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Review

Selenium and agricultural crops

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Higher plants have different capacities to accumulate and tolerate selenium, referred to as accumulative and non-accumulative plants. Selenium-accumulators plants may contain hundreds of times more selenium than non-accumulators even when grown in the same soil, or can also grow in soils with low and medium selenium reserves; while selenium non-accumulator plants present low accumulation and tolerance to high selenium levels in the culture medium. Several studies have demonstrated the protective role of selenium in relation to oxidative stress in plants. Depending on the dose used, Se can activate certain enzymes such as superoxide dismutase, glutathione reductase and glutathione peroxidase. These enzymes are activated in the presence of Se, reducing the rate of lipid peroxidation and formation of hydrogen peroxide in plant tissue cells, which results in reduced senescence. Symptoms of selenium toxicity include reduced growth, chlorosis of leaves and pink coloration of the roots, yellowing of leaves and black spots. Studies provide evidence on a beneficial role of Se in plants and for environmental phytoremediation. However more research is needed to consolidate the beneficial effects of Se in plants.

Key words: Selenium, accumulating plants, metabolism, functions plant, toxicity.

INTRODUCTION

Selenium (Se) is an essential mineral micronutrient for the health of humans, animals, archaea and some other microorganisms (El-Ramady et al., 2016), occurring naturally in almost every part of the earth (Feng et al., 2013). Selenium was considered a toxic element until being recognized as an essential element for animals in 1957 (Schwarz and Foltz, 1957).

Regarding the role of Se in plants, several studies have shown that at low concentrations this element has beneficial effects on growth and stress tolerance by increasing its antioxidant capacity (Pilon et al., 2003).

Some studies have demonstrated the benefits of adding small amounts of Se, including increased tuber yields and greater concentration of starch in young potato leaves (Turakainen et al., 2004). This response was associated with inhibition of lipid peroxidation vi the increase in GSH- Px (Xue and Hartikainen, 2000).

However, at high concentrations Se is toxic to plants due to its incorporation in molecules which contain S (Pilon et al., 2003).

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The amount of available Se in soil determines the amount of Se in foods from plants that are grown in the soil. Awareness that ingestion of plants with desirable, non-toxic levels of Se is the first step for input of Se into the food chain may explain why biofortification with this element has received great attention (Mora et al., 2015).

However, when using this technology the question is often raised: What is the ideal dosage of Se? Selenium often has a dual effect on plant growth. At low doses it may stimulate plant growth and neutralize various types of environmental stresses, including those of heavy metals, whereas at higher dosages it can also act as a pro-oxidant and cause damage to plants (Feng et al., 2013).

In this context, this literature review aims to report on the functions, benefits and toxicity of selenium in agricultural crops.

SELENIUM ACCUMULATING AND NON-ACCUMULATING PLANTS

Higher plants have different capacities to accumulate and tolerate Se, therefore they are classified as accumulating and non-accumulating plants. Non-accumulating Se plants can be indicative of selenium-rich soils (White et al., 2004) and some plant species are classified as selenium hyperaccumulators, where the genus *Astragalus* is one of the largest hyperaccumulators, groups of this element (Terry et al., 2000).

Selenium hyperaccumulators, plants are divided into two groups, the first being primary selenium accumulators, which are able to accumulate 100 to 10000 mg selenium per kg^{-1} dry matter. This group includes the species *Astragalus, Machaeranthera, Haplopappus* and *Stanleya*. These species grow in selenium-contaminated soils with selenium contents greater than 5 mg kg^{-1} soil (Gupta and Gupta, 2000) and are responsible for selenosis in grazing animals. These selenium accumulating plants may contain hundreds of times more selenium than non-accumulating plants growing in the same soil (Kopsell and Kopsell, 2007).

Secondary selenium-accumulators can grow in soils with low and medium selenium reserves and can adsorb 25 to 100 mg selenium kg^{-1} of dry matter. This group includes different genera, such as *Aster, Astragalus, Atriplex, Brassica, Castilleja, Comandra, Grindelija, Machaeranthera* and others (Kopsell and Kopsell, 2007). Furthermore, these plants are tolerant to soil salinization (Terry et al., 2000).

According to White et al. (2004), selenium non-accumulating plants present low accumulation and tolerance to high levels of selenium in the culture medium, which usually contains less than 25 mg kg^{-1} of selenium in the dry mass. This group includes most crops such as cereals, potatoes, herbs, fruits and many natural plant species growing in the same soil as cultivated plants (Kopsell and Kopsell, 2007).

In non-accumulating plants, selenium is mainly found in the form of proteins; however, accumulating plants have the ability to synthesize it at non-protein amino acids, which prevents toxicity (Bergmann, 1982).

FUNCTIONS OF SELENIUM IN PLANTS

Selenium is involved in the metabolism of transfer RNA, as a radical 5-methylamino-7-seleno uridine, which acts in protein synthesis from the incorporation of amino acid analogs containing S, and via this radical becomes part of proteins. Selenocysteine ($CH_2SeHCHNH_2COOH$) is considered the 21^{st} amino acid in terms of protein synthesis mediated by ribosomes (Stadman, 1990).

When a large selenoprotein was discovered in mung bean seedlings (*Vigna radiata* L.) supplemented with 2 mg L^{-1} selenite, the function of Se in the mitochondrial membrane was discovered (Easwari and Lalitha, 1994). Another role of Se in plants was indicated by the discovery that a cysteine desulfurase (NIF) as a protein may be engaged in selenoprotein synthesis in chloroplasts (Pilon Smits et al., 2010), which together with the mitochondria are subject to high levels of oxidative stress.

Several studies have demonstrated the protective role of Se in relation to oxidative stress in plants, wherein the presence of this element increases glutathione peroxidase activity (GSH-PX) and decreases the activity of lipid peroxidation (Hartikainen and Xue, 1997; Cartes et al., 2005; Djanaguiraman et al., 2005).

Studies have shown that the addition of low concentrations of Se decreased the oxidative stress caused by ultraviolet radiation in lettuce and ryegrass (Hartikainen and Xue, 1999) and strawberry (Valkama et al., 2003). Suitable levels of Se were sufficient to increase the antioxidant capacity and delay senescence in leaves of lettuce, rye and soybean (Hartikainen and Xue, 1999; Xue et al., 2001; Xue and Hartikainen, 2000; Pennanen et al., 2002; Djanaguiraman et al., 2005; Hartikainen, 2005).

In potatoes, Xue et al. (2001) and Pennanen et al. (2002) showed that the addition of Se in the culture had an effect on the mesophyll of leaves, affecting the integrity of the cell membranes (Kong et al., 2005).
Accordingly, soybean plants when sprayed with sodium selenate at a concentration of 50 mg L^{-1} after 78 days of planting in tests on the ability of the culture to retard senescence related to oxidative stress, showed that plants can incorporate selenium in their physiological reactions so that it can act as an antioxidant agent, preventing degradation of chlorophyll (Djanaguiraman et al., 2004). This process occurs in many other plants by association of increased enzymatic activity of superoxide dismutase (SOD) and GSH-PX (Djanaguiraman et al., 2005).

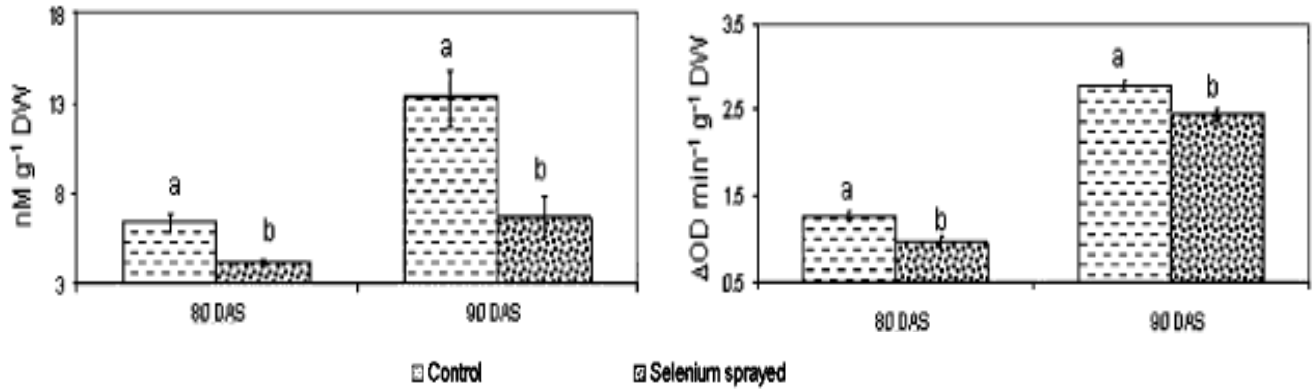

Figure 1. Effect of Se spray on stability of the cell membrane in soybeans at 80 and 90 days after sowing (DAS) (Djanaguiraman et al., 2005).

It was observed that Se promoted growth and acted as antioxidant for inhibition of lipid peroxidation and the percentage of injury to the cell membrane. These enzyme contents were positively correlated with the selenium content.

Work performed by Djanaguiraman et al. (2005) when studying selenium as a protective antioxidant in soybeans during senescence of the culture in India, noted that in plants control the content of superoxide and hydrogen peroxide was higher at 80 and 90 days after planting compared to treatment with Se (Figure 1). This result can be explained by the presence of two selenoproteins, GSH- Px and thioredoxin reductase induced by Se acting to protect the cells against oxidative stress.

Regarding the activity of SOD and GSH-Px, there is increased activity of these enzymes in treatments with the application of Se (Figure 2). Although the SOD did not present in its composition, Se may have altered the transcription levels of SOD thereby altering gene expression (Noctor and Foyer, 1998).

The reduction of the SOD activity in control plants may be due to increased superoxide and hydrogen peroxide, which destroy the SOD enzyme. It is possible that there is elimination of superoxide and hydrogen peroxide by increasing the activity of GSH-Px. The reason could be that GSH-Px, which is present throughout the cell and substrate, has a higher affinity in the presence of glutathione as a reductant (Noctor and Foyer, 1998).

The antioxidative action of Se also can be confirmed in the studies performed by Ríos et al. (2008), who observed the form of selenium accumulation in lettuce plants and the time of leaf antioxidative capacity. After different rates of selenite and sodium selenate were applied (5, 10, 20, 40, 60, 80 and 120 μ mol L^{-1}); the results showed that the least toxic form for this culture was selenate which induce the same production time for a larger amount of biomass, increased accumulation of selenium and a larger quantity of antioxidant compounds compared to selenite.

The treatment of 40 μmol L^{-1} was best suited for the lettuce plants, where the antioxidant capacity and selenium accumulation increased without decreasing the biomass, and making these plants appear healthier in comparison with the control plants.

Some studies have demonstrated benefits of adding small amounts of Se, including increased tuber yield and greater concentration of starch in young potato leaves (Turakainen et al., 2004). This response was associated with inhibition of lipid peroxidation through increase of GSH- Px (Xue and Hartikainen, 2000).

Lyons et al. (2009) studied increasing selenium on seed production in Brassica in Australia, and found that the pollen of plants control showed an average 14% non-viable grains compared to an average of 2% non-viable grains in treated plants. However electron microscopy revealed no apparent morphological differences in pollen grains of the treatments.

They observed that plants treated with Se presented higher total respiratory activity in leaves and flowers (Figure 3), which may have contributed to higher seed production. This response of the fertilized plants with Se is primarily due to an increase in capacity via the cytochrome, mediated by cytochrome oxidase (COX) (Lyons et al., 2009).

Immunoblot analysis of protein extracts from flowers of the control and treated plants with Se showed an increase in the relative amount of the protein COX II in flowers to which Se was applied (Figure 4). This observation indicates an increase in the amount of COX complex (Lyons et al., 2009).

An increase in the total respiratory activity of leaves and flowers of the treatment to which Se was applied compared to the control suggests that mitochondrial activity in plants treated with Se is greater. This may be due to protection of the mitochondria in plants treated with Se against damage caused by reactive oxygen species (ROS), for an up-regulation of the cellular antioxidant defense system. The increase observed in

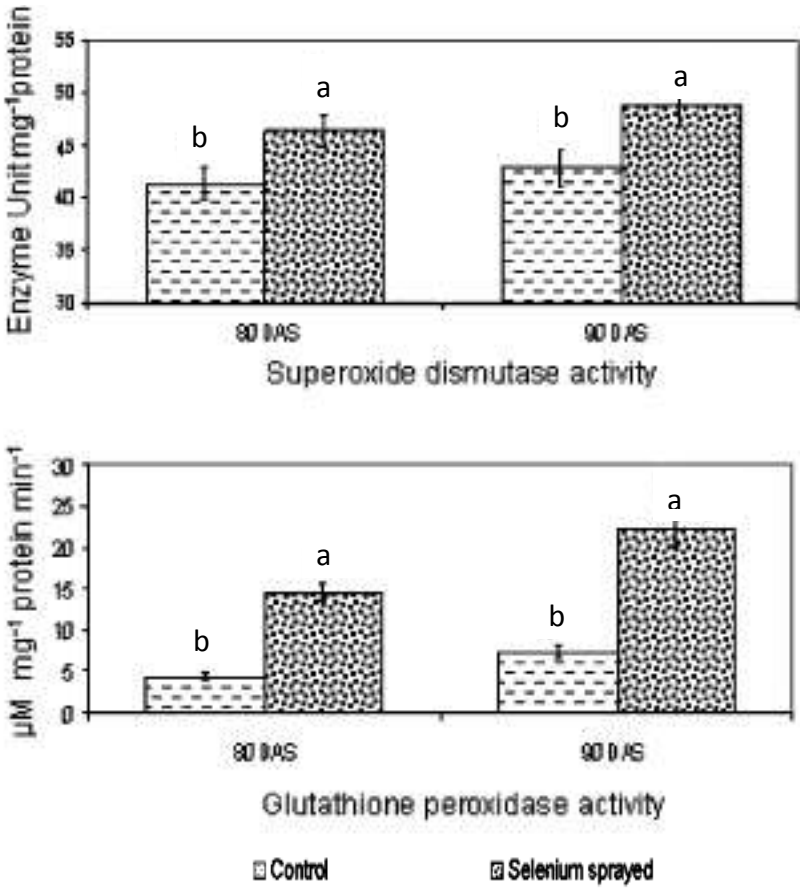

Figure 2. The effect of Se spraying on superoxide dismutase and glutathione peroxidase in the soybean crop at 80 and 90 DAS (Djanaguiraman et al., 2005).

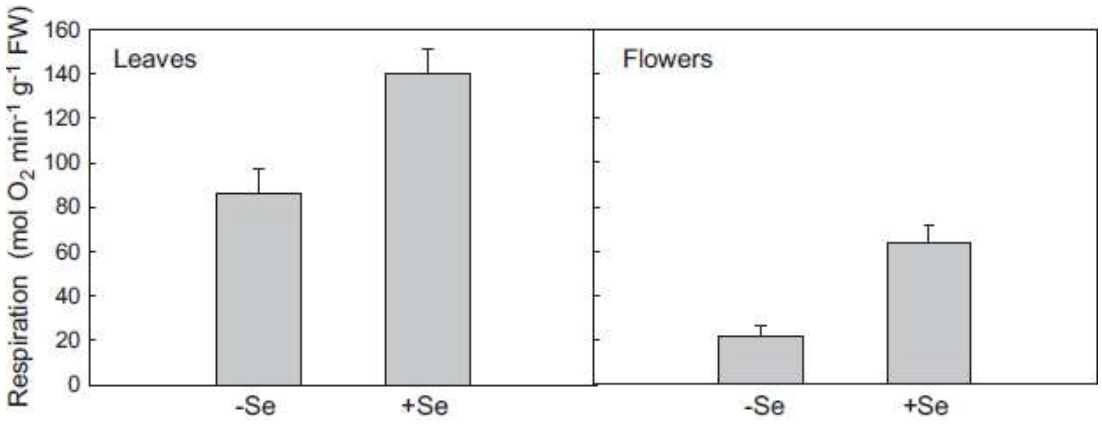

Figure 3. Respiration rate in Brassica leaves and flowers grown in nutrient solutions with or without sodium selenite (Lyons et al., 2009).

respiration is not likely to be a partial response to oxidative stress, since the Se concentrations were below toxic levels (Lyons et al., 2009).

Regarding the germination of seeds, 92% germination was observed for seeds from plants treated with Se in relation to the control treatment which showed 81% (Lyons et al., 2009).

This study therefore provides further evidence on the

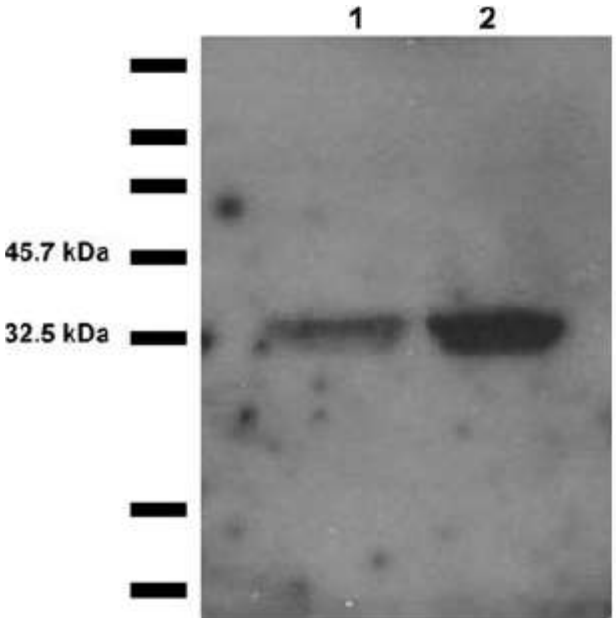

Figure 4. Immunoblot analysis of protein extracts COX from flower tissues treated with Se and untreated plants. Track 1 is the control plant while track 2 is the plant treated with Se (Lyons et al., 2009).

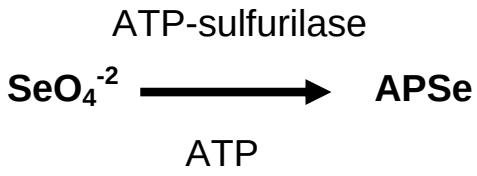

Figure 5. Reaction controlled by the ATP-sulfurylase enzyme, which activates the SeO_4^{-2} in adenosine phospho-selenate (APSe), similar to active sulfate (APS).

