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Review

## Integrated crop-livestock farming system for sustainable agricultural production in Nigeria

Ezeaku, I. E. \*, Mbah, B. N., Baiyeri, K. P. and Okechukwu, E. C.

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Integrated crop-livestock is advocated to be very promising in boosting food productivity and soil fertility in Nigeria owing to its numerous synergistic benefits as outlined in this review. Particularly, the system will be very suitable in the savannah regions of the country where livestock production is predominant. Although integrated crop-livestock farming has been in existence in these regions, it is typically cereal based-livestock system resulting in perennial depletion of soil fertility. Introduction of legumes into the system to form cereal-legume-livestock system can function as a key integrating factor through improvement of soil fertility, provision of healthy protein in the human diet and fodder for livestock consumption. This paper therefore, aimed to review the benefits of introduction of legumes like cowpea into the long existing cereal based livestock farming in Nigeria, particularly in the savannah regions of the country in order to boost food security and income of the farmers. The review showed that incorporation of cowpea into the system will in greater measure increase the overall food productivity, ensures sustainability of the soil fertility and substantially improves the income of the farmers in the country.

**Key words:** Cowpea fodder, soil fertility, food security, nutrient cycling.

### INTRODUCTION

Agriculture in West Africa is intensifying in response to increasing population of humans and livestock. Consequently, increased productivity demand is placed upon integrated crop-livestock systems and more emphasis is on the roles of legumes such as cowpea and groundnut (Tarawali et al., 2003). Legumes can function as a key integrating factor in intensifying crop-livestock farming systems through supply of healthy plant protein in the human diet, fodder for livestock, and bringing nitrogen into the farming system through nitrogen fixation (Sanginga et al., 2003).

In this context, cowpea for example, has a wide role in contributing to food security, income generation, and the maintenance of resource base for millions of small scale farmers who grow it in the region. Raising goats, sheep and cattle for human consumption is the dominant form of livestock production by smallholder farmers in Nigeria. Besides providing animal products, livestock offer a means to store wealth and a form of insurance in the absence of properly functioning financial institutions (Moll, 2005). The introduction of livestock in the predominantly cereal-based system of the Guinea

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may stimulate farmers to increase the area cropped with legumes, breaking the cycle of continuous cereal cultivation with its negative impact on soil fertility and the control of biotic pressures (Alvey et al., 2001; Bagayoko et al., 2000). Legume haulms represent high-valued feed for ruminants and their presence on or near the farm could increase the profitability of legume cultivation. The cultivation of dual-purpose legumes such as groundnut, soybean and cowpea for the purpose of providing both edible grain and animal fodder, has rapidly grown in popularity among farmers of the Guinea savannah (Sanginga et al., 2003).

Integrated crop-livestock farming system offers opportunities to promote organic agriculture; and carry-over of carbon and nutrients from one cropping season to the next. In northern guinea savannas of Nigeria, almost all above ground biomass disappears from the field during the dry season but the common practice among farmers in collecting plant residues, feeding them to ruminants over the period of dry season, and returning the manure of the ruminants to the field at subsequent planting helps to reduce carbon and nutrient losses (Franke et al., 2008).

This paper considers the imperatives of agricultural intensification, benefits of integrated crop-livestock including nutrient cycling and soil fertility restoration with particular reference to the features of the Nigerian savannas where these scenarios are prominent. Also highlighted in the review are the potential roles that cowpea play in the system as well as strategies to optimize cowpea fodder yield.

## **CHARACTERIZATION OF THE REGION AND OF THE CROP AND LIVESTOCK PRODUCTION**

Annual rainfall is less than 1000 mm with a growing period of 180 days or less meaning that much of the region experiences a longer (7-9 months) harsh dry season. The growing period shortens on a south-north axis. The sandy soil which is prevalent in this region is generally poor with low organic carbon and cation exchange capacity, and is also deficient in nutrients, especially nitrogen and phosphorus. Cropping is cereal based with maize, sorghum and millet dominating, and the maize and sorghum decreasing in prominence towards the north. Intercropping cereals with grain legumes is common in over 90% of fields, with cowpea and groundnut being the most common legume components. Both grain and residues from cropping and the ruminant livestock are important components of the farming system. Cattle, sheep, goats and to a lesser extent camels, provide milk, meat, traction, manure and cash (Bagayoko et al., 2000).

Farm sizes in Nigeria for example are generally small, ranging from about 3 to 6 ha; each field is usually 1 ha or less and one farmer rarely owns contiguous fields

(Ogungbile et al., 1999). A typical cropping pattern shows that at the onset of the rainy season, cereals are sown in rows with wide inter row spacing. Two-three weeks later, a grain type cowpea (short duration) is sown in alternate inter row spacing, followed by a fodder type cowpea (or dual purpose, late maturing) in the remaining inter rows about three weeks later. Cereals will be harvested first, together with the grain type cowpea. Grain type cowpea will give a reasonable grain yield, but virtually no crop residue (Tarawali et al., 2003).

The remaining dual-purpose/fodder type of cowpea is left to grow over the rest of the field, until the rain ceased and the leaves begin to show signs of wilting. At this stage, any grain on the plants is harvested, and the residue is cut and rolled up for storage on house roofs or in tree forks. The stored residue is fed to ruminants during the dry season, or in some cases, sold in local market leading to substantial contribution to farmer's income during this period of scarcity. The leaves of cereal stalks are stripped off after harvesting and fed to ruminants, while the stalks are used for building or as fencing materials. Ruminants within farm compounds are supplemented with the cowpea or groundnut residues and manure is collected with household wastes. At the start of the next cropping season, the "compost" of manure and house hold wastes are spread on the crop fields before land preparation (Singh et al., 2003).

## **OPPORTUNITIES AND CHALLENGES OF CROP-LIVESTOCK INTENSIFICATION**

In sub-Saharan Africa, the population may reach 1.2 billion by 2025, with a demographic shift from about 30% urban population to at least 50% (Tarawali et al., 2004). These changes will mean an increasing demand for crops and livestock and even if production expands at the rate of 3% annually, it is likely that at least 21% of the children, about 39 million, will remain under nourished (ILRI, 2000). Some studies have indicated that through changes in dietary requirements in response to urbanization (Ehui et al., 1998), livestock demand in particular is likely to increase dramatically, from an increase of 2.5% for mutton, pork, and poultry to 4.2% for beef between 1993 and 2020 (Delgado et al., 1999).

Within sub-Saharan Africa, more than 40% of the current regional population is in West Africa (FAO, 2000), meaning that the opportunities and challenges presented by the intensification scenario will be heightened in the region. One of the responses of farming systems to agricultural intensification is the integration of crop and livestock production (McIntire et al., 1992). As crop farmers seek to increase production, their cropping activities spread into marginal land, fallow periods become reduced or absent, and consequently, the demand for nutrient inputs is raised. In the absence of reliable and cheap supplies of inorganic fertilizers, manure

from transhumant livestock becomes more important. At the same time as livestock keepers enlarge their herds, crop residues from crop farmers increasingly become the major feed resources because there is no longer marginal or fallow land for grazing. Estimates have shown that ignoring crop residues as feed resources would result in serious feed shortages (Naazie and Smith, 1997). Fodder scarcity owing to climate change is already triggering conflict between herd's men and settled farmers in various parts of Nigeria (APS, 2008).

In these scenarios, crop farmer may begin to have their own livestock for ready access to manure and simultaneously sell off some of the marginal lands to livestock keepers, who settle and begin crop farming, using the manures from their animals (and possibly traction) as an input (Okike et al., 2001). In the dry savannas of West and Central Africa, integrated crop-livestock is already a common feature of the farming system. For example, the dry savannas consisting of the drier part of the northern Guinea Savannas, plus the Sudan Savannas representing more than 50% of the total land area of sub-Saharan Africa, with a significant proportion located in West Africa are practicing crop-livestock integration. Over 40% of the total ruminant livestock in West and Central Africa are in this region (Winrock, 1992).

Major constraints to agricultural productivity in the region include the long dry season, which results in crop stress due to drought at the beginning and, end of the wet season and a shortage of ruminant fodder during the harsh, dry period. The capture and storage of excess rainfall and the use of resource-efficient irrigation remain the only guaranteed means of maintaining cropping intensification. Other strategies that can increase water productivity directly or have indirect water saving benefits include reducing soil evaporation through use of cover crops and conservation agriculture practices, planting more water-efficient crop varieties, enhancing soil fertility to increase yields per unit of water utilized, decreasing runoff from cultivated land, reducing crop water requirements through microclimatic changes, reusing wastewater for agricultural purposes. Currently, about 2 million hectares are irrigated by reused wastewater (Knox et al., 2012). Conservation agriculture, precision-irrigated agriculture and the resulting improved water productivity require specialized tools and equipment; incentives are needed to ensure that these inputs are adopted in areas where the expansion of commercial agricultural is desirable (Pretty et al., 2006). The poor soils and incidences of pests and diseases also have negative effects on crop production (both grain and fodder). Moreover, inputs such as fertilizers and pesticides to counteract these negative forces are generally scarce or priced well above the means of the small holder farmers.

In the dry savannas, crop and livestock enterprises are closely integrated, with reciprocal benefits from crop residues as fodder for livestock, while livestock provide

manure and traction that contribute directly to crop production. The mixed crop-livestock farming systems currently contribute to over 50% of the world's meat and over 90% of milk (ILRI, 2000). This system is recognised to have the greatest potential for intensification (De Haan et al., 1997). Increasing food demand due to expanding population places increased pressure on these systems to raise productivity.

In some cases, where production of mixed farming system has intensified, the full implications of use of non-leguminous crops have not been considered, as for example, soil is mined and severely degraded and livestock waste products become less in quality (Delgado et al., 1999). In this context, the situation in the dry savannas of West Africa, where integrated crop and livestock production systems have existed for many decades, but now face the pressure to produce more is ripe for interventions that address these opportunities. Cowpea which can contribute both to crop-livestock production system and directly to soil fertility through better quality manure has the potential to make major contribution to the system.

## **CONTRIBUTIONS OF COWPEA TOWARDS INCREASED AND SUSTAINABLE PRODUCTIVITY IN MIXED SYSTEMS**

### **Soil fertility attributes**

As legume, cowpea can contribute to soil fertility, mainly through its nitrogen fixing abilities. Part of the nitrogen fixed will remain in the soil in the roots, and thereby contribute to the soil fertility for subsequent crops. Some nitrogen fixed in the crop will eventually return to the soils as manure after residues are fed to livestock. In terms of the direct effects of cowpea in rotation with cereals, Manu et al. (1994) reported a comparison of on-station and on-farm studies in Niger where cowpea-millet intercrop and cowpea-millet rotations were used. On farmer's field, rotation with cowpea gave 2.6 times more millet grain and 3.3 times more residues, than the intercropped, non-rotated treatment. Bagayoko et al. (2000) reported that cowpea can supply 35 to 40 kg N ha<sup>-1</sup> in a cowpea-millet rotation, and Carsky and Berner (1995) presented similar figures for cowpea rotation with maize.

In addition to the direct benefits of improved livestock production and health that results from feeding cowpea fodder, the quantity and quality of manure from such better fed animals will be improved and therefore, when returned to the soil at the beginning of the growing season, contribute more towards the maintenance of soil fertility. Schlecht et al (1995) reported that the manure nitrogen in gramme nitrogen/tropical livestock unit/day (g /TLU/ day) was on average 25% higher in animals receiving supplements. Feed quality affects nitrogen and phosphorus quality of manure with dietary legume addition



having the potential to improve manure quality. Inclusion of cowpea and other feeds could be expected to alter dietary nitrogen relative to the needs of rumen microbes and the ruminant animal itself. The addition of small levels of cowpea to a diet of cereal stover could be expected to meet the needs of rumen microbes to improve digestion and intake (De Haan et al., 2010). Completely replacing cereals stover with cowpea haulms as the forage source with wheat bran increases dietary nitrogen well above the needs of both the rumen microbes and the animal. This diet would likely result in excess nitrogen excretion by the animal. Because of the relatively high availability of nitrogen, it is expected that much would be excreted in the urine rather than faeces. Combining cereal stover with legumes, grains and or by product feeds creates diets which match nitrogen needs for growth while supplying energy for good rates of gain and other soil nutrients and reducing excessive nitrogen that might lead to negative environmental consequences (Koralagama et al., 2008).

### Weed dynamics

There is some evidence that cowpea may help to reduce the number of viable *Striga hermonthica* seeds in the soil through stimulating suicidal germination of the seed. *S. hermonthica* is parasite on cereal plants, and causes huge crop losses (Berner et al., 1996). Carsky and Berner (1995) reported that rotation with selected cowpea varieties has a substantial and rapid effect on reducing *S. hermonthica*, with the number of attached *Striga* plants per maize plant being reduced by at least 50% when maize was grown after cowpea. The potential impact of reduced *S. hermonthica* population because of rotation with cowpea will result in better soil fertility arising from higher stover of cereals which will ultimately be converted into manure. Farmers' awareness of the roles of cowpea for soil fertility and *S. hermonthica* reduction is to some extent, demonstrated by the fact that they usually interchange the legume and cereal rows each year, and the cereal will benefit at the "micro level" from the cowpea grown in the previous year.

The system affect weed composition and its occurrence by changing the pool of management practices applied to the area, which will change the nature and amount of resources available for weeds, and help excluding from the system those weed species highly specialized in exploring a single or a few environmental resources, leaving room for less specialized and more flexible plant species (Gurevitch et al., 2009), which are usually not troublesome weeds. Understanding not only the level of occurrence but also the composition of the weed community under different cropping system is important to achieve efficient weed control. Management systems with low soil disturbance allow formation of a more diverse weed seed bank in soil. Rotation of crops with

livestock help to break weed cycles thus reducing production costs and environmental risks posed by the use of agro-chemicals (Germani et al., 2015).

### Animal feed and animal production

Cowpea residue is one of the most nutritious fodder resources for ruminant livestock (Tarawali et al., 1997). Farmers in the dry savannas deliberately grow cowpea varieties and use management practices/cropping systems which favor forage production, even at the expense of grain production (Steiner, 1982). Harvesting at the end of the wet season, before the dry season becomes severe, gives the best quality fodder, and this is preserved throughout the storage period. If the fodder is harvested late, when dry season is already underway quantity is reduced, and quality becomes poorer (Tarawali et al., 1997).

Cowpea fodder as a feed supplement increases animal live weight gain during the dry season. Schlecht et al. (1995) reported an experiment where Zebu cattle (bulls of about 250 kg live weight) were supplemented with 1 kg cowpea hay at night and 0.5 kg fresh rice feed meal in the morning per day per animal during the second half of the dry season. The animals were allowed to graze as usual for the rest of the day. It was found that the supplemented group gained 95 kg compared to 62 kg for the unsupplemented group. In many regions, cowpea fodder is particularly valued as a supplement for fattening livestock in the period leading up to Muslim festivals when sheep are traditionally slaughtered. Most farmers sell cowpea fodder during the dry season when feed shortage is critical and there is the belief that income from fodder sales is comparable to that of grains there by contributing substantially to farmer's annual income (ICRISAT, 1991).

From 1 ha of improved cowpea, a farmer could benefit by an extra 50 kg meat per annum from better nourished animals, with over 300 kg more cereal grain as a result of improved soil fertility directly from the cowpea and better manure from the animals (Tarawali et al., 1997). Other benefits include, better fed traction animals would work harder, timely land preparation and better crop yields. Relwani et al. (1970) recommended the use of cowpea in combination with cereals for lactating cows, to maintain milk yields of 5 L day<sup>-1</sup>. Better fed ruminants would give more milk and are likely to be more productive (that is, increased weight gains mean that young animals will come into oestrus earlier). Providing more nutritious fodder also means that the comparatively indigestible parts of cereals (stalk) that are used as fodder are likely to be better consumed. Intake of more fibrous materials usually improves with the addition of better quality material to the diet.

Livestock systems reaching the highest levels of intensification may begin to show an increasing reliance

on forage grown specifically as livestock feed. Cowpea can also play a role in these systems and forage-type cowpeas have been tested and used for both grazing and hay production. Researchers in Florida (Foster et al., 2009) have tested cowpea forage as a supplement for sheep fed bahiagrass hay compared to other legume hays or soybean meal. Cowpea hay had lower crude protein concentration than groundnut hay but sheep gained between 32 and 51 g/d when cowpea was supplemented with soybean (Chakeredza et al., 2002).

### Crop production

Bhatti et al. (1983) recommended forage cowpea for use in Pakistan, recording dry-matter yields of 5.7 Mg ha<sup>-1</sup> for the best variety. Dry matter yields can be positively associated with days to flower. The longer vegetative period, the more forage was produced (Tyagi et al., 1978). The number of leaves and branches were positively correlated with green fodder yield (Ram et al., 1990). In Australia cowpea is regarded primarily as a fodder crop with grain harvest being an exception (Tarawali et al., 1997). Imrie and Butler (1983) found that seed yield is positively correlated with forage yield in determinate cowpea accessions. In eastern and southern Africa cowpea is grown for human consumption of its leaves and beans, whereas in West Africa cowpea fodder plays a major role in the drier areas. Singh et al. (1997) reported that early and medium maturing varieties yielded higher grain but lower fodder than late maturing and fodder-type cowpea varieties which yielded 5 Mg ha<sup>-1</sup> of fodder and less grain. This informed the farmer's practice of growing different cowpea varieties for grain and fodder production.

Only a limited number of studies have reported the specific variety of cowpea used and animal response which have been found to differ with cowpea variety and its associated forage quality (Anele et al., 2010). Singh et al. (2003) reported higher weight gain in rams supplemented with the cowpea haulms of variety IT90K-277-2 compared to Dan Ila. Akinlade et al. (2005) reported increased milk yield in cows supplemented with cowpea haulms of variety IT96D-716 compared to 994-DP. Residues of cereal crops are generally nutritionally inadequate to produce high yields of meat and milk. The greater nutritional quality of legume residues allows them to be used as a supplement to livestock diets based on cereal stover and other low-quality forage. One benefit of the use of cowpea and other legume fodders as a supplement is the provision of nitrogen to the rumen microbes, allowing them to improve utilization of the low quality forage. Energy intake is improved by both the addition of a higher energy feed (cowpea) and by increasing the availability of energy through increased digestibility of the lower quality forage (Baloyi et al., 2006).

### CONCLUSION AND RECOMMENDATIONS

Integrated crop-livestock is frequently advocated as one of the most promising solutions to soil fertility decline and productivity losses in intensifying systems in Nigeria. Crop residues make up a major component of livestock diets in mixed crop-livestock systems and, therefore, improving the use and nutritional quality of crop residues is important to enhancing farm productivity and profitability. Residues of cereal crops are generally nutritionally inadequate to produce high quality and quantity manure, meat and milk. Introduction of legume fodder as a supplement not only provide nitrogen to the rumen microbes, allowing them to improve utilization of the low quality forage but also increase the availability of energy through increased digestibility. Development of dual purpose cowpea varieties that better feed both human and livestock will give farmers new and better choices for improving levels and efficiency of livestock production. Farming practices that encourages rotation between cereals and legumes as well as application of manure are recommended strategies which will turn around the fortune of agriculture in the region, and hence improve the livelihoods of the ever increasing populations now afflicted by climate change. The entire system is currently being threatened by unpredictable climate challenge. The capture and storage of excess rainfall and the use of resource-efficient irrigation remain the only guaranteed means of maintaining cropping intensification. By restoring soil fertility and reducing weed population yields increases to a much greater extent at both farm and regional levels than by using purchased agro-inputs. Increased livestock productivity in terms of weight gain, milk production and traction make the system not only profitable but also supply the protein requirements of the ever increasing urban populations. Furthermore, one way of tackling the incessant clashes between Fulani cattle rearers and settled farmers in Nigeria could be through the promotion of integrated crop-livestock concept among Fulani herders.

### Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

## Effect of potassium doses fertirrigated in the nutritional contents of tomato fruit and leaves in their early development

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Tomato is the most commonly consumed and marketed vegetables in the world and one of the plants that respond more to fertilization, and particularly in demanding potassium, which is responsible for stomata opening, sugar translocation, protein synthesis and enzymatic activation. Analysis of nutrients contents made available to the plant and its relationship with what is present in plant tissue is a great alternative for the correct handling of mineral nutrient solutions. The objectives of this study are to evaluate the influence of increased K doses on the growth of tomato and its relationship with other nutrients. For the study, two experiments of fertirrigated tomatoes in pots were performed with sand as substrate, and five different doses of potassium, grown in a greenhouse. Nutritional content in the fruit in early development and leaf immediately above each bunch was evaluated. Data were submitted to polynomial regression analysis to the second degree. Results showed that increased potassium levels significantly influenced K, S and N contents. Increasing potassium doses significantly affect nutritional contents of the plant tissue.

**Key words:** Plant tissue, nitric, nitric-perchloric perchloric digestion, *Lycopersicon esculentum* Mill.

### INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) originated in South America and is one of the most consumed vegetables in the world; it occupies second place in Brazil in economic importance, second only to potatoes (Paula Júnior and Venzon, 2007). It is consumed fresh or dried in the forms of sauces, pastes, jams and juices.

It is mainly produced in greenhouses by providing production conditions throughout the year, adjusting cropping seasons to market requirements. As an

expensive structure that needs an intensive production to have expected financial return, such a system is subject to an increase in the number of pathogens that affect production over time.

In any form of cultivation, the use of technology to reduce costs and increase profit margin is a great alternative to producers. Tomatoes cultivated directly on the ground are often contaminated with pathogens and salinated by intensive fertilization. But, tomato produced

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in a greenhouse using fertirrigated vases are free from such hazard; thus, it is a viable alternative to prevent the proliferation of pests and diseases. In addition, fertigation provides nutrients in adequate quantities for plant growth.

Tomato is among the highest vegetables in nutrients and one of the species that best responds to high doses of chemical fertilizers; nutrient absorption by tomato plants is low until the appearance of the first flowers (Alvarenga, 2000).

All nutrients are fundamental in plant nutrition, but Potassium (K) is the most absorbed nutrient and required by tomato crop. It has an important role in its development and production, acting in the synthesis of carotenoids, particularly lycopene, responsible for red fruit, and also in the biosynthesis, organic acids, sugars and vitamin C (Marschner, 1995).

Knowledge about the nutritional content of plants is important to assess the ability of removal of nutrients for each crop, and the amount that must be provided at every stage of cultivation to ensure high yields. Tracking the nutrient content and especially K in fertirrigated cultivation is of paramount importance for nutritional supplementation according to crops' needs during their cycle.

This study aims to evaluate the influence of increased K doses absorption and nutritional content of tomato fruit and leaves during its development.

## MATERIALS AND METHODS

For the analysis, two experiments were conducted in a greenhouse: arch type, with 6 m wide, 30 m long, 3 m high and transparent polyethylene cover. The samples were analyzed in the Laboratory of soils from the State University of Londrina, Londrina, PR, (latitude 23° 23'S, longitude 51° 10'W, at an altitude of 580 m).

These experiments consisted of increased K rate in tests with two varieties of fertirrigated tomato grown in pots with sand. They were done in a randomized block design with five treatments and 10 repetitions, totaling 50 pots side by side and 60 cm between rows, with borders around and fertirrigated drip.

Plants were grown in plastic pots with 9 L of capacity (23.5 × 26 × 19.5 cm), using sand as coarse substrate. The sand test results were obtained:  $H^+ + Al^{3+} = 1.89 \text{ cmolc dm}^{-3}$ ; organic matter = 0 g  $\text{dm}^{-3}$ ;  $K^+ = 0 \text{ cmolc dm}^{-3}$ ; Mehlich P = 0.02  $\text{cmolc dm}^{-3}$ ;  $Mg^{+2} = 1.44 \text{ cmolc dm}^{-3}$ ;  $Ca^{+2} = 0.29 \text{ cmolc dm}^{-3}$  and  $Al^{3+} = 0.08 \text{ cmolc dm}^{-3}$ .

Tomato seedlings used for the first experiment were Pizzadoro type (E1) and for the second experiment, Carina (E2) from commercial vivarium certificates that were transplanted to pots with 25 to 30 cm on March, 23<sup>rd</sup> 2013 and terminated on July 29<sup>th</sup> 2013.

The pest control was performed with preventive measures and the following insecticides were applied from the start of cultivation: Cypermethrin (pyrethroid), one  $\text{ml}^{-1}$ , every 15 days; large fruit borer (*Helicoverpa zea*) and Dipel® (biological), one  $\text{ml}^{-1}$  once a week for tomato leafminer (*Tuta absoluta*). The fungicides applied from the reproductive stage were: Chlorothalonil, five  $\text{mL L}^{-1}$  once a week for early blight, septoria and powdery mildew; and Amistar Top®, one  $\text{ml}^{-1}$  once every 15 days also to early blight.

The experiments were conducted with five treatments consisting of five concentrations of K in the nutrient solution (60, 120, 180, 240 and 300  $\text{mg dm}^{-3}$  K). It was applied after the opening of the first flowers, at 29 days after transplanting. Up to this stage of development, the nutrient solution was standard for all treatments.

These doses were established from prior knowledge of the mean dose of K which is recommended for tomato (180  $\text{mg dm}^{-3}$ ). From this information, it was decided to test doses, starting from 60 to 300  $\text{mg dm}^{-3}$ .

The doses were of essential nutrients ( $\text{mg dm}^{-3}$ ): N: 198, P: 43.6, C: 152.4, Ca 233, Mg: 27 S: 39; the followings were used as fertilizers: MAP (200 g  $1000\text{L}^{-1}$ ); Ca (NO<sub>3</sub>)<sub>2</sub> (800 g  $1000\text{L}^{-1}$ ); CaCl<sub>2</sub> (300 g  $1000\text{L}^{-1}$ ); MgSO<sub>4</sub> (300 g  $1000\text{L}^{-1}$ ) and KNO<sub>3</sub> (400 g  $1000\text{L}^{-1}$ ). Micronutrients were supplied through REXOLIN BRA® (11.6% K<sub>2</sub>O, 1.28% S, 0.86% Mg, 2.1% B, 0.36% Cu, 2.66% Fe, 2.48% Mn, 0.036% Mo and 3.38% Zn) and REXOLIN M48® (65% to chelated Fe EDDHMA); both at a concentration of 25 g  $1000\text{L}^{-1}$  (Table 1).

