement,

Table 1. Chemical and physical characterization of commercial fields with carrot cultivation.

Chemical and physical analysis	Cultivar			
	Brasilia - Site 1	Brasilia - Site 2	Suprema Max - Site 4	Juliana - Site 5
pH water	6.27	6.43	5.97	6.73
P-rem (mgL^{-1})	11.13	10.27	12.25	7.06
O. M. (dag kg^{-1})	3.48	3.21	3.70	3.14
P (mg dm^{-3})	47.77	43.87	45.69	11.76
K ($mgdm^{-3}$)	123.00	176.00	81.00	86.00
Ca ($cmoldm^{-3}$)	4.49	3.83	4.01	3.87
Mg ($cmoldm^{-3}$)	0.83	0.95	0.87	0.88
Al (cmol dm^{-3})	0.04	0.04	0.04	0.04
H+Al (cmol dm^{-3})	3.10	2.71	4.04	2.08
BS (cmol dm^{-3})	5.63	5.23	5.09	4.97
CEC (t) ($cmoldm^{-3}$)	5.67	5.27	5.13	5.01
CEC (T) (cmol dm^{-3})	8.32	7.94	9.13	7.05
m (%)	0.70	0.76	0.78	0.80
V (%)	64.54	65.87	55.72	70.54
B (mg dm^{-3})	0.31	0.22	0.13	0.18
Cu (mg dm^{-3})	1.40	7.70	4.30	1.10
Fe (mg dm^{-3})	28.50	32.40	28.30	20.90
Mn (mg dm^{-3})	8.10	13.40	8.80	6.10
Zn ($mgdm^{-3}$)	11.10	25.70	22.40	8.50
S (mg dm^{-3})	13.97	5.85	3.77	5.66
Clay (g kg^{-1})	416.00	446.00	424.00	418.00
Silt (gkg^{-1})	160.00	174.00	172.00	176.00
Sand (gkg^{-1})	424.00	380.00	404.00	406.00
TOC ($dagkg^{-1}$)	2.02	1.86	2.15	1.82

P, K, Fe, Zn, Mn, Cu: Mehlich I extractor. Ca, Mg, Al: KCl 1 mol L^{-1} extractor. CEC (t): Effective cation exchange capacity. CEC (T): Cation exchange capacity at pH 7.0. Texture: Pipette Method. V: Base saturation. m: Aluminum saturation. H + Al: SMP extractor. B: Hot water extractor. P-rem: Remaining phosphorus, concentration of P of the equilibrium solution after stirring the air-dry soil during 1 h with $CaCl_2$ solution at 10 mmol L^{-1}, containing 60 mg L^{-1} of P (1:10). S: Monocalcium phosphate in acetic acid. Organic matter: Oxidation - $Na_2Cr_2O_7$ 4N + H_2SO_4 10N. TOC - Total Organic Carbon: Oxidation with $Na_2Cr_2O_7$ 4N + H_2SO_4 10 mol L^{-1}.

Table 2. Fresh weight of commercial carrot roots (kg m^{-2}) after different applications of *Bacillus* spp. isolates.

Treatment	Cultivar			
	Brasilia - Site 1	Brasilia - Site 2	Suprema Max - site 3	Juliana - site 4
SF 202 (Rizos®)	5.8550[a]	9.0880[a]	6.5030[b]	6.5038[a]
SF 264(*Bacillus* spp.)	6.1770[a]	8.5900[a]	6.6260[b]	6.1788[b]
SF 266 (Quartz®)	6.0760[a]	8.5740[a]	6.1780[b]	6.6262[a]
SF 267 (Onix®)	6.4090[a]	8.5070[a]	7.2650[a]	7.2650[a]
SF 268(*Bacillus* spp.)	6.2130[a]	9.0990[a]	5.6580[c]	5.6588[b]
Control(water)	5.2758[b]	6.9890[b]	5.8910[c]	5.8916[b]
CV(%)	8.79	9.54	8.88	7.39

Means followed by the same letter, in the columns, are not different at 5% probability by Scott-Knott test. CV: coefficient of variation.

besides indirect benefits by environmental suppression against pathogens. Additionally, the beneficial association provides an increase in physiological metabolites that unleash the root system sensitivity to external conditions, which may improve the nutrient uptake (Manjula and Podile, 2005).

Isolates of *B. subtilis* also has the ability to induce hormonal regulation in plants, as reported by Tsavkelova et al. (2006), Persello-Cartieaux et al. (2003) and Lanna Filho (2010) controlling the root growth by auxin, gibberellin and cytokinin synthesis. It may also explain the responses of *B. subtilis* isolates in all experiments. Besides the genetic differences among isolates and their possible influence on root colonization, the interaction between isolates and carrots cultivar may also influenced the plant growth (Romeiro et al., 2005; Choudhary et al., 2007).

For the cultivar Brasilia, all isolates increased the carrot production. The SF 264 isolate in the cultivar Juliana and the SF 268 in the cultivar Suprema Max and Juliana did not have the same beneficial effect. It is possible that the production and root exudates composition among cultivars are different; therefore, the SF 268 and SF 264 isolates have not been able to colonize efficiently the rhizosphere of Suprema Max®and Juliana®cultivars, and consequently act as growth promoter.