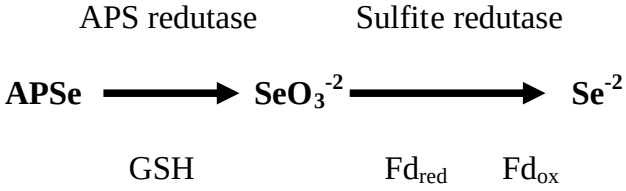

Figure 6. Selenium (Se^{-2}), receiving electrons supplied by ferredoxin, mediated by action of the enzyme sulfite reductase.

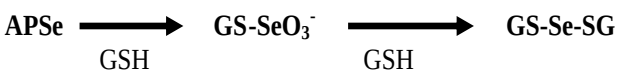

Figure 7. Selenite reduced via GSH into seleno-diglutathione (GS-Se-SG).

beneficial role of Se in plants. However more research is needed to consolidate the beneficial effect of Se in plants.

PARTICIPATION IN PLANT METABOLISM

Selenium is mainly absorbed in the oxidized form, selenate (SeO_4^{-2}) and, similar to sulfur, must be reduced to the selenium ion (Se^{2-}) either enzymatically or non-enzymatically for subsequent incorporation into organic compounds such as amino acids and proteins. Thus, these reactions have been studied in non Se accumulating plants (> 25 mg Se kg^{-1} DM) when grown in soils with high concentrations of this element (Terry et al., 2000).

In Se reduction via the enzymatic route, when allocated in the leaves by xylem, it enters the chloroplasts to be metabolized. Even in the sulfate reduction process (Prado, 2008), the first reaction is controlled by the ATP-sulfurylase enzyme, which activates the SeO_4^{-2} in adenosine phospho-selenate (APSe), similar to active sulfate (APS) (Figure 5).

The produced active selenate is reduced to selenite (SeO_3^{-2}) using reduced glutathione (GSH reducer) and the enzyme APS reductase, as in the reduction of sulfate to sulfite (Bick and Leustek, 1998). Thus, the selenite formed is reduced again to form the ion selenium (Se^{-2}), receiving electrons supplied by ferredoxin, mediated by action of the enzyme sulfite reductase (Figure 6).

Reduction through a non-enzymatic enzyme, or selenium reduced by ATP-sulfurylase the APSe

In 1977 Gregory and collaborators demonstrated the non-enzymatic reduction of this active selenium by reaction with GSH ($GS\text{-}SeO_3^-$) in bacteria of the genus *Saccharomyces*. Thus, the conjugate selenite is reduced again via GSH into seleno-diglutathione (GS-Se-SG) (Figure 7):

Finally, GS-Se-SG is reduced to selenol (GS-SEH) and the combined selenium ion ($GS\text{-}Se^-$) with the reducing power of NADPH and the GSH reductase enzyme.

However, for incorporation of the Se absorbed in amino acids, and subsequently in proteins, non-specific enzymes are needed that act on previous products ($GS\text{-}Se^-$ and Se^{-2}). Thus, selenium-cysteine (Cys-If) and selenium-methionine (Se-Met) are synthesized by cysteine synthase (Cys synthase) and methionine synthase (Met synthase), respectively (Figure 8).

Brown and Shrift (1981) found high contents of Se in proteins of non-accumulating species when subjected to sodium selenate. The authors attributed the results obtained in these plants to the rapid incorporation of the element in proteins. However, in tolerant species their lower Se contents could be caused by the synthesis of

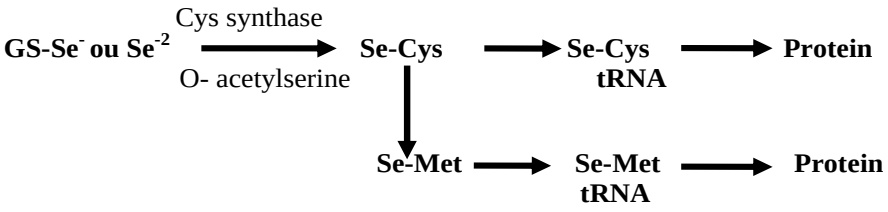

Figure 8. Selenium-cysteine (Cys-If) and selenium-methionine (Se-Met) synthesized by cysteine synthase (Cys synthase) and methionine synthase (Met synthase).

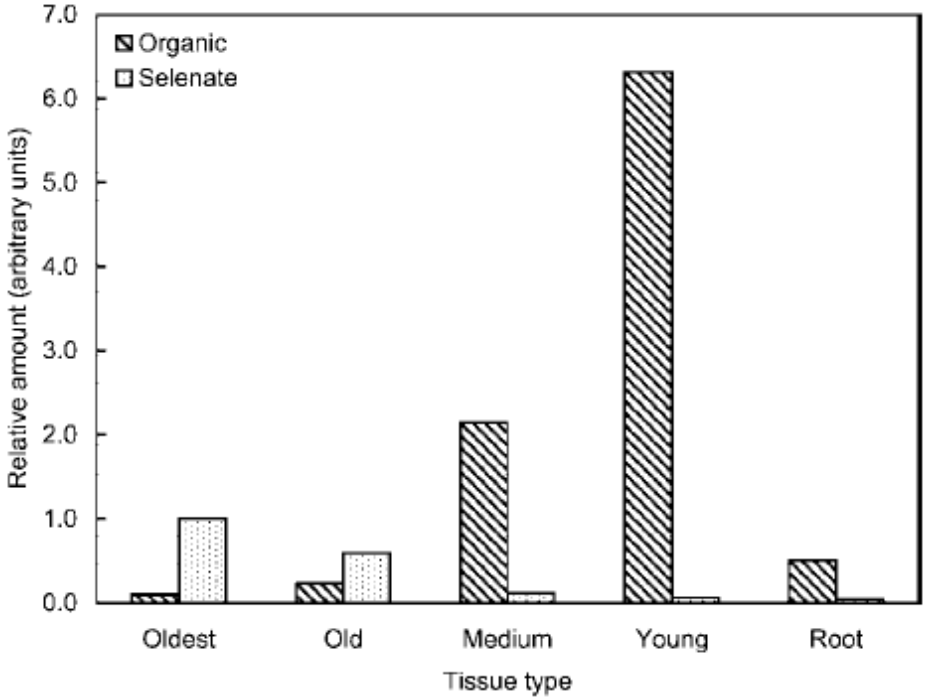

Figure 9. Relative quantities of Se in tissues of *A. bisulcatus* (Pickering et al., 2003).

other Se non-protein amino acids such as Se-metil-SeCys (MeSeCys).

Thus, the capture of Se and the formation of these metabolites reduces the integration of Se-Cys and Ser-Met in proteins. Accordingly, Pickering et al. (2003) observed larger contents of organic Se (MeSeCys) in different plant tissues of the tolerant species, *Astragalus bisulcatus* (Figure 9).

The beneficial effect of Se on environmental phytoremediation is observed when passing through a volatilization process in the cytosol of plant cells, being converted to the gas dimethyl selenide (DMSE), whose toxicity is much smaller than that of the ion Se^{-2}, and is also the primary volatile compound of Se in non-accumulating plants (Lewis et al., 1974). These authors observed the primary metabolic pathway for the production of DMSE in brassicas leaves, where the source was methyl selenomethionine (methyl-SeMet), which is produced by breakdown of the Se-Met amino acid and the action of the enzyme methyltransferase methionine (MMT), which is possibly the same enzyme responsible for the production of S-methylmethionine (SMM) in the metabolic pathway of sulfur (Sors et al., 2005). Finally, the methyl-SeMet is converted to the DMSe gas by the enzyme DMS hydrolase (Figure 10):
Another route is by carboxylation of methyl-SeMet and production of an intermediate product called dimethylsulfoniopropionate (DMSeP), which is then transformed to DMSe by the DMSP-lyase enzyme. Thus, Souza et al. (2000) compared the percentage of volatilization of Se absorbed in *Brassica juncea* L. When submitted to different sources of Se, and showed the greatest results when using DMSeP. This makes the volatilization process more efficient, thus demonstrating the existence of this alternative pathway in the volatilization of Se (Table 1).

In the case of accumulating plants it was observed that the incorporation of selenate in SeCys occurs in a

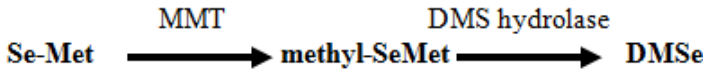

Figure 10. Methyl-SeMet converted to the DMSe gas by the enzyme DMS hydrolase.

Table 1. Absorption and volatilization percentage of Se by *B. juncea* subjected to various Se sources (adapted from Souza et al., 2000).

Source of Se	Total absorption (µg Se)	% volatilized (From being absorbed)
Selenate	382 ± 151	1.8
Selenite	157 ± 61	6.3
Se-Met	529 ± 114	21.5
DMSeP	953 ± 375	59.6

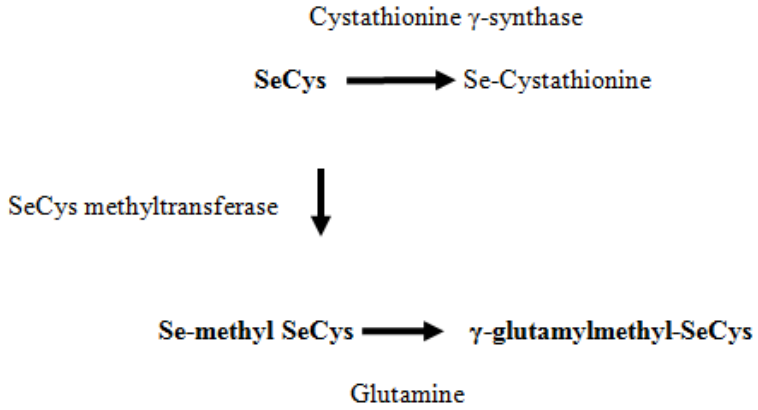

Figure 11. γ-glutamylmethyl-SeCys formed by the combination of the Se-methyl SeCys with glutamine in the peptides.

manner similar to non-accumulating plants, as previously mentioned. However, there are plants capable of volatilizing Se from a compound called di-methyl di-selenide (DMDSe), the main volatile compound of Se hyperaccumulator plants (Terry et al., 2000).

The SeCys is substituted by the action of the SeCys methyltransferase enzyme to form methylselenocysteine (Se-methyl SeCys), the first compound (non-protein amino acid selenium) that could be accumulated in plants and that could explain their higher tolerances to stress conditions (Pilon-smits and Quinn, 2010). Moreover, some plants have the ability to convert SeCys in non-protein compounds, to Se-cystathionine to be accumulated. Finally, the third compound to be accumulated by hyperaccumulators plants is γ-glutamylmethyl-SeCys formed by the combination of the Se-methyl SeCys with glutamine in the peptides; however the enzyme that catalyzes this reaction is still not well understood (Figure 11).

Finally, there is evidence which suggests the oxidation of Se-methyl SeCys for formation of MeSeCysSeO and subsequent methylation by the enzyme Cys-sulfoxide lyase and the consequent formation of the volatile compound DMDSe (Sors et al., 2005).

TOXICITY

At high concentrations, Se is toxic to plants due to its incorporation in the molecules which contains S and especially the indiscriminate substitution of cysteine for selenocysteine (Pilon et al., 2003). In this sense the non-specific integration of selenoamino acids, selenocysteine and selenomethionine in proteins are considered the largest contributor of Se toxicity in plants (Brown and Shrift, 1981).

High Se levels depress growth, protein synthesis and nucleic acid synthesis (Terry et al., 2000). High Se levels can damage the photosynthetic apparatus inhibiting photosynthesis, and result in excessive starch production (Vitová et al, 2011; Wang et al., 2012).

Symptoms of selenium toxicity are reduced growth, chlorosis of the leaves and pinkish coloration of the roots (Bergmann, 1982; Neal, 1990), yellowing of leaves and

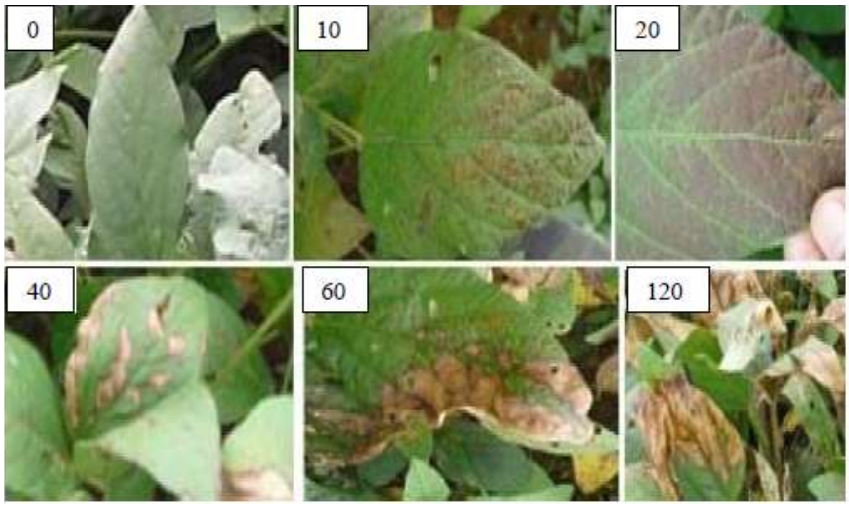

Figure 12. Increasing phytotoxic effect on soybean plants with increasing doses of sodium selenite applied to the leaves (Martinez, 2013).

black spots (Jacobs, 1989; Wu, 1994).

It is common that leaves present Se concentrations in regions of growth and in seed may reach 1500 ppm. However, there is variation in the ability of plants to absorb Se, presented in descending order: Crucifer, forage grasses, legumes and cereals, which is associated with a distinct metabolic capacity to divert Se, preventing its participation in protein synthesis (Brown and Shrift, 1981) for detoxification, linking it to non-protein amino acids (Correia, 1986).

According to Brown and Shrift (2008), the toxicity of selenate and selenite to most plants can be attributed to the combination of three factors. Firstly, selenate and selenite are readily absorbed from the soil by the roots and translocated to other plant parts. Secondly, metabolic reactions convert these anions in organic forms of selenium; and thirdly, organic selenium metabolites act as analogous essential sulfur compounds and interfere with cellular biochemical reactions.

The selenite is rapidly converted to organic forms which are incorporated into proteins in place of S, causing toxicity (Hopper and Parker, 1999). Sharrer and Schropp reported that 1.3 ppm of selenium and 25 ppm of sulfur in a soil solution is toxic to wheat, Barley and oats.

The absence of phytotoxicity symptoms has been reported in the USA, but experimental evidence has shown a negative correlation between the increase in selenium in the soil and growth (dry weight, root length and shoot height). In alfalfa, a decrease in yield was observed when Se extraction exceeded 500 mg kg^{-1} in the soil.

In China, phytotoxicity caused by high Se concentrations in the soil promoted pink discoloration of corn embryos, where the pink color is attributed to the presence of elemental selenium. Yang et al. (1983) observed that levels of 2 and 1.25 mg kg^{-1} of selenium are harmful to the growth and yield of wheat and pea, respectively.

With respect to food crops, the present relatively low tolerance to selenium toxicity and most crops have the potential to accumulate the element in amounts which are toxic to animals and humans. In general, tubers contain selenium concentrations higher than other organs and leaves often contain higher concentrations than the tuber.

In this context Yang et al. (1983) observed in seleniferous soils of China that selenium concentrations in plants (0.3 to 81.4 mg kg^{-1}) were higher in cereal crops (0.3 to 28.5 mg kg^{-1} rice and maize). The turnip showed high Se content with an average of 457 compared to an average of 12 mg kg^{-1} in tubers.

In moderate to low selenium content environments, alfalfa accumulated more Se in relation to other forage crops, which may be due to greater rooting causing more alkaline conditions, thus more selenium is available at greater depths. However, in general the species grown in soils high Se levesl present little difference in selenium content (Jacobs, 1989), by an exception was reported in New Zealand.

According to Marschner (1995) and Lyons et al. (2004), different plant species vary widely in both selenium accumulation capacity and in the ability to tolerate high concentrations of this element in the soil solution. These results corroborated with those of other studies in literature, which show that tobacco and soybean plants are sensitive to selenium and may be affected by this element (Lyons et al., 2004; Martin and Trelease, 1938).

In this context, Martinez (2013) evaluated the effects of foliar fertilization with sodium selenite in biofortification of the soybean culture cv BRS Favorita RR in the municipality of Itutinga-MG under field conditions. A phytotoxic effect was observed in all foliar application levels (0, 10, 20, 40, 60 and 120 g ha^{-1} Se) (Figure 12).

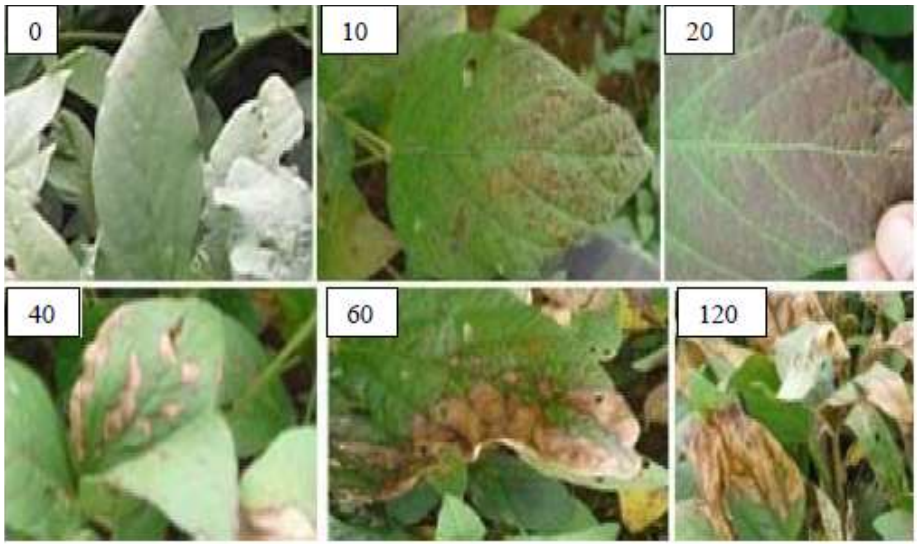

Figure 12. Increasing phytotoxic effect on soybean plants with increasing doses of sodium selenite applied to the leaves (Martinez, 2013).