The concentration of each nutrient present was monitored by periodically measuring the electrical conductivity of the solution in water tanks and the resulting eluviation. The nutrient solution was passed through the pot and retained on the plate below it, leaving the system conductivity to exceed three  $\text{dS m}^{-1}$  which could adversely affect plant development. When the conductivity exceeded three  $\text{dS m}^{-1}$ , fertigation was ceased and the system was irrigated for one day only with water to prevent salinization of the system.

The fertigation system consisted of submersible pumps; it has an operating pressure of up to 1.9 mca and power of 38 watts, model AT 203 Atman® in water tank with 80 L capacity for each treatment. The pumps were connected to a timer, driven by a contactor to avoid damage due to oscillation of the amperage.

The fertilizer applications were made through irrigation water with variable frequency so that the losses did not exceed 10% by irrigation interval. Each dripper was set for maximum flow of 300  $\text{ml min}^{-1}$ . The irrigation interval was defined based on climatic conditions - temperature, relative humidity, which were measured inside the greenhouse during the experiment, with datalogger Instrutherm® ht-500 model. The characteristics of culture range from 1 to 5 times a day shift.

Average monthly temperatures were 33.6°C on 27 to 31 March; 26°C in April; 27.5°C in May; 23.8°C in June and 27.9°C in July 29. The relative humidity was 42% in 27 to 31 March; 64.4% in April; 57% in May, 72.6% in June and 54.1% in July 29.

Plants were conducted with three tomato bunches, and after the third bunch, five leaves were counted and plants were pruned to cut the apical dominance. After the onset of fruiting, a green fruit in each repetition, the basal part of the bunch, with a diameter between four and five centimeters were collected.

The leaf immediately above the same cluster was also collected for analysis and both suitably packaged in kraft paper bags and labeled. They were kept under air forced circulation stove at 65°C for three days and then ground in mill type Willey.

All collected plant materials were prepared for determination of nutrients. They were obtained by sulfuric acid digestion N content using Kjeldahl microdestilador method (Bremner and Keeney, 1965). Certain nutrients and P content underwent nitroperclórica digestion. Phosphomolybdate was reduced by ascorbic acid (Braga and Defelipo, 1974; Blanchar et al., 1965), S turbidimetrically sulfate, K photometrically flame and Ca, Mg and micronutrients were reduced by atomic absorption spectrophotometry.

The results of nutritional contents of leaves and fruits of the crop were submitted to polynomial regression analysis to the second degree to verify the influence of increased K doses on them.

## RESULTS AND DISCUSSION

Results show that the behavior of nutrients in the two varieties of tomato was noticed at the beginning of fruit development (Tables 2 and 3). There was significant increase in K levels and reduction in N, P, Fe and Zn in



**Table 1.** Nutrient concentration (mg dm<sup>-3</sup>) of the nutrient solutions and electrical conductivity (EC) (dS m<sup>-1</sup>) of the nutrient solutions used in the treatments.

Nutrient	Complete solution (100%)*	Treatments containing K (mg dm <sup>-3</sup> ) and of the nutritive solution Conductivities ***					
		Solution * (70%)**	60 (2.07)***	120 (2.14)***	180 (2.15)***	240 (2.26)***	300 (2.42)***
N	198	138.6	198.0	198.0	198.0	198.0	198.0
P	43.6	30.52	43.6	43.6	43.6	43.6	43.6
K	152.4	106.68	60.0	120.0	180.0	240.0	300.0
Ca	233	163.1	233.0	233.0	233.0	233.0	233.0
Mg	27	18.9	27	27	27	27	27
S	39	27.3	39	39	39	39	39
B	0.5	0.35	0.5	0.5	0.5	0.5	0.5
Fe	5	3.5	5	5	5	5	5
Cu	0.07	0.049	0.07	0.07	0.07	0.07	0.07
Mn	0.1	0.07	1	1	1	1	1
Mo	0.075	0.0525	0.075	0.075	0.075	0.075	0.075
Zn	0.4	0.28	0.4	0.4	0.4	0.4	0.4

\* Sarruge (1975) modified and used in the UEL Soils Laboratory. \*\* Nutrient solution used for 15 days for adaptation of seedlings. \*\*\* Means of electrical conductivity (EC) measured in the nutrient solution water tanks (dS m<sup>-1</sup>).

**Table 2.** Levels of nutrients in g kg<sup>-1</sup> and micronutrients mg kg<sup>-1</sup> in early fruit development of the first, second and third bunch of Experiment 1 with Pizzadoro tomato.

	mg.dm <sup>-3</sup>	N	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn
		g.kg <sup>-1</sup>						mg.kg <sup>-1</sup>			
1 <sup>st</sup> bunch	60	39.22	11.74	54.84	3.58	2.88	5.48	21.38	396.41	115.32	54.27
	120	40.16	16.77	83.50	3.37	2.79	6.09	24.76	233.40	127.32	46.38
	180	38.96	12.72	78.21	3.39	2.85	6.15	21.44	213.75	89.75	31.36
	240	36.39	13.43	71.54	3.10	2.74	6.43	24.57	255.94	168.35	42.89
	300	36.55	11.60	75.66	3.34	2.65	5.90	29.60	159.71	88.33	46.43
	CV(%)	6.49	18.97	19.48	38.38	16.80	22.19	27.62	62.05	82.77	20.94
	p>F*	*	*	*	ns	ns	ns	ns	*	ns	*
2 <sup>sd</sup> bunch	60	32.95	7.91	65.88	3.17	1.92	4.01	11.53	140.29	128.80	29.08
	120	32.34	10.57	69.17	3.47	2.07	4.08	15.93	185.76	98.36	38.80
	180	29.44	9.58	70.64	4.36	2.01	5.06	16.52	178.72	113.28	75.78
	240	30.39	10.45	72.64	4.02	2.23	5.42	16.94	118.53	167.14	69.50
	300	29.50	9.60	74.39	4.50	2.01	5.79	18.69	144.66	129.24	77.28

**Table 2.** Contd.

	CV(%)	7.63	17.99	12.48	41.82	20.06	16.41	27.23	50.43	44.01	28.66
	p>F*	*	*	ns	ns	ns	*	*	ns	ns	*
	60	52.39	9.89	54.88	3.53	1.82	4.69	11.23	175.96	86.53	48.50
	120	44.03	9.50	66.00	3.29	1.96	5.59	8.50	220.81	59.68	29.07
	180	29.11	10.00	67.62	3.27	2.30	5.45	6.72	186.62	69.13	25.20
	240	34.15	9.60	70.20	2.97	2.04	5.82	6.55	256.02	84.20	24.58
3 <sup>th</sup> bunch	300	34.42	9.29	73.32	3.16	1.79	6.01	2.34	280.20	69.58	20.86
	CV(%)	5.79	20.70	7.77	38.74	22.73	11.01	17.44	75.35	30.60	29.42
	p>F*	*	ns	*	ns	ns	*	*	ns	ns	*

\*: Significant data to 5% by regression testing. ns: not significant data for 5% by regression testing.

**Table 3.** Levels of nutrients in g kg<sup>-1</sup> and micronutrients in mg kg<sup>-1</sup> in early fruit development of the first, second and third bunch of Experiment 2, tomato Carina.

	mg.dm <sup>-3</sup>	N	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn
		g.kg <sup>-1</sup>						mg.kg <sup>-1</sup>			
	60	33.49	12.25	67.16	2.95	2.84	6.84	23.46	168.14	82.08	48.84
	120	30.83	11.31	80.88	3.73	3.01	7.14	22.26	240.55	76.46	48.73
	180	44.71	11.11	78.64	2.65	2.95	8.11	22.75	179.65	34.62	45.90
	240	36.38	10.59	82.17	5.03	2.79	8.21	22.77	149.85	20.28	41.51
1 <sup>st</sup> bunch	300	33.28	10.98	88.59	5.14	2.93	8.79	27.57	280.54	41.29	40.50
	CV(%)	6.52	18.27	16.59	41.04	13.74	18.52	33.30	50.86	44.34	18.27
	p>F*	*	ns	*	*	ns	*	ns	*	*	ns
	60	33.37	8.96	50.46	1.66	1.92	3.90	10.09	161.73	93.49	34.64
	120	41.93	10.57	69.62	2.09	2.53	4.29	8.63	185.76	109.77	39.19
	180	36.68	9.74	74.29	5.90	2.49	4.98	15.20	176.24	110.72	76.67
	240	35.54	10.40	81.39	1.86	2.17	3.83	13.72	118.53	101.80	70.20
	300	38.79	9.44	84.22	1.35	2.13	4.48	12.84	132.29	118.88	76.14
2 <sup>sd</sup> bunch	CV(%)	11.40	20.57	14.48	152.52	16.14	15.34	47.85	61.59	24.48	28.74
	p>F*	*	ns	*	ns	*	*	ns	ns	ns	*
	60	36.42	9.60	59.51	3.51	1.61	5.43	7.27	158.51	93.53	20.60
	120	32.38	7.62	65.09	3.25	1.71	5.58	10.76	224.71	78.65	21.54
3 <sup>th</sup> bunch	180	29.66	10.29	69.32	3.24	2.19	5.85	11.65	207.44	83.14	30.30

Table 3. Contd.

240	30.83	8.80	76.00	2.94	2.11	5.85	12.15	111.68	64.24	19.40
300	30.50	7.69	80.53	3.13	1.56	6.34	14.24	130.97	67.22	20.64
CV(%)	8.35	13.92	7.39	37.77	11.93	9.20	15.05	53.29	25.68	21.34
p>F*	*	*	*	ns	*	*	*	*	*	*

\*: Significant data to 5% by regression testing. ns: not significant data for 5% by regression testing.

the fruits of the first bunch in line with an increase of K levels in the nutrient solution in E1 (Table 2). For the second bunch, there are increases in the levels of P, S, Cu and Zn and reduced levels of N. In the third bunch, there was an increase in the levels of K and S and reduced N, Cu and Zn (Table 2).

E2 consisted of the fruits of the first bunch; there was significant increase in K, Ca, S and Fe and decrease in Mn content and quadratic fit for the contents of N. In the second bunch, there was an increase in levels of N, K, Mg, and Zn.

For the third bunch, there was an increase in content K, S and Cu quadratic fit for P and Mg and Zn, and reduction in content of N, Fe and Mn (Table 3). As they are immature fruits, in the beginning of development; they still transpire and fully absorb nutrients; also, as there are some immovable or slightly mobile nutrients in the plant, there is an initial fructification period when the greatest increase of concentration in the fruits occurs.

The slightly mobile nutrients are transported by the xylem and this type of transport only occurs while these fruits still have the permeable membrane, which enables the transpiration. This fact can result in the increased concentration of immobile or slightly mobile nutrients, as the Ca is absorbed in large quantities in this period; consequently it can inhibit the absorption of other nutrients, such as Mg, Zn, and Cu, as they compete for the same absorption sites. But as

soon as the membrane becomes impermeable, the Ca concentration is diluted in the fruit and the concentration of other nutrients increases, becoming balanced again. It is important to emphasize that the dosages of K in the experiments did not harm the nutritional concentration of the fruits.

Prado et al. (2011), in the research work on mineral absorption in growing "Raissa" tomato plants in a hydroponic culture, also uncovered a decreased concentration of Cu and B in fruits as time went on, a quadratic adjustment for Ca, Mn, and Zn and increased concentrations of N, P, K Mg, and S.

The accumulation of P by the organs that compose the fruit branch began to increase after the twelfth week while sprouting. This was accompanied by the increase of the dry mass by the fruits and this was also observed in the studies by Clark and Smith (1990). Miller et al. (1979) reported that in field conditions phosphorus absorption rate was higher in the time interval from 84 to 98 days after transplantation.

K constituted the increased quantity of absorbed macro-nutrient. A proportional growth in the increase of the dosages of nutritive solution was observed when analyzing the accumulation curve of this nutrient, throughout the development cycle in the fruits. For the Ca in our experiments, there was an increase in the beginning of the development and afterwards a decrease. Clark and Smith (1990) verified in the persimmon fruit

that there was a significant accumulation during the initial development and 12 weeks after pollination; approximately 88% of the final contents had been acquired, while only 35% of the dry mass had accumulated during the same period.

For the nutritional concentrations of the leaves above each cluster in E1, for "Pizzadoro" tomato, there was an increase in K dosages, a decrease in the concentrations of N and Ca and a quadratic increase for the concentrations of P (Table 4).

There was an increase in the concentrations of S and Mn. The values found in the leaves above the first cluster coincide with those found by Fernandes et al. (2002). In the leaves collected above the second cluster, there was an increase in the concentrations of N, K, Cu, and Mn, as well as a decrease in the concentrations of Ca. This coincides with Fernandes et al. (2002) for N, P, and S and also with Raji et al. (1996) for the other analyzed nutrients.

The leaves above the third cluster displayed a decrease in the concentrations of N, P, Ca, and Mn and an increase in the concentrations of K, S, and Cu. This is in line with Fontes et al. (2000) in the concentrations of K and Ca, and also with Ribeiro et al. (1999), who worked on the analyses of the leaf and petiole opposed to the third cluster, in N and Mg and Fernandes et al. (2002) for the other nutrients (Table 4).

In E2 consisting of "Carina" tomato, when the nutritional concentrations were analyzed for the

**Table 4.** Levels of macronutrients in g kg<sup>-1</sup> and micronutrients in mg kg<sup>-1</sup> of the leaves just above the first, second and third cluster at the beginning of fruit development of Experiment 1, tomato Pizzadoro along with reference levels (Londrina, 2014).

	mg.dm <sup>-3</sup>	kg.ha <sup>-1</sup> *2					mg.kg <sup>-1</sup>				
		N	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn
1 <sup>st</sup> bunch	60	32.81	13.34	27.64	40.49	5.04	16.24	21.65	474.37	568.32	78.85
	120	35.87	15.97	50.25	38.69	5.25	18.96	19.08	500.87	578.26	56.83
	180	34.59	12.24	52.05	32.70	5.17	19.38	18.83	403.76	578.00	37.81
	240	33.16	14.23	63.12	20.71	5.37	23.38	20.58	445.69	726.49	75.02
	300	32.03	12.62	65.08	19.96	5.05	23.99	19.02	395.54	786.93	76.62
	CV(%)	5.07	20.30	17.48	30.79	25.20	6.51	22.25	24.54	12.04	12.85
	p>F*	*	*	*	*	ns	*	ns	ns	*	*
2 <sup>sd</sup> bunch	60	33.83	9.77	30.97	34.67	3.00	11.87	21.11	480.89	655.78	38.76
	120	32.17	10.63	35.01	36.54	3.05	14.95	24.75	511.55	693.80	40.50
	180	29.82	10.68	37.69	30.12	2.71	14.41	28.55	556.70	697.06	35.70
	240	32.28	10.55	48.84	19.27	2.93	16.43	41.33	542.06	900.83	49.94
	300	34.30	9.57	48.41	18.76	2.73	14.65	40.39	608.33	839.39	48.56
	CV(%)	14.32	13.76	25.17	32.66	21.21	23.42	28.83	16.91	22.43	32.76
	p>F*	ns	ns	*	*	ns	ns	*	ns	*	ns
3 <sup>th</sup> bunch	60	34.59	16.71	27.73	33.83	3.22	19.48	8.63	368.31	796.51	48.80
	120	36.39	15.32	49.69	36.10	3.13	17.05	10.52	417.39	619.11	38.60
	180	32.71	11.13	56.04	28.83	3.09	18.78	12.05	419.81	599.08	30.32
	240	31.81	11.62	61.09	18.26	2.90	21.07	14.38	279.48	775.85	61.73
	300	31.35	11.33	83.50	17.59	2.78	22.89	15.77	295.88	731.96	46.48
	CV(%)	5.49	24.53	11.93	32.49	13.91	10.99	13.41	41.03	20.72	23.65
	p>F*	*	*	*	*	ns	*	*	ns	*	*
Fontes (2000)		56	3.1	47-70	31.6	8.4	9.8	798	183	258	25
Ribeiro (1999)		26-40	5.9	91-80	27.4	4.9	-	41	66	103	134
Raij et al. (1996)		40-60	4-8	30-50	14-40	4-8	3-10	5-15	100-300	50-250	30-100
Fernandes (2002)		32	13	51	45	9	18	10	209	665	96

\*: Significant data to 5% by regression testing. ns: not significant data for 5% by regression testing.

first leaf above the first cluster, a quadratic relationship was observed based on the increase of the dosages of K for N and P (Table 5).

There was an increase in the concentration of K

and S and a decrease in the concentrations of Ca, based on the increase in the dosages of K, coinciding with Fernandes et al. (2002). There was a reduction in the concentrations of N, Ca,

and Fe, an increase in the concentrations of K, S, and Mn and a quadratic response for the Cu in the leaves of the second cluster. The results coincide with Fernandes et al. (2002) for N, P, and S and

**Table 5.** Levels of macronutrients in g kg<sup>-1</sup> and micronutrients in mg kg<sup>-1</sup> of the leaves just above the first, second and third cluster at the beginning of fruit development of the Experiment 2, tomato Carina, with reference levels (Londrina, 2014).

	mg.dm <sup>-3</sup>	N	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn	
		kg.ha <sup>-1</sup> *2						mg.kg <sup>-1</sup>				
1 <sup>st</sup> bunch	60	34.47	11.27	46.39	40.45	4.51	11.62	20.59	503.35	505.46	82.54	
	120	29.69	18.11	50.66	38.66	5.40	21.13	19.16	527.44	512.44	62.03	
	180	36.70	11.95	62.53	34.86	5.12	20.42	18.71	420.51	530.13	53.85	
	240	34.99	14.15	63.06	24.73	5.33	23.29	19.05	416.23	733.52	75.09	
	300	34.22	12.83	64.92	19.94	5.01	21.33	17.22	303.04	683.60	76.81	
	CV (%)	7.71	25.06	18.52	34.28	24.67	13.24	24.83	28.51	20.04	13.53	
	p>F*	*	*	*	*	ns	*	ns	*	*	*	
2 <sup>sd</sup> bunch	60	43.00	12.44	43.52	33.51	2.71	6.66	6.08	363.29	637.95	79.26	
	120	37.49	10.02	65.55	36.50	3.01	12.42	10.00	359.91	564.44	83.66	
	180	27.20	11.27	71.35	31.90	2.35	12.26	11.24	253.71	603.37	93.08	
	240	32.34	10.21	73.39	20.41	2.77	17.70	11.56	167.69	785.45	58.81	
	300	31.89	9.68	75.50	18.74	2.88	18.57	7.86	153.07	874.04	58.02	
	CV (%)	10.70	27.78	16.50	32.88	26.38	24.06	41.50	38.83	27.30	28.06	
	p>F*	*	ns	*	*	ns	*	*	*	*	*	
3 <sup>th</sup> bunch	60	36.53	11.37	25.87	33.79	2.39	12.59	10.21	273.88	745.53	30.21	
	120	31.75	10.97	51.21	36.06	2.85	17.27	11.92	388.09	626.03	38.79	
	180	31.24	11.60	63.81	27.64	2.77	23.28	16.64	332.18	696.26	47.74	
	240	30.23	9.78	69.17	18.21	2.97	20.41	15.14	310.74	585.10	35.67	
	300	34.82	11.20	72.15	17.57	2.96	23.05	13.67	181.29	605.54	47.32	
	CV (%)	6.65	14.23	14.62	30.21	18.82	13.46	19.20	30.76	27.13	23.73	
	p>F*	*	ns	*	*	ns	*	*	*	*	*	
Fontes (2000)	56	3.1	47-70	31.6	8.4	9.8	798	183	258	25		
Ribeiro (1999)	26-40	5.9	91-80	27.4	4.9	-	41	66	103	134		
Raij et al. (1996)	40-60	4-8	30-50	14-40	4-8	3-10	5-15	100-300	50-250	30-100		
Fernandes (2002)	32	13	51	45	9	18	10	209	665	96		

\*: Significant data to 5% by regression testing. ns: not significant data for 5% by regression testing.

with Raij et al. (1996) for all the other analyzed nutrients (Table 5).

When the leaves were analyzed right above the third cluster, there was a decrease in the

concentration of N and Mn and an increase in the concentration of K, S, and Zn. There was a quadratic response for Ca and Fe. The results coincide with Fontes et al. (2000) and the

concentrations of K and Ca, and with Ribeiro et al. (1999), who worked on the leaf and petiole opposite to the third cluster, in N and Mg and Fernandes et al. (2002) for all the other nutrients.

When analyzed jointly, the behavior of the nutrients above the three clusters, with increased dosages of K, an increased concentration of Mn was verified in the leaves of the first and second cluster in both experiments and a decrease in the leaves of the third cluster. This behavior was mainly attributed to the excess of K that caused Mn deficiency, as confirmed by Silva et al. (1995) and Carvalho et al. (2001) in the study on the increase of the dosages of K in passion fruit plant. It was also verified the reduction in the concentrations of Ca based on the increase of the dosages of K in the leaves above all the clusters in both experiments. This is another case of competition among cations, verified since the beginning of the development of the plant. It coincides with the reports of Mascarenhas et al. (2000) and Oliveira et al. (2001).

According to Malavolta (2006), based on the increase in the concentrations of K in the nutritive solution, there is competition in the absorption of Ca and Mg as during the absorption process, these nutrients use the same loading sites.

There was a quadratic response in the leaves of the first cluster and the concentration of Cu increased in the leaves of the second and third cluster of E1 in both experiments on the concentration of P. There was quadratic behavior in the leaves of the same clusters in E2, based on the increase in the dosages of K. This fact is related to the decrease in the concentrations of Ca whose increase leads to a decrease in the concentrations of Cu.

A decrease in the concentrations of N was also observed in the leaves above the third cluster in both experiments. This is attributed to the increase in the dosages of K that caused an increase in the synthesis of proteins in the plant, consuming the N for the synthesis of proteins. Due to the increase in the synthesis of proteins, an increase in the concentration of S in the leaves was also observed, above the three clusters, in both experiments. This led to a high demand for this macro-nutrient because of the increase of the dosages of K.

The increase in the dosages of potassium significantly influenced the nutritional concentration in the vegetable tissue, as there were increases in the concentrations of K and S in the fruits and K, Mn, and Cu in the leaves, with decreased concentrations of Ca, in both experiments.

## Conflict of Interests

The authors have not declared any conflict of interests.

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## Full Length Research Paper

## Comparison of stability methods in elephant-grass genotypes for energy purposes

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Elephant grass is a plant of tropical origin with high biomass-production potential that stands out today as an alternative energy source. The potential of its genotypes depends on the genotype × environment interaction. The objective of this study was to estimate the genotype × environment interaction and compare stability methods in elephant grass for biomass production in a biannual cutting regime. The experiment was conducted in a randomized block design with two replicates and evaluations of 73 elephant-grass genotypes in six cuts. The trait dry matter yield was utilized for the analysis of the genotype × environment interaction and the stability. The stability analysis methods employed were those of Yates and Cochran, Plaisted and Peterson, Wricke, Annicchiarico, Lin and Binns, and Huehn. Kang and Phan's ranking was adopted for all the methods. Spearman's coefficient was utilized to evaluate the degree of agreement between the different methods employed. Significant differences were observed for the genotype × environment interaction. Non-parametric Lin and Binns' and Annicchiarico's methods were more discriminating than the analysis of variance methods in the evaluation of stability and productivity of the tested genotypes.

**Key words:** *Pennisetum purpureum*, energy alternative, biomass production, genotype by environment interaction.

### INTRODUCTION

In the last decades, energy demand has become a global problem, and the search for alternative energy sources is ever increasing (Rossi et al., 2014). Because the biomass combustion recycles the CO<sub>2</sub> taken from the atmosphere by photosynthesis, in the long term, it will be

one of the energy alternatives to overcome the environmental crisis and the dependence on oil faced by the world today (Morais et al., 2009).

Elephant-grass species has desirable qualitative traits with regard to its percentage of fiber, this fiber's

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components (cellulose, hemicellulose, and lignin) and the carbon-to-nitrogen (C:N) ratio of these materials, because the higher this ratio is, the better the plant is for combustion. These features validate it as an alternative energy source (Morais et al., 2009). The breeding of elephant grass has always aimed at forage qualities, high protein contents, and low fiber levels, which requires a change in the selection of genotypes of this species for use as a source of bioenergy (Flores et al., 2013).

The genotype  $\times$  environment interaction (G  $\times$  E) is one of the greatest challenges faced by breeders of any species. Among the alternatives to optimize it, in the phase of selection or recommendation of cultivars, is the choice of varieties with high adaptability and good stability (Cruz et al., 2012). Therefore, different methodologies should be applied for safer genotype recommendations (Peluzio et al., 2010).

Several methods, based on different principles, have been described to evaluate the G  $\times$  E interaction and to determine the phenotypic stability of cultivars. Among the most commonly used methods are those based on analysis of variance, the non-parametric, and the regression-based ones. In those based on analysis of variance, the stability estimates are expressed in quadratic components, whereas those based on non-parametric statistics evaluate the performance of each genotype in relation to the maximum response of each environment. Lastly, in the regression-based methods, the dependent variable is expressed as a function of an environmental index that measures the quality of the evaluated environments (Cruz et al., 2014).

This study aimed to estimate the genotype  $\times$  environment interaction (biannual cuts) and compare stability methods based on analysis of variance and non-parametric methods in elephant grass for biomass production in a biannual cutting regime.

## MATERIALS AND METHODS

### Location and characterization of the experimental area

This experiment was conducted in an area belonging to the partnership between Centro Estadual de Pesquisas em Agroenergia and Aproveitamento de Resíduos, from PESAGRO-Rio, in Campos dos Goytacazes-RJ, Brazil, and the State University of Norte Fluminense "Darcy Ribeiro" (UENF), located at 21°19'23" S and 41°19'40" W, at an altitude varying from 20 to 30 m. The climate of the region is classified as a hot wet tropical, Aw Köppen type, with annual precipitation around 1,152 mm (Köppen, 1948). The soil is classified as a Yellow Latosol, and the analysis showed the following characteristics: pH 5.5; phosphorus (mg dm<sup>-3</sup>) 18; potassium (mg dm<sup>-3</sup>) 83; Ca (cmolc dm<sup>-3</sup>) 4.6; Mg (cmolc dm<sup>-3</sup>) 3.0; Al (cmolc dm<sup>-3</sup>) 0.1; H + Al (cmolc dm<sup>-3</sup>) 4.5; and C (%) 1.6.

