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*Full Length Research Paper*

# The influence of several irrigation water depths in the growth and productivity of coffee shrubs in the Muzambinho Region, Southern Minas Gerais, Brazil

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**Coffee crop, highly important worldwide, is one of the main commodities in Brazilian exports. However, due to climate changes, its cultivation is currently demanding irrigation techniques. Current assay evaluates the effect of different irrigation water levels on the development and productivity of coffee shrubs in predominant soil and climate conditions of the town of Muzambinho, in the southern region of the state of Minas Gerais, Brazil. The experiment was performed in July 2013 on a coffee plantation, planted with coffee shrubs, cultivar Red Catuaí IAC/144, by drip irrigation. Experiment design consisted of randomized blocks with four replications and four treatments, or rather, different irrigation water depths: 0 (without irrigation), 50, 100 and 125% of $ET_0$. Productivity, vegetal growth and distribution of the radicular system were evaluated after 12 months. Treatments did not affect vegetal growth. Better quantity and distribution of the radicular system were detected at 100% irrigation level, with a productionof 55 sacks.$ha^{-1}$ or a 45% gain when compared to that in non-irrigated areas.**

**Key words:** Management, coffee, drip system.

## INTRODUCTION

It is a well-known fact that the coffee shrub is greatly affected by water deficit which causes a subsequent fall in production. Required irrigation has been employed to stimulate the shrub's vegetal development, increase production and harvest better grains. Much research is needed to discover the best form to supply water demands in coffee plantations. In fact, there are no definite criteria for irrigation management with regard to two factors: when irrigation is required or irrigation schedule, fixed or variable, and the amount of irrigation or

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the water depth necessary to supply water to the shrub (Silva et al., 2011).

The pressurized irrigation system allows drip distribution in the region, though the water must be of good quality so that no clogging or damage to the system's efficiency occurs (Valipour, 2012a, b, c; Valipour, 2014a, b).

When compared to the aspersion irrigation system, the great advantage in the drip system lies in the fact that the water applied on the soil surface does not moisten the leaves or the stem. The method reduces the occurrence of fungus diseases in the cultivation. Another advantage is the water amount, or rather, the great efficiency in its application and in the application of fertilizers (Boas et al., 2011). According to Evangelista (2011), success in irrigated coffee culture depends on the proper management of the natural resources soil-water-plant which interacts with the air and determine the potential conditions of maximum productivity of a culture in full phytosanitary and nutrition conditions.

Oliveira et al. (2010) reported the effect of drip irrigation on the production of coffee plantation in the first six harvests and verified that the productivity of the irrigated coffee shrubs averaged 50% higher than that without any irrigation. Drip irrigation in coffee culture is economically viable since a 33.48% increase in productivity, caused by irrigation, provides better income. Irrigation-caused productivity rise is an asset in investing in coffee production, with a considerable rise in economic levels and a decrease in the time for stock return.

Silva et al. (2011) applied water depth levels according to pre-defined percentages of the coefficient rates of the culture (Kc), namely, 60, 80, 100, 120 and 140% of Kc rates, plus a treatment without any irrigation. In the 2007 and 2008 harvests, the yearly productivity was higher than that in non-irrigated parcels. Further, the highest productivity in both harvests occurred with treatment at 100% water depth of Kc.

Rezende (2006) evaluated irrigation water levels 0% (L0, without irrigation), 40% (L1), 80% (L2) and 120% (L3) of evaporation of Classe A Tank (ECA) of 2002/2003 and 2003/2004 coffee harvest (cv. Topazio MG-1190). Accumulated productivity was higher for irrigated treatments, with increase when compared to non-irrigated ones; varying between 23.68% (L1) and 68.23% (L2) when compared to non-irrigated coffee shrubs.

Bruno et al. (2007) researched 3 to 5-year-old coffee shrubs, cv. Catuaí, and reported that the climatological water balance based on evapotranspiration estimates by the Thornthwaite and Penman-Monteith method replaced adequately field measurements and made possible a more practical irrigation management. Reference evapotranspiration ($ET_0$) is the evapotranspiration on a reference surface, with no lack of water. $ET_0$ may be obtained by direct and precise techniques with specific equipments, such as lysimeters, or estimated by mathematical models, with satisfactory results (Alves Sobrinho, 2011).

Due to the difficulties in the management of coffee shrubs, current experiment verified the effect of different irrigation levels, calculated by reference evapotranspiration, with drip irrigation, on the growth and productivity of coffee culture.

## MATERIALS AND METHODS

### Features of the experimental area

The experiment was conducted on the coffee culture experimental area of the Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais – Campus Muzambinho (IFSULDEMINAS), in the municipality of Muzambinho, in the southern region of the state of Minas Gerais, Brazil. According to Koppen's classification, the region's predominant climate is Cwb, with mean annual temperature at 18°C and mean yearly rainfall 1605 mm. The central point lies at 21° 21' 01.07''S and 46° 31' 21.10''W, at a mean altitude 1100 m.

Planting of 360 six-month-old coffee seedlings (*Coffea arabica* L.) var. Red Catuaí (IAC/144), with excellent phytosanitary conditions, occurred on January 2012. There was 3.0 spacing between the rows and 1.00 between the holes, totally 3333 plants per hectare, in an area 64.5 m long and 21.5 m wide, approximately totaling 1344 $m^2$.

### Irrigation

The plants were irrigated three times a week by a localized irrigation system, with auto-compensating drip emitters, at a discharge of 1.3 $L.h^{-1}$ at every 30 cm, with a single irrigation row under the surface of each coffee shrub row.

Conditions tested in current experiment did not allow the application of FAO Peaman-Monteith method. However, empirical methods comprising mass transference, temperature and evaporation-based methods have been applied to estimate ET0. In fact, several research works show the efficiency of such methods (Valipour, 2014c, d, e, f, g, h, i, j, k; Valipour, 2015a, b, c). Applied water levels were calculated by Equation (1) with 0.9 efficiency, following Montovani (2011). Water excess in the soil was not reported during the experiment. Reference Evapotranspiration ($ET_0$) was calculated by the Penman-Monteith. Depths were calculated according to rainfall and water balance of the soil (Allen et al., 1998) (Figure 3b). The sum of $ET_0$ was performed every three days subtracting rainfall amounts.

$$Li = \frac{ETo * Kc}{Ei}, \tag{1}$$

where $Li$ is the irrigation water depth [mm]; $ETo$ is the reference evapotranspiration (Penman-Monteith) [mm]; $Ei$ is the efficiency of irrigation; $Kc$ is the crop coefficient.

### Experimental design and treatments

The experimental design comprised randomized blocks with four treatments and four replications. Treatments were water levels applied as percentages of reference evapotranspiration ($ET_0$), namely: Li01 = 0 (without any irrigation); Li02 = 50% $ET_0$; Li03 = 100% $ET_0$; Li04 = 125% $ET_0$, totaling 16 parcels. Each block was

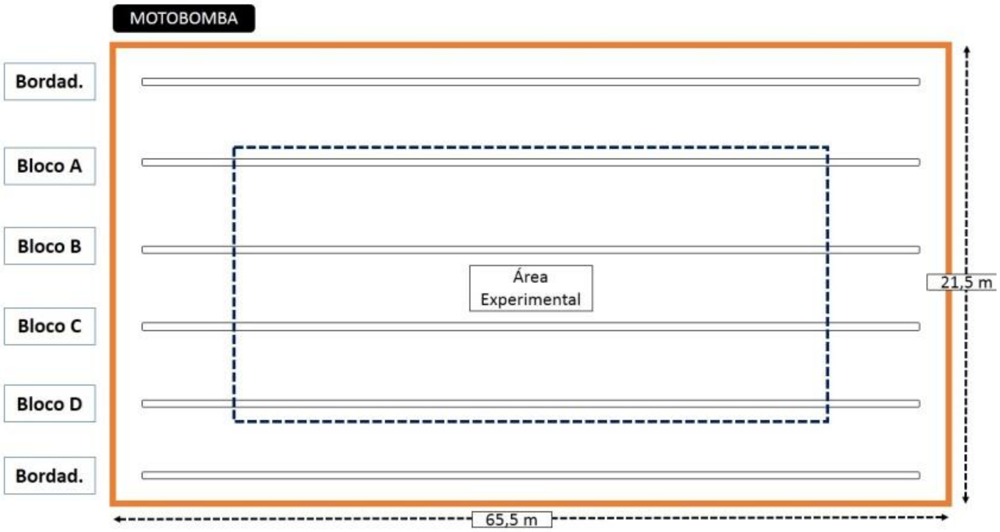


**Figure 1.** Experimental scheme.

composed of a row with 60 plants, totaling 240 plants. Each parcel comprised one row with 6 plants, but only the two central plants were evaluated; the other plants on the row were kept at the margins (Figure 1).

### Estimates of reference evapotranspiration (ETo)

Reference evapotranspiration ($ET_0$) was estimated by the Penman-Monteith method, FAO 1998 standard, following Allen et al. (1998),

$$ET_0 = \frac{s}{(s+\gamma^*)}(R_n - G)\frac{1}{\lambda} + \frac{\gamma}{(s+\gamma^*)}\frac{900}{(T+273)}U_2(e_s - e_a) \quad (2)$$

where $s$ is the curve declivity of vapor saturation pressure [kPa °C$^{-1}$]; $R_n$ $R_n$ is the radiation balance [MJ m$^{-2}$ d$^{-1}$]; $G$ is the heat flow in the soil [MJ m$^{-2}$ d$^{-1}$]; $\lambda$ is the evaporation latent heat [MJ kg$^{-1}$]; $e_a$ is the partial pressure of the vapor [kPa]; $e_s$ is the pressure of vapor saturation [kPa]; $\gamma$ is the psychrometric coefficient [kPa ºC$^{-1}$], $\gamma^*$ is modified psychrometric coefficient [kPa °C$^{-1}$]; $T$ is mean air temperature [ºC]; $U_2$ is the mean speed of wind at a height of 2 m [m s$^{-1}$].

Climate variables for the estimation of $ET_0$ during the experiment were obtained from a Davis Vantage Pro 2 meteorological station, at 21°18'00"S and 46°30'00"W, mean altitude 1033 m.

### Vegetal characteristics

After the start of treatment applications, the monthly evaluations on the vegetal growth of the plants were undertaken throughout the experiment, between July 2013 and July 2014, comprising height of plant (HP), measured by a graded bar from the soil surface to the tip of the plant, in cm; diameter of canopy (DC), measured by a graded bar at the height of the third middle of the plant and perpendicular to the row [cm]; number of primary plagiotropic branches (NPPB), measured all the plagiotropic branches in the plants. Radicular growth was measured by an auger drill at depths 20, 40 and 60 cm and at two distances from the trunk, at 20 and 50 cm, on July 2014.

### Productivity and quality of the coffee plants

After the harvest of the experimental parcels, samples of coffee from cloth-sieve were retrieved and dried daily until moisture reached 11 to 12%. After drying, the samples were weighed, processed and weighed again. Data from all the process phases were used to calculate productivity, expressed in 60 kg of coffee per hectare.

Samples were removed from the processed volume for type and sieve classification. Grain size classification was undertaken in samples of 300 g and obtained by grain percentages in circular sieves (16 mm).

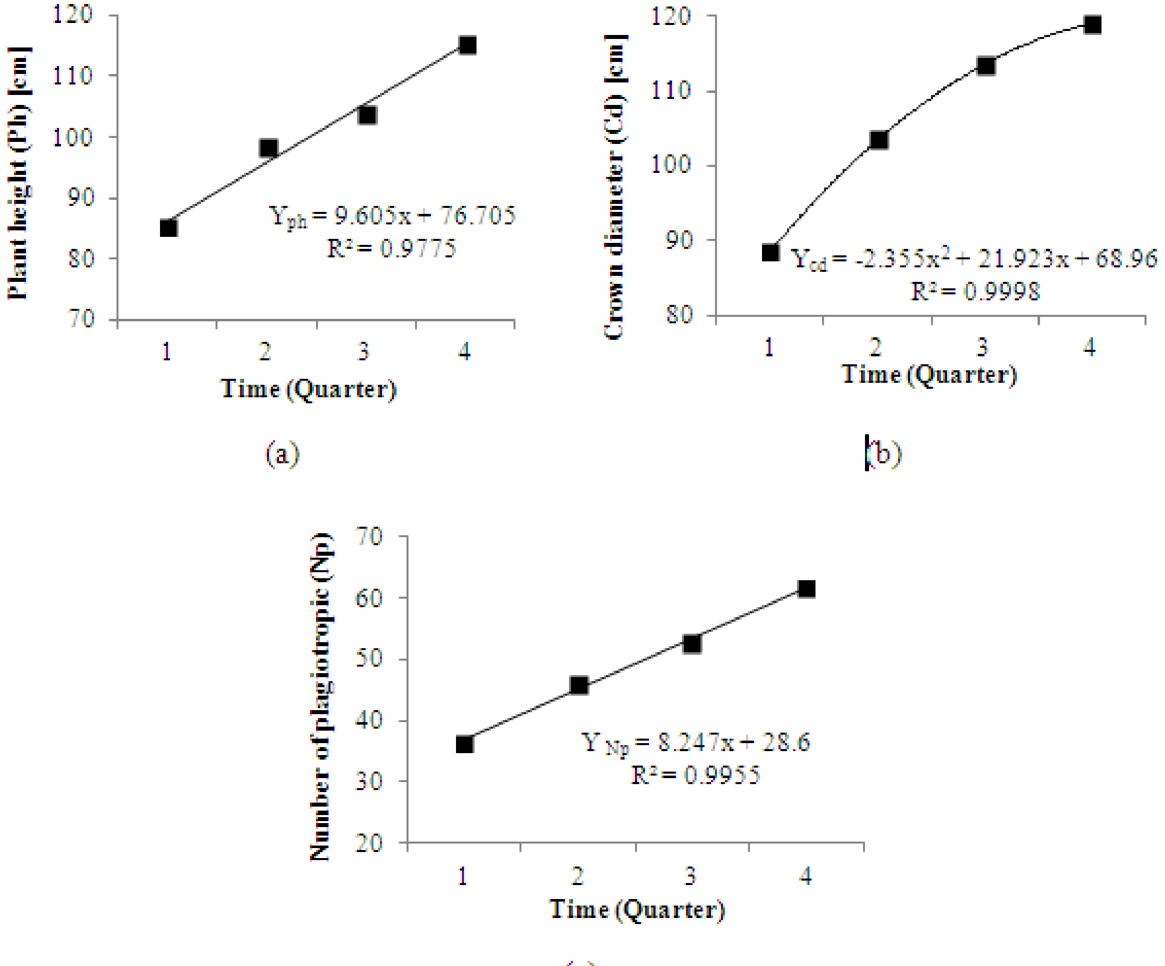
### Statistical analyses

Evaluation results underwent analysis of variance (ANOVA) by F-test; when results were significant, means were compared by Scott & Knott test at 5% significance, with SISVAR (Ferreira, 2011).

## RESULTS AND DISCUSSION

Vegetal growth was not affected by treatments, or rather, there were no significant effects on plant height, number of plagiotropic branches and crown diameter by F-test ($p<0.05$). The variables height of plant, the number of plagiotropic branches and crown diameter did not behave differently with different water regimes.

Figure 2 gives details on the equations of vegetal growth for treatment Li 3 = 100% $ET_0$, taking into consideration height of plants, crown diameter and number of plagiotropic branches versus time, coupled to the result of the regression test for this parameter with coefficient of determination at 97.7, 99.9 and 99.5%.



(a)

(b)

(c)

**Figure 2.** Vegetal growth of the coffee shrub between July 2013 and July 2014. Height of plants (A); diameter of crown (B); number of plagiotropic branches (C).

Linear behavior and higher growths were reported for the variable height and number of plagiotropic branches; a linear and quadratic adjustment was registered for the crown diameter.

Height of plant reached 115 cm, with a 35% growth during the year. The crown diameter reached 120 cm with 62 plagiotropic branches during the same period, featuring respectively a yearly growth of 35 and 70%. In their research on different cultivars of irrigated coffee shrubs in the savannah of Goiás, Brazil. Oliveira et al. (2004) reported heights between 111 and 120 cm and 47 to 50 plagiotropic branches per plant of the 24-month-old cultivar Red Catuai. Vegetal growth was similar to that in current analysis.

Rezende et al. (2010) reported a positive irrigation effect on plant growth with regard to height and crown diameter in 457-day-old Obatã-IAPAR-59 cultivar. Carvalho et al. (2006) also registered that the crown diameter of the coffee shrub cultivar Rubi MG-1192 was also affected by irrigation, underscoring its benefits in the development of coffee cultivation.

Figure 3 gives data on rainfall, evapotranspiration (ET), water storage and irrigation depth between July 2013 and June 2014. Irrigation depths did not influence the vegetal growth, probably due to the good water conditions of the soil assessed between July and mid-September 2013. On the other hand, the soil suffered a water deficit from October till the end of November. In December, the soil's water deficit decreased significantly due to rainfall increase, with good water conditions during approximately four months, from July to December 2013. This fact may have contributed towards the development of the plants within the crop´s vegetal growth phase.

Irrigation affected the distribution of the radicular system ($p<0.05$). As a rule, roots had a greater concentration at a distance of 20 cm from the trunk, or rather, practically for the projection of the coffee plants' crown (Figure 4). A decrease in the number of roots

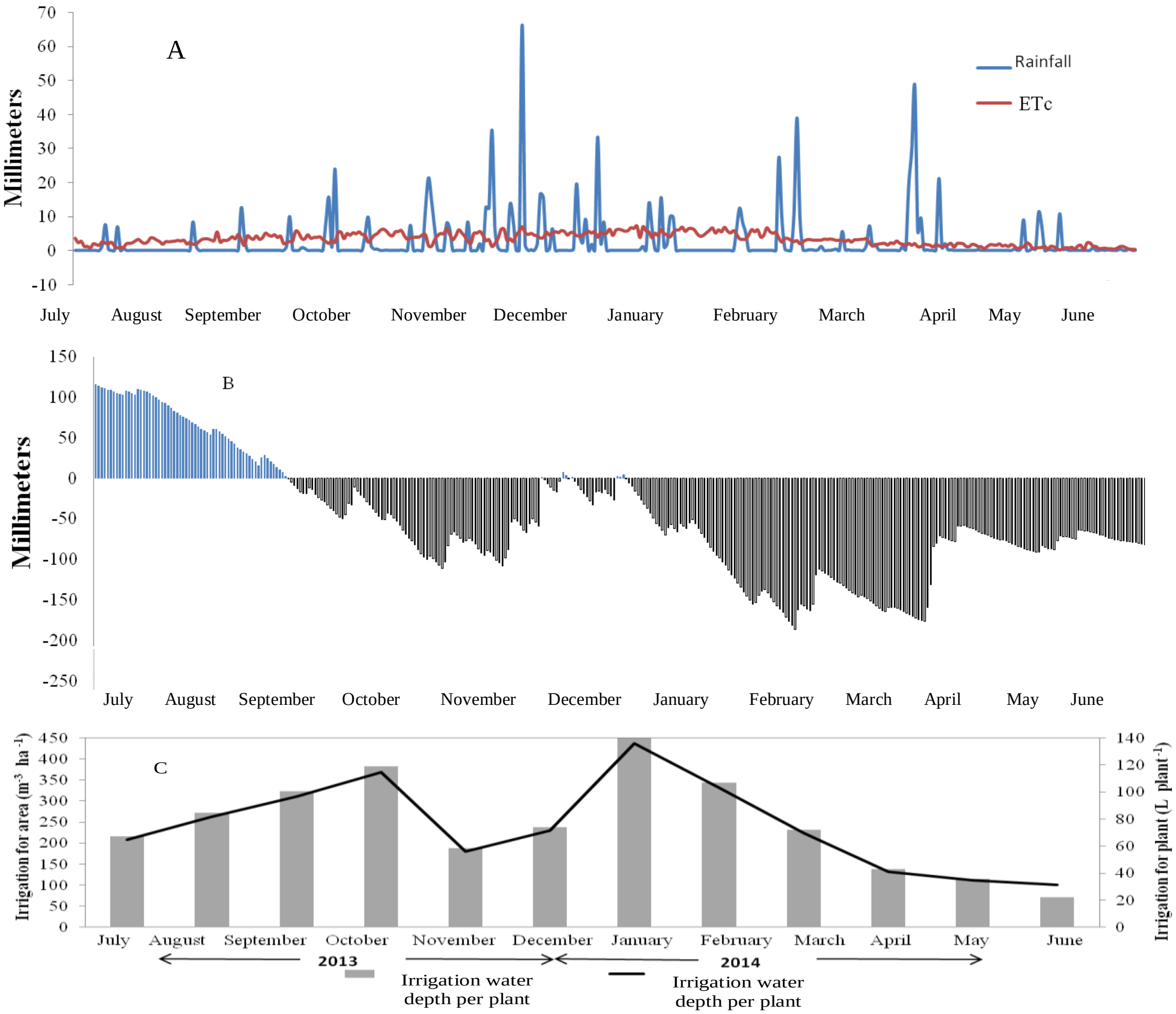


**Figure 3.** A) Rainfall and evapotranspiration (ET); B) water storage; C) Irrigation water depth between July 2013 and June 2014.

occurred in most treatments when distance increased to 50 cm from the trunk. At a depth up to 60 cm, there was a decreasing trend in the quantity of roots in the treatment with plants growing without any irrigation water.

According to Carducci et al. (2014), the greatest concentration of the coffee shrub's radicular system occurs with the crown projection band, predominantly at depths between 20 and 34 cm, corroborating results obtained in control as those with different irrigation water depths.

The treatment with 100% irrigation water depth

**Table 1.** Summary of the analysis of variance for productivity of the 2012 harvest of coffee plant (*Coffea arabica* L.) cv. Red Catuaí (IAC/144) and the test for the comparison of means of harvested and processed coffee, yield, sieve over 16, coffee productivity, according to different treatments.

| Irrigation depth (%) | Harvested coffee (kg plant$^{-1}$) | Processed coffee (kg plant$^{-1}$) | Yield (%) | Sieve over 16 (%) | Productivity (sacks ha$^{-1}$) |
|---|---|---|---|---|---|
| 0 | 3.262$^{B}$ | 0.6317$^{B}$ | 23.12$^{B}$ | 61.21$^{B}$ | 39.39$^{C}$ |
| 50 | 3.536$^{B}$ | 1.0515$^{A}$ | 31.10$^{A}$ | 61.65$^{B}$ | 57.18$^{A}$ |
| 100 | 4.267$^{A}$ | 1.0117$^{A}$ | 24.29$^{B}$ | 74.23$^{A}$ | 55.36$^{A}$ |
| 125 | 3.238$^{B}$ | 0.8045$^{B}$ | 24.49$^{B}$ | 64.88$^{B}$ | 45.38$^{B}$ |
| | | | **Statistical parameters** | | |
| Treatment | 22.431** | 11.268** | 5.242* | 18.17* | 67.02** |
| Block | 0.260$^{NS}$ | 0.687$^{NS}$ | 1.275$^{NS}$ | 3.075$^{NS}$ | 0.121$^{NS}$ |
| CV (%) | 5.68 | 13.27 | 12.27 | 4.32 | 11.17 |

Means followed by the same capital letter on the vertical line do not differ by Scott-Knott test at 5% probability; ** - significant by F-test 1% probability level; $^{ns}$ – not significant by F-test at 5% probability level.

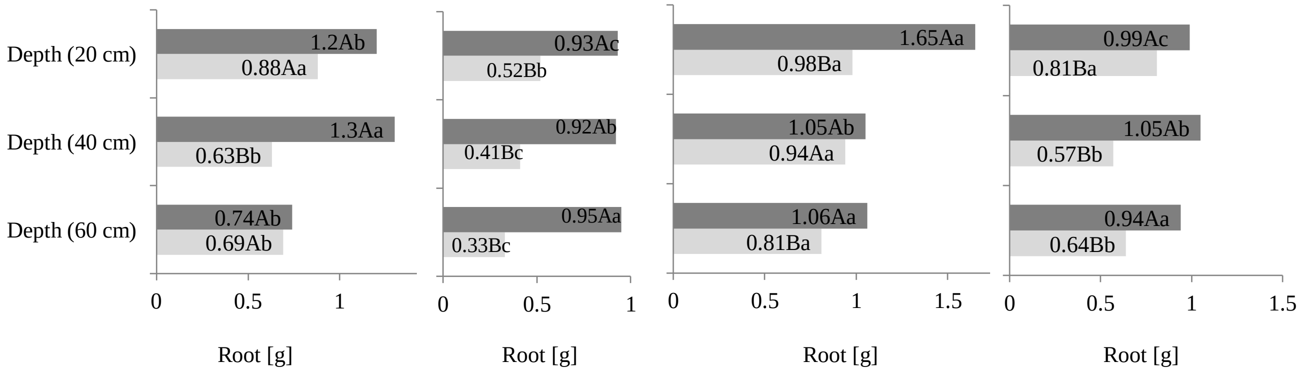


**Figure 4.** Distribution of radicular system according to different irrigation water depths, at different distances from the trunk and soil depths. Means followed by the same capital letter on the vertical line and by a small letter on the horizontal line did not differ by Scott-Knott test at 5% probability.

(Li 03) tended towards a greater number of roots distributed at a 20 to 50 cm distance from the trunk and at a 20 to 60 cm in depth. The treatment with the combination 50 cm distance and 40 to 60 cm depth was better than the others; similarly, at a distance of 20 cm from the trunk and at a depth of 20 cm.

There was a decrease in the radicular system in treatment with 50% irrigation water depth at a distance of 50 cm from the trunk for all soil depth evaluated and in all treatments studied. Growth decrease in the radicular system, even when compared to control treatment (without any irrigation water), may be related to the re-translocation process of photo-assimilates for the aerial section of the coffee shrub in this treatment, aiming to maintain the high productivity reported (57 sacks ha$^{-1}$), statistically equal to treatment with 100% of water irrigation depth (Table 1).

There was probably better water distribution in the treatment 100% irrigation water depth (Li 03) at a 20 cm distance from the trunk. This was due to the dripper which formed a moist bulb with an amount of water close to the ideal for the coffee shrub and which contributed towards a higher radicular growth with the sampled band. In the sample 50 cm from the trunk, there occurred a natural decrease in water availability, with a decrease in the radicular system. Similar results were verified by Barreto et al. (2006) in a drip irrigated coffee plantation. According to these authors, high water rate in the soil interferes in radicular aeration and respiration, besides making difficult the passage of ethylene produced by the radicular system, and by soil pores, jeopardizing root growth. The above may explain the behavior of radicular growth in treatment 125% of irrigation water depth (Li 04) in current analysis (Figure 4).

## Characteristics of production

Table 1 shows a summary of the analysis of variance for the productivity of processed coffee for the 2012 harvest.

There was a significant effect ($p<0.05$) for all evaluated production attributes.

Table 1 reports the analysis of variance for granulometry and demonstrates that irrigation water depths applied according to $ET_0$ percentage affected significantly the granulometry of the coffee grains.

Irrigation at 100% water irrigation depth provided 74% of over 16-sieve coffee grains, registering the best result for the variable. It is actually about 8% more grain than that of other treatments, which is highly beneficial from the commercial point of view, since over 16-sieve grains are better classified on the market.