CONCLUSION

Several studies on the growth of companies have beneficial effects on plant growth and stress tolerance by increasing their antioxidant capacity. However in high concentrations, the Se is toxic to plants. Thus, this literature review was developed based on studies of Se in plants, mainly for its role in metabolism, functions, benefits and toxicity in agricultural crops. This information may contribute to a better understanding of the role of Se in plants and to encourage future research in this area of study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Interaction of biological nitrogen fixation and fertilization: Effects on growth and yield of common bean in the dry season

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The inoculation with *Rhizobium* together with nitrogen (N) fertilization during sowing can maximize common bean yield cultivated in the rainy season, but this interaction was not studied in the dry season cultivation. Therefore, the objective of this study was to evaluate the effects of biological nitrogen fixation (BNF) and or N fertilization on growth and yield of common bean cultivated in the dry season. Two experiments were conducted in a randomized block design with four replications. The first experiment, in 2013, had three treatments: F-25 (only fertilized with 20 kg of N ha^{-1} at sowing and with 40 kg of N ha^{-1} at 25 days after emergence - DAE), I-25 (only inoculated with *R. tropici* at sowing and fertilized with 40 kg N ha^{-1} at 25 DAE) and IF-25 (inoculated with *R. tropici* and fertilized with 20 kg N ha^{-1} at sowing and with 40 kg N ha^{-1} at 25 DAE). The second experiment, in 2014, had the same three treatments and an additional treatment I (inoculated with *R. tropici* with no N fertilization). Three plants were collected randomly weekly, for growth analysis, which showed the highest biomass and leaf area accumulation and, consequently, highest grain yield of common bean in the treatment IF-25. The results indicated that in the dry season, the inoculation with *Rhizobium tropici* might replace the N fertilization (20 kg ha^{-1}) at sowing without yield loss for common bean cultivation in a low-cost agriculture. Nevertheless, the N fertilization (20 kg ha^{-1}) together with inoculation with *Rhizobium tropici* at sowing did not inhibit root nodulation, increasing growth and yield of common bean for a high-cost agriculture. However, more studies are required with other cultivars and sites, to recommend these agronomic practices in the cultivation of common bean in the dry season.

Key words: Inoculation, fertilization, *Rhizobium, Phaseolus vulgaris,* growth.

INTRODUCTION

Brazil is the largest world's producer and consumer of common bean (*Phaseolus vulgaris* L.), an important source of protein for the increasing world population (Vieira et al., 2006), but its average yield is one of the

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Table 1. Soil chemical and physical characteristics.

pH	P (mg dm^{-3})	OM (g dm^{-3})	K^+	Na^+	Ca^{+2}	Mg^{+2}	H+Al	Al^{+3}	V (%)
			……..…$cmol_c$ dm^{-3}……						
5.8	86	10.7	115	0.03	1.5	1.4	0.6	0.0	84

lowest in the world (Hungria and Kaschuk, 2014). However, the common bean yield has significantly increased from 500 kg ha^{-1} in the late 1970's to 1050 kg ha^{-1} in 2015. This increase in yield occurred mainly due to a larger participation of big farmers, who use high-cost technologies for obtaining high yields, especially in the rainy season (first crop), but also in the dry season (second crop) and winter season (third crop), both in a less extent (Conab, 2015).

However, low technology of small farmers is one of the factors still reducing common bean crop yield in Brazil (Grange et al., 2007), due to their low input agriculture and environmental stresses, especially of water and N deficiency in the dry season (Pimentel, 2006), forcing them to concentrate their production in the rainy season (first crop) (Cardoso et al., 2012; Hungria and Kaschuk, 2014). On the other hand, if the efficiency of plant N use is improved, the yield of common bean cultivated in the dry season can be increased for a low-cost small farming, if there is no other environmental stress (Pimentel, 2006).

The common bean is a C_3 plant and thus, it is more sensitive to N deficiency than C_4 plants (Pimentel, 2006). The C_3 plants use more than 50% of the leaf N content for synthesizing the enzyme ribulose-1.5-bisphosphate carboxylase/oxygenase (RubisCO), which is responsible for photosynthetic assimilation of CO_2 to maintain plant growth and consequently, grain yield (Long et al., 2006). Nowadays, the efficient use of N is essential for yield increases, decreasing production costs and lowering risks of environmental pollution, due to the losses of N in the soil, especially in the tropics, where they are generally poor in organic matter (Cardoso et al., 2012; Hungria and Kaschuk, 2014). Mineral N fertilizers are produced from non-renewable reserves using large quantities of fossil fuel energy and thus, have a high cost for food production. Therefore, research to improve the efficiency of N use by crops is a new paradigm for modern agriculture (Pimentel, 2006; Remigi et al., 2016).

Nevertheless, common bean crop can benefit from BNF, through symbiotic relations with rhizobia air-nitrogen fixers (Cardoso et al., 2012). However, the BNF does not meet the common bean N requirements for a high yield, thus needing an addition of mineral N fertilizer (Cardoso et al., 2012; Hungria and Kaschuk, 2014). Hungria et al. (2000) have shown that common bean crops in suitable environmental conditions in the rainy season can reach yields higher than 4 t ha^{-1} only with the use of inoculation with rhizobia. Thus, technologies for increasing crop yield at lower costs are necessary, such as the use of cultivars with greater BNF potential or inoculation with more competitive and better-adapted rhizobia strains (Hungria and Kaschuk, 2014). The BNF process is important for the Brazilian agriculture sustainability, generating organic N at low cost and with low environmental impact when compared with mineral N fertilizers use (Grange et al., 2007). Therefore, the objective of this study was to assess the effects of biological nitrogen fixation and or N fertilization on growth and yield of common bean cultivated in the dry season.

MATERIALS AND METHODS

Site and climate description

Two experiments were conducted in the field, at the Experimental Station of the Department of Crop Science, Federal Rural University of Rio de Janeiro (Universidade Federal Rural do Rio de Janeiro, UFRRJ), Seropédica, RJ, Brazil (22°44' S, 43°42' W and 40 m of altitude), in May (dry season) of 2013 and 2014. The climate in the region is an Aw, according to the Köppen classification, with hot and rainy summers and dry winters.

The soil of the experimental area was classified as a Kanhapudalf soil with sandy loam texture. The chemical and physical characteristics of the soil layer 0-20 cm was presented in Table 1. During the experiments, total precipitation were of 153.5 (2013) and 139.2 mm (2014), evapotranspiration of 217.9 (2013) and 235.1 mm (2014), average maximum temperature of 28 (2013) and 28.3°C (2014) and minimum of 18.1 (2013) and 18.6°C (2014).

Crop management

The common bean cultivar Carioca, used in the experiments, has a type-III indeterminate growth habit with an intermediate cycle (85 days) and seeds are beige with brown strips. The seeds were sowed manually, using 20 seeds per meter in rows spaced 0.5 m apart, which was thinned to a density of 12 plants per linear meter at 7 DAE. All the treatments of both experiments were fertilized with 30 kg P_2O_5 ha^{-1} (superphosphate) and 45 kg K_2O ha^{-1} (potassium chloride) (Vieira et al., 2006). The inoculant for common bean SEMIA 4080, selected in low pH soils under high air temperatures, produced by the Embrapa Agrobiology with viable *Rhizobium tropici* cells was used to inoculate the seeds of the treatments with inoculation. During the experiments, the area was maintained free from weeds by manual control and irrigation was provided when necessary.

Plant analysis

Three random plants were weekly collected in each plot for growth analysis, from 14 DAE to the end of the crop cycle. The leaf area was determined using a portable leaf area meter (Li-3000C, LI-COR Biosciences, USA) and the leaves, stems and roots collected were

dried at 65°C for 72 h. According to the methodology described by Hunt (1978), the TDW data was transformed to biomass per land area and the leaf area data were transformed to LAI. The CGR and NAR were determined from the TDW and LAI data (Pereira and Machado, 1987).

In addition, samples of three plants per plot were collected at pollination stage (50% of plants in the plots with flower buds), when plants attain maximal growth and BNF (Vieira et al., 2006), which occurred at 33 DAE in 2013 and 31 DAE in 2014. In these plants, their BNF potential was assessed using the variables NN and NDW per plant, as stated by Hungria et al. (2003), and plant growth by the SDW and RDW per plant. The nodules were removed from the roots counted immediately to determine the NN and then dried at 65°C for 72 h to determine the NDW. The shoot and root were also dried at 65°C for 72 h to determine the SDW and RDW.

Furthermore, the central leaflet of the youngest fully expanded leaf of three plants were collected in each plot at the four development stages, as described by Vieira et al. (2006): vegetative (20 DAE), before N fertilization at 25 DAE; pollination (P); flowering (F) and grain filling (GF) for the first and second year of cultivation. The leaflets were immediately wrapped in foil and immersed in liquid N. These samples were used to quantify LSPC, according to the Bradford (1976) method.

Finally, the plants of the two central rows of each plot were collected at physiological maturity, excluding 0.5 m from each borders, to determine the NP, NGP, DW100G and GY.

Experimental design and statistical analysis

The two experiments were conducted in a randomized block design, with three treatments in 2013 and four treatments in 2014, with four replications for both years. Each plot consisted of five rows of 5 m spaced 0.5 m apart, with a total area of 10 m^2 per plot. The treatments of the first experiment were: F-25 (only fertilized with 20 kg of N ha^{-1} at sowing and with 40 kg of N ha^{-1} at 25 days after emergence - DAE), I-25 (only inoculated with *Rhizobium tropici* at sowing and fertilized with 40 kg N ha^{-1} at 25 DAE) and IF-25 (inoculated with *R. tropici* and fertilized with 20 kg N ha^{-1} at sowing and with 40 kg N ha^{-1} at 25 DAE). The second experiment was conducted in 2014 with four treatments, F-25, I-25, IF-25 and an additional treatment I (inoculated with *R. tropici* with no N fertilization). Data were subjected to analysis of variance (ANOVA), and the means were compared and segregated by the Tukey's test at 5% of significance level (P<0.05).

RESULTS

Total plant dry weight and leaf area accumulation

The TDW of the treatments with nitrogen fertilization at sowing (IF-25 and F-25) were significantly higher than those without sowing nitrogen fertilization (I-25 and I), in some samplings from the vegetative stage to maturity (Figure 1). In 2013, differences in TDW among treatments were observed from 28 DAE (Figure 1A), and especially the TDW in IF-25 treatment was significantly higher than in F-25 and I-25 from 35 DAE until the last sampling day (77 DAE). The maximal TDW occurred at 70 DAE for both years (Figure 1). In 2014, differences in TDW were observed from 49 DAE (Figure 1B), and TDW for IF-25 and F-25 treatments were significantly higher than the other two treatments until 77 DAE.

In addition, significant differences of the LAI among the treatments were found in both years. The treatment IF-25 presented higher LAI in both years (Figure 2), with a peak at 63 DAE in 2013 and at 56 DAE in 2014, that is, the beginning of the grain filling stage. The treatment I-25 had a significant lower LAI than the others treatments in 2013, especially from 21 to 70 DAE (Figure 2A). In 2014, the LAI of the IF-25 and F-25 treatments were significant higher than the treatments without sowing N fertilization from 42 to 70 DAE (Figure 2B), with significantly higher LAI values than especially the I treatment, but also for I-25 treatment in several samplings.

The CGR had maximum values before the LAI peak, at 56 (2013) and 49 (2014) DAE, with significant differences between treatments in both years (Figure 3). As for TDW and LAI, the treatment IF-25 had significant higher CGR than the others treatments in the initial and final samplings of 2013 (Figure 3A). While in 2014, this treatment IF-25 showed significant differences only on samplings at 28 and 42 DAE (Figure 3B), with significantly higher CGR than the treatment I, but the treatments F-25 and I-25 did not differ from IF-25 and I (Figure 3B). The TDW peaked (Figure 1) at the end of the cycle (70 DAE), when the CGR reached negative values (Figure 3).

The NAR was high in the first sampling (14 DAE) in both years (Figure 4), and gradually decreased from this time, however, it was similar in all treatments and years evaluated, with values near zero or negative from 63 DAE (beginning of grain filling stage), together with the LAI decrease (Figure 2).

Nodulation and plant dry weight at the pollination stage

The BNF potential was evaluated by the NN and NDW, and the plant dry weight accumulation at the pollination stage was evaluated by the SDW and RDW, which occurs at 33 (2013) and 31 (2014) DAE (Table 2). The NN and NDW of the treatments were different in both years, with significantly higher values in the treatment I-25 than in the others two treatments with N fertilization at sowing (F-25 and IF-25) in 2013. In 2014, the treatments with only inoculation without N fertilization at sowing (I and I-25) presented significantly higher NN values than the treatments F-25 but not for IF-25, as shown in Table 2. The NDW of the treatments were also significantly different (Table 2), with higher values in the treatment I-25 than F-25 and IF-25 in 2013 and in 2014, the treatment I was significantly higher as compared to the F-25, and the treatments I-25 and IF-25 did not differ from I and F-25. On the other hand, the treatment IF-25 had SDW significantly higher than the I-25 in 2013, without any significant differences in 2014 as for RDW in both years (Table 2).

During ontogeny (LSPC)

The LSPC was similar for all treatments at the vegetative

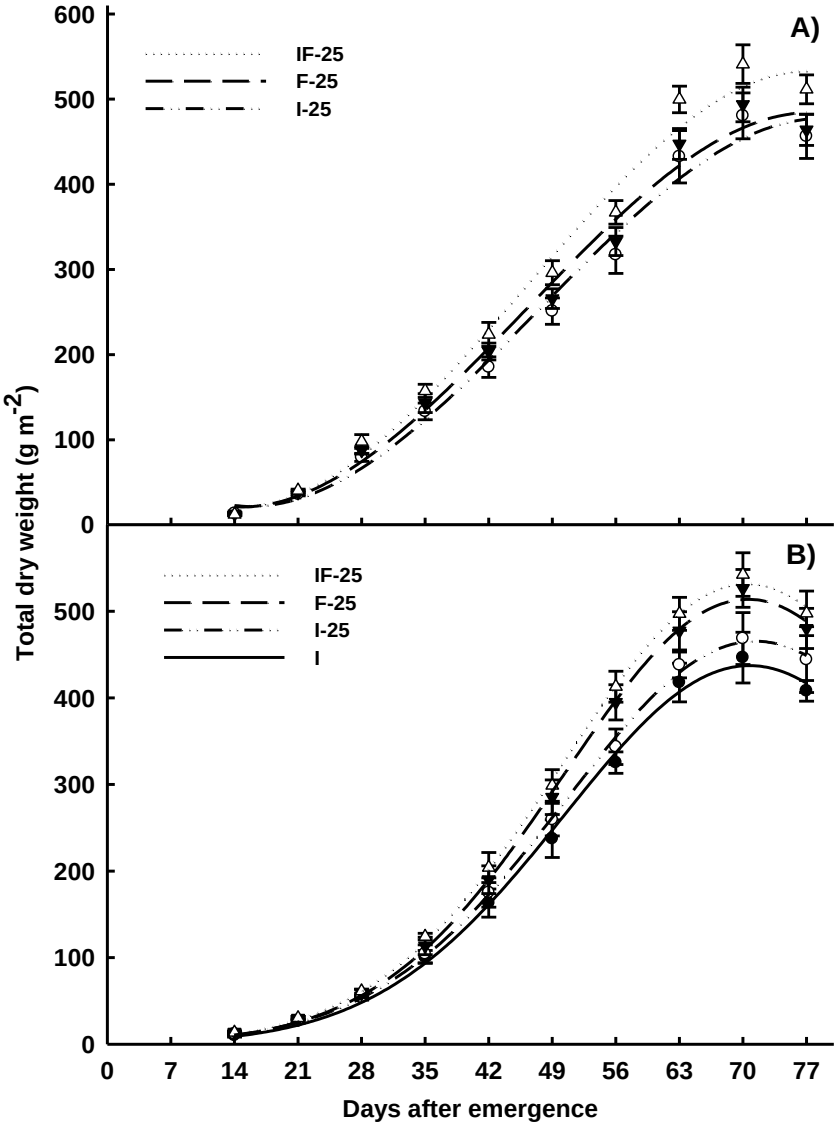

Figure 1. TDW of common bean, under the treatments F-25 (▼), I-25 (○), and IF-25 (Δ) in the first year (A), and a fourth treatment I (●) included in the second year (B).

stage, before the fertilization with 40 kg N ha^{-1} at 25 DAE, at the pollination stage and at the grain filling stage, in both years (Figure 5). However, the treatments were significantly different at the flowering stage, at 39 (2013) and 38 DAE (2014), in which the treatment IF-25 had significantly higher LSPC than the treatment I-25 in 2013 (Figure 5A) and I in 2014 (Figure 5B). At the grain filling stage, the LSCP was much lower than in the stages before indicating an increased N remobilization and leaf senescence (Figure 5).

Yield components

The yield components NP, NGP and DW100G of the treatments were similar in both years (Table 3), while GY of the treatments was significantly different. The treatment IF-25 had significantly higher GY than the others in 2013 and higher than the I in 2014, however, the treatments I-25 and F-25 did not differ from IF-25 and I (Table 3).