### Design and genotypes evaluated

The experimental design was organized as randomized blocks with two replicates. The plot was formed by one 5.5 m row spaced 2 m apart, totaling 11 m<sup>2</sup>. Each replicate contained 73 elephant-grass

genotypes from the Active Germplasm Bank (AGB) of UENF (Table 1). Planting was on February 23 and 24, 2011, using whole stems arranged with the base touching the apex of the other plant, distributed into the furrows at the rate of two per furrow. After the establishment phase, on December 15, 2011, all treatments were cut near the soil level (plot-leveling) and another planting was made concomitantly to reduce flaws in the planting rows. The environments consisted of six cuts that were made in June 2012, December 2012, August 2013, February 2014, August 2014, and February 2015. The evaluated characteristic was dry matter yield (DMY) per cut, in t.ha<sup>-1</sup>. Shortly after, two tillers were collected and placed in a 5 kg paper bag to be dried in an oven at 65°C for 72 h, until they reached constant weight (air-dried sample). The dried material (leaf and stem) was ground in a Wiley mill with 1-mm sieve and conditioned in a plastic bottle. Next, the samples were dried again in an oven at 105°C for 12 h (oven-dried sample).

During fertilization at planting, each row received 60 g of single superphosphate, and 50 days after planting, each row was top-dressed with 70 g urea and 40 g potassium chloride (KCl), corresponding to 28.6 kg nitrogen (N) and 24 kg potassium oxide (K<sub>2</sub>O) per hectare. This topdressing was also performed after each one of the evaluation cuts. The fertilization practices adopted were based on the results of the soil chemical analyses and recommendations for culture in Rio de Janeiro State.

### Statistical analyses

The computer resources of the GENES program version 1.0 were used for the genetic-statistical analysis (Cruz, 2013). The analysis of variance for the evaluated trait was conducted based on the average of the plots considering all effects random (random model), employing the following statistical model:

$$Y_{ij} = \mu + g_i + b_j + \varepsilon_{ij},$$

where  $Y_{ij}$  is phenotypic value of observation  $ij$  referring to genotype  $i$  in block  $j$ ;  $\mu$  is the overall constant of the trait;  $g_i$  is the effect of genotype  $i$ ;  $b_j$  is the effect of block  $j$ ; and  $\varepsilon_{ij}$  is the average experimental error.

In the case of perennial plants, the combined analysis of variance is performed based on the performance of some harvests (cuts). The statistical model, according to Steel and Torrie (1996), is given by:

$$Y_{ijk} = \mu + G_i + B_j + \varepsilon_a + C_k + \varepsilon_b + GC_{ik} + \varepsilon_c$$

where  $Y_{ijk}$  is the observed value relative to genotype  $i$  in block  $j$  in cut  $k$ ;  $\mu$  is the overall constant of the trial;  $G_i$  is the random effect of genotype  $i$ ;  $B_j$  is the effect of block  $j$ ;  $\varepsilon_a$  is the effect of error associated with genotype  $i$  in block  $j$ ;  $C_k$  is the random effect of cut  $k$ ;  $\varepsilon_b$  is the effect of error  $b$  associated with block  $j$  in cut  $k$ ;  $GC_{ik}$  is the effect of the interaction between genotype  $i$  and cut  $k$ ; and  $\varepsilon_{ijk}$  is the effect of error  $c$  associated with genotype  $i$  in block  $j$  in cut  $k$ .

### Stability methodologies

The stability methods adopted were based on analysis of variance and non-parametric.

**Table 1.** Genotypes present in the Active Germplasm Bank (AGB) of elephant grass of UENF, in Campos dos Goytacazes-RJ, Brazil, 2015.

Genotype	Identification	Genotype	Identification
1	Elefante da Colômbia	38	T 241 Piracicaba
2	BAGCE 2	39	BAGCE 51
3	Três Rios	40	Elefante Cachoeiro Itapemirim
4	Napier Volta Grande	41	Capim Cana D'África
5	Mercker Santa Rita	42	Gramafante
6	Pusa Napier N° 2	43	Roxo
7	Gigante de Pinda	44	Guaçu/I.Z.2
8	Napier Goiano	45	Cuba-115
9	Mercker S. E. A	46	Cuba-116
10	Taiwan A-148	47	King Grass
11	Porto Rico 534-B	48	Roxo Botucatu
12	Taiwan A-25	49	Mineirão IPEACO
13	Albano	50	Vruckwona Africano
14	Pusa Gigante Napier	51	Cameroon
15	Elefante Híbrido 534-A	52	BAGCE 69
16	Costa Rica	53	Guaçu
17	Cubano Pinda	54	Napierzinho
18	Mercker Pinda	55	IJ 7125
19	Mercker Pinda México	56	IJ 7136
20	Mercker 86 México	57	IJ 7139
21	Napier S.E.A.	58	Goiano
22	Taiwan A-143	59	CAC 262
23	Pusa Napier N° 1	60	Ibitinema
24	Elefante de Pinda	61	Australiano
25	Mineiro	62	13 AD
26	Mole de Volta Grande	63	10 AD IRI
27	Porto Rico	64	07 AD IRI
28	Napier	65	Pasto Panamá
29	Mercker Comum	66	BAGCE 92
30	Teresópolis	67	05 AD IRI
31	Taiwan A-46	68	13 AD IRI
32	Duro de Volta Grande	69	03 AD IRI
33	Mercker Comum Pinda	70	02 AD IRI
34	Turrialba	71	08 AD IRI
35	Taiwan A-146	72	BAG 86
36	Taiwan A-121	73	BAG 87
37	Vruckwona	-	-

**Yates and Cochran's (traditional) method (1938)**

The method consists of the combined analysis of the experiments, considering all environments and the subsequent breakdown of the sum of squares of the environment effects and of the genotype × environment interaction into effects of environments within each genotype. The genotypes that show the lowest  $\theta_i$  values are the most stable. Its estimator is:

$$MS(E/G_i) = \frac{r}{a-1} \left[ \sum_j Y_{ij}^2 - \frac{(Y_i)^2}{a} \right]$$

where  $Y_{ij}$  is the mean of genotype  $i$  ( $i = 1, 2, \dots, g$ ) in environment  $j$  ( $j = 1, 2, \dots, n$ );  $r$  is the number of replicates associated with the genotype; and  $a$  is the total number of environments.

**Plaisted and Peterson's (1959) method**

The method proposed by Plaisted and Peterson (1959) quantifies the relative contribution of each genotype to the G × E interaction and identifies those of highest stability.

The estimate was obtained by the following expression:

$$\theta_i = \frac{\sum_i \alpha^2 g a_{ii'}}{g-1} \text{ with } i \neq i'$$

in which:

$$\alpha^2 g a_{ii'} = \frac{SS_{(G_{ii'} \times A)}}{r} RMS,$$

where

$$SS'(g'_{ii'} \times A) = \frac{r}{2} \left[ d^2_{ii'} \frac{1}{a} (y_i - y_{i'})^2 \right] \quad \text{and}$$

$$d^2_{ii'} = j (y_{ij} - y_{i'j})^2 \quad (j=1, 2, \dots, n).$$

$n$  is the number of environments.

The relative contribution of each genotype was calculated as follows:

$$\theta_i (\%) = \frac{\theta_i \times 100}{g \alpha^2_{ga}}$$

#### Wricke's (1964) method

The ecovalence ( $\omega_i$ ) or stability of genotype  $i$  is given by:

$$\omega_i = \left[ Y_{ij} - \bar{Y}_i \bar{Y}_j + \bar{Y}_{..} \right]^2,$$

where  $Y_{ij}$  is the mean response of genotype  $i$  in environment  $j$ ;  $\bar{Y}_i$  and  $\bar{Y}_j$  are the mean deviations of genotypes and environments, respectively; and  $\bar{Y}_{..}$  is the overall mean.

Thus, genotypes with low  $\omega_i$  values have lower deviations in relation to the environments and are more stable.

#### Annicchiarico's (1992) method

Annicchiarico's method is based on the so-called genotypic confidence index, estimated by:

$$I_{i(g)} = \mu_{i(g)} - Z_{(1-\alpha)} \sigma_{zi(g)}$$

Considering all environments, where  $\mu_{i(g)}$  is the average percentage of genotypes  $i$ ;  $Z_{(1-\alpha)}$  is the percentage of the standard normal distribution function; and  $\sigma_{zi(g)}$  is the standard deviation from the  $Z_{ij}$  values, associated with genotypes  $i$ . The confidence coefficient adopted was 75%, that is,  $\alpha = 0.05$ .

#### Lin and Binns' (1988) method

In this method, the parameter  $P_i$  defines the stability of a genotype and is defined as the mean-squared distance between the mean of a genotype and the mean maximum response for all sites, such that genotypes with lower values correspond to those of better performance. Thus, the estimator is given as:

$$P_i = \sum_{j=1}^n (Y_{ij} - M_j)^2 / 2n$$

where  $P_i$  is the estimate of the stability parameters of genotype  $i$ ;  $Y_{ij}$  is the response of genotype  $i$  in environment  $j$ ;  $M_j$  is the maximum response observed among all genotypes in environment  $j$ ; and  $n$  is the number of environments.

#### Huehn's (1990) method

Huehn (1990) suggested the non-parametric evaluation of phenotypic stability based on the classification of genotypes in each environment, utilizing the principle of homeostasis to characterize the genotype. In this method, a genotype is considered stable if the classification presented by the genotype  $\times$  environment interaction effect is similar. In this case, the parameters that measure the stability ( $S_1$ ,  $S_2$ , and  $S_3$ ) are equal to zero.

The stability parameters were estimated from:

(i)  $S_1$ : means of the absolute differences between the classifications of genotype " $i$ " in the environments, after the removal of the effects of genotypes ( $Y'_{ij}$ ):

$$S_1 = \frac{\sum_{j < j'} [rij - rij']}{a \frac{(a-1)}{2}}$$

where  $r_{ij}$  is the classification of genotype  $i$  in environment  $j$ ;  $r_{ij'}$  is the classification of genotype  $i$  in environment  $j'$ ;  $a$  is the number of environments.

(ii)  $S_2$ : variance of the classifications of genotype  $i$  in the environments, after the removal of the effects of genotypes:

$$S_2 = \frac{\sum_j j (\bar{r}_{ij} - \bar{r}_j)^2}{a-1}$$

where

$$\bar{r} = \frac{\sum_j j r_{ij}}{a}$$

(iii)  $S_3$ : sum of the absolute deviations of each classification, in relation to the average of the classifications, that is,

**Table 2.** Summary of the individual analyses of variance for dry matter yield in 73 elephant-grass genotypes in six cuts.

Cut	MS Block	MS Genotype	MS Residual	Overall mean	CV (%)
1	152.87	105.73*	69.93	22.89	36.53
2	42.23	19.6*	11.83	10.83	31.78
3	247.83	42.91**	21.04	18.36	24.98
4	1.9292	54.17*	35.49	16.85	35.35
5	0.053	55.5**	22.5	16.38	28.97
6	2.17	23.6*	14.82	11.9	32.37
DF	1	72	72	-	-

MS, Mean square; DF, degree of freedom; CV, coefficient of variation. \*\*Significant at the level of 1% probability; \*Significant at 5% probability.

$$S_3 = \frac{\sum_j [r_{ij} - \bar{r}_j]}{\bar{r}_i}$$

By this method, the genotype with maximum stability will express  $S_1$ ,  $S_2$ , and  $S_3$  estimates equal to zero.

**Kang and Phan’s method**

The genotypes were ranked based on the estimators of Yates and Cochran (1938) and Plaisted and Peterson (1959); Wricke’s (1964) ecovalence; Annicchiarico (1992); Lin and Binns (1988); and Huehn (1990).

For the ranking of the genotypes, they were classified in ascending order based on the aforementioned stability estimators, except for Annicchiarico’s method, in which the clones were ranked in descending order, and subsequently descending order, based on the estimates of the dry-matter-yield means. The ranking values of each genotype were summed, generating the sum of classifications, which constituted Kang and Phan’s (1991) estimator.

Thus, the genotypes with the lowest values in the sum of classifications are the most stable and productive.

**Spearman’s correlation coefficient**

Spearman’s correlation coefficient ( $\rho$ ) was utilized to evaluate the degree of agreement between the different methods employed. This approach considers the ranking of the clones according to each one of the parameters of the stability methods.

The expression for the calculation of Spearman’s coefficient is given by:

$$\rho = 1 - \frac{6 \sum_{i=1}^n d_i^2}{n(n^2 - 1)2}$$

where  $\rho$  is the Spearman’s correlation coefficient;  $d_i$  is the difference between the rankings; and  $n$  is the number of ranking parts.

**RESULTS AND DISCUSSION**

The results of the individual analyses of variance detected significant differences at 1 and 5% probability between the genotypes in all the evaluated cuts for dry matter yield. These different performances between the elephant-grass genotypes indicate that there is genetic variability in the Active Germplasm Bank of elephant grass of UENF (Table 2). In the study conducted by Oliveira et al. (2014), *Pennisetum purpureum* genotypes at 12 months of age showed significant differences at 1% probability for dry matter yield. Menezes et al. (2014) also found significant differences in dry matter yield in 40 genotypes of *P. purpureum*.

The experimental coefficients of variation ranged from 24.98 to 36.53%, because dry matter yield is a quantitative trait largely influenced by the environment. In other studies with elephant grass (Oliveira et al., 2014; Rossi et al., 2014), the coefficients of variation were high for dry matter yield: 22.96 to 36.95%.

The coefficient of variation, obtained from the analysis of variance of an experimental trial, indicates its degree of precision. However, the particularities of the studied culture should be considered, and one should especially distinguish the nature of the evaluated trait (Costa et al., 2002). This classification may vary depending on the soil-climatic conditions or reproductive cycle of the culture (Scapim et al., 2010).

The values obtained in the individual analyses of variance (per cut) of dry matter yield in t.ha<sup>-1</sup> resulted in a ratio between the highest and lowest residual mean squares (RMS) of 5.91 (Table 2). This ratio is in agreement with Daher et al. (2003), who evaluated *P. penissetum* in eight environments whose ratio of homogeneity of variances (Hartley’s test) was 4.94, which allowed the inclusion of all environments in the combined analysis.

Pimentel-Gomes and Garcia (2002) commented on the use of the maximum F test, concluding that if the ratio between the highest and the lowest RMS is lower than seven, the combined analysis can be performed with no major problems. However, when this ratio is greater than

**Table 3.** Summary of the combined analysis of variance for dry matter yield in 73 elephant-grass genotypes in six cuts.

Source of variation	DF	RMS
Block	1	199.21
Genotype	72	112.15*
Error A	72	41.33
Cut	5	2843.24**
Error B	5	50.14
Genotype × Cut	360	37.9*
Error C	360	26.2
RMS (highest)/RMS (lowest)	-	5.91
Total	875	-

DF, Degree of freedom; RMS, residual mean square. \*\*Significant at the level of 1% probability; \*Significant at 5% probability.

seven, it is recommended to consider the subgroups of the experiments with not-very-heterogeneous RMS separately.

The combined analysis of variance demonstrated significant effects of cuts ( $p < 0.01$ ), genotypes ( $p < 0.05$ ), and cut × genotype interaction ( $p < 0.05$ ) on dry matter yield, indicating that the genotypes had different performances in the biannual cuts evaluated (Table 3). The significant effect of the genotype × environment interaction indicates inconsistent performance of the genotypes according to the environmental variables. The evaluation of this interaction is essential for plant breeding, because the best genotype in a certain environment may not have the same response in another, so it would be necessary to evaluate the stability of the genotypes.

The genotype × environment interaction is unfavorable to researchers' work because of the magnitudes of differences between the genotypes and cuts, and so the classification of the genotypes is changed with the cuts (Daher et al., 2003). Thus, a more detailed study of the performance of genotypes in view of these variations was undertaken by stability analysis.

The stability parameter of Yates and Cochran's method is shown in Table 4. This methodology indicated genotypes 8, 14, 70, 15, 45, 58, 43, 17, 30, and 62 as the 10 most stable genotypes of the evaluated group. On the other hand, the corresponding classifications of these genotypes concerning the mean in the six evaluation cuts were not satisfactory (70th, 34th, 41st, 62nd, 20th, 59th, 71st, 17th, 57th, and 47th), corroborating Cruz et al. (2014) assumption that genotypes with a consistent response in a number of environments are, in general, not very productive.

The evaluation of genotype performance stability by Plaisted and Peterson (1959) method (based on analysis of variance) demonstrated that, because they showed lower values for the estimate of  $\theta$  (%), the 10 most stable genotypes were 63, 41, 6, 2, 21, 49, 33, 30, 57 and 7 in

ascending order of magnitude.

According to this method, in general, there was no agreement between stability and productivity, that is, the most productive genotypes were not necessarily the most stable. Daher et al. (2003), who evaluated 17 clones of elephant grass for forage production, stated that the stability estimates of Plaisted and Peterson also proved that there was no agreement between stability and productivity; in other words, the most productive genotypes were not necessarily the most stable ones.

Wricke's method considers the genotype with the lowest estimate of  $w_i$  (%) as the most stable, similarly to Plaisted and Peterson. The conclusions were identical for both stability methods.

The results of the stability analysis obtained by Annicchiarico's (1992) method indicated genotypes 47, 31, 11, 7, 61, 44, 3, 42, 65, and 32 as superior, with confidence indices higher than 100% when all environments were considered, which shows that they have good stability, with a predictable response in different cuts.

The methodology of Annicchiarico (1992) expresses the genotypic stability, facilitating the decision-making process (Cruz et al., 2014). Considering the dynamics and recurrence of the processes in breeding programs, it is a methodology that can be applied in the moment of determining the permanence or removal of a certain genotype from the program, safely and quickly.

The application of Lin and Binns' (1988) method made it possible to identify individuals with high dry matter yield and phenotypic stability (lower  $P_i$  values). Table 4 shows that genotype 47 is the most adaptable and stable, with the lowest  $P_i$  value in the six cuts, followed by genotypes 31, 11, 44, 65, 32, 54, 7, 46, and 45, with respective increases in dry matter yield. These results agree with Daher et al. (2003), who found an inverse relationship between the stability parameter  $P_i$  and the clones' dry-matter-yield means, indicating the applicability of these stability estimates for the evaluation of perennial-cycle



**Table 4.** Mean values for dry matter yield (M) and the estimates of the methods of Yates and Cochran (YC), Plaisted and Peterson (PP), Wricke (W), Annicchiarico (A), Lin and Binns (LB), and Huehn (HU) with their respective positions (P) for the 73 elephant-grass genotypes (G).

G	M	P	YC		PP		W		A		LB			HU				
			MS	P	$\theta(\%)$	P	wi(%)	P	l(%)	P	Pi	P	S <sup>1</sup>	P	S <sup>2</sup>	P	S <sup>3</sup>	P
1	18.80	19	30.39	18	0.54	33	0.85	33	113.45	11	99.49	20	22.53	32	349.60	34	2.33	29
2	13.89	56	31.81	21	-0.45	4	0.25	4	83.29	52	166.18	50	18.33	24	237.37	22	2.87	45
3	20.02	9	31.14	19	0.09	19	0.58	19	121.67	7	87.02	15	24.40	44	413.07	42	4.26	68
4	17.20	31	475.74	73	17.17	73	11.13	73	81.84	54	127.63	34	15.93	16	301.37	27	1.13	6
5	19.01	16	209.30	71	9.74	72	6.54	72	101.04	29	115.13	25	12.00	7	96.00	7	0.70	4
6	12.96	63	41.34	29	-0.54	3	0.19	3	75.23	61	179.27	60	14.07	11	134.57	11	2.87	46
7	20.83	5	51.26	38	-0.34	10	0.31	10	125.80	4	74.28	8	23.67	39	373.77	38	3.40	59
8	11.40	70	5.67	1	0.69	38	0.95	38	68.77	66	225.06	72	26.07	50	442.70	50	2.56	35
9	13.94	55	55.33	42	1.39	52	1.38	52	79.38	59	176.27	58	24.00	43	440.80	49	2.65	39
10	18.86	18	82.08	52	1.33	50	1.34	50	111.10	15	85.71	14	23.47	38	387.47	39	2.36	30
11	21.51	4	71.34	47	0.72	40	0.97	40	128.75	3	56.74	3	24.80	45	433.47	45	2.61	38
12	15.60	38	53.03	39	-0.20	13	0.40	13	90.14	37	134.84	36	23.33	36	415.47	43	3.48	60
13	17.51	27	193.56	70	4.50	67	3.30	67	96.23	30	101.07	22	35.80	73	856.17	73	3.86	64
14	16.10	34	8.82	2	4.16	66	3.09	66	91.43	35	160.22	45	16.73	19	190.57	18	1.26	9
15	13.04	62	14.10	4	1.56	55	1.49	55	78.27	60	206.10	66	28.80	62	551.87	59	3.19	54
16	13.31	60	28.44	15	-0.32	12	0.33	12	79.45	58	179.63	61	26.40	51	468.00	51	4.23	67
17	18.92	17	17.16	8	4.94	70	3.58	70	104.97	22	92.52	18	8.13	4	49.47	3	0.46	3
18	14.40	50	40.81	27	0.10	21	0.59	21	84.68	50	160.86	48	27.87	57	573.20	62	4.62	71
19	17.19	32	45.79	32	0.31	26	0.72	26	102.20	27	126.53	33	31.80	71	76.14	5	4.95	72
20	13.62	58	87.07	54	0.26	25	0.69	25	75.17	62	166.54	51	23.67	40	398.30	41	2.51	32
21	11.92	67	44.68	31	-0.41	5	0.27	5	69.54	65	198.77	65	13.80	10	141.37	12	2.19	27
22	13.21	61	76.13	50	0.40	29	0.77	29	71.54	64	180.16	62	25.47	48	434.27	46	2.60	37
23	19.46	11	239.95	72	4.93	69	3.57	69	101.35	28	79.17	11	23.00	35	439.37	48	1.83	18
24	10.91	73	53.96	40	0.09	20	0.58	20	61.35	72	222.23	69	17.87	22	242.80	23	2.00	23
25	18.09	23	34.44	23	0.79	42	1.01	42	107.51	18	121.55	30	24.80	46	437.47	47	2.18	26
26	18.04	24	119.05	64	0.92	44	1.09	44	102.26	26	91.49	17	16.40	18	196.27	19	1.41	12
27	11.94	66	65.35	45	0.46	31	0.81	31	65.95	70	197.33	64	29.40	65	619.77	67	3.66	63
28	11.72	68	54.81	41	1.02	45	1.16	45	64.84	71	220.58	68	29.20	63	565.20	61	3.20	55
29	11.57	69	25.45	14	0.44	30	0.80	30	67.88	68	222.57	71	20.47	27	304.57	29	2.51	33
30	13.85	57	18.19	9	-0.35	8	0.31	8	84.43	51	174.21	55	12.40	8	101.87	8	2.31	28
31	22.09	2	75.48	49	0.06	18	0.56	18	132.57	2	53.13	2	16.20	17	209.10	20	1.53	13
32	19.79	10	92.07	57	0.04	17	0.55	17	117.31	10	72.32	6	11.33	5	89.07	6	1.19	7
33	15.05	43	29.06	16	-0.38	7	0.29	7	90.35	36	146.58	38	25.80	49	574.97	64	6.95	73
34	15.12	42	74.83	48	0.57	35	0.88	35	86.17	46	150.12	42	31.20	69	662.67	70	4.17	66
35	14.94	45	144.98	68	5.49	71	3.91	71	80.10	56	160.45	47	13.00	9	116.30	10	0.85	5
36	19.01	15	56.05	44	-0.33	11	0.32	11	113.13	12	87.45	16	27.67	56	532.17	56	4.54	70
37	17.36	29	157.94	69	2.33	62	1.96	62	95.80	31	100.86	21	16.87	20	187.77	16	1.35	11
38	17.87	25	87.66	55	1.41	53	1.40	53	102.87	25	111.51	23	31.07	68	654.67	69	3.60	62
39	13.99	54	41.13	28	0.58	36	0.88	36	80.82	55	175.71	57	18.40	25	269.47	25	1.62	15
40	14.97	44	36.46	26	2.94	63	2.34	63	86.16	47	174.98	56	27.07	53	523.20	54	2.09	25
41	12.90	64	42.46	30	-0.66	2	0.11	2	74.93	63	179.02	59	11.47	6	103.47	9	2.81	42
42	20.12	8	46.02	33	0.11	22	0.59	22	120.48	8	85.63	13	22.73	34	371.90	37	2.96	47
43	11.15	71	17.09	7	0.40	28	0.77	28	66.30	69	226.34	73	23.80	42	395.77	40	3.14	53
44	20.75	6	97.51	59	0.15	23	0.62	23	122.52	6	60.22	4	14.27	12	190.00	17	1.60	14
45	18.73	20	15.33	5	2.21	60	1.89	60	104.23	24	77.79	10	30.00	67	633.20	68	3.35	58
46	19.07	14	141.82	67	1.70	56	1.57	56	109.25	17	77.42	9	28.33	58	529.90	55	2.85	44
47	23.08	1	136.04	66	1.27	49	1.31	49	134.56	1	41.58	1	27.27	55	541.90	57	2.81	43
48	14.05	53	50.55	37	-0.14	14	0.44	14	82.44	53	158.25	43	20.40	26	325.87	31	3.01	49

Table 4. Contd.

