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EXOGENOUS L-TRYPTOPHAN IMPROVES SEED GERMINATION AND AGING TOLERANCE IN *GLYCINE MAX* (L.) MERRILL

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ABSTRACT

In crop production, achieving maximum yield potential requires the use of technologies, management systems and inputs to maximize optimal conditions, such as the application of certain amino acids. The present experiment was carried out with the use of soybean (*Glycine max* (L.) Merrill) seeds of cultivar LG60163IPRO from the 2022/2023 growing season, in a completely randomized factorial experimental design with two factors and four replications. The first factor consisted of either the absence or the presence of the accelerated aging test, while the second factor consisted of four doses (0, 0.01, 0.02, and 0.04 g) of L-tryptophan per kg of seeds. The seeds were sown in Germitest® paper and maintained in a germinator at a temperature of 25 ± 1°C. Evaluations were conducted for germination, primary root length, shoot length and seedling dry matter. The application of L-tryptophan in soybean seed treatment positively influenced germination, abnormal seedlings, root/shoot length, and total dry matter, demonstrating the beneficial effects of this specific amino acid for seed treatment. However, seeds subjected to the accelerated aging process, even with the application of L-tryptophan, were unable to achieve results similar to those of non-aged seeds.

Keywords: Amino acid; Accelerated aging; Seedling.

INTRODUCTION

According to data from the Brazilian Institute of Geography and Statistics[(1)], in 2024, soybean production in the state of Santa Catarina reached approximately 2.9 million metric tons with seed quality considered one of the main factors affecting crop productivity. Seeds generally exhibit a

ISSN: 2455-6939

Volume: 11, Issue: 04 "July-August 2025"

high genetic potential, nevertheless, this potential does not guarantee a uniform establishment in commercial fields[2]. Therefore, various agricultural inputs, often applied to seeds, including L-tryptophan, have emerged as tools to improve seed germination, emergence and establishment. L-tryptophan is an amino-acid that has been associated with increased resistance to abiotic and biotic stresses, among other benefits [3,4,5,6,7].

The benefits attributed to the exogenous application of L-tryptophan include the regulation of plant metabolism, improved photosynthesis, reduced phytotoxicity caused by certain pesticides, and increased tolerance to pests and diseases. Additionally, it enhances nutrient absorption and translocation, promotes a more developed and vigorous root system, regulates plant hormonal activities, and improves the quality of harvested products [3,4,7].

In plants, L-tryptophan is a well-known precursor of indole-3-acetic acid (IAA) [4,8]. The conversion of L-tryptophan into IAA occurs through different pathways. The most common route for this conversion is through the indole-pyruvic acid pathway. In this pathway, L-tryptophan undergoes transamination, generating indole-pyruvic acid, which is then decarboxylated to form indoleacetaldehyde. Upon oxidation, indoleacetaldehyde is converted into IAA. Alternatively, some plants may reduce indoleacetaldehyde to indole-ethanol, which can influence the regulation of IAA biosynthesis [9]. IAA is a plant hormone involved in cell elongation and division. This process occurs through the acidification of the cell wall, which is triggered by the release of protons outside the plasma membrane. Proton extrusion induces enzymes present in the plasma membrane to break glycosidic bonds between polysaccharides in the cell wall, leading to its loosening and a reduction in mechanical resistance [8].

Vigor tests performed with the use of different conditions, such as water stress, salinity, flooding, cold, controlled deterioration, and accelerated aging, were developed to identify differences in the physiological potential of seed batches [10]. The accelerated aging test was developed by Delouche to estimate the relative storage potential of clover and fescue seeds [11]. This test is based on the principle that seed deterioration rates increase significantly when seeds are exposed to extreme temperature and different relative humidity levels, which are considered the primary environmental factors influencing deterioration intensity and speed [12].

In the present study, the accelerated aging test was used to expose *Glycine max* seeds to adverse conditions to assess the protective effect of L-tryptophan on germination and seedling growth, as well as its impact on variables such as germination speed, root length, shoot length, and ultimately, dry matter content.

