

## Application of glycine on soybean plants submitted to water deficit

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### Abstract

Soybean is one of the most important crops in the world. Studies are necessary to improve its productivity, especially in stress environments. Therefore, the objective of this study was to evaluate the effect of glycine as seed treatment to soybean plants submitted to water deficiency, using twelve replicates per treatment. Glycine was applied at a dose of 9 mg kg<sup>-1</sup> of seeds under high water deficit (performed at stage V4) and without water deficiency. Root development, antioxidant metabolism and dry mass accumulation of plants were evaluated. Results showed that the application of glycine to plants that were not subjected to water deficiency, promoted the increase of root development, accumulation of mass and reduction of stress in plants. This reflected in 10% increase in productivity compared to the control treatment. On the other hand, plants with glycine application subjected to water deficiency showed a reduction in dry mass accumulation and root development, indicating that these plants suffered the effect of stress. Untreated plants submitted to water deficiency showed symptoms of stress such as reduced accumulation of mass and productivity by 12%. Therefore, the present study reports that the application of glycine on seeds is not very efficient for attenuating stress in soybean plants submitted to water deficiency. However, in environments without water deficiency, the application of glycine on seeds affects the greater development of the plant and increased productivity.

**Keywords:** Glycine max (L.) Merrill, seed treatment, amino acids, drought.

### Introduction

Soybean is among the most economically important crops in the world, both as a commodity that participates in the future market and for its transformation into food and fiber. After a great evolution of cultivated area and increased productivity since the 90s (Cattelan and Dall’Agnol, 2017), Brazil consolidated itself as the country with the highest world production (134 million tons) and cultivated area (38.6 million hectares) in the world in the 20/21 harvest (USDA, 2021). One important aspect that still to be considered is to improve soybean productivity in stress environments. Drought stands out for affecting the productivity of many regions where irrigation is not practiced or not feasible. In Brazil, research shows that the soybean yield gap due to drought can reach up to 1600 kg.ha<sup>-1</sup> (Sentelhas et al., 2015; Reis et al., 2020). In China, soybean productivity was lost up to 21.8% at severe drought (Wang et al., 2020).

Research shows that the application of glycine seems to be a promising practice to stimulate growth or mitigate plant stresses (Mosa et al., 2021, Shooshtari et al., 2020; Mohammadipour and Sour, et al., 2019, Noroozlo et al., 2019a, Noroozlo et al., 2019b), although some reports have shown a reduction in development of plants (Matysiak et al., 2020; Khan et al., 2019). Glycine, one of the 20 essential amino acids for plants, is a neutral, non-polar amino acid, synthesized from serine during photorespiration. Part of the glycine can be converted into glutamate, which can then be

metabolized to  $\gamma$ -aminobutyric acid (GABA) (Igamberdiev and Kleczkowski, 2018). It is already known that GABA acts as an important signal for the regulation of plant growth and development, mediating response to stress tolerance due to low light, salinity, nitrogen deficiency, water deficit, high temperatures and regulates antioxidant defense systems (Ramos-Ruiz et al., 2019). It is known that glutamate, via glutamate receptors (GLRs), is a fundamental signal for tolerance to biotic and abiotic stresses (Qiu et al., 2020).

Although not yet consolidated, there are two other plant mechanisms that may be associated with the effect of glycine on stress tolerance. First, the well-known effect of glycine betaine (GB) against plant stresses (Annunziata et al., 2019; Shehzadi et al., 2019; Xu et al., 2018). GB is synthesized from choline, which originates from the same metabolism that produces glycine (Xu et al., 2018). Some studies show that choline is synthesized from methionine (Buchanan et al., 2015), while others show that ethanolamine, the basis for choline synthesis, is synthesized from serine (Lin et al., 2020). Therefore, it is possible that there is some interaction between the glycine metabolism and glycine betaine synthesis in plants. Second, it has recently been shown that proteins rich in glycine (GRP) are involved in stress-related metabolisms. GRP are characterized by a high concentration of glycine (more than 70%). The expression of these proteins is involved in tolerance to stresses at low and high

temperatures, salinity, wounds, drought, oxidative stress and biotic stresses (Czolpinska and Rurek, 2018).