G	YC			PP		W		A		LB			Hu					
	M	P	MS	P	$\theta$ (%)	P	wi(%)	P	I(%)	P	Pi	P	S <sup>1</sup>	P	S <sup>2</sup>	P	S <sup>3</sup>	P
49	17.37	28	33.09	22	-0.38	6	0.29	6	105.29	21	116.90	26	17.60	21	221.47	21	3.53	61
50	17.31	30	23.36	12	0.74	41	0.98	41	104.36	23	120.66	29	20.60	28	296.57	26	1.97	22
51	15.51	39	76.28	51	3.32	65	2.57	65	88.11	40	160.37	46	7.87	3	53.07	4	0.42	2
52	14.88	46	35.75	25	1.25	48	1.30	48	87.48	42	168.63	53	29.80	66	606.97	66	3.11	51
53	15.72	37	35.58	24	3.28	64	2.55	64	93.83	32	166.58	52	31.60	70	666.00	71	2.98	48
54	19.28	13	106.01	62	0.55	34	0.86	34	112.41	13	72.78	7	14.80	14	180.67	15	1.34	10
55	18.25	21	29.92	17	-0.02	16	0.51	16	110.60	16	112.70	24	14.47	13	161.77	14	1.94	21
56	15.96	35	83.83	53	0.25	24	0.68	24	92.25	34	120.60	28	26.67	52	518.80	53	4.36	69
57	14.10	52	23.51	13	-0.35	9	0.31	9	85.13	49	165.15	49	21.60	31	323.60	30	3.04	50
58	13.33	59	15.80	6	1.22	47	1.28	47	79.79	57	191.25	63	21.00	29	344.30	33	1.63	16
59	15.93	36	100.39	60	0.69	39	0.95	39	90.00	38	129.99	35	25.20	47	423.20	44	2.67	40
60	17.63	26	31.39	20	0.66	37	0.93	37	106.28	19	124.59	32	22.60	33	339.37	32	2.70	41
61	21.82	3	135.53	65	4.65	68	3.40	68	124.21	5	83.61	12	4.80	1	17.07	1	0.30	1
62	14.75	47	21.16	10	0.81	43	1.02	43	87.99	41	159.59	44	23.73	41	368.27	36	2.08	24
63	14.48	49	46.11	34	-0.78	1	0.04	1	86.41	43	148.83	40	7.73	2	44.27	2	3.13	52
64	16.57	33	93.70	58	0.49	32	0.83	32	93.79	33	122.76	31	29.33	64	592.67	65	3.32	57
65	20.65	7	112.32	63	1.21	46	1.27	46	118.75	9	67.58	5	21.40	30	302.57	28	1.83	19
66	14.24	51	22.86	11	0.33	27	0.73	27	85.31	48	169.09	54	17.87	23	250.27	24	1.74	17
67	18.15	22	70.09	46	2.23	61	1.90	61	105.60	20	119.14	27	14.93	15	148.27	13	1.22	8
68	10.96	72	105.56	61	1.79	58	1.63	58	56.53	73	222.50	70	28.73	61	547.10	58	2.53	34
69	19.38	12	91.81	56	1.88	59	1.68	59	111.29	14	94.70	19	23.33	37	351.47	35	1.86	20
70	15.37	41	10.64	3	1.71	57	1.58	57	86.19	45	145.45	37	28.67	60	573.47	63	2.48	31
71	12.03	65	55.95	43	1.47	54	1.43	54	67.96	67	207.02	67	32.33	72	680.17	72	3.29	56
72	14.72	48	48.77	36	-0.06	15	0.49	15	86.23	44	146.89	39	27.20	54	495.60	52	4.00	65
73	15.46	40	47.03	35	1.36	51	1.36	51	88.89	39	149.87	41	28.53	59	563.20	60	2.56	36

genotypes subjected to successive cuts.

The ability of this parameter to detect the genotypic behavior of clones is based on the use of deviations between the evaluated genotype and the maximum productivity in each environment. Thus, low  $P_i$  values for a given genotype demonstrate that it was near the maximum in the cuts made (Daher et al., 2003).

The results obtained for the stability parameters, according to Huehn's (1990) methodology, for dry matter yield, are shown in Table 4. According to the results, genotype 61 was considered the most stable of all, with the lowest estimate of parameters  $S_1$ ,  $S_2$ , and  $S_3$ , and good classification of the mean in all cuts.

Genotypes 63, 17, 51, 32, and 5 also obtained good parameter estimates for dry matter yield, in which genotype 63 was the second most stable, according to  $S_1$  and  $S_2$ . By this methodology, the genotypes that showed the lowest variance in the ranks are considered the most stable.

The results referring to the ranking method, according to the performance of the genotypes and their respective estimates of the phenotypic-stability parameters, are shown in Table 5.

The stability parameter of Yates and Cochran's method

indicated genotypes 8, 14, 70, 15, 45, 58, 43, 17, 30, and 62 (Table 4) as the most stable and with unsatisfactory means. With Kang and Phan's ranking associated with Yates and Cochran's method (Table 5), these genotypes were better ranked with their means as 35th, 4th, 10th, 25th, 2nd, 24th, 43rd, 1st, 26th, and 16th, respectively. In general, it can be observed that the most stable clones started to occupy the means positions after Kang and Phan's weighting.

The 10 best genotypes resulting from the methodology of Kang and Phan (1991) associated with the methods of Plaisted and Peterson (1959) and Wricke (1964) were 7, 31, 36, 32, 3, 44, 42, 49, 55, and 11. Among them, genotypes 31, 11, 7, 44, and 42 stood out as the most productive. The results for the methods are equal, because they are perfectly correlated with each other.

For Annicchiarico's method, the clones with the highest confidence indices were those of the greatest stability. This method, associated with that of Kang and Phan (1991), did not show alterations in the ranking of genotypes. Therefore, groups 47, 31, 11, 61, 7, 44, 3, 54, and 45 prevailed as the most productive and stable. Thus, both methodologies displayed good agreement in identifying the cultivars of greater stability and dry matter

**Table 5.** Mean values for dry matter yield (M) and estimates of Kang and Phan's method (KP) applied to the methods of Yates and Cochran (KP+YC), Plaisted and Peterson (KP+PP), Wricke (KP+W), Annicchiarico (KP+A), Lin and Binns (KP+LB), and Huehn (KP+HU) with their respective positions (P) for the 73 elephant-grass genotypes (G).

G	M	P	KP+YC	P	KP+PP	P	KP+W	P	KP+A	P	KP+LB	P	KP+HuS <sub>1</sub>	P	KP+HuS <sub>2</sub>	P	KP+HuS <sub>3</sub>	P
1	18.80	19	37	5	52	16	52	16	30	14	39	19	51	21	53	23	48	20
2	13.89	56	77	40	60	20	60	20	108	54	106	54	80	41	78	38	101	53
3	20.02	9	28	3	28	5	28	5	16	7	24	13	53	23	51	20	77	37
4	17.20	31	104	65	104	63	104	63	85	41	65	33	47	16	58	27	37	13
5	19.01	16	87	51	88	48	88	48	45	23	41	20	23	6	23	5	20	4
6	12.96	63	92	58	66	27	66	27	124	62	123	61	74	35	74	36	109	64
7	20.83	5	43	9	15	1	15	1	9	5	13	6	44	14	43	12	64	27
8	11.40	70	71	35	108	68	108	68	136	67	142	70	120	68	120	68	105	60
9	13.94	55	97	61	107	66	107	66	114	57	113	58	98	54	104	58	94	47
10	18.86	18	70	32	68	30	68	30	33	16	32	17	56	26	57	26	48	21
11	21.51	4	51	14	44	10	44	10	7	3	7	3	49	17	49	18	42	16
12	15.60	38	77	41	51	15	51	15	75	37	74	36	74	36	81	42	98	51
13	17.51	27	97	62	94	55	94	55	57	29	49	24	100	57	100	55	91	46
14	16.10	34	36	4	100	61	100	61	69	33	79	38	53	24	52	22	43	18
15	13.04	62	66	25	117	71	117	71	122	61	128	64	124	69	121	69	116	66
16	13.31	60	75	37	72	37	72	37	118	59	121	59	111	64	111	63	127	72
17	18.92	17	25	1	87	47	87	47	39	18	35	18	21	5	20	3	20	5
18	14.40	50	77	42	71	33	71	33	100	50	98	49	107	61	112	65	121	68
19	17.19	32	64	21	58	18	58	18	59	30	65	34	103	60	37	11	104	58
20	13.62	58	112	70	83	45	83	45	120	60	109	55	98	55	99	54	90	44
21	11.92	67	98	63	72	38	72	38	132	65	132	66	77	38	79	39	94	48
22	13.21	61	111	68	90	49	90	49	125	65	123	62	109	63	107	60	98	52
23	19.46	11	83	47	80	43	80	43	39	19	22	11	46	15	59	30	29	9
24	10.91	73	113	71	93	54	93	54	145	72	142	71	95	50	96	50	96	49
25	18.09	23	46	11	65	24	65	24	41	20	53	27	69	31	70	33	49	22
26	18.04	24	88	52	68	31	68	31	50	26	41	21	42	11	43	13	36	12
27	11.94	66	111	69	97	57	97	57	136	68	130	65	131	70	133	72	129	73
28	11.72	68	109	67	113	69	113	69	139	70	136	68	131	71	129	70	123	70
29	11.57	69	83	48	99	59	99	59	137	69	140	69	96	51	98	51	102	55
30	13.85	57	66	26	65	25	65	25	108	55	112	57	65	30	65	31	85	39
31	22.09	2	51	15	20	2	20	2	4	2	4	2	19	4	22	4	15	2
32	19.79	10	67	27	27	4	27	4	20	10	16	8	15	2	16	2	17	3
33	15.05	43	59	17	50	12	50	12	79	38	81	39	92	48	107	61	116	67
34	15.12	42	90	54	77	40	77	40	88	43	84	41	111	65	112	66	108	63
35	14.94	45	113	72	116	70	116	70	101	51	92	47	54	25	55	24	50	23

Table 5. Contd.

36	19.01	15	59	18	26	3	26	3	27	13	31	15	71	33	71	34	85	40
37	17.36	29	98	64	91	52	91	52	60	31	50	26	49	18	45	15	40	14
38	17.87	25	80	44	78	41	78	41	50	27	48	23	93	49	94	49	87	42
39	13.99	54	82	46	90	50	90	50	109	56	111	56	79	39	79	40	69	30
40	14.97	44	70	33	107	67	107	67	91	46	100	51	97	52	98	52	69	31
41	12.90	64	94	59	66	28	66	28	127	64	123	63	70	32	73	35	106	61
42	20.12	8	41	7	30	7	30	7	16	8	21	10	42	12	45	16	55	25
43	11.15	71	78	43	99	60	99	60	140	71	144	73	113	67	111	64	124	71
44	20.75	6	65	22	29	6	29	6	12	6	10	4	18	3	23	6	20	6
45	18.73	20	25	2	80	44	80	44	44	22	30	14	87	44	88	46	78	38
46	19.07	14	81	45	70	32	70	32	31	15	23	12	72	34	69	32	58	26
47	23.08	1	67	28	50	13	50	13	2	1	2	1	56	27	58	28	44	19
48	14.05	53	90	55	67	29	67	29	106	53	96	48	79	40	84	45	102	56
49	17.37	28	50	13	34	8	34	8	49	25	54	28	49	19	49	19	89	43
50	17.31	30	42	8	71	34	71	34	53	28	59	30	58	28	56	25	52	24
51	15.51	39	90	56	104	64	104	64	79	39	85	42	42	13	43	14	41	15
52	14.88	46	71	36	94	56	94	56	88	44	99	50	112	66	112	67	97	50
53	15.72	37	61	19	101	62	101	62	69	34	89	44	107	62	108	62	85	41
54	19.28	13	75	38	47	11	47	11	26	11	20	9	27	7	28	7	23	7
55	18.25	21	38	6	37	9	37	9	37	17	45	22	34	8	35	8	42	17
56	15.96	35	88	53	59	19	59	19	69	35	63	31	87	45	88	47	104	59
57	14.10	52	65	23	61	21	61	21	101	52	101	52	83	42	82	43	102	57
58	13.33	59	65	24	106	65	106	65	116	58	122	60	88	46	92	48	75	34
59	15.93	36	96	60	75	39	75	39	74	36	71	35	83	43	80	41	76	35
60	17.63	26	46	12	63	22	63	22	45	24	58	29	59	29	58	29	67	28
61	21.82	3	68	29	71	35	71	35	8	4	15	7	4	1	4	1	4	1
62	14.75	47	57	16	90	51	90	51	88	45	91	46	88	47	83	44	71	32
63	14.48	49	83	49	50	14	50	14	92	47	89	45	51	22	51	21	101	54
64	16.57	33	91	57	65	26	65	26	66	32	64	32	97	53	98	53	90	45
65	20.65	7	70	34	53	17	53	17	16	9	12	5	37	9	35	9	26	8
66	14.24	51	62	20	78	42	78	42	99	49	105	53	74	37	75	37	68	29
67	18.15	22	68	30	83	46	83	46	42	21	49	25	37	10	35	10	30	10
68	10.96	72	133	73	130	73	130	73	145	73	142	72	133	72	130	71	106	62
69	19.38	12	68	31	71	36	71	36	26	12	31	16	49	20	47	17	32	11
70	15.37	41	44	10	98	58	98	58	86	42	78	37	101	58	104	59	72	33
71	12.03	65	108	66	119	72	119	72	132	66	132	67	137	73	137	73	121	69
72	14.72	48	84	50	63	23	63	23	92	48	87	43	102	59	100	56	113	65
73	15.46	40	75	39	91	53	91	53	79	40	81	40	99	56	100	57	76	36

**Table 6.** Spearman correlations among the stability parameters of the different methods utilized in 73 elephant-grass genotypes.

Parameter	YC	PP	W	A	LB	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	KP+YC	KP+PP	KP+W	KP+A	KP+LB	KP+S <sub>1</sub>	KP+S <sub>2</sub>	KP+S <sub>3</sub>
M	-0.38**	-0.23*	-0.23*	-0.97**	0.97**	0.14 <sup>ns</sup>	0.15 <sup>ns</sup>	0.23 <sup>ns</sup>	0.53**	0.58**	0.58**	0.11 <sup>ns</sup>	0.99**	0.74**	0.74**	0.78**
YC	-	0.30**	0.30**	0.25*	-0.43**	-0.06 <sup>ns</sup>	-0.03 <sup>ns</sup>	-0.21 <sup>ns</sup>	0.55**	-0.08 <sup>ns</sup>	-0.08 <sup>ns</sup>	-0.56**	-0.41**	-0.30**	-0.28*	-0.36**
PP	-	-	1	0.10 <sup>ns</sup>	-0.13 <sup>ns</sup>	0.16 <sup>ns</sup>	0.17 <sup>ns</sup>	-0.45**	0.07 <sup>ns</sup>	0.64**	0.64**	-0.54**	-0.18 <sup>ns</sup>	-0.04 <sup>ns</sup>	-0.03 <sup>ns</sup>	-0.43**
W	-	-	-	0.10 <sup>ns</sup>	-0.13 <sup>ns</sup>	0.16 <sup>ns</sup>	0.17 <sup>ns</sup>	-0.45**	0.07 <sup>ns</sup>	0.64**	0.64**	-0.54**	-0.18 <sup>ns</sup>	-0.04 <sup>ns</sup>	-0.03 <sup>ns</sup>	-0.43**
A	-	-	-	-	-0.94**	-0.13 <sup>ns</sup>	-0.15 <sup>ns</sup>	-0.15 <sup>ns</sup>	-0.64**	-0.68**	-0.68**	0.10 <sup>ns</sup>	-0.96**	-0.71**	-0.73**	-0.71**
LB	-	-	-	-	-	0.15 <sup>ns</sup>	0.14 <sup>ns</sup>	0.17 <sup>ns</sup>	0.46**	0.66**	0.66**	0.12 <sup>ns</sup>	0.99**	0.73**	0.72**	0.72**
S <sub>1</sub>	-	-	-	-	-	-	0.92**	0.70**	0.05 <sup>ns</sup>	0.23 <sup>ns</sup>	0.23 <sup>ns</sup>	-0.04 <sup>ns</sup>	0.14 <sup>ns</sup>	0.76**	0.70**	0.52**
S <sub>2</sub>	-	-	-	-	-	-	-	0.63**	0.09 <sup>ns</sup>	0.25*	0.25*	-0.12 <sup>ns</sup>	0.14 <sup>ns</sup>	0.69**	0.76**	0.48**
S <sub>3</sub>	-	-	-	-	-	-	-	-	0.03 <sup>ns</sup>	-0.21 <sup>ns</sup>	-0.21 <sup>ns</sup>	0.26*	0.19 <sup>ns</sup>	0.61**	0.56**	0.78**
KP+YC	-	-	-	-	-	-	-	-	-	0.45**	0.45**	-0.45**	0.50**	0.37**	0.40**	0.37**
KP+PP	-	-	-	-	-	-	-	-	-	-	1	-0.38**	0.62**	0.54**	0.55**	0.23*
KP+W	-	-	-	-	-	-	-	-	-	-	-	-0.38**	0.62**	0.54**	0.55**	0.23*
KP+A	-	-	-	-	-	-	-	-	-	-	-	-	0.12 <sup>ns</sup>	0.05 <sup>ns</sup>	0.002 <sup>ns</sup>	0.23 <sup>ns</sup>
KP+LB	-	-	-	-	-	-	-	-	-	-	-	-	-	0.74**	0.73**	0.75**
KP+S <sub>1</sub>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.95**	0.85**
KP+S <sub>2</sub>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.82**
KP+S <sub>3</sub>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

\*\*Significant at 1% probability; \*Significant at 5% probability, by the t test. Means for dry matter yield (M), Yates and Cochran (YC), Plaisted and Peterson (PP), Wricke (W), Annicchiarico (A) and Lin and Binns (LB), Huehn (S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub>) and Kang and Phan associated with Yates and Cochran (KP+YC), Plaisted and Peterson (KP+PP), Wricke (KP+W), Annicchiarico (KP+A), Lin and Binns (KP+LB) and Huehn (S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub>).

yield.

Genotypes 47, 31, 11, 44, 65, 32, 54, 7, 46, and 45 stood out as very promising according to Lin and Binns' method. Nevertheless, the association between Kang and Phan's and Lin and Binns' methods led to a slight change in the ranking of 47, 31, 44, 65, 7, 61, 32, 54, and 42, keeping the same positions with high stability and dry matter yield (Table 5). These results indicate that these genotypes showed high stability, and most importantly for elephant-grass breeders, high dry matter yield. Therefore, the non-parametric Annicchiarico's and Lin and Binns's methods, associated with Kang and Phan's method, were efficient in indentifying genotypes with high stability and dry matter yield.

Kang and Phan's (1991) approach, associated with Huehn's method, kept genotypes 61, 31, 32, 17, 44, and 54 in the best positions for stability. Despite the simplicity in obtaining the statistics that evaluated stability, Huehn's (1990) method is criticized for not taking into account the magnitude of the obtained mean values, which is another aspect that stability comprehends, regardless of whether the classification was good or bad. Thus, the statistics will only be useful if the mean performance of the evaluated genotypes is considered simultaneously (Cruz et al., 2014).

The correlations between the different stability methods for the trait dry matter yield, according to Spearman's correlation coefficient (r), revealed statistical significance at 5 and 1% of probability

by the t test, indicating that these methods agree partially (Table 6).

The mean was highly correlated with Lin and Binns' and Annicchiarico's methods, positively and negatively, respectively. Regarding Kang and Phan's (1991) associated method, there was a change in the ranking of Yates and Cochran (1938), in which Plaisted and Peterson (1959), Wricke (1964) and Huehn became positively correlated, but with a low coefficient.

The methods that were highly correlated with each other were Plaisted and Peterson and Wricke (r = 1), and Kang and Phan associated with the latter. Daher et al. (2003) also obtained the same result for dry matter yield in studies on the stability of elephant grass.

Kang and Phan's method associated with Lin and Binns' had high negative and positive correlations, respectively, with Annicchiarico's ( $r = -0.96$ ) and Lin and Binns' methods ( $r = 0.99$ ). The results of the methodology of Lin and Binns and Annicchiarico were similar, which is in agreement with the results obtained by Cunha (2012) regarding the similarity in the recommendation of the genotypes by these methodologies.

Also in the comparison of the estimates of the algorithms of the non-parametric methods, it is observed that Huehn's (1990) parameters  $S_1$ ,  $S_2$ , and  $S_3$  have a high agreement ( $P < 0.01$ ) with each other, and associated with Kang and Phan's (1991). Additionally,  $S_1$  and  $S_2$  ( $r = 0.92$ ) and Kang and Phan's associated with  $S_1$  and  $S_2$  ( $r = 0.95$ ) showed a noteworthy high correlation. Scapim et al. (2010) investigated the correlations between stability parameters of some methods such as those of Huehn (1990) and Kang (1988), aiming to identify the most reliable method to select popcorn cultivars. According to these authors,  $S_1$ ,  $S_2$ , and  $S_3$  were correlated positively and significantly, indicating that only one of these statistics is sufficient for the selection of stable genotypes.

## Conclusion

The genotypes that showed the highest dry matter yields were those of the greatest stability by Lin and Binns' and Annicchiarico's method. These methods displayed a strong association with each other and produced similar genotypic classifications as to phenotypic stability, so it is recommended to use one or the other. Plaisted and Peterson's (1959) and Wricke's (1964) methods had a Spearman correlation of 1, indicating the same stable genotypes. Of the 73 genotypes with the greatest productivity and good stability parameters, it is concluded that the genotypes that showed to be the most promising for possible uses were King Grass, Taiwan A-46, Porto Rico 534-B, Gigante de Pinda, Australiano, and Guaçu/IZ.

## Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

## Correlations between chemistry components of caryopsis in oat genotypes cultivated in different environments

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The study of trait correlations can allow the identification of occurrence, intensity and direction of associations between characters, providing information to elect the most correlated to the phenotypic performance of main chemical composition components in caryopsis of oat genotypes (*Avena sativa* L.). Thus, this study aimed to estimate the correlation between traits of chemical composition of caryopses in oat cultivars grown in different environments. In the 2007, 2008 and 2009 seasons, 15 cultivars were grown in Augusto Pestana, Capão do Leão and Passo Fundo, Rio Grande do Sul State, Brazil, following the randomized block design with four replications, with 3.0 m<sup>2</sup> plots. Eleven characters related to chemical composition of caryopsis were evaluated. For the set of environments, the phenotypic, genetic and environmental correlations among traits were estimated, followed by the partitioning of genetic correlations into direct and indirect effects on protein, lipid, nitrogen-free extract, and total dietary fiber content through path analysis. The results of this study suggest there is great difficulty in associating simultaneously high chemical quality of oat caryopsis - protein, lipid and total dietary fiber - with high content of carbohydrates. However, since there is no remarkable relationship, it seems to be possible to achieve high contents of  $\beta$ -glucan, important dietary fiber fraction, without interference of other chemical component of oat caryopsis.

**Key words:** *Avena sativa* L., path analysis, protein, lipid, nitrogen-free extract, total dietary fiber.

### INTRODUCTION

The oat grain (*Avena sativa* L.) is considered high quality food for human and animal consumption. The caryopsis has high concentration of starch and lipids, with predominance of unsaturated fatty acids, and high protein

content of excellent nutritional value, with balanced amino acid profile of high digestibility (Pedó et al., 1999; Beber et al., 2002). Besides, oat grains represent an important source of dietary fiber, arranged in soluble and

insoluble fractions. The  $\beta$ -glucan is a dietary fiber constituent, and it is the main component responsible for human health benefits conferred by regular oat consumption, reducing serum cholesterol, and thus decreasing the risks of cardiovascular diseases (Butt et al., 2008). Because of its high chemical quality, the oat grain is recognized as functional food (FDA Food Labeling, 1997; ANVISA, 1999).

The increasing demand of oats grains proper for different niches of market, as food industrialization and feed animals, has required the adoption of new attributes as target of selection, besides the grain production components that usually has been adopted. So, the study of expression dynamics and the selection considering chemical components of oat grain has become a major trend in worldwide breeding programs (Ahokas and Manninen, 2000; Loskutov, 2000; Doehlert et al., 2001; Peterson et al., 2005; Chernyshova et al., 2007), also in Brazil (Beber et al., 2002; Silva et al., 2006, 2008; Crestani et al., 2010, 2012; Hawerth et al., 2013). Grain-producing oat genotypes suitable for human diet, in general, need to present low calorie, lipid and nitrogen-free extract, and high protein and dietary fiber contents (Holland, 1997; Peterson et al., 2005; Chernyshova et al., 2007). However, genotypes proper to feed animals need to produce high caloric grains, characterized by high lipid and nitrogen-free extract, associated with high protein and low  $\beta$ -glucan contents, in order to promote the body mass gain (Marinissen et al., 2004; Martinez et al., 2010; Hizbai et al., 2012).

In oat improvement, genetic gains to be obtained via indirect selection are heavily dependent on correlation of characters considered (Carvalho et al., 2004; Lorencetti et al., 2006). The correlation and path analysis can be performed aiming to understand the dynamic of characters related to chemistry quality of caryopsis of oat genotypes. Correlation is a measure of the degree to which variables vary together or a measure of intensity of association (Steel and Torrie, 1980). However, path analysis studies permit to identify the direct effect of independent variables on dependent variables after removing the influence of all other independent variables included in the analysis (Wright, 1921). These evaluations can help electing the most interfering traits in phenotypic performance of main oat caryopsis chemistry quality components and to assist in developing strategies to achieve genetic gains.

Few studies have been performed or are available to scientific community considering the correlations between the most important chemistry components of caryopsis of oat genotypes cultivated in different environments. Thus,

this study aimed to estimate the correlations among traits of chemical composition of caryopses in oat cultivars in different environments, and partition the genetic correlation coefficients in direct and indirect effects on protein, lipid, nitrogen-free extract, and total dietary fiber content by path analysis.

## MATERIALS AND METHODS

In the 2007, 2008 and 2009 crop seasons, 15 Brazilian oat cultivars (*A. sativa* L.) were tested: Albasul, Barbarasul, Brisasul, FAPA Louise, UPF 15, UPF 16, UPF 18, UFRGS 14, UFRGS 19, UPFA 20, UPFA 22, URS 20, URS 21, URS 22 and URS Guapa. The experiments were conducted in Augusto Pestana (28°27'S and 53°54'W, altitude 280 m) in a Typic Dystrophic Red Latosol (Santos et al., 2006); in Capão do Leão (31°45'S and 52°29'W, altitude 13 m) in a Dystrophic Yellow Red Argisol (Santos et al., 2006); and in Passo Fundo (28°15'S and 52°24'W, altitude 687 m) in a Dystrophic Red Latosol (Santos et al., 2006), Rio Grande do Sul State, Brazil (Table 1). All experiments were conducted following the randomized block design with four replications, adopting a density of 300 viable seeds per square meter, with replicates formed by plots with five rows of 5.0 m long spaced in 0.20 m, with the measurements made from the product in the three core lines, featuring 3.0 m<sup>2</sup> plots. The soil preparation and fertility correction followed the oat technical recommendations of Brazilian Oat Research Committee (2006). The fertilization corrections with macronutrients were performed according to the levels observed in the soil chemical quality analysis in each year in order to supply the demands for a grain yield of 2.0 t ha<sup>-1</sup> (Table 1). Tebuconazole fungicide applications were performed at a dosage of 0.75 L ha<sup>-1</sup> according to the need for shoot disease control during the development cycle.