DISCUSSION

Total plant dry weight and leaf area accumulation

The TDW of all treatments were significantly different in both years (Figure 1A, B). The treatments with N fertilization at sowing (F-25 and IF-25), showed

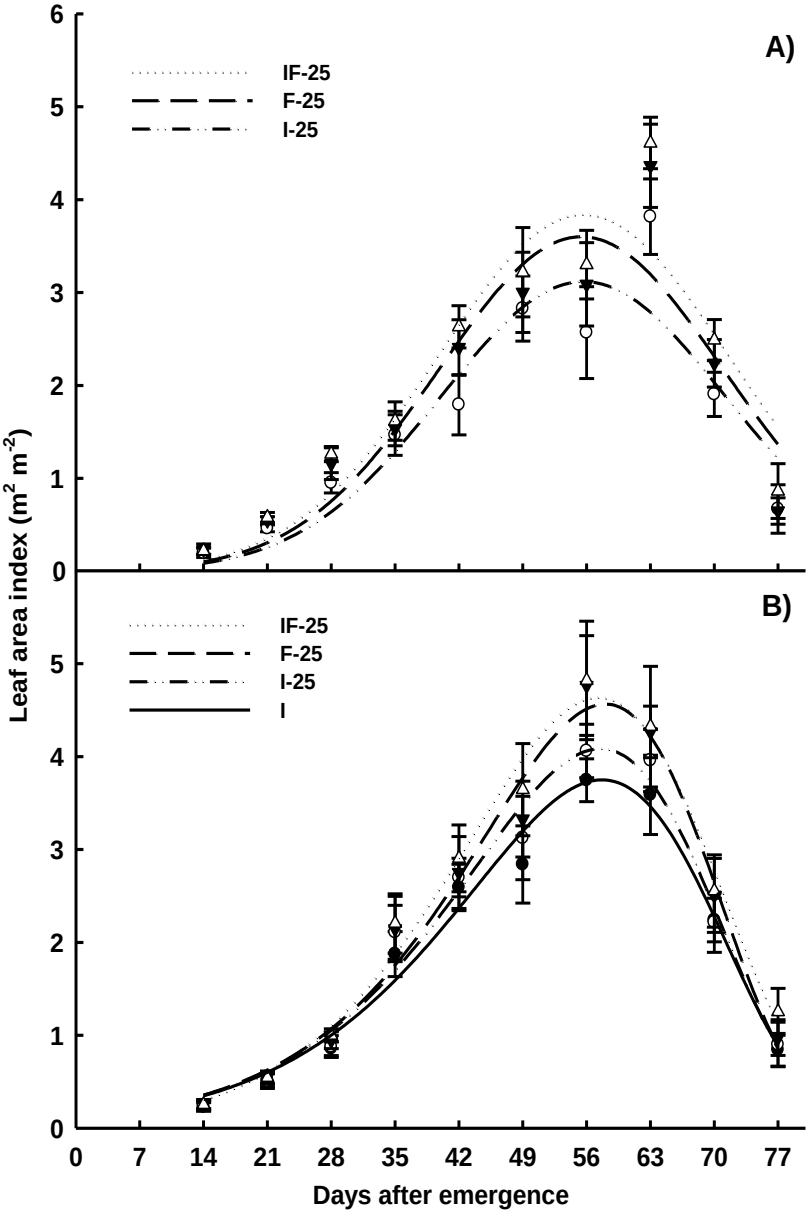

Figure 2. LAI of common bean, under the treatments F-25 (▼), I-25 (○), and IF-25 (Δ) in the first year (A), and a fourth treatment I (●) included in the second year (B).

significantly higher TDW from the vegetative stage to the end of the cycle, in both years. This result was probably due to an incomplete nodulation in the vegetative stage (Hungria et al., 2000), resulting in some samplings with lower TDW in the treatments that was only inoculation at sowing (I-25 and I). The treatment IF-25 presented significantly higher TDW than the others treatments in most samplings, with a peak at 70 DAE for all treatments during the grain filling stage in both years, in accordance with Gomes et al. (2000). Therefore, the start dose of 20 kg N ha^{-1} at sowing in the dry season cultivation induced higher plant growth at initial developmental stages before the increase in BNF, as stated by Hungria et al. (2003).

In addition, the LAI of all treatments was also significantly different in both years (Figure 2A, B). As for TDW, the treatment IF-25 presented higher LAI in both years. The LAI peak occurred at 63 DAE in 2013 (Figure 2A) and at 56 DAE in 2014 (Figure 2B), during the beginning of the grain filling stage, indicating a decrease in the LAI (leaf senescence) and photosynthesis from this stage (Pimentel et al., 1999), when photoassimilate requirements for grains is high to maintain embryo growth

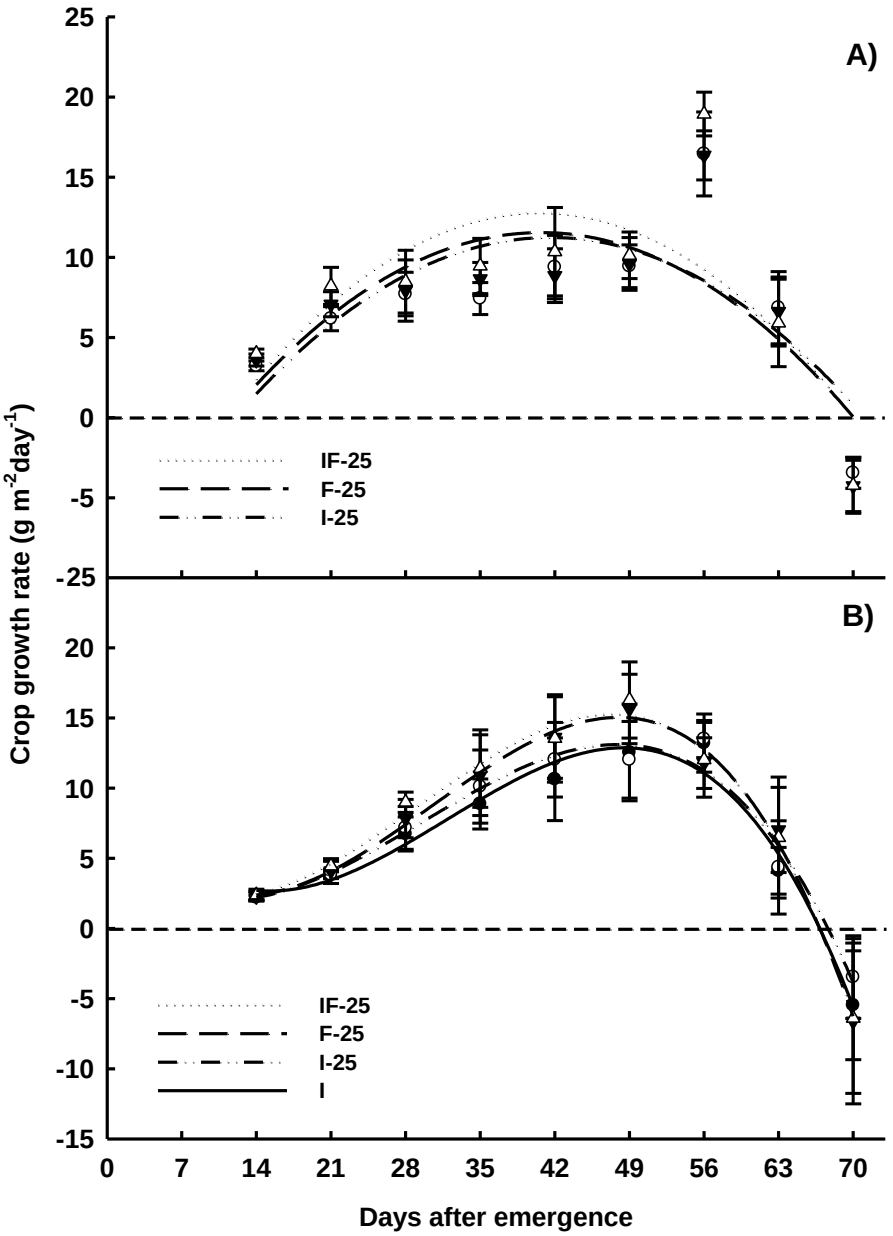

Figure 3. CGR of common bean, under the treatments F-25 (▼), I-25 (○), and IF-25 (Δ) in the first year (A), and a fourth treatment I (●) included in the second year (B).

(Pimentel, 2006). The treatment I-25 had a significant lower LAI than the others treatments in 2013 (Figure 2A), especially from 21 to 70 DAE, while in 2014, there were more significant differences of LAI between the treatments from 42 to 70 DAE (Figure 2B). Therefore, the treatments IF-25 and F-25 had significantly higher LAI than especially the treatment I but they were also higher than the I-25 in several samplings of both years. Thus, the increase in TDW of the treatment IF-25 and F-25 promoted an increased leaf expansion and consequently LAI, which will increase total leaf photosynthesis to produce more photoassimilates for growth.

The evaluation of CGR during plant development (Figure 3) showed that a maximal accumulation of biomass per area of soil occurred at 56 (2013) and 49 (2014) DAE, before the LAI peak, with significant differences between treatments in both years. To ensure higher TDW and LAI, the treatment IF-25 had significant higher CGR than the others treatments in the initial and final samplings of 2013 (Figure 3A), while in 2014 (Figure 3B), this treatment IF-25 showed significant differences only on samplings at 28 and 42 DAE, with significantly

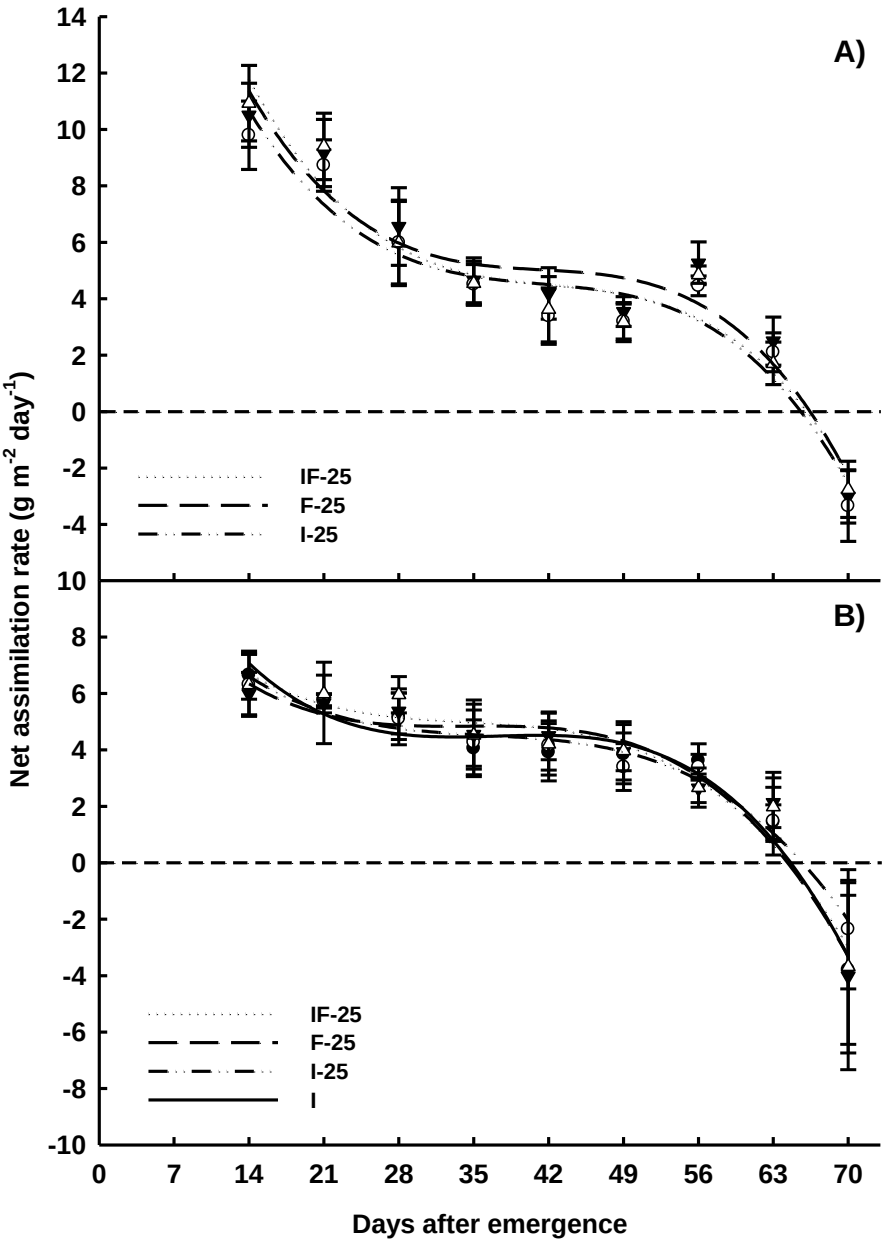

Figure 4. NAR of common bean, under the treatments F-25 (▼), I-25 (○), and IF-25 (Δ) in the first year (A), and a fourth treatment I (●) included in the second year (B).

higher CGR than the treatment I, but the treatments F-25 and I-25 did not differ from IF-25 and I in 2014. The TDW peaked at the end of the cycle (70 DAE), when the CGR reached negative values, and this is probably related to the well-known high leaf senescence rate of common bean from the flowering stage (Pimentel et al., 1999; Vieira et al., 2006), resulting in a leaf area decrease at the grain filling stage from 63 (2013) and 56 (2014) DAE, when the need of photoassimilates for the grain is high.

The NAR is a growth variable that represents the biomass produced per leaf area and time, that is, the biomass accumulation from the photosynthesis (Pereira and Machado, 1987). The NAR was high in the first sampling (14 DAE) in both years (Figure 4A and B), and gradually decreased after this date; however, it was similar in all treatments and years evaluated, with values near zero or negative from 63 DAE (beginning of grain filling stage), along with the LAI decreasing (Figure 4). The annulment of NAR in the grain filling stage confirm the high rate of abortion of last formed reproductive organs in common bean (Vieira et al., 2006) due to a decrease in photosynthesis in this final stage (Pimentel et

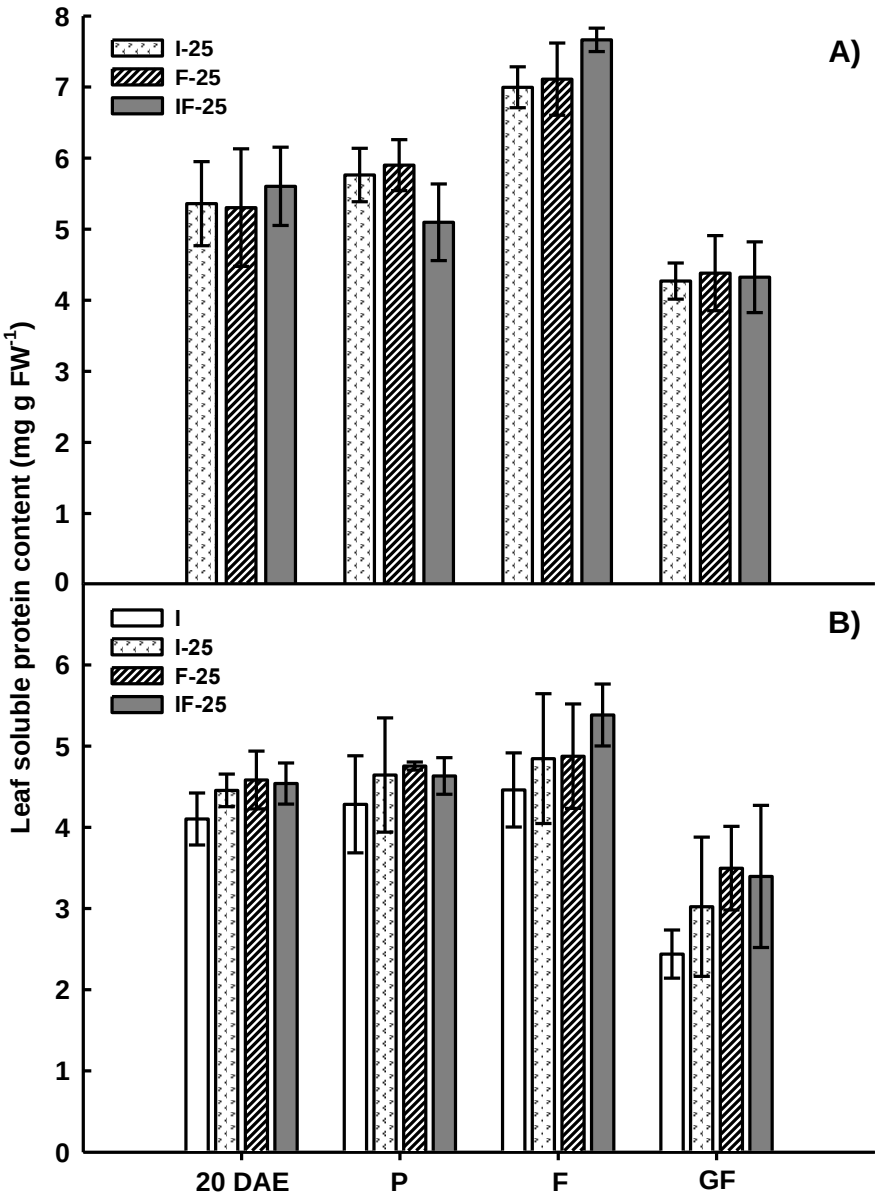

Figure 5. LSPC of common bean, under the treatments F-25 (▼), I-25 (○) and IF-25 (Δ) in the first year (A), and a fourth treatment I (●) included in the second year (B).

al., 1999). The treatment IF-25, which showed higher values of TDW and LAI did not show significant differences of NAR as compared to the other treatments (Figure 4A and B).

Nodulation and plant dry weight at the pollination stage

At the pollination stage of common bean, at 33 (2013) and 31 (2014) DAE, when the BNF is considered maximal (Vieira et al., 2006), the BNF potential was estimated by the NN and NDW (Table 1), which are considered proportional to the nitrogenase activity, as stated by Hungria et al. (2003). At this time, the plant dry weight accumulation was also evaluated by the SDW and RDW (Table 2). The NN and NDW of the treatments were significantly different in both years, with significantly higher values in the treatment I-25 than in the others two treatments with N fertilization at sowing (F-25 and IF-25) in 2013. In 2014, the treatments only inoculated at sowing (I and I-25) presented significantly higher NN values than the treatment F-25, but not for IF-25. The NDW of the treatments were different, with significantly higher values in I as compared to the F-25, however, the treatments I-25 and IF-25 did not differ from F-25 and I

Table 2. NN, NDW, SDW and RDW per plant of common bean at the pollination stage, under the treatments F-25, I-25, and IF-25 in the first year, and a fourth treatment I included in the second year.

Treatment	NN	NDW	SDW	RDW
		mg	g	g
2013				
I-25	57.75[a]	170.19[a]	7.37[b]	0.640[a]
F-25	38.00[b]	129.03[b]	7.67[ab]	0.537[a]
IF-25	40.50[b]	130.24[b]	8.850[a]	0.594[a]
Pr>Fc	0.0071	0.0109	0.0348	0.2467
CV%	12.98	10.52	7.87	14.05
		2014		
I	70.25[a]	182.75[a]	6.74[a]	0.70[a]
I-25	65.00[a]	165.50[ab]	7.45[a]	0.63[a]
F-25	41.75[b]	111.78[b]	7.86[a]	0.58[a]
IF-25	54.64[ab]	158.52[ab]	8.22[a]	0.61[a]
Pr>Fc	0.0107	0.0151	0.1089	0.1317
CV%	17.21	15.91	10.23	10.04

Mean values followed by the same letter in the column do not differ by the Tukey's test ($p < 0.05$). Pr>Fc = F probabilities.

Table 3. NP, NGP, DW100G and GY of common bean, under the treatments F-25, I-25, and IF-25 in the first year, and a fourth treatment I included in the second year.

Treatment	NP	NGP	DW100G	GY
			g	kg ha^{-1}
2013				
I-25	13.75[a]	5.75[a]	25.55[a]	2346.00[b]
F-25	13.25[a]	5.50[a]	25.76[a]	2248.69[b]
IF-25	15.00[a]	5.50[a]	25.69[a]	2509.42[a]
Pr>Fc	0.7327	0.9190	0.7609	0.2437
CV%	22.51	17.66	1.55	15.64
2014				
I	15.00[a]	5.25[a]	25.68[a]	2207.52[b]
I-25	18.50[a]	6.50[a]	26.44[a]	2529.85[ab]
F-25	17.25[a]	6.00[a]	26.17[a]	2474.77[ab]
IF-25	20.00[a]	5.50[a]	26.00[a]	2642.58[a]
Pr>Fc	0.3178	0.7733	0.1059	0.0137
CV%	20.35	19.25	1.47	17.97

Mean values followed by the same letter in the column do not differ by the Tukey's test ($p < 0.05$). Pr>Fc = F probabilities.

(Table 2). Therefore, the N fertilization at sowing reduced BNF potential in 2013 for F-25 and IF-25, but in 2014, the N fertilization associated with inoculation of the treatment IF-25 produced the same BNF potential as compared to the only inoculated at sowing treatments I and I-25.

At this pollination stage, the treatment IF-25 had SDW significantly higher than the I-25 in 2013; however, in 2014, the treatment F-25 did not differ from IF-25 and I-25 (Table 2). The SDW of all the treatments in 2014 were similar, indicating that the application of 20 kg N ha^{-1} at sowing reduced, but did not inhibit the BNF potential and plant growth. Hungria et al. (2003) also found the same results in the rainy season cultivation of common bean, applying 15 kg N ha^{-1} at sowing. On the other hand, the RDW of all the treatments were similar in both years (Table 2), in agreement with Pimentel. (2006) considering root growth of annual crops.

LSPC during ontogeny

The LSPC is proportional to the leaf RubisCO content and thus, it regulates the net photosynthetic rate and crop growth and yield (Long et al., 2006). The LSPC was similar in all treatments at the vegetative stage, before the fertilization with 40 kg N ha^{-1} at 25 DAE and in the pollination stage, in both years (Figure 5). Therefore, the inoculation without the N fertilization (20 kg ha^{-1}) at sowing, in the treatments I-25 in 2013 and I-25 and I in 2014, provided enough N by BNF for the initial LSPC synthesis in the same level as with sowing N fertilization (20 kg ha^{-1}) in the treatments F-25 and IF-25, for both years (Figure 5). Nevertheless, the treatments with N fertilization (20 kg ha^{-1}) at sowing, F-25 and especially IF-25, had the highest TDW (Figure 1), LAI (Figure 2) and high CGR (Figure 3) than the only inoculated at sowing treatments I-25 and I. Consequently, the total LSPC in a plant with increased LAI, as in the treatments F-25 and

IF-25, will be higher than in the treatments only inoculated at sowing treatments with lower LAI.

However, in the flowering stage, at 39 (2013) and 38 DAE (2014), the treatment IF-25 with high TDW had also a significantly higher LSPC than the treatment I-25 in 2013 (Figure 5A) and I in 2014 (Figure 5B). The higher LSPC probably ensured an increased RubisCO activity and photoassimilates production per plant (Pimentel et al., 1999; Long et al., 2006) increasing TDW (Figure 1), LAI (Figure 2) and CGR (Figure 3). At the grain filling stage, there were no significant differences for LSPC in both years (Figure 5). The LSPC was lower at this stage than for the stages before confirming the increased leaf senescence and reduced photosynthesis (Pimentel et al., 1999) in this important stage causing an accentuated abortion of reproductive organs well-known in common bean reducing its potential yield (Vieira et al., 2006).

Yield components

The yield components NP, NGP and DW100G of the treatments were similar in both years (Table 3), while the GY of the treatments was significantly different. The treatment IF-25 had significantly higher GY than the others in 2013 but only higher than I in 2014, when the treatments I-25 and F-25 did not differ from IF-25 and I (Table 3). Thus, the treatment IF-25, which had significantly higher TDW, LAI, CGR and LSPC for both years, produced a significantly higher GY than the others treatments in 2013 and a high GY in 2014.

The results of these experiments indicate that in the dry season, the use of inoculation in the treatments I and I-25 resulted in a similar yield as compared to the treatment with N fertilization at sowing (20 kg N ha^{-1}) and at 25 DAE (40 kg N ha^{-1}), but at lower cost. Therefore, these treatments can be recommended to small farmers with limited technological resources, as proposed by Pimentel. (2006), and it can increase common bean yield and production by these small farmers especially in the dry season (second crop). On the other hand, the mineral N fertilization (20 kg ha^{-1}) combined with inoculation and 40 kg N ha^{-1} at 25 DAE (treatment IF-25) resulted in the highest yield, and thus, this treatment can be recommended for farmers with higher technological resources to have high yield and annual production. However, further studies evaluating other cultivars and others locations are needed to support these recommendations.

Conclusions

In the dry season, the inoculation with *R. tropici* may replace the N fertilization (20 kg ha^{-1}) at sowing without yield loss for a low-cost agriculture for small farmers. However, the N fertilization (20 kg ha^{-1}) combined with inoculation with R. tropici at sowing and 40 kg N ha^{-1} at 25 DAE did not inhibit the root nodulation of common bean and increased its growth and grain yield for a high-cost agriculture for big farmers. In addition, the LSPC at the flowering stage was higher in the treatment with higher growth and yield, and thus, this physiological parameter can be used for selection of more productive common bean genotypes. However, more studies are required with other cultivars and sites, before recommending these agronomic practices of inoculation with or without sowing N fertilization to improve common bean yield in the dry season.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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Abbreviations

NN, Number of nodules; **NDW,** nodules dry weight; **SDW,** shoot dry weight; **RDW,** root dry weight; **NP,** number of pods per plant; **NGP,** number of grains per pod; **DW100G,** dry weight of 100 grains; **GY,** grain yield; **TDW,** total plant dry weight; **LAI,** leaf area index; **CGR,** crop growth rate; **NAR,** net assimilation rate; **LSPC,** leaf soluble protein content; **DAE,** days after emergence.

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Soybean yield of degraded pasture after reimplantation with and without phosphating

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The objective of this work was to evaluate the effect of different forms of reimplantation of pasture with and without phosphatation aiming to contribute to the increase of soybean yield. The experiment was conducted at the Experimental Farm of Universidade do Oeste Paulista (Unoeste), located in the municipality of Presidente Bernardes - SP. The design was done with split plot scheme, containing four replicates. The plots were constituted with 4 kg ha^{-1} of *Urochloa brizantha* (Marandu grass), BNS + Seeding in haul, BNS + Seeding in line consortium with soybean as subplots (with and without phosphatation). The following were analyzed: number of tillers and dry mass yield (PMS); analysis of plant tissue from pasture; foliar diagnosis analysis; and components of production and production of soy. Analyzed variables were submitted to analysis of variance ($p < 0.05$) and means were compared by the Tukey test ($p < 0.05$). In this context, it can be concluded that a higher quality production with a reimplantation of pasture intercropped with a soybean crop yielding an increase of 276 kg ha^{-1} compared to the treatment that did not have pasture reimplantation (BNS). Phosphate increased soil phosphorus content in the production of soybean dry matter and no leaf phosphorus content and higher soybean yield.

Key words: Natural seed bank, no-tillage, *Urochloa brizantha.*

INTRODUCTION

Soybean (*Glycine max* L.) is the most important oilseed crop in the world. It is currently one of the most important products in the Brazilian economy, occupying a prominent place in the supply of oil for domestic consumption, in animal feed as the main source of protein, as well as in the export agenda of the country (Val, 2014). Brazilian soybean production in the years 2014/2015 was equivalent to 45 million tons of grains (Conab, 2015). Soybean has been indicated as an alternative for disease prevention and use in the manufacture of flour, milk, textured protein, biodiesel, paints and varnishes, among others (Ávila et al., 2010).

Another great importance of soybeans is that it stands out as one of the main alternatives in crop rotation in no-tillage systems and, more recently, no crop-livestock integration system, as a consequence of the great

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efficiency in fixing N2 (Santos et al., 2006).

The results of different regions indicate that no-tillage can also favor a biological nitrogen fixation (BNF) in legumes (Alves et al., 2003).

However, it is often observed that the lack of management of the soil-plant-animal system, in association with the inadequate management of the enterprise, has led to the degradation of pastures which, lately, is the greatest obstacle to the establishment of a cattle ranch sustainable in agronomic, economic and environmental terms (Martha Júnior and Vilela, 2002).

Pasture degradation can be seen as an evolutionary process of loss of vigor, yield and natural recovery capacity of pastures to sustain economically, production levels and quality required by animals, as well as to overcome the harmful effects of pests, diseases and weeds culminating in the advanced degradation of natural resources due to inadequate management (Macedo, 2001). For this reason, the importance of the rotation of crops and pastures, which is technically and economically feasible strategy for the recovery and renewal of degraded pastures has been demonstrated (Cezar et al., 2000; Vilela et al., 2002).

The highest proportion of pasture area cultivated in Brazil is composed by plants of the genus *Urochloa* (Soares Filho, 1994). *Urochloa* species have high dry mass yield, excellent fertilizer response, are perennial, and remain green during moderate periods of water restriction. Among these species, marandu grass is the most used (Zimmer et al., 1998). The reasons for this preference are that this cultivar is tolerant to low soil fertility and grasshopper, presenting high yield when properly fertilized and managed.

Urochloa brizantha is characterized by its diversity of uses such as direct grazing, forage for fencing and silage, and more recently, for crop-livestock integration or as a straw-growing crop under no-tillage system (NTS) (Dias and Alves, 2008). In the present study, the use of biomass in tropical regions (Rodrigues and Rodrigues, 1987) is important, especially when it receives cultural treatments, soil correction, fertilization, and in some cases, irrigation.

The use of pastures in farming areas, for a period of time, can contribute to the improvement of the physical quality of soils. The herbage legume consortium, that is, the diversification of production, mainly in the use of forage, provides several advantages in the production system, both in the physical properties and in the chemical part of the soil (nitrogen fixation). It also reduces the use of pesticides, promotes the breakdown of diseases and pests, reduces the population of invasive plants, and has a great influence on the increase of farmers' profitability (Cobucci, 2001; Oliveira and Vidor, 2001). The dry mass residues of the pastures allow to recover the organic matter contents of the soil to values close to the original (Freitas et al., 2000; Wendling et al., 2005). Furthermore, plant residues are indispensable to increase the size and stability of the aggregates, favoring erosion control and soil resistance to compaction (Costa et al., 2015). Therefore, Igue (1984) reports that *Poaceae* contains a greater amount of root, also contributing to the improvement of soil porosity and aggregation.

In the case of phosphorus, since phosphorus (P) has low mobility (Barber, 1984) and low availability in oxidic soils (Novais et al., 2007), the amount of P available to plants can be modified, since the absorption of P is related to the amount of available nutrient (Anghinoni, 1992; Model and Anghinoni, 1992; Klepker and Anghinoni, 1995) and the different plant species and different soil textures that cause variations in the critical levels of phosphorus.

The most commonly used forms of phosphate fertilization in the production of grain crops are the haul on the surface with or without incorporation in the soil and in the sowing groove in strips (Sousa et al., 2004). Nunes et al. (2011) report that the use of surface phosphating is advantageous in a production system that has a higher response rate at planting, where fertilization occurs before or after planting. Therefore, in order to be able to perform highly efficient phosphating, its application must take place in the best way, allowing a better positioning in relation to the roots (Anghinoni and Barber, 1980), thus reducing the fixation by iron and aluminum oxides (Sousa and Volkweis, 1987).

The objective of this work was to evaluate the effect of different forms of reimplantation of pasture with and without phosphatation aiming to contribute to the increase of soybean yield.

MATERIALS AND METHODS

The experiment was conducted at the Experimental Farm of the Universidade do Oeste Paulista (Unoeste) in Presidente Bernardes - SP, at 22°17'27 "S, 51°40'51". The 385 m altitude in the period from January 2014 until February 2015. According to the Brazilian Soil Classification System (Embrapa, 2006), the soil of the experimental area is classified as dystrophic Red Argissolo, with smooth undulating relief.

The experiment was done with *Urochloa brizantha* (culture Marandu) with five years of implantation, but with low forage production capacity due to degraded pastures. The experimental design was done in bands in a subdivided plot scheme, with four replications. The plots consisted of four systems of pasture implantation and the subplots, with and without phosphate fertilization (Table 1).

For the implementation of T3 and T7 treatments, the John Deere seeder was used, seven rows with a 0.45 m spacing. For T4 and T8 implantations, two operations were carried out, the first for sowing the forage and the other for soybean sowing.

Before the implementation of the experiment, the soil chemical characterization was performed at a depth of 0 to 20 cm.

The following parameters were determined: organic matter, P (resin), K, Ca, Mg, pH and (H + Al), total cation exchange capacity (CTC) and base saturation. In January 2014, 1.0 Mg ha^{-1} of limestone and 1.0 Mg ha^{-1} of gypsum (Raij et al., 1997) were applied after chemical characterization of the soil. Phosphating was performed after limestone application with 500 kg of single superphosphate ha^{-1}.

Table 1. Systems of pasture implantation and subplots with and without phosphate fertilization.

Treatment	Plot-Systems	Subplot-Fertilizer With P
T1	NSB (control)	
T2	NSB + Seeding	Without phosphating
T3	NSB + Seeding	
	In line	
T4	NSB + Seeding in line Intercropped soybean	-
T5	NSB	
T6	NSB + Seeding	With phosphating
T7	NSB + Seeding	
	In line	
T8	NSB + Seeding in line Intercropped soybean	-

NSB: Natural seed bank; In the sowing of *Urochloa Brizantha* (cv. Marandu) were sowed 4 kg ha^{-1}.

Table 2. Chemical analysis of the soil before the implantation of the experiment.

Prof.	pH	M.O	P	SO_4^{-2}	Al^{+3}	H+Al	K	Ca	Mg	SB	CTC	m	v
		g dm^{-3}	mg dm^{-3}			$mmol_c$ dm^{-3}						%	
0-20 cm	5.2	11.5	1.7	6.2	0	19.6	0.9	10.1	9.1	20.1	39.6	0	50.6

The desiccation of the degraded pasture was carried out in December 2013 and the re-planting of the *U. brizantha* grass together with the soybean occurred at the beginning of January 2014. At the beginning of November 2014 the entire pasture of the area was desiccated and the line consorted with soybean, which served as a vegetable cover for soybean farming. The soybean cultivar used was TMG 1264 RR, which seeded 17 m^{-1} seeds at the end of November 2014. The treatment of the soybean seed was carried out with the agrochemical Fipronil in the dosage 40 ml 100 kg of seed, containing germination: 80% and minimum purity: 99%. The fertilization of sowing was 260 kg ha^{-1}, using formulation 04 30 10, being 10 kg ha^{-1} of N, 78 kg ha^{-1} of P_2O_5 and 26 kg ha^{-1} of K_2O, respectively, also performed A potassium chloride coverage fertilizer, the first at 20 DAE (days after emergence) at the dosage of 125 kg ha^{-1}, 75 of K_2O ha^{-1}. The equipment used to apply the liquid inoculant doses was coupled to the header of the seed drill, it has a tank with a capacity of 200 L and has a constant stirrer, providing a better homogenization of the solution. The inoculant was released when the seedlings touched the soil and started the sowing process, done in eight doses of the product. In this way, the solution was injected into all the sowing grooves at the same time. This solution was applied at a dosage of 50 L ha^{-1}.

The parameters evaluated were soil chemical analysis at a depth of 0 to 20 cm, a soil sampling of each treatment was carried out. After homogenization, 300 g were retained for the chemical analysis of fertility in the Unoeste second soil analysis laboratory (Raij, 2011). Samples were collected in an area of 0.15 m^2 (four replicates) for the determination of shoot dry matter yield. The collection was carried out five months after sowing of the pasture, randomly within the useful area of the plots. The plants were dried in an oven with forced circulation of air and temperature of 60 to 70°C until reaching constant mass (dry matter determination). After drying, the samples were ground in a Willey type mill to perform the nutritional analysis. In the R1 stage, the 3rd trifolia were collected from the apex on the main stem of 30 plants per plot. The leaves were dried in the forced circulation oven at 60°C for 48 h and then were ground and sent to the Laboratory of Analysis of foliar tissues of the Faculty of Agrarian Sciences of the University of the West (Paulista University) for macronutrient leaf analysis. At the end, soy production and productivity components were evaluated.

The variables analyzed in each treatment were submitted for analysis of variance ($p<0.05$) and the means were compared by the Tukey test ($p<0.05$) using the SISVAR software (Ferreira, 2011).

RESULTS AND DISCUSSION

None of the soil attributes were influenced by the interaction between pasture reimplantation systems and phosphate fertilization (Table 2). Grassland reimplantation systems influenced only the pH and organic matter (MO), potential acidity (H + Al) and magnesium (Mg) levels of the soil (Table 3). The highest values of pH were verified in the seeding system of the forage intercropped with soybean, in relation to the systems with natural seed bank and sowing of the forage to the haul (Table 3). However, in tropical soils under

Table 3. Chemical analysis of soil with and without phosphatization in diferente forms of pasture reimplantation.

Treat.	pH	M.O.	P	S	Al	H+Al	K	Ca	Mg	CTC	V
		g dm^{-3}	mg dm^{-3}		mmolc dm^{-3}						%
NSB	5.0^{b}	13.4^{b}	2.8	6.4	0.3	22.7^{a}	1.2	10.4	6.1^{b}	40.4	43.2
Seeding(S)	5.0^{b}	15.0^{a}	3.1	6.7	0.1	22.5^{ab}	1.2	11.3	6.3^{ab}	41.4	45.3
S + line	5.1^{ab}	15.5^{a}	2.9	5.9	0.0	23.0^{a}	1.0	12.0	7.0^{ab}	42.5	46.0
S + line + soybean	5.2^{a}	14.4^{ab}	3.4	3.3	0.0	20.3^{b}	1.2	13.0	9.0^{a}	43.3	52.1
CV	1.6	7.7	25.4	45.0	380.1	7.6	31.9	24.8	22.1	8.6	14.4
Phosp.											
with P	5.1	14.3	3.5^{a}	5.89	0.0	21.1^{b}	1.1	12.4	7.2	42.0	49.0
withoutP	5.1	14.8	2.7^{b}	5.25	0.2	23.0^{a}	1.2	11.0	7.0	42.0	44.3
CV	1.8	7.5	32.3	44.8	434.0	7.2	16.2	30.0	28.8	11.0	14.0
Probability (P≥F)											
Sist. (S)	0.013	0.021	0.505	0.481	0.436	0.044	0.513	0.379	0.031	0.590	0.116
Phosp. (P)	0.208	0.261	0.043	0.484	0.217	0.005	0.021	0.273	0.552	0.927	0.096
S × P	0.619	0.694	0.380	0.153	0.534	0.953	0.916	0.910	0.926	0.930	0.989

Means with the same letter in the columns do not differ by the Tukey test (P≤0.05). NSB, Seeding, Seending in line and Seeding in line + Soybean: natural seed bank, sowing of the forage to the haul, sowing of the forage in line and sowing of the forage in line consorciated with soybean, respectively.

Table 4. Dry mass yield (DMY) of forages in different pasture reimplantation systems, with and without phosphating.

Treatment	DMY (Mg ha$^{-1)}$
NSB	4.7^{b}
Seeding(S)	5.5^{ab}
S + line	6.1^{a}
S + line + soybean	6.6^{a}
CV	14.4
Phosphorus	
with P	5.6
withoutP	5.8
CV	18.5
Probability (P≥F)	
Sist. (S)	0.006
Phosp. (P)	0.554
S × P	0.706

Means with the same letter in the columns do not differ by the Tukey test (P≤0.05). NSB, Seeding, Seending in line and Seeding in line + Soybean: natural seed bank, sowing of the forage to the haul, sowing of the forage in line and sowing of the forage in line consorciated with soybean, respectively.

pasture it is rare to find excessively high values of pH, even if liming is frequent, based on technical recommendations (Oliveira et al., 2008).

The highest values of MO were verified in forage systems in line and in the haul, in relation to the system with natural seed bank. The forage seeding system in line with soy reduced consortium H + Al content compared to systems with natural seed bank and seeding forage increased in line and Mg content compared to the system with natural seed bank. The levels of H + Al were reduced with the use of phosphate fertilization in relation to the absence of this fertilization (Table 3).

According Raij (1991), Brazilian soils are poor in phosphorus as a result of its source material and the strong interaction of P with the ground, so the match can be considered the most limiting nutrient of biomass of tropical soils (Novais and Smyth, 1999). Prior to redeployment pasture together with the phosphorus fertilization was the value of P (1.7 mg dm^{-3}) after reimplantation of sowing and phosphorus fertilization systems was increased to P (3.5 mg dm^{-3}) (Table 3). However, the use of phosphate fertilization contributed to the increase of phosphorus levels, since there were no significant differences in P content (Table 3). Sa (2004) reports that the application of phosphate fertilizers haul without incorporation into tillage, it is a viable and fertilizer maintenance practice and/or refund for soils that have been fertilized and have average levels to high P (Table 3).

The highest yields of dry matter (DMY) of the forages were verified in forage sowing in line (6.1 Mg ha^{-1}) and forage in line with soybean (6.6 Mg ha^{-1}) and BNS (4.7 Mg ha^{-1}) (Table 4). Phosphate fertilization did not influence forage PMS. According to Kluthcouski et al. (2003), good soil protection requires about 7 Mg ha^{-1} of dry matter mass, even if the following work did not reach this value (7 Mg ha^{-1}), the dry mass yield found was very

Table 5. Mineral composition of pasture with and without phosphatization in different forms of pasture reimplantation.

Treatment	N	P	K	Ca	Mg	S
	g kg^{-1}					
NSB	15.1^{a}	2.1^{a}	19.0^{ab}	5.2	5.9	1.1
Seeding(S)	14.5^{ab}	2.0^{ab}	24.0^{a}	4.8	6.6	1.2
S + line	11.7^{bc}	1.5^{bc}	12.3^{bc}	4.6	4.8	1.1
S+line+ soybean	11.0^{c}	1.2^{c}	14.4^{c}	4.7	4.6	1.2
CV	22.2	23.2	21.3	18.5	26.1	27.7
Phosp.						
with P	11.9b	1.8	16.1	4.8	5.5	1.1
withoutP	14.3a	1.6	18.7	4.8	5.3	1.2
CV	9.6	20.1	21.1	28.8	30.6	24.5
Probability (P≥F)						
Sist. (S)	0.503	0.006	0.000	0.549	0.030	0.954
Phosp. (P)	0.000	0.047	0.071	0.911	0.780	0.466
S x P	0.058	0.099	0.490	0.284	0.216	0.557

Means with the same letter in the columns do not differ by the Tukey test (P≤0.05). NSB, Seeding, Seending in line and Seeding in line + Soybean: natural seed bank, sowing of the forage to the haul, sowing of the forage in line and sowing of the forage in line consorciated with soybean, respectively.

close to this (6.6 Mg ha^{-1}).

None of the attributes of the pasture mineral composition was influenced by the interaction between pasture reimplantation systems and phosphate fertilization (Table 5). In the table of mineral composition of the pasture in relation to the reimplantation systems of the pasture, the nitrogen content (N) was higher in the natural seed bank system (NSB) and lower in the seeding system of the forage intercropped with soybean (Table 5). Corsi and Nússio (1992) found that the increase of forage production has as one of the promoters, the adequate availability of nutrients, among which nitrogen stands out.

Thus, the same occurred for the phosphorus (P). As for potassium (K), the highest content was in the sowing system to the haul and the lowest in the sowing of the forage in line consorciada with soybean.In relation to phosphate, the difference was only observed for the N content, and when N fertilized with phosphorus, N was lower than without phosphate fertilization (Table 5).

The P contents of the soybean shoot were higher in the sowing system of the forage in relation to the sowing in line (Table 6). The use of phosphate fertilization also increased the levels of P of the aerial part of the soybean in relation to the absence of this fertilization, demonstrating the positive effect of this fertilization on the availability of P for the soybean crop, in a pasture reimplantation area. The levels of K, Ca, Mg and S were influenced by the interaction between pasture reimplantation and phosphatic fertilization systems (Tables 6 and 7). The highest levels of K were verified with the use of phosphatization in the forage sowing system to the haul. In the sowing system of forage intercropped with soybean, the highest levels of K were verified in the presence of phosphate fertilization, whereas in the absence of fertilization, the lowest value was verified in the sowing system of forage in the haul and superior in sowing of the forage in line consorted with.

In the sowing system of the forage to the haul the absence of the phosphate fertilization contributed to the value of Ca which was superior. The content of Ca in the presence of phosphate fertilization was higher in sowing of the forage in line, while in the sowing system of the forage to the lace was lower (Table 7).

In the forage sowing and sowing systems of in-line forage, phosphate fertilization contributed to lower Mg content in both systems of pasture reimplantation. In the absence of phosphatization, the highest Mg content was verified in the sowing system of the forage harvested and the lowest in the sowing of forage intercropped with soybean.

Malavolta (2006) established sufficiency ranges for nutrients P (4-5 g kg^{-1}), K (22-25 g kg^{-1}), Ca (9-10 g kg^{-1}), Mg (3,5), S (2.5-3.5 g kg^{-1}), in order to better understand and interpret the results of foliar diagnosis, therefore the contents of P and S are all below, both in the pasture reimplantation system variable and with and without phosphate fertilization. K has the ideal content only in the sowing system of forage intercropped with soybean. The Ca and Mg contents are all mentioned earlier.

Phosphate contributed to the decrease of S values

Table 6. Mineral composition of the aerial part of the soybean with and without phosphatization in different forms of pasture reimplantation.

Treatment	N	P	K	Ca	Mg	S
			g kg^{-1}			
NSB	30.9	2.3^{ab}	11.3^{b}	24.3	10.3^{ab}	1.6
Seeding(S)	29.3	2.4^{a}	12.7^{b}	26.6	11.7^{a}	1.5
S + line	29.8	2.1^{b}	14.1^{b}	29.0	10.4^{ab}	1.7
S+line+ soybean	32.6	2.2^{ab}	27.9^{a}	26.0	9.4^{b}	1.6
CV	8.1	9.6	11.5	14.5	9.9	6.7
Phosphorus						
with P	31.0	2.5^{a}	17.5^{a}	25.0^{b}	10.0^{b}	1.6
withoutP	30.3	2.0^{b}	15.5^{b}	28.0^{a}	10.9^{a}	1.6
CV	11.0	8.5	15.2	11.6	11.1	13.4
Sist. (S)	0.101	0.045	0.000	0.214	0.013	0.028
Phosp. (P)	0.519	0.000	0.049	0.034	0.059	0.628
S × P	0.083	0.064	0.012	0.023	0.020	0.043

Means with the same letter in the columns do not differ by the Tukey test (P≤0.05). NSB, Seeding, Seending in line and Seeding in line + Soybean: natural seed bank, sowing of the forage to the haul, sowing of the forage in line and sowing of the forage in line consorciated with soybean, respectively.

Table 7. Deployment of the mineral composition of the aerial part of the soybean with and without phosphatization in different forms of reimplantation of pasture.

Fosfatagem	NSB	Seeding	S+Line	S+Line+ Soybean
		K (g kg^{-1})		
With	12.67^{aB}	16.50^{aB}	13.87^{aB}	26.92^{aA}
Without	9.95^{aBC}	9.00^{bC}	14.32^{aB}	28.92^{aA}
		Ca (g kg^{-1})		
With	21.95^{aA}	24.02^{bA}	26.17^{aA}	27.87^{aA}
Without	26.65^{aAB}	29.12^{aAB}	31.12^{aA}	23.55^{aB}
		Mg (g kg^{-1})		
With	10.62^{aA}	10.20^{bA}	9.50^{bA}	9.77^{aA}
Without	10.02^{aBC}	13.15^{aA}	11.27^{aAB}	9.07^{aC}
		S (g kg^{-1})		
With	1.40^{bA}	1.47^{aA}	1.70^{aA}	1.72^{aA}
Without	1.82^{aA}	1.50^{aAB}	1.67^{aAB}	1.45^{bB}

Means with the same letter in the columns do not differ by the Tukey test (P≤0.05). NSB, Seeding, Seending in line and Seeding in line + Soybean: natural seed bank, sowing of the forage to the haul, sowing of the forage in line and sowing of the forage in line consorciated with soybean, respectively.

within the NSB system, whereas the opposite was verified in the sowing system of the forage intercropped with soybean, and the phosphate fertilization contributed to the increase of S in this system of reimplantation of pasture. In the absence of phosphate fertilization, the highest value of S was verified in the NSB system and the lowest value in the sowing system of the forage intercropped with soybean (Table 7).

None of the soil attributes was influenced by the interaction between pasture reimplantation systems and phosphate fertilization (Table 8). Regarding soybean yield, the highest value was verified in the system of sowing of forage intercropped with soybean and lower in the NSB and sowing of the forage to the haul. Phosphate fertilization contributed to the increase of shoot dry mass (MSPA) and soybean yield (Yield).

Table 8. Production and productivity components of soybeans with and without phosphate in different forms of pasture reimplantation.

Treatment	Pop. Plant m^{-1}	Pod n° $plant^{-1}$	Grain n° vagem	Weight 100 grãos	Yield Kg ha^{-1}
NSB	13.1	34.4	2.0	14.0	1.848[b]
Seeding(S)	12.7	31.0	2.2	14.2	1.886[b]
S + line	13.1	35.2	2.1	14.4	2.070[ab]
S+line+ soybean	13.4	32.7	2.0	14.5	2.124[a]
CV	12.1	34.5	10.9	7.8	7.4
Phosp.					
with P	13.0	32.9	2.0	14.8	2.106[a]
withoutP	13.1	33.6	2.0	13.9	1.854[b]
CV	15.9	48.5	10.8	8.7	8.5
Probability (P≥F)					
Sist. (S)	0.847	0.878	0.195	0.700	0.009
Phosp. (P)	0.947	0.901	0.702	0.071	0.001
S × P	0.728	0.491	0.061	0.089	0.394

Means with the same letter in the columns do not differ by the Tukey test (P≤0.05). NSB, Seeding, Seending in line and Seeding in line + Soybean: natural seed bank, sowing of the forage to the haul, sowing of the forage in line and sowing of the forage in line consorciated with soybean, respectively.

According to Malavolta et al. (1997), the P sufficiency range indicated for soybean is 2 to 5 g kg^{-1}, therefore, P levels are suitable for soybean cultivation.

In many areas under the ecosystem, soybeans have been shown to have higher yields on straw of *Brachiaria* genus, mainly in succession to *U. brizantha* (Pitol et al., 2001; Kluthcouski and Stone, 2003)

Conclusions

Soybean yield was higher with reimplantation of intercropped pasture with soybean crop. This treatment provided an increase of 276 kg ha^{-1} in relation to treatment that did not have pasture reimplantation.
Phosphate fertilization provided an increase in soil phosphorus content, production of soybean dry matter, on leaf phosphorus content, and higher soybean yield.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Organic matter sources in the composition of pelletized organomineral fertilizers used in sorghum crops

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Organomineral fertilizers have been used to meet plants' nutritional needs and reduce producers' reliance on mineral fertilizers. This study aimed to determine the effect of organic matter sources for organomineral fertilizers and traditional mineral fertilizers to the sorghum initial development. The experiment followed a randomized complete block design in a '4 x 3 + 2' factorial arrangement, with four fertilizer doses (50, 75, 100 and 125%) of the recommended dose for sorghum crops (450 kg ha^{-1}), three organic matter sources to compose the organomineral fertilizers (sewage sludge, filter cake, peat), a control (mineral fertilizer), and an untreated check (no fertilizers). Each experimental plot consisted of four plants divided into two pots. Plant height, stem diameter, chlorophyll *a*, chlorophyll *b*, and leaf area were performed at 30 and 60 days after seeding (DAS) when shoot dry mass was also measured. Organomineral fertilizers outperformed both control and untreated check plots for most variables at 30 DAS. Sorghum fertilized with organomineral fertilizers also showed positive results at 60 DAS, even with dose reductions. Considering the variables herein reported, organomineral fertilizers can replace mineral fertilizers in the development of sorghum, even with dose reductions.

Key words: Biofertilizer, sewage sludge, filter cake, peat, plant nutrition.

INTRODUCTION

High grain yields require high agronomic inputs, and among these, mineral fertilizers represent major investments, with approximately 13 and 24% of the total investment on sorghum (*Sorghum bicolor* (L.) Moench) crop production cycle (Wylie, 2008; USDA, 2016). However, despite the large costs, appropriate management of fertilizers and consequently of the soil fertility increases considerably the productivity of crops (Lopes and Guilherme, 2007; Hawkesford et al., 2014).

The fertilization with organic compounds is an option to the exclusive use of mineral fertilizers in agricultural production systems. Organic fertilizers are any product derived from plants, animals, urban or industrial residues, which is composed of degradable carbon, and may also be any substance that is present in the soil and has as source plants, microorganisms, excretions of fauna and everything that turns into humus after the decomposition (Silva and Mendonça, 2007; Chem, 2015). However, the

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Table 1. Chemical characterization of the Red Latosol (Oxisoil) used.

pH H_2O	Pmeh^{-1}	K^{+1}	Ca^{+2}	Mg^{+2}	Al^{+3}	H+ Al	BS	t	T	V	m	O.M.	C.O
----mg dm^{-3}----			----------cmolc dm^{-3}----------							----%-----		--dag Kg^{-1}---	
6.2	2.3	0.31	2.3	0.8	0	2.8	3.41	3.41	6.21	55	0	2.7	1.6

Water pH (1:2.5); P, K: extractor (HCl 0.05 mol L^{-1}); Al, Ca, Mg: extractor (KCl 1 mol L^{-1}); SB: base sum; t: effective CTC; T: CTC at pH 7; V: base saturation; m: Al saturation; O.M.: organic matter; O.C.: organic carbon.

exclusive organic fertilization is technically feasible only for some crops or in small areas; usually, the great amount of organic fertilizer needed to accomplish the nutritional requirement of most cultures would raise the cost of freight and turn organic fertilization impracticable.

The association of organic fertilizers with mineral fertilizers is an alternative for the production of organomineral fertilizers which have characteristics of both sources. Organomineral fertilizer formulation is variable as it is influenced by the amount of organic and mineral source used for its composition. Organomineral fertilizers have some characteristics in common, such as the gradual release of nutrients, increased agronomic efficiency of soil fertilization, can correct soil acidity and improvement of its physical characteristics (Kiehl, 2008).

The organic fraction mineralization of organomineral fertilizers can greatly contributes to increase the levels of nitrogen, phosphorus and sulfur in soil (Vezzani et al., 2008; Antille et al., 2013). This organic matter present in organomineral fertilizers also helps to reduce phosphorus fixation by oxides of iron and aluminum that are abundant in weathered soils (Rheinheimer et al., 2008; Castro et al., 2015). Moreover, the advantages of organomineral fertilization are not limited only to the crop season that receive the application, there is a cumulative residual effect in subsequent years, favoring the chemical, physical and biological properties of the soil (Ghosh et al., 2009). The organomineral fertilizers also have environmental benefits because they reduce the amount of organic wastes placed incorrectly on the environment, which could pollute water, soil and air.

Despite the benefits cited for organomineral fertilizers, information about its benefits in several cultures are incipient, important agricultural crops, as sorghum are still in need of studies with organomineral fertilizers. Sorghum is a C4 plant of tropical origin, adapted to conditions of high temperature and drought, and tolerant to various conditions of soil fertility. These features allow sorghum to be cultivated in a wide range of latitude, including areas where other cereals have low economic production (Smith and Frideriksen, 2000).

The sorghum high productivity is dependent upon a good initial plant development and the availability of nutrients during its crop cycle. Therefore, the adequate management of fertilization, and its sources, is one of the main reasons for proper establishment and productivity of sorghum. The study and development of options of fertilizers for the proper management of plant nutrition must be constant to allow the sorghum producers to use appropriately the fertilizers available.

Due to the need to find alternative sources to reduce production costs related to mineral fertilization, also, the lack of information on organomineral fertilizer application in sorghum and the possibility of correctly allocate an environmental waste produced by different sectors, this study aimed to evaluate the ability of organomineral fertilizers made from different sources of organic compounds to replace the application of mineral fertilizers on sorghum crop.

MATERIALS AND METHODS

The experiment was conducted from March to May 2015, in a greenhouse of the Universidade Federal de Uberlândia (UFU), located in Uberlândia, Minas Gerais state, Brazil (18°54' S, 48°15' W, 843 meters above sea level). The predominant climate of the region is subtropical climate type Cwa according to Köppen's (1948) classification.

The experimental design consisted of randomized blocks with four replications in a factorial structure '4 x 3 + 2', corresponding to four levels of organomineral fertilizer (50, 75, 100 and 125% of the dose of 450 kg ha^{-1} of NPK 5-17-10, according to the recommendation of Ribeiro et al., 1999), three sources of organic matter for the organomineral fertilizer (sewage sludge, filter cake, peat), and two additional treatments, being a treatment with mineral fertilization corresponding to 100% of the dose of organomineral fertilizer, and a control treatment with no fertilization. All fertilizers were produced with the formulation 0.1% of B, 3% of Si, 0.4% of Zn and 8% of total organic carbon (TOC).

The treatments plots were composed of two 5 liters pots, where four sorghum seeds (single-cross hybrid 1G100) were sown, at 3 centimeters depth. After 14 days, thin was performed to two plants per pot. Fertilization treatments were applied and mixed with soil prior to sown. The soil used was the Red Ratosol (Oxisoil) according to the classification of EMBRAPA (2013). Table 1 presents the soil chemical attributes. Analyses were performed at the laboratory of soil analysis (LABAS-UFU).

At 30 and 60 DAS after sowing, plant height, stem diameter, chlorophyll *a*, chlorophyll *b* and leaf area were analyzed. At 60 DAS, the plant shoot was harvested and dried in an air driven oven to obtain dry mass after observation of constant weight, about 72 h after drying. For plant height measuring ruler was used, being considered the distance of the neck until the end of the last leaf completely developed. The stem diameter was measured 1 cm above ground level with the aid of a digital caliper. For evaluation of chlorophyll *a* and *b*, chlorophyll meter was used (ClorofiLog Falker CFL 1030, Brasil) to evaluate the last two fully developed leaves, totalizing eight samples per plot.

Leaf area assessment were considered only by the leaves fully

Table 2. ANOVA of plant height (cm), stem diameter (mm), chlorophyll *a*, chlorophyll *b* and leaf area (cm^2), according to the source of organic matter (Source) and levels of organomineral fertilizer (Level) at 30 DAS of sorghum.

Source of variation	DF	Square mean				
		Height	Diameter	Chlorophyll *a*	Chlorophyll *b*	LA
Source	2	8.53*	0.66^{ns}	30.39^{ns}	5.46^{ns}	73.18^{ns}
Level	3	10.85**	0.88^{ns}	25.81^{ns}	2.14^{ns}	5667.78**
S x L	6	2.64^{ns}	0.05^{ns}	8.13^{ns}	0.56^{ns}	618.71^{ns}
Error	39	2.29	0.32	17.55	2.08	1020.60
CV%	-	10.34	13.58	16.28	21.91	28.20

** = Significant at 0.01 (p≤0.01); * = Significant at 0,05 significance (p≤0,05); ns = non significant; LA = leaf area.

Table 3. ANOVA of plant height (cm), stem diameter (mm), chlorophyll *a*, chlorophyll *b*, leaf area (cm^2) and dry mass (g), depending on the sources of organic matter in the composition (S) and levels of organomineral fertilizer (L) at 60 DAS of sorghum.

Source of variation	DF	Square mean					
		Height	Diameter	Chlorophyll *a*	Chlorophyll *b*	LA	SDM
Source	2	1.95^{ns}	0.68^{ns}	3.23^{ns}	0.54^{ns}	2966^{ns}	1.40^{ns}
Level	3	4.88**	2.44**	0.98^{ns}	0.51^{ns}	69260**	11.06**
S x L	6	0.43^{ns}	0.43^{ns}	2.85^{ns}	0.43^{ns}	12703^{ns}	2.20^{ns}
Error	39	0.91	0.50	3.50	0.60	6518	8.79
CV%	-	4.44	9.56	7.70	13.30	14.76	15.78

** = Significant at 0.01 (p≤0.01); ns = non significant; LA = leaf area; SDM = shoot dry mass.

submitted to the formula: leaf height × greatest leaf width × 0.75.

After attending the ANOVA assumptions (normality of residues (Kolmogorov-Smirnov), and homocedascity (Levene), both at p≤0.01), all data proceed its respective ANOVA analysis. When differences among organomineral sources were significant (ANOVA, p≤0.05), their averages were compared to each other by Tukey's test (p≤0.05); when differences among organomineral doses were significant (ANOVA, p≤0.05), their averages were compared by regression models.

The treatments including organomineral fertilization were compared to the additional treatments (mineral fertilizer and untreated check) by Dunnet's test (p≤0.05) when differences were significative (ANOVA, p≤0.05). Pearson correlation coefficient was calculated between plant height and shoot dry mass at 60 DAS only for treatments including the organominerals, excluding the data from the two additional treatments.

The statistical programs used were the SPSS 19.0 for Windows, Assistat (Silva and Azevedo, 2002), SISVAR (Ferreira, 2010) and SigmaPlot (Systat, 2008).

RESULTS AND DISCUSSION

The summary of the analysis of variance for the sorghum variables were analyzed at 30 DAS as presented in Table 2. The analysis of variance showed that the different sources of organic matter for the organomineral fertilizer composition significantly affected only plant height, while, for the levels of organomineral fertilizer, height and leaf area were significant. The interaction between the sources of organic matter and the levels of organomineral fertilizer was not significative for all variables at 30 DAS sorghum.

The summary of the analysis of variance for plant height, stem diameter, chlorophyll *a*, chlorophyll *b*, leaf area and dry mass as a function of sources of organic matter and levels of organic material fertilizers at 60 DAS for the sorghum crop is presented in Table 3. The different levels of fertilization significantly influenced the plant height, stem diameter, leaf area and dry mass. No significant interaction for any variables analyzed was detected.

Differing from what was observed at 30 DAS (Table 2), the evaluations at 60 DAS of sorghum presented no significant differences between the sources of organic matter used to compose the organomineral fertilizer (Table 3). Dereje et al. (2016) found parallel results by evaluating the development of sorghum in Ethiopia. The sorghum grain yield evaluated presented similar results among inorganic and organic fertilizers, and their combinations.

Stem diameter, chlorophyll *a*, chlorophyll *b* and leaf area, analyzed at 30 DAS of sorghum did not differ among organomineral sources (p≤0.05). However, at 30 DAS, the organomineral fertilizers formulated with peat resulted in the greater plant height (16.1 cm) than sewage sludge (14.73 cm). The plant height of the organomineral formulated with filter cake (14.97 cm) did not differ from the others (p≤0.05).

Table 4. Test of Dunnet for plant height (cm), chlorophyll *a*, chlorophyll *b*, stem diameter (mm) and leaf area (cm^2) of sorghum at 30 DAS with different levels of organomineral fertilizer composed with sewage sludge, filter cake and peat.

Treatments (%)	Height	Diameter	Chlorophyll *a*	Chlorophyll *b*	LA
Sewage sludge (50)	13.18	26.60	7.30*	3.77	88.70*
Filter cake (50)	13.49	23.40	6.08	3.81	97.94*
Peat (50)	15.81*[1]	27.25	6.54	4.27	105.41*[1]
Sewage sludge (75)	14.26*	26.11	7.31*	3.85	117.10*[1]
Filter cake (75)	14.96*[1]	23.30	6.11	4.24	109.37*[1]
Peat (75)	15.56*[1]	26.81	6.43	4.28	128.38*[1]
Sewage sludge (100)	15.13*[1]	29.08*[1]	7.56*	4.20	142.58*[1]
Filter cake (100)	16.18*[1]	27.60	7.03	4.37*	155.16*[1]
Peat (100)	15.47*[1]	25.81	5.99	4.56*	124.76*[1]
Sewage sludge (125)	16.37*[1]	30.49*[1]	8.15*[1]	4.49*	151.56*[1]
Filter cake (125)	15.25*[1]	27.00	6.93	4.42*	146.63*[1]
Peat (125)	17.56*[1]	27.83	7.49*	4.78*	133.46*[1]
Control	10.49	19.48	4.29	3.21	31.64
Mineral	11.39	19.45	5.00	4.04	53.21

* = Averages that differ from the control by Dunnet's test at 0.05 significance; [1] = Averages that differ from the mineral by Dunnet's test at 0.05 significance. LA = leaf area.

The height of the plant is of great morphophysiological importance, by directly reflecting on plant growth and differentiation (Taiz and Zeiger, 2010). This parameter influences all process that is involved with the soil-plant system and indicates that the sources of peat and filter cake are more appropriate to sorghum cropping. Comparisons of plant height, chlorophyll *a*, chlorophyll *b*, stem diameter and leaf area, at 30 DAS of sorghum, between the treatments with organomineral fertilizers and the treatment without fertilization (control) or with mineral fertilizer (Table 4).

There was a superiority of the organomineral fertilizers in relation to mineral for plant height and leaf area. Even reducing the application in 50% of the recommended dose, the use of organomineral fertilizers based on peat (T3) was enough to have a greater average height of plants when compared with mineral fertilization. The organomineral fertilizers, even at 50% of the recommended dose, also stood out as good sources to increase leaf area in sorghum in relation to exclusive mineral fertilization. For the other sources of organic matter, only doses from 75% of the recommended achieved the same effect (Table 4).

For chlorophyll *a*, two levels of organomineral with sewage sludge managed to overcome the control, being 100% or 125% of the recommended dose of 450 kg ha^{-1} for sorghum crop (Ribeiro, 1999). However, to achieve similar results of the mineral fertilization, any level of organomineral fertilizer can be used, regardless of the organic matter source used for its composition (Table 4).

The chlorophyll *b* contend was superior to control for the filter cake and peat at 100% dose, or for any of the organic source at 125%. Sources or doses did not differ from the mineral fertilizer. Both chlorophylls are important for sorghum plant development. Chlorophyll *a* is essential to the photochemistry phase of the photosynthesis, and while this photosynthetic phase is ongoing, other pigments assists light absorption and radiation transferring to the centers of reaction, and among these pigments there is chlorophyll *b* (Taiz and Zeiger, 2010). In this way, it can argued that there is great importance for the evaluation of these parameters during the development of sorghum.

In Table 5, comparisons of plant height, chlorophyll *a*, chlorophyll *b*, stem diameter, leaf area and sorghum dry mass at 60 DAS of sorghum, between the treatments with organomineral fertilizers and the treatments without fertilization (control) and with mineral fertilizer are presented.

At 60 DAS of sorghum, the organomineral fertilizers showed results similar to those found by mineral fertilizer for the variables plant height, chlorophyll *a* and *b*, and stem diameter, even at levels below the recommended for the crop. The exception was the lowest application (50%) for sewage sludge which showed lower chlorophyll content than with the use of mineral fertilizer. For leaf area, doses equal or superior to 75% of sewage sludge showed leaf area greater than exclusive mineral fertilizer treatment.

Despite the low concentrations of N, P and K in organic fertilizers, there is a complement of their concentration by the mineral fraction present in the organomineral. Therefore, there is a great efficiency in the use of organomineral fertilizer due to the slow release of nutrients during the plant growth (Ramesh at al., 2009; Hazra, 2016). Thus, even with the application of small

Table 5. Test of Dunnet for plant height (cm), chlorophyll *a*, chlorophyll *b*, stem diameter (mm), leaf area (cm^2) and sorghum shoot dry mass (g) at 60 DAS of sorghum with different levels of organomineral fertilizer composed with sewage sludge, filter cake and peat.

Treatments (%)	Height	Diameter	Chlorop. *a*	Chlorop. *b*	LA	SDM
Sewage sludge (50)	20.84[1]	22.21*[1]	4.75[1]	6.70	420.11	15.35
Filter cake (50)	20.53[1]	24.55	5.69	6.82	429.76	14.15
Peat (50)	21.32	25.02	5.90	7.74	514.20	19.45*
Sewage sludge (75)	20.74[1]	23.36[1]	5.63	6.96	619.91*	18.60*
Filter cake (75)	20.88[1]	23.73	5.69	7.72	543.71	19.25*
Peat (75)	20.91[1]	23.36[1]	5.60	7.37	495.06	17.00
Sewage sludge (100)	21.80	24.24	5.86	7.66	598.56*	19.50*
Filter cake (100)	21.90	23.25[1]	5.49	8.32*[1]	679.02*	24.00*[1]
Peat (100)	22.22	24.76	5.95	7.70	564.44	19.80*
Sewage sludge (125)	21.38	24.06	5.80	7.89*	617.25*	21.15*
Filter cake (125)	21.69	23.89	5.94	7.77	629.99*	24.13*[1]
Peat (125)	22.84	24.32	6.06	8.14*	604.00*	22.80*[1]
Control	21.34	26.09	6.06	6.69	398.19	11.35
Mineral	23.28	27.40	7.06	6.40	545.89	16.55

* = Averages that differ from the control by Dunnet's test at 0.05 significance; [1] = Averages that differ from the mineral by Dunnet's test at 0.05 significance. LA = leaf area. Chlorop. = Chlorophyll; SDM = shoot dry mass.

amounts of organomineral fertilizer, there is a great advantage for the plant due to the nutrient slow release.

The plants fertilized with the lowest levels of organomineral fertilizers composed of filter cake or sewage sludge showed an average plant height lower than those found with the mineral fertilization. The stem diameter of the s sewage sludge 50% dose was lower when compared to mineral and control treatment. The sorghum plants fertilized with organomineral fertilizers composed of peat at low level, 50% of recommended presented plant height and stem diameter equal to that found with exclusive mineral fertilizer (Table 5). No treatment was different from plant height from the control at 60 DAS of sorghum. Santana (2012) observed the same results presented in this work for maize crop, because the organomineral fertilizer showed no difference from the control even when varying the dose of fertilizer applied. For the variable chlorophyll a, the two lower levels of filter cake and the lowest level of peat organomineral were the treatments that stood out, which not differ from the mineral fertilization despite the lower quantity of nutrients applied (Table 5).

Reducing the amount of fertilizer applied may be due to increased cation exchange capacity of organic matter present in the organomineral fertilizer. This characteristic leads to greater availability of mineral nutrients for plants and reduction of losses by leaching (Troeh and Thompson, 2005). Neumann et al. (2005) observed promising results using a fertilizer organomineral, with lower concentrations of nutrients in relation to mineral fertilizer, managing to reduce in 5.72% the total cost of sorghum crop.

The chlorophyll *b* presented lower values when compared to mineral fertilization, for low doses of organomineral fertilizer (Table 5). Santana (2012) also found that there is no influence of organomineral fertilizers on the rates of chlorophyll *a* and *b* in maize, because none of the organomineral fertilizer showed no difference with respect to the control or even between different sources and forms of application.

In this study an increase in sorghum shoot dry mass at 100 and 125% organomineral fertilizer dose of from all organic matter source was observed (Table 5). Audu and Samuel (2015) also found good results for rice growth and yield mass with the use of organomineral fertilizer when compared with the exclusive mineral fertilization. The lower doses of organomineral fertilizers did not differ from the mineral fertilization for leaf area. However, with the use of fertilizer organomineral composed of sewage sludge, it was possible to verify greater leaf area with doses from 75% of recommended for the crop. The 125% dose of all organic matter source presented greater leaf area when compared to control, where no fertilizer was applied.

The plant height data at 30 DAS of sorghum were submitted to a regression analysis for the different levels of organomineral fertilizer based on sewage sludge, filter cake and peat (Figure 1A, B and C). The increased levels of sewage sludge as a source of organic matter caused a linear and positive response over the sorghum plant height at 30 DAS of sorghum (Figure 1A). Being observed that for every kilogram of organomineral fertilizer added, with up to 125 kg ha^{-1}, there was an increase of 0.0462 cm in height.

Using filter cake (Figure 1B) and peat (Figure 1C) as sources for production of organomineral fertilizers, the

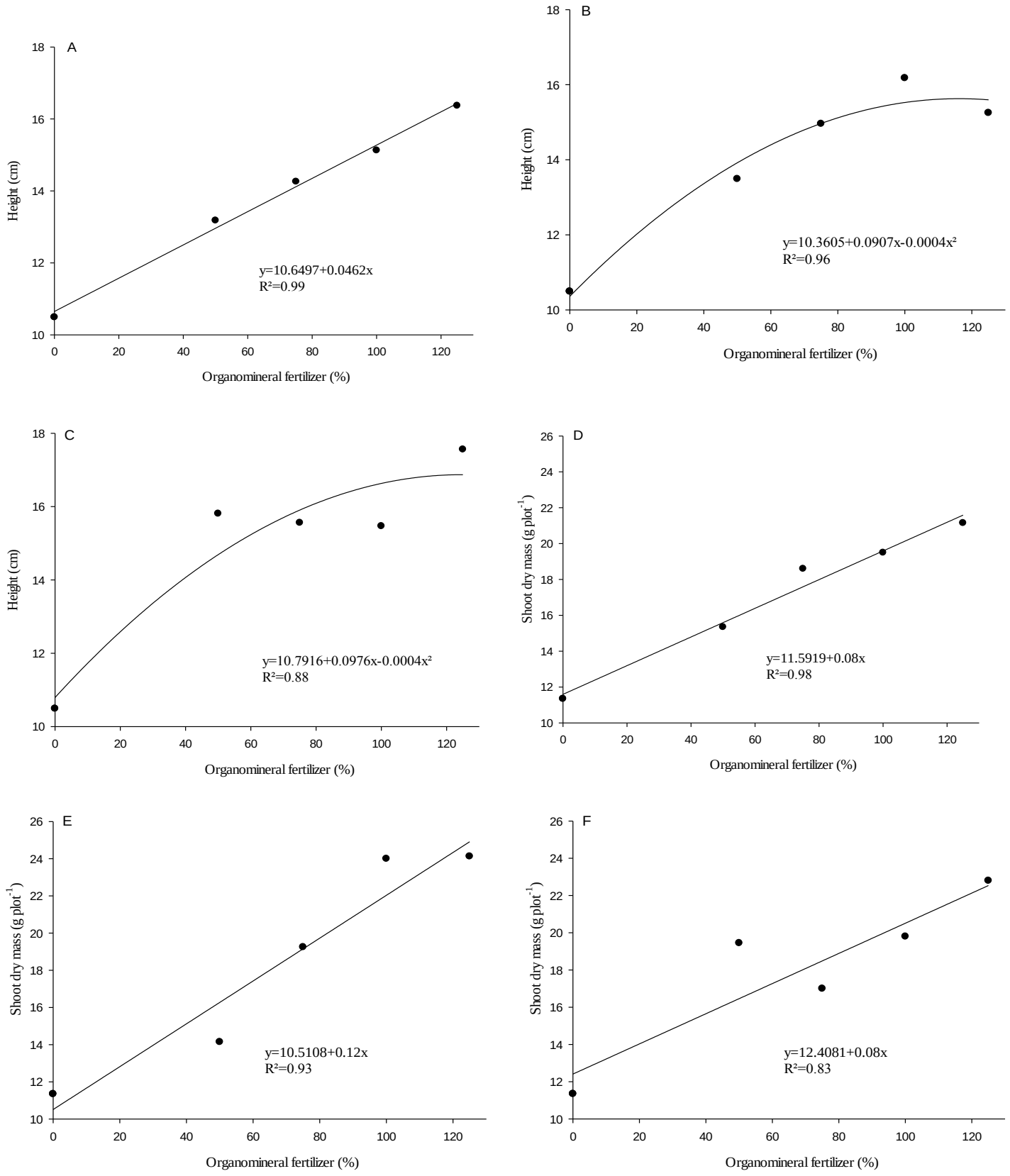

Figure 1. Sorghum plant height at 30 DAS and shoot dry mass at 60 DAS for sewage sludge (A, D), filter cake (B, E) and peat (C, F) based organomineral.

increase in height, at 30 DAS of sorghum, was positive until the doses between 113.38 and 122 kg ha^{-1}, resulting in a height of 15.5 and 16.7 cm, respectively. After these doses, there was a reduction of growth until the maximum dose of 125 kg ha^{-1}. As occurred with plant height, the diameter increase with the use of peat was linear until the dose of 125 kg ha^{-1}, with an increase in diameter of 0.012 mm for each kilogram of peat applied to the soil. However, Makinde (2015) studding amaranthus (*Amaranthus caudatus* L.) found that exclusive mineral nutrition produced taller plants, while a combination of organomineral with mineral fertilizer sources (50:50%) originated similar results to exclusive mineral fertilization regarding stem diameter, leaf area and mass yield.

The data of sorghum shoot dry mass at 60 DAS were submitted to a regression analysis for the different levels of organomineral fertilizer composed of sewage sludge, filter cake and peat (Figure 1D, E and F). At 60 DAS of sorghum, all sources of organomineral fertilizer presented linear increase on sorghum shoot dry mass as it increased the doses (Figure 1D, E and F).

Similar to this study findings, Smith et al. (2015) studding the use of organomineral as a replacement for mineral fertilizers found that in barley, wheat, maize and silage maize the dry silage matter production from both sources did not differ. In this study, the filter cake presented the best results at the highest dose, reaching the highest shoot dry weight part among the sources (Figure 1E).

The Pearson correlation coefficient involving plant height and shoot dry mass of the organomineral treatments at 60 DAS indicated a significative and positive correlation between these traits (p<0.000; r=0.506). Other studies also reported similar correlations between sorghum dry mass and plant height (Abubakar and Bubuche, 2013; Perazzo et al., 2014; Castro et al., 2015), suggesting that the use of organomineral fertilizers affects favorably sorghum plant development. The dry mass of plants is an indication of plant development. This parameter is affected mainly by plant shoot - depending on, for example, the number of leaves and leaf area - responsible for the interception of solar energy and, therefore, by the assimilation of carbon, which acts on the accumulation of dry mass.

Conclusions

The organomineral fertilizers formulated from sewage sludge, peat and filter cake can be used to replace exclusive mineral fertilization. Under greenhouse conditions, the organomineral fertilizers showed increase of plant biomass, plant height, stem diameter, chlorophyll *a* and *b* and leaf area in relation to control (no fertilizer) or exclusive mineral fertilization, what indicate organomineral fertilizers as feasible replacement for exclusive inorganic fertilization. This study demonstrate that it is possible to reduce the dose of organomineral fertilizer recommended for sorghum crop, and still reaching the same results or exceeding the values found in areas fertilized with the recommended mineral fertilizer dose. The use of organic residues for the production of organomineral fertilizer also is an alternative to the correct allocation of those.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Symbiotic efficiency of pea (*Pisum sativum*) rhizobia association under field conditions

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The objective of this study was to evaluate the symbiotic efficiency of rhizobia under field conditions for peas (*Pisum sativum* L.). Ten treatments were evaluated and divided into eight strains of rhizobia and two uninoculated strains as controls (with and without the addition of mineral N). The variables analyzed were: Nodulation, dry mass of the aerial part, total N of the aerial section, pea production and symbiotic efficiency. The inoculated rhizobia strains had effects on the number and mass of nodules, accumulated N in the aerial part and pea grains production. The strain EEL7802 presented the highest symbiotic efficiency for peas. The inoculation may allow cost reduction due to the equivalence with nitrogen fertilization. Studies in other soil types are needed to confirm the efficiency of this strain.

Key words: *Pisum sativum*, *Rhizobium leguminosarum*, nodulation, nitrogen biological fixation.

INTRODUCTION

Pea (*Pisum sativum* L., Fabaceae) is a grain legume, characterized by its high nutritional value and its potential use for human and animal feeding. The pea grains can be used for immediate consumption or canned for long-term storage, and can also be used for the preparation of instant soups. The huge industrial demand for peas has increased the cultivated area in Brazil by 36% between 2001 (1893 ha) and 2010 (2569 ha). In addition, pea production was 4442 and 5909 tonnes in 2001 and 2010, respectively. Despite this increase in production, Brazil still imports more than 80% of the consumed peas (FAO, 2012).

Pea crops, like other legume crops, are able to establish symbiosis with rhizobia (Van Rhijn and Vanderleyden, 1995). The pea plant absorbs nitrogen (N) obtained by the biological fixation by *Rhizobium leguminosarum* bv. *viceae* through symbiosis. Under conditions of low N availability in the soil, symbiosis can provide up to 80% of the N amount required for the growth of the pea plant (Voisin et al., 2002).

Before the 1990s, the number of rhizobial strains recommended for association with pea plant in Brazil was

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Table 1. Pea rhizobia strains by Epagri collection.

Strain	Source	Collection year
SEMIA 3007	*Embrapa, Londrina, Paraná state	-
USA 212-7	*Embrapa, Londrina, Paraná state	-
EEL 5501	São Jorge, Rio Grande do Sul state	2001
EEL 3001	São Jorge, Rio Grande do Sul state	2001
EEL 7802	Raul Basso farm, Vacaria, Rio Grande do Sul state	2002
EEL 1002	Rodrigo Barison Farm, Vacaria, Rio Grande do Sul state	2002
EEL 6802	Raul Basso farm, Vacaria, Rio Grande do Sul state	2002
EEL 13402	Bonanza farm, Lagoa Vermelha, Rio Grande do Sul state	2002

*Embrapa = Brazilian Agricultural Research Corporation, National Rhizobia Collection.

Table 2. Soil analysis of the experimental area at Triunfo Farm, Lages-SC.

Clay	Water:pH	SMP Index	P	K	OM	Al	Ca	Mg	H+Al	CEC
%	1:1		mg.dm^3		%			cmol$_c$/dm^3		
62	4.5	4.7	1.4	108	5.4	3.2	0.5	0.8	19.4	21.0

*OM = Organic matter; CEC = Cation exchange capacity; cmol = centimole.

few, and this was based on the studies conducted in Europe and Brazil (Conceição et al., 1981; Jensen, 1987). The isolation and selection of efficient N fixing rhizobial strains was initiated in Santa Catarina in 2001 (Brose and Muniz, 2008). However, most of the studies were performed under controlled conditions that use sterile substrates (sand and vermiculite) in pots and nutritious solution for the growth of the pea plants. However, this procedure is not recommended for the inoculation and culture of the rhizobial strains by the Ministry of Agriculture, Government of Brazil. The Agriculture Ministry requires field trials for the official recommendation of the rhizobial strains (SEMIA 3007 and USA 212-7). Therefore, the objective of this study was to evaluate the symbiotic efficiency of several rhizobial strains on pea plant under field conditions.

MATERIALS AND METHODS

The experiment was conducted through September to November 2005 in an Argisol. The Argisol represented the soil type specific for the growth of the pea plant in Brazil. Six strains of *R. leguminosarum* bv. *viceae*, which were previously selected through greenhouse experiments were obtained and evaluated from the diazotrophic bacterial collection of Epagri (Table 1) (Brose and Muniz, 2008).

Rhizobial strains, SEMIA 3007 and USA 212-7, which were previously recommended for the production of pea inoculants, were used as controls (Brasil, 2009). In addition, two non-inoculated controls were used: One without N fertilization and the other with a dose of 200 kg N ha^{-1} applied as urea by broadcasting method. Soil fertilization and correction were performed by using phosphorous pentoxide (220 kg P_2O_5. ha^{-1}) and potassium oxide (90 kg K_2O. ha^{-1}) according to the recommendations of the Brazilian Society of Soil Science (2004). This recommendation was based on the chemical analysis presented in Table 2. The chemical analysis was performed according to Embrapa (1997).

Pea seeds (cultivar: Spencer) were inoculated with the rhizobial bacterial broth in plastic packaging containing sterile peat. The inoculant was prepared at a ratio of 3:1 of peat and log phase cultures in 79 liquid media (Vincent, 1975). Two hundred grams of the inoculant was used per 20 kg of pea seeds. Planting was carried out on September 23, 2005 at the Triunfo Farm in the municipality of Lages, Santa Catarina. After 60 days of germination, the pea plants were collected to evaluate nodulation, dry mass of the aerial part, and the total N accumulated in the aerial part. After 60 days of flowering, the pea crop was harvested to determine the yield and the total N accumulated in the grain. The relative efficiency of the biological N fixation was calculated according to the following formula (Brockwell et al., 1966):

$$\mathrm{Ef}(\%) = \frac{\mathrm{Ninoc} - \mathrm{TestSN}}{\mathrm{TestCN} - \mathrm{TestSN}} \times 100$$

Ef (%) = Relative efficiency in biological nitrogen fixation; N_{inoc} = Total nitrogen in the inoculated treatment grains; TestCN = Total nitrogen in the control grains with addition of nitrogen; TestSN = Total nitrogen in the grains of the control treatment without addition of nitrogen. The Ef classes considered for strains selection were: 0 to 20% = inefficient; 21 to 40% = very low; 41 to 60% = low; 61 to 80% = average; 81 to 100% = high; and> 100% = very high.

The experimental design consisted of six randomized blocks. The area of each plot was 24 m^2 with a space of 40 cm between the rows. The results obtained were analyzed for variance analysis and Tukey's mean separation test. Statistical analysis was performed using the Matlab software. The values were considered statistically significant at $p < 0.10$, which was adopted as per the recommendations of the Brazilian Ministry of Agriculture (Brazil, 2009).

RESULTS AND DISCUSSION

The number of nodules was higher in the pea plants

Table 3. Number of nodules (NNOD), dry mass of nodules (MNOD), total nitrogen of aerial part (NTPA), dry mass of aerial part(MSPA), total nitrogen of grain (NTG), grain mass (MG) and relative efficiency (EF) in pea cv. 'Spencer'.

Strain	NNOD	MNOD	MSPA	NTPA	NTG	MG	EF
	N° planta^{-1}	mg planta^{-1}		mg N planta^{-1}	Kg N ha^{-1}	Kg ha^{-1}	%
SEMIA 3007	36.5^{bc}	83.1^{cb}	3400^{a}	105.9^{ab}	188.4^{a}	1040^{ab}	66.4
USA 212-7	30.9^{bcd}	165.3^{a}	2838^{a}	80.2^{b}	188.5^{a}	1016^{ab}	31.4
EEL 5501	50.8^{ab}	139.2^{ab}	3352^{a}	108.3^{ab}	186.6^{a}	1059^{ab}	64.6
EEL 3001	21.9^{cd}	21.6^{d}	4217^{a}	100.0^{ab}	163.9^{a}	1006^{ab}	41.9
EEL 7802	25.3^{cd}	36.9^{cd}	3298^{a}	91.7^{ab}	206.0^{a}	1170^{a}	84.1
EEL 1002	53.5^{ab}	42.7^{cd}	4033^{a}	108.0^{ab}	175.7^{a}	1066^{ab}	53.7
EEL 6802	67.4^{a}	46.6^{cd}	4603^{a}	129.1^{a}	181.7^{a}	1049^{ab}	59.7
EEL 13402	45.5^{abc}	49.4^{cd}	4037^{a}	113.1^{ab}	174.2^{a}	1063^{ab}	52.2
TEST. SN	10.6^{d}	26.4^{cd}	3125^{a}	86.1^{b}	122.1^{a}	762^{b}	0.0
TEST. CN	10.3^{d}	41.2^{cd}	3812^{a}	113.3^{ab}	221.9^{a}	1177^{a}	100.0
CV (%)	41.00	53.75	27.73	22.19	19.73	20.73	

*Averages with the same letter in the column do not statistically differ from each other by the Tukey's test ($p < 0.10$). CV = Coefficient of variation.

inoculated with the rhizobial strain EEL 7802 than in those inoculated with other strains (Table 3). The mass of the pea nodules ranged from 21.6 to 165.3 mg plant^{-1} and was higher with the USA 212-7 and EEL 5501 rhizobial strains than with other strains. Pea nodulation was also observed in the non-inoculated samples, indicating the presence of autochthonous noduliferous bacterial population in the soil, since there was no previous record of pea cultivation in the study area. The dry mass of the aerial plant part did not vary much between inoculated treatments and controls. The total N of the aerial plant part varied between 80.2 and 129.1 mg plant^{-1}. Inoculation of the EEL 7802 strain resulted in higher N fixation in the aerial part than in the non-inoculated plants and plants without N fertilization. It was found that the number and mass of nodules of the inoculated pea plants had no correlation with pea grain production, which is in accordance with the observation that the efficiency of N fixation and the competitive capacity of a strain are not necessarily correlated (Romdhane, 2007).

Variations were observed in the N content of the aerial plant part and in the grain yield of the pea plants inoculated with different rhizobial strains. However, no differences were observed in the dry mass production of the aerial plant parts. These results could be attributed to the interaction between the soil and the symbiotic performance of the strains inoculated with the studied pea variety (He et al., 2011). Consequently, soil N levels have an effect on the nodulation and biological N fixation, which can be promoted at relatively low levels; however, soil N levels are suppressed at high nutrient concentrations (Eaglesham, 1989). Therefore, efficient strain selection should also consider the adaptation of rhizobia to the N availability conditions and the soil environment (Chen et al., 2004). Therefore, relatively high levels of organic matter (Table 2) in the experimental area might have been a source of N in the soil, and hence influenced N accumulation in the aerial part of the pea plant.

Grain production in the pea plants that received N fertilization did not differ much from that obtained in the plants inoculated with any rhizobial strain. These results suggest that similar grain yield can be obtained without the need of nitrogen fertilization. Hence, inoculation of pea seeds with rhizobia can be regarded as a more economical form of pea production. Grain productivity in the pea plants inoculated with the EEL 7802 rhizobial strain was 53% higher than in the control without N application, although no differences were observed in the N content of the grains. Experiments with N application in different varieties of pea plants have shown contrasting results (Mckenzie et al., 2001), where the production of grains in different cultivars ranged from 390 kg ha^{-1} in the 'Kodama' cultivar (Moreira et al., 2006) to 1.266 kg ha^{-1} (De Souza Romero et al., 2008) in the 'Maria' cultivar. Furthermore, experiments with inoculation of pea seeds with *Rhizobium* did not consistently reveal increase in the grain yield (Mckenzie et al., 2001; Ahmed et al., 2007). However, previous study had shown that inoculation with *Rhizobium* increased pea productivity (Rani et al., 2016; Huang et al., 2017).

The relative efficiency of the symbiotic N fixation of the rhizobial strains was high with respect to inoculation of the EEL7802 strain, which demonstrated highest relative efficiency of N fixation during symbiosis with the Spencer pea variety (Table 3).

Conclusions

The inoculation of pea seeds with rhizobial strains produced grain amounts similar to the amount produced upon the addition of 200 kg N. ha^{-1}, and hence, was considered a more economical practice. The EEL7802

strain showed the highest symbiotic efficiency with the pea plant under field conditions in the tested environment, and field-efficiency studies in other ecosystems are necessary to confirm the efficiency of the EEL7802 strain.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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