When a microorganism is introduced into a new environment, it must overcome the competition with other microorganisms (microbiostase), in order to develop and perform its ecological functions (Tsavkelova et al., 2006).There are reports that show the high specificity of growth - promoting bacteria to colonize specific hosts. This specificity was not observed in the present study. Raasch et al. (2013) stated that eucalyptus production with Rizolyptus® (*B. subtilis*) varied according to the tested clones, suggesting a high specificity of root colonization regarding the host.

Mello et al. (2002) reported the lack of specificity of the C210, ENF10, ENF16 and RAB9 isolates, obtained from cabbage, beans and radish, respectively, regarding growth promotion in seedlings of pineapple micro-propagation. In another study, isolates of C116 (*Bacillus pumilus*) and C25 (*Bacillus thuringiensis* subvar. *kenyae*), from cabbage were effective in promoting the growth of lettuce seedlings (Gomes et al., 2003).

Besides the specificity of bacterial isolates to the host, stands out the differences in the soil of rooting for rhizobacteria colonization, once abiotic factors (pH, nutrient availability, moisture retention, aeration etc.) and biotic (qualitative and quantitative composition of the microbiota and others) may favor or not the colonization, survival and beneficial activity of rhizobacteria (Mafia et al., 2009).

Such variables may explain the difference in the production of commercial carrot when using a same bacterial isolate and in different experiments, i.e. under different environmental conditions. This was observed for the cultivar Brasilia - site 1, where the SF 267 isolate (*B. methylotrophicus*, Onix®) was used, the fresh weight was 6.41 kg while in the site 2 it was 8.51 kg.

In the cultivation fields of the cultivar Suprema Max and Juliana, the potassium content in the soil was around 40% lower than the other fields as highlighted in the Table 1. There may be some relationship between potassium and phosphorus contents in the soil and the capacity of the SF 268 isolate colonize the rhizosphere of carrot plants and promote the plant growth. Moreover, the SF 267, SF 266 and SF 202 isolates do not seem to depend on the presence of high amounts of these nutrients in the soil, considering that some PGPR solubilize phosphates and/or provide potassium to the plants (Gomes et al., 2003; Viruel et al., 2014). Further studies are recommended to investigate whether the beneficial effect of the SF 267, SF 266 and SF 202 isolates in increasing the production of commercial carrot roots is due to their action as nutrients solubilizers in the soil.

Conclusions

The application of *Bacillus* spp., present in the products Rizos®, Quartz® and Onix®, enhanced the yield of commercial carrot roots.

Conflict of Interests

The authors have not declared any conflict of interest.

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REFERENCES

Alves E (2007). Mecanismos estruturais na resistência de plantas a patógenos. Summa Phytopathol. 33:154-156.

Andreani DRK, Agiado JC, Junior RA (2014). Efeito de bactérias rizosféricas sobre o desenvolvimento da cenoura. Rev. da Universidade Vale do Rio Verde 12: 211-220.

CEASAMINAS. Centros de abastecimento de Minas Gerias. Informações de mercado: Cenoura. (2015). Available at: http://www.ceasaminas.com.br/informacoesdemercado/contador_procedenciaprodutos.asp (accessed in June 2016).

Choudhary DK, Prakash A, Johri BN (2007). Induced systemic resistance (ISR) in plants:mechanism of action. Indian J. Microbiol 47:289-297.

Dev Sharma SC, Shovon MS, Jahan MGS, Asaduzzaman AKM, Rahaman MA, Biswas KK, Abe NR (2013). Antibacterial and cytotoxic activity of *Bacillus methylotrophicus*-scs2012 isolated from soil. J. Microb. Biotech. Food Sci. 2:2293-2307.

Empresa Brasileira de Pesquisa Agropecuária – Embrapa (2010). Sistemas de Produção: Cenoura [internet]. Brasília, DF; 2010 [accessed on Jul. 25, 2016]. Available at: http://www.cnph.embrapa.br

Ferreira DF (2007). SISVAR: Sistema de análise de variância para dados balanceados, versão 5.1 Build 72. Lavras: DEX/UFLA.

Gomes AMA, Mariano LRR, Silveira EB, Mesquita JCP (2003). Isolamento, seleção de bactérias e efeito de *Bacillus* spp. na produção de mudas orgânicas de alface. Hort. Bras. 21:699-703.

Harthmann OEL, Mógor AF, Wordell FJA, Luz WC (2010). Rizobactérias no crescimento e produtividade de cebola. Cienc. Rural 40:462-465.

Konusny-Andreani DI, Agiado JC, Andreani JR (2014). Efeito de bactérias rizosféricas sobre o desenvolvimento da cenoura. Rev. da Universidade Vale do Rio Verde12:211-220.

Lanna FR, Ferro HM, Pinho RSC (2010). Controle biológico mediado por *Bacillus subtilis*. Rev. Trópica – Ciênc. Agrárias e Biol. 4:12-20.

Mafia RG, Alfenas AC, Ferreira EM, Binoti DHB, Mafia GMV, Mounteer AH (2009). Root colonization and interaction among growth promoting *rhizobacteria* isolates and eucalypts species. Rev. Árvore 33:1-9.

Manjula K, Podile AR (2005). Increase in seedling emergence and dry weight of pigeon pea in the field with chitin-supplemented formulations of *Bacillus subtilis* AF 1. World J. Microb.Biot. 21:1057-1062.

Mello MRF, Mariano RLR, Menezes M, Câmara TR, Assis SMP (2002). Seleção de bactérias e métodos de bacterização para promoção de crescimento em mudas de abacaxizeiro micropropagadas. Summa Phytopathol. 28:222-228.