The evaluated characters aiming to assess the oat grain chemical composition were protein (Prot),  $\beta$ -glucan ( $\beta$ -glu), lipid (Lip), nitrogen-free extract (NFE), total dietary fiber (TDF), insoluble dietary fiber (IDF), soluble dietary fiber (SDF), neutral detergent-soluble fiber (NDSF), acid detergent-soluble fiber (ADSF), ash (Ash) and crude fiber (CF) contents in the caryopsis. For these analyses, samples of manually dehulled 300 grains larger than 2 mm were used, originating from a bulk harvest of each plot. The caryopses were ground in Willey type mill (brand Marconi, Piracicaba, Brazil) with a 0.25 mm sieve opening. The milled material was analyzed in the Food Research Center - University of Passo Fundo, adopting the near infrared reflectance spectrometer (NIRS - brand Perstorp Analytical, model 5000, Maryland, USA). The calibration curves for chemical quality traits determination adopting the NIRS were built by the Laboratory of Physical Chemistry of the Food Research Center, University of Passo Fundo. The New Infracsoft International software (ISI, 1996) was used to obtain these curves, considering the analysis of 100 oat samples following the methods specified by AACC (1999) and AOAC (1997). The protein content was obtained by multiplying the correction factor 6.25 for N content identified in the sample. NIRS readings were performed in triplicate and the results expressed in g 100 g<sup>-1</sup> in dry weight basis.

For the group of nine environments tested the coefficients of phenotypic ( $r_p$ ), genetic ( $r_G$ ) and environmental ( $r_E$ ) correlations

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**Table 1.** Soil chemical properties and climate conditions of oat cultivars grown in three locations of Rio Grande do Sul State, Brazil, in the crop seasons 2007, 2008 and 2009.

Crop season	Location	Soil chemical characteristics				
		Clay (%)	SMP index	O.M. (%)	P (mg dm <sup>-3</sup> )	K (mg dm <sup>-3</sup> )
2007	Augusto Pestana	50.0	6.6	3.2	31.0	385.0
	Capão do Leão	24.0	6.8	2.2	29.1	143.0
	Passo Fundo	56.0	5.4	5.5	15.0	183.8
2008	Augusto Pestana	54.0	6.2	2.9	26.0	292.0
	Capão do Leão	16.0	6.8	1.5	8.6	64.0
	Passo Fundo	46.0	6.0	3.3	5.0	204.0
2009	Augusto Pestana	56.0	6.4	2.8	25.0	215.0
	Capão do Leão	21.0	6.5	2.1	19.7	80.0
	Passo Fundo	57.4	5.4	5.4	15.1	186.0

Crop season	Location	Average climatic conditions in the growing period <sup>(1)</sup>			
		Maximum temperature (°C)	Minimum temperature (°C)	Medium temperature (°C)	Accumulated precipitation (mm)
2007	Augusto Pestana	23.3	11.4	17.3	863.0
	Capão do Leão	19.5	10.8	15.7	798.8
	Passo Fundo	21.3	11.3	16.3	1263.5
2008	Augusto Pestana	23.1	11.3	17.2	963.6
	Capão do Leão	20.4	11.7	16.0	575.3
	Passo Fundo	20.7	11.5	16.1	1118.8
2009	Augusto Pestana	22.8	9.7	16.2	1407.4
	Capão do Leão	20.0	11.0	15.0	952.0
	Passo Fundo	20.5	11.3	15.4	1425.7

<sup>(1)</sup>Data concerning the period from June to November of each year. O.M = organic matter.

among all traits were estimated using the expected mean square [E(MS)] by variance analysis, as described by Falconer and Mackay (1996). The hypotheses were tested at 5% probability by t test described by Steel and Torrie (1980). A total of n-2 degrees of freedom were used, following the model:  $t=r/\sqrt{[(1-r^2)/(n-2)]}$ , "r" being the coefficient of correlation between traits X and Y, and "n" the degrees of freedom in the generation considered. The  $r_G$  were partitioned into direct and indirect effects of traits (independent variables in the regression model) on protein, lipid, NFE and TDF (basic variables) through path analysis (Wright, 1921). The degree of multicollinearity of the singular matrix X'X was established based on its number of conditions, which is the ratio between the highest and the

lowest eigenvalue of the matrix of genetic correlations (Montgomery and Peck, 1981). The analysis of the genetic correlation matrix eigenvalues was performed to identify the nature of the linear dependence among the traits, detecting those that contributed to the occurrence of multicollinearity (Belsley et al., 1980). All procedures and analysis were run on the GENES software (Cruz, 2013).

## RESULTS AND DISCUSSION

The environment of cultivation showed a significant effect in the phenotypic expression of

all characters evaluated considering the average behavior of the oat cultivars (Table 2). The results observed in this evaluation are in agreement with the literature since studies show that the phenotypic behavior of chemical components of caryopsis in oats are interfered by environment conditions and the traits correlations tend to be likewise variable (Peterson, 2005; Martinez et al., 2010; Crestani et al., 2012). So, evaluating oat genotypes along a considerable number of environments is valuable in order to permit the analysis of which relationships between traits are

**Table 2.** Variation of performance (average  $\pm$  standard deviation) in relation to different characters related to chemical quality of caryopsis of oat cultivars grown in three locations of Rio Grande do Sul State, Brazil, in the crop seasons 2007, 2008 and 2009.

Character (%)	Location of cultivation/ Crop season								
	Augusto Pestana			Capão do Leão			Passo Fundo		
	2007	2008	2009	2007	2008	2009	2007	2008	2009
Protein*	18.33 $\pm$ 1.19	18.09 $\pm$ 1.11	18.56 $\pm$ 1.46	18.26 $\pm$ 1.14	21.26 $\pm$ 1.29	17.58 $\pm$ 1.15	17.26 $\pm$ 1.20	19.85 $\pm$ 1.15	19.17 $\pm$ 1.13
$\beta$ -glucan*	4.44 $\pm$ 1.02	4.04 $\pm$ 1.09	4.51 $\pm$ 0.86	5.38 $\pm$ 0.83	5.94 $\pm$ 0.97	5.12 $\pm$ 0.94	3.74 $\pm$ 0.81	5.15 $\pm$ 0.87	5.70 $\pm$ 0.83
Lipid*	7.43 $\pm$ 0.70	8.21 $\pm$ 0.57	7.59 $\pm$ 0.66	8.02 $\pm$ 0.70	7.96 $\pm$ 0.62	8.10 $\pm$ 0.82	8.08 $\pm$ 0.88	7.99 $\pm$ 0.56	7.50 $\pm$ 0.66
NFE*	69.06 $\pm$ 1.91	66.84 $\pm$ 1.78	67.90 $\pm$ 2.24	68.94 $\pm$ 1.94	63.92 $\pm$ 2.06	69.01 $\pm$ 2.26	70.33 $\pm$ 2.11	64.98 $\pm$ 2.03	67.09 $\pm$ 2.03
TDF*	9.47 $\pm$ 0.40	9.54 $\pm$ 0.39	9.40 $\pm$ 0.43	9.54 $\pm$ 0.44	9.72 $\pm$ 0.42	9.04 $\pm$ 0.54	9.55 $\pm$ 0.46	9.66 $\pm$ 0.39	9.40 $\pm$ 0.43
IDF*	5.88 $\pm$ 0.25	5.98 $\pm$ 0.28	5.83 $\pm$ 0.30	5.66 $\pm$ 0.31	5.57 $\pm$ 0.26	5.47 $\pm$ 0.40	6.04 $\pm$ 0.28	5.80 $\pm$ 0.25	5.72 $\pm$ 0.29
SDF*	3.59 $\pm$ 0.22	3.57 $\pm$ 0.18	3.58 $\pm$ 0.27	3.88 $\pm$ 0.24	4.16 $\pm$ 0.24	3.57 $\pm$ 0.26	3.51 $\pm$ 0.29	3.87 $\pm$ 0.23	3.68 $\pm$ 0.25
NDSF*	10.63 $\pm$ 0.26	10.52 $\pm$ 0.27	10.63 $\pm$ 0.40	10.44 $\pm$ 0.26	11.19 $\pm$ 0.37	10.29 $\pm$ 0.27	10.22 $\pm$ 0.29	11.00 $\pm$ 0.31	10.80 $\pm$ 0.35
ADSF*	2.86 $\pm$ 0.38	2.87 $\pm$ 0.33	2.75 $\pm$ 0.36	3.08 $\pm$ 0.30	3.49 $\pm$ 0.46	2.64 $\pm$ 0.41	2.68 $\pm$ 0.29	3.15 $\pm$ 0.31	2.92 $\pm$ 0.40
Ash*	2.22 $\pm$ 0.06	2.20 $\pm$ 0.04	2.24 $\pm$ 0.05	2.29 $\pm$ 0.05	2.35 $\pm$ 0.05	2.25 $\pm$ 0.05	2.21 $\pm$ 0.05	2.26 $\pm$ 0.04	2.27 $\pm$ 0.05
CF*	2.17 $\pm$ 0.42	2.75 $\pm$ 0.53	2.39 $\pm$ 0.52	2.23 $\pm$ 0.36	2.97 $\pm$ 0.69	2.09 $\pm$ 0.52	1.75 $\pm$ 0.50	3.23 $\pm$ 0.76	2.38 $\pm$ 0.50

\*Significant at 1% of probability by F test in relation to the factor environment of cultivation (location x crop season). NFE, nitrogen-free extract; TDF, total dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; NDSF, neutral detergent-soluble fiber; ADSF, acid detergent-soluble fiber; Ash, ash content, CF, crude fiber.

more stable across environments and in fact significant, and thus able to assist breeder decisions aimed at genetic improvement of oat.

Considering the performance of oat cultivars cultivated in Capão do Leão, Augusto Pestana and Passo Fundo in the 2007, 2008 and 2009 seasons, the environmental correlations ( $r_E$ ) between the caryopsis chemical components are presented on Table 3. It is possible to observe that most of significant environmental correlations were positive, with the highest magnitudes showed between protein and the characters NDSF ( $r_E = 0.89$ ), Ash ( $r_E = 0.78$ ), SDF ( $r_E = 0.72$ ), and by the relation of TDF with IDF ( $r_E = 0.78$ ) and SDF ( $r_E = 0.70$ ), so, both characters were harmed or benefited by the same variations in environment (Falconer and Mackay, 1996). However, the most expressive negative environmental correlations were observed between

NFE and the character protein ( $r_E = -0.86$ ), NSDF ( $r_E = -0.83$ ), SDF ( $r_E = -0.70$ ), TDF ( $r_E = -0.69$ ), and ash ( $r_E = -0.62$ ), indicating that the environment favored one character over another.

In study, the most part of phenotypic correlations ( $r_P$ ) presented magnitude slightly superior than the genotypic correlations ( $r_G$ ) (Table 3). This behavior can be attributed to environmental modifying effects on associations between characters, reflecting in the reduction of its expression front of environmental influences. However, many significant correlations of low magnitude were detected and identified by t test at 5% ( $\geq 0.09$ ) and 1% ( $\geq 0.11$ ). Plan strategies of indirect selection based on these associations can lead to inefficient process of genetic gains, since it can be result of a weak genetic correlation (genetic linkage and pleiotropy). Thus, the main associations between characters are particularly

highlighted.

In Table 3, the character protein showed strong positive correlations with ash ( $r_P = 0.90$ ;  $r_G = 0.93$ ), NDSF ( $r_P = 0.88$ ;  $r_G = 0.87$ ), SDF ( $r_P = 0.75$ ;  $r_G = 0.75$ ), CF ( $r_P = 0.47$ ;  $r_G = 0.48$ ), TDF ( $r_P = 0.48$ ;  $r_G = 0.47$ ), and with ADSF ( $r_P = 0.42$ ;  $r_G = 0.42$ ), meanwhile, presented negative correlations with NFE ( $r_P = -0.87$ ;  $r_G = -0.87$ ). As regards the caryopsis component lipid, positive correlations were revealed in relation to IDF ( $r_P = 0.94$ ;  $r_G = 0.98$ ), TDF ( $r_P = 0.94$ ;  $r_G = 0.97$ ), SDF ( $r_P = 0.75$ ;  $r_G = 0.78$ ), and with ash ( $r_P = 0.45$ ;  $r_G = 0.52$ ). Also, expressive negative correlations were observed between lipid and NFE ( $r_P = -0.60$ ;  $r_G = -0.62$ ). The fraction of NFE corresponds to non-structural carbohydrates present in the oat caryopsis, formed mainly of starch, sucrose and pectin present in the cell content, featuring an indicative of caryopsis energy. The starch content in oat

**Table 3.** Estimates of phenotypic ( $r_P$ ), genetic ( $r_G$ ) and environmental correlations ( $r_E$ ) between of characters related to chemistry quality of caryopsis of oat cultivars grown in Augusto Pestana, Capão do Leão and Passo Fundo, Rio Grande do Sul State, in the seasons 2007, 2008 and 2009.

Character	$\beta$ -glucan	Lipid	NFE	TDF	IDF	SDF	NDSF	ADSF	Ash	CF
<b>Phenotypic correlation coefficient (<math>r_P</math>)</b>										
Protein	0.01 <sup>ns</sup>	0.20**	-0.87**	0.48**	0.22**	0.75**	0.88**	0.42**	0.90**	0.47**
$\beta$ -glucan	-	-0.30**	0.05ns	-0.21**	-0.31**	-0.03 <sup>ns</sup>	0.25**	0.20**	0.03ns	0.21**
Lipid		--	-0.60**	0.94**	0.94**	0.75**	-0.18**	-0.33**	0.45**	0.29**
NFE			--	-0.80**	-0.60**	-0.91**	-0.67**	-0.27**	-0.89**	-0.66**
TDF				--	0.94**	0.88**	0.11**	-0.19**	0.68**	0.34**
IDF					--	0.67**	-0.15**	-0.41**	0.42**	0.15**
SDF						--	0.44**	0.14**	0.90**	0.53**
NDSF							--	0.70**	0.69**	0.58**
ADSF								--	0.41**	0.59**
Ash									--	0.47**
<b>Genotypic correlation coefficient (<math>r_G</math>)</b>										
Protein	-0.04 <sup>ns</sup>	0.21**	-0.87**	0.47**	0.22**	0.75**	0.87**	0.42**	0.93**	0.48**
$\beta$ -glucan	--	-0.31**	0.09*	-0.27**	-0.38**	-0.09*	0.22**	0.22**	-0.07 <sup>ns</sup>	0.26**
Lipid		--	-0.62**	0.97**	0.98**	0.78**	-0.18**	-0.34**	0.52**	0.28**
NFE			--	-0.81**	-0.62**	-0.93**	-0.66**	-0.29**	-0.94**	-0.68**
TDF				--	0.95**	0.90**	0.08**	-0.22**	0.70**	0.36**
IDF					--	0.72**	-0.17**	-0.44**	0.45**	0.19**
SDF						--	0.43**	0.12**	0.94**	0.53**
NDSF							--	0.74**	0.71**	0.61**
ADSF								--	0.37**	0.65**
Ash									--	0.54**
<b>Environmental correlation coefficient (<math>r_E</math>)</b>										
Protein	0.44**	-0.08 <sup>ns</sup>	-0.86**	0.64**	0.26**	0.72**	0.89**	0.38**	0.78**	0.26**
$\beta$ -glucan	--	-0.25**	-0.33**	0.46**	0.29**	0.38**	0.46**	0.10*	0.55**	-0.21**
Lipid		--	-0.21**	0.32**	0.10*	0.39**	-0.19**	-0.32**	-0.22**	0.47**
NFE			--	-0.69**	-0.34**	-0.70**	-0.83**	-0.15**	-0.62**	-0.40**
TDF				--	0.78**	0.70**	0.54**	0.13**	0.59**	0.13**
IDF					--	0.09*	0.22**	-0.14**	0.23**	-0.23**
SDF						--	0.60**	0.36**	0.67**	0.47**
NDSF							--	0.31**	0.64**	0.29**
ADSF								--	0.66**	0.13**
Ash									--	-0.03 <sup>ns</sup>

NFE, Nitrogen-free extract; TDF, total dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; NDSF, neutral detergent-soluble fiber; ADSF, acid detergent-soluble fiber; Ash, ash content; CF, crude fiber. <sup>ns</sup> Non significant, \* and \*\* Significant at 0.05 and 0.01 probability levels, respectively, by t test.

grains tends to be positively correlated with the hectolitre weight (Martinez et al., 2010). In this sense, evaluating the performance of oat cultivars in different environments, Peterson et al. (2005) verified that the lipid content in oat grains was negatively associated with kernel and groat size and groat percentage.

Besides the relations already presented earlier, the TDF presented positive correlations with IDF ( $r_P = 0.94$ ;  $r_G = 0.95$ ), SDF ( $r_P = 0.88$ ;  $r_G = 0.90$ ), and with ash ( $r_P = 0.68$ ;  $r_G = 0.70$ ), and negative correlation with NFE ( $r_P = -0.80$ ;  $r_G = -0.81$ ). The NFE also showed high negative correlations with Ash ( $r_P = -0.89$ ;  $r_G = -0.94$ ), SDF ( $r_P = -0.91$ ;  $r_G = -0.93$ ), CF ( $r_P = -0.66$ ;  $r_G = -0.68$ ), IDF ( $r_P = -0.60$ ;  $r_G = -0.62$ ) and with NDSF ( $r_P = -0.67$ ;  $r_G = -0.66$ ). In agreement with these results, Martinez et al. (2010) observed negative correlation between starch and NDSF. The negative correlations of NFE with all other characters suggest a general tendency that the higher the mass of caryopsis forming the oat caryopsis, represented mainly by the non-structural carbohydrates, the lower will be the protein, lipid, and the dietary fiber content, agreeing with results in the literature (Peterson et al., 2005; Martinez et al., 2010; Crestani et al., 2012).

The  $\beta$ -glucan is a structural carbohydrate (hemicellulose) that constitutes about 75% of the endosperm cell walls in oat caryopsis (Butt et al., 2008). Also, the  $\beta$ -glucan is an important dietary fiber component of the oat caryopsis responsible for the reduction of serum cholesterol, and thus reducing the risk of cardiovascular disease, conferred by the regular oat grains consumption when it is associated to proper diet and healthy lifestyle (Andon and Anderson, 2008; Butt et al., 2008). In this evaluation, the  $\beta$ -glucan has not presented strong correlations with any chemical component of caryopsis evaluated. However, it were detected positive relationships of  $\beta$ -glucan with CF ( $r_P = 0.21$ ;  $r_G = 0.26$ ), NDSF ( $r_P = 0.25$ ;  $r_G = 0.22$ ) and ADSF ( $r_P = 0.20$ ;  $r_G = 0.22$ ), and negative associations with lipid ( $r_P = -0.30$ ;  $r_G = -0.31$ ), TDF ( $r_P = -0.21$ ;  $r_G = -0.27$ ) and IDF ( $r_P = -0.31$ ;  $r_G = -0.38$ ) (Table 3). The positive relationship between  $\beta$ -glucan and CF, NDSF and ADSF components is probably explained by the fact of these components are also structural constituents of the cell wall, whose global content is closely related to the number and size of cells in the caryopsis.

The genetic gains to be obtained via artificial selection are heavily dependent on the combining ability of parents used in the hybridization (Benin et al., 2003, 2005; Bertan et al., 2007), which implies a need for an accurate assessment of genotype potentials in oat breeding programs. The characters with high level of genotypic and phenotypic correlation can be considered in the selection strategies. However, only the genetic correlations involving an association of heritable nature may therefore contribute to the effective targeting of improvement programs (Falconer and Mackay, 1996). As consequence, the genotype correlation coefficients were

partitioned into their direct and indirect effects on protein, lipid, nitrogen-free extract, and total dietary fiber content through path analysis aiming to assess if the correlation between two variables was due to a cause and effect or was determined by the influence of other variables (Tables 4 and 5).

The coefficients of determination of the path analysis model ( $R^2$ ) presented high values in relation to protein (0.98), lipid (0.99), nitrogen-free extract (0.99) and total dietary fiber content (0.99), indicating that the explanatory variables explained a major part of the variation observed in the basic variable considered. The causal diagram illustrating the direct and indirect effects of the most important explanatory variables on the basics variables protein, lipid, nitrogen-free extract, and total dietary fiber content considering the cultivation of oat cultivars in different environments are shown in Figure 1.

In general, it is observed that the most part of direct effects magnitudes were lower than the genotypic correlations magnitudes (Figure 1, Tables 3 and 4). So, much of the genetic correlation detected between the characters is not directly explained by cause and effect relationship, but it is resultant of the set of indirect effects that are exercised via other variables related to the main characters considered.

Confirming the strong genotypic correlations with Protein (Table 3), the characters SDF, NDSF and ash presented positive direct effects on protein expression (0.25; 0.48 and 0.31, respectively), while NFE showed significant negative direct effects (-0.30) (Table 4). Also, the most significant indirect effects of independent variables on protein were performed via SDF, NDSF, ash and NFE. However, despite of positive genetic correlations with protein, the components lipid and ADSF (0.21 and 0.42, respectively) showed negative direct effects on protein content (-0.20 and -0.20, respectively). The negative causal and effect relationship between lipid and protein are reinforced by direct effects of protein on lipid content (-0.11) (Table 4).

Based on the literature, there is no consistent relationship between oil percentage and protein content in oat caryopsis, since in the different studies are reported the occurrence of positive and negative correlations, or even no significant correlations (Peterson et al., 2005; Silva et al., 2008; Martinez et al., 2010). These inconsistencies may be attributed to the evidences that these traits have polygenic inheritance, with strong contribution of additive effects, and the environmental contribution in the phenotypic definition is variable (Zhu et al., 2004; Orr and Molnar, 2007; Crestani et al., 2012; Hizbai et al., 2012).

A weak negative direct effect of  $\beta$ -glucan in relation to Protein content (-0.12) was detected, despite the absence of genotypic correlation between these traits. This way, the selection based on high protein content can lead to obtain genotypes with lower content of Lipid, NFE and  $\beta$ -glucan concentrations, but with higher content of

**Table 4.** Direct effects and indirect effects (values presented along the table lines) of characters related to chemistry quality of caryopsis on protein content and lipid content in oat genotypes cultivated in different environments.

Indirect effects of x on Protein via y	Protein content									
	<sup>(y)</sup> Lipid	$\beta$ -glucan	NFE	TDF	IDF	SDF	NDSF	ADSF	Ash	CF
<sup>(x)</sup> Lipid	--	0.04	0.19	0.03	-0.14	0.20	-0.09	0.07	0.16	-0.02
$\beta$ -glucan	0.06	--	-0.03	-0.01	0.05	-0.02	0.11	-0.04	-0.02	-0.02
NFE	0.13	-0.01	--	-0.02	0.09	-0.24	-0.32	0.06	-0.29	0.05
TDF	-0.20	0.03	0.24	--	-0.14	0.23	0.04	0.05	0.22	-0.03
IDF	-0.20	0.04	0.19	0.03	--	0.18	-0.08	0.09	0.14	-0.01
SDF	-0.16	0.01	0.28	0.02	-0.10	--	0.21	-0.02	0.29	-0.04
NDSF	0.04	-0.03	0.20	0.00	0.03	0.11	--	-0.15	0.22	-0.05
ADSF	0.07	-0.03	0.09	-0.01	0.06	0.03	0.35	--	0.12	-0.05
Ash	-0.11	0.01	0.28	0.02	-0.06	0.24	0.34	-0.08	--	-0.04
CF	-0.06	-0.03	0.20	0.01	-0.03	0.14	0.29	-0.13	0.17	--
Direct effect of y on Protein	-0.20	-0.12	-0.30	0.03	-0.14	0.25	0.48	-0.20	0.31	-0.07
r <sub>G</sub> with Protein	0.21**	-0.04 <sup>ns</sup>	-0.87**	0.47**	0.22**	0.75**	0.87**	0.42**	0.93**	0.48**

Indirect effects of x on Lipid via y	Lipid content									
	<sup>(y)</sup> Protein	$\beta$ -glucan	NFE	TDF	IDF	SDF	NDSF	ADSF	Ash	CF
<sup>(x)</sup> Protein	--	0.00	-0.09	0.14	0.11	0.02	-0.31	0.01	0.37	0.08
$\beta$ -glucan	0.00	--	0.01	-0.08	-0.18	0.00	-0.08	0.00	-0.03	0.05
NFE	0.09	0.00	--	-0.24	-0.30	-0.03	0.24	-0.01	-0.37	-0.12
TDF	-0.05	0.00	-0.09	--	0.45	0.03	-0.03	0.00	0.28	0.06
IDF	-0.02	0.00	-0.07	0.29	--	0.02	0.06	-0.01	0.18	0.03
SDF	-0.08	0.00	-0.10	0.27	0.35	--	-0.15	0.00	0.37	0.09
NDSF	-0.10	0.00	-0.07	0.03	-0.08	0.01	--	0.01	0.28	0.11
ADSF	-0.05	0.00	-0.03	-0.07	-0.21	0.00	-0.26	--	0.15	0.11
Ash	-0.10	0.00	-0.10	0.21	0.21	0.03	-0.25	0.01	--	0.09
CF	-0.05	0.00	-0.07	0.11	0.09	0.02	-0.22	0.01	0.21	--
Direct effect of y on Lipid	-0.11	0.00	0.11	0.30	0.48	0.03	-0.36	0.02	0.40	0.17
r <sub>G</sub> with Lipid	0.21**	-0.31**	-0.62**	0.97**	0.98**	0.78**	-0.18**	-0.34**	0.52**	0.28**

NFE, Nitrogen-free extract; TDF, total dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; NDSF, neutral detergent-soluble fiber; ADSF, acid detergent-soluble fiber; Ash, ash content; CF, crude fiber. <sup>ns</sup> Non significant, \* and \*\* Significant at 0.05 and 0.01 probability levels, respectively, by t test. Protein content: coefficient of determination ( $R^2$ ) = 0.98, residual variable effect = 0.15; Lipid content: coefficient of determination ( $R^2$ ) = 0.99, residual variable effect = 0.06.

minerals (Ash), SDF and NDS. The NDSF showed negative direct effect on lipid expression, even higher than genotypic correlation magnitude

( $r_{G \text{ Lipid} \times \text{NDSF}} = -0.18$ ; direct effect = -0.36) (Table 4). However, the NFE showed positive direct effects on lipid content (direct effect = 0.11),

despite the negative genotypic correlation (-0.62).