Thus, the glycine pool in plants may be involved in several mechanisms related to the tolerance of biotic and abiotic stresses. Although information about this is scarce, the application of glycine can also be a strategy to mitigate the effects of water deficit in plants. Teixeira et al. (2019) demonstrated that the application of proline via leaf is an efficient alternative to mitigate the effect of moderate water stress in soybeans, while under high water deficit, the application of glutamate via leaf or in the treatment of seeds is more efficient to minimize the effects of stress. However, information on the effect of glycine application on soybean crops is still scarce, especially under stressful situations.

## Results

### *Sampling performed at the V<sub>3</sub> stage*

This analysis showed that the application of glycine provided increases of 54, 53, 161, 55 and 110% in the variables: projection area (PA), volume (Vol), main root length (MLR), total root length (TLR) and number of secondary roots (NSR), respectively.

However, despite these positive effects observed from the application of glycine in the parameters related to the root, there was no difference in the accumulation of dry mass in relation to the control, except in the dry mass of the stem, which increased 17% in relation to the control treatment (Table 1). The application of glycine on seeds reduced activity of some enzymes, among them the nitrate reductase (NR), and the enzymes of the antioxidant metabolism superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD). This reduction reached 57% for CAT and 47% for SOD. The lower activity of antioxidant enzymes increases plant lipid peroxidation (LP) by 50%.

Plants submitted to seed treatment with glycine also showed a higher concentration of proline (Prol) in the leaves (Table 2).

### *Sampling performed at the V<sub>6</sub> stage*

There was a reduction in the projection area (PA), total root length (TLR) and number of secondary roots (NSR), in plants that were treated with glycine and subjected to water deficiency (Table 3). The same treatment led to a reduction in dry mass of root (RDM), stem (SDM), leaves (LDM) and total (TDM). The decrease reached 27, 25 and 24% for the parameters of LDM, TDM and SDM, respectively, in relation to the control treatment without water deficiency. The activity of the enzymes nitrate reductase and urease was higher in plants treated with glycine and submitted to water deficiency (Table 4). Plants without treatment and subjected to water stress showed a 27% reduction in the relative leaf water content (RWC), compared to plants without water deficiency. As a consequence, there was an increase of 48% in the lipid peroxidation (LP) of these plants. Plants submitted to seed treatment with glycine and submitted to water deficiency did not show a reduction in RWC and an increase in LP, at stage V<sub>6</sub>.

### *Sampling performed at the V<sub>8</sub> stage*

No statistical difference was observed in the dry mass of plants at the V<sub>8</sub> stage. (Table 5). The same was observed in number of pods (NP). The pod dry mass (PDM) was higher in plants treated with glycine at non-stress conditions. The same

plants showed higher productivity (Prod), with an increase of 10% compared to control. Plants submitted to water deficiency, with and without glycine treatment, showed lower productivity compared to other treatments.

## Discussion

The application of glycine as seed treatment promoted an increase in root development at stage V<sub>3</sub> (Table 1). Glycine can play the role of signaling in plants, as they could bind to GLRs (Glutamate Receptors), initially so named because glutamate is the first amino acid discovered for these receptors (Dubos et al., 2003). GLRs activate calcium signals, which in turn, signal various processes in other parts of the plant, such as alteration of carbon and nitrogen metabolism (Price et al., 2012; Forde and Roberts, 2014), characteristics that can have an impact on further development of roots, as observed in our experiment. Teixeira et al. (2019), also showed that the application of glycine in seed treatment increases the development of roots in soybean plants. Some benefits of applying glycine were also observed at stage V<sub>6</sub> (Table 3), with an increase in RDM, TDM, SOD and a reduction in LP. Characteristics that may have had an impact on the increase in the PDM and productivity are shown in Table 5. Teixeira et al. (2018), reported an increase in productivity of plants under application of glycine at the rate of 9 mg kg<sup>-1</sup> of seed, as observed in this experiment. The activation of GLRs provided by glycine can also lead to increased antioxidant metabolism and the consequent reduction in plant stress (Weiland et al., 2015). In addition, glycine is part of the structure of several proteins, mainly those linked to the formation of the cell wall, whereas about 70% of these proteins are formed by glycine (Buchanan et al., 2015, Ringli et al., 2001). Glycine can act in the stress response since it is part of the formation route of glycine betaine, a compatible solute that acts as an osmoprotector in plants, especially under salt stress conditions (Demiral and Turkan, 2006). According to Hu et al. (2012) from the production of glycine betaine, various signaling processes begin in plants, such as increased activity of antioxidant enzymes and the consequent reduction in lipid peroxidation, which was observed in this experiment. However, plants that were subjected to seed treatment with glycine and water stress showed a reduction in root parameters and mass accumulation (Table 3), with reduced root development and accumulation of total dry mass in plants. Cell growth is the most sensitive process to low water availability, and cell division and expansion are directly inhibited by water stress. For this reason, reduced growth is considered the first consequence of water restriction in vegetables (Taiz et al., 2017). The effect of glycine has been possibly faded up throughout the development of the plant, under stress condition and the signaling potential of this amino acid was not effective to alleviate water stress to reduce plant development. Therefore, it is believed that direct application of glycine on leaves is more effective under stress conditions, as this type of application activates the antioxidant metabolism of plants and improves the defense system (Teixeira et al., 2017). The control treatment (no-glycine) plants, subjected to water stress showed increased NSR (Table 3), possibly with the objective of increasing the water catchment area in the soil and trying to survive under stress conditions. Under water deficit conditions, the hormones abscisic acid (ABA) and