Despite a considerable number of studies on the exogenous application of amino acids in plants, data regarding the effects of this amino acid on the germination of cultivated plants remain scarce

ISSN: 2455-6939

Volume: 11, Issue: 04 "July-August 2025"

and inconclusive. There is still a need to elucidate various metabolic aspects, justifying the use of L-tryptophan on soybean seeds to deepen the existing knowledge on this subject.

MATERIAL AND METHODS

The experiment was carried out in the Seed and Grain Laboratory at the Federal University of Fronteira Sul, Chapecó, Santa Catarina. The soybean seeds used belonged to Limagrain Brasil S/A, cultivar 60163IPRO, from the 2022/2023 growing season, category S1, with a germination potential of 80% and a purity of 99%. The trial was carried out as a completely randomized factorial experimental design with two factors and four replications (13,14). The first factor (treatments) consisted of either the absence (AAT) or presence (PAT) of the accelerated aging test. The second factor consisted of the use of four different doses (0, 0.01, 0.02, and 0.04) of L-tryptophan per kg of seed.

Soybean seeds were divided into four batches of 400 g each and stored in transparent plastic bags. Each batch received a solution containing L-tryptophan at the concentrations shown above. Once the solutions were added to the plastic bags, air was injected, and the bags were vigorously shaken until the solution was evenly distributed. The batches were then left to dry in the shade at a temperature of 25±2°C for 24 hours (15).

For the accelerated aging test (PAT), 220 seeds from each batch were placed in Gerbox® plastic containers with mesh screens (11.0 × 11.0 × 3.0 cm), forming a single layer of seeds (16). At the bottom of the plastic boxes, 40 mL of distilled water was added, and the boxes were placed in a BOD chamber under 41±1°C for 48 hours (12). For the germination test, seeds subjected either to PAT or AAT were divided into batches of 8 replications of 50 seeds each. Each replication was sown in Germitest® paper substrate previously moistened with a water volume corresponding to 2.5 times the weight of the paper. The papers were rolled, and the rolls were maintained in a germinator at a constant temperature of 25±1ºC (17). Germination assessments were carried out on the fifth and eighth days after sowing, and the data were converted into percentage for the variables analyzed (16, 17).

On the eighth day, using a millimeter ruler, the length of the primary root and shoot was measured, and the results were expressed in cm (16, 17). The determination of dry matter content was performed on normal seedlings by quantifying the shoot and root portions. After weighing, the seedlings were placed in paper bags and kept in a forced-air circulation oven at a constant temperature of 80±2°C for 24 hours (16). Thereafter, the seedlings were kept at room temperature for a few hours and then, weighed again for the quantification of the dry matter content, which was expressed in g.

All ANOVA assumptions were checked (normality, homogeneity and independence) for all data collected during the trials. When all assumptions were satisfied, ANOVA was used to analyze the

ISSN: 2455-6939

Volume: 11, Issue: 04 "July-August 2025"

variables (F-test at 5%). A multi comparison test (Tukey's HSD) or a regression analysis, both at 95% confidence level, was used to compare means that were identified as significantly different [14, 18].

RESULTS AND DISCUSSION

According to the analysis of variance (F-test), no significant interaction was observed between the treatment factors (AAT and PAT) and L-tryptophan doses for the variables normal and abnormal seedlings (Table 1), indicating the absence of dependency between these factors. However, a significant effect was observed for the treatment and L-tryptophan dose factors individually. The means for the treatment factor levels are presented in Table 1 (normal and abnormal seedlings), while the mean comparisons for the L-tryptophan dose factor levels are represented by the estimated linear regression equations shown in Figure 1A (normal seedlings) and Figure 1B (abnormal seedlings). Conversely, the variable "ungerminated seeds (dead or hard seeds)" showed a significant interaction between the treatment factors and L-tryptophan doses (Table 1), indicating a dependency between these factors. By analyzing the interaction effect through an additional analysis of variance (F-test), where the levels of the L-tryptophan factor were compared within each treatment factor (and vice versa), a significant effect was observed for the treatment factor within each L-tryptophan dose. The mean comparisons for this factor are presented in Table 1. A significant effect was also observed for the L-tryptophan dose factor within the AAT treatment, which can be visualized through the estimated linear regression equation presented in Figure 2.