In the study performed by Banás et al. (2007), the majority of the total lipid in the oat caryopsis

**Table 5.** Direct effects and indirect effects (values presented along the table lines) of characters related to chemistry quality of caryopsis on nitrogen-free extract content (NFE) and total dietary fiber content (TDF) in oat genotypes cultivated in different environments.

Indirect effects of x on NFE via y	Nitrogen-free extract content (NFE)									
	(y) Protein	$\beta$ -glucan	Lipid	TDF	IDF	SDF	NDSF	ADSF	Ash	CF
(x) Protein	--	0.00	0.03	-0.11	-0.08	-0.02	-0.17	0.00	-0.17	-0.11
$\beta$ -glucan	0.01	--	-0.04	0.06	0.13	0.00	-0.04	0.00	0.01	-0.06
Lipid	-0.05	0.00	--	-0.22	-0.34	-0.02	0.04	0.00	-0.10	-0.06
TDF	-0.11	0.00	0.13	--	-0.33	-0.02	-0.02	0.00	-0.13	-0.08
IDF	-0.05	0.00	0.13	-0.21	--	-0.02	0.03	0.00	-0.08	-0.04
SDF	-0.18	0.00	0.10	-0.20	-0.25	--	-0.08	0.00	-0.17	-0.12
NDSF	-0.21	0.00	-0.02	-0.02	0.06	-0.01	--	0.00	-0.13	-0.13
ADSF	-0.10	0.00	-0.04	0.05	0.16	0.00	-0.14	--	-0.07	-0.14
Ash	-0.22	0.00	0.07	-0.16	-0.16	-0.02	-0.14	0.00	--	-0.12
CF	-0.11	0.00	0.04	-0.08	-0.07	-0.01	-0.12	0.00	-0.10	--
Direct effect of y on NFE	-0.24	0.01	0.13	-0.23	0.35	-0.02	-0.19	0.01	-0.19	-0.22
r <sub>G</sub> with NFE	-0.87**	0.09*	-0.62**	-0.81**	-0.62**	-0.93**	-0.66**	-0.29**	-0.94**	-0.68**

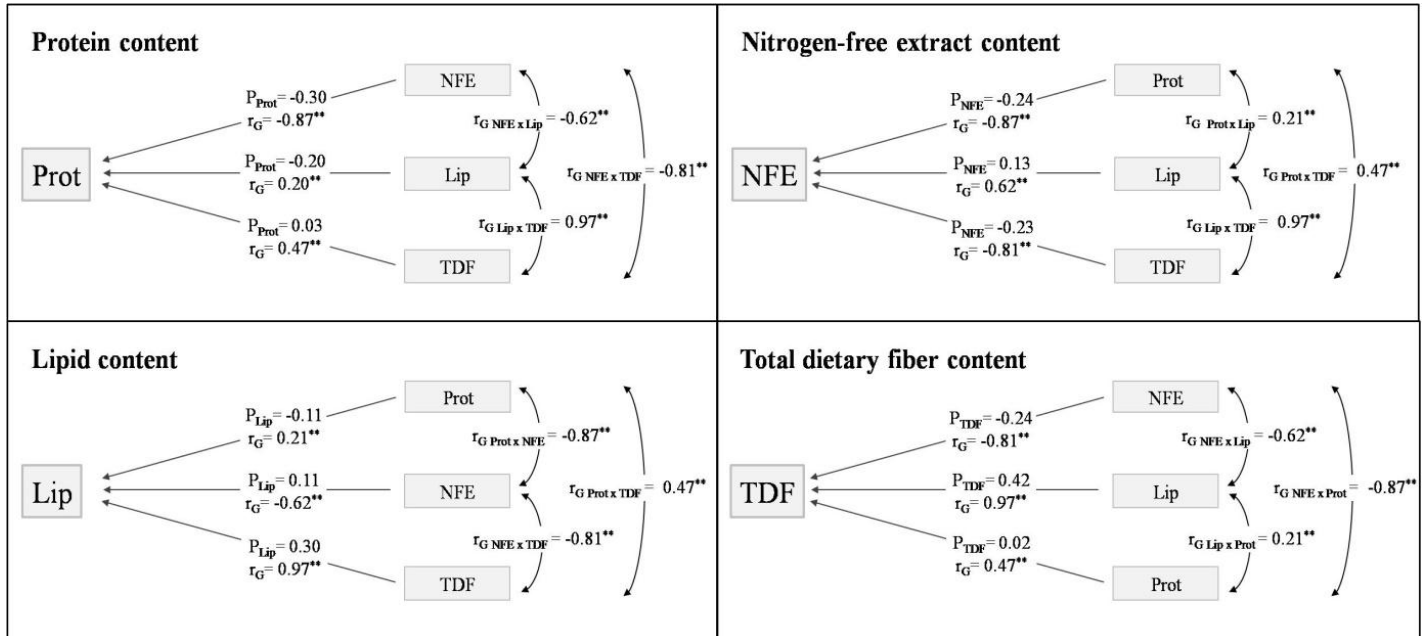
Indirect effects of x on TDF via y	Total dietary fiber content (TDF)									
	(y) Protein	$\beta$ -glucan	NFE	Lipid	IDF	SDF	NDSF	ADSF	Ash	CF
(x) Protein	--	0.00	0.21	0.09	0.05	0.16	0.02	-0.03	-0.02	-0.02
$\beta$ -glucan	0.00	--	-0.02	-0.13	-0.08	-0.02	0.00	-0.02	0.00	-0.01
NFE	-0.02	0.00	--	-0.26	-0.13	-0.20	-0.01	0.02	0.02	0.02
Lipid	0.00	0.00	0.15	--	0.20	0.17	0.00	0.03	-0.01	-0.01
IDF	0.00	0.00	0.15	0.41	--	0.15	0.00	0.03	-0.01	-0.01
SDF	0.01	0.00	0.23	0.33	0.15	--	0.01	-0.01	-0.02	-0.02
NDSF	0.02	0.00	0.16	-0.08	-0.04	0.09	--	-0.06	-0.01	-0.02
ADSF	0.01	0.00	0.07	-0.14	-0.09	0.03	0.01	--	-0.01	-0.02
Ash	0.02	0.00	0.23	0.22	0.09	0.20	0.01	-0.03	--	-0.02
CF	0.01	0.00	0.16	0.12	0.04	0.11	0.01	-0.05	-0.01	--
Direct effect of y on TDF	0.02	-0.01	-0.24	0.42	0.21	0.21	0.02	-0.07	-0.02	-0.03
r <sub>G</sub> with TDF	0.47**	-0.27**	-0.81**	0.97**	0.95**	0.90**	0.08**	-0.22**	0.70**	0.36**

NFE, Nitrogen-free extract; TDF, total dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; NDSF, neutral detergent-soluble fiber; ADSF, acid detergent-soluble fiber; Ash, ash content; CF, crude fiber. <sup>ns</sup> Non significant, \* and \*\* Significant at 0.05 and 0.01 probability levels, respectively, by t test. Nitrogen-free extract content: coefficient of determination ( $R^2$ ) = 0.99, residual variable effect = 0.08; Total dietary fiber content: coefficient of determination ( $R^2$ ) = 0.99, residual variable effect = 0.06.

was deposited during the first half of grain development when the endosperm was still liquid,

whereas both protein and starch deposition proceeded with the same rate to a late stage of

development. According to these authors, the endosperm switches glucose utilization to favour



**Figure 1.** Illustrative diagram showing the correlations between explanatory variables on the basic variable (Prot, Lip, NFE and TDF) in oat cultivars grown in different environments. Prot, protein content; Lip, lipid; NFE, nitrogen-free extract; TDF, total dietary fiber;  $r_G$  = genotypic correlation; P, direct effect on the basic variable.

starch synthesis over lipid synthesis after de mid-stages of grain development. Meanwhile, Morrison (1988) has suggested that the positive correlations between lipid content and amylase content in isolated oat starches may be explained by the regulation of starch biosynthesis by lipids.

The positive relationship of TDF, IDF and Ash with Lipid was confirmed by their positive direct effects on lipid concentration (0.30, 0.48 and 0.40, respectively) (Table 4). Through the action of the neutral detergent on caryopsis fiber fraction is dissolved the cellular content, formed mainly of protein, lipids, pectin, starch and sugars (non structural carbohydrates), getting the neutral detergent fiber content (NDF), composed basically by cellulose, hemicellulose and lignin. With the sample treatment with acid detergent is separated the content of cellulose and lignin, getting the acid detergent fiber content (ADF). Thus, the basic difference between NDF and FDA fractions corresponds to sample's hemicellulose content. Therefore, the different magnitudes of direct effects of ASDF and NDF on caryopsis chemical components protein, lipid, NFE and TDF (Tables 4 and 5) can be associated with the hemicellulose synthesis (ADSF - ADF = hemicellulose).

Except by  $\beta$ -glucan, all other characters showed strong negative correlation with NFE, however, the strongest negative direct effects on NFE expression were performed by protein, TDF and CF (-0.24, -0.23 and -0.22, respectively) (Table 5). Also, IDF and lipid presented positive direct effects on NFE (0.35 and 0.13,

respectively) despite of the negative correlations ( $r_{G\ NFE \times IDF} = -0.62$ ;  $r_{G\ NFE \times Lipid} = -0.62$ ). The higher magnitudes of indirect effects of independent variables on NFE were performed by protein, TDF and IDF. So, the increase of NFE in the oat caryopsis can lead to lower content of protein and TDF, however, is possible to achieve simultaneously higher contents of IDF and lipid concentrations.

Ash content in oat caryopsis contemplates its inorganic fraction composed of minerals. Peterson et al. (1975) found significant levels of phosphorus, calcium, potassium, copper, manganese, iron, sodium and magnesium present in oats caryopsis. However, despite the importance of many of these minerals in food and feed, its content in the grain has not been considered as a target of selection by oat breeding programs, neither in Brazil nor other countries. Therefore, the observed results were expected, since there is direct positive effect of ash content (mineral matter) on protein and lipid present in the oat caryopsis (0.31 and 0.40, respectively) and negative direct effect on NFE (-0.19), as shown in Tables 4 and 5.

As expected, IDF and SDF showed positive direct effects on TDF (0.21 and 0.21, respectively), however, with magnitude much lower than the genotypic correlations ( $r_{G\ TDF \times IDF} = 0.95$ ;  $r_{G\ TDF \times SDF} = 0.90$ ) (Table 5). Part of the relationship between these traits is explained by indirect effects of these independent variables (IDF and SDF) by NFE, lipid, SDF and IDF. The negative relationship between NFE and TDF are

reaffirmed by the negative direct effect of NFE on TDF content (-0.24). However, the lipid content showed to have strong direct effects on TDF phenotypic expression (0.42). So, these results suggest that genotypes rich in TDF tend to show concomitantly high contents of lipid as well rather smaller contents of NFE. So, since lipid are strongly positive correlated with TDF while NFE shows negative correlation with TDF ( $r_G = 0.97$  and  $-0.81$ , respectively) indirect selections aiming higher TDF contents can be successfully performed based on phenotype higher lipid content or even based on smaller NFE concentration in the oat caryopsis.

The expression dynamic of  $\beta$ -glucan in oat grains has been further studied lately, and, the same way, has been considered as a differential target of selection by breeding programs (Cervantes-Martinez et al., 2001; Zhu et al., 2004; Chernyshova et al., 2007; Crestani et al., 2010; Crestani et al., 2012). In this evaluation no remarkable direct or indirect effects of  $\beta$ -glucan was detected on protein, lipid, NFE and TDF expression, despite of  $\beta$ -glucan genotypic correlations with these characters ( $r_G = -0.04$ ,  $-0.31$ ,  $0.09$  and  $-0.27$ , respectively) (Tables 4 and 5). These results suggest that the selection based on  $\beta$ -glucan will not interfere substantially in the protein, lipid, NFE and TDF caryopses content. Likewise, Crestani et al. (2010) found significant positive genotypic correlations between  $\beta$ -glucan content with yield of grains and with industrial yield of grains considering oat genotypes cultivated in different environments. So, it suggests the possibility to achieve genotypes rich in  $\beta$ -glucan content and concomitantly highly productive via genetic improvement. Brennan and Cleary (2005) highlight that the relationship between  $\beta$ -glucan levels in cereal grains and grain quality characters, or yield parameters, appear to vary greatly depending upon genetic background of the cereal line being examined.

Similarly, it is noteworthy that the relationships observed are suitable to the analyzed group of genotypes in this study, since the correlations coefficients depend on the gene pool and the environment effects. However, the behavior observed for this set of genotypes can represent trends that can be repeated in other situations, mainly those correlations with higher magnitudes, and could be adopted efficiently in the indirect selection during breeding procedures. Thus, breaking undesirable correlations in segregating populations aiming to attend oat breeding programs demands should be sought by conducting crossings between complementary gene pool associated with generating large populations aiming to increase the opportunities of desired crossing-overs.

## Conclusion

In general, content of lipid is positively correlated with total dietary fiber and nitrogen-free extract in the caryopsis, and negatively with protein. Meanwhile, high contents of nitrogen-free extract tended to be

accompanied by lower concentrations of protein and total dietary fiber. Thus, it can be a tough work to involve different characters simultaneously in genetic improvement process aiming to reach appropriate genotypes for different market niches of oat grains. The results suggest there is great difficulty in associating simultaneously high chemical quality of oat caryopsis - protein, lipid and total dietary fiber - with high content of carbohydrates. However, since there is no remarkable relationship, it seems to be possible to achieve high contents of  $\beta$ -glucan, important dietary fiber fraction, without interference of other chemical component of oat caryopsis.

## Conflict of interests

The authors have not declared any conflict of interests.

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## Full Length Research Paper

# Effects of three levels of mycorrhiza and four levels of planting bed on total wet and dry weight of shoots and the number of lateral branches of white periwinkle (*Catharanthus alba*)

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Medicinal and ornamental plant of periwinkle (*Catharanthus alba*) contains the valuable alkaloids of vinblastine and vincristine in vegetative parts and Ajmalicine in its root that play a role in the chemotherapy types of cancers. This study was conducted for evaluating the effects of three levels of biological fertilizer of mycorrhiza (control (m0), *Glomus mosseae* (m1) and *Glomus intraradices* (m2)) and four levels of seed bed (the bed a; included the typical agricultural soil, bed b; contained a mixture of agricultural soil and sheep manure and rotten leaf and Aeolian sand, bed c; included the normal agricultural soil mixed with vermicompost and bed d; contained the normal agricultural soil mixed with compost) and their interactions on total shoot wet and dry weight and number of side branches containing the alkaloids of medicinal and ornamental plant of periwinkle (*C. alba*) as potted and in a factorial randomized complete block design with three replications and each replication including 12 treatments and each treatment including 4 pot with a total of 144 pots and each pot including two plants that were performed as the indirect culture (first in the nursery and then transfer to the pot), in Tehran. According to the results, the mycorrhizal type of *G. interaradices* (treatment m2) showed the highest significant effect on the characteristics of total wet and dry weight of shoots and the number of lateral branches. In studying the effect of planting beds, the bed b (agricultural soil mixed with sheep manure, rotten leaves and Aeolian sand) had the highest effect on all the characteristics. But in the interactions of mycorrhiza and planting bed, the treatment bm1 (bed b inoculated with mycorrhizal type of *G. mosseae*) and bm2 (bed b inoculated with mycorrhizal type of *G. interaradices*) showed the highest effect on total wet and dry weight of shoots, respectively. Finally, the bed b showed more favorable results in the yield of the medicinal plant of periwinkle.

**Key words:** *Catharanthus alba*, shoot wet and dry weight, mycorrhiza, animal manure, vermicompost, compost.

## INTRODUCTION

A significant number of drugs originally derived from plants (Gaines, 2004) since the mid-twentieth century and after identifying the adverse effects resulting from the use of chemical medicines, the medicinal plants and

herbal medicines research and production developed and soon replaced chemical medicines (Aminpoor and Mousavi, 1995). Since the climate of Iran is very diverse, it is considered as a good place for growing a variety of

plants. So a variety of medicinal plants can be grown as wild and by planting in large quantities (Ghasami, 2001). Periwinkle (*Catharanthus alba*), from the family of oleander, is a shrub and perennial plant that is annually cultivated in the cold areas (Dobelis, 1989). The plants of this family include about 300 genera and 1,300 species that mainly grow in the tropical areas. Among these species, only a few medicinal plants have been found that periwinkle is one of them (Omidbeigi, 2009). Almost all plants belonging to the family contains a variety of alkaloids and glycosides (Bruneton, 1995). According to studies conducted by Mukhopadhyay et al. (1983) and Maloney et al. (2006), *Catharanthus* plants contain more than 85 alkaloids. The plant contains important alkaloids like, vinblastine and vincristine in shoots (to treat some kinds of cancers) and ajmalicine in the root (hypertension) (Sajjadi, 2000).

Now a days, these alkaloids (vinblastine, vincristine, and vinorelbine) are producing synthetically and used as medication to treat the cancers and as a controlling agent for normal reactions of immune system. The extracts and derivatives of Periwinkle plant such as vinpocetine are also known as non-harmful medicines (Hassan, 2012). The alkaloids of vinblastine and vincristine are produced in the young leaves and the alkaloids of ajmalicine are produced in the roots of the plant. Proper use of nutrients during the medicinal plant cultivation, has main role in increasing the yield, and also affect the quality and quantity of their active ingredients (Omidbeigi, 2009).

Organic-biological agriculture is one of the new methods for producing the products with minimal residues of chemical fertilizers in its own texture (Hassan, 2012). One of the ways to achieve the sustainable agriculture is using the microorganisms which have an important role in meeting the nutritional requirements of plants (Nadian, 1998).

Mycorrhizal symbiosis provides a better competitive strategic for the host plant (Kothamasi and Babo, 2001). It was also reported that vermicomposts contain the active biological substances that act as growth regulators (Tomati et al., 1988). The first advantage of municipal waste compost is having high organic material and low mass (Soumare et al., 2003). In addition to compensating the food shortages, helping stabilize soil aggregate in the light soil and entering the useful micro-organisms in soil, the animal manure will lead to save water.

## MATERIALS AND METHODS

The mentioned experiment was conducted as a factorial randomized block design with three replicates and 12 treatments each (totally 36 treatments). This research has two factors. The first

factor (A) includes:

**A) Typical agricultural soil (a)** that is without the use of any additives with pH= 7.74, total nitrogen= 0.137 ppm, phosphorus= 24 ppm, K= 428 ppm, percent of organic carbon= 1.37% and CEC= 19.52 Cmol/kg and EC= 2.43 Ds/m.

**B) A mixture of agricultural soil and sheep manure and rotten leaves (b)** that is a mixture of agricultural soil, completely rotten sheep manure, rotten leaves, Aeolian sand with the equal proportions with pH= 7.49, total nitrogen= 0.464 ppm, phosphorus= 122 ppm, K= 1494.9 ppm, percent of organic carbon= 6.46% and CEC= 16 Cmol/kg and EC= 7.96 Ds/m.

**C) A mixture of agricultural soil with vermicompost (c)** which includes two-thirds of agricultural soil and one-third of vermicompost with pH= 7.27, total nitrogen= 0.983 ppm, phosphorus= 94 ppm, K= 578.6 ppm, percent of organic carbon= 9.83% and CEC= 19.2 Cmol/kg and EC= 5.34 Ds/m.

**D) A mixture of agricultural soil with compost (d)** that includes two-thirds of agricultural soil and one-third of compost with pH= 7.47, total nitrogen= 0.143 ppm, phosphorus= 24 ppm, K= 277.3 ppm, organic carbon= 1.43% and CEC= 2.148 Cmol/kg and EC= 2.77 Ds/m.

The second factor (B) includes three levels of mycorrhiza. First level: control (m0), second level (m1): the bed inoculated with mycorrhiza of *Glomus mosseae* with the amount of 100 gm<sup>-2</sup> and the third level (m2): the bed inoculated with mycorrhiza of *Glomus intraradices* with the amount of 100 gm<sup>-2</sup> and 105 (CFU) each.

After preparing the planting beds, the mycorrhiza fungi with the amount of 100 gm<sup>-2</sup> was added to the planting beds (except for the control treatment) and then the seeds were planted. In each treatment, hundred seeds of periwinkle with the distance of 2 cm from each other were planted. After 76 days (3 to 4 leaves), the seedlings were transferred from nursery to pots. Each of seedlings were planted in the pots containing its planting bed soil with respective treatments (control (m0) and inoculated mycorrhizal types, m1 and m2). The amount of Mycorrhiza used was 10 g per pot that were treated at a depth of 5 cm from the surface of pot soil. The height of each pot was 40 cm and the diameter of each pot opening was 20 cm span. Until two months after the transfer to the pot, irrigation of pots was conducted every 12 h and each time with the amount of 960 cc. But after two months and considering the cooler weather and shortness of day and completion of plant growth, it was reduced to once every 24 h. After completion of vegetative and reproductive growth the number of lateral branches was counted (the lateral branches with flowers and capsules were counted). At the end of the experiment, the plants were cut from the soil surface and from the rosette area (Mahbobi Khomami, 2006) to take the wet weight of all samples.

At the peak of flowering, the samples were taken from the young plant leaves with the highest amount of alkaloids (vinblastine and vincristine). According to the reports, harvesting the plant at the time of full bloom had the highest amount of alkaloids, particularly the alkaloids of vincristine (Lata, 2007). The samples were placed in the oven in 45°C for one week (Zarezadeh et al., 1997) to measure the total dry weight of shoots. Yanavan method was used to measure total alkaloids (Zarezadeh, 1997). To measure vinblastine and vincristine, HPLC method was used (British Pharmacopoeia, 2008; Skoog and West, 1994). The statistical analysis was conducted by using statistical software (Mstatc) and the Duncan's multiple-range test was used to compare the mean

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**Table 1.** Variance analysis of the effect of two factors of mycorrhizal biological fertilizer and four planting bed levels and their interaction on total shoot wet and dry weight and number of lateral branches.

S O V	df	Wet weight of whole aerial organs	Dry weight of whole aerial organs	Number of lateral branches
Replication	2	3495.72	146.601	83.621
Mycorrhiza	2	206.02**	50.14**	9.718*
Seedbed soil	3	183.89**	37.37**	20.174**
Mycorrhiza* Seedbed soil	6	26.43*	4.86*	1.695 <sup>ns</sup>
Error	22	10.27	1.81	2.402
CV%		6.45	9.74	17.63

ns: not significant, \*\* significant in 1% level, \* significant in 5% level.

comparison.

## RESULTS AND DISCUSSION

The results obtained from study of the effects of mycorrhiza and planting bed and their interactions on total shoot dry and wet weight and number of lateral branches (Table 1).

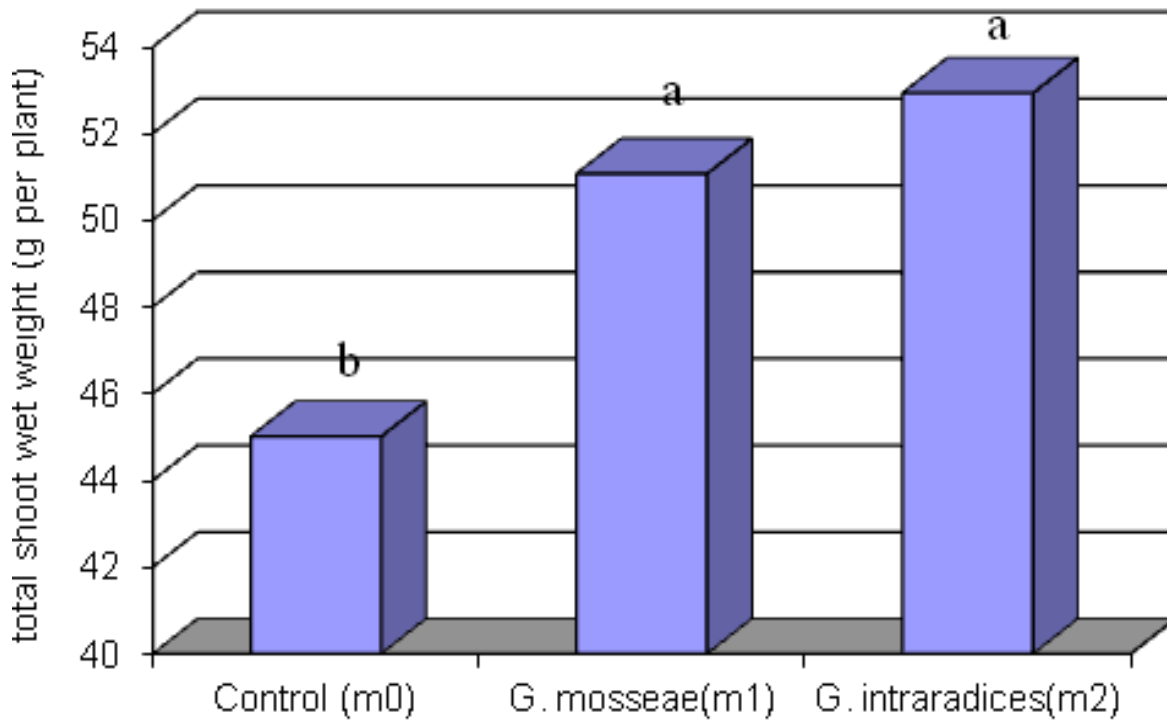
### Total wet weight of shoots

The analysis of variance (Table 1) indicate statistically significant difference at the level of 1% in using two factors of mycorrhiza and four levels of planting bed, and a significant difference at the level of 5% in the interactions between the different levels of mycorrhiza and planting bed on the total wet weight of shoots. The results of mean comparison (Figure 1) shows that the highest yield of total wet weight of shoots with the mean of 52.93 g per plant related to the treatment m2 (mycorrhiza type of *G. intraradices*), and then treatment m1 (mycorrhiza type of *G. mosseae*) with the mean of 51.07 g per plant had a significant difference with the treatment m0 (control, 45.01 g). However, no significant difference was statistically observed between the two types of mycorrhiza regarding total wet weight of shoots. The plants with mycorrhizal symbiosis, drain the water from the soil faster and more efficiently than non-mycorrhizal plants and lead to more decrease of the soil water potential, because in the mycorrhizal plants, the shoots usually develop more, the leaf area increases and this increases the need for evapotranspiration in mycorrhiza.