Processed coffee production derived from irrigation Li 03 (100% $ET_0$) was higher when compared to treatment without irrigation and to treatment 120% of $ET_0$. It was statistically equal to coffee with 50% $ET_0$, with rates between 1.01 and 1.05 kg plant$^{-1}$, with no difference by Scott-Knott test at 5% probability. In fact, irrigation enhanced an approximately 60% increase of processed coffee per plant when compared to plants without irrigation. The performance of the treatments 50 and 100% irrigation water depths for the variable processed coffee provided the highest productivity rates which varied between 55 and 57 sacks ha$^{-1}$, respectively between 16 and 18 surplus coffee sacks, or a 45% gain when compared to the productivity of plants without any irrigation water.

Harvest provided a mean productivity of 55.36 sacks per hectare (Li 03), which is excellent productivity, when the importance of coffee productivity for the coffee producer´s economic return is so relevant. It should also be verified whether the productivities obtained are the first produce of the coffee plantation.

Productivity decreases as the irrigation water depth increases, till it reaches 45.38 sacks per hectare with Li 04 (125% $ET_0$). Decrease in the productivity of irrigated plants at depth over 100% $ET_0$ may be due to the excessive water application at the region of the radicular system of the culture and, consequently, the leaching of nutrients with the irrigation water at the soil's deepest layers. Therefore, increase in water volume to the coffee plants does not necessarily mean an increase in productivity.

In current analysis, the non-irrigated treatment had the lowest productivity and showed that irrigation in coffee plants in the region of Muzambinho MG Brazil is advantageous, corroborating results by Oliveira et al. (2010) and Silva et al. (2011).

## Conclusions

(i) Irrigation under soil and climate conditions of the experimental area does not affect the vegetal growth of plants;

(ii) 100% irrigation water depth provided a higher growth and distribution of the plants' radicular system.

(iii) Irrigation enhances a significant increase to the production of coffee plants (*Coffea arabica* L.) cv. Catuaí when compared to non-irrigated treatments.

## Conflict of Interest

The authors have not declared any conflict of interest.

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# Trace gas fluxes from intensively managed rice and soybean fields across three growing seasons in the Brazilian Amazon

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**The emission of gases that may potentially intensify the greenhouse effect has received special attention due to their ability to raise global temperatures and possibly modify conditions for life on earth. The objectives of this study were the quantification of trace gas flux ($N_2O$, $CO_2$ and $CH_4$) in soils of the lower Amazon basin that are planted with rice and soybean, and the relation of this flux to soil physical and chemical parameters and to precipitation. This study was conducted in agricultural fields planted with rice (*Oryza sativa*) and soybean (*Glycine max*), located near the cities of Belterra and Santarém in western Pará State, Brazil, during the production years of 2005 to 2007. Measurements were done using static chambers in the field, and samples were analyzed by gas chromatography in the laboratory. Statistical analysis was conducted to determine variation in gas flux in both crops, and the results show that $CO_2$ flux varied between 305 and 227 mg-C $m^{-2}$ $h^{-1}$ under rice, and 243 and 156 mg-C $m^{-2}$ $h^{-1}$ under soybean. Flux of $N_2O$ under rice varied between 4.5 and 20.4 µg-N $m^{-2}$ $h^{-1}$, and under soybean flux variation was between 4.0 and 9.4 µg-N $m^{-2}$ $h^{-1}$. Variation in flux of $CH_4$ under rice was between 5.1 and 14.0 µg-C $m^{-2}$ $h^{-1}$, and under soybean it was 0.4 and 1.2 µg-C $m^{-2}$ $h^{-1}$. These results demonstrate that, during the study period, the rice crop had higher flux for all trace gases than the soybean crop.**

**Key words:** Amazon region, trace gas, crops.

## INTRODUCTION

Land use change, the anthropogenic clearing of forest for agriculture, and agricultural practices are three of the main contributors to the increase in atmospheric greenhouse gas (GHG) concentrations (Houghton, 1991; Fearnside, 2005; IPCC, 2007) and, thus; climate changes. The main gases that contribute to the

*Corresponding author. E-mail: raimundo.oliveira-junior@embrapa.br greenhouse effect (GHG) are carbon dioxide ($CO_2$), methane ($CH_4$), nitrous oxide ($N_2O$), ozone ($O_3$) and water vapor ($H_2O$) (Ostermayer, 2004). Without these gases, which act as a natural blanket around the earth, thermal infrared radiation absorbed by the earth´s surface would dissipate into space and the temperature of the planet would be approximately 20°C colder than the current global average of 15°C (Houghton, 2004). However, increased concentrations of GHG lead to retention of heat in the atmosphere, provoking disequilibrium in terrestrial ecosystems due to the increase in temperature. According to estimates of the Intergovernmental Panel on Climate Change (IPCC), the average global temperature will increase by 1.8ºC by the end of this century (IPCC, 2007). The consequences of this increase are affecting the quality of life currently experienced around the world and also for long-term agricultural sustainability.

The increase in the concentration of these gases in the atmosphere is principally due to anthropogenic actions manifested directly through fossil fuel burning, industrial production, and fires, and indirectly through the irrational use of natural resources (Cardoso et al., 2001).

Combined $CO_2$, $CH_4$, and $N_2O$ contribute to more than 90% of anthropogenic climate warming gasses (Hansen et al., 2000). The fluxes of $CO_2$, $CH_4$, and $N_2O$ are influenced by multiple factors, such as climate, nitrogen deposition, and land management practices. Agriculture releases to the atmosphere significant amounts of $CO_2$, $CH_4$, and $N_2O$ (Smith et al., 2007; Metay et al., 2007). As a proportion of global anthropogenic emissions in 2005, agriculture contributed approximately 20% of $CO_2$ (Smith et al., 2007; Johnson et al., 2007) 58% of $N_2O$ (Smith et al., 2007; Syakila and Kroeze, 2011), and 47% of $CH_4$, (Smith et al., 2007). Agricultural emissions of $CH_4$ and $N_2O$ have increased globally by nearly 17% from 1990 to 2005 (IPCC, 2007), and agricultural $N_2O$ emissions are projected to increase by 35 to 60% by 2030 due to increased nitrogen fertilizer use and increased animal manure production (FAO, 2003). Assuming that $CH_4$ emissions will grow in direct proportion to increases in livestock numbers, then global emissions from livestock is expected to increase by 60% by 2030 (FAO, 2003).

Land use change releases greenhouse gases when the forest is burned to clear the land (Houghton, 1991; Crutzen and Andreae, 1990; Goreau and Mello, 1988). Deforestation rates in the tropics have reached 2% of the total area, annually (Williams, 1990; Meyers, 1991). These deforested areas in the tropics are converted principally to plantations and pastures (Hecht, 1992; Fearnside, 2005). After land use change, agricultural practices such as soil tillage and application of mineral fertilizer affect soil gas emissions. Soil tillage accelerates decomposition of soil organic matter (SOM), whereas application of nitrogen-based fertilizer to the soil increases emissions of $N_2O$ and NO (Davidson et al., 1996; Matson et al., 1996; Veldkamp et al., 1998; Crill et al., 2000; Mosier, 2001; Yan et al., 2001; Steudler et al., 2002).

Due to recent agricultural expansion in the Amazon evaluation of impacts by this land use on greenhouse, gas emissions have become a research priority. Many studies have documented the emissions from pastures (Verchot et al., 1999; Cerri and Cerri, 2007; Neil et al., 2005; Wick et al., 2005; Garcia-Montiel et al., 2001), and forests (Luizão et al., 1989; Melillo et al., 2001, Keller et al., 2005), but few studies have investigated emissions from croplands in the Amazon region. The variability in edaphic and climatic conditions in the Amazon basin is enormous, which makes research on this topic even more important. Within the region, there are many different scenarios of agricultural expansion occurring simultaneously, and these diverse situations will have different impacts on the way in which they alter natural processes and the rates at which these impacts occur. Within the region of western Pará State, for example, there has been a large front of expansion of mechanized agriculture during recent years, and few if any studies have been conducted in an attempt to evaluate the impacts caused by these activities and their effects on areas adjacent to these new centers of production. The area dedicated to soybean production in the region of Santarém, Pará State tripled between 1999 and 2002 (Lameira and Alencar, 2002), and was 28,150 ha in 2009, and was projected to reach 86,900 ha in 2010 (CONAB, 2012). The majority of this expansion has been in areas of secondary forest and pasture, with just 10% resulting from deforestation of primary forest (Venturieri et al., 2007). The area under soybean in the State of Pará, which was 86,900 ha in 2010 (CONAB, 2012), could triple by 2014 as a function of current market tendencies (FBOMS, 2004).

In light of this situation, this work had as its principal objectives the quantification of trace gas fluxes of $N_2O$, $CO_2$ and $CH_4$ in soils of the lower Amazon basin planted with soybean and rice, and the relation of this flux with soil chemical and physical parameters and also with precipitation. It was expected to find that $N_2O$ emissions under soybean would be higher than those from soils under rice–because soybean is a legume with N-fixing ability and therefore increasing the nitrogen abundance in the soil. It was also expected that methane emissions would be higher in soils under rice cultivation, because of the lower oxygen content of these soils due to expected higher water content.

## MATERIALS AND METHODS

This study was conducted in agricultural fields planted rice (*Oryza sativa*) and soybean (*Glycine max*), located near the cities of Belterra and Santarém in western Pará State, Brazil, during the production years of 2005 to 2007. The areas were initially planted with rice, then with soybean, both crops using a conventional management system, in the same area. During dry season this area was in fallow.

The region's climate is type Am in the Köppen classification

**Table 1.** Some physical and chemicals characteristics of clayey Yellow Latossols in Belterra municipality, Pará State (Two experimental sites).

| HOR | Depth |  | Sand | ADA | Clay | C | $Fe_2O_3$ | $H_2O$ | ΔpH | Ca | Mg | K | S | Al | T | Silt/ clay | Ki | V | m | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | cm |  | (g/kg of soil) |  |  |  | pH |  |  | (cmol$_c$ kg$^{-1}$ soil) |  |  |  |  |  |  | % |  |  | (mg/kg) |
| **Typic Hapludox – coordinates: 02º54'S e 54º56'W** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A1 | 0 - | 11 | 30 | 640 | 890 | 19.8 | 61 | 3.7 | -0.1 | 0.40 |  | 0.05 | 0.50 | 2.70 | 10.5 | 0.09 | 1.91 | 5 | 84 | 4 |
| AB |  | 23 | 20 | 510 | 920 | 13.6 | 63 | 4.1 | -0.3 | 0.30 |  | 0.03 | 0.45 | 2.10 | 7.8 | 0.07 | 1.86 | 5 | 84 | 2 |
| BA |  | 45 | 20 | 0 | 930 | 9.6 | 66 | 4.3 | -0.4 | 0.40 |  | 0.02 | 0.44 | 1.80 | 5.9 | 0.05 | 1.86 | 7 | 82 | 1 |
| Bw1 |  | 91 | 20 | 0 | 930 | 6.4 | 67 | 4.4 | -0.4 | 0.30 |  | 0.01 | 0.32 | 1.70 | 4.4 | 0.05 | 1.88 | 7 | 85 | 1 |
| Bw2 |  | 160 | 10 | 0 | 930 | 4.2 | 67 | 4.7 | -0.6 | 0.40 |  | 0.01 | 0.44 | 1.00 | 3.6 | 0.06 | 1.92 | 11 | 71 | 1 |
| **Typic Hapludox – coordinates: 02º 45'S e 54º54'W** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| O | 3 - | 0 | 160 | 50 | 680 | 55.9 | 41 | 4.8 | -0.8 | 6.8 | 3.2 | 0.23 | 10.3 | 0.3 | 22.9 | 0.24 | 2.03 | 13 | 89 | 1 |
| A1 | 0 - | 15 | 100 | 50 | 820 | 18.1 | 49 | 4.5 | -0.5 | 0.5 | 0.5 | 0.04 | 1.1 | 1.6 | 8.4 | 0.10 | 1.99 | 5 | 92 | <1 |
| AB |  | 29 | 70 | 68 | 840 | 11.3 | 52 | 4.5 | -0.5 | 0.4 |  | 0.02 | 0.44 | 1.7 | 6.4 | 0.11 | 1.98 | 1 | 90 | <1 |
| BA |  | 44 | 60 | 0 | 870 | 7.9 | 52 | 4.7 | -0.6 | 0.50 |  | 0.02 | 0.53 | 1.5 | 4.6 | 0.08 | 1.94 | 1 | 88 | <1 |
| Bw1 |  | 79 | 70 | 0 | 870 | 5.1 | 52 | 4.9 | -0.9 | 0.40 |  | 0.01 | 0.42 | 1.3 | 4.0 | 0.07 | 1.94 | 1 | 91 | <1 |
| Bw2 |  | 122 | 60 | 0 | 890 | 5.0 | 55 | 5.0 | -1.0 | 0.40 |  | 0.01 | 0.42 | 1.3 | 3.5 | 0.06 | 1.92 | 1 |  |  |
| Bw3 |  | 200 | 50 | 0 | 880 | 4.0 | 54 | 5.0 | -1.0 | 0.40 |  | 0.01 | 0.43 | 1.1 | 3.5 | 0.08 | 1.95 | 1 |  |  |

ADA, Clay dispersed in water.

system (IBAMA, 2004), and the soils are predominately clayey Oxisols on slightly undulating terrain, classified by the Brazilian Soil Classification System (Embrapa, 1999) as Typic Hapludox, with an average clay content above 850 g kg$^{-1}$ (Table 1) (Moraes et al., 1995; Rodrigues et al., 2001).

In the city of Santarém, annual average air temperature varies between 25.4 and 27.1°C, and relative humidity is high year-round with an average of 86.7%. Precipitation indices present large annual variation with an average of 1920 mm (INMET, 2010), and there are two distinct seasons: a rainy season from December to May, and a dry season from July through November. In the city of Belterra, annual average temperature varies between 25 and 28°C with an average annual relative humidity of 86%. Precipitation also occurs in two distinct periods, with a dry season from July to January presenting a monthly average of 62.5 mm, and a rainy season from February to June with an average of 770 mm (INMET, 2010).

## Experimental design

Sampling was done in two 1 ha monoculture fields of soybean and of rice. In each of the 4 fields, 10 static chambers were randomly distributed for each sampling event. During the crop cycle (and concurrent with sampling), mineral fertilizer was applied to both crops at a rate of 400 kg ha$^{-1}$ (2% N, 45% P, and 60% K), and nitrogen fertilizer was applied at a rate of 60 kg ha$^{-1}$ of urea (60% N) 60 days after plant emergence. Soybean productivity was 3,180 kg ha$^{-1}$ (± 400 kg) per year; rice productivity was 3,000 kg ha$^{-1}$.

Sampling was divided into 3 stages: Period 1: From initial seedling planting (day zero) to day 7, with daily sampling to verify the effects of fertilizer application; Period 2: Day 8 up to harvest day, with sampling conducted once a week; Period 3: Harvest day to day 5 after harvest, with sampling done each day.

## Gas sample collection protocol

The static chamber method was used to sample $N_2O$ and $CH_4$, following the protocol described in Keller et al. (2005). Chambers were randomly installed in the field and inserted into the soil to a maximum depth of 3 cm. For each sampling event, 10 chambers were sampled and at each chamber 4 samples were taken. Sampling began by placing a lid on top of the base 5 min after the chamber base was put in the soil. Samples were taken using a nylon syringe at 1, 10, 20, and 30 min after the lid was put in place. Air temperature at the height of the chamber and at 2 cm in the soil were measured a digital thermometer and the chamber height was measured at 3 internal points to calculate gas concentration.

Collected samples were transported to the gas chromatography laboratory at Embrapa Amazônia Oriental in Santarém and analyzed using a Shimadzu (GC 8A) gas chromatograph within 24 h. $N_2O$ and $CO_2$ were determined using an electron capture detector (ECD), with injector,

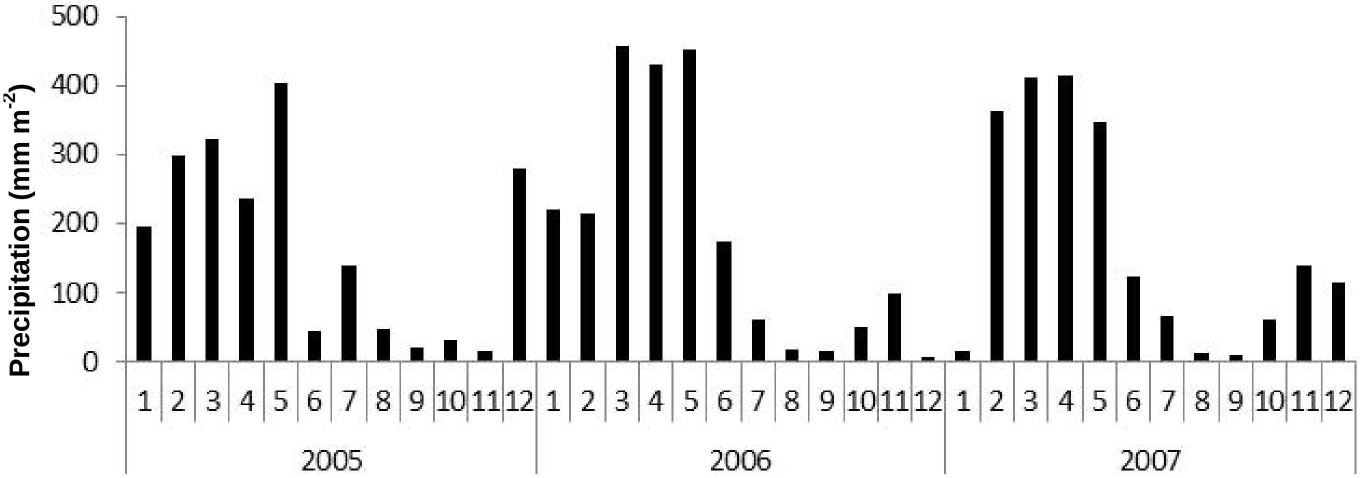

**Figure 1.** Total precipitation during entire study period.

detector, and column temperatures of 330, 330 and 80°C, respectively. Peaks of gas concentration were compared to primary standards (calibrated to National Institute of Standards and Technology) containing known concentrations of $N_2O$ (308.1 and 753 ppb) and $CO_2$ (969 and 30000 ppm). Concentrations of $CH_4$ were determined using a flame ionization detector (FID), injector, detector, and column temperatures of 125, 125 and 40ºC, respectively. Peaks of $CH_4$ concentration were compared to primary standards (calibrated to National Institute of Standards and Technology) containing known concentrations of 1.75 and 0.884 ppm.

Gas fluxes emitted from the soil surface (F) were calculated from the linear correlation of gas concentration over the sampling time using Equation 1:

$$F = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \frac{m}{Vm} \qquad (1)$$

Where: $\Delta C$ = change in gas concentration within the chamber; ($\Delta t$) = time that the chamber remained closed; *V* and A are, the volume and the area of the soil covered by the chamber, respectively, and m = molecular mass of each gas ($N_2O$, $CH_4$ and $CO_2$)

### Soil sampling

Soil was sampled at 10 cm depth from inside the area covered by the chamber immediately after gas collection was finished. Samples were placed in labeled plastic bags and transported to the laboratory for weighing on a precision balance to obtain wet mass. Soils were dried at 105ºC for 48 h in an oven and then weighed again to obtain dry mass.

Water filled pore space (WFPS; Linn and Doran, 1984) was calculated using soil bulk density (dg), soil particle density (dp) and the concentration of water in the soil (u), according to Equations 2 to 4.

(2)

(3)

(4)

Where: θ = soil moisture ( %); α = soil pore space ( %); dg =soil bulk density (g cm$^{-3}$); dp = soil particle density (g cm$^{-3}$), and u= concentration of water in the soil (%).

### Statistical analysis

Data analysis was done using the program Statistica 8.0 (Statsoft Inc.). Normality was tested, and when necessary data were log transformed ($CO_2$= log ($CO_2$); $N_2O$ = log ($N_2O$ + 60), $CH_4$= log ($CH_4$ + 650), in mg m$^{-2}$ h$^{-1}$ ($CO_2$), µg m$^{-2}$ h$^{-1}$ ($N_2O$ and $CH_4$), respectively. Differences were tested using one-way analysis of variance (ANOVA) and the Tukey post-hoc test. A probability level of $\alpha = 0.05$ was used for all tests. Linear regression was used to investigate possible relationships between soil moisture content and gas flux.

## RESULTS

### Precipitation and soil moisture

Rainfall is highly seasonal in the research area and the monthly totals during the study period (January 2005 to December 2007) are shown in Figure 1. Most agricultural activities took place in the rainy season with 70% of annual precipitation occurring during this season.

In the rice fields, WFPS displayed an increasing trend throughout the study, with the second period often being the wettest. During the 2005 rice crop cycle, WFPS values averaged 39% and the highest value (43%) during the second period. Year 2006 was a wetter, with annual soil moisture above 58%, and with the highest value (69%) again during the second sampling period. Year 2007 was the wettest of the three years, with annual WFPS values above 55% and period 3 being the wettest this year (61%; Table 2).

In the soybean fields WFPS decreased over time with no period being consistently wetter. In 2005, WFPS was 61, 64 and 63% for periods 1-3, respectively, while in 2006, it was 47, 33 and 28% for periods 1-3, respectively.

**Table 2.** Average flux of $N_2O$, $CO_2$ and $CH_4$ and the standard error of the mean (±SEM) for the years 2005-2007 in mechanized soybean and rice fields. The periods (1, 2, and 3) represent cultivation phases.

| Year | Treatment | $N_2O$ ± SEM (µg-N m$^{-2}$ h$^{-1}$) | $CO_2$ ± SEM (mg-C m$^{-2}$ h$^{-1}$) | $CH_4$ ± SEM (µg-C m$^{-2}$ h$^{-1}$) | WFPS (%) |
|---|---|---|---|---|---|
| **Soybean** | | | | | |
| 2005 | Period 1 | 1.9 ± 0.5 | 500± 57 | -0.2 ± 0.3 | 60.6±0.9 |
| | Period 2 | 1.6 ± 0.4 | 131 ± 15 | -0.8 ± 0.4 | 64.0±1.0 |
| | Period 3 | 2.6 ± 2.8 | 120 ± 34 | -0.2 ± 0.7 | 62.8±1.3 |
| 2006 | Period 1 | 7.4 ± 2.3 | 178 ± 22 | -0.1 ± 0.4 | 47.1±1.9 |
| | Period 2 | 7.9 ± 1.0 | 155 ± 24 | -1.3 ± 0.7 | 32.8±1.8 |
| | Period 3 | 6.2 ± 1.6 | 143 ± 24 | -0.5 ± 0.1 | 28.3±5.6 |
| 2007 | Period 1 | 3.6 ± 0.9 | 104 ± 13 | 0.5 ± 2.9 | 22.6±2.0 |
| | Period 2 | 2.6 ± 0.7 | 407 ± 36 | 0.1 ± 2.5 | 31.1±1.3 |
| | Period 3 | 21.5 ± 3.3 | 122 ± 18 | 0.6 ± 5.6 | 34.2±3.7 |
| **Rice** | | | | | |
| 2005 | Period 1 | 77.8±18.8 | 350 ± 32 | -1,0 ± 0.4 | 35.0±2.1 |
| | Period 2 | 5.3 ± 2.0 | 607 ± 54 | -0.8 ± 0.9 | 43.0±2.7 |
| | Period 3 | 1.8 ± 0.8 | 205 ± 17 | 6.6 ± 4.7 | 41.8±5.3 |
| 2006 | Period 1 | 8.2 ± 1.6 | 135 ± 12 | -1.2 ± 1.4 | 46.4±2.1 |
| | Period 2 | 6.7 ± 1.4 | 245 ± 44 | 0.4 ± 0.3 | 69.3±2.1 |
| | Period 3 | 5.8 ± 2.1 | 181 ± 41 | -1.9 ± 2.9 | 60.2±1.3 |
| 2007 | Period 1 | 3.3 ± 1.1 | 180 ± 22 | -8.6 ± 12.3 | 51.2±1.4 |
| | Period 2 | 3.4 ± 0.8 | 350 ± 45.3 | 0.1 ± 2.6 | 55.2±2.0 |
| | Period 3 | 2.9 ± 0.6 | 427 ± 48.8 | 0.04 ± 2.0 | 61.6±1.1 |

In 2007, the WFPS values were lowest, with periods 1-3 showing 22.6, 31.1 and 34.2%, respectively (Table 2).

In the rice fields, $N_2O$ had an average cycle flux of 28.3 µg m$^{-2}$ in 2005, with a steep decrease from period 1 (seedling planting to 7 days afterward) to the other periods (Table 2). In 2006, the cycle average decreased to 6.9 µg m$^{-2}$, followed by a slight decrease through the end of the cultivation cycle. In 2007, the average of the three periods was even lower (3.2 µg m$^{-2}$) remaining stable during the three periods.

In the soybean plantation, in 2005, $N_2O$ had little variation during periods 1-3 (Table 2). In 2006, $N_2O$ fluxes were 4 times greater. While in 2007, $N_2O$ fluxes decreased in the first two periods followed by a large increase in period 3.

In the rice plantation, $CH_4$ had the largest variation of the trace gas fluxes during the 3 years of the study with a 3-year average of -0.006 µg m$^{-2}$, and the values increased from period 1 to 3 with period 3 (harvest) showing the largest value (6.6 µg m$^{-2}$). In 2006, the fluxes for periods 1-3 were -1.1, 0.4 and -1.9 µg m$^{-2}$, respectively. In period 1 of 2007 the values for $CH_4$ flux was highly negative (-8.62 µg m$^{-2}$), with periods 2 and 3 having a flux of 0.1 and 0.04 µg m$^{-2}$, respectively.

In the soybean plantation $CH_4$ flux displayed similar trends. Period 1 for years 2005 and 2006 had a flux of -0.17 and -0.09 µg m$^{-2}$, respectively; decreasing in period 2 to -0.85 and -1.33 µg m$^{-2}$, for years 2005 and 2006, respectively. During period 3, the flux was -0.21 µg m$^{-2}$ (2005) and -0.49 µg m$^{-2}$ (2006). In 2007, the fluxes were 0.47, 0.11 and 0.63 µg m$^{-2}$ for periods 1-3, respectively.

During the cultivation cycle of rice in 2005, $CO_2$ had an average annual flux of 387.1 mg m$^{-2}$, with periods 1-3 having fluxes of 349.8, 607.0 and 205.4 mg m$^{-2}$, respectively. Flux values for 2007 were lower with an annual average of 318.6 mg m$^{-2}$, while period 1 had a flux of 179.7 mg m$^{-2}$ and period 2 had 349.5 mg m$^{-2}$. In the soybean plantation $CO_2$ in 2005 had an annual average flux of 500.36 mg m$^{-2}$, diminishing to 130.87 and 119.52 mg m$^{-2}$ for periods 2 and 3, respectively. The same pattern between periods was evident in 2006, with a flux of 177.6, 155.3 and 142.7 mg m$^{-2}$, for periods 1-3, respectively. In 2007, period 2 had a very high flux of 406.7 mg m$^{-2}$, while period 1 had 103.7 and period 3 had 122.3 mg m$^{-2}$.