Mena-Violante HG, Olalde-Portugal V (2007). Alteration of tomato fruit quality by root inoculation with plant growth-promoting *rhizobacteria* (PGPR): *Bacillus subtilis*BEB-13bs. Sci. Hort.113:103-106.

Persello-Cartieaux F, Nussaume L, Robaglia C (2003).Tales from the underground: Molecular plant-rhizobacteria interactions. Plant Cell Environ. 26:186-199.

Raasch LD, Bonaldo SM, Oliveira AAF (2013). *Bacillus subtilis*: enraizamento e crescimento de mini estacas de eucalipto em Sinop, Norte de Mato Grosso, Brasil. Biosci. J. 29:1446-1457.

Romeiro RS, Lanna-Filho R, Vieira JR, Silva HSA, Baracat-Pereira MC, Carvalho MG (2005). Macromolecules released by a plant growth-promoting *rhizobacterium* as elicitors of systemic resistance in tomato to bacterial and fungal pathogens. J. Phytopathol. 153:120-123.

Singh JS, Pandey VC, Singh DP (2011). Efficient soil microorganisms: a new dimension for sustainable agriculture an environmental development. Agric. Ecosys. Environ. 140:339-353.

Sottero NA, Freitas SS, Melo AMT, Trani PE (2006). Rizobactérias e alface: colonização rizosférica, promoção de crescimento e controle biológico. Rev. Bras. Ci. Solo 30:225-234.

Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI (2006). Microbial Producers of Plant Growth Stimulators and Their Practical Use: A Rev. Appl. Biochem Micro 42:117-126.

Vafadar F, Amooaghaie R, Otroshy M (2014). Effects of plant-growth-promoting rhizobacteria and arbuscularmycorrhizal fungus on plant growth, stevioside, NPK, and chlorophyll content of Stevia rebaudiana. J. Plant Interactions 9:128-136.

Viruel E, Erazzú LE, Calsina LM, Farrero MA, Lucca ME, Siñeriz F (2014). Inoculation of maize with phosphate solubilizing bacteria: effect on plant growth and yield. J. Soil Sci. Plant Nut. 14:819-831.

Yan X, He L, Song G, Wang R (2011). Antagonistic bioactivity of endophytic strains isolated from *Salvia miltiorrhiza*. Afr. J. Biotechnol. 10:15117-15122.

Zhang QL, Liu Y, Ai GM, Miao L, Zheng HY, Liu ZP (2012). The characteristics of a novel heterotrophic nitrification–aerobic denitrification bacterium, *Bacillus methylotrophicus* strain L7. Bioresour. Technol. 108:35-44.

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Full Length Research Paper

Phosphorus fractionation in sediments of the ypacarai lake basin

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The aim of this paper was to determine the phosphorus fractions in the sediment, at different points of the tributaries, on the lake, and on the Ypacarai lake basin. There was a collection of 23 bottom sediment (SF) samples of 0 to 0.05 m of tributaries of the Ypacarai lake basin and 10 samples from the lake. The sand, silt and clay fractions and textural class were determined; in addition to the P forms, grouped in labile, moderately labile and nonlabile. The bottom sediments of the tributaries and of the lake presented scarce clay content, predominantly containing sand on the textural class. There is a greater amount of phosphorus in every evaluated fraction in the fine sediment of the tributaries than in the sediment of the lake. The greatest fractions of moderately labile phosphorus were obtained from the lake, presenting values considered to be adequate in the tributaries. However, in the tributaries, the determinations showed hagh variability between the points which run through densely populated regions, being that the three forms labile, moderately labile and nonlabile were found distributed in equal parts.

Key words: Available phosphorus, eutrophication, environmental contamination, erosion.

INTRODUCTION

The state of the watercourses in densely populated regions suffers the effects of human activities in the watershed, with phosphorus as one of the key players for the eutrophication process of the water (Pellegrini, 2005). In rural areas, the application of high doses of P via inorganic mineral fertilizers or organic amendments leads to the saturation of the soil surface functional groups of higher affinity (Rheinheimer, Anghinoni, 2003). In urban areas, the contamination is caused by the improper disposal of the organic material, such as sewage waste, industrial residues and household garbage, mainly from the use of detergents with polyphosphates. Based on the aforementioned studies of chemical elements fractionations, the sedimentary compartment applied to

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watercourses, are of utmost importance, recording the occurring processes in the aquatic ecosystem, serving as a great tool to understand the processes of the behaviour of phosphorus and other nutrients (Zhang et al., 2010).

The extraction of sedimentary phosphorus is grouped into five main fractions; it has been widely applied in soils, marine, lake and estuary sediments, and provides a more comprehensive analysis of the dynamic phosphorus than simply investigating the total phosphorus (Aquino, 2014). The labile P is the most important factor when the quality of the lake water is considered, as it is an essential nutrient for the occurrence of the eutrophication (Sharpley et al., 1987). The entry of the sediment in the body of water plays a fundamental role in P availability, it can work as source or drain of the element, the more the basin suffers the effects from men, the greater the entry of the sediment and of P in the particulate form (Schenatto, 2009). The quantity and the form of P found in the sediments vary widely among watercourses (Zhou et al., 2001) and the ability to release the P depends on the way it is adsorbed in its functional groups, specially on the silt and clay fractions (Zhu et al., 2013).