**Table 1.** Projection area (PA), volume (Vol), main root length (MLR), total root length (TLR), number of secondary roots (NSR), root dry mass (RDM), leaf dry mass (LDM), stem dry mass (SDM) and total dry mass (TDM) at the V<sub>3</sub> stage, for soybean cultivar RK6813RR, submitted to the application of glycine (Gly) and control on seed treatment (ST).

Treatments	PA	Vol	MLR	TLR	NSR	RDM	LDM	STD	TDM
	cm <sup>2</sup>	cm <sup>3</sup>	cm		-	g plant <sup>-1</sup>			
Control	56.65	0.17	92.30	277.0	34.33	0.129	0.059	0.052	0.305
Glycine	87.30*	0.26*	241.19*	430.5*	72.00*	0.111	0.065	0.061*	0.299

Asterisks indicate statistically significant difference between treatments, using the Student's t-test, p<0.05.

**Table 2.** Activity of the enzymes nitrate reductase activity (NR), lipid peroxidation (LP), activity of the enzymes superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), peroxide hydrogen content (H<sub>2</sub>O<sub>2</sub>) and proline content (Prol), at the V<sub>3</sub> stage, for soybean cultivar RK6813RR, submitted to the application of glycine (Gly) and control on seed treatment (ST).

Treatments	NR	LP	SOD	POD	CAT	H <sub>2</sub> O <sub>2</sub>	Prol
	µg [N-NO <sub>2</sub> <sup>-</sup> ] g <sup>-1</sup> [fresh biomass] h <sup>-1</sup>	nmol [TBARS] g <sup>-1</sup> [fresh biomass]	U mg [protein]	µmol min <sup>-1</sup> mg [protein]		µmol g <sup>-1</sup> [fresh biomass]	
Control	4.40*	38.58	313.4*	11.98*	612.1*	4.71	0.04
Glycine	1.57	57.99*	164.2	1.65	261.1	4.61	0.10*

Asterisks indicate statistically significant difference between treatments, using the Student's t-test, p<0.05.

**Table 3.** Projection area (PA), volume (Vol), main root length (MLR), total root length (TLR), number of secondary roots (NSR), root dry mass (RDM), leaf dry mass (LDM), stem dry mass (SDM) and total dry mass (TDM) at the V<sub>6</sub> stage, for soybean cultivar RK6813RR, submitted to the application of glycine (Gly) and control on seed treatment (ST), associated to water deficit levels (WD): high WD (40% of field capacity - FC) and without WD (80% FC).

Treatments	Water deficit level	PA	Vol	MLR	TLR	NSR	RDM	LDM	SDM	TDM
		cm <sup>2</sup>	cm <sup>3</sup>	Cm		-	g plant <sup>-1</sup>			
Control	Without	85.06 a	2.46 a	33.10 a	2306.1 a	2765.7 b	0.60 b	0.25 ab	0.24 a	1.08 b
	Hight	87.83 a	2.46 a	33.22 a	2616.6 a	3348.3 a	0.55 b	0.29 a	0.25 a	1.09 b
Glycine	Without	82.88 a	2.31 a	33.34 a	2298.7 a	2399.5 bc	0.80 a	0.29 a	0.26 a	1.35 a
	Hight	60.30 b	1.91 a	32.74 a	1528.5 b	2078.3 c	0.43 c	0.21 b	0.19 b	0.82 c

Means followed by the same letters do not differ significantly from each other, using the Duncan test at 5% significance.