ISSN: 2455-6939

Volume: 11, Issue: 04 "July-August 2025"

Table 1: Mean percentage of normal and abnormal seedlings, ungerminated seeds, root length, shoot length, and dry matter content of soybean seeds and seedlings with and without the application of the accelerated aging test and treated with different doses of L-tryptophan.

Variables	Treatment	L-tryptophan concentration (g kg^{-1} of seeds)				
		0.0g	0.01g	0.02g	0.04g	Mean
Normal seedlings (%)	AAT	76.0	80.8	81.5	83.5	80.4a
	PAT	0.0	2.0	18.8	26.3	11.8b
		Standard deviation = 19.821%				
Abnormal seedlings (%)	AAT	12.0	12.0	14.0	14.0	13.0a
	PAT	11.3	14.0	5.8	6.0	9.3b
		Standard deviation = 13.32%				
Ungerminated seeds (%)	AAT	12.0b	7.3b	4.5b	2.5b	6.6
	PAT	88.7a	84.0a	74.5a	67.8a	78.7
		Standard deviation = 5.09%				
Root length (cm)	AAT	11.198	11.019	11.840	13.873	12.0a
	PAT	0.001	0.525	1.650	3.302	1.4b
		Standard deviation = 7.37%				
Shoot length (cm)	AAT	0.5269a	0.5421a	0.6493a	1.0976a	0.7040
	PAT	0.0003b	0.0001b	0.0001b	0.0001b	0.0001
		Standard deviation = 17.23%				
Dry matter (g)	AAT	0.8410	0.8264	0.8994	1.0165	0.958a
	PAT	0.0010	0.0394	0.1239	0.2480	0.1031b
		Standard deviation = 14.86%				

AAT – absence of the accelerated aging test; PAT – presence of the accelerated aging test.
Means followed by the same letter do not differ among themselves within the same column for the same variable, according to Tukey's HSD test at 95% confidence level.

The results obtained demonstrated that increasing L-tryptophan doses led to a higher percentage of germinated seeds, both in those subjected to AAT (7%) and those subjected to PAT (26%). This had a significant impact on the percentage of ungerminated seeds, reducing it by 20.9% on average. These findings indicate that L-tryptophan positively modulated seed germination. Regarding abnormal seedlings, although there was variation in the percentages between the sub-factors (AAT and PAT) of the dose factor, no interaction between these factors was observed. However, when comparing the results obtained for the AAT sub-factor, it was noted that the PAT sub-factor resulted in a lower rate of abnormal seedlings. It is important to consider that, at the same time, it led to an increase in the percentage of ungerminated seeds.

ISSN: 2455-6939

Volume: 11, Issue: 04 "July-August 2025"