The organic P and the P connected to iron and aluminum oxy-hydroxides are the most abundant (Monbet et al., 2010), and the P connected to calcium is of lower availability (Kerr et al., 2011).

Once P is found in the water, there is a balance between the P fractionated in the solution and in the sediment, resulting into a quick balance on the surface of the particles; however, within its interior the balance is slow and the release of P can only occur when there is a resuspension of the particles found at the bottom of the water mass (Correl, 1999). In environments with low oxygen concentrations, there is an increase of P release due to the iron reduction and the surface of oxy-hydroxides are modified (Schenatto, 2009). In order to study the availability of P in the sediment, the study makes use of the P fractionation, which consists in performing successive extraction of the same sample of the sediment through different chemical extractors. In this context, the aim of this paper was to determine the phosphorus fractions in the sediment, in different points of the tributaries, in the lake, and the Ypacarai lake basin.

MATERIALS AND METHODS

The Ypacarai lake is located to the East side of the city of Assunção (25° 19' 42,87" and 57° 20' 35,57" to 60 meters of altitude), with approximately 60 km^2. The geological formation is composed of basic conglomerates and quartz crystals. There was a collection of 23 samples of bottom sediment (SF) of the tributaries of the lake basin and 10 samples of the sediment of the lake, coming to a total of 33 watershed points of the Ypacarailake (CHY) (Figure 1). At each point two samples were collected at a 0 to 05 m depth. The experiment was conducted in a completely randomized design, with 2 treatments, with samples of SF of the tributaries, with 23 repetitions and SF sample of the lake, with 10 repetitions. At each point there was a collection of two samples composed of various sub-samples, with two repetitions of laboratory, coming to a total of 132 analyzed samples. The SF samples were dried in greenhouse at 50°C and sieved with a sieve of 63 μm to separate silt and clay from the sand fraction. The texture of the samples was determined through the volumetric pipette method (USDA, 1972).

The most commonly used methodology to perform the P fractionation is the one proposed by Hedley (Gatiboni et al., 2007), from which the P of the sediment can be grouped into labile, or P extracted through anion exchange resin and bicarbonate, or moderately labile, which is the P extracted through sodium hydroxide, and nonlabile which refers to the P extracted through strong acids (Rheinheimer et al., 2008). The samples of lime plus clay of the SF were used to determine the fractionation of P described by Hedley et al. (1982) with modifications proposed by Rheinheimer (2000). The forms of P were sequentially extracted through: Anion exchange resin (AMI 7001S) (P_{RIA}); $NaHCO_3$ 0.5 mol L^{-1} at pH 8,5 (P_{NaHCO3}), NaOH 0.1 mol L^{-1} (P_{NaOH01}); HCl 1.0 mol L^{-1}eNaOH 0.5 mol L^{-1}($P_{NaOH0,5}$). All the extractors remained in contact with the samples for 16 h in agitator type "endover" 33 rpm followed by centrifugation at 2510 spin for 20 min. In the alkaline extracts of the solution of $NaHCO_3$ and NaOH, the available P was determined through the method from Dick andTabatabai (1977), modified by He and Honeycutt (2005) and the total P performing the extraction through digestion with ammonium persulfate and H_2SO_4 in autoclave at 121°C and subsequently determined by Murphy and Riley (1962).

Afterwards, the residue was dried, ground and had the residual P extracted by the method H_2SO_4 + H_2O_2 + $MgCl_2$ (Olsen and Sommers, 1982). The P determinations extracted were estimated by the method of Murphy and Riley (1962). The inorganic P forms extracted through the RTA and through NaHCO3 were considered "labile" forms. The P extracted through the NaOH was considered a "moderately labile" form. The P extracted through HCl and the residual inorganic P were considered little labile and nonlabile, respectively. The total P was estimated by the sum of all extractions. The statistical analysis was performed by the statistical software Assistat (Silva and Azevedo, 2009). The obtained data were submitted to a variance analysis. In this finding, significant effect on the averages were submitted to the Tukey test at 5% of probability.

RESULTS AND DISCUSSION

From all the analyzed samples, corresponding to the tributaries from the YpacaraiLake, numbered from P1 to P23, (Figure 1) 91% of them presented sandy textural class, with high sand content which ranged from 92.2 to 94.9% and low clay percentages of only 0.3 to 1%. The remaining samples 9% had loamy sand texture. In the samples collected at the Ypacarai Lake, corresponding from P24 to P33, the clay content was greater, ranging from 6 to 15%. The adsorption capacity of P is inversely related to the size of the particles, whose bioavailability is difficult to determine, because of its high affinity with the lost particles (sediments) and the reversibility of the adsorption/desorption process (Zhu et al., 2013). The way in which the adsorbed P is released, will depend on the physical-chemical conditions of the water interface on the soil and on how P is connected to particles of the sediment (Pettersson and Istvanovics, 1988).