**Table 4.** Activity of the enzymes nitrate reductase activity (NR) and urease (U), leaf relative water content (RWC), lipid peroxidation (LP), activity of the enzymes superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), peroxide hydrogen content (H<sub>2</sub>O<sub>2</sub>) and proline content (Prol), at the V<sub>6</sub> stage, for soybean cultivar RK6813RR, submitted to the application of glycine (Gly) and control on seed treatment (ST), associated to water deficit levels (WD): high WD (40% of field capacity - FC) and without WD (80% FC).

Treatments	Water deficit level	NR	U	RWC	LP	SOD	POD	CAT	H <sub>2</sub> O <sub>2</sub>	Prol
		µg [N-NO <sub>2</sub> <sup>-</sup> ] g <sup>-1</sup> [fresh biomass] h <sup>-1</sup>	µmol [N-NH <sub>4</sub> <sup>+</sup> ] g <sup>-1</sup> [fresh biomass] h <sup>-1</sup>	%	nmol [TBARS] g <sup>-1</sup> [fresh biomass]	U mg [protein]	µmol min <sup>-1</sup> mg [protein]		µmol g <sup>-1</sup> [fresh biomass]	
Control	Without	23.97 b	3.83 b	23.30 a	48.44 b	127.2 b	1.70 a	80.00 b	8.14 a	0.15 a
	Hight	15.19 b	4.58 b	17.05 b	71.55 a	165.1 ab	2.36 a	196.7 a	8.35 a	0.17 a
Glycine	Without	16.95 b	3.71 b	26.25 a	48.90 b	176.7 a	2.44 a	43.29 b	7.12 a	0.16 a
	Hight	38.03 a	6.11 a	25.05 a	63.87 ab	144.2 ab	2.04 a	38.58 b	7.21 a	0.25 a

Means followed by the same letters do not differ significantly from each other, using the Duncan test at 5% significance.

**Table 5.** Root dry mass (RDM), leaf dry mass (LDM), stem dry mass (SDM), pod dry mass (PDM), total dry mass (TDM) and number of pod (NP) at the R<sub>8</sub> stage, and productivity (Prod), for soybean cultivar RK6813RR, submitted to the application of glycine (Gly) and control on seed treatment (ST), associated to water deficit levels (WD): high WD (40% of field capacity - FC) and without WD (80% FC).

Treatments	Water deficit level	RDM	LDM	STD	PDM	TDM	NP	Prod
		g plant <sup>-1</sup>						-
Control	Without	3.39 a	2.09 a	1.79 a	2.31 ab	9.58 a	69.75 a	1.85 b
	Hight	3.97 a	2.36 a	1.80 a	2.41 ab	10.54 a	69.25 a	1.62 c
Glycine	Without	3.81 a	2.55 a	2.05 a	2.54 a	10.96 a	70.75 a	2.03 a
	Hight	4.47 a	2.59 a	2.30 a	1.92 b	11.28 a	68.50 a	1.54 c

Means followed by the same letters do not differ significantly from each other, using the Duncan test at 5% significance.

ethylene balance that mediates different responses in plants. ABA induces stomatal closure to prevent water loss in plants. In addition, this hormone inhibits the increase of ethylene in the root, which stimulates root growth. On the other hand, the increase in ethylene in the aerial part provides a reduction in leaf growth (Salazar et al., 2015; Taiz et al., 2017). Therefore, these characteristics explain the increase in plant NSR. In addition, the stress resulted in greater lipid peroxidation and a reduction in the relative water content in the leaves, characteristics that reflected in the reduction of the productivity of these plants (Table 5).