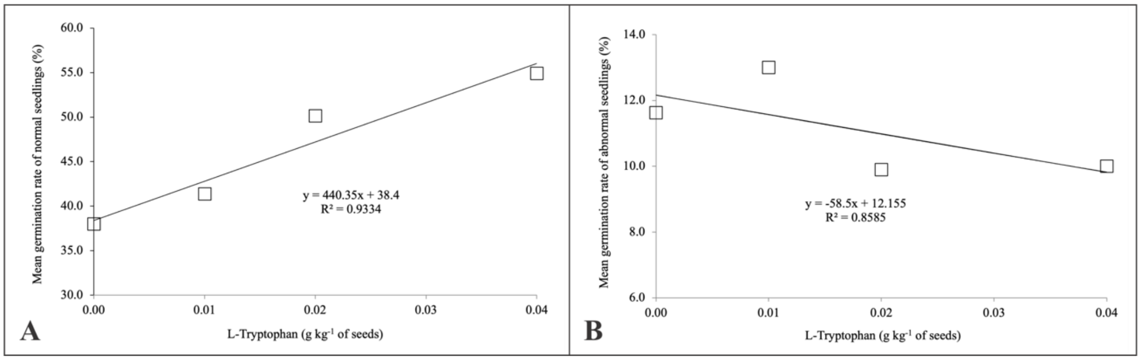

Figure 1: Linear equations referring to (A) mean germination rate of normal seedlings and, (B) mean germination rate of abnormal seedlings of soybean seeds with and without the application of the accelerated aging test and treated with different doses of L-tryptophan.

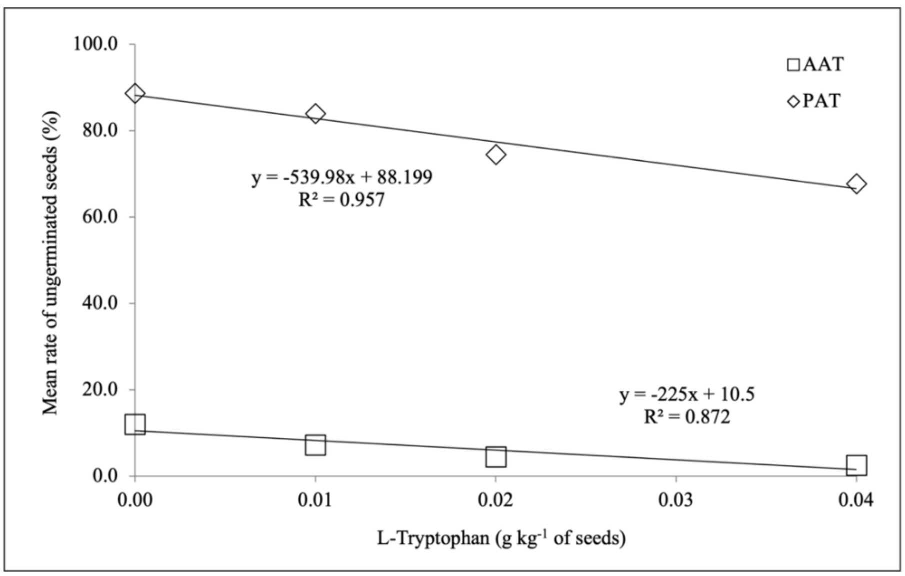

Figure 2: Linear equations referring to the mean rate of ungerminated soybean seeds with and without the application of the accelerated aging test and treated with different doses of L-tryptophan

According to Srivastava et al.(19), when amino acids are applied exogenously, they enhance plant tolerance to stress factors by increasing the efficiency of the antioxidant system, upregulating gene expression, and regulating osmolytes. The authors state that L-tryptophan protects cell membranes from damage caused by free radicals by enhancing the activity of antioxidant enzymes. Cabrini et al.(20) observed that exogenous application of L-tryptophan was directly related to germination, growth, induction of the enzymatic antioxidant system, and the biosynthesis of osmoregulatory molecules in maize (*Zea mays* L.) plants.

ISSN: 2455-6939

Volume: 11, Issue: 04 "July-August 2025"

This trend had already been reported by several authors. Kormaz et al.[21] demonstrated that pepper seeds treated with L-tryptophan showed a significant improvement in germination rates under salt stress. Coelho[5] highlighted several positive effects of amino acid application on maize seeds, noting that the seeds initially exhibited a 92% germination rate and 89% vigor. According to the author, treatment with the product Aminol Forte® at a dose of 2 mL kg^{-1} of seed resulted in a higher emergence rate compared to the control treatment. Since the experiment was conducted until the end of the crop cycle, it was possible to assess productivity, which increased by 14%. Queiroz[22] demonstrated that a dose of 0.2042 mg L^{-1} of tryptophan significantly increased the germination rate, measured on the 5th and 8th days after sowing, in soybean seeds compared to the control. The author reported that, by the first count (5 days), 47 out of 50 seeds had already germinated, indicating that L-tryptophan enhanced germination speed and, consequently, soybean seedling vigor. Furthermore, the author suggested that these results were due to L-tryptophan inducing oxidative stress in seeds.