In the rice crop, there were no statistical differences between periods of cultivation, but significant differences were found between years of cultivation (F=25, 8; $p < 0.01$) for $CO_2$ flux (Table 3). $N_2O$ on the contrary, showed significant differences between years and also between periods; with period 1 different than 2 and 3, and 2 equal to 3. There were no differences found for $CH_4$ flux

**Table 3.** Average trace gas ($N_2O$, $CO_2$ and $CH_4$) flux values and the respective WFPS values (%) with standard error of the mean (±SEM) for years 2005 to 2007 in an area of mechanized cultivation of soybean and rice.

| Crop | Period (years) | $CO_2$ (mg-C $m^{-2}$ $d^{-1}$) | $N_2O$ (μg.$m^{-2}$) | $CH_4$ (μg.$m^{-2}$) | WFPS (%) |
|---|---|---|---|---|---|
| **Rice** | 2005 | $443.9 \pm 30.6^a$ | $38.0 \pm 9.2^a$ | $-0.006 \pm 0.7^a$ | $39.2 \pm 1.6^a$ |
| | 2006 | $197.5 \pm 23.2^b$ | $7.0 \pm 0.9^b$ | $-0.6 \pm 0.8^a$ | $60.3 \pm 1.4^b$ |
| | 2007 | $310.8 \pm 24.4^c$ | $3.2 \pm 0.5^b$ | $-3.0 \pm 4.3^a$ | $55.5 \pm 1.0^b$ |
| **Soybean** | 2005 | $305.1 \pm 32.2^a$ | $1.8 \pm 0.4^a$ | $-0.5 \pm 0.2^a$ | $62.3 \pm 0.6^a$ |
| | 2006 | $166.8 \pm 15.0^b$ | $7.5 \pm 1.3^a$ | $-0.6 \pm 0.3^a$ | $40.4 \pm 1.4^b$ |
| | 2007 | $254.1 \pm 22.5^{ab}$ | $6.6 \pm 11.5^a$ | $0.3 \pm 0.3^a$ | $29.0 \pm 1.2^c$ |

*Different letters in the same columns and crop represent statistically significant differences ($p < 0.05$).

between years or periods. There were significant differences for WFPS between years and periods (F=56, 4; $p < 0.01$); with period 1 different than 2 and 3, and 2 equal to 3 (Table 3).

In the soybean crop, $CO_2$ showed significant differences for years (F=10, 4; $p < 0.01$) and between periods (F=3.7; $p < 0.05$), with period 1 equal to 2 and different than 3, and 2 equal to 3. $N_2O$ had significant differences between years (F=12, 4; $p < 0.01$) and between periods (F=14.4; $p < 0.01$), with period 1 equal to 2 and different than 3, and 2 equal to 3. Trends for $CH_4$ and WFPS followed those for the rice crop.

## DISCUSSION

The high frequency of sample collection during the period of crop management was important to be able to evaluate the effects of rice and soybean crop systems on the emission of $N_2O$. Furthermore, the soil preparation management in the soybean crop, together with high temperatures and rainfall, represent a unique situation to measure $N_2O$ emission in the soil-atmosphere continuum.

With respect to $N_2O$ flux, there was a significant difference in the soybean plantation in relation to period (F=10.4; $p < 0.01$) and also for year (F=21.8; $p < 0.01$), which could be explained by the greater availability of N in period 1 compared to the other periods. This greater availability of N is probably associated with good conditions for nitrifying and denitrifying bacterial activity (Firestone and Davidson, 1989). This is related to soil tilling under conventional management prior to seedling emergence that stimulates organic matter cycling, $O_2$, temperature, and moisture conditions (Dobbie and Smith, 2001) that favor N availability, which is reflected in greater $N_2O$ emission (Table 3). These results are similar to those for young pastures in the Amazon rich in available N (Davidson et al., 2001). Greater availability of N and conditions of WFPS > 60% (Table 2) favor denitrifying bacterial activity in this system which should result in greater emission of $N_2O$ (Oenema et al., 1997, Davidson et al., 2001). This is one of the causes of the positive and significant relationship between $N_2O$ and WFPS in the soybean crop. Anaerobic conditions associated with high concentrations of N, principally in the form of nitrate (N-$NO_3^-$) represent ideal conditions for high denitrifying bacterial activity (Smith et al., 2003), which could also explain the elevated flux found in period 1 in soybean in all the years studied.

The data for emission of $N_2O$ in this study are lower than those related in studies done in primary and secondary forests in the Amazon. Palm et al. (2002) reported an annual $N_2O$ flux of 9.1 μg $m^{-2}$ in secondary forest in Peru. Keller et al. (2005) found average annual $N_2O$ fluxes of about 75 μg $m^{-2}$ on a clay soil and 16 μg $m^{-2}$ on a sandy soil in primary forest in the Brazilian Amazon.

Various studies have reported elevated $N_2O$ emissions in soybean crops during 20 days before and after harvest (Yang and Cai, 2005; Ciampitti et al., 2005, 2007). These high levels of emissions are apparently related to greater concentrations of labile C as a product of node senescence, which is used as a primary substrate by the microbial population, favor the growth of all microbes but in particularly the nitrifying and denitrifying bacteria (Ciampitti et al., 2005). Escobar et al. (2010) found that $N_2O$ emissions after soybean harvest were three times greater in no-till compared to a conventional management system. In the Peruvian Amazon, Palm et al. (2002) found that $N_2O$ fluxes in high and low input annual agricultural systems were 2 to 3 times higher than those in secondary forest and 3 to 10 times higher compared to perennial tree-based systems. Greater $N_2O$ emission could be related to a greater proportion of soil pores being filled with water in this clayey soil, a product of the property of having many small pores and high levels of moisture (Skiba and Ball, 2002), which would reduce $O_2$ diffusion, thus, through microbial competition for $O_2$, creating anaerobic sites (Baggs et al., 2006), favoring $N_2O$ emission through denitrification. Therefore, we can conclude that, in 2005 and 2006, the maximum emission of $N_2O$ in these conventional agricultural management systems occurred in period 1, decreasing in period 2, and then increasing in period 3 (Table 2).

High levels of WFPS lead to an increase in anaerobic microsites causing an exponential increase in $N_2O$ emissions (Smith et al., 2003). In the present study, $N_2O$ emissions from the soil under conventional agriculture increased with increasing WFPS, which is in agreement with other studies that reported a greater denitrification rate in soils with higher water content (Bateman and Baggs, 2005; Liu et al., 2006). Interestingly, in the Amazon, Wick et al. (2005), found no relationship between soil $N_2O$ emissions and WFPS in pastures. In the current study, when WFPS exceeded 50% in this system, $N_2O$ emissions dramatically increased. This is consistent with the results of Keller et al. (2005) in primary forest in the Brazilian Amazon. Palm et al. (2002) found large increases in $N_2O$ flux with a doubling or tripling of WFPS in soybean, rice, and corn cropping systems in the Peruvian Amazon. In this case denitrification would be the dominant process (Moreira and Siqueira, 2006; Aita et al., 2007). The transition point between the processes that operate aerobically (nitrification) and anaerobically (denitrification) is frequently cited as 60% WFPS (Sehy et al., 2003; Webb et al., 2010). During the 2005 and 2007 crop cycles, after period 2 the soy crop had an increase in $N_2O$ emission, probably due to the increase in available N in the soil caused by the fall of senescent leaves and the senescence of roots and rhizome nodules, as shown by Yang and Cai (2005) in soybeans.

During the 3 years of soy cultivation, soil respiration, which is determined by root and microbial activity (Cattani et al., 2002), could have been influenced by soil preparation and addition of N fertilizer which may have provoked the high $CO_2$ flux in period 1 compared to the 3 years of flux (Table 2). However, other factors such as WFPS and soil temperature have been discussed as being possible controllers of $CO_2$ flux from the soil to the atmosphere (Carvalho, 2005; Kuzyakov, 2006).

After seedling planting, the high availability of N could stimulate microbial activity leading to an increase in soil respiration. Furthermore, it is likely that soil preparation through tillage increased soil aeration and increasing the availability of labile organic matter that was previously protected by aggregates (Follett, 2001). High mineral N availability could have been responsible for the high $CO_2$ flux in period 1 during N fertilization activities, as compared to $CO_2$ flux for all years (Table 1). It is probable that fertilization had stimulated the absorption of N in the initial phase of soybean cultivation, and as a consequence, increased root and microbial respiratory activity. Carvalho (2005) demonstrated that N availability explained 66% of the flux of $CO_2$ in savannah soils cultivated with corn under different management systems.

In period 2, compared to all 3 years, there were high fluxes of $CO_2$, just as in period 1. This pattern could be due to the growth cycle of soybean, being accelerated just after N fertilizer application, thus stimulating the autotrophic component of the soil and increasing soil $CO_2$ flux (Table 2). The same pattern in flux is comparable to that obtained in an Oxisol in São Paulo State by La Scala et al. (2006). Additionally, the highest flux determined after soil preparation (Table 2) could be explained by the liberation of $CO_2$ previously produced through microbial action decomposing residual organic matter exposed by the physical fracturing of the soil or by root respiration (D’Andréa et al., 2006).

In 2007, $CO_2$ flux in period 1 was inferior compared to period 2, and although N fertilizer was applied in period 1; this difference could be in response to the limited addition of N associated with a smaller C: N of soluble material and also to the capacity of microbes to adapt to conditions of low availability of N (Marques et al., 2000). The high $CO_2$ flux values in period 2, when compared to the other periods could possibly be due to soil preparation during seedling planting. Various studies have attributed the increase in $CO_2$ flux during the initial phase of cultivation to the destruction of soil structure and the exposition of organic matter to microbial action (Reicosky and Lindstrom, 1993).

The two biggest peaks of $CH_4$ flux during the rice crop cycle could be related to the availability of C for methanogenesis from residues from the previous crop, from production of organic matter by the growing crop itself, from soil organic matter, and from root exudates. Additionally, morphogenic changes in rice plants that occur in the final growth phase wherein plants reach the maximum number of tillers and panicles in the reproductive phase (Lindau et al., 1991), phases in which these peaks occurred. These results for methane production are similar to those found by Palm et al. (2002) in the Peruvian Amazon (range = 5.2 - 33 $\mu g\ m^{-2}.h^{-1}$) who attributed the positive flux of methane to high-input agricultural practices in a rice/soybean/corn system. Furthermore, these authors showed an inverse relationship with WFPS wherein the high-input agriculture system continued to produce methane even at just 45% WFPS. Interestingly, in the study by Palm et al. (2002) all the other treatments (low-input agriculture, shifting cultivation, agroforestry, peach palm plantations, and forest fallow) had $CH_4$ consumption.

The pulse in the $CH_4$ flux in the rice crop in period 3 of 2005 (6.58 $\mu g\ m^{-2}$) is a significant result when compared to annual values reported for forests in the Amazon (-30 $\mu g\ m^{-2}$) (Palm et al., 2002); (-12.5 $\mu g\ m^{-2}$) (Keller et al., 2005), and this result could be due to the production of organic compounds from the anaerobic decomposition of organic matter such as leaves and dried panicules. This could also be due to catabolism of labile organic compounds by methanogenic microbes (Neue et al., 1996), as well as to the increase in the exudation of organic compounds by roots, a process that increases as the plant develops. These exudates serve as substrate for methanogenic bacteria metabolism, thus increasing the production of $CH_4$ (Aulakh et al., 2001). According to these authors, there is a positive correlation between the

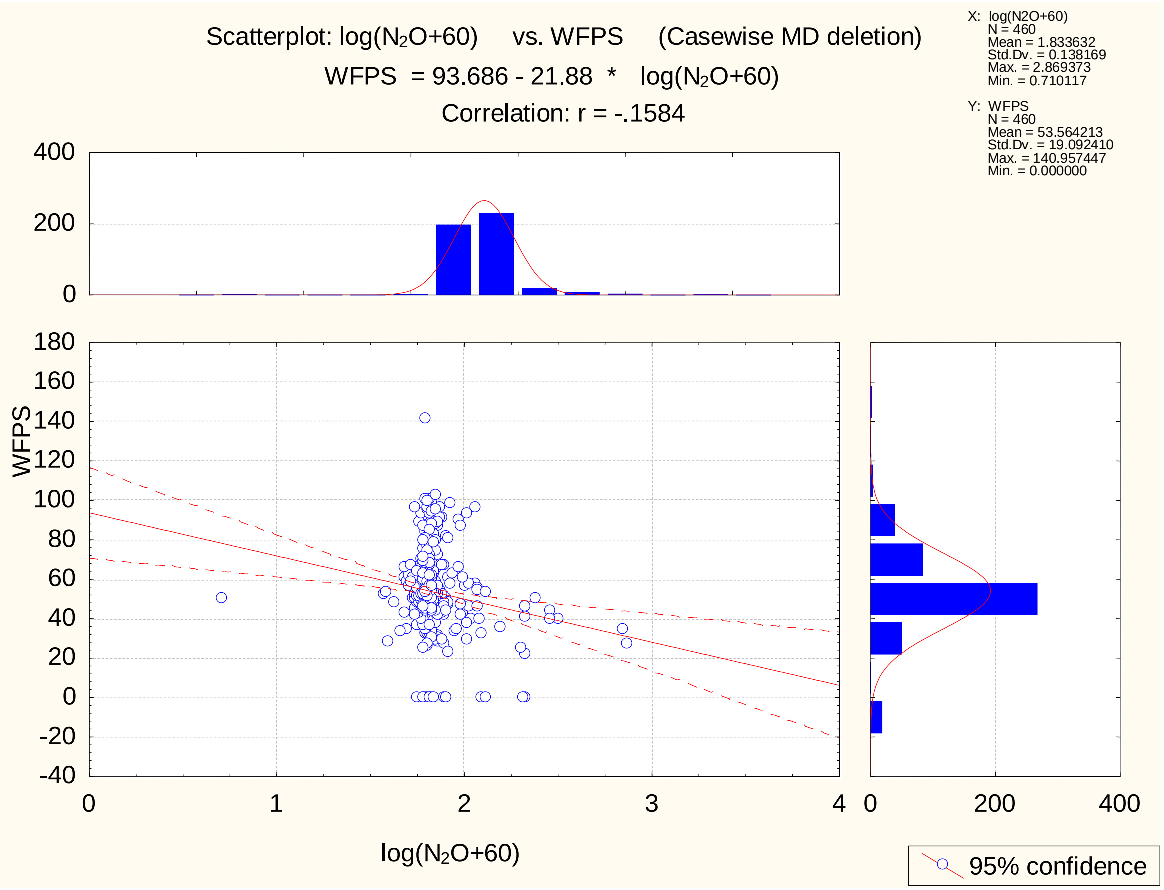

**Figure 2.** Correlation between WFPS x $CO_2$ (A), and WFPS x $N_2O$ (B) in rice.

dry mass of roots and shoots in rice plants with the exudation of organic compounds by roots, so as the plant grows exudation increases, a process that is dependent on the photosynthetic rate of the plant, which in turn is dependent of available sunlight and the nutritional status of the plant (Taiz and Zeiger, 1991). However, in the initial phase of growth of rice cultivated in soil that has received residual plant matter, the largest contribution of C to methanogenesis is from the decomposition of these residuals and from the labile soil organic matter fractions, with less contribution from root exudates (Watanabe, 1999). In the present study, the conventional system of cultivation demonstrates the importance of the incorporation of crop residues and vegetation before the start of the next crop cycle, a practice that favors the accumulation of C, thus initially permitting greater fluxes of $CH_4$ and $CO_2$.

The increase of tillers and leaves, and consequently, the number of canals of escape of $CH_4$ from the soil through the plant, also could have contributed to the intensity of the first peak of $CH_4$ (0.44 µg $m^{-2}$) in period 2 of 2006 (Huang et al., 1997; Nouchi et al., 1990). This peak could also be related to the intense liberation of root exudates during the phases of panicule differentiation, booting stage, panicule emission, and finally, florescence in the reproductive phase of rice (Aulakh et al., 2001). Furthermore, senescent leaves begin to fall at this stage forming a litter layer on the soil surface, and tillers that do not produce a panicule, senescent roots and their exfoliates, dead rice plants, and root exudates have a significant role in providing C to methanogenic organisms (Watanabe, 1999). Anaerobic decomposition of these materials reduced the redox potential and is a source of C for methanogenesis in inundated soils thus increasing the production of $CH_4$ (Lauren and Duxbury, 1993; Cai et al., 1997; Chidthaisong and Watanabe, 1997).

With respect to the flux of $N_2O$, the low values registered in rice soils in period 3 of 2005, and compared with the 3 periods of 2007, could be related to a low availability of N and to aerobic conditions since WFPS was <60% (Table 1). In general, Oxisols are characterized as being N-limited and present low nitrification rates (Nardoto and Bustamante, 2003), and only rarely the production of N-$NO_3^-$ exceeds the demand of microorganisms and roots.

Eichner (1990) found $N_2O$ emission rates in agricultural soils cultivated with leguminous plants to be between 0.34 and 4.6 kg $N_2O$-N ha.year$^1$. In the present study,

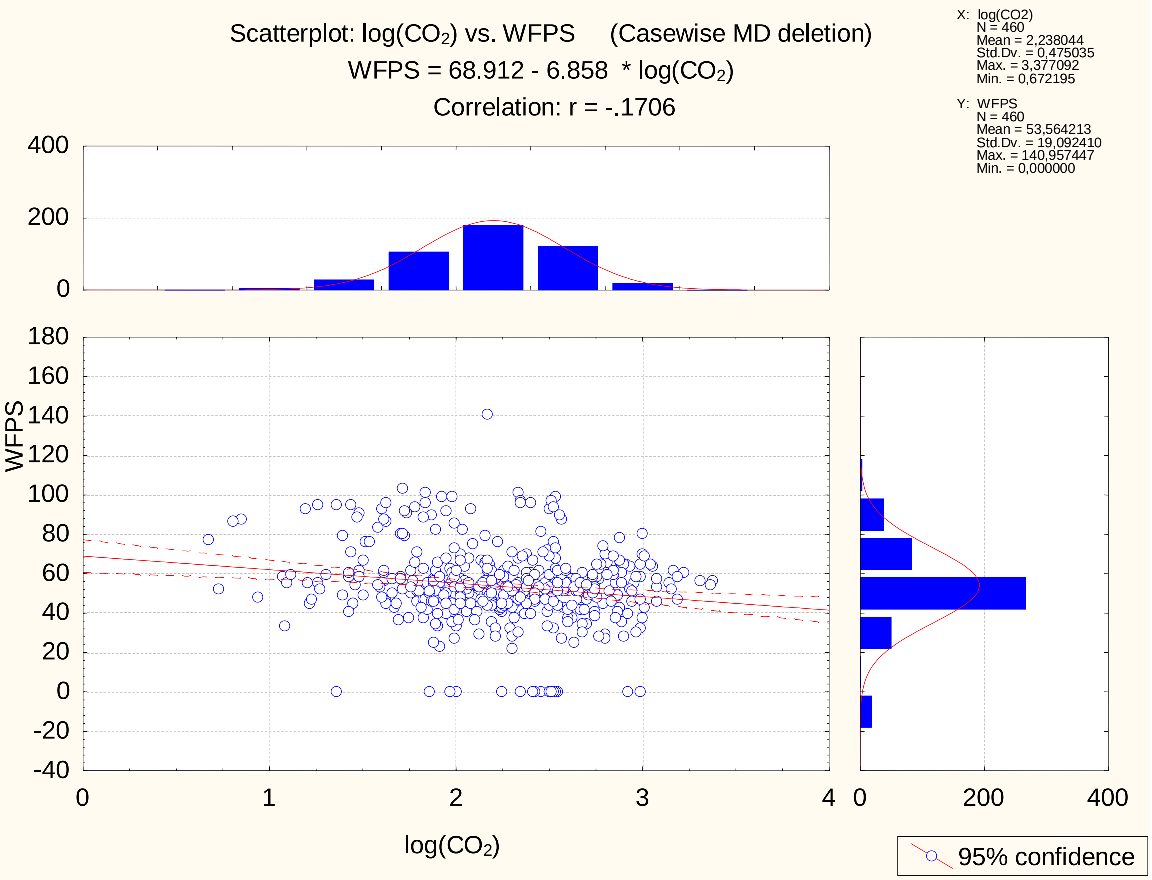

**Figure 3.** Correlation between WFPS x $N_2O$ in soybean.

fluxes varied between 0.53 kg ha year$^1$ and 0.90 kg $N_2O$-N ha year$^1$; values that are lower than 2.3 and 1.27 kg $N_2O$-N ha year$^1$ in high- and low-input annual soybean/rice/corn agricultural systems in the Peruvian Amazon (Palm et al., 2002).

In Table 2, the rice crop had larger fluxes than the soybean crop in all study years, corroborating previous studies (Motschenbacher et al., 2014) that described the role of greater organic matter production by rice as compared to soybean and its relation to the gases analyzed in the present study. Furthermore, in the rice crop there was a significant correlation between the flux of $CO_2$ and $N_2O$ with WFPS ($p<0.05$), as reported by other authors (Keller et al., 2005; Weitz et al., 2001), which can also be visualized in Figure 2.

In the soybean crop, this correlation was significant only for WFPS × $N_2O$ (Figure 3).

## Conclusions

There was annual variation in the rice and soybean crops for carbon dioxide and nitrous oxide, but there was no variation for methane between crops. There was positive and significant correlation between $N_2O$ nitrous oxide and WFPS in the soybean crop. During the period from initial seedling planting to day 7 (period 1), the rice crop emitted more nitrous oxide than in the period from day 8 up to harvest day or in the period from harvest day to 5 days after harvest the other two periods; in the soybean crop this happened more frequently in from harvest day to 5 days after harvest, (period 3). In general the gas fluxes (carbon dioxide, nitrous oxide, and methane) were greater in the rice than the soybean crop.

## Conflict of Interest

The authors have not declared any conflict of interest.

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# Grain sorghum intercropping with *Brachiaria brizantha* cultivars in two sowing systems as a double crop

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**The intercropping of grain sorghum with forage grasses is a promising cultivation technique for the production of grains and forage in the Midwest region of Brazil. However, few studies have been performed with the goal of evaluating the development of the two species when cultivated simultaneously in the same area. The goal of the present study was to evaluate agronomic characteristics and grain yield of grain sorghum intercropped with *Brachiaria brizantha* cultivars under two sowing systems (row and inter-row) as a double crop in the midwest region of Brazil. The experiment was conducted at the Federal Institute of Goiás (*Instituto Federal Goiano*), Rio Verde Campus. A randomized block design was used, with a 3x2+1 factorial scheme, with three replicates. Three cultivars of *B. brizantha* (*Marandu palisadegrass*, *Xaraes palisadegrass* and *Piata palisadegrass*) were row or inter-row (sowing systems) intercropped with grain sorghum. In addition, there was one treatment of sorghum monoculture. The inter-row intercropping of grain sorghum with *B. brizantha* cultivars had no effect on sorghum agronomic characteristics and grain yield. However, row intercropping with *X. palisadegrass* affected the sorghum stem diameter, dry mass production and grain yield. The *M. palisadegrass* and *P. palisadegrass* cultivars are therefore better recommended for intercropping with sorghum. The intercropping of sorghum with the tested *B. brizantha* cultivars was shown to be a viable cultivation practice for grain production as a double crop in the Midwest region of Brazil.**

**Key words:** *Marandu palisadegrass, Piata palisadegrass, Xaraes palisadegrass*, integrated crop-livestock farming systems, *Sorghum bicolor*, grain yield.

## INTRODUCTION

Over the last few years, the intercropping of annual crops with tropical forage grasses, used in integrated crop-livestock farming systems, has been increasingly adopted by farmers from the Cerrado region (Pacheco et al., 2008; Petter et al., 2011). This system allows the cultivation of crops for grain production and the stocking of cattle, for either meat or milk production, in the same area. The integrated crop-livestock farming system

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creates an increase in production and a decrease in the risk of pasture degradation, while improving chemical, physical and biological soil characteristics and the grain, forage grasses and silage yield potential (Silva et al., 2010).

Grain sorghum has been used as an alternative crop in integrated crop-livestock farming systems in the off season (Horvathy et al., 2012, Silva et al., 2013). From the agronomical point of view, the use of sorghum intercropped with forage grasses, especially those belonging to the genus *Brachiaria*, is mainly justified by the potential for the production of sorghum grain and for the production of dry mass of both of the crops. Thus, sorghum has been found to be an excellent option for the production of grain, forage and silage when water deficit and low soil fertility result in higher risk for the cultivation of other crops, such as corn (Embrapa Milho and Sorgo, 2012).Of the forage grasses used in crop rotation, succession or intercropping systems in the Cerrado (tropical savanna) region, grasses of the genus *Brachiaria* should be highlighted. The use of species of this genus in integrated systems is advantageous due to their abundant root system, which contributes to water infiltration as well as higher soil aggregation and aeration (Kluthcouski et al., 2004). In addition, these forage grasses present good adaptation, tolerance and resistance to biotic factors and high production of dry mass that has good nutritional value and is able to meet animal demands, especially during the dry season (Brighenti et al., 2008).

The previous studies of integrated crop-livestock farming systems have used *Brachiaria brizantha* cv. Marandu, *Brachiaria decumbens* and *Brachiaria ruziziensis* (Paris et al., 2010, Horvathy Neto et al., 2012, Silva et al., 2013; Maia et al., 2014). With the emergence of new *B. brizantha* cultivars, new studies of the intercropping of sorghum with the cultivars *Xaraes palisadegrass* and *Piata palisadegrass* are needed to identify the associations that result into in higher sorghum grain yields. Given that the association of sorghum with *Brachiaria* cultivars is largely unexplored, especially as a double crop, there is a need for more information, especially in terms of the recommendations for implementation methods as alternatives for diversification of crops in farms.The goal of the present study was to evaluate agronomic and grain yield characteristics of grain sorghum intercropped with *B. brizantha* cultivars according to two sowing systems (row and inter-row) cultivated as a double crop in the midwestern region of Brazil.

## MATERIALS AND METHODS

The experiment was conducted in the field (17° 48' S, 50° 55' W, and 748 m altitude), at the Federal Institute of Goiás (*Instituto Federal Goiano*), Rio Verde Campus, in an area of dystroferric Red Latosol (Embrapa, 2013). The soil of the experimental area, in the 0 to 20 cm depth instead, before the setting of the experiment had the following chemical and physical characteristics: 510 g kg$^{-1}$ clay; 160 g kg$^{-1}$ silt; 330 g kg$^{-1}$ sand; pH in $CaCl_2$: 5.10; Ca: 2.88 cmol$_c$ dm$^{-3}$; Mg: 1.27 cmol$_c$ dm$^{-3}$; Al: 0.01 cmol$_c$ dm$^{-3}$; Al+H: 4.0 cmol$_c$ dm$^{-3}$; K: 0.39 cmol$_c$ dm$^{-3}$; CEC: 8.54 cmol$_c$ dm$^{-3}$; P: 6.72 mg dm$^{-3}$; Cu: 3.4 mg dm$^{-3}$; Zn: 1.5 mg dm$^{-3}$; Fe: 43.0 mg$^{-3}$; O.M.: 26.76 g dm$^{-3}$.