## Materials and Methods

### Experimental design and conditions

The experiment was carried out in a greenhouse located in the University Center of Patos de Minas (Unipam), municipality of Patos de Minas, MG, Brazil (18° 34' S, 46° 31' W and 815 m asl). Soybean plants [*Glycine max* (L.) Merrill], cultivar RK6813RR of medium cycle and indeterminate growth habit were used in a randomized block design, consisting of the application of glycine (pure amino acid Sigma Aldrich®, with optical isomerism levogiro - L-amino acid) and one control (distilled water), applied as seed treatment (ST), using twelve replicates for each treatment. These treatments were associated to water deficit levels, ranging from conditions of no water deficit to high water deficit. Plants were grown in pots of 10 dm<sup>3</sup> capacity, filled with washed sand (washed sand of medium texture, with grain sizes between 0.05mm and 0.8mm). During the conduction of the experiment, the pots were irrigated daily according to the water requirement. A weekly application of nutrient solution was also applied as proposed by Johnson et al. (1957). Glycine was diluted in distilled water and applied to seeds at the concentration of 9 mg kg<sup>-1</sup> [seed] (40 mM), with a volume of 4 mL kg<sup>-1</sup> [seed]. Before sowing, seeds were treated with fungicide and insecticide (fipronil + piraclostrobin + methyl thiophanate) at the rate of 1 mL kg<sup>-1</sup> [seeds]. The control treatment consisted of distilled water as a substitute for the diluted glycine. For the application of water deficit levels, it was necessary to determine the field capacity (FC) of the substrate (sand) in the pots. 80% of the field capacity was adopted (taken as a water potential of -0.05 MPa) as the minimum soil water content for the treatment without water deficit. The treatments with high water deficit corresponded to vessels conducted at 40% of FC (-1.5 MPa). The water deficit started when the plants were at V<sub>4</sub> stage (four nodes on the main stem) and maintained for a period of 15 days. The monitoring of field capacity was carried out through daily weighing of the pots and with the aid of tensiometers. The two ways of control were used together, because

tensiometers do not operate for water potentials below -1 MPa.

### Evaluation of characteristics

For the biochemical determinations, samplings were performed at the V<sub>3</sub> (three nodes on the main stem) and V<sub>6</sub> (six nodes on the main stem) growth stages. Completely expanded leaves were collected from the middle third of the plants. The fresh material was used to determine the activity of nitrate reductase – NR (Mulder et al., 1959), urease - U (Hogan et al., 1983; McCullough 1967), quantification of hydrogen peroxide – H<sub>2</sub>O<sub>2</sub> (Alexieva et al., 2001), lipid peroxidation - LP (Heath and Packer 1968) and proline levels (Bates et al., 1973). Quantification of antioxidant enzymes was performed with fresh leaves and frozen in liquid nitrogen shortly after collection. This material was extracted according to the protocol proposed by Kar and Mishra (1976). Then, the determination of total soluble protein content (Bradford 1976) and the enzymes catalase - CAT (CAT, Peixoto et al., 1999), peroxidase – POD (Teisseire and Guy 2000) and superoxide dismutase – SOD (Beauchamp and Fridovich 1971) were performed. The relative water content in the leaves (RWC) was analyzed at the V<sub>6</sub> stage, according to the method proposed by Barrs and Weatherley (1962). Root growth was evaluated at stages V<sub>3</sub> and V<sub>6</sub>, using two plants per replicate. The analyses were performed using the Winrhizo® software, version 4.1, coupled to an Epson XL 10000 scanner, and followed the procedures proposed by Bouma et al. al. (2000). The program establishes a gray tonality value automatically, from which it is possible to identify each plant tissue. The main length root (MLR, cm), total root length (TLR, cm), projection area (PA, cm<sup>2</sup>), root volume (cm<sup>3</sup>) and number of secondary roots (NSR) were obtained. Root (RDM), stem (SDM), leaf (LDM) and pod (PDM) dry matter mass determinations were performed at the V<sub>3</sub>, V<sub>6</sub> and R<sub>8</sub> growth stages. Two plants were used per replicate, when each organ of the plant was separately packed in paper bags, and then dry mass was evaluated using an oven with forced air circulation and with a temperature of 65°C, until constant mass. For the yield, plants were harvested manually considering three plants per replicate. The harvested grain from each plant were weighed on a digital scale with an accuracy of 0.01 grams. The water content of the grains was determined, and the productivity was calculated with the water content corrected to 13% (0.13 g g<sup>-1</sup>). The result was presented in grams per plant.

### Statistical analysis

Statistical analysis was performed using the Student's t-test, in the evaluations carried out at the V<sub>3</sub> stage, which had not yet undergone water deficit treatments. The other evaluations were compared using the Duncan Test. All tests

performed at 5% level of significance. All analyzes were carried out with the help of statistical software SAS 9.3 (SAS Institute 2011).

## Conclusions

This study showed that the application of glycine (9 mg kg<sup>-1</sup>) as seed treatment provides an increase in root growth and accumulation of dry mass in the early development of plants only at normal conditions (without water deficiency). However, these characteristics do not perpetuated when the plants were subjected to water deficiency conditions. On the other hand, in environments without water deficiency, the application of glycine in the treatment of seeds affected the greater development of the plant and increased productivity.

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