According to the analysis of variance (F-test), there is no significant interaction between the treatment factors and L-tryptophan doses regarding the root length variable (Table 1), indicating the absence of dependence between the factors. However, a significant effect was observed for both the treatment factors and L-tryptophan doses independently. The means between the treatment factor levels are presented in Table 1, while the comparison of means between the L-tryptophan dose levels is represented by the estimate of the linear regression equation shown in Figure 3.

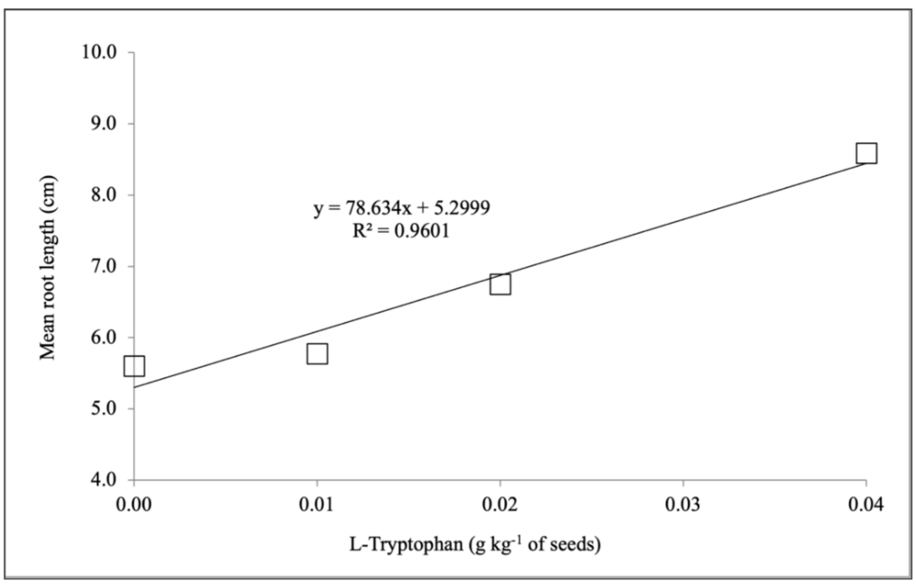

Figure 3: Linear equation referring to mean root length of soybean seedlings with and without the application of the accelerated aging test and treated with different doses of L-tryptophan.

ISSN: 2455-6939

Volume: 11, Issue: 04 "July-August 2025"

It was observed that the increase in L-tryptophan concentrations led to an increase in root length, both for seeds subjected to accelerated aging and for those that did not undergo this treatment.

Guerra(6) conducted a study analyzing the variables plant height, dry matter accumulation, and root length in cotton, comparing plants in normal systems and plants subjected to water deficit. Regarding the root system volume, in all cultivars tested under water stress, the application of amino acids resulted in the largest root volumes. According to the author, the amino acids made the plants' defense system more efficient as a result of an increase in antioxidant levels.

Teixeira(23) demonstrated that the use of the amino acids glutamine, glycine, cysteine, and phenylalanine, either individually or in combination, promoted increases in the parameters of primary root length, total root length, root surface area, root projected area, root volume, root diameter, number of lateral roots, and lateral root length in soybean plants at 10 and 25 days after sowing. However, the author emphasized that the application of all the amino acids did not show a cumulative effect of the benefits provided by each amino acid alone, possibly due to the cumulative effect causing negative responses in plants.