On average, the different P forms were greater in the sediment of tributaries on the lake (Table 1). The P resin is extracted twice in SF on tributaries and SF of the lake, considering that this one is the most available and can be

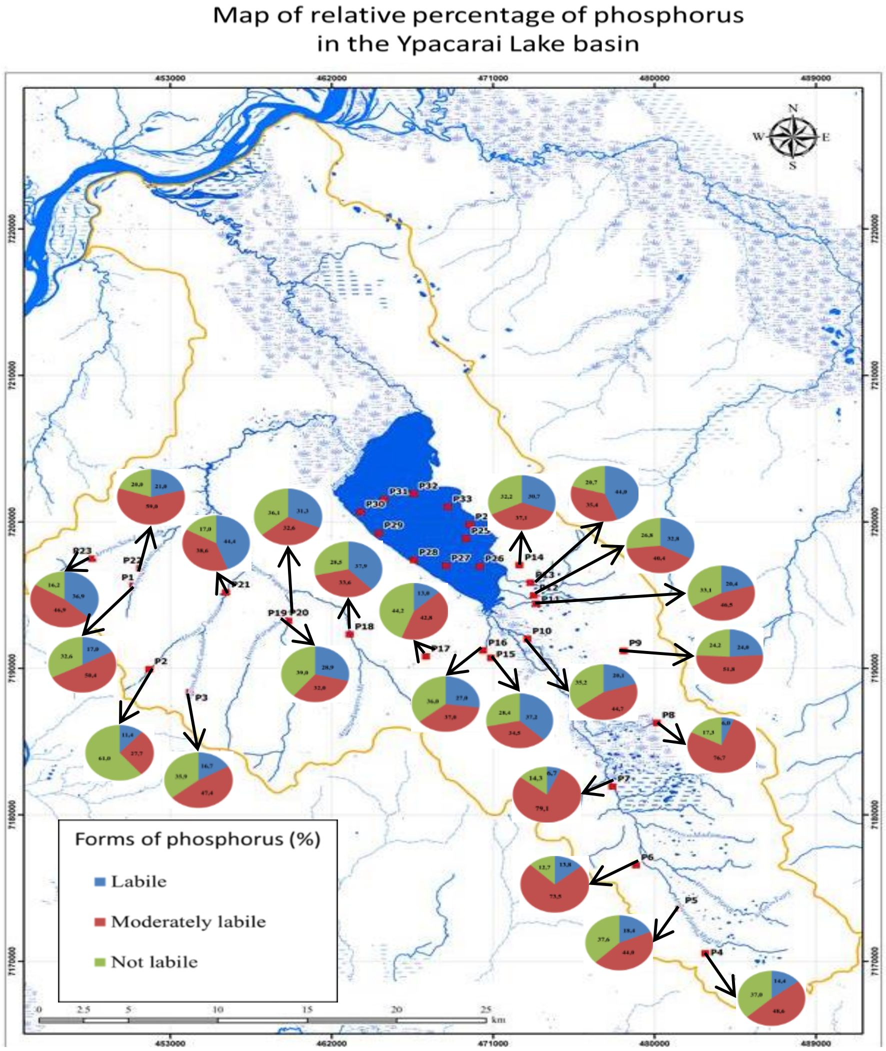

Figure 1. Relative percentage of the labile, moderately labile and nonlabile phosphorus forms, in the bottom sediment of the tributaries, obtained by phosphorus fractionation in the watershed of the Ypacarailake, Paraguay. Red: moderately labile; green: nonlabile; blue: labile.

Table 1. Phosphorus forms in the bottom sediment of the tributaries and the lake, obtained by phosphorus fractionation in the watershed of the Ypacarailake, Paraguay.

Site	Phosphorus fractionation								Residual P	Total P
	Resi	$NaHCO_3$		NaOH 0.1 M		HCl 1 M	NaOH 0.5 M			
	Pi	Pi	Po	Pi	Po	Pi	Pi	Po		
	mg kg^{-1}									
Tributaris	7^a	33^a	135^a	54^a	103^a	113^a	156^a	29^a	90^a	692^a
Lake	3^b	8^b	22^b	34^b	54^b	10^b	31^b	18^b	56^b	219^b
CV	16.6	17.2	31.2	42.5	43.9	31.2	17.4	56.5	65.4	19.4

[1]Means followed by the same letter in are not significantly different (Tukey> 0.05). Pi = inorganic phosphorus; Po = organic phosphorus.

easily used by aquatic organisms due to its availability. The $NaHCO_3$, both in the biological (Po) and inorganic (Pi) form is from 4 to 6 times, respectively, on the tributaries of the lake SF. Phosphorus extracted through bicarbonate can usually become available for the organisms, which occurs when there are environments where there is pH variation, redox or reduction of P content soluble in water (Schenatto, 2009). NaOH 0.1 M and NaOH 0.5 M, represent P which is connected mainly to iron and aluminum oxy-hydroxide, in addition to being on the organic matter, if water enters on the course of some organic matter from water erosion or decomposing organic materials, there is availability of this P (Schenatto, 2009; Monbet et al., 2010).

However, under conditions with low or zero oxygen concentration, there is release of P connected to the iron, due to the reduction of this element and the surface of the oxy-hydroxides is modified, releasing P (Vahl, 1999; Rannotodos, 2007). A large part of P is on the biological form and can represent up to 25% of P potentially bioavailable when they are mineralized (Correl, 1999; Bhadha and Jawitz, 2010). The P extracted through hydrochloric acid (HCl) (P-HCL), as well as other P forms, make is in past tense greater on the SF of tributaries, eleven times higher than on the SF of the lake. Usually, the amount of P-HCl present in lake sediments is below the water resources, especially in areas where acidic soils are found, because the extracted P-HCl is connected to the calcium of minerals, such as calcite, apatites or neoformation of calcium phosphate of Pi. As it is important on alkaline soils, or in rural areas where phosphate fertilizers is applied in the form of natural phosphate, or when there is the application of high doses of agricultural limestone, which allows the neoformation of calcium phosphate (Gatiboni et al., 2007), which is not observed on the basin under study.