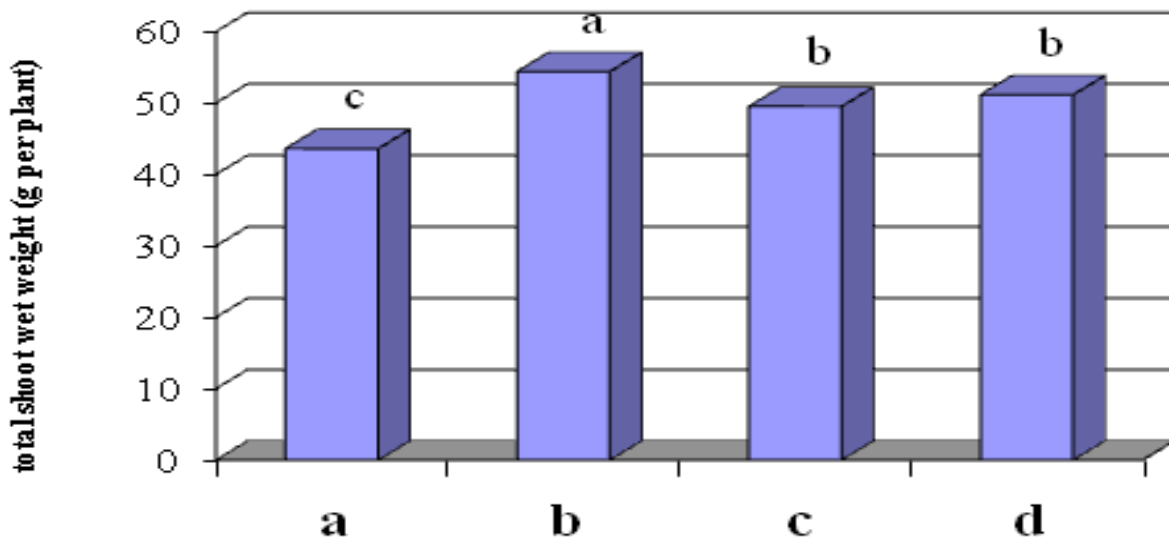
On the other hand, the root system of mycorrhizal plants is developed more and branches off more than mycorrhizal root and the diameter of lateral roots in them decreases and root length increases. All of these factors lead the mycorrhizal roots to have more contact level with the soil and so absorb the water from the soil faster.

Arbuscular mycorrhizal fungi are different in efficiency for increasing the water uptake from soils. This ability seems to be related to the amount of external mycelium produced by each arbuscular mycorrhizal fungi generated and the amount of root colonization according to living and active structures of fungus (Marulanda et al., 2003). Hassan (2012) stated that VAM mycorrhizal fungus significantly increases the wet weight of the branches and leaves of periwinkle (*Catharanthus roseus* (L.) G. DON). In another experiment on pink flower periwinkle (*Catharanthus roseus* (L.) G. DON), Sepehri et al. (2012) stated that using two mycorrhizal factors of *G. mosseae* and *G. intraradices* caused a significant difference at the level of 1% in the yield of total wet weight of shoots and top branches containing alkaloids. On a white flower periwinkle (*C. alba*), Sepehri et al. (2013) stated that using two mycorrhizal factors led statistically significant difference at the level of 1% in the yield of wet weight of top branches containing alkaloids. Research on wheat inoculated with mycorrhiza by Panwar (1992) was proved that mycorrhizal fungus increases the weight of root and shoot. In the research on the kinds of planting bed, the results showed the plants cultivated on the planting bed b with the mean of 54.4 g per plant had the highest total wet weight of shoots followed by the plants cultivated on the planting bed d with the mean of 51.15 g per plant and then the plants cultivated on the planting bed c with the mean of 49.51 g per plant had statistically significant difference with the planting bed a with the mean of 43.61 g per plant.

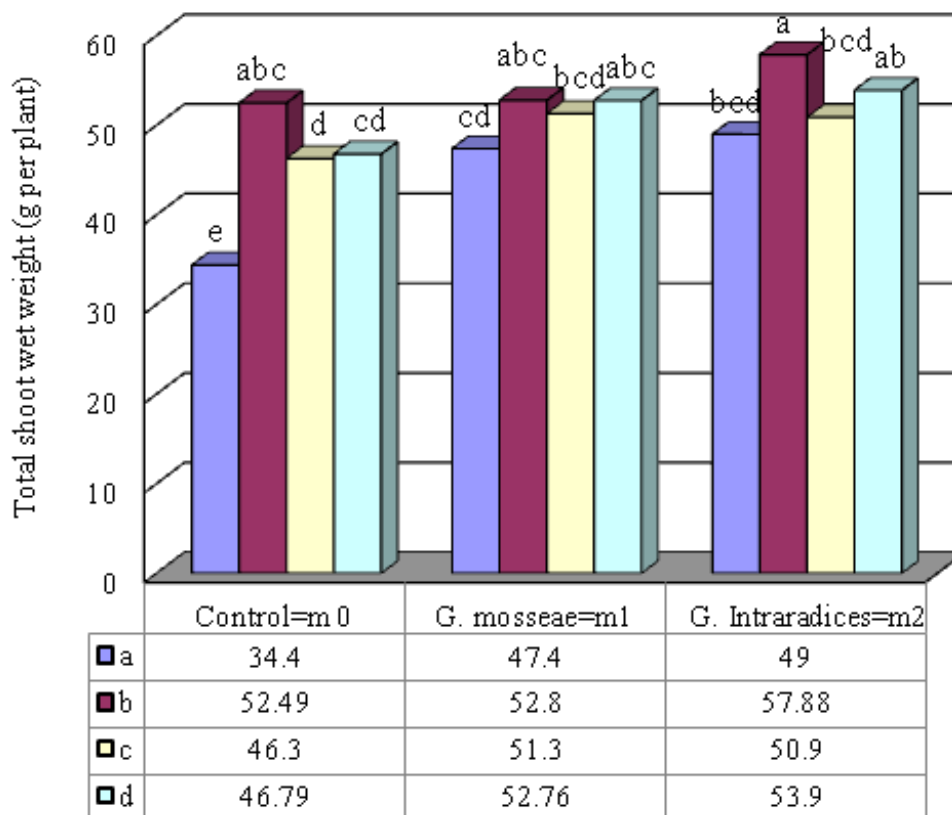
Also, the bed b showed a significant difference compared to the beds c and d, but two beds of c and d did not have any significant difference (Figure 2). While improving the soil texture, organic fertilizers (manure, compost and vermicompost) lead to increase the soil permeability, decrease the soil bulk density, capability of holding soil water and also increase the soil nutrient and makes it easy to absorb water and nutrients through the roots to the plant that it causes to increase plant growth and total wet weight of shoots. Khalil et al. (2007) reported that the highest wet and dry weight of single



**Figure 1.** Effect of two kinds of mycorrhiza on total shoot wet weight [m0= Control m1= *Glomus mosseae*; m2= *Glomus intraradices*]; [a= typical agricultural soil; b= agricultural soil mixed with rotten sheep manure, rotten leaves and Aeolian sand; c= agricultural soil mixed with vermicompost; d = agricultural soil mixed with compost].



**Figure 2.** Effects of planting beds on total shoot wet weight. a= typical agricultural soil (control) [am0= the bed of a without mycorrhiza; am1= the bed of a inoculated with mycorrhiza of *Glomus mosseae*; am2= the bed of a inoculated with mycorrhiza of *Glomus intraradices*]; b = agricultural soil mixed with rotten sheep manure, rotten leaves and Aeolian sand [bm0= the bed of b without mycorrhiza;bm1= the bed of b inoculated with mycorrhiza of *Glomus mosseae*;bm2= the bed of b inoculated with mycorrhiza of *Glomus intraradices*]; c = agricultural soil mixed with vermicompost;cm0= the bed of c without mycorrhiza;cm1= the bed of c inoculated with mycorrhiza of *Glomus mosseae*;cm2= the Aeolian bed of c inoculated with mycorrhiza of *Glomus intraradices*]; d = agricultural soil mixed with compost;dm0= the bed of d without mycorrhiza;dm1= the bed of d inoculated with mycorrhiza of *Glomus mosseae*;dm2= the Aeolian bed of d inoculated with mycorrhiza of *Glomus intraradices*].



**Figure 3.** Interaction of mycorrhiza and planting bed on total organ wet weight.[ m0= Control;m1= *Glomus mosseae*;m2= *Glomus intraradices*].

plant of *Dracocephalum moldavica* was obtained under the condition of using the organic fertilizer especially animal manure. In other experiment, Sepehri et al. (2013) also stated that using the manure, compost and vermicompost causes a significant difference at the level of 1% on the yield of wet weight of top branches containing alkaloid white flowers periwinkle (*C. alba*).

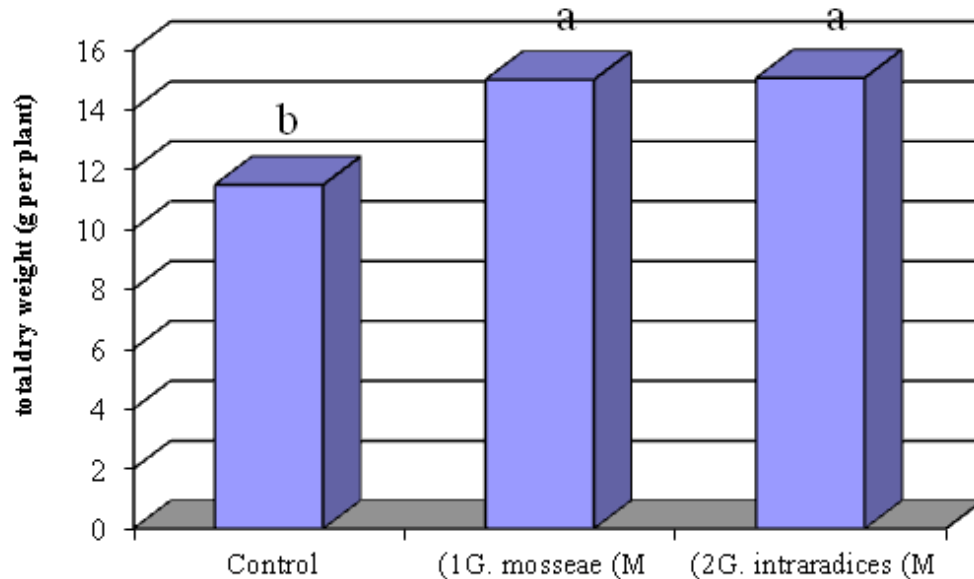
In another experiment on pink flower periwinkle (*Catharanthus roseus* (L.) G. DON), Sepehri et al. (2012) reported that the animal manure, compost and vermicompost caused a statistically significant difference at the level of 1% on total wet weight of shoots and the wet weight of top branches containing alkaloids. In the interactions study, it was observed that in the control treatment (m0), the plants cultivated on the planting bed b with the mean of 52.49 g per plant had the highest total wet weight of shoots followed by the planting bed d with the mean of 46.79 g per plant and then the planting bed c with the mean of 46.3 g per plant had a statistically significant difference with the planting bed a with the mean of 34.4 g per plant.

Also, the beds b and d as well as the beds c and d did not have any significant difference with each other (Figure 3). But in the treatment m1 (inoculated with the mycorrhizal type of *G. mosseae*), it was observed

that none of planting beds had statistically significant difference with each other on total wet weight of shoots. In the treatment m2 (inoculated with the mycorrhizal type of *G. intraradices*), it was observed that the plants cultivated on the planting bed b with the mean of 57.88 g per plant had the highest total wet weight of shoots and this bed had significant difference with the two beds a and d but it did not show any significant difference with the planting bed d. also, no statistically significant difference were observed between the beds a, c and d. while, by study and compare of the three treatments m0, m1 and m2, it was observed that in the beds of a, the plants cultivated in the treatments am1 and am2 had a statistically significant difference with the plants cultivated in the treatment am0 which the highest amount of it, was related to treatment am2 with the mean of total wet weight of 49 g per plant.

However, no significant difference was observed between the treatments am1 and am2. Three planting beds of b, the treatments bm0, bm1 and bm2, did not have any statistically significant differences with each other. Also, in the planting beds of c, three treatments cm0, cm1 and cm2 did not have any significant differences with each other. But, in three planting beds of d, the plants in the bed dm2 with the mean of total wet





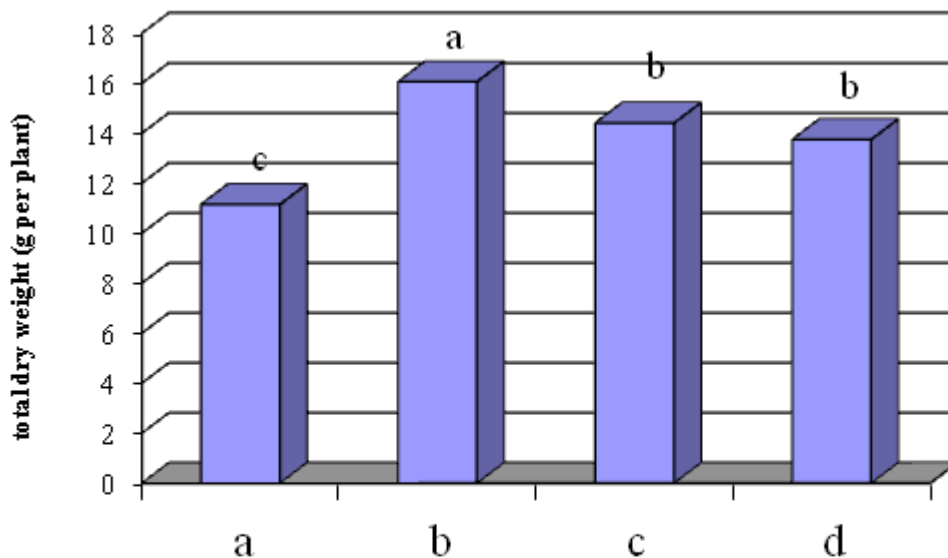
**Figure 4.** Effect of planting bed on total dry weight of shoots.[a= typical agricultural soil; b= agricultural soil mixed with rotten sheep manure, rotten leaves and aeolian sand;c= agricultural soil mixed with vermicompost; d = agricultural soil mixed with compost].

weight of 53.9 g per plant, showed statistically significant difference compare to the bed of dm0, but no significant difference was seen between the beds of dm1 and dm2 as well as between two beds of dm0 and dm1. It seems that these results were not due to the direct effect of manure, vermicompost and compost on the percentage of mycorrhizal symbiosis but they resulted from the effects of nutrients of above organic fertilizers on the direct and indirect development of fungus network and its effect on stimulating the root growth of host plant. Also using the vermicompost improves the growth and finally increases the yield of total wet weight of shoots by a positive effect on the percentage of mycorrhizal symbiosis and development of external hyphae and subsequently by the effect of mycorrhizal fungi on the development and prosperity of the host plant root growth. In a similar experiment, the interactions of different levels of mycorrhizal and planting beds on the wet weight of top branches containing alkaloid of white flower periwinkle (*C. alba*) did not report any significant effect (Sepehri et al., 2013). But in other research on pink flower periwinkle (*C. roseus* (L.) G. DON), the same researchers stated that the interactions between different levels of mycorrhiza and planting bed caused a significant increase of 5% in wet weight of top branches containing alkaloid and a significant increase of 1% in total wet weight of shoots (Sepehri et al., 2012).

#### Total dry weight of shoots

Using two factors of mycorrhiza and four levels of

planting bed caused a statistically significant difference at the level of 1% and in the interactions of different levels of Mycorrhiza and planting bed caused significant difference at the level of 5% on the yield of total dry weight of shoots (Figure 4; Table 1). Both treatments of m1, m2 showed statistically significant difference with control treatment (m0). The treatment of m2 (mycorrhizal type of *G. intraradices*) with a mean of 15.05 g per plant had the highest effect on total dry weight of shoots but no significant difference was observed between two treatment (m1 and m2). Increased uptake of water and nutrients required for plant led to increase the growth and photosynthesis of plants and improve the yield and increase the dry matter in plants. In the research on white flower periwinkle (*C. alba*), Sepehri et al. (2013) found that using two mycorrhizal types of *G. mosseae* and *G. intraradices* caused a statistically significant difference of 5% in the yield of dry matter of top branches containing alkaloids. Also in other research on pink flower periwinkle (*C. roseus* (L.) G. DON), Sepehri et al. (2012) stated that using two mycorrhizal types of *G. mosseae* and *G. intraradices* caused statistically significant difference of 5% in the yield of dry matter of top branches containing alkaloids and 1% difference in total dry weight of shoots. Hassan (2012) stated that VAM mycorrhizal fungi significantly increased the dry weight of branches and leaves in periwinkle (*C. roseus* (L.) G. DON). Also, in the research on corn, Subramanian and Charest (1997) observed that corn aerial biomass inoculated with mycorrhiza (*Glomus intraradices*) increased. In a research on single-cross corn, Sajedi and Sajedi (2009) found that mycorrhiza increased the biological yield and



**Figure 5.** Effects of planting bed on total dry weight of shoot [a= typical agricultural soil (control); am0= the bed of a without mycorrhiza; am1= the bed of a inoculated with mycorrhiza of *Glomus mosseae* ; am2= the bed of a inoculated with mycorrhiza of *Glomus intraradices* ;b = agricultural soil mixed with rotten sheep manure, rotten leaves and Aeolian sand; bm0= the bed of b without mycorrhiza; bm1= the bed of b inoculated with mycorrhiza of *Glomus mosseae* ; bm2= the bed of b inoculated with mycorrhiza of *Glomus intraradices* ; c = agricultural soil mixed with vermicompost; cm0= the bed of c without mycorrhiza; cm1= the bed of c inoculated with mycorrhiza of *Glomus mosseae* ; cm2= the Aeolian bed of c inoculated with mycorrhiza of *Glomus intraradices*; d = agricultural soil mixed with compost; dm0= the bed of d without mycorrhiza; dm1= the bed of d inoculated with mycorrhiza of *Glomus mosseae* ; dm2= the Aeolian bed of d inoculated with mycorrhiza of *Glomus intraradices*].

total dry weight of shoots and roots compared to the control treatment.

The results obtained from the effect of planting beds suggest that the plants cultivated on the planting bed b with the mean of 16.06 g per plant, the plants cultivated on the planting bed c with the mean of 14.41 g per plant and the plants cultivated on the planting bed d with the mean of 13.74 g per plant showed statistically significant difference with the planting bed with the mean of 11.15 g per plant. Also, the bed b showed a significant difference with other three planting beds in total dry weight of plants but two other beds of c and d do not have any significant difference with each other (Figure 5). While improving the soil and adding nutrients to the soil, using the organic fertilizers (manure, compost and vermicompost) lead to root development, optimal absorption of water and nutrients, and also improving the efficiency of photosynthesis and making matters in the plant and thus increasing the shoot growth and dry matter in the plant (Sepehri et al., 2012).

In a research on *D. moldavica*, Rahbarian et al. (2009) reported that using the animal manure increases the dry weight of plant. The results of research conducted by McCallum et al. (1998) on the use of compost in Cotton cultivation show that compost improves the germination of seed and increases the dry matter produced compared

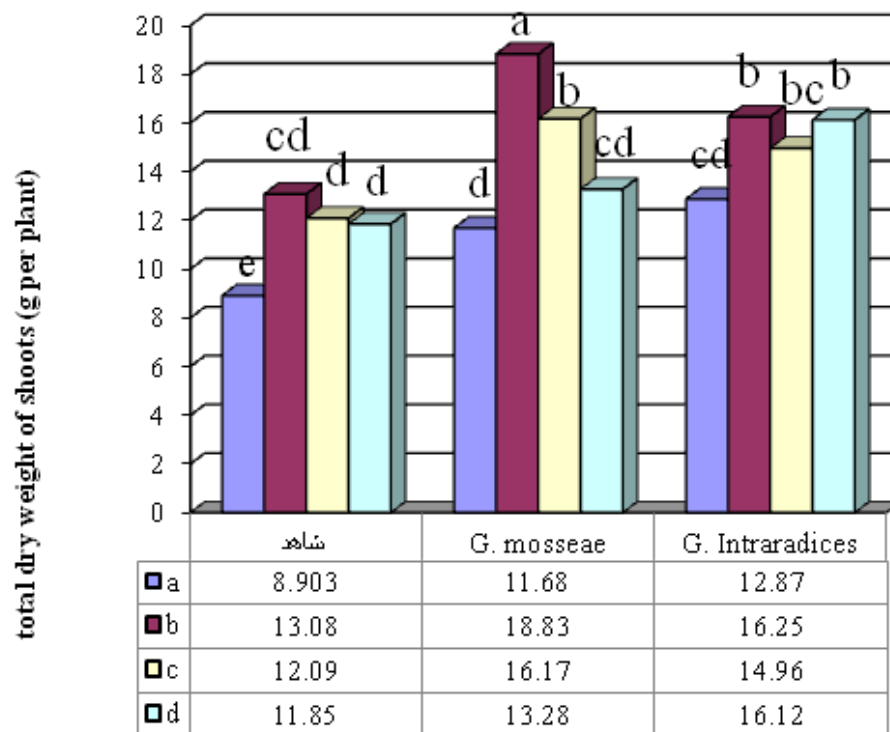
with the treatments without the use of compost. In a research on single-cross hybrid corn, Jahani et al. (2011) found that using vermicompost increased total dry weight of shoots compared to the control treatment.

In another experiment, the use of animal manure, compost and vermicompost caused a statistically significant difference of 1% in the yield of the dry weight of top branches containing alkaloid of white flowers periwinkle (*C. alba*) (Sepehri et al., 2013).

In study of the interactions of mean comparison result (Figure 6), it was observed that in the control treatment (m0) the planting bed of b, c and d had a significant difference with the planting bed a, but three mentioned planting beds did not show any significant difference in total dry weight of shoots. In this treatment, the highest total dry weight was related to the bed b with the mean of 13.08 g per plant and the lowest was related to the bed a, with the mean of 8.903 g per plant.

In the treatment m1 (inoculated with the mycorrhizal type of *G. mosseae*), it was observed that the planting beds of a, b and c as well as the beds of b, c and d had statistically significant difference with each other but no significant difference was observed between the planting beds of a and d. In this treatment, the highest total dry weight of shoots was related to the planting bed of b with the mean of 18.83 g per plant and the lowest was related





**Figure 6.** Interactions of different levels of mycorrhiza and planting bed on total dry weight of shoots (g per plant).

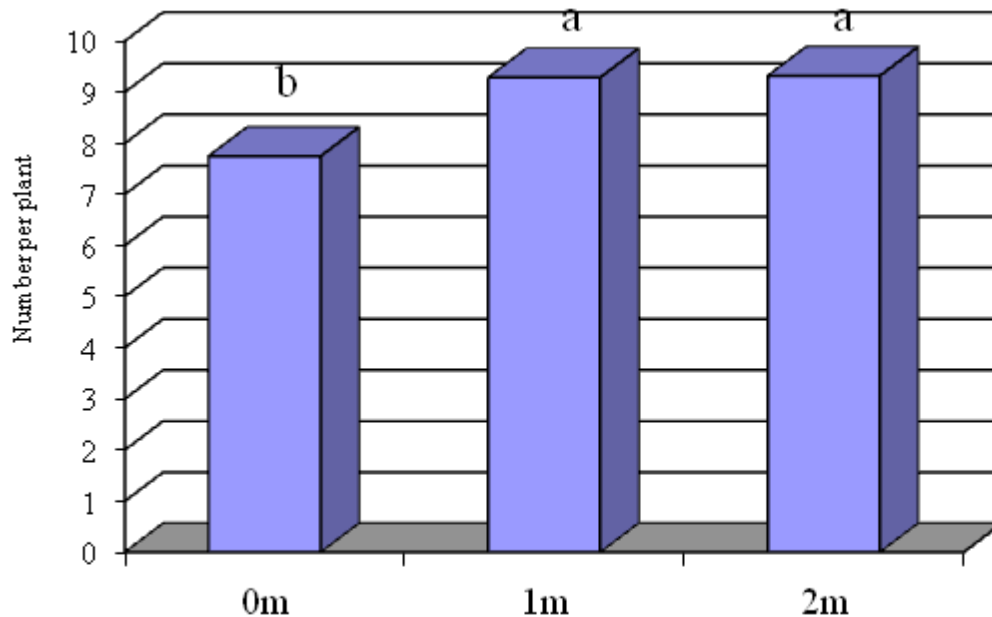
to the planting bed of a, with the mean of 11.68 g per plant. In the treatment m2 (inoculated with the mycorrhizal type of *G. intraradices*), it was observed that the planting beds of b and d had significant difference with the bed of a, but no statistically significant difference in total dry weight of shoots was observed between three planting beds of b, c and d. Also, no significant difference was observed between two planting beds of a and c. In this treatment, the plants cultivated in the planting bed of b with the mean dry weight of 16.25 g per plant and with negligible difference with the plants cultivated in the planting bed of d with the mean dry weight of 16.12 g per plant had the highest total dry weight of shoots and the plants cultivated in the planting bed of a, with the mean of 12.87 g per plant had the lowest total dry weight of shoots. But, in studying and comparing three treatments m0, m1, m2, it is observed that the plants cultivated in the beds of am1 and am2 have statistically significant difference with the plants cultivated in am0 that the highest amount of it is related to treatment am2 with the mean of total wet weight of 12.87 g per plant.

However, there is no significant difference between two beds of am1 and am2. Also, a significant difference was observed between three beds of bm0, bm1, bm2 with each other. The bed of bm1 with the mean of 18.83 g per plant, the bed of bm2 with the mean of 16.25 g per plant and the bed of bm0 with the mean of 8.903 g per plant included total dry weight of shoots. Also in three planting

beds of cm0, cm1 and cm2, it was observed that two beds of cm1 and cm2 have a statistically significant difference with the beds of cm0 in the control treatment. But no statistically significant difference was observed between two beds of cm1 and cm2. The bed of cm1 with the mean of 16.17 g per plant had the highest significant effect on total dry weight of shoots compared to control treatment. In three planting beds of dm0, dm1 and dm2, the bed of dm2 with the mean of total dry weight of 16.12 g per plant showed significant difference with two beds of dm0 and dm1, but no significant difference was seen between two beds of dm0 and dm1.