According to the analysis of variance (F-test), there is a significant interaction between the treatment factors (PAT and AAT) and L-tryptophan doses regarding the shoot length variable (Table 1), indicating the existence of dependence between the factors. Through the breakdown of the interaction effect, by conducting a new analysis of variance (F-test), where the levels of the L-tryptophan dose were compared within the levels of the treatment factor (and vice versa), a significant effect was observed for the treatment factor within each L-tryptophan dose. The comparison of means between the levels of this factor is presented in Table 1. A significant effect was also observed for the L-tryptophan dose within the AAT treatment, and this effect can be observed through the estimates of the linear regression equation presented in Figure 4.

It is possible to observe a significant increase in the shoot length as L-tryptophan concentrations increased, compared to seeds not subjected to stress. Guerra(6) suggests that the elongation of the shoot and root system in cotton plants may be related to the influence of serine levels (1.49%) present in the commercial product Proteins®. This result can be explained by the fact that serine is one of the precursors of auxin, a plant hormone responsible for cell elongation in growth zones of the plant.

ISSN: 2455-6939

Volume: 11, Issue: 04 "July-August 2025"

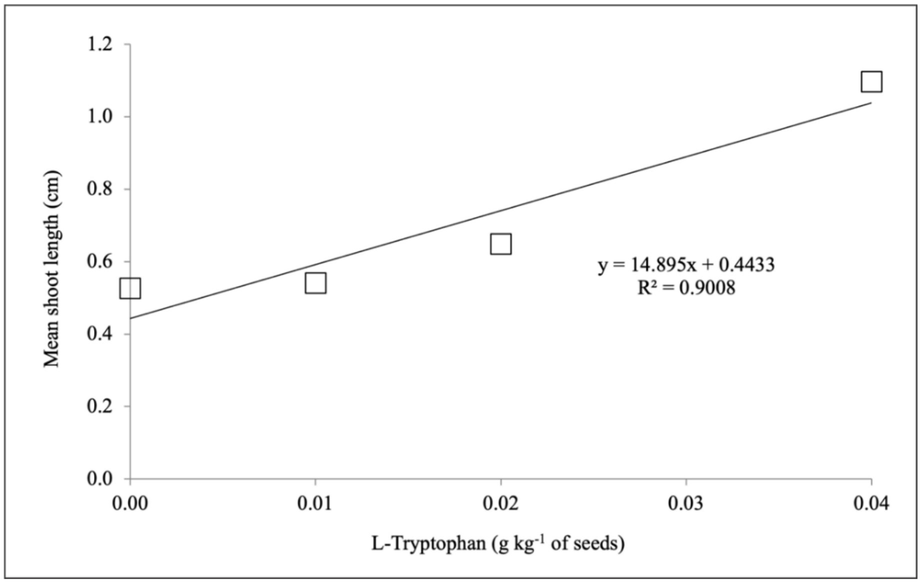

Figure 4: Linear equation referring to mean shoot length of soybean seedlings without the application of the accelerated aging test (AAT) and treated with different doses of L-tryptophan.

Neto(24) details that the foliar application of isolated amino acids in soybean plants promoted increases in plant height. The use of aspartic acid, arginine, cysteine, cystine, taurine, tyrosine, threonine, L-tryptophan, and valine resulted in an average increase of 20.63% compared to the control.

According to the analysis of variance (F-test), there is no significant interaction between the treatment factors and L-tryptophan doses regarding the dry matter variable (Table), indicating the absence of dependence between the factors. However, a significant effect was observed for the treatment factors and L-tryptophan doses independently. The means between the treatment factor levels are presented in Table 1, while the comparison of means between the L-tryptophan dose levels is shown through the estimate of the linear regression equation presented in Figure 5.

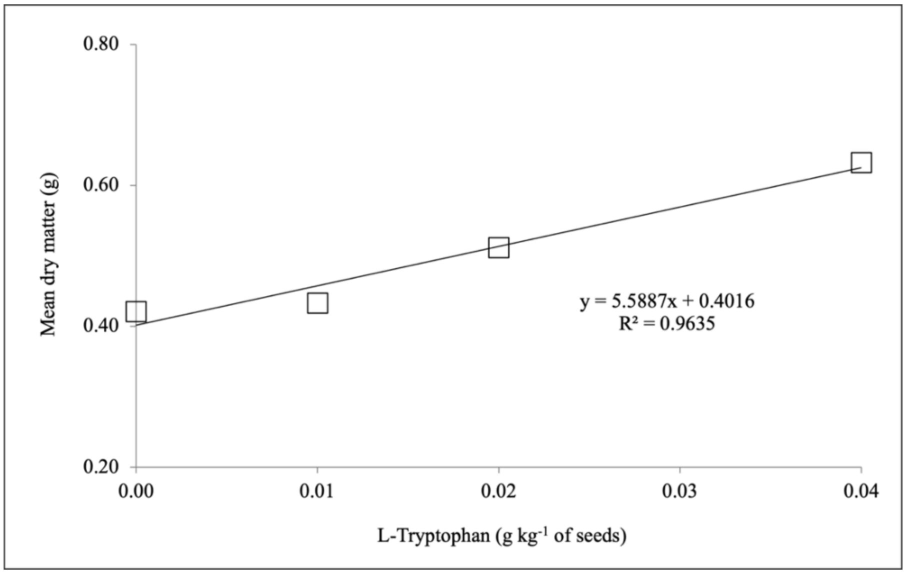

Figure 5: Linear equation referring to mean dry matter of soybean seedlings with and without the application of the accelerated aging test and treated with different doses of L-tryptophan.

Upon observing the results, it is possible to notice an increase in the dry matter content with the increase in the concentrations of L-tryptophan to which the seeds were subjected.

Several authors have already documented this trend. Guerra(6), working with cotton cultivation, highlighted that regarding the accumulation of total dry matter, the application of amino acid doses resulted in higher accumulation. Teixeira(23) demonstrated that the accumulation of dry matter in different plant organs was positively affected by the application of the amino acids cysteine and phenylalanine in seeds. Queiroz(22), working under water stress conditions, observed increases in dry matter content when plants were treated with proline. Hussain *et al*.(7) reported increases in dry matter content when seeds were treated with L-tryptophan under various levels of cadmium toxicity. On the other hand, Teixeira(25) emphasized that no significant effect of glycine application on dry matter accumulation was observed at 25 days, although significant increases were noted at 40 days after sowing.

By integrating statistical reports from ChemAnalyst(26), DataIntelo(27) and the World Integrated Trade Solution (WITS) (28) website it is shown that in 2023 the World's total market value for L-tryptophan was of approximately US$700 million (2% of total amino acids trades) with a trade volume of roughly 67 thousand metric tons, corresponding to about US$10.44 per kilogram of product. Therefore, considering that seed companies and farmers usually perform seed treatments(29) with nematicides, fungicides, insecticides, micronutrients and biological products, such as the application of *Bradyrhizobium*, the practical and economical aspects of treating seeds

ISSN: 2455-6939

Volume: 11, Issue: 04 "July-August 2025"

with exogenous L-tryptophan lies on adding an extra product to the process of seed treatment and spending and extra of less than US$0.01 per kilogram of seed submitted to the treatment process.

CONCLUSIONS

Although no interaction was observed between the factors for the percentage of normal and abnormal seedlings, root length, and dry matter content, it can be concluded that the use of the amino acid L-tryptophan in soybean seed treatment positively influenced all the evaluated variables.

The indices obtained under accelerated aging conditions could not be equated to those observed in the absence of this stress. Nevertheless, a reduction in the percentage of abnormal seedlings and ungerminated seeds was observed across all treatments, demonstrating a stimulatory effect of L-tryptophan on the germination process.

Additional studies involving the tested doses of L-tryptophan for soybean seed treatment ought to be developed in field conditions to address the potentials of these doses on the development and yield of plants.

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