There is on average three times the P total on the SF of tributaries 692 mg kg^{-1} than on the lake 219 mg kg^{-1} which is a concern, considering that the sediment will end up deposited on the lake and that currently presents problems due to the eutrophication of its waters; if corrective measures are not taken on the Ypacaraí lake to prevent the entry of sediments rich in P into the lake, these problems will get worse. Previously, Ritterbusch (1988), in a limnological study of the Ypacarai Lake, found high concentrations of nitrogen and phosphorus available, especially P, significantly exceeding 30 mg $L\text{-}^{1}$, which is considered the limit for the eutrophication process.

The same author observed that the total P is larger on the outputs of the system that is being retained on the lake bed, and a very high level of total P in areas influenced by domestic and industrial sewage in the districts of Areguá and San Bernardino. In studies in southern Brazil, on sampling carried out on rivers that are part of the Mirim lake basin in RS, contents above the level of 0.1 mg L^{-1} (Coradi et al., 2009) were observed. Diel et al. (2007) found that, on average, the content of total P in the water destined for rice irrigation was of 0,041 mg L^{-1}, while in dams, ponds and rivers the average values of P were 0,027; 0,035; 0,060 mg L^{-1}. When the samples of SF tributaries are compared, a lot of variation is noted on the total P value (Figure 2), with a higher total amount of P on the sample of SF collected in the (P23) flow and the San Lorenzo flow Tayuazape (P1, P22), which runs to the same water flow. These points are located in streams with high anthropic occupation, so that the high levels of total P can be found through removal of domestic and industrial waste. In addition, the P16, P17, and P18 points which crosses the cities of Ypacarai and Itaugua have high levels of total P, although, almost half of the total P, when compared to the tributaries run through San Lorenzo.

The tributaries located on the South of the basin, from Arroyo ZanjaMoroti (P4, P5, and P6 points), which has its source in the Paraguarí district and disgorges in the Pirayú (P7) stream, until its mouth in Ypacarai lake and tributaries located to the East side of the Ypacarai lake, which has two headsprings in the districts of Caacupé (P8), Ypacarai (P9 and P10) and San Bernardino (P11, P12, P13 and P14) usually has low levels of total P (Figure 2). These watershed areas are sparsely populated, so the entries of P would come from the pollution from diffuse sources, as agriculture and livestock that takes place in a large part of the basin (Figure 3). The results obtained at the Capiatá stream can be highlighted, alongside the headspring (P2 and P3) possessing relatively high amounts of P (776 and 681mgkg^{-1}). However, at P21 point there is less, with 50% of total P on the header 278 mg kg^{-1}, so it would be advisable to carry out a new SF sampling, to check if there was any collection of error or if there actually is any factor that allows total reduction of P content, as gallery forests and marshes. On average, to the south of the Ypacarai Lake there is a lower P total than the SF of the tributaries (Table 1); however, at different points of comparison sampling Ypacarai Lake shows that there is a variation in total amount of P at its SF, and to the south of the lake it is more than total P, especially at the nearest point to the city of Ypacarai (P26), which presented 356 mg kg^{-1}. In the middle of the lake there is less total P, especially at the shores located in the Areguá district (P30, P29, P31 and P32).

Conclusion

The concentration of populations and anthropogenic activities in the shores of the Ypacaraylake, the available P in the lake sediments increased up to 0.05 m deep. The saturation of the absorption sites of P in the lake sediments; evaluated by the determination of the labile, moderately labile and nonlabile forms, presented variability, being higher in the lake than in the tributaries. Eutrophication is found both in the lake as in the tributaries in all the evaluated points.

Conflict of Interests

The authors have not declared any conflict of interests.