A research on grain sorghum by Alizadeh et al. (2009) was observed, the combined application of mycorrhiza and vermicompost cause a significant increase in biological yield. They stated that this increase is not due to a direct effect of vermicompost on mycorrhizal symbiosis but it is due to the effect of nutrients of vermicompost on direct and indirect development of fungus network and its effect on stimulating the root growth of host plant. It seems that the use of vermicompost leads to improve the growth and ultimately increase the seed yield in the plant through the positive effect that it has on the percentage of mycorrhizal symbiosis and development of external hyphae and subsequently the effect that mycorrhizal fungi has on the development of host plant root growth.

In another research, Hassan (2012) stated that the



**Figure 7.** Effects of two kinds of mycorrhiza on the number of lateral branches. m0= Control;m1= *Glomus mosseae*;m2= *Glomus intraradices*[a= typical agricultural soil;b= agricultural soil mixed with rotten sheep manure, rotten leaves and aeolian sand;c= agricultural soil mixed with vermicompost;d= agricultural soil mixed with compost].

interactions between VAM mycorrhizal fungi and bacteria *Bacillus* and rabbit droppings caused a significant increase in dry weight of branches and leaves of periwinkle (*C. roseus* (L.) *G. DON*) two consecutive seasons. In other research on the medicinal plant of anise (*Pimpinella anisum* L.) conducted by Darzi et al. (2010) found the interactions of Vermicompost and biological phosphate fertilizer caused a significant increase in biological yield of plant.

In a research on the periwinkle from the variety of Alba (*C. alba*), Sepehri et al. (2013) did not report any significant effect of different levels of mycorrhiza and planting bed on the dry weight of top branches containing the active ingredient. Also, in the research on the interactions of different levels of mycorrhiza and planting bed in the pink flowers periwinkle (*C. roseus* (L.) *G. DON*), no significant effect on the yield of total dry weight of shoots and the dry weight of top branches containing active ingredient was reported (Sepehri et al., 2012).

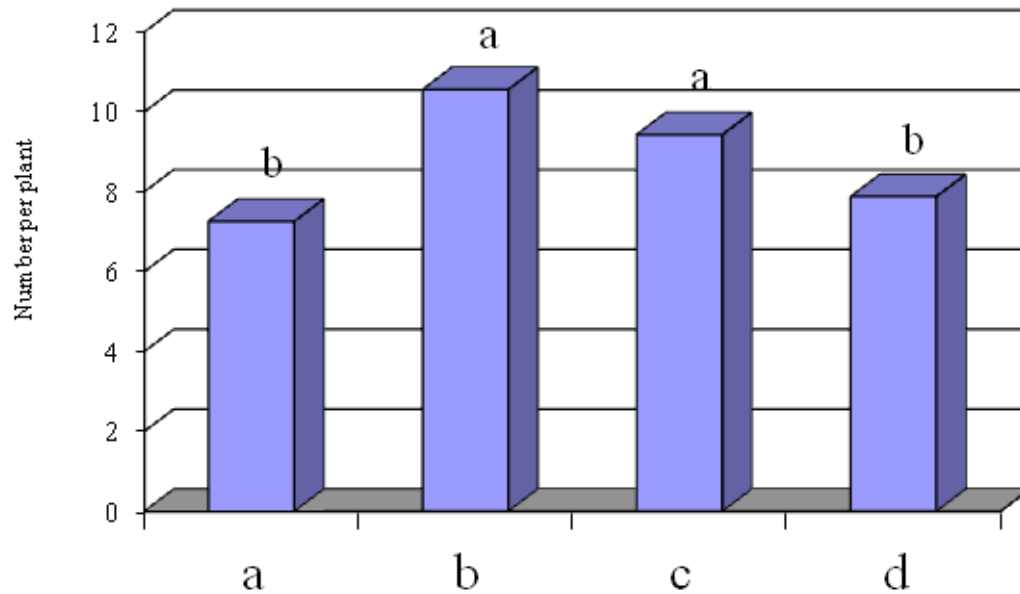
### Number of lateral branches

The results of research (Table 1) indicate a significant difference at the level of 5% in using two factors of mycorrhiza, at the level of 1% in using four levels of planting bed and lack of statistically significant differences in the interactions between the different levels of mycorrhiza and planting bed on total wet weight of the shoots. Both types of mycorrhiza showed a significant

difference in the yield of number of lateral branches with the control (m0).

However, no significant difference was observed between the two types of mycorrhiza. In treatment m2 (mycorrhiza *G. intraradices*) with the mean of 9.32 number per plant and treatment m1 (mycorrhiza type *G. mosseae*) with the mean of 9.288 number per plant included the number of lateral branches. But in many references, the positive effects of organic fertilizers on the development of mycorrhizal fungi, the composition of microbial communities, fauna, flora, soil, and the intensification of metabolic processes in the soil, root, branches and leaves has been emphasized (Fay and Osborne, 1996). The results suggested that mycorrhizal symbiosis increases the absorption of immobile nutrients in the soil such as phosphorus and zinc significantly (Al-Karaki, 2000).

One of the most important properties of mycorrhiza is absorption of important and essential elements in the soil such as nitrogen and phosphorus that in this research has led to increase the number of lateral branches. Phosphorus causes the strength of the roots and improvement of element absorption by the root as well as an increase in diameter growth and strength of stem and the number of lateral branches of the plant. In a research on soybean, Taherianfard et al. (2011) stated that the simple effect of mycorrhizal causes a significant increase in the number of lateral branches. In studying the interactions between manure, mycorrhiza and irrigation, they also observed a significant increase in the number of



**Figure 8.** Effects of planting bed on the number of lateral branches of white flower periwinkle.

lateral branches of soybean compared to the control.

In the research on the effect of different levels of planting beds, two beds of b and c had significant difference compared to planting beds of a and d, but no statistically significant difference was observed between two beds of b and c. Also, no significant difference was observed between two beds of a and d in the number of lateral branches. In the research, the bed of b with the mean of 10.56 number per plant and then the bed of c with the mean of 9.43 number per plant had the highest significant effect on the yield of number of lateral branches while the lowest amount was related to the soil of a, with the mean of 7.26 number per plant.

The animal manure and vermicompost increase the vegetative growth and the number of lateral branches in the plant by improving the soil and making it lighter and by increasing the water holding capacity of the soil as well as adding the necessary nutrients to the soil while the lighter soil leads to increase the root development and better activity of roots in the soil. The rotten leaves of sycamore and Aeolian sand are also used in the soil of b that help to making the soil lighter and improving it more and are much needed to the medicinal plant of periwinkle (Sepehri et al., 2012). In another study on pink flower periwinkle (*C. roseus* (L.) G. DON), Hassan (2012) found that fertilizing the plants with compost or rotted manure of rabbits at all levels resulted in a significant increase in the number of branches compared to untreated plants in two experimental seasons. Study on pink flower periwinkle (*C. roseus* (L.) G. DON), Sepehri et al. (2012) found that organic fertilizers (manure, compost and vermicompost) caused a significant increase of 1% in the number of lateral branches containing alkaloids compared to control treatment.

Rahbarian et al. (2009) stated that the use of animal manure and regular irrigation increased the flowering branches in the medicinal plant of *D. moldavica*. Padasht (2004) found that the use of compost had the best effect on growth indices of *Tagetes erecta* and increased the wet weight, dry weight, accelerating the flowering, increasing the number of flowers and the number of lateral branches in the plant. In the interaction of mycorrhiza and planting bed, no significant effect was seen on the lateral branches. In a study on soybean conducted by Mostajeran and Khozoei (1999), it was determined that in plants grown in soil with low phosphorus, the mycorrhizal infection resulted in high concentrations of phosphorus in the leaves and branches and more dry weight of stem and node compared to non-mycorrhizal treatment. The results of some researches indicate that mycorrhizal efficiency is reduced by increasing the fertility of the soil.

## Conclusion

In this study, according to the results, the mycorrhizal type of *G. interradices* (treatment m2) showed the highest significant effect on the characteristics of total wet and dry weight of shoots and the number of lateral branches. In studying the effect of planting beds, the bed b (agricultural soil mixed with sheep manure, rotten leaves and Aeolian sand) had the highest effect on all the characteristics. But in the interactions of mycorrhiza and planting bed, the treatment bm1 (bed b inoculated with mycorrhizal type of *G. mosseae*) and bm2 (bed b inoculated with mycorrhizal type of *G. interradices*) showed the highest effect on total wet and dry weight of

shoots, respectively. Finally, the bed b showed more favorable results in the yield of the medicinal plant of periwinkle.

### Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

## Losses of corn in the harvest, loading and transportation at rural property level

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Corn belongs to the family of Poaceae, and the corn grain is used mainly for human and animal consumption. This study aims to assess the total losses of maize grain in the harvest, losses in loading from the harvester to the truck and transportation losses up to its first point of reception. The experiment was conducted in the city of Itaipulândia-PR, Brazil. To perform the sampling of the losses in the crop methods of total loss provided by EMBRAPA, for the losses in loading we use samples of 24.5 m<sup>2</sup>, for the evaluation of losses in transportation, the methodology was the determination of fixed points of 15 m<sup>2</sup> where the mass of the lost grains was obtained, the obtained values were extrapolated to the distance up to the first point of reception. The obtained results were subjected to analysis of variance and the averages were compared with the Tukey's test at 5% probability using the Assisat Beta program. It was observed that the lowest loss on the harvest was at the speed of 4.5 km / h, already on loading the smallest loss it was at low rotation per minute of the harvester, for loading were statistically equal.

**Key words:** *Zea mays*, Embrapa, fixed points.

### INTRODUCTION

Corn (*Zea mays* L.) is a cereal which belongs to the family of Poaceae. It can be considered one of the main sources of nourishment nowadays and it is used as a source of carbohydrates to feed both humans and animals (Borém and Giúdice, 2004).

In the past corn was relevant to the function of subsistence; today its production is associated to commercial crops based on the use of modern technologies. It is a crop planted throughout the entire Brazilian territory forming the main input to the production of animal feed (Souza and Braga, 2004). The corn is one of the most important crops for Brazilian agriculture, due

to its wide variety of periods for planting, it stays on the field practically the whole year (Silva and Schipanski, 2006). A good amount of the food produced in the world is usually lost due to the neglect with which it is treated, occurring in the whole productive chain, from implementation of cultivation to its final consumption (Germiro, 2003). A significant part of the losses happen during the mechanized harvesting, decreasing productivity and yield of the operation, resulting in losses to the producer, as this is the final interference in the productive procedure, moment in which the grain has the highest added value (Sgarbi, 2006).

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Mantovani (1989) reported that the losses in the corn harvesting occur in three fundamental ways: in the pre-harvest, at the cutting deck and in the internal mechanisms of the harvester. These three factors combined bring extremely severe losses in production, in a state and national scale. Even though the origins of the losses are varied and occur both before and during harvest about 80 to 85% of them occur by the action of the cutting deck mechanisms for harvesters (Embrapa, 1998). According to Mesquita et al. (2001), grain losses are independent of brands and age of harvesters up to 15 years from 15 years losses can be high.

After the harvesting of the grains in the field, there is the need of properly storing them for future sales or consumption, done by institutes that may refer to the government, cooperatives or individuals (Silva and Rübénich, 2004). But until arriving to their destiny, there is a wide distance to be covered and expressive losses occur in the Brazilian crop, illustrated by the Agricultural Indicators 1996-2003 conducted by the Brazilian Institute of Geography and Statistics (IBGE) (2005). In Brazil, around 70% of Brazilian loads are moved by road transportation.

As described by Christopher (2011), the mission of logistics should be to plan and coordinate the activities in order to achieve the desired quality levels while having the lowest possible cost. According to the Brazilian Confederation of Agriculture and Livestock, the damage done by the spillage of grains during road transportation goes up to R\$ 2,7 billions in each harvest, being an amount to be considered as it causes a strong impact in the quotation of prices to the producer and the consumer (Perdas, 2005). During transport, part of the load is being lost from the truck due to vibrations of the truck, and also due to bad load seal. Also, your old vehicle fleet causes serious damage (Carvalho et al., 2012).

Semprebom (2009) shows that the losses occurred due to the logistics activities must be studied, measured, and controlled, so they are sustained at acceptable levels. Regarding the losses in transportation, Semprebom (2009) and Anes (2003) claim that these can represent 0.25% per moved ton. This problem affects practically all producing regions of the country.

Considering the importance of transportation for agriculture, Gallimore (1981) states that as important as the skill in production there must be an ability of moving it to consumer cores. Michaels et al. (1982) stress that "road transportation by trucks has the capacity to adjust to the variations in demand".

To Faria (2005), the road system is applied to small and medium loads, to short and medium distances, with collecting and delivery point to point, offering an extensive coverage, it can be described as flexible and versatile, being more related to the needs of customer service than other means of transportation.

Koo and Larson (1985) point out the importance of effectiveness of the transporting system for producers

and consumers, besides its essential connecting function between the production and the agricultural trading of products and inputs. The authors connect the prosperity of agriculture to the transporting system that enables the distribution of products and inputs at the lowest possible cost. According to Girardi (2011), the Brazilian harvest is on the road. Thousands of trucks carrying grain to silos, cooperatives and ports and lose part of the cargo on the way. The prejudice is for those who produce and those who carry.

According to work done by Caneppele and Sardine (2014) losses at the time of loading of trucks on farms occur due to the high rotation of the system of the harvester, because the grains hit the truck arches and end up falling out. According to the authors at the time it was proposed measure these losses, It was observed reduction of loss in cargo trucks.

According to the considerations above the objective of this paper was to evaluate the total losses of grains found in the harvest, the setting of the harvester will be in accordance to the conditions encountered. In addition, it was also evaluated the losses that occur in transportation of property up to the first point of reception, and the losses on the unloading from the harvester to the truck, in order to report where the mistakes possibly are, and show the most important points to try to prevent these losses that exist in Brazil.

## MATERIALS AND METHODS

The experiment was conducted in the city of Itaipulândia/PR, Brazil. In an area of 20 há<sup>1</sup>. The area was measured with a GPS Garmin Etrex 10® before initiating the experiment. The area had 40 collection points in the tillage, distributed by completely randomized design. For the losses in loading the evaluation was according to each discharge conducted from the harvester to the truck. The data collection in transportation was according to 20 evaluated trucks, being 10 sealed with pieces of tarp on the corners of the truck body and 10 without sealing, up until the reception point. The soil of the experimental area was classified as typic dystrophic Red Latosol (Embrapa, 2006).

The current study assessed the total losses in the harvest, the loading and the transportation up until the reception point. The total losses in the harvest were obtained in only one stage, the harvester used was an NH TC 57, the platform used was an NH 19 lines. After the operation of the harvester, random points were chosen with a 2 m<sup>2</sup> frame set in such a way that covered the whole width of the route taken by the harvester. All the grains within the frame were collected, including the ones that were on the cob, thus the mass of the grains was determined and this mass was converted into kg há<sup>-1</sup>, by using the following equation: loss (kg há<sup>-1</sup>) = mass of grains (g) x 10/ demarcated area (m<sup>2</sup>), this way the total loss per hectare in the harvest was obtained.

For the assessment of loading the methodology was a little more complex due to the lack of written papers found in literature about the subject. The methodology used for this assessment was: after each unloading through the discharging pipe from the harvester to the truck, the truck was removed from the road, as this job was done by an Mercedes Benz 1513 truck with a body made of wood, 6.80 m long, in good conditions. The methodology for this evaluation is given as follows after each discharged from the harvester to the truck the truck was moved, the grains were

**Table 1.** Tukey's test 5% of significance for speed variable.

Treatment	Grain mass (kg ha <sup>-1</sup> )
3.0 km/h	86.74 <sup>ab</sup>
4.5 km/h	72.50 <sup>b</sup>
6.0 km/h	85.26 <sup>ab</sup>
8.0 km/h	109.58 <sup>a</sup>
Overall average	88.52
P- value	0.46
CV (%)	27.98

Averages followed by the same letter are not statistically different from each other. The Tukey's test was applied at 5% level of probability.

**Table 2.** Tukey's test 5% of significance for low and high rotation variable.

Treatment	Grain mass (g)
Low rotation 425 RPM	33.43 <sup>b</sup>
High rotation 1150 RPM	57.57 <sup>a</sup>
Overall average	45.50
P- value	0.072
CV(%)	17.98

Averages followed by the same letter are not statistically different from each other. The Tukey's test was applied at 5% level of probability.

**Table 3.** Tukey's test 5% of significance for sealed and unsealed variable.

Treatment	Lost mass (kg/5 km)
Unsealed	24.92 <sup>a</sup>
Sealed	21.98 <sup>a</sup>
Overall average	23.45
P-value	0.37
CV (%)	14.94

Averages followed by the same letter are not statistically different from each other.

collected considering the area where the truck he was. Whereas the truck body had 6.8 m we use a margin of 10 cm in front the truck body and 10 cm behind the truck body, and 25 cm on each side the truck body, that is, we collect 7.0 m x 6.8 m long considering the truck body more margin 20 cm. And wide 3.5 m whereas the width of road 3.0 m more 50 cm margin. These margins were considered because it has been observed that many grains beat the arches of the truck and fall a few centimeters outside the truck perimeter, the collected grains were weighed to evaluate the mass of the same and at the end we observed losses on high speed 1150 RPM speed 425 rpm with 30 repetitions of each, totaling 60 samples, considering that the ability in the grain tank is 3300 kg of corn. For the evaluation of the transport losses also it was an evaluation with difficulty, due to the lack of

methodologies found in the literature to perform it, so the implemented methodology was as follows: within the property evaluated were chosen points on the roads, the width that was considered for these roads was of 3.0 m, each evaluated had an area of 5.0 m x 3.0 m<sup>-1</sup> (width of the road), these points were chosen where the condition of the roads was worse as curves and areas declined, were collected grains within the points and thus determined the mass of grain, the figure was extrapolated for the mileage to the first point of reception, evaluated 20 trucks and 10 sealed with tarps in the truck vertices and 10, not every truck had 5 points with 5 linear meters, in each truck had 25 linear meters of collection, the mass found in these 25 linear meters was extrapolated to 5000 linear meters distance this until the first point of reception, therefore it was possible to quantify the losses in transportation to the first reception by truck, considering sealed and unsealed. With all these data it was possible to quantify how much is lost at each step. The results of the variables were subjected to the analysis of variance and the averages were compared with the Tukey's test at 5% of probability, using the Assistant Beta program version 7.7.

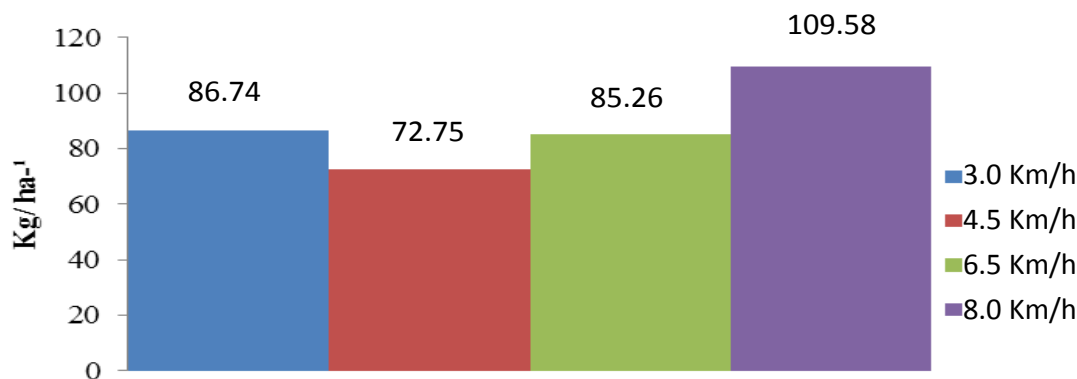
## RESULTS AND DISCUSSION

Analysing Table 1 we can observe that the losses in the harvest are different at a significance level of 5% according to the speed adopted by the harvester. On Table 2 we can observe the mass of the lost grains at the moment of unloading from the harvester to the track, taking into consideration the low and high rotation at the moment of unloading, the values are statistically different when compared to the significance of 5% adopted on the test.

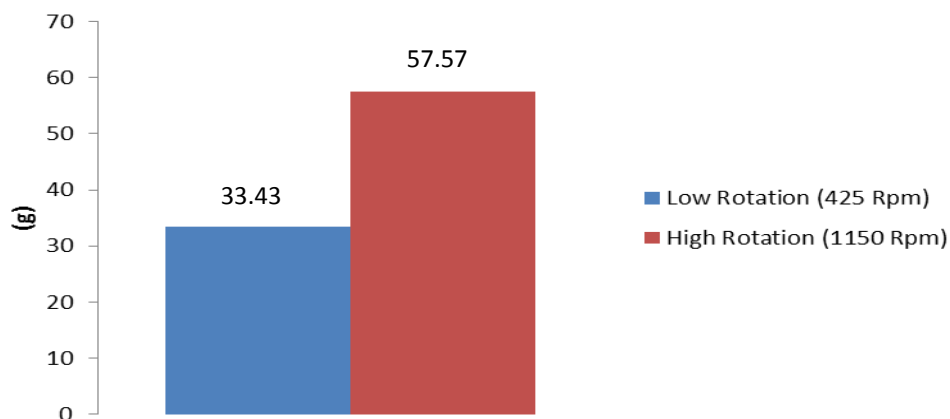
On Table 3 it is possible to visualize the losses during transportation, 1 truck was used in this assessment taking into consideration the sealed and unsealed cargoes coming to a total of 20 assessed truck. The collected averages of each truck were obtained, as 5 fixed points of each truck were collected and thus it was obtained the lost grains mass extrapolated up to the first reception point which was 5000 m away. We can see that the trucks did not differ at the 5% of significance to which they were subjected.

When analysing Figure 1 we can observe that the speeds too far below or too far above the recommended speed are more susceptible to losses, showing that there are statistical differences among the assessed speeds, the one with the lowest loss being the one at 4.5 Km/h, but all of them among the acceptable parameters of not more than 120 kg/ha (Embrapa, 2002). The indicated limits to working speed are from 4 to 7 km/h. When not respected, the rail system of the harvester gets overloaded, thus increasing the amount of not threshed grains (Cunha and Zandbergen, 2007). As shown on Figure 2, the losses of high and low speed are significantly different compared to the significance level of 5%, as these values are obtained in grams by bulk carriers taking into consideration 55sc/bulk carrier, that is, 3300 kg, being that the best way of unloading is with the machine at low rotation.





**Figure 1.** Crop losses as a function of the speed of the harvester.



**Figure 2.** Unloading losses due to the harvester rotation, by bulk Carrier.

This loss was assessed according to each performed unloading and thus the average for both low and high rotation was obtained, taking into consideration that 6 unloadings were necessary to fill up the truck; the losses per truck were on average from 200.62 to 345.42 g, respectively. It was possible to observe that these losses in loading usually happen due to the hurry of the operator who increases the rotation of the machine causing the product to hit the truck arches and to fall out of its back, or even when the person does not wait for the complete unloading of the product and closes the discharging pipe in advance causing an unnecessary loss at this moment.

This chart from Figure 3 shows the losses obtained on the trucks. As we can see their values are very close to each other, not being statistically significant at the 5% level. But it is significant for the income of the producer who ended up losing part of his production up to the delivery point, factors which often happen by filling up the truck over its capacity or by a badly sealed truck body. The grains logistics in Brazil is currently outdated, as it did not have the expected development and success. The foundations for the consolidation of logistic concepts

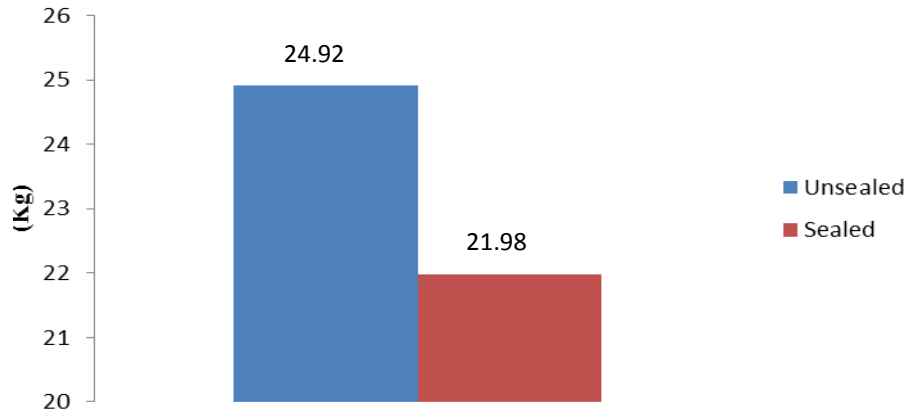
are fragile and their primary process, transportation, is currently dealing with a poor infrastructure with no conditions of support (Carvalho et al., 2012).

A solution for the upper sealing of bulk carrier trucks was presented by Tsiloufas et al. (2011). It is an innovating product, which puts on the tarp in an automated way, in a simple and robust design. From the point of view of the responsible ones for the carrier, the product is economically viable, once that the savings provided by the product exceeds the initial investment in a reduced number of trips.

## Conclusion

Based on the results of this paper we can conclude that regarding the losses on harvest the best option is the speed of 4.5 km/h which caused a loss of 72 kg/há<sup>-1</sup> the lowest one among the assessed speeds value which is within the parameters described in literature. The results of losses on unloading are significantly different showing that the best way of unloading is at low rotation, thus





**Figure 3.** Transportation losses from the property to the first reception point 5 km away.

avoiding unnecessary losses. The losses on transportation were not statistically significant, but they are relevant for the producer, as they can go up to 31.72 kg of lost grains mass in 5 km, which is equivalent to 6.34 kg/km, facts that can be avoided if the weight limits of the truck are respected and if the truck is properly sealed.

### Conflict of Interests

The authors have not declared any conflict of interests.

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A person in a light blue shirt and dark vest is working in a field, possibly harvesting oranges. The background shows trees and a clear sky. The foreground is filled with ripe, bright orange fruits.

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