The area was prepared by clearing the weeds by applying 3 L ha$^{-1}$ glyphosate. Thirty days after desiccation, harrowing was performed using a disk plough, followed by a disk harrow. One week before sowing, a second harrowing was performed, and sowing furrows were opened using a seeder.

The experimental design was of randomized blocks, with three replicates, in a 3x2+1 factorial scheme, with three *B. brizantha* cultivars (*Marandu palisadegrass, X. palisadegrass* and *P. palisadegrass*) row and inter-row intercropped with grain sorghum Buster (an early hybrid with grains without tannin and with reddish color). An additional treatment of sorghum in monoculture was included in the study. 50 cm spacing between rows was used for all the treatments. The plots with monoculture and with row intercropping were composed of eight three-meter-long lines. For the inter-rowintercropping, fifteen rows were used (eight with sorghum and seven with *Brachiaria*) for a total of 12 m². A usable plot area of 6 m² was considered by excluding one row on each side of each plot and 0.5 m at the ends of each row.

Sowing was performed in February 2013, with 80 kg ha$^{-1}$ $P_2O_5$ and 20 kg ha$^{-1}$ FTE BR 12 fertilization, using single superphosphate and silicon dioxide as sources, respectively. At 20 and 40 days after seedling emergence (DAE), 50 kg ha$^{-1}$ nitrogen and 40 kg ha$^{-1}$ $K_2O$ in the form of urea and potassium chloride, respectively, were applied by broadcasting.

For the row intercropping, *B. brizantha* cultivars were sown at a depth of 6cm, in combination with the fertilizers, and sorghum was sown at 2cm depth. For the inter-rowintercropping and for the sorghum monoculture, all the seeds were sown at 2cm depth.The Buster sorghum was thinned two weeks after seedling emergence, leaving 240.000 plants ha$^{-1}$, in accordance with the recommended density for this cultivar for the region of study. For the forage grasses, 5 kg of pure viable seeds were used per hectare. Weeding was performed during the experiment to avoid problems with weeds and pests that could compromise production. The daily rainfall and monthly average temperature were monitored (Figure 1).

For sorghum, both intercropped and as a monoculture, the plant height (measured from the stem base to the tip of the last expanded leaf, for the first measurement, and to the tip of the panicle for measurements at 60 and 90 days after sowing (DAS) for one plant per row in the usable plot area), stem diameter and number of fully expanded leaves were measured at 30, 60 and 90 DAS.Grain was harvested 148 days after seedling emergence, and the following characteristics were evaluated: plant stand (counting of the number of plants and extrapolating to plants per hectare), panicle index (ratio between the number of harvested panicles and the initial plant population), dry mass production, thousand grain weight (weight of one thousand grains, in grams, corrected for 13% moisture) and grain yield (panicle threshing, grain weighing and conversion of the data into kg ha$^{-1}$ corrected for 13% moisture).

An analysis of variance followed by a Tukey's test, at $p<0.05$, was performed. Two analyses were performed: one using only the data for intercropping (sorghum intercropped with the *B. brizantha* cultivars and the two sowing systems), and one using the data for both of the plants in association and in monoculture. The Dunnett test, at $p<0.05$, was also used to compare data for intercropped plants with the sorghum in monoculture. All the analyses were performed using the ASSISTAT 7.6 beta software

## RESULTS AND DISCUSSION

No significant differences were observed in plant height

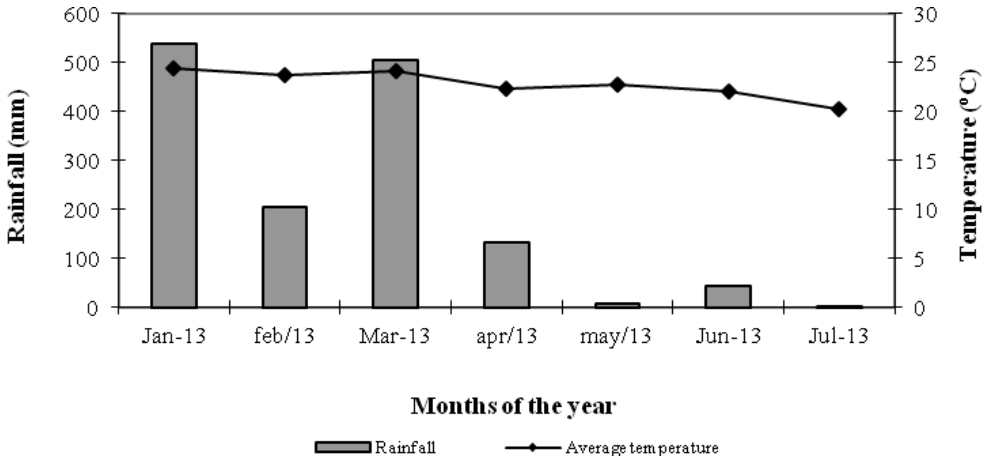

**Figure 1.** Rainfall and average temperature from January to July 2013 in Rio Verde-GO, Brazil.

**Table 1.** Plant height at 30, 60 and 90 DAS of sorghum in monoculture and intercropped with *B. brizantha* cultivars under two different sowing systems.

| Integrated systems | Sowing Systems | | Average |
|---|---|---|---|
| | **Row** | **Inter-row** | |
| | **Plant height at 30 DAS (m)** | | |
| Sorghum x *Marandu palisadegrass* | 0.41 | 0.42 | 0.41$^{a}$ |
| Sorghum x *Xaraes palisadegrass* | 0.40 | 0.44 | 0.42$^{a}$ |
| Sorghum x *Piata palisadegrass* | 0.42 | 0.47 | 0.44$^{a}$ |
| Average | 0.41$^{a}$ | 0.44$^{a}$ | |
| Sorghum in monoculture | 0.41 | | |
| CV (%) | ..........................10.52............................ | | |
| | **Plant height at 60 DAS (m)** | | |
| Sorghum x *Marandu palisadegrass* | 0.92 | 0.82 | 0.87$^{a}$ |
| Sorghum x *Xaraes palisadegrass* | 0.88 | 0.89 | 0.88$^{a}$ |
| Sorghum x *Piata palisadegrass* | 0.89 | 0.79 | 0.83$^{a}$ |
| Average | 0.89$^{a}$ | 0.83$^{a}$ | |
| Sorghum in monoculture | 0.98 | | |
| CV (%) | ............................8.52............................ | | |
| | **Plant height at 90 DAS (m)** | | |
| Sorghum x *Marandu palisadegrass* | 1.35 | 1.28 | 1.32$^{a}$ |
| Sorghum x *Xaraes palisadegrass* | 1.39 | 1.26 | 1.33$^{a}$ |
| Sorghum x *Piata palisadegrass* | 1.31 | 1.28 | 1.29$^{a}$ |
| Average | 1.35$^{a}$ | 1.27$^{a}$ | |
| Sorghum in monoculture | 1.33 | | |
| CV (%) | ………..................8.28.......................... | | |

Means followed by the same lowercase letters within the same column, and uppercase letters within the same row, were not significantly different according to Tukey's and F tests, at$p<0.05$. Averages followed by (*) were significantly different from the sorghum monoculture according to the Dunnett test, at $p<0.05$.

at 30, 60 and 90 DAS between the different integrated systems (sorghum intercropped with *B. brizantha* cultivars) and sowing systems (row and inter-row), nor were there significant interactions between factors ($p>0.05$) (Table 1). This finding indicates that *B. brizantha* did not affect sorghum development, which is in

**Table 2.** Stem diameter at 30, 60 and 90 DAS of sorghum plants in monoculture and intercropped with *Brachiaria brizantha* cultivars under two different sowing systems.

| **Integrated systems** | **Sowing Systems** | | **Average** |
|---|---|---|---|
| | **Row** | **Row** | |
| | **Stem diameter at 30 DAS (mm)** | | |
| Sorghum x *Marandu palisadegrass* | 10.09 | 10.77 | $10.38^{a}$ |
| Sorghum x *Xaraes palisadegrass* | 9.57 * | 11.26 | $10.42^{a}$ |
| Sorghum x *Piata palisadegrass* | 10.48 | 11.66 | $11.07^{a}$ |
| Average | $10.01^{a}$ | $11.23^{a}$ | |
| Sorghum in monoculture | 12.47 | | |
| CV (%) | ...................12.09 ................. | | |
| | **Stem diameter at 60 DAS (mm)** | | |
| Sorghum x *Marandu palisadegrass* | 15.93 | 17.06 | $16.50^{a}$ |
| Sorghum x *Xaraes palisadegrass* | 15.11 * | 16.32 | $15.71^{a}$ |
| Sorghum x *Piata palisadegrass* | 16.27 | 16.21 | $16.24^{a}$ |
| Average | $15.77^{a}$ | $16.53^{a}$ | |
| Sorghum in monoculture | 19.50 | | |
| CV (%) | .................. 11.60 .................. | | |
| | **Stem diameter at 90 DAS (mm)** | | |
| Sorghum x *Marandu palisadegrass* | 16.73 | 17.57 | $17.15^{a}$ |
| Sorghum x *Xaraes palisadegrass* | 15.67 * | 17.01 | $16.34^{a}$ |
| Sorghum x *Piata palisadegrass* | 17.37 | 16.74 | $17.0^{6a}$ |
| Average | $16.59^{a}$ | $17.11^{a}$ | |
| Sorghum in monoculture | 19.97 | | |
| CV (%) | ................. 10.71 .................. | | |

Means followed by the same lowercase letters within the same column, and uppercase letters within the same row, were not significantly different according to Tukey's and F tests, at p<0.05. Averages followed by (*) were significantly different from the sorghum monoculture according to the Dunnett test, at p<0.05.

accordance with Almeida et al.(2012) and Horvathy et al. (2012). At 90 DAS, the average sorghum height was 1.31 m in monoculture and 1.33 m intercropped (Table 1). These values were higher than those reported by Silva et al. (2013), who evaluated sorghum inter-row intercropped with *Brachiaria*, as a double crop, and obtained 1.14 and 1.15 m plant height. This difference may be due to the different hybrids used in the two studies and the sowing time, which affects plant development.

The sorghum row intercropped with *X. palisadegrass* exhibited lower stem diameters at 30, 60 and 90 DAS (Table 2). This difference was likely due to greater competition for water, light, nutrients and physical space because both of the species were sown in the same row. In addition, *X. palisadegrass* may have affected sorghum development because it possesses wider leaves and greater height (Costa et al., 2009), resulting in more competition for light. These findings differ from those of Crusciol et al. (2011), who observed that sorghum hybrids row intercropped with *M. palisadegrass* exhibited greater stem diameters than sorghum grown in monoculture. In turn, when *B. brizantha* cultivars were inter-row intercropped with sorghum, the association had no effect on stem diameter compared to sorghum grown in monoculture (p>0.05) (Table 2). These findings confirm the viability of inter-rowintercropping because it results in less competition with the sorghum plants.

The number of leaves at 30 DAS was not significantly different between the different integrated and sowing systems tested (p>0.05) (Table 3). However, sorghum exhibited a lower number of leaves when intercropped with *Marandu palisadegrass* than in monoculture at 60 DAS (p>0.05). This difference may be attributed to the fact that plants with fewer leaves develop greater leaf area to maintain the photosynthesis levels necessary for development. No significant differences were observed in plant populations between the sorghum intercropped with *B. brizantha* cultivars and the sorghum in monoculture, thus confirming the absence of competition between species under different forage systems and indicating that the association with *Brachiaria* was not detrimental for the sorghum plant stand (p>0.05) (Table 3).

However, the sowing system influenced (p>0.05) the sorghum population. Sowing *B. brizantha* as an inter-row

**Table 3.** Number of leaves, plant population and panicle index at 30 and 60 DAS for sorghum grown in monoculture and intercropped with *Brachiaria brizantha* cultivars under two different sowing systems.

| Integrated systems | Sowing Systems | | |
|---|---|---|---|
| | **Row** | **Inter-row** | **Average** |
| | **Number of leavesat 30 DAS** | | |
| Sorghum x *Marandu palisadegrass* | 4.17 | 4.41 | 4.29 $^{a}$ |
| Sorghum x *Xaraes palisadegrass* | 3.83 | 4.50 | 4.17 $^{a}$ |
| Sorghum x *Piata palisadegrass* | 4.08 | 4.58 | 4.33 $^{a}$ |
| Average | 4.03 $^{a}$ | 4.50 $^{a}$ | |
| Sorghum in monoculture | 4.92 | | |
| CV (%) | ....................... 16.28 .......................... | | |
| | **Number of leavesat 60 DAS** | | |
| Sorghum x *Marandu palisadegrass* | 7.42 * | 7.91 | 7.67 $^{a}$ |
| Sorghum x *Xaraes palisadegrass* | 7.58 | 7.50 | 7.54 $^{a}$ |
| Sorghum x *Piata palisadegrass* | 7.63 | 7.67 | 7.63 $^{a}$ |
| Average | 7.53 $^{a}$ | 7.69 $^{a}$ | |
| Sorghum in monoculture | 8.50 | | |
| CV (%) | .................... 6.25 ...................... | | |
| | **Plant population** | | |
| Sorghum x *Marandu palisadegrass* | 243.333 | 185.000 | 214.117 $^{a}$ |
| Sorghum x *Xaraes palisadegrass* | 236.667 | 205.000 | 220.833 $^{a}$ |
| Sorghum x *Piata palisadegrass* | 233.889 | 198.333 | 216.111 $^{a}$ |
| Average | 237.963 $^{a}$ | 196.111 $^{b}$ | |
| Sorghum in monoculture | 216.667 | | |
| CV (%) | ........................ 17.08 ....................... | | |
| | **Panicle index (%)** | | |
| Sorghum x *Marandu palisadegrass* | 94.50 | 94.89 | 94.70 $^{a}$ |
| Sorghum x *Xaraes palisadegrass* | 87.11 | 91.45 | 89.28 $^{a}$ |
| Sorghum x *Piata palisadegrass* | 85.92 | 93.38 | 89.65 $^{a}$ |
| Average | 89.18 $^{a}$ | 93.24 $^{a}$ | |
| Sorghum in monoculture | 94.65 | | |
| CV (%) | ............................ 4.98............................ | | |

Means followed by the same lowercase letters within the same column, and uppercase letters within the same row, were not significantly different according to Tukey's and F tests, at$p<0.05$. Averages followed by (*) were significantly different from the sorghum monoculture according to the Dunnett test, at$p<0.05$.

crop resulted in a smaller sorghum population. This result is in contrast with previous studies of the intercropping of grain sorghum with *Brachiaria*, in which no differences in population were observed either for intercropping at the row (Horvathy et al., 2012) or inter-row (Lara, 2011; Silva et al., 2013).

The forage and sowing systems did not affect (p>0.05) the panicle index (Table 4). It should be noted that this variable represents the tillering capacity of sorghum. Even with differences in plant population between the tested sowing systems, the panicle indexes were similar between treatments, which indicates that the tillering index did not change with the decrease in plant population observed with inter-row intercropping (approximately 18%). This lack of change in the tillering index was most likely due to the high plant density recommended for the Buster hybrid. Therefore, even with high plant populations, the values obtained in intercropping and in monoculture were similar to those reported by Borghi et al. (2013) for sorghum grown in monoculture (91%) and intercropped with *M. palisadegrass* (99%).

No differences were observed in dry mass production among the sorghum crops grown in association with the different *B. brizantha* cultivars and under different sowing systems (p>0.05) (Table 4). However, there was a 45%

**Table 4.** Dry mass production, total dry mass production, thousand-grain weight and grain yield of sorghum in monoculture and in association with *Brachiaria brizantha* cultivars under two different sowing systems.

| Integrated systems | Sowing Systems | | |
|---|---|---|---|
| | **Row** | **Inter-row** | **Average** |
| | **Dry mass production of sorghum (kg ha$^{-1}$)** | | |
| Sorghum x *Marandu palisadegrass* | 4.964 | 4.615 | 4.964 |
| Sorghum x *Xaraes palisadegrass* | 3.850* | 4.385 | 3.850* |
| Sorghum x *Piata palisadegrass* | 4.979 | 4.068 | 4.979 |
| Average | 4.598 $^{a}$ | 4.356 $^{a}$ | 4.598 $^{a}$ |
| Sorghum in monoculture | 5.618 | | |
| CV (%) | 16.12 | | |
| | **Total dry mass production (kg ha$^{-1}$)** | | |
| Sorghum x *Marandu palisadegrass* | 8.568* | 8.474* | 8.568* |
| Sorghum x *Xaraes palisadegrass* | 8.507* | 9.647* | 8.507* |
| Sorghum x *Piata palisadegrass* | 8.755* | 9.061* | 8.755* |
| Average | 8.610 $^{a}$ | 9.060 $^{a}$ | 8.610 $^{a}$ |
| Sorghum in monoculture | 5.618 | | |
| CV (%) | 18.11 | | |
| | **Thousand-grain weight (g)** | | |
| Sorghum x *Marandu palisadegrass* | 29.41 | 30.74 | 29.41 |
| Sorghum x *Xaraes palisadegrass* | 30.74 | 31.99 | 30.74 |
| Sorghum x *Piata palisadegrass* | 27.62 | 31.87 | 27.62 |
| Average | 29.26 $^{a}$ | 31.53 $^{a}$ | 29.26 $^{a}$ |
| Sorghum in monoculture | 31.52 | | |
| CV (%) | 6.25 | | |
| | **Grain yield (kg ha$^{-1}$)** | | |
| Sorghum x *Marandu palisadegrass* | 5.872 | 4.940 | 5.872 |
| Sorghum x *Xaraes palisadegrass* | 3.729 * | 4.075 | 3.729* |
| Sorghum x *Piata palisadegrass* | 4.622 | 4.035 | 4.622 |
| Average | 4.741 $^{a}$ | 4.350 $^{a}$ | 4.741 $^{a}$ |
| Sorghum in monoculture | 6.953 | | |
| CV (%) | 14.63 | | |

Means followed by the same lowercase letters within the same column, and uppercase letters within the same row, were not significantly different according to Tukey's and F tests, at p<0.05. Averages followed by (*) were significantly different from the sorghum monoculture according to the Dunnett test, at p<0.05.

decrease in dry mass production of the sorghum row intercropped with *X. palisadegrass* compared to sorghum grown in monoculture. This finding may be due to greater competition between plants in this treatment. In addition to being in the same sowing row as the sorghum, using the same resources (water, light and nutrients), the *X. palisadegrass* has wider leaves and is taller than the remaining cultivars (Costa et al., 2009); therefore, there may have been more competition between the *X. palisadegrass* and sorghum plants.

One of the great advantages of intercropping is the complementation of the production of both of the species without resulting in decreases in the yield of the main crop. The absence of significant differences in dry mass production of the sorghum intercropped with *M. palisadegrass* and *P. palisadegrass,* compared to the sorghum in monoculture, indicates that there was no competition with these cultivars to the point of decreasing production.

For the total dry mass production (sorghum + *B. brizantha* cultivars) (Table 4), all the intercropping systems and both of the sowing systems exhibited higher values (p>0.05) than the sorghum in monoculture. The intercropping of sorghum with *B. brizantha* cultivars contributed to increase the total dry mass production by 53 and 61% for the row and inter-row sowing systems, respectively, which shows the efficiency of *B. brizantha* cultivars in supplementing the dry mass production in integrated systems.

These results confirm the viability of using grain sorghum intercropped with *B. brizantha* cultivars. This system allows the straw from sorghum and *B. brizantha* to remain in the area following the grain harvest. The straw can then be grazed by animals during the off-season period (Maia et al., 2014) or used for desiccation for the establishment of the summer crop (Silva et al., 2013).No significant differences were observed in the thousand-grain weight between the sowing systems (p>0.05) (Table 4). This pattern is explained by the absence of competition of sorghum with *B. brizantha* at the maturation stage, that is, following sorghum flowering. In addition, the results of the thousand-grain weight were similar to the remaining parameters, especially the plant height. This result indicates that the association with *B. brizantha* cultivars did not influence the formation of sorghum panicles and consequently the thousand-grain weight, which were determined at the reproductive (30 to 60 DAS) and maturation (after 60 DAS) stages of sorghum (Vanderlip, 1993).

Grain yield was only affected by the sorghum rowintercropping with *X. palisadegrass* for consistency, which resulted in decreased sorghum grain yield compared to the monoculture (p>0.05), similar to what was observed for the stem diameter and dry mass production (Table 4). The stem is one of the structures that stores reserve substances in plants; wider stem diameters provide the plant with greater capacity for storage. A larger diameter considerably contributes to grain filling (Gimenes et al., 2008).

The sorghum grain yield was similar when it was intercropped with *M. palisadegrass* and *P. palisadegrass* and in monoculture, for the two tested sowing systems (Table 4). This result indicates that the association of the Buster hybrid with these cultivars is viable for cultivation as a double crop because intercropping did not result in grain yield decrease. This finding is in accordance with a previous study of the inter-row intercropping of grain sorghum with *M. palisadegrass* (Silva et al., 2013).

Due to the different region of study and genetic differences between sorghum cultivars, the present grain yields were lower than those reported by Borghi et al. (2013) and higher than those reported by Horvathy et al. (2012) and Silva et al. (2013). The higher yields obtained in the present study are most likely due to the earlier time of sorghum sowing (February) together with the early onset of the Buster hybrid. Because of this relation, the crop reached the flowering stage at the time of rainfall occurrence (Figure 1), which is desirable to obtain higher grain yields (Baumhardt et al., 2005; Fornazieri Filho and Fornasieri, 2009).

## Conclusion

The inter-row intercropping of grain sorghum with *B. brizantha* cultivars did not interfere with the sorghum agronomic characteristics and grain yield. However, the association of sorghum with *X. palisadegrass* in the rows affected the stem diameter, sorghum dry mass production and grain yield. Intercropping with *M. palisadegrass* and *P. palisadegrass* is therefore recommended more. The intercropping of sorghum with *B. brizantha* cultivars was a viable cultivation system for grain production as a double crop in the midwest region of Brazil.

## Conflict of Interest

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

# Performance evaluation of a tractor mounted pneumatic planter for sorghum in dryland

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**Performance of a pneumatic planter was studied in both laboratory and actual field conditions using sorghum seeds in context to justify its use in dry land. Pneumatic planter consists of frame, aspirator blower, seed hopper, metering unit, multi-groove metering plate, vacuum retaining plate, furrow opener, pair of ground wheels with power transmission system. The multi groove metering plate having seed hole of diameter 3 mm and vacuum pressure of 2 kPa were used throughout the experiments for picking of single seed. Based on the results of laboratory tests the performance of the pneumatic planter was carried out in field. Data on performance parameters of the pneumatic planter was collected, analyzed and found that, average values of plant to plant spacing, miss index and multiple index, actual field capacity and field efficiency were found to be 101 mm, 2.07%, 3.8%, 0.773 ha/h and 79.7%, respectively.**

**Key words:** Pneumatic planter, sorghum, seed rate, performance indices.

## INTRODUCTION

Horizontal seed metering devices were popular and widely accepted but the problems occurred with higher seed damage, missing and multiple drops. To reduce these losses, inclined and vertical plate planters were developed (Shafii and Holmes, 1990; Guarella et al., 1996). Horizontal plate planters with cells on the periphery, as a seed metering devices for precision planting of seeds were the first precision planters developed in India (Datta, 1974). Literature reveals that the difficulty was felt with developed planters to plant irregular and spherical shaped seeds. Therefore, pneumatic metering devices were tried which has the advantage of metering irregular shaped seeds, besides spherical seeds. Pneumatic planting devices could be suitable for planting of groundnut, cotton, sorghum, maize, soybean, sorghum, mustard, okra and radish but its use has to be justified by conducting the field experiments.

Use of conventional seeding devices has higher seed rate application which leads to wastage of costly seeds, adds the cost of thinning and results in increased production cost. Dixit et al. (2011) compared the

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**Figure 1.** Pneumatic planter during in laboratory evaluation. 1. Frame; 2. Aspirator blower; 3. Seed hopper; 4. Metering unit; 5. Multi groove metering plate; 6. Vacuum retaining plate; 7.Furrow opener; 8. Ground wheel; 9. Cordon shaft.

performance of inclined plate planter and pneumatic planter and found that pneumatic planter performed much better for cotton. Using pneumatic planter, seed germination efficiency has increased many folds at reduced seed rate compare to conventional planters. Inter row and intra row spacing for sorghum is an important factor in order to achieve optimum crop yield. The parameters for the evaluation of performance of the planter include spacing between seeds or plants (Hollewell, 1992; Parish et al., 1991), percent multiples and misses (Brooks and Church, 1987; Singh et al., 2005; Singh et al., 2007; Sun et al., 2012; yasir et al., 2012) and precision in spacing (Hofman, 1988; Jasa and Dickey, 1982). Important factor of the pneumatic seed-metering device is its uniformity of seed spacing. Besides the design of the metering devices, field and operational parameters affect the precision distribution of seeds.

Karayel and Ozmerzi (2001) stated that variability in the seed spacing with a precision vacuum seeder increased with increasing forward speed. Use of conventional planting machines does not maintain precise plant spacing and seed rate (Khambalkar et al., 2014). Therefore, an attempt was made to evaluate the pneumatic planter developed at CIAE (Central Institute of Agricultural Engineering), Bhopal both in laboratory and field conditions to justify its use in planting of sorghum seeds in dry land cultivation of sorghum.

## MATERIALS AND METHODS

The pneumatic planter consists of main frame, aspirator blower, disc with cell type metering plate, individual hopper, furrow openers, PTO driven shaft, ground drive wheel etc. Pictorial view of the pneumatic planter is shown in Figure 1. Specifications of the pneumatic planter are given in Table 1.

