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L-Tryptophan Improves Germination and Early Growth of *Glycine max* Seedlings Subjected to Cold Test

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***Abstract*— *The genetic potential of seeds does not inherently ensure uniform establishment and optimal stand quality in commercial crop fields. This challenge has led to the development of various agricultural inputs, often applied directly to seeds, including L-tryptophan. The present study was conducted using a completely randomized factorial experimental design with two factors and four replications. The first factor comprised two levels: the presence (CT) and absence (ACT) of a cold test. The second factor included four concentrations of L-tryptophan: 0, 0.02, 0.04, and 0.08 g kg^{-1} of seeds. Soybean seeds were sown on Germitest® paper substrate and maintained in a germination chamber. Evaluations included germination rate (normal and abnormal seedlings, as well as hard or dead seeds), primary root length, shoot length and dry matter. The application of L-tryptophan exhibited a protective effect on soybean seeds subjected to cold stress, indicating its potential as an effective seed treatment for crops expected to encounter low temperatures and high soