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

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

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

INTRODUCTION

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

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

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

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

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

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

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

MATERIALS AND METHODS

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

Physiological aspects of the crop under partially controlled conditions

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

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

Effect on emergency and productivity of the soybean in the field

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

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

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

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

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

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

*mL kg^{-1} of seeds.

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

Statistical analysis

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

RESULTS

Physiological aspects of the crop under partially controlled conditions

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

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

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

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

Effect of micronutrientes, amino acids and hormones in the field

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Conclusions

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

Conflict of Interests

The authors have not declared any conflict of interests.

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