### Power transmission system of the pneumatic planter

Power transmission system with different gear arrangement of the pneumatic planter is shown in the Figure 2. Various gear ratios were used to get desired seed rate. Two gears were mounted on the axle of the ground wheel having 14 and 20 teeth. The power from the ground wheel was then transferred through chain to the primary driving shaft having gears with 20 and 14 teeth at each ends. The primary driving shaft had five gears 26, 22, 20, 16 and 14 in first, second, third, fourth and fifth, respectively. The power from primary driving shaft is transferred to the secondary driving shaft with the help of chain and idler gears. Seven idle gears were of 14 teeth in the secondary driving shaft. One gear on the secondary driving shaft is connected to one of the five gears on the primary driving shaft through chain. The power from the secondary driving shaft is transferred to the seed metering plate. The other gears on the secondary driving shaft were attached to the gear on the axle of seed metering device with the help of chain. The gears on the seed

**Table 1.** Specifications of the pneumatic planter.

| Parameter | Value |
|---|---|
| Overall dimensions, mm | 1450 × 2450 ×1250 |
| Weight, kg | 200 |
| Number of rows | 6 |
| Row spacing, mm | 250 and above |
| Type of blower | Aspirator |
| Seed metering | Pneumatic disc suction principle |
| Change of seed spacing | By changing appropriate size of sprocket |
| Number of holes on disc | 16 |
| Seed hole diameter, mm | 3 |
| Working width, mm | 2700 |
| Power source | Tractor 25 kW and above |

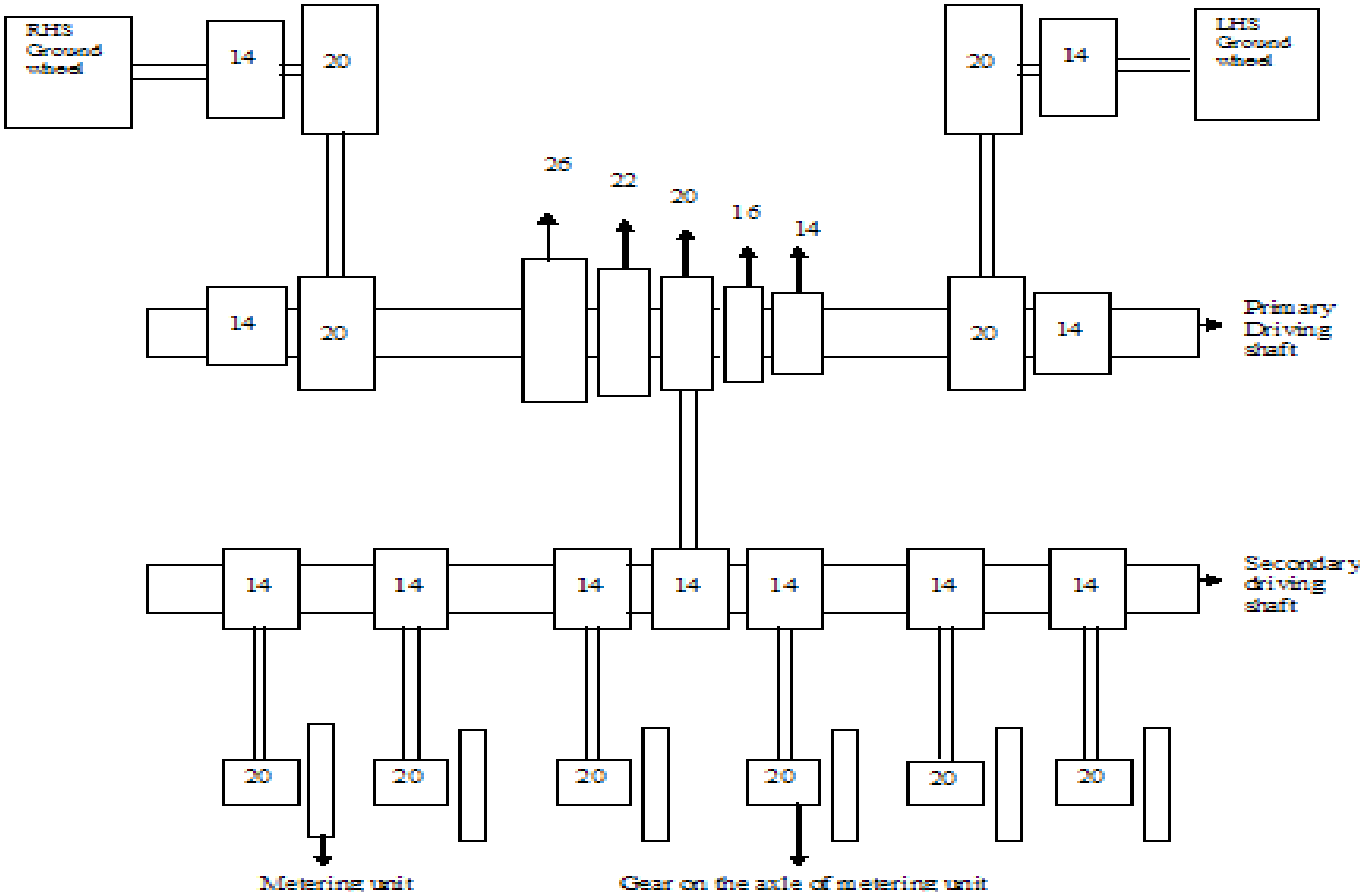

**Figure 2.** Power transmission system of the pneumatic planter.

metering mechanism had 20 teeth, as shown in Figure 1.

### Working principle of pneumatic planter

The metering device was powered by a pair of ground wheels through chain and sprocket. Power to the aspirator blower was given by PTO shaft of the tractor with the help of cordon shaft. The disc was mounted to a vacuum retaining plate made of Bakelite material having outer diameter 295 mm and thickness 40 mm. Suction pressures inside the metering unit was created by connecting it to a vacuum pump. The vacuum retaining plate was

**Table 2.** Parameters and their values of field, seed and operational parameters.

| Parameter | Value |
|---|---|
| Plot size, $m^2$ | 24 m × 12 m |
| Type of soil | Sandy clay loam |
| Moisture content, % | 9±2 |
| Bulk density, g/cc | 1.31±0.05 |
| Forward speed, km/h | 3.2-3.6 |
| Slip of driving wheels, % | 8-10 |
| Row to row spacing, mm | 450 |
| Seed to seed spacing, mm | 100 ± 5 |
| Depth of placement of seed, mm | 30 ± 5 |

equipped with a baffle to release the vacuum pressure of the seed disc. The rotating seed disc carried the seeds attached to the seed holes under negative pressure and dropped only when the holes passed through the baffle that released the suction pressure. The dropped seed fall in the furrow opened by furrow opener and cover with the soil. To view the movement of the seeds inside the metering disc, the seed disc was provided with a protective cover made of mild steel and transparent acrylic plastic.

### Laboratory evaluations

The pneumatic planter was calibrated with sorghum seeds in the laboratory. PTO was connected to the aspirator air blower of the pneumatic planter. The gear combination at left and right hand side of ground wheel was set and the main driving shaft of gear was laid on the first gear. Then the PTO was operated at 550 rpm resulting in the rotation of the blower at 2720 rpm to create proper vacuum in the seed metering unit for proper metering of the seed. Then the ground wheel of the pneumatic planter was rotated 20 times with constant speed manually. Seeds were collected in the poly-ethylene bag underlying the furrow opener. Simultaneously the rpm of PTO shaft was taken by the tachometer to about 550 rpm. The seed quantity was measured in grams. Similar procedure was adopted for the different gears of main driving shaft at same setting of side gears of ground wheel. The side gear combination of ground wheel was changed and again the observations were taken at different gears of main driving shaft as stated earlier. Three replications were taken for each setting and average values are given Table 2.

### Performance parameters measured during field evaluation

#### *Speed of operation*

The time taken (*s*) to distance travelled (m) during operation was determined using stopwatch. The forward speed of tractor (km/h) was calculated by following equation.

$$\text{Forward speed of tractor} = \frac{Distance}{time} \times 3.6 \quad (1)$$

#### *Field capacity and field efficiency*

The actual field capacity, theoretical field capacity and field efficiency was calculated as follows:

$$AFC = \frac{Ac}{Tt} \quad (2)$$

$$TFC = \frac{W \times S}{10} \quad (3)$$

$$FC = \frac{AFC}{TFC} \times 100 \quad (4)$$

Where AFC = actual field capacity (ha/h), TFC = theoretical field capacity (ha/h), Ac = actual area covered (ha), Tt = time taken (hr), FC = field efficiency (%), W = width of machine (m), and S = forward speed (km/h).

#### *Miss index*

The miss index ($I_{ms}$) is the ratio of number of spacing (Nms) greater than 1.5 times of set spacing and total number of measured spacings (N):

$$I_{ms} = \frac{N_{ms}}{N} \times 100 \quad (5)$$

#### *Multiple index*

The multiple index ($I_{mt}$) is the ratio of number of spacing (Nmt) ≤ 0.5 times of set spacing and total number of measured spacings (N):

$$I_{mt} = \frac{N_{mt}}{N} \times 100 \quad (6)$$

Pneumatic planter was set based on the results obtained in laboratory. The procedure outlined in RNAM Test code and procedure (1983) for seedling equipment was followed. Parameters and their values of field, seed and operational parameters is shown in Table 2.

The pneumatic planter was powered by 29.84 kW tractor (TAFE 585 DI). The tractor was operated at an average forward speed of 3.6 km/h. Pre-experimental trials had been undertaken to adjust the working parts such as speed of PTO shaft, furrow openers, depth adjustment. A mark was made on the lever of the three point linkage to set the depth of operation of the furrow opener at 30 mm. Pneumatic planter during field operation is shown in Figure 3.

**Figure 3.** Pneumatic planter during actual field operation.

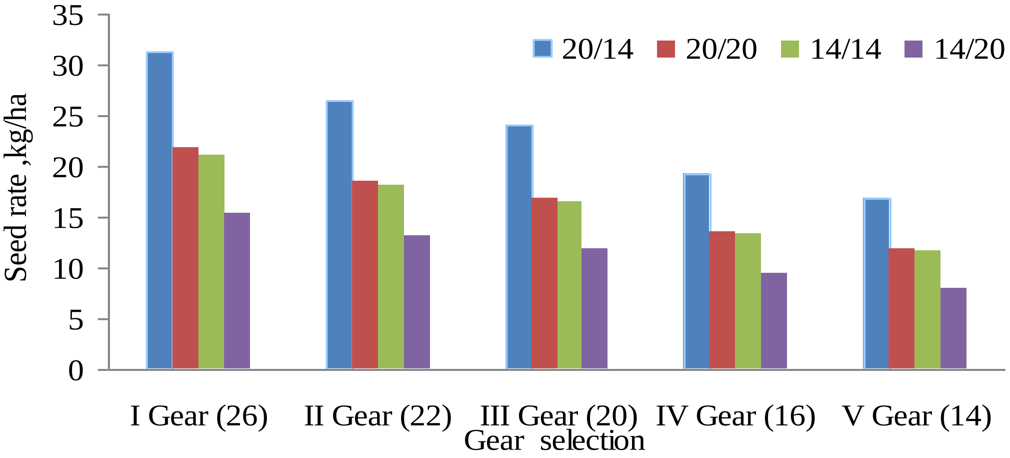

**Figure 4.** Sorghum seed rate obtained at different gear combinations.

## RESULTS AND DISCUSSION

The pneumatic planter was tested in the laboratory as well as in field conditions of Department of Farm Machinery and Power Engineering and Crop Research Center of GBPUA and T Pantnagar, respectively. The tests were carried out for sorghum seeds at different gear combinations to obtain the recommended seed rate. The seed rate obtained for different combination and for different gears of primary driving shaft is shown in the Figure 4.

Gear having 14 teeth mounted on the axle of ground wheel is attached to the gear having 20 teeth mounted at the ends of the primary driving shaft, and second gear, that is, having 14 teeth mounted on the middle of the primary driving shaft gives the required seed rate of 7.98 kg/ha and was within the recommended seed rate of 7.5 to 8 kg/ha. Based on the results obtained in laboratory, performance of the pneumatic planter was carried out in field. Data on performance parameters of the planter such as the plant to plant spacing, miss index and multiple index, actual field capacity and field efficiency was collected and analyzed. The average values of the field trials conducted were found as 101 mm, 2.07%, 3.8%, 0.773 ha/h, 79.7%, respectively. The average values of all the performance parameters are given in

**Table 3.** Average values of all the performance parameters.

| Parameter | Plot 1 | Plot 2 | Plot 3 | Average |
|---|---|---|---|---|
| Plant spacing, mm | 100.35 | 99.98 | 102.28 | 100.87 |
| Miss index, % | 1.99 | 2.10 | 2.13 | 2.07 |
| Multiple index, % | 3.8 | 3.9 | 3.7 | 3.8 |
| Field capacity, ha/h | 0.775 | 0.785 | 0.760 | 0.773 |
| Field efficiency, % | 80 | 81 | 78 | 79.7 |

Table 3. Standard deviation and standard error was 5.8, 3.4, 9.2 and 1.7, 0.7, 1.5 for miss index, multiple index and field capacity respectively.

## Conclusions

The pneumatic planter was found suitable for sorghum with the best suited gear combination, that is, gear having 14 teeth mounted on the axle of ground wheel and 20 gear teeth mounted at the ends of the primary driving shaft with 14 gear teeth mounted at the middle of the primary driving shaft of pneumatic planter with tractor PTO shaft speed of 550 rpm. Performance of the pneumatic planter was evaluated in the field and the average values of plant to plant spacings, mean miss index and multiple index, actual field capacity and field efficiency were found to be 101 mm, 2.07%, 3.8%, 0.77 ha/h, 79.7%, respectively. All the observed values were within the recommended levels. Hence, this planter was found suitable for planting of sorghum seeds in dry land.

## Conflict of Interest

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

# The effect of nitrogen dose on the yield indicators of oats

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**Studies on the effect of nitrogen dose on the yield indicators of oats can help cultivation recommendations. This study aims to study the simulation of the productivity indicators of oats based on optimal dose of nitrogen on grain yield expression, under the agricultural stand conditions of high and low availability of nitrogen and favorable and unfavorable weather conditions. Studies were conducted in 2011 and 2012 years, in a complete randomized block design with four replications in a 2 x 4 factorial arrangement to cultivars (Barbarasul and Brisasul) and nitrogen doses (0, 30, 60 and 120 kg ha$^{-1}$) in the soybean/oat and corn/oat succession system. In each succession system, two experiments were conducted, one for quantifying the production of biomass and another to estimate grain yield. The use of nitrogen introduced quadratic regression model on the grain yield and harvest index, showed linear behavior in the biological yield and straw in the two crop succession system. The difference of this behavior is caused by the biological, straw and also by year favorable or unfavorable. The simulation of the nitrogen optimal dose for the production of grain confirms major efficiency of Brisasul cultivar in the expression of grain yield indicators.**

**Key words:** *Avena sativa* L., crop succession, nitrogen rates, yield indicators, simulation.

## INTRODUCTION

Climatic changes on the planet have provided a new challenge in the world agricultural production, where breeding of more productive and tolerant to stresses cultivars (Araus et al., 2008), and efficiency in the use of

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light and nutrients (Oliveira et al., 2011) are required. In this context, the improper management of nitrogen, which efficiency depends on the quality of fertilizer, cultivation techniques, utilization of genetic efficiency and edaphoclimatic conditions become reasons for the low grain production of oat (Silva et al., 2006; Veloso et al., 2009; Costa et al., 2013). Increasing the nitrogen dose and the optimal application time under favorable climatic conditions shows significant effects on grain yield (Dencic et al., 2011; Flores et al., 2012). However, in unfavorable years of cultivation, utilization efficiency may be compromised, reducing productivity and increasing production costs, besides the nitrogen losses by volatilization and leaching generating environmental pollution (Benin et al., 2012; Silva et al., 2015).

The biological yield (grain yield + straw yield) is closely related to the processes of photosynthesis and respiration during the vegetative and reproductive stages (Demétrio et al., 2012). The relationship between grain and biological yields allows the determination of the harvest index, important parameter in defining the rate with which photo assimilates are transported to straw and grains (Silva et al., 2012). Studies of Demétrio et al. (2008), show that these traits are influenced by genotype, cultivation technique, water and nutrients availability, and edaphoclimatic conditions, which highlights the importance of these variables on the efficiency of nitrogen uptake and utilization (Silva et al., 2015). Thus, the studies of nitrogen use efficiency based on grain yield indicators can help towards the recommendations on better adjusted technologies, economically satisfactory, lower impact on the environment and decisive conditions in the search for more sustainable agricultural biosystems (Parry et al., 2011; Prando et al., 2013).

The objective of this study was the simulation of the productivity indicators of oats based on optimal dose of nitrogen on grain yield expression, under the agricultural stand conditions of high and low availability of nitrogen and favorable and unfavorable weather conditions.

## MATERIALS AND METHODS

The field experiments with oats were conducted in Augusto Pestana, RS, Brazil (28° 26’ 30’’ South latitude and 54° 00’ 58’’ West longitude), in 2011 and 2012 years. The soil of the area is classified as Oxisol Distroferric Typical and the climate, according to Köppen classification (Kuinchtner and Buriol, 2011), is temperate humid with hot summer, without dry season. Soil analysis ten days before oats sowings identified the following chemical characteristics of the local: i) crop succession corn/oat (pH = 6.5; P = 34.4 mg $dm^{-3}$; K = 262 mg $dm^{-3}$; Organic Matter = 3.5%; Al = 0.0 $cmol_c$ $dm^{-3}$; Ca = 6.6 $cmol_c$ $dm^{-3}$ e Mg = 3.4 $cmol_c$ $dm^{-3}$) and ii) crop succession soybean/oat (pH = 6.2; P = 33.9 mg $dm^{-3}$; K = 200 mg $dm^{-3}$ Organic Matter = 3.4%; Al = 0.0 $cmol_c$ $dm^{-3}$; Ca = 6.5 $cmol_c$ $dm^{-3}$ e Mg = 2.5 $cmol_c$ $dm^{-3}$). In both experimental years, oats was sown in optimal time, that is, in the first week of June with seeder-fertilizer. Each plot consisted of 5 rows of 5 m length with 0.20 m line space, forming the experimental unit of 5 $m^2$. During the vegetation period, oats was protected from diseases by FOLICUR® CE tebuconazole fungicide applications at the dose of 0.75 l $ha^{-1}$ and from weeds control by ALLY® metsulfuron-methyl herbicide, at the dose of 2.4 g $ha^{-1}$ of the active ingredient and manual weeding when necessary. At the time of oat sowing, NPK formulation was used (5-20-20), with nitrogen base of 10 kg $ha^{-1}$ (except in the standard experimental unit) and 60 and 50 kg $ha^{-1}$ of $P_2O_5$ and $K_2O$ applied, respectively, based on the levels of organic matter, P and K in the soil to expect grain yield of about of 3 t $ha^{-1}$. The rest of the nitrogen to contemplate the remaining doses was applied in coverage with urea (45% N), in four oat leaf stage.

The experimental design was randomized blocks with four repetitions, in factorial scheme 4 × 2 to doses of N-fertilizer (0, 30, 60, 120 kg $ha^{-1}$) and oat cultivars (Barbarasul and Brisasul), respectively, totaling 64 experimental units for crop succession. Therefore, in each crop succession (corn/oat and soybean/oat system), two experiments were conducted to quantify the total biomass production and the other targeting exclusively to estimate grain yield. For that purpose, oats were harvested manually from three central rows of each plot at the maturity stage (grain moisture about 22%). Then threshed with a stationary thresher and dried to the 13% grain moisture, in addition to weighing to estimate the grain yield (GY, kg per $ha^{-1}$). The grain yield from each plot was determined and then recalculated to Kg per ha, the same for the other parameters.

In the experiments for the total biomass estimations, plant material was cut close to the soil, from the collection of a linear meter of the three central rows of each plot. Green biomass samples were dried in forced air oven at 65°C and weight for biological yield (BY, kg per $ha^{-1}$). Straw yield (SY, kg per $ha^{-1}$) was calculated by subtraction BY - GY and the harvest index (HI, kg per $kg^{-1}$) according to $\frac{GY}{BY}$ equation.

The homogeneity and normality was checked by Bartlett and Lilliefors test, after the analysis of variance which was performed to detect the main and interaction effects. The weather conditions and values average of grain yield were used to characterize the years in favorable and unfavorable. It was performed to the adjustment equations of degree two $GY = b_0 \pm b_1 x \pm b_2 x^2$ for the estimation of maximum technical efficiency ($MTE = -\frac{b_1}{2b_2}$) of grain yield. Equations that describe the biological yield, straw yield and harvest index to simulate this parameters in the use of ideal dose of nitrogen by grain yield were obtain. All analyzes were performed using the GENES software (Cruz, 2006).

In 2011, there was more pluviometric precipitation before N-fertilization (Figure 1). Maximum temperatures observed at the beginning of oat development were higher in 2012. From the fertilization, variations of temperature not shown marked change to oat crop (Figure 1). In Table 2, the minimum were not below 5°C in the months of May and June. The maximum temperature, even in the hottest months of the cycle, does not exceed 27°C. In 2011, expressive yield indicators of oat (Table 1) showed that total accumulated pluviometric precipitation was similar to the average precipitation over 25 years (Table 2). Moreover, the reduced expression of these parameters in 2012 indicated smaller rainfall compared to the historical average. Based in these results (Table 2), the 2011 year was classified as favorable (FY) and 2012 as unfavorable year (UY) for the oat production.

## RESULTS AND DISCUSSION

In soybean/oat crop succession system (Table 1), nitrogen rates indicate change on the yield indicators of oat, regardless of year of cultivation. Genetic differences were observed for grain yield and harvest index in 2011.

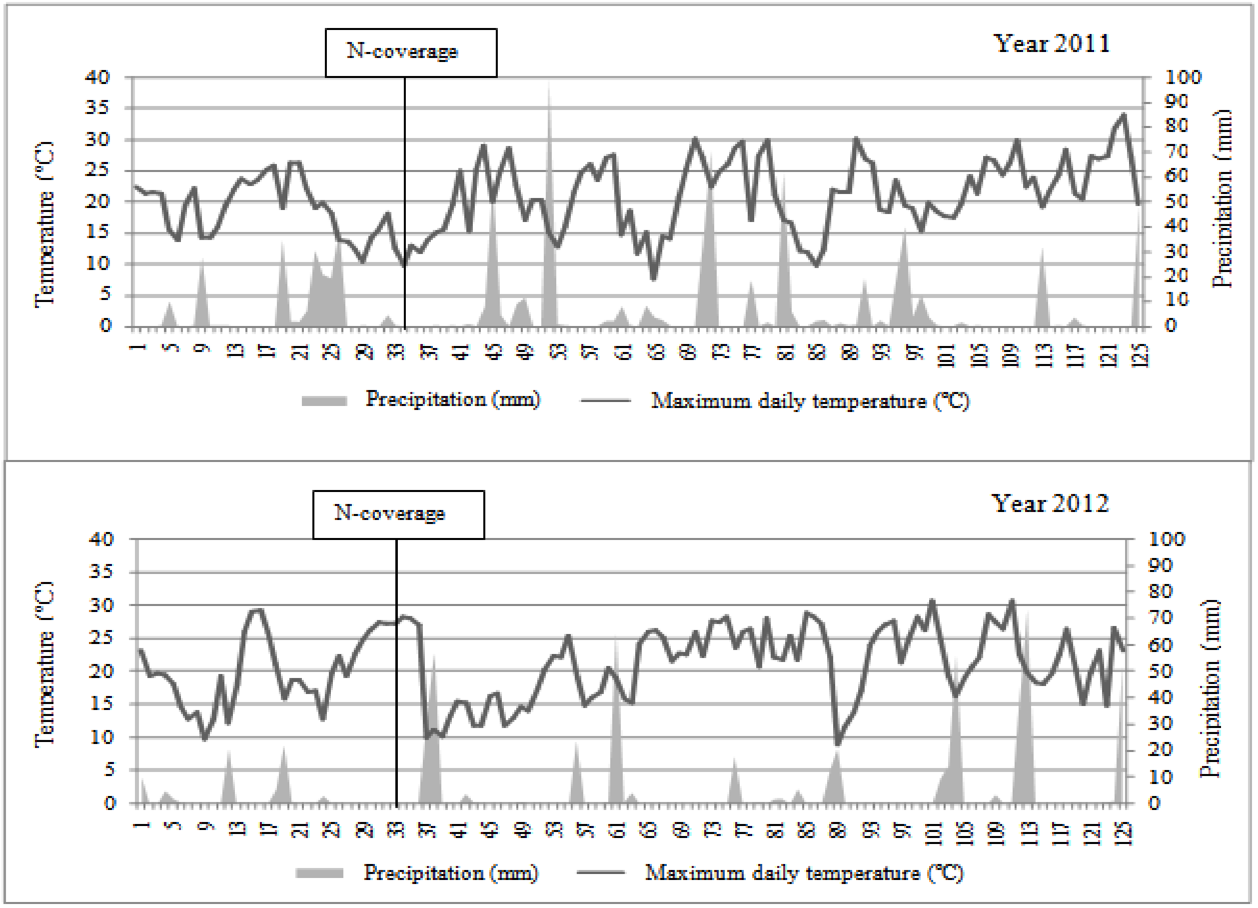

**Figure 1.** Pluviometric precipitation and maximum temperature in oats cycle.

Although, the genetic differences on the yield indicators of oat in 2012 did not show differences, however, the interaction genotype *versus* doses confirm differences on nitrogen use by cultivars, condition similar to the previous year. In crop succession corn/oat system (Table 1), the nitrogen levels were also shown on the yield indicators of oat regardless of the year of analysis. In this condition, genetic differences were more evident, except for grain yield in 2011 which did not differed. The mean difference in general between the years in crop succession soybean/oat system were significant, with 2011 surpassing 2012 in 0.7, 3 and 2.5 t ha$^{-1}$ grain yield, biological and straw, respectively.

In corn/oat crop succession system, even with lower average soybean/oat condition, also identified differences between the years, highlighting 2011 as the most favorable, with higher values of 0.2, 1.2 e 1 t ha$^{-1}$ grain yield, biological and straw, respectively. There is the reduction of the harvest index in the years that were more effective for biomass production and grain, independent succession system. Therefore, the increase in grain yield in favorable years does not follow the same way the expression of the biological yield, leading to reduced harvest index by higher biomass straw. These results confirm in oat, in general, the biological yield and straw must show linear behavior and grain yield and harvest index with quadratic trend. The genetic differences edaphoclimatic conditions and different management in crops affect the use of nitrogen (Dencic et al., 2011; Viola et al., 2013).

The cultivation of cereals such as wheat and oats on the residue of soybeans and corn as a fore crops, show different development, mainly by differences in N-residual availability and their interaction with the nitrogen supplied in coverage (Wendling et al., 2007; Mantai et al., 2015). Studies with oat in different succession systems indicated a greater magnitude of alteration in the biological yield and grain than on income of straw and harvest index (Schaedler et al., 2009; Silva et al., 2015). Moreover, the quadratic trend in the expression of grain yield and oat harvest index has been observed (Mantai et al., 2015; Silva et al., 2015).