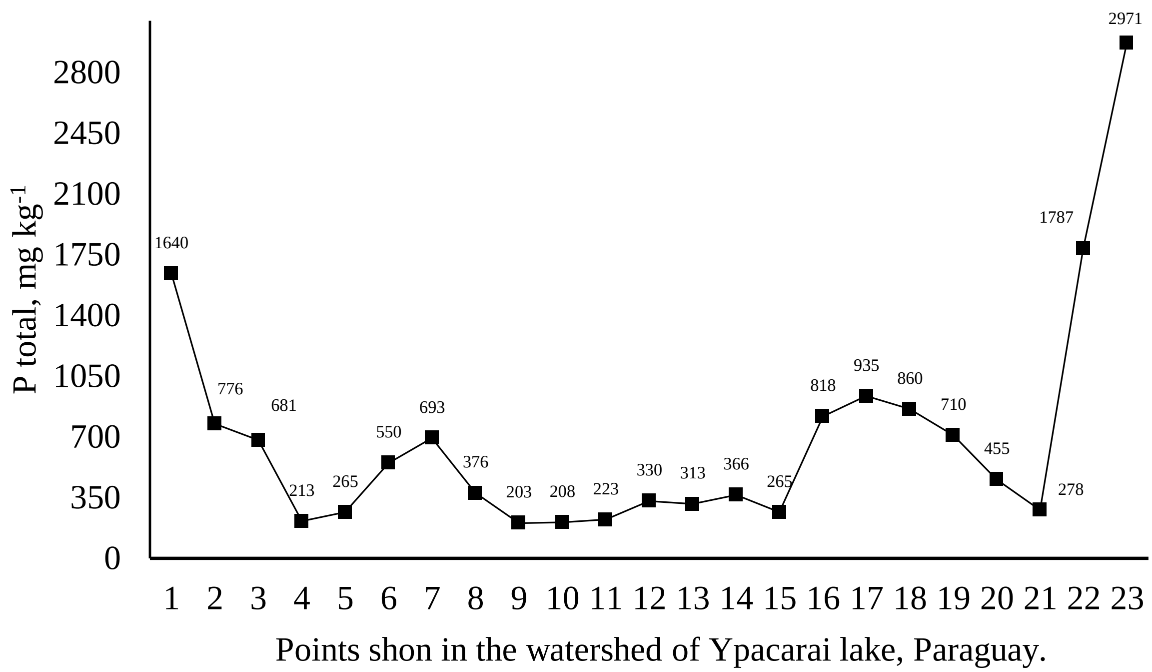

Figure 2. Total phosphorus in the bottom sediment of the tributaries obtained by the sum of fractions on the watershed of Ypacarailake, Paraguay.

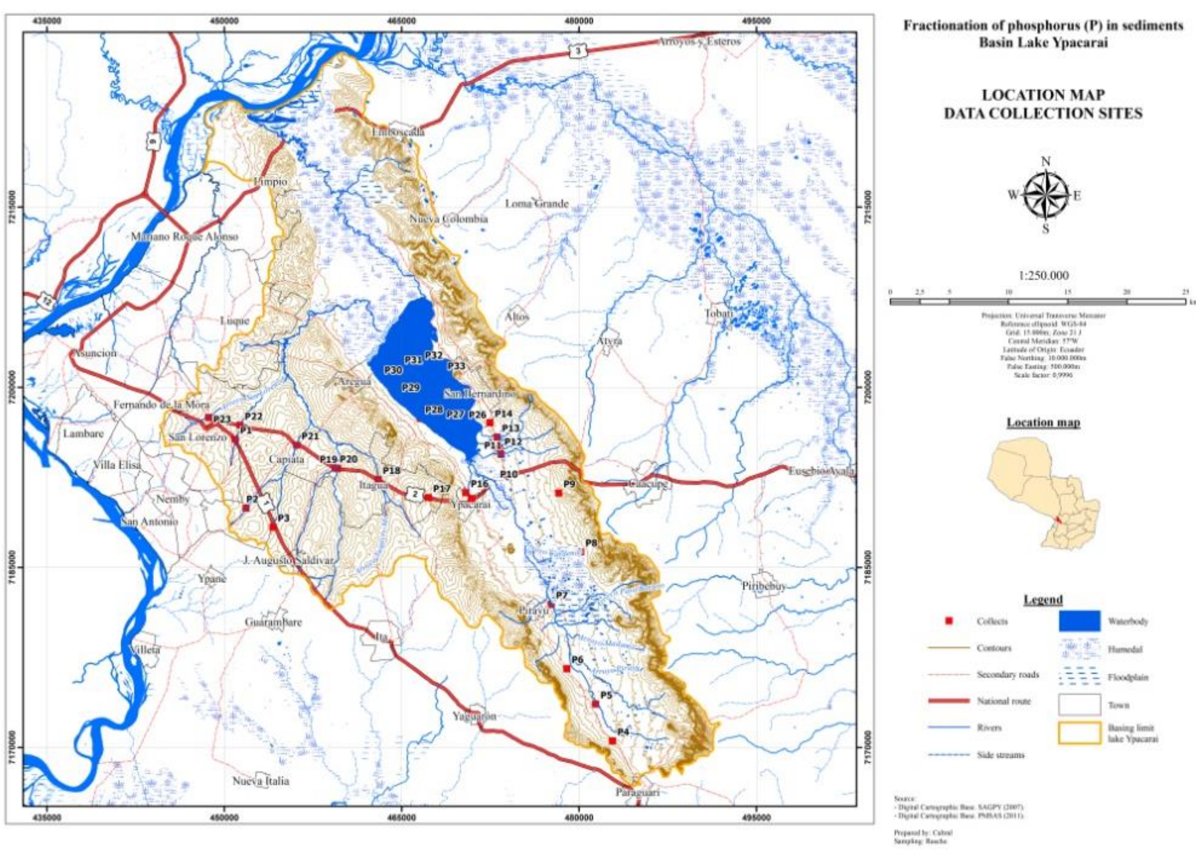

Figure 3. Geographical distribution of the collection points of the bottom sediment of the tributaries and the Ypacarai lake, in the watershed of the Ypacarailake, Paraguay.