The cultivation soybean/wheat or soybean/oat crop succession compared to corn/wheat crop succession increased grain yield with the same fertilizer due to the greater availability of N-residual in favorable years (Bredemeier et al., 2013; Mantai et al., 2015). It is noteworthy that the set of favorable year effect (2011)

**Table 1.** Summary of variance analysis of N-fertilizer on the yield indicators of oat cultivars and of mean comparison between cultivation years in biosystems of high and reduced of N-residual release.

| Source of variation | DF | Mean Square – Physiologic parameters | | | |
|---|---|---|---|---|---|
| | | GY (kg ha$^{-1}$) | BY (kg ha$^{-1}$) | SY (kg ha$^{-1}$) | HI (kg kg$^{-1}$) |
| | | **Soybean/oat system** | | | |
| | | **2011** | | | |
| Block | 3 | 44194 | 182552 | 152819 | 0.00038 |
| Dose (D) | 3 | 1352671* | 5715876* | 3681997* | 0.00672* |
| Cultivar (C) | 1 | 1303305* | 212063$^{ns}$ | 463925$^{ns}$ | 0.00845* |
| D x G | 3 | 225638* | 1946987* | 1128018* | 0.00111* |
| Error | 21 | 29097 | 244796 | 154992 | 0.00012 |
| CV (%) | | 7.81 | 11.89 | 10.54 | 6.29 |
| Mean | | 3621 $^{a}$ | 10725 $^{a}$ | 7104 $^{a}$ | 0.33 $^{b}$ |
| | | **2012** | | | |
| Block | 3 | 27547 | 9658 | 28167 | 0.00035 |
| Dose (D) | 3 | 345496* | 10229110* | 9124200* | 0.01989* |
| Cultivar (C) | 1 | 220$^{ns}$ | 361675$^{ns}$ | 343827$^{ns}$ | 0.00015$^{ns}$ |
| D x G | 3 | 29279* | 1323114* | 994165* | 0.00111* |
| Error | 21 | 18421 | 128323 | 76005 | 0.00019 |
| CV (%) | | 6.74 | 8.62 | 6.38 | 5.43 |
| Mean | | 2920 $^{b}$ | 7368 $^{b}$ | 4447 $^{b}$ | 0.40 $^{a}$ |
| | | **Corn/oat system** | | | |
| | | **2011** | | | |
| Block | 3 | 64143 | 541745 | 245360 | 0.00006 |
| Dose (D) | 3 | 1468904* | 13310303* | 6722848* | 0.00388* |
| Cultivar (C) | 1 | 21218$^{ns}$ | 4166662* | 4781005* | 0.01445* |
| D x G | 3 | 128645* | 2708766* | 1729137* | 0.00096* |
| Error | 21 | 17827 | 179581 | 111029 | 0.00014 |
| CV (%) | | 9.12 | 12.47 | 9.49 | 7.82 |
| Mean | | 2606 $^{a}$ | 7735 $^{a}$ | 5129 $^{a}$ | 0.34 $^{b}$ |
| | | **2012** | | | |
| Block | 3 | 41989 | 342191 | 161748 | 0.00014 |
| Dose (D) | 3 | 4683712* | 26277787* | 9589247* | 0.01212* |
| Cultivar (C) | 1 | 1060332* | 3603270* | 8571870* | 0.08303* |
| D x G | 3 | 36261* | 524507* | 385751* | 0.00183* |
| Error | 21 | 8481 | 104874 | 81369 | 0.0002 |
| CV (%) | | 8. 64 | 10.08 | 13.85 | 9.87 |
| Mean | | 2403 $^{b}$ | 6464 $^{b}$ | 4050 $^{b}$ | 0.37 $^{a}$ |

* = Significant at the 5% level of probability the test F; $^{ns}$ = not significant; Means followed by the same letter in the column in the culture system does not differ between the years by Tukey model in 5% probability of error; CV = coefficient of variation; DF = Degrees of freedom; GY = grain yield; BY = biological yield; SY = Straw yield; HI = Harvest index.

with the N-residual biosystem greater release (soybean/oat) allowed increasing grain yield around 3.6 t per ha$^{-1}$ (Table 1). Years of favorable and unfavorable climate change to nitrogen availability and use of efficiency by the plant (Espindula et al., 2010). Benin et al. (2012), observed in wheat greater response to grain yield by nitrogen when the rains were not limiting. The strong variation of grain yield is associated with greater variability of cultivation conditions, and the agricultural year being the largest contributing factor to the instability of production (Storck et al., 2014). This conditions support the use of more productive cultivars, tolerant to stresses and efficient in the use of light and nutrients (Araus et al., 2008; Oliveira et al., 2011).

The genotype *versus* nitrogen dose interaction tested in biosystems signal the breakdown of equations for each genotype in both the favorable and unfavorable cultivation years. In Table 3, the soybean/oat crop succession system, the Barbarasul genotype showed grain yield of expression and harvest index with quadratic

**Table 2.** Temperature and precipitation data in the months and years of oat cultivation.

| Year | Month | Temperature (ºC) | | | Precipitation (mm) | | Class |
|---|---|---|---|---|---|---|---|
| | | Minimum | Maximum | Mean | Mean 25 year* | Occurred | |
| 2011 | May | 10.5 | 22.7 | 16.6 | 149.7 | 100.5 | FY |
| | June | 7.9 | 18.4 | 13.15 | 162.5 | 191 | |
| | July | 8.3 | 19.2 | 13.75 | 135.1 | 200.8 | |
| | August | 9.3 | 20.4 | 14.85 | 138.2 | 223.8 | |
| | September | 9.5 | 23.7 | 16.6 | 167.4 | 46.5 | |
| | October | 12.2 | 25.1 | 18.65 | 156.5 | 211.3 | |
| | Total | - | - | - | 909.4 | 973.9 | |
| 2012 | May | 11.1 | 24.5 | 17.8 | 149.7 | 20.3 | UY |
| | June | 9.3 | 20.7 | 14.5 | 162.5 | 59.4 | |
| | July | 7.4 | 17.5 | 12.4 | 135.1 | 176.6 | |
| | August | 12.9 | 23.4 | 18.1 | 138.2 | 61.4 | |
| | September | 12.0 | 23 | 17.5 | 167.4 | 194.6 | |
| | October | 15.0 | 25.5 | 20.2 | 156.5 | 286.6 | |
| | Total | - | - | - | 909.4 | 798.9 | |

***** = Mean of precipitation pluviometric obtained the months from May to October 1982-2007; Class = classification suggested by the authors; FY = Favorable year; UY = Unfavorable year.

**Table 3.** Regression equation and significance parameters to estimate the optimal dose of nitrogen in the simulation of the physiological characters in the production of soybean/oat system.

| Variable | Mean square | Equation ($Y=b0\pm b1x\pm b2x^2$) | P ($b_i$) | $R^2$ | N/MTE (kg $ha^{-1}$) | $Y_E$ |
|---|---|---|---|---|---|---|
| | | | **Barbarasul 2011 (FY)** | | | |
| GY | 1724526* | $2997 + 26.5x - 0.20x^2$ | * | 0.95 | 66 | 3875 |
| BY | 2154331* | $10210 + 8.27x$ | $^{ns}$ | 0.92 | | 10210 |
| SY | 1703510* | $7210 - 18.10x + 0.20x^2$ | * | 0.99 | | 6887 |
| HI | 0.01520* | $0.29 + 0.002x - 0.000019x^2$ | * | 0.04 | | 0.34 |
| | | | **2012 (UY)** | | | |
| GY | 192484* | $2668 + 11.05x - 0.06x^2$ | * | 0.97 | 92 | 3176 |
| BY | 21562772* | $6101 + 26.16x$ | * | 0.86 | | 8507 |
| SY | 17624335* | $3309 + 23.65x$ | * | 0.77 | | 5485 |
| HI | 0.02103* | $0.40 + 0.0019x - 0.00002x^2$ | * | 0.99 | | 0.4 |
| | | | **Brisasul 2011 (FY)** | | | |
| GY | 1746813* | $3202 + 30.44x - 0.20x^2$ | * | 0.84 | 76 | 4360 |
| BY | 17032950* | $9586 + 23.25x$ | * | 0.82 | | 11353 |
| SY | 10835333* | $6010 + 18.54x$ | * | 0.98 | | 7419 |
| HI | 0.003507* | $0.35 + 0.0008x - 0.000009x^2$ | * | 0.92 | | 0.36 |
| | | | **2012 (UY)** | | | |
| GY | 501201* | $2636 + 15.34x - 0.11x^2$ | * | 0.79 | 70 | 3171 |
| BY | 8465599* | $6401 + 16.39x$ | * | 0.86 | | 7548 |
| SY | 6935548* | $3565 + 14.83x$ | * | 0.91 | | 4603 |
| HI | 0.005556* | $0.41 + 0.0008x - 0.00001x^2$ | * | 0.99 | | 0.42 |

$Y_E$ = Expected value from the ideal dose of N on grain yield; P($b_i$) = Slope parameter of the trend line; * = Significance of the slope parameter to 5% of probability; $R^2$ = Coefficient of determination; N/MTE = Nitrogen dose for maximum technical efficiency of grain yield; GY = grain yield; BY= biological yield; SY = Straw yield; HI = Harvest index; FY = Favorable year; UY = Unfavorable year.

trend, and the biological yield linear behavior, independent of the agricultural year. Brisasul genotype cultivated after the soybean as a fore crop (Table 3) showed the same response of nitrogen in grain, biological yield and harvest index, in both years. It is noteworthy that, although the Barbarasul genotype indicate this system in unfavorable condition of growing a linear trend, similar to Brisasul in 2011 and 2012, the favorable agricultural year is revealed in straw yield as a quadratic trend. The results indicate quadratic behavior in grain yield and harvest index and the trend of linearity by biological yield, however, the interactions genotype *versus* favorable and unfavorable year seem to bring more dynamism to instability in straw yield.

The favorable year (2011) in soybean/oat crop succession in Barbarasul indicated maximum nitrogen use efficiency for grain yield with 66 kg ha$^{-1}$. In unfavorable year maximum, efficiency was obtained with 92 kg ha$^{-1}$, including, the grain yield reducing drastically compared to the previous year. In the simulation of biological yield, the difference between the years was intensified, primarily by strong change in straw yield (Table 3). It is noteworthy that the observed reduction in harvest index in the favorable year does not express the efficiency obtained for the productivity of grain due to lowest use of nitrogen in comparison with 2012.

Therefore, a condition reports inefficiency of these parameters to indicate more genotypes adjusted to reduced fertilizer in comparing crop years. In Brisasul cultivar, it showed the same management conditions (Table 3), but favorable and unfavorable cultivation year, the use of nitrogen to the maximum grain yield showed similar result with 76 and 70 kg ha$^{-1}$ fertilizer. Furthermore, in the favorable year grain, biomass and straw yields of barbarasul cultivar increased.

Therefore, identifying the brisasul behavior of higher agronomic efficiency to the preparation of both biomass and grain was reported. This fact is evidenced in the more restrictive cultivation year, because, it showed similarity in grain yield, why the use of nitrogen by Barbarasul demanded an increase by 20 kg per hectare. This is in agreement with the results obtained in the adjusted model for simulating the harvest index. The use of the optimal dose, confirms the higher grain yield efficiency of this cultivar, independent of the agricultural year. Besides genetic efficiency of each cultivar, the largest nitrogen utilization is directly linked to environmental stimuli such as photoperiod, temperature, radiation and water availability (Almeida et al., 2011).

The use of harvest index while indicating the efficiency of the photo assimilates conversion for the production of straw and grains, but cannot identify cultivars more efficient, because an expressive grain yield also requires an appropriate minimum of expression of leaves and stems (Silva et al., 2015). Oliveira et al. (2011), comment the importance of detecting the genetic differences among cultivars in the production of straw and grains, because, confer gains that qualify economic performance and the benefits of high biomass are directed to the soil. Emphasizes on the viability of the tillage system is directly dependent on the volume and quality of biomass on soil (Silva et al., 2006). Schaedler et al. (2009), studying the genetic variability of yield indicators of oat, observed harvest index between 0.33 and 0.45. The results found by these authors, conforms with the result obtained in this study, that the use of optimal dose of nitrogen directed to grain yield is between 0.34 to 0.42.

In corn/oat crop succession system, the Barbarasul cultivar evidenced grain yield and harvest index quadratic, and the behavior of the biological yield and linear straw (Table 4). This behavior of indicators of oat production occurred during favorable or unfavorable condition year of cultivation. Therefore, showing adjusted results obtained on theses parameters. In Brisasul cultivar, in corn/oat crop succession system (Table 4), the use of nitrogen in the expression of grain yield and harvest index showed a quadratic behavior, independent of the year. On the other hand, the biological yield and straw indicated quadratic adjustment of expression in the year 2011 (favorable crop), but linearity observed in the more restrictive year (2012). These results evidenced that crop succession system slower the release of N-residual, part of the nitrogen applied as fertilizer has been used in microbial action in straw, especially in the favorable year, provided that the best volume and distribution of rainfall is observed (Figure 1 and Table 2), giving better benefit to the biological activity.

Thus, greater action of microorganisms in straw degradation leads to greater competition in nitrogen use, hypothesis which can elucidate the stability of nutrient absorption oats obtained by this system. Although, it has not been quantified, visible differences in degradation time straw in a year indicated in 2012, still in the oat harvest period, was observe volume of corn straw on the soil cover. Moreover, in 2011 (favorable year), the intensified degradation process did not permit visualization of straw after oats harvest. In general, independent of the year, the quadratic behavior in the expression of grain yield and harvest index was similar among cultivars and years of study, whereas differences of the year in corn/oat crop succession system major instability of the biological yield and straw (Table 4).

This similar condition was also obtained in soybean/oat crop succession system by instability of expression biomass straw (Table 3). The favorable year (2011) in corn/oat cropping succession system showed in Barbarasul maximum nitrogen use for grain yield with 96 kg ha$^{-1}$, with similarity when compared to unfavorable year with maximum use to 93 kg ha$^{-1}$. Although, the simulations using of the optimal dose in this cultivar, evidence similar expression to the grain yield, and favorable year indicated higher nitrogen contribution in the expression of biological yield and straw in relation with harvest index.

**Table 4.** Regression equation and significance parameters to estimate the optimal dose of nitrogen in the simulation of the physiological characters in the production of corn/oat system.

| Variable | Mean square | Equation ($Y=b0\pm b1x\pm b2x^2$) | P ($b_i$) | $R^2$ | N/MTE (kg $ha^{-1}$) | $Y_E$ |
|---|---|---|---|---|---|---|
| | | | **Barbarasul 2011 (FY)** | | | |
| GY | 727753* | $1942 + 25.17x - 0.13x^2$ | * | 0.99 | 96 | 3160 |
| BY | 34905064* | $5627 + 33.28x$ | * | 0.99 | | 8822 |
| SY | 19259724* | $3444 + 24.72x$ | * | 0.97 | | 5817 |
| HI | 0.009118* | $0.35 + 0.002x - 0.000014x^2$ | * | 0.99 | | 0.41 |
| | | | **2012 (UY)** | | | |
| GY | 1817920* | $1178 + 39.06x - 0.21x^2$ | * | 0.99 | 93 | 2994 |
| BY | 34526115* | $5061 + 33.10x$ | * | 0.87 | | 8193 |
| SY | 12977790* | $3502 + 20.29x$ | * | 0.93 | | 5389 |
| HI | 0.135586* | $0.26 + 0.002x - 0.000016x^2$ | * | 0.98 | | 0.31 |
| | | | **Brisasul 2011 (FY)** | | | |
| GY | 1259308* | $2061 + 25.71x - 0.17x^2$ | * | 0.99 | 76 | 3033 |
| BY | 6027717* | $6635 + 62.45x - 0.38x^2$ | * | 0.97 | | 9186 |
| SY | 1778050* | $4573 + 36.74x - 0.20x^2$ | * | 0.95 | | 6210 |
| HI | 0.001755* | $0.30 + 0.00076x - 0.000006x^2$ | * | 0.88 | | 0.32 |
| | | | **2012 (UY)** | | | |
| GY | 1758868* | $1560 + 38.42x - 0.20x^2$ | * | 0.95 | 96 | 3405 |
| BY | 35868825* | $4357 + 33.74x$ | * | 0.88 | | 7759 |
| SY | 14087486* | $2423 + 21.14x$ | * | 0.89 | | 4452 |
| HI | 0.020574* | $0.38 + 0.002x - 0.00002x^2$ | * | 0.96 | | 0.39 |

$Y_E$ = Expected value from the ideal dose of N on grain yield; P($b_i$) = Slope parameter of the trend line; * = Significance of the slope parameter to 5% of probability; $R^2$ = Coefficient of determination; N/MTE = Nitrogen dose for maximum technical efficiency of grain yield; GY = grain yield; BY = biological yield; SY = Straw yield; HI = Harvest index; FY = Favorable year; UY = Unfavorable year.

Therefore, different from what occurred in the soybean/oat cropping succession system, the release condition of the N-residual by corn showed higher harvest index in the favorable year than in the more restrictive condition. This happened because although the values of the total biomass and straw have been incremented, there was also an increase in grain yield when using optimal dose. In the analysis, the Brisasul cultivar slow release of the N-residual biosystem (Table 4) favoring the growth conditions in 2011 showed dose adjusted to maximum grain yield with 76 kg $ha^{-1}$. There is reduction in fertilizer in comparison with the Barbarasul cultivar in the same crop year.

In 2012, there was greater restriction on oat cultivation, although nitrogen consumption show similarity between the two cultivars, grain yield was maximized by Brisasul cultivar, confirming its potential as an ecologically being a more sustainable genotype by higher grain yield by less use of fertilizer. The simulation parameters of production in Brisasul cultivar using the optimal dose in grain yield showed in 2011 high biological yield and straw, including, results obtained in the same year of Barbarasul cultivar.

Similar behavior was also obtained in these variables both in condition of high release of N-residual (soybean/oat) and cultivars (Table 3). The biochemical composition of plant residues affect the nitrogen release rate contained in the straw and the use of N-fertilizer by the plants due to competition with soil microorganisms (Siqueira et al., 2010; Nascimento et al., 2012). Moreover, water deficiency hinders the process involved in the use of fertilizer and residual nitrogen, showing that nitrogen to grain yield and biomass can be considerably increased with adequate moisture in the soil (Cazetta et al., 2008).

In wheat, the best results of nitrogen fertilization were obtained with nitrogen doses ranging from 70 to 120 kg $ha^{-1}$ (Espindula et al., 2010; Teixeira Filho et al., 2010). Kolchinski and Schuch (2003), found in oat grain yield nitrogen of 75 kg $ha^{-1}$. Mantai et al. (2015), studying the nitrogen use efficiency in oat showed a linear behavior on biomass production rate with the increment of fertilizer, condition not always accompanied by the highest grain yield by evidence quadratic behavior. It is noteworthy that oats biomass and grain yields tend to be favored by crop succession system of smaller C/N ratio, generating cost savings and less environmental pollution by nitrogen fertilizers (Silva et al., 2006).

Studies realized by Silva et al. (2015), showed that the use of the analysis of the yield indicators was decisive in the most sustainable management system suggesting changes in population density in oat.

## Conclusions

The use of N-fertilizer in oats presented quadratic response of grain yield and harvest index, however, linear behavior in the biological yield and straw in soybean/oat and corn/oats crop succession systems were obtained. Changes of the response were caused by the biological yield, straw and also by year of favorable or unfavorable cultivation. The simulation of the optimal dose of nitrogen for grain yield confirmed higher efficiency of Brisasul cultivar in the expression of yield indicators.

## Conflict of Interest

The authors have not declared any conflict of interest.

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*Full Length Research Paper*

# The rhizosphere effect of some wheat cultivars on inorganic phosphorus fractions in a phosphorus-deficient calcareous soil

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**Knowledge of the distribution and changes of soil phosphorus fractions in the rhizosphere, helps to determine efficiency of different crop cultivars in phosphorus acquisition. This study was conducted in order to evaluate the rhizosphere effect of some wheat cultivars on inorganic phosphorus fractions. A greenhouse experiment as a factorial in completely randomized design was conducted with 10 treatments and 3 replications. Experimental factors were different plant cultivars (4 wheat cultivars and control) and soil-sampling zone (rhizosphere and non-rhizosphere). A rhizobag technique was used to separate rooted and non-rooted inside each pot representing the two soil sampling zones. Based on fractionation results, the order of inorganic phosphorus fractions in the studied soil were apatite-P> OCP-P> DCP-P> Al-P> Fe-P> O-P which were found to be in the order of 830, 123, 17, 16, 14 and 0 g kg$^{-1}$, respectively. The concentration of the most inorganic phosphorus fractions between rhizosphere and non-rhizosphere soil was significantly different. All plant cultivars decreased inorganic phosphorus forms significantly. This difference was not equal for all cultivars and all fractions. Organic P was significantly higher in the rhizosphere soil compared to non-rhizosphere soil. Soluble phosphorus was not significantly differed between rhizosphere and non-rhizosphere soil. Root induced chemical changes; root morphology, microbial populations, pH changes etc. can be determining factors on phosphorus depletion differences among plant cultivars and soil sampling zone.**

**Key words:** Phosphorus, fractionation, rhizosphere, rhizobag, wheat.

## INTRODUCTION

Phosphorus (P) is one of the most important essential macronutrients for plant growth in agricultural systems (Raghothama, 1999; Zhao et al., 2007). It plays a primary role in many of the physiological processes such as energy metabolism and biosynthesis of nucleic acids in plants and functions in very important process like photosynthesis, respiration, and regulation of a number of enzymes (Vance et al., 2003; Caldecott, 2009).

Due to limited sources of P in the world and regarding to P character as a low mobile and low available element in soil, P is a major limiting factor of plant growth in cropping systems (Hinsinger, 2001; Lambers et al.,

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2006). Owing to this restricted movement, the utilization efficiency of P fertilizer by plant is very low and its recovery ranges from 15 to 25% (Nisar, 1985). A part of applied P fertilizer in soil, goes to soil solution and taken up by plants, while the rest either precipitates or is adsorbed by exchange sites in the soil (Hinsinger, 2001). The most amount of P reaches to plant roots by diffusion (Rengel, 1993; Jaillard et al., 2000) and P diffusion coefficient ($D_e$) is very low ($10^{-12}$ to $10^{-15}$ $m^2$ $s^{-1}$ otherwise its fixation rate in soil is high (Morgan, 1997; Sylvia et al., 2005). In fact, not only P can be dominantly adsorbed by Al/Fe oxides and hydroxides in acid soils, but also can be adsorbed on the surface of Ca carbonate and clay minerals in neutral-to-calcareous soils (Lindsay et al., 1989; Parfitt, 1989). Concentration gradient and diffusivity of P in the soil near the roots determines P absorption by plants. One of the most important factors in generating this concentration gradient is root-soil interaction in the rhizosphere (Marschner, 1995; Hinsinger, 2001). Chemical and biological changes in the rhizosphere induced by roots, is a determining factor in bioavailability of soil P and an important key for optimizing P management to improve P-use efficiency in crop production. Therefore, better perspective of P dynamic in the soil-rhizosphere-plant complex continuum is necessary for achieving this purpose (Shen et al., 2011).

Sequential fractionations methods can help to access some information about the bounded forms of soil inorganic P (BaRančíková et al., 2007). It is an attempt to separate P pools according to their lability (Abekoe, 1996). It can be used to study the effect of management system on soil P (Negassa and Leinweber, 2009). Single or combined chemical reagents are used to extract particular P fractions. There are not many studies that relate these fractions directly to their bioavailability (Brezonik et al., 2000).

Soil properties, source and amount of applied P fertilizer, plant species, crop rotation etc. are some factors that affect or redistribute all P fractions in soil. Possible difference among species or cultivars in P uptake and recognizing plants potential to change soil inorganic P and its acquisition will expectantly be effective on reducing cost of P fertilization and will increase applied fertilizer recovery as well as plant productivity with using more P efficient plants (Gahonia et al., 1999).

Distribution of inorganic and organic P fractions in two soils as affected by crop species (soybean, white lupin, and maize) and nitrogen applications was investigated by Qiao (2012) and was reported that the percentage of total P present as inorganic P was affected by crop species, soil type, and N source but the moderately labile organic P was not affected significantly. This investigator mentioned that correct choice of crop species and the application of a suitable N source may increase crop yield and P uptake by plant in P-deficient soils. Changes in P fractions in the rhizosphere of some crop species under glasshouse conditions was investigated by Safari Sinegani and Rashidi (2011). These authors reported a decrease in all inorganic P forms by all plant species in rhizosphere compared to non-rhizosphere soil. Similarly, it was reported that inorganic P forms decreased in the rhizosphere of three tea cultivars, while increasing in microbial activity and transformation of inorganic dissolved P to organic phosphorus caused an increase at the amount of organic P (Zoysa et al., 1999).

Mineral nutrition of plants, specially P and micronutrients as low mobile elements in soils, can be affected by rhizosphere processes through modifying nutrient solubility in soil close (1-2 mm) to absorbing roots (Gahoonia and Nielsen, 1991).

Study of rhizosphere soil mainly is technically difficult due to the thin soil layer directly influenced by roots and the wide root distribution (Hylander, 2002). Based on the aims and facilities provided, different methods (gentle shaking and using a soft brush) and techniques (different types of devices such as rhizobox and rhizobag), had been proposed for studying chemical changes in the rhizosphere (Riley and barber, 1969; Cappy and Brown 1980; Kuchenbuch and Jungk, 1982; Youssef and Chino 1988; Gahoonia and Nielsen 1991; Wenzel et al., 2001).

The aim of study, type of plants investigated and whether they are cultivated in containers or in the field are some determining factors to choose the procedure followed to obtain rhizosphere and bulk soils (Corti et al., 2005). Restricting the root growth to a certain soil volume will create a higher root density and facilitate sampling of rhizosphere soil. However, each method has its own advantages and disadvantages and the differences and precision of each method for sampling the rhizosphere soil need to be studied more.

Understanding the quantity and significance of different soil P forms is important for proper soil fertility management especially for calcareous soils. Making long term environmentally sound agricultural decisions requires knowledge of the availability of soil P forms for plant species and cultivars. The objective of this study was to investigate the status of different inorganic P forms in the rhizosphere. Soil inorganic P around the roots of four wheat cultivars, as rhizosphere soil, were compared to P inorganic forms in non-rhizosphere soil using rhizobag technique.

## MATERIALS AND METHODS

### Soil sampling

A surface soil (0-30 cm) with low available P (<5 mg $kg^{-1}$) was sampled from the agricultural research station fields (36° 15´ 17´´ N, 49° 54´ 28´´ E) in Qazvin province (Northern Iran) with annual rainfall 310 to 320 mm and annual average temperature 9°C. The soil used was a Coarse loamy, mixed, thermic, Typic Xerofluvents. Illite, Colorite, Smectite and fewer amounts of Kaolinite and Quartz were the dominant clay minerals in the site.

### *Soil physical and chemical analyses*

The soil sample was air-dried and ground to pass through a 2 mm sieve for laboratory experiments. Selected soil properties were determined according to standard methods (Sparks, 1996). Soil particles contents (sand, silt and clay) were separated using hydrometer method; Equivalent calcium carbonate (ECC) was measured by back-titration procedure. Soil pH and electrical conductivity (EC) was measured in a saturated paste and saturated extract, respectively. Organic carbon (OC) was analyzed by dichromate oxidation and titration with ferrous ammonium sulfate. Total nitrogen (N) was determined by the Kjeldahl method. Available Potassium (K) was extracted from the soil by ammonium acetate 1 normal pH=7 and measured by flame photometer.

Available P (Olsen-P) was extracted using 0.5 M sodium bicarbonate solution at pH=8.5 and determined using spectrophotometer as blue molybdate-phosphate complexes under partial reduction with ascorbic acid (Jackson, 1958). Soluble P in water, total P by perchloric acid ($HClO_4$) digestion and organic P according to Kou (1996) were also determined according to standard methods.

Method described by Jiang and Gu (1989) was used to determine different forms of soil inorganic P. Sequentially soil di-Ca-phosphates (DCP-P) in 0.25 M $NaHCO_3$ (pH=7.5), octa-Ca-phosphates (OCP-P) in $NH_4OAC$ 0.5 M (pH=4.2), Al phosphates (Al-P) in 0.5 M $NH_4F$ (pH=8.2), Fe phosphates (Fe-P) in 0.1 N NaOH-0.1 N $Na_2CO_3$, occluded P (O-P) in 0.3 M $Na_3$-Cit-$Na_2S_2O_4$-NaOH, and P as apatite (apatite-P) in 0.5 N $H_2SO_4$ were extracted and determined with spectrophotometer.

### *Plant culture and rhizosphere-study technique*

Plastic pots with 15 cm height and 10 cm opening mouth diameter were selected. Each pot was filled with approximately 1.7 kg of air-dried soil passed through a 4 mm sieve. The soil moisture was held constant at field capacity by weighting pots and adding appropriate volumes of di-ionized water. The soil was amended with basal nutrients such as 100 mg $kg^{-1}$ N (as Urea) in two splits, 50 mg $kg^{-1}$ K (as $K_2SO_4$ ), 30 mg $kg^{-1}$ Mg (as $MgSO_4$, $7H_2O$), 5 mg $kg^{-1}$ Fe (as sequestrene-138) and 0.5 mg $kg^{-1}$ B (as $H_3BO_3$).

A rhizobag technique was used for separating rhizosphere soil. In this technique, certain content of the prepared soil was filled into a nylon bag with a certain mesh size, restricting growth of roots from the outer compartment. Then, these rhizobags were placed in the center of plastic buckets and surrounded by the soil so that soil surfaces inside and outside the bags were at equal levels (McGrath, 1997; Silva Gonzaga et al., 2006). In this study, a cylindrical rhizobags (12×6 cm) was made of nylon with a mesh size of 60 µm and used to sample rhizosphere and non-rhizosphere soil. The content of soil inside of each rhizobag was about 380 g.

In each pot five seeds were sown in the rhizobag. Four days after germination seedling were reduced uniformly to three per pot. Four cultivars of *Triticum aestivum* (Azadi, Yavarus, Karaj 1 and Marvdasht) were grown in a controlled greenhouse conditions and were grown in total for 6 weeks. The control treatment was unplanted soil.

At harvest (after six weeks), plant roots were separated carefully and soil material inside and outside the rhizobag was taken as rhizosphere and non-rhizosphere soil, respectively. Soluble and Olsen P, DCP-P, OCP-P, Al-P, Fe-P, apatite-P, and total P were measured in the rhizosphere and non-rhizosphere soils. The rhizosphere and non-rhizosphere soil pH were measured in a 1:5 soil to water extract after shaking 30 min (Hesse, 1971). Moreover, two series of pots with and without P fertilizer application were prepared. Six weeks After planting of four above mentioned wheat cultivars, some root morphological properties (total root length, root surface area, root volume, wet and dry weight of roots) were measured and shoot to root ratio was calculated (Newman, 1966; Bohm, 1979). Furthermore, shoot P concentration (PC) was assayed using the vanadomolybdate method (Westerman, 1990) and shoot total P uptake (TP), P acquisition efficiency (PAE) and P efficiency (PE) was calculated using following formula.

$$TP = PC \times SDW$$
$$PAE = [TP \text{ in } P_0 / TP \text{ in } P_{20}] \times 100$$
$$PE = [SDW \text{ in } P_0 / SDW \text{ in } P_{20}] \times 100$$

### Statistical analyses

The experiment was considered a completely randomized design as factorial in 3 replicates. In fractionation experiment, the factors were plant cultivars (4 wheat cultivars and control) and soil sampling zone (soil inside and outside the rhizobag as rhizosphere and non-rhizosphere soil). In experiment related to root morphological properties and plant efficiency indexes, the factors were plant cultivars (4 wheat cultivars) and P fertilizer application (no fertilizer and 20 mg $kg^{-1}$ P fertilizer considered as efficient amount). Data were statistically analyzed by SAS software. Duncan's new multiple range tests were performed to assess the effect of plant cultivars and sampling zone on soluble and Olsen P, DCP-P, OCP-P, Al-P, Fe-P, apatite-P, and total P.

## RESULTS AND DISCUSSION

### Soil properties

Table 1 shows some physical and chemical characteristics of soil used in the experiment. According to soil fractionation results determined by Jiang and Gu (1989) method, the contents of different P forms in the studied soil were 15.53 mg $kg^{-1}$ for DCP-P, 114.5 mg $kg^{-1}$ for OCP-P, 14.67 mg $kg^{-1}$ for Al-P, 13.02 mg $kg^{-1}$ for Fe-P, 770.1 for P as apatite (apatite-P), and 0 mg $kg^{-1}$ for O-P. The amount of total P, soluble P and organic P in studied soil were 1091.9 and 2.78, 123.5 mg $kg^{-1}$, respectively.

As mentioned previously, apatite-P (83%) and OCP-P (12.3 %) were the predominant soil inorganic P forms in this soil. The amount of O-P fraction in the studied soil was negligible. With respect to the sampling location, this result is in accordance with other studies (Mostashari et al., 2008; Safari Sinegani and Rashidi, 2011). The percentages of P fractions in the planted and control soils from Hydare fields in Hamadan province (NW Iran) studied by Safari Sinegani and Rashidi (2011) were near 640 g $kg^{-1}$ apatite-P, 240 g $kg^{-1}$ OCP-P, 70 g $kg^{-1}$ Fe-P, 40 g $kg^{-1}$ DCP-P, 10 g $kg^{-1}$ Al-P, and 0 g $kg^{-1}$ O-P. Mostashari et al. (2008) examined the amount of different P forms, that is, DCP-P, OCP-P, Al-P, Fe-P, O-P, and P as apatite in some calcareous soils of Qazvin province and reported to be in the range of: 1.6-42.3, 72-314, 14.5-54.8, 8.4-34.8, 5.9-33.4 and 262-697 mg $kg^{-1}$, respectively.

### *The effects of plant cultivars on soil inorganic P fractions*

The effect of plant cultivars on soil soluble P, total P,

**Table 1.** Some physical and chemical properties of used soil

| ECC | OC | EC | pH in saturated mud | FC | SP | Soil | Clay | Silt | Sand |
|---|---|---|---|---|---|---|---|---|---|
| (g kg$^{-1}$) | | (ds m$^{-1}$) | | (%) | | texture | | (%) | |
| 70 | 4.7 | 1.489 | 7.45 | 17 | 34 | Sandy loam | 19 | 24 | 57 |
| **Cu** | **Mn** | **Zn** | **Fe** | **Mg** | **Ca** | **K** | **P** | **Total N** | |
| | | **(mg kg$^{-1}$)** | | **(mg l$^{-1}$)** | | **(mg kg$^{-1}$)** | | **(g kg$^{-1}$)** | |
| 1.1 | 10.4 | 0.76 | 2.07 | 16.92 | 229.2 | 298.8 | 4.65 | 0.5 | |

**Table 2.** Analysis of variance of the effect of plant cultivars (PC) and soil-sampling zone (SSZ) on inorganic P fractions, Olsen-P, soluble P and total P after plant harvest.

| | DF | Olsen-P | Soluble P | DCP-P | OCP-P | Al-P | Fe-P | Apatite-P | Total P |
|---|---|---|---|---|---|---|---|---|---|
| | | MS | | | | | | | |
| SSZ | 1 | 0.9575** | 0.0110$^{ns}$ | 25.2397** | 3799.7893** | 0.6714$^{ns}$ | 37.3572** | 1578.9071$^{*}$ | 4483.0** |
| PC | 4 | 5.6162** | 0.1988** | 110.8305** | 1105.4501** | 27.6873** | 29.7321** | 4731.0161** | 4544.0** |
| SSZ*PC | 4 | 0.1933$^{ns}$ | 0.0002$^{ns}$ | 3.5249** | 309.5875** | 0.0445$^{ns}$ | 2.4054$^{ns}$ | 402.8216$^{ns}$ | 2232.7** |
| Error | 20 | 0.0304 | 0.0069 | 0.7633 | 22.2904 | 0.5322 | 0.9825 | 217.7186 | 72.398 |

*, Mean square (MS) of the treatment is significant at the 0.05 level; **, significant at the 0.01 level; ns, not significant.

**Table 3.** Soil inorganic P fractions, Olsen P, soluble P and total P as affected by plant cultivars (n=6).

| | Olsen-P | Soluble P | DCP-P | OCP-P | Al-P | Fe-P | Apatite-P | Total P |
|---|---|---|---|---|---|---|---|---|
| | (mg kg$^{-1}$) | | | | | | | |
| Azadi | 1.15$^{d}$ | 1.43$^{b}$ | 4.98$^{d}$ | 79.30$^{d}$ | 8.58$^{c}$ | 7.13$^{d}$ | 694.60$^{d}$ | 1018.0$^{c}$ |
| Yavarus | 1.50$^{c}$ | 1.73$^{a}$ | 7.59$^{c}$ | 90.61$^{c}$ | 12.69$^{a}$ | 8.62$^{c}$ | 723.52$^{bc}$ | 1073.1$^{b}$ |
| Karaj 1 | 1.43$^{c}$ | 1.46$^{b}$ | 6.54 | 83.77$^{d}$ | 10.34$^{b}$ | 8.22$^{cd}$ | 711.77$^{cd}$ | 1071.0$^{b}$ |
| Marvdasht | 2.05$^{b}$ | 1.76$^{a}$ | 12.51$^{b}$ | 97.41$^{b}$ | 13.25$^{a}$ | 10.51$^{b}$ | 732.63$^{b}$ | 1068.2$^{b}$ |
| Control | 3.57$^{a}$ | 1.83$^{a}$ | 15.17$^{a}$ | 113.95$^{a}$ | 13.57$^{a}$ | 12.78$^{a}$ | 769.78$^{a}$ | 1091.6$^{a}$ |

Means followed by the same letter in each column are not significantly different ($p<0.05$).

Olsen-P and soil inorganic P fractions (Table 2) revealed that the effect of plant cultivars on soluble P, total P, Olsen-P, DCP-P, OCP-P, Al-P, Fe-P, and apatite-P was significant ($p<0.01$).

The results of Duncan's new multiple range tests of means of soil soluble P, total P, Olsen-P and soil inorganic P fractions as affected by plant cultivars has been shown in Table 3. Unplanted soil (control) had the highest amount of soluble P, Olsen-P, total P, and different soil inorganic P fractions, that is, DCP-P, OCP-P, Al-P, Fe-P, and P as apatite. Soil soluble P, total P, Olsen-P and soil inorganic P fractions were decreased significantly by plants ($p<0.05$). Although, the decrease of these P forms in the rhizosphere soil was not equal for each fraction.

Soil planted with Azadi and Marvdasht cultivars had the lowest and the highest amount of mentioned P forms, respectively (Table 3). Azadi as a P-efficient cultivar had higher ability to decrease different soil P forms. For example, soil P as apatite decreased from 769.78 mg kg$^{-1}$ in unplanted soil to 694.60 mg kg$^{-1}$ in soil planted with Azadi cultivar (Table 3).

However, all plant cultivars decreased soluble P and Al-P, but the amounts of these fractions in soil planted with Yavarus and Marvdasht cultivars were not significantly ($p<0.05$) different from that in control soil.

Apatite-P had the most considerable decrease (from 769.78 mg kg$^{-1}$ in unplanted soil to about 673.36 mg kg$^{-1}$ in Azadi cultivar) in the rhizosphere soil in comparison to DCP-P, OCP-P, Al-P and Fe-P (Table 7). The ability of Azadi cultivar to take up P from different forms through redistribution was the highest among the tested cultivars.

Reversely, the lowest amount (120.73 mg kg$^{-1}$) of organic P was observed in unplanted soil (control) and all wheat cultivars increased organic P, significantly ($p<0.05$). Azadi cultivar had the highest amount (139.31 mg kg$^{-1}$) of organic P and mean of organic P in Yavarus, Karaj 1 and Marvdasht cultivars were 138.77, 136.48 and

**Table 4.** Root morphological properties as affected by plant cultivars (n=6) and phosphorus fertilizer levels (n=12).

| | Root wet weight (g) | Root dry weight (g) | Shoot dry weight (g) | Shoot to root ratio | Total root length (cm) | Root surface area (cm$^2$) | Root volume (cc) |
|---|---|---|---|---|---|---|---|
| Azadi | $2.269^{a}$ | $0.313^{a}$ | $0.727^{a}$ | $2.319^{b}$ | $278.42^{a}$ | $90.17^{a}$ | $2.333^{a}$ |
| Yavarus | $1.784^{b}$ | $0.252^{b}$ | $0.695^{a}$ | $2.770^{a}$ | $223.84^{b}$ | $75.92^{b}$ | $2.050^{b}$ |
| Karaj 1 | $1.967^{b}$ | $0.260^{b}$ | $0.611^{b}$ | $2.313^{b}$ | $231.55^{b}$ | $79.39^{b}$ | $2.167^{ab}$ |
| Marvdasht | $1.283^{c}$ | $0.201^{c}$ | $0.588^{b}$ | $3.041^{a}$ | $179.04^{c}$ | $56.06^{c}$ | $1.417^{c}$ |
| No P fertilizer | $1.311^{b}$ | $0.182^{b}$ | $0.447^{b}$ | $2.507^{a}$ | $161.54^{b}$ | $54.39^{b}$ | $1.467^{b}$ |
| Optimum level of P fertilizer | $2.340^{a}$ | $0.331^{a}$ | $0.864^{a}$ | $2.714^{a}$ | $294.89^{a}$ | $96.38^{a}$ | $2.517^{a}$ |

Means followed by the same letter in each column are not significantly different ($p<0.05$).

**Table 5.** Efficiency indexes as affected by plant cultivars (n=6) and phosphorus fertilizer levels (n=12).

| | Shoot P concentration (mg/g) | Shoot total P uptake (mg/pot) | Phosphorus efficiency (%) | Phosphorus acquisition efficiency (%) |
|---|---|---|---|---|
| Azadi | $3.294^{a}$ | $2.572^{a}$ | $57.32^{a}$ | $32.90^{a}$ |
| Yavarus | $2.608^{c}$ | $1.946^{b}$ | $56.51^{a}$ | $32.75^{a}$ |
| Karaj 1 | $2.746^{b}$ | $1.822^{c}$ | $47.48^{ab}$ | $29.10^{ab}$ |
| Marvdasht | 2.046d | 1.348d | $44.60^{b}$ | $23.44^{b}$ |
| No P fertilizer | $1.953^{b}$ | $0.892^{b}$ | - | - |
| Optimum level of P fertilizer | $3.394^{a}$ | $2.951^{a}$ | - | - |

Means followed by the same letter in each column are not significantly different ($p<0.05$).

131.21 mg kg$^{-1}$, respectively.

Assessing root morphological properties (total root length, root surface area, root volume, wet and dry weight of roots) of studied cultivars with and without P fertilizer application, revealed stronger and more extensive root system in Azadi cultivar compared to the others (Table 4). Also, this cultivar had higher dry weight of shoots, shoot to root ratio, shoot P concentration and shoot total P uptake as well as phosphorus acquisition efficiency and phosphorus efficiency (Tables 4 and 5).

Soil pH values in the rhizosphere of all four studied wheat cultivars were significantly ($p<0.05$) lower than those in the corresponding non-rhizosphere soil (Figure 1). The decrease of soil pH in the rhizosphere of studied wheat cultivars was between 0.12 and 0.38. In particular, decrease of soil pH in the rhizosphere of Azadi cultivar (0.38 units) was sharper than those in the rhizosphere of the other three cultivars. The decrease of soil pH in the rhizosphere of Yavarus and Karaj 1 cultivar were about 0.25 and 0.15 units respectively. Finally, Marvdasht cultivar had the least effect on soil pH (0.12 units). Mean of soil pH in the rhizosphere and non-rhizosphere soil were about 8.19 and 8.37, respectively.

Based on Jiang et al. (2010) investigation, plant species differ in their ability to take up P from the soil and these differences were attributed to the morphology and physiology of plants. Adventitious root angle, root surface area and root volume were defined as some indexes for low-P tolerant genotypes during the seedling stage.

Root secretions and associated soil microorganisms can directly affect the narrow region of soil which is defined as rhizosphere. The biological, chemical and physical activities in this region are different from bulk soil (Ma et al., 2009). The effect of root-associated factors such as ability to modify the rhizosphere using different mechanisms (root exudation of amino acids, organic acids, proton, acid phosphatase etc.), root morphology and architecture, root-hair density on inorganic P acquisition has been reported by many researchers (Hinsinger, 2001; Vance et al., 2003; Lambers et al., 2006; Richardson et al., 2009; Zhang et al., 2010; Shen et al., 2011). The results of investigation about the status of inorganic P fractions in the rhizosphere of some xerophytic shrubs in the Alxa desert showed soil inorganic P fractions were significantly affected by root activities. A decrease in soil pH in the rhizosphere ranges from 0.4-0.8 units has been reported in this investigation. These shrubs had different ability in acquiring P based on their ability to acidify the rhizosphere soil (Ma et al., 2009).

### *The effects of sampling zone on inorganic P fractions*

The effect of soil sampling zone on soil soluble P, total P, Olsen-P and soil inorganic P fractions (Table 2) revealed

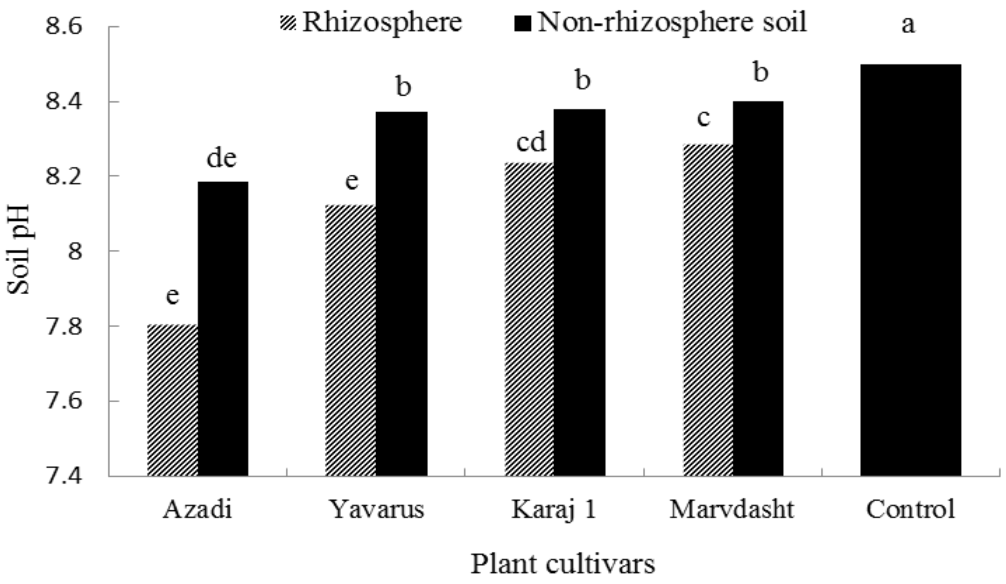

**Figure 1.** Soil pH in the rhizosphere and non- rhizosphere soil of different wheat cultivars. Means followed by the same letter are not significantly different (p<0.05).

**Table 6.** Soil inorganic P fractions, Olsen-P, soluble P and total P as affected by soil-sampling zone (n=15).

| Sample zone | Olsen-P | Soluble P | DCP-P | OCP-P | Al-P | Fe-P | Apatite-P | Total P |
|---|---|---|---|---|---|---|---|---|
| | | | | (mg kg$^{-1}$) | | | | |
| Rhizosphere soil | 1.76$^{b}$ | 1.62$^{a}$ | 8.44$^{b}$ | 81.75$^{b}$ | 11.54$^{a}$ | 8.34$^{b}$ | 719.20$^{b}$ | 1052.2$^{b}$ |
| Non-rhizosphere soil | 2.12$^{a}$ | 1.66$^{a}$ | 10.28$^{a}$ | 104.26$^{a}$ | 11.84$^{a}$ | 10.57$^{a}$ | 733.71$^{a}$ | 1076.61$^{a}$ |

Means followed by the same letter in each column are not significantly different (p<0.05).

**Table 7.** The amounts of different soil P fractions in the rhizosphere and non-rhizosphere soil of studied cultivars.

| | DCP-P | | OCP-P | | Al-P | | Fe-P | | Apatite-P | | Organic P | | Total P | | Available P | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | (mg kg$^{-1}$) | | | | | | | | |
| | R | B | R | B | R | B | R | B | R | B | R | B | R | B | R | B |
| Azadi | 4.6 | 5.3 | 70 | 88 | 8.4 | 8.8 | 5.7 | 8.6 | 673 | 716 | 144 | 135 | 972 | 1064 | 1.08 | 1.22 |
| Yavarus | 6.7 | 8.5 | 72 | 109 | 12.5 | 12.9 | 7.2 | 10.0 | 720 | 727 | 143 | 135 | 1065 | 1081 | 1.04 | 2.00 |
| Karaj 1 | 5.1 | 8.0 | 71 | 97 | 10.2 | 10.5 | 6.7 | 9.7 | 705 | 718 | 138 | 135 | 1065 | 1077 | 1.29 | 1.57 |
| Marvdasht | 10.6 | 14.4 | 82 | 113 | 13.0 | 13.5 | 9.3 | 11.7 | 727 | 738 | 133 | 129 | 1067 | 1069 | 2.84 | 3.27 |
| Control | 15.2 | | 114 | | 13.6 | | 12.8 | | 770 | | 121 | | 1092 | | 3.57 | |

R and B show rhizosphere and non-rhizosphere soil, respectively.

that the effect of soil sampling zone on Olsen-P, total P, DCP-P, OCP-P, and Fe-P was significant (p<0.01). In the case of apatite-P this effect was significant at the 0.05 level and there was no significant effect in the case of soluble P and Al-P.

The concentrations of total P and Olsen-P were significantly (p<0.05) lower in the rhizosphere than those in corresponding non-rhizosphere soils. There was no significant difference in the case of soluble P between rhizosphere and non-rhizosphere soil (Table 6).

Ca-P fractions (apatite-P and OCP-P) were the highest proportions in the rhizosphere. Compared to Fe-P, the content of Al-P was higher in the rhizosphere soil, as well. The concentrations of all soil inorganic P fractions, except Al-P, varied significantly (p<0.05) between rhizosphere and non-rhizosphere soils (Table 6). The results of mean comparison have shown that, although the amounts of soluble P and Al-P in different sampling zones were not significantly different, same as the other inorganic P forms, these forms of soil P were lower in the rhizosphere soil compared to non-rhizosphere soil. This indicates the considerable influence of the rhizosphere of plant cultivars on different inorganic P forms.

Orders of inorganic P fractions in the non-rhizosphere soil were apatite-P> OCP-P> Al-P> Fe-P> DCP-P> O-P with 842.7, 119.8, 13.6, 12.1, 11.8and 0 g kg$^{-1}$, respectively and orders of inorganic P fractions in the rhizosphere soil were apatite-P> OCP-P> Al-P> DCP-P> Fe-P> O-P which was found to be in the order of 867.3, 98.6, 13.9, 10.2, 10.0 and 0 g kg$^{-1}$, respectively.

Mean of organic P in the rhizosphere soil was higher compared to non-rhizosphere soil. Mean of this fraction in the rhizosphere and non-rhizosphere soil were 136 and 131 mg kg$^{-1}$, respectively. The decrease of Olsen-P in the rhizosphere which is in accordance with the results of other researchers (Jianguo and Shuman, 1991; Hanafi and Ng, 1996; Shen et al., 2004) showed Olsen-P depletion in the rhizosphere and it is related to absorption of Olsen-P by plants. In general, the mean of Olsen-P in the rhizosphere and non-rhizosphere soils were 1.8 and 2.1 mg kg$^{-1}$, respectively (Table 6). Total plant available P in soil can be considered as the remained available P in soil after plant harvest plus absorbed P by plants (roots and aerial bodies). Bagayoko et al. (2000) cultivated some cereals and legumes species in severely P-deficient acid sandy soils to measure changes in nutrient availability as affected by distance from the root surface and reported that available P in the rhizoplane was between 190 and 270% higher compared to the non-rhizosphere soil.

The decrease of acid extractable P (apatite-P) in the rhizosphere can be related to root induced chemical changes and microbial activities that contribute to transformation of this form of P to moderately or highly soluble and available P forms such as DCP-P and OCP-P. Najafi and Towfighi (2006) investigated the effects of rhizosphere of rice plant on the inorganic P fractions in the paddy soils and reported the decrease of apatite in treatments without P fertilizer application in most studied soils.

Soil pH decrease in the rhizosphere that has been reported by other researchers (Neumann and Römheld, 1999; Wang et al., 2006; Ma et al., 2009) can solve Ca phosphates such as DCP-P and OCP-P. The decrease of recent P forms in the rhizosphere shows that these P forms can be available as inorganic P forms for wheat. Based on Zhang et al. (2004) reports, reduction rate of DCP-P was the highest among all fractions while soil pH was decreased. Ma et al. (2009) also reported the decrease of DCP-P in the rhizosphere of some studied shrubs.

The decrease in Fe-P in the rhizosphere which is in accordance with the result of Safari Sinegani and Rashidi (2011) revealed that despite low content of Fe-P in calcareous soils, plant species can also use these forms of soil P. P and Fe uptake by plants decreases their concentration in the rhizosphere which follows by Fe-P release in solution, then recent P is absorbed by plant roots.

No significant difference in soluble P between rhizosphere and nonrhizosphere soil has been reported by Najafi and Towfighi (2006) and Safari Sinegani and Rashidi (2011). It seems there is a dynamic relationship among different P forms that buffer soluble P in the rhizosphere and compensate the soluble P uptake by plants. Otherwise soluble P, which is very low in the soil solution, cannot cover plants P requirements.

Generally has been reported that pH changes in the rhizosphere has a strong effect on bioavailability of soil P dynamics. Also, because of the possible effect of organic compounds on the rate and forms of inorganic P in calcareous soils, the effects of organic matter in the rhizosphere should be taken into account (Hinsinger, 2001). Moreover, microbial populations, phosphatase activity, root exudations and root morphology (root density, root surface area, root-hair length and density) and root-soil interactions in the rhizosphere can be determining factors on P depletion differences among plant species and different cultivars (Lambers et al., 2006; Richardson, 2009). As mentioned before, more root density was observed in Azadi cultivar in this study. This means more soil accessibility by the roots of this cultivar and more P efficiency.

## Conclusion

Changes in different P forms in the rhizosphere of different wheat cultivars were observed in this study compared to the non-rhizosphere soil. However, these changes were not equal for all cultivars and all forms. Generally, Azadi cultivar with greater root development and higher phosphorus uptake depleted more inorganic P from the rhizosphere compared to other cultivars. Karaj 1 and Yavarus were the next cultivars which had the most effect on redistribution of inorganic P forms in the rhizosphere and finally, Marvdasht cultivar had the lowest effect on inorganic P forms in the rhizosphere. The changes in different P forms in the rhizosphere of studied cultivars might be a response of the cultivar differences in respect to root morphology and soil accessibility. Apatite-P and OCP-P had the highest proportion in both rhizosphere and non-rhizosphere soil. Compared to non-rhizosphere soil, most inorganic P fractions decreased significantly in the rhizosphere soil. Understanding P dynamics in the soil-rhizosphere-plant complex continuum can be useful in better management of P nutrition to save limited P resources and to maintain the quality of our environment. The right selection of the species or cultivars can also improve crop production and the uptake of P in P-deficient soils.

## Conflict of Interest

The authors have not declared any conflict of interest.