REFERENCES

Aquino RF (2014). Acumulo e fracionamento de fósforo nos sedimentos do estuário do Rio Coreaú (Ceará) para avaliação do impacto da carcinicultura. Dissertação (Mestrado). Universidade Federal do Paraná Instituto de geociências, programa de pós-graduação em Geologia e Geoquímica. 61 p.
Bhadha JH, Jawitz JW (2010). Characterizing deep soils from an impacted subtropical isolated wetland: implications for phosphorus storage. J. Soils Sediments 10:514-525.
Coradi CP, Pereira RO, Fia R, Matos AT (2009). Qualidade da água superficial da bacia hidrográfica da lagoa Mirim. Rev. de Ciênc. Ambientais. Canoas 3(1):53-64.
Correl DL (1999). Phosphorus: A Rate Limiting Nutrient in Surface Waters. Poult. Sci. Maryland 78:674-682.
Dick WA, Tabatabai MA (1977). Determination of orthophosphate in aqueous solutions containing labile organic and inorganic phosphorus compounds. J. Environ. Qual. Madison 6:82-85.
Diel M, Castilhos RM, Sousa RO, Valh LC, Silva JB (2007). Nutrientes na água para irrigação de arroz na Região Sul do Rio Grande do Sul, Brasil. Ciên. Rural. Santa Maria 37(1):102-109.
Gatiboni LC, Kaminski J, Rheinheimer DS, Flores JP (2007). Biodisponibilidade de formas de fósforo acumuladas em solo sobre sistema plantio direto. Rev. Bras. Ciênc. do Solo. Viçosa 31:691-699.
He Z, Honeycutt CW (2005). A Modified Molybdenum Blue Method for Orthophosphate Determination Suitable for Investigating Enzymatic Hydrolysis of Organic Phosphates. Communications in Soil Science and Plant Analysis. Abingdon 36:1373-1383.
Hedley MJ, Stewart JWB, Chauhan BS (1982). Changes in the inorganic and organic phosphorus fractions induced by cultivation practices and by laboratory incubation. Soil Sci. Soc. Am. J. Madison 46:970-976.
Kerr JG, Burford MA, Olley JM, Stuart E, Bunn ES, Sudy J (2011). Examining the link between terrestrial and aquatic phosphorus speciation in a subtropical catchment: The role of selective erosion and transport of fine sediments during storm events. Water Res. 45:3331-3340.
Monbet P, Mckelvie ID, Worsfold PJ (2010). Sedimentary pools of phosphorus in the eutrophic Tamar estuary (SW England). J. Environ. Monit 12:296-304.
Murphy J, Riley JP (1962). A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27:31-36.
Olsen SR, Sommers LE (1982). Phosphorus. In: Page AL, Miller RH, Keeney QR (Eds.) Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. 2. ed. SSSA. Madison pp. 403-430.
Pellegrini JB (2005). Fósforo na água e no sedimento na microbacia hidrográfica do arroio Lino - Agudo – RS. Disertação (Mestrado em Ciência do Solo) - Curso de Pós-graduação em Ciência do Solo, Universidade Federal de Santa Maria, Santa Maria. Santa Maria 98p.
Pettersson K, Istvánovic V (1988). Sediment phosphorus in Lake Balaton forms and mobility. Arch. Hydrobiol. Suppl. 30:25-46.
Ranno SK (2007). Estimativa da disponibilidade de fósforo para a cultura do arroz irrigado em solos do RS. Disertação (Mestrado em Ciência do Solo) - Curso de Pós-graduação emCiência do Solo, Universidade Federal de Santa Maria, Santa Maria 159 p.
Rheinheimer DS, Anghinoni I (2003). Accumulation of soil organic phosphorus by soil tillage and cropping systems in subtropical soil. Commun. Soil. Sci. Plant Anal. 34:2339-2354.
Rheinheimer DS, Gatiboni LC, Kaminski J (2008). Fatores que afetam a disponibilidade do fósforo e o manejo da adubação fosfatada em solo sob sistema plantio direto. Ciênc. Rural Santa Maria 38(2):576-586.
Rheinheimer DS (2000). Dinâmica do fósforo em sistemas de manejo de solos. Tese (Doutorado em Ciência do Solo). Universidade Federal do Rio Grande do Sul, Porto Alegre. 210 p.
Ritterbusch B (1988). Estudio Limnologico del Lago Ypacarai. Rev. de la Asociación de Cienc. Nat. del Litoral 19(1):11-26.
Schenatto RB (2009). Dinâmica do fósforo de sedimentos de uma pequena bacia hidrográfica de cabeceira. Disertação (Mestrado em Ciência do Solo) - Curso de Pós-graduação em Ciência do Solo, Universidade Federal de Santa Maria. Santa Maria 105 p.
Sharpley AN, Smith SJ, Naney JW (1987) Environmental impact of agricultural nitrogen and phosphorus use. J. Agric. Food Chem. 35(5):812-817.
Silva FAS, Azevedo CA (2009). Principal Components Analysis in the Software Assistat-Statistical Attendance. In: World congress on computers in agriculture, 7, Reno-NV-USA: Am. Soc. Agric. Biol. Eng. P. 1.
USDA (1972). Soil Conservation Service.Soil Survey Investigations.Washington, (Report, n.1) 63 p.
Vahl LC (1999). Fertilidade de solos de várzea. In: Gomes, A. S.; Pauletto, E. A. (eds.) Manejo do solo e da água em áreas de várzea (1999). Pelotas: Embrapa Clima Temperado pp. 119-162.
Zhang JZ, Guo L, Ficher C (2010). Abundance and chemical speciation of phosphorus in sediments of the Mackenzie riverDalta, the chukchi seat and the Bering sea: Importance of Detrial apatite. Aquatic Geochem. 16(3):353-371.
Zhou Q, Gibson CE, Zhu Y (2001). Evaluation of phosphorus bioavailability in sediments of three contrasting lakes in China and the UK. Chemosphere 42:221-225.
Zhu Y, Zhang R, Wu F, Qu X, Xi F, Fu Z (2013). Phosphorus fractions and bioavailability in relation to particle size characteristics in sediments from Lake Hongfeng, Southwest China. Environ. Earth Sci. 68:1041-1052.

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