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# Physiological attributes, growth and expression of vigor in soybean seeds under soil waterlogging

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**Soil waterlogging in lowland areas is detrimental to many rainfed crops in all regions of the world, affecting the growth and development of plants. Thus, this study aimed to evaluate the growth, assimilate partitioning and expression of vigor in the soybean cultivar BMX Potência when subjected to waterlogging. The treatments consisted of 2- and 4-day periods of waterlogging during the vegetative growth stage, V5. Control plants were maintained at the field capacity. At regular intervals of 14 days after sowing until the end of the cycle, plants were collected for the determination of dry mass and leaf area. We evaluated growth according to simple logistics, assimilate partitioning and the expression of seed vigor in each period. Plants in different periods of growth showed a reduced efficiency in converting solar energy over time in response to waterlogging, yielding seeds with an increased mass and vigor. Thus, it was observed that an increase in the period of waterlogging reduces plant growth but does not affect the vigor of soybean seeds.**

**Key words:** *Glycine max* (L.) Merrill, dry matter, leaf area, seed vigor.

## INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) belongs to the family Fabaceae and is grown on an acreage of 8.3% over the previous crop, with a productivity of 2.9 t ha$^{-1}$ (Conab, 2014). The products and by-products of soybean used by the chemical and food industries exhibit great versatility (Fante et al., 2010), and soybean is therefore one of the main commodities in the southern region of Brazil.

In recent years, the Rio Grande do Sul has shown unfavorable environmental conditions influencing soybean yields, causing many problems during the vegetative and reproductive growth of cultivated plants. The state of Rio Grande do Sul encompasses an area of 5.4 million hectares of lowland soils (Embrapa, 2005), most of which is used for rice cultivation and as pasture. Thus, employing other cultures in lowland areas is essential to improve the use and the yield of these areas.

Soil waterlogging affects the growth and development of various organs of plants, causing variations in respiration and photosynthesis (Alaoui-Sosse et al., 2005). These effects are reflected in changes in the leaves (Bailey-Serres and Voesenek, 2008), stomatal conductance (Alaoui-Sosse et al., 2005), decreased root

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permeability and alterations of hormonal balance (Moura et al., 2008).

With soil waterlogging, there is a decrease in oxygen in the root system, activating anaerobic metabolism (Kumutha et al., 2008; Sairam et al., 2009) and resulting in morphological and anatomical changes (Fukao and Bailey-Serres, 2004; Yin et al., 2010). The reduction of oxygen levels in the soil affects nitrogen fixation and other minerals, reducing root growth (Amarante and Sodek, 2006), the growth of leaves and relative growth (Almeida et al., 2003). The occurrence of these factors can cause lower rates of production and the transport of assimilates (Parent et al., 2008) as well as cessation of vegetative and reproductive growth (Moura et al., 2008) and may affect seed vigor.

Growth analysis is the first step for interpreting crop production, and it is important to evaluate the effects of different environmental conditions on the morphophysiological attributes of plants during ontogeny (Pedó et al., 2013). Through the examination of leaf area and organ dry matter, this method evaluates the effect of genotype x environment interactions and the influence of agronomic practices on plant growth (Barreiro et al., 2006). Furthermore, the evaluation of attributes related to seed vigor is important to estimate their performance in adverse field situations (Peske et al., 2012).

This study aimed to evaluate growth, assimilate partitioning and expression of vigor in soybean seeds subjected to periods of soil waterlogging.

## MATERIALS AND METHODS

The experiment was conducted in a chapel model greenhouse, arranged in the north-south direction, coated with polycarbonate and equipped with temperature and relative humidity controls, at a geographical location of 31°52 'S and 52°21' W, at the Federal University of Pelotas. The climate of the region is characterized as temperate with well-distributed rainfall and hot summers, corresponding to Köppen classification Cfa.

Sowing was performed on 01/06/2014, employing seeds from the BMX Potência soybean cultivar, which were allowed to germinate and develop in black polyethylene pots with a volume of 12 L, containing the A1 horizon of a Planossolo Haplic Eutrophic Solódico as a soil substrate, which was previously corrected in accordance with a prior soil analysis based on a fertilization manual (Cqfs RS / SC 2004).

The experiment was set up as a factorial experiment with a completely randomized 3x10 design (three periods and 10 collection times), with three replications. The treatments consisted of periods of flooding of two and four days during the vegetative stage - V5. To enable the establishment of treatments, holes were drilled at the bottom of polyethylene pots containing soil to facilitate the drainage of excess water and maintenance of the field capacity. The field capacity was determined using the voltage table method (Embrapa, 1997), maintaining a 20 mm blade of water on the soil surface by fitting a second black polyethylene vessel, without perforation of the vessels containing soil, seeking to ensure gas exchange and soil aeration. For drainage, the soaked soil was carried in the superimposed perforated vessel and imperforated vessel, allowing water to drain to the field capacity. As a control treatment, a set of plants that were maintained at the field capacity throughout the experimental period was used.

Data on the maximum, minimum and average temperature and solar radiation were obtained with a mercury thermometer and pyranometer, respectively, installed at a height of 1.5 m inside the greenhouse; the results are shown in Figure 1a and b. Evaluations were performed through the collection of primary growth data in successive harvests at regular intervals of 14 days after sowing, throughout the crop development cycle. At each harvest, the plants were cut close to the ground and divided into different organs (leaves, stems, roots and pods), which were packed separately in brown paper envelopes. To determine dry matter contents, the material was placed in a forced air oven at a temperature of 70 ± 2°C until constant weight.

Leaf area ($A_f$) was determined with a Licor LI-3100 model area meter, and the leaf area index (LAI) was estimated with the formula LAI = $A_f/S_t$, where $A_f$ is the leaf area, and $S_t$ is the vessel surface area occupied by the plant. The raw data for the accumulated total dry matter ($W_t$) were adjusted using the simple logistic equation $W_t = W_m / (1 + Ae^{-Bt})$, where $W_m$ is an asymptotic estimate of the maximum growth; A and B are constants used for adjustment; e is the base natural logarithm (Naperian logarithm); and t is the time in days after sowing (Richards, 1969). The primary of leaf area ($A_f$) data were adjusted by means of orthogonal polynomials (Richards, 1969).

Instantaneous values for the dry matter production rate ($C_t$) were obtained from the derivative of the equation for total dry matter and the relative growth rate ($R_w$) using the equation $R_w=1/W_t.dW/dt$, and instantaneous values for the net assimilation rate ($E_a$), leaf area ratio ($F_a$) and leaf weight ($F_w$) were estimated with the equations $E_a=1/A_f.dW/dt$, $F_a=A_f/W_t$ and $F_w=W_f/W_t$, respectively (Radford, 1967). The conversion efficiency of solar energy (ξ) was determined using the equation $\xi\ (\%) = (100.C_t.\delta)/R_a$, where $R_a$ is the mean value of the incident solar radiation (cal $m^{-2}$ $day^{-1}$) recorded over fourteen days prior to the corresponding $C_t$, and the calorific value (δ) of 4460 cal $g^{-1}$ cited by Silva Neto et al. (1991) was used.

The dry matter partitioning between different plant structures (roots, stems, leaves and pods) was determined separately from the measurement of the weight allocated to each plant structure, followed by transformation of the primary mass allocation data for each organ to a percentage.

From the harvested seeds, the thousand seed weight in grams was determined from eight repetitions in which the mass of 1000 seeds was measured with an analytical balance, as indicated by the Seed Analysis Rules guidelines (Brasil, 2009). Seedling emergence was carried out using eight replicates of 50 seeds per treatment, which were allowed to germinate in black polyethylene trays containing characterized above ground, kept at field capacity according to the methodology described above in a greenhouse environment. The seedling emergence speed index was determined from the daily count of the number of seedlings that emerged from the substrate, as proposed by Nakagawa (1994). Leaf area and total dry matter were measured based on the mass of eight samples from 10 seedlings at the end of the emergence test.

Data on the emergence and the emergence speed index of the seedlings, the leaf area and the area of organs were subjected to analysis of variance, and for significant F values, we applied Tukey's test at 5% probability. Primary data on total dry matter, the leaf area and the dry matter contents of the leaves, roots, stems and pods were subjected to analysis of variance, with data for the growth analysis being analyzed with a simple logistic equation (Radford, 1967). The statistical program used was Winstat.

## RESULTS AND DISCUSSION

The production total dry matter ($W_t$) in the soybean plants

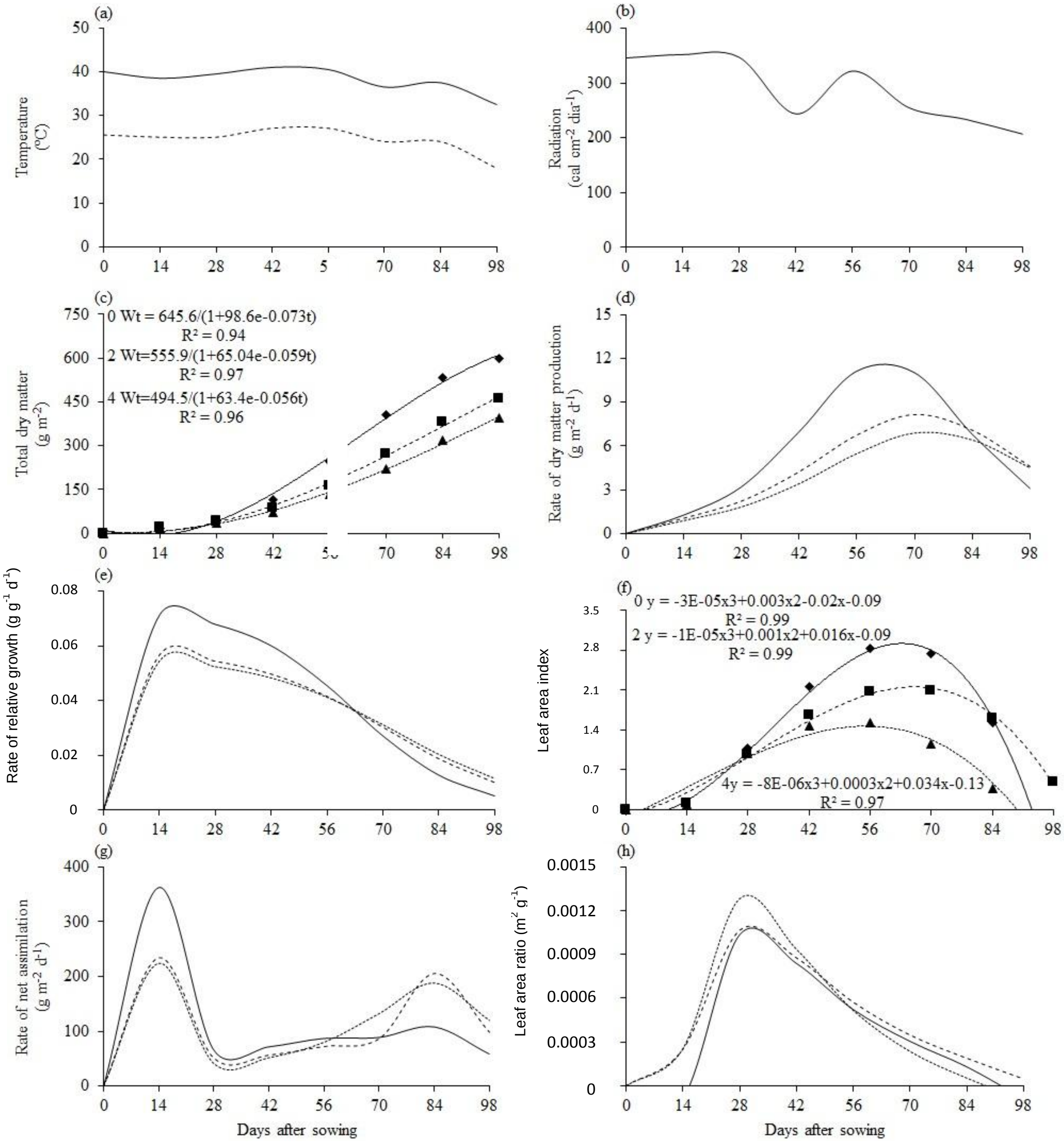

**Figure 1.** Maximum and minimum temperature (a), solar radiation (b), total dry matter (c), dry matter production rate (d), relative growth (e), leaf area index (f), rate of net assimilation (h) and leaf area ratio (g) in soybean plants exposed to flooding. Control ( ━━ ), two ( ‒ ‒ ‒ ) and four ( ---- ) days of soil waterlogging.

showed a logistic trend, with a high coefficient of determination ($R^2 \geq 0.94$). Growth was slow until 28 days after sowing (DAS), and the maximum was observed at 98 DAS (Figure 1c). The increased periods of soil waterlogging reduced the production of total dry matter in the soybean plants compared with the control plants. According to Pires et al. (2002), in soybean, the level of tolerance to soil waterlogging can be represented by the dry matter content.

The increase in growth during ontogeny can be explained by the increase in the leaf area and the consequent increase in the net production of assimilates (Aumonde et al., 2011). However, hypoxic conditions reduce stomatal conductance, reducing the photosynthetic rate, consequently leading to reduced growth of plants subjected to soil waterlogging.

The dry matter production rate ($C_t$), representing the accumulation of dry matter as a function of time, peaked at 63 DAS in the control plants, whereas in the flooded plants, the maximum dry matter production rate was obtained at 70 DAS (Figure 1d). The plants subjected to two and four days of soil waterlogging exhibited a higher dry matter production rate up to 98 DAS, showing that this type of stress increases the growing season of plants. Among the crop species, soybean is particularly susceptible to stress due to flooding (Komatsu et al., 2012). In the early stages of growth, soybean plants show differential regulation of hormone and carbohydrate metabolism (Nanjo et al., 2010, 2011), which contributes to the growth retardation of this species in soil waterlogging conditions.

The relative growth rate ($R_w$) presented the highest value in the early growth stages (14 and 28 DAS), with a further decrease being observed by the end of the crop cycle in the soybean plants (Figure 1e). However, at 65 DAS, there was a significant reduction of the relative growth rate in the plants that were not waterlogged compared with those subjected to soil waterlogging. In this case, the stress caused by soil waterlogging did not influence $R_w$ at the end of the cultivation cycle. The high photosynthetic capacity observed in young fully expanded leaves contributes to the high Rw observed in early development (Aumonde et al., 2011), in addition to increasing the respiratory activity of other growth bodies (Barreiro et al., 2006), as the consequent decrease in this variable may be due to self-shading (Lopes et al., 1986).

The leaf area index (LAI), a variable dependent on the leaf area (Melges et al., 1989), showed a high coefficient of determination ($R^2 \geq 0.96$). The maximum growth occurred at 63, 70 and 56 DAS in the control plants and the plants subjected to two and four days of flooding, respectively (Figure 1f). An increase in the LAI results in greater interception of solar radiation, which may be reflected in the net photosynthesis (Heiffig et al., 2006). A decrease in the LAI is expected at the end of the cycle due to the increase in leaf senescence (Aumonde et al., 2011), and in soybean plants, this is enhanced by seed maturation.

The net assimilation rate ($E_a$) observed in control plants was higher compared with the plants subjected to periods of soil waterlogging (Figure 1g). The maximum values of the net assimilation rate were obtained at the beginning of vegetative growth (14 DAS) and subsequently decreased. A second peak occurred at 84 DAS, and the highest values were obtained in the plants subjected to two and four days of soil waterlogging, followed by the control plants. At the beginning of the development cycle, when self-shading is reduced, $E_a$ tends to present high values (Gondim et al., 2008). The second peak can be explained by the establishment of the reproductive phase (Lopes et al., 1986), which demands greater photosynthetic activity for the maintenance of new bodies.

Although it is only an estimate, $E_a$ express the rate of photosynthesis in terms of the dry matter produced, for the leaf area and time (Benincasa, 2003). Under soil waterlogging, the permeability of the root system decreases, and the absorption of water and minerals is therefore also impaired. Then, stomatal closure can be observed, accompanied by decreasing net photosynthesis (Liao and Lin, 2001). Under favorable conditions, plants with a high net assimilation rate tend to exhibit fast initial growth and greater interception of solar radiation (Vivian et al., 2013), which may have resulted in greater total dry matter production in the control plants.

The relationship between the leaf area and the total dry weight or the leaf area ratio ($F_a$) corresponds to the useful area for photosynthesis (Aumonde et al., 2011). The maximum values were obtained at 28 DAS in all treatments (Figure 1h), with a subsequent decrease being observed until the end of the crop cycle (98 days). Similar to $E_a$, the behavior of $F_a$ can be explained in part by the development of new leaves and the consequent self-shading (Peixoto and Peixoto, 2009; Barreiro et al., 2006). The high initial $F_a$ of plants under soil waterlogging may be due to the higher efficiency of the photosynthetic apparatus, regardless of the leaf area (Campos et al., 2008).

The leaf weight ratio ($F_w$), representing the estimated fraction of assimilates retained in the leaves and not exported to other organs of the plant, was high under all periods of soil waterlogging at 28 DAS. The control plants showed greater allocation of dry matter in the leaves at 28 DAS and at the end of the crop cycle compared with the soil waterlogging treatments. However, during ontogeny, the dry matter content tends to decrease due to an increase in the source / sink ratio (Linzmeyer Junior et al. 2008), whereas increased leaf senescence increases the need for assimilates to produce pods and for seed filling.

The solar energy conversion efficiency ($\xi$), which is related to the photosynthetic process and consequently the synthesis of assimilates, showed increasing curves up to 70 DAS (Figure 2b), with maximum values of 1.9, 1.4 and 1.2% being obtained for the control plants and the plants subjected to two and four days of soil waterlogging, respectively, with a subsequent decline being observed until the end of the crop cycle. This decrease is a result of the leaf senescence rate (Melges et al., 1989). Similar trends for $\xi$ were reported in soybean by Melges et al. (1989) and Silva Neto et al. (1991).

The dry matter partitioning between different structures in the soybean plants was modified during the development of the plants, under all periods of soil waterlogging (Figure 2). The control plants exhibited the greatest partitioning of dry matter in the leaves up to 28 DAS, followed by the stems and roots (Figure 2c). Beginning at 42 days, dry matter accumulation was observed in the pods, which increased at 70 DAS, reducing the allocation to the leaves and roots.

In the plants subjected to two days of soil waterlogging, dry matter accumulation in the leaves showed a maximum at 14 DAS and was reduced by the end of the crop cycle (Figure 2d). At 56 DAS, dry matter accumulation in the pods was increased, while that in the leaves and stem was reduced. A similar trend in the partitioning of dry matter between different organs was

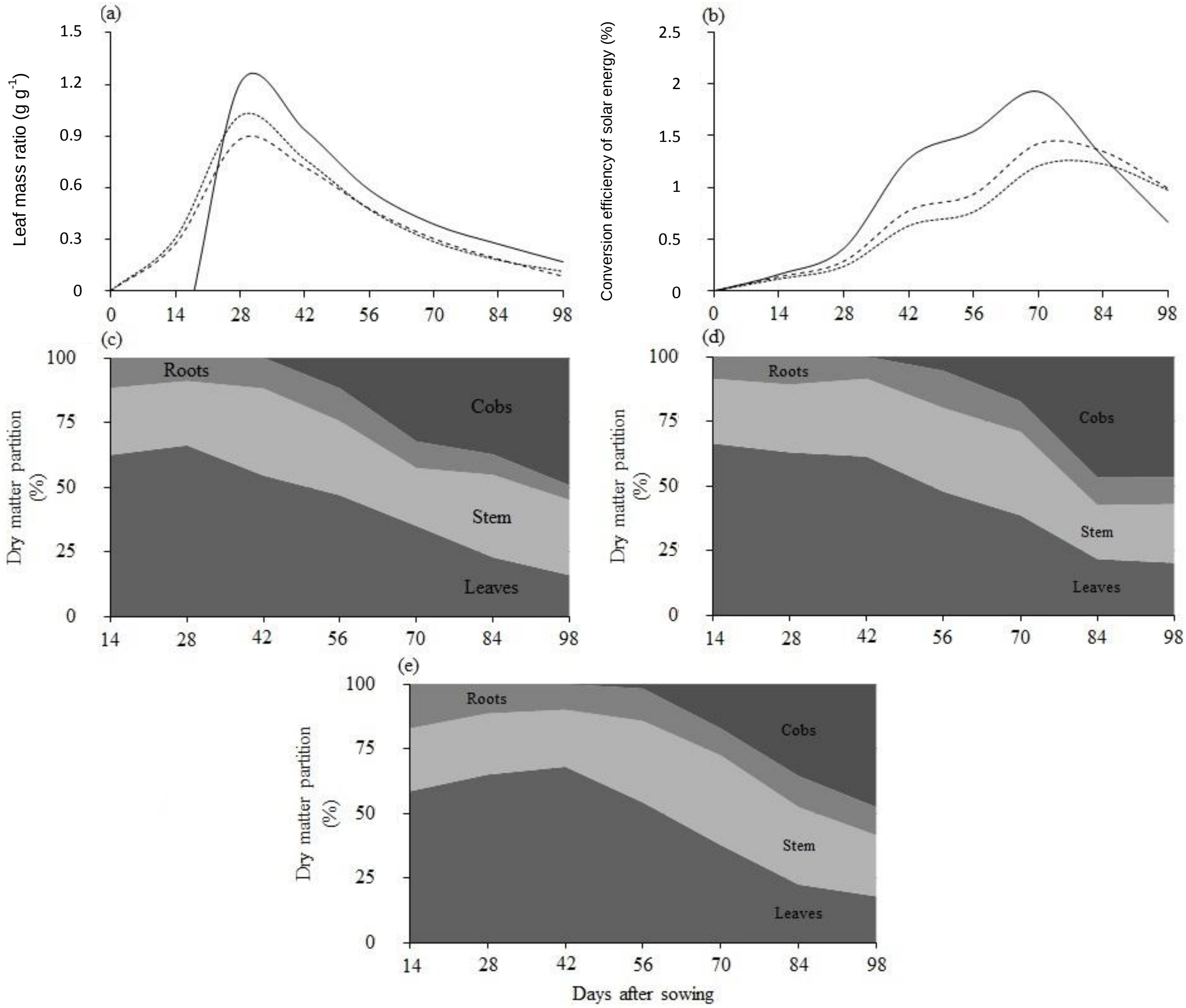

**Figure 2.** Leaf mass ratio (a) of the solar energy conversion efficiency (b) and the partitioning of dry matter in the soybean plants in the control treatment (c) and those subjected to two (d) and four (e) days of flooding. Control (——), two (– – –) and four (----) days of soil waterlogging.

observed in the plants subjected to four days of flooding (Figure 2e). However, at 42 DAS, there was an increase in dry matter accumulation in the leaves, which had decreased at 98 days. Thus, it was observed that the conditions of soil waterlogging increased the vegetative growth stage of the soybean plants, causing a delay in the early reproductive stage.

Soil waterlogging can reduce the rate of carbohydrate translocation to the leaves, causing accumulation of assimilates in the form of starch (Liao and Lin, 2001). The delay in dry matter accumulation in the pods of plants subjected to two and four days of flooding could be a result of reduced production and translocation of assimilates from the leaves to other organs and chemical changes in the soil caused by the flooding of the soil (Yordanova et al., 2004; Parent et al., 2008). The number of pods was greater in the control plants compared with those subjected to flooding. These results reflected the thousand seed weight, which was lower in the control plants (Table 1). The lower pod production observed in plants grown under soil waterlogging conditions may have been a reflection of the retardation of the vegetative cycle of the plants, which resulted in the lowest solar energy conversion efficiency (Figure 2b) for the production of pods. However, the increase of $E_a$ detected in plants under soil waterlogging during seed filling (84 DAS) may have resulted in a greater thousand seed weight compared with control plants.

Obtaining a good yield in soybean plants depends on a large intake of carbohydrates, which is in turn dependent on the photosynthetic rate guaranteed by the leafiness of plants (Pereira, 1989). The weight of seeds can be used as an indicator of physiological conditions, where seeds with a high weight exhibit greater amounts of reserve

**Table 1.** Number of seeds per plant (Ns), weight of 1000 seeds (M1000), emergence (E), leaf area (Af), and total dry matter content ($W_t$) in soybean plants obtained from seeds produced under soil waterlogging UFPel, (Pelotas, 2014).

| Treatment | $N_s$ | M1000 (g) | E (%) | $A_f$ (m²) | $W_t$ (g) |
|---|---|---|---|---|---|
| Control | 70.25$^{a1}$ | 102.15$^{c}$ | 82$^{b}$ | 0.0016$^{c}$ | 1.11$^{b}$ |
| 2 days of soil waterlogging | 44.75$^{b}$ | 120.16$^{b}$ | 89$^{a}$ | 0.0021$^{b}$ | 1.11$^{b}$ |
| 4 days of soil waterlogging | 44.25$^{b}$ | 158.99$^{a}$ | 92$^{a}$ | 0.0024$^{a}$ | 1.14$^{a}$ |
| CV (%) | 6.27 | 1.56 | 3.94 | 4.92 | 7.38 |

[1]Values with the same letter in the column do not differ significantly by the Turkey test (≤5%). Level of significance, P = 5%.

**Table 2.** Summary of the analysis of variance for the leaf area ($A_f$) and the dry matter contents of the leaves (Wf), stems (Wc), roots (Wr) and cobs ($W_{vg}$).

| Fv | GL | Mean squares | | | | |
|---|---|---|---|---|---|---|
| | | $A_f$ | $W_f$ | $W_c$ | $W_r$ | $W_{vg}$ |
| Conditions | 2 | 1115100* | 16.059* | 13.69* | 0.68* | 21.88* |
| Seasons | 6 | 1637848* | 27.92* | 34.25* | 5.12* | 153.71* |
| CON x SEA | 12 | 144258* | 2.88* | 2.13* | 0.31* | 5.78* |
| Residue | 40 | 30423 | 0.93 | 0.67 | 0.19 | 0.64 |
| Total | 62 | | | | | |
| Average | | 590.29 | 3.11 | 2.67 | 1.04 | 3.08 |
| CV(%) | | 29.55 | 30.94 | 30.64 | 42.20 | 26.04 |

Level of significance, P = *5%.

substances and a consequent increase in the probability of success in seedling establishment (Peske et al., 2012).

When seedling emergence was evaluated, it was found to be greater in seeds produced under flooding (Table 1). Similarly, the leaf area was greater in the plants obtained from seeds produced under four and two days of soil waterlogging, followed by the control.

However, the plants from seeds produced under four days of soil waterlogging exhibited a higher total dry matter content, possibly due to the greater seed weight (Table 1), which is indicative of seeds with adequate reserves that may contribute to the increased expression of seed vigor and superior performance of seedlings (Peske et al., 2012). The analysis of variance for the primary growth data is shown in Table 2. From the analysis of the mean squares for the leaf area and dry matter data for organs, it was observed that there was a high degree of significance between the conditions of soil waterlogging and the seasons of collection.

## Conclusions

Soil waterlogging affects the growth and partitioning of dry matter in soybean plants. Moreover, it reduces the efficiency of solar energy conversion over time and increases the thousand seed weight and the expression of seed vigor.

## Conflict of Interest

The authors have not declared any conflict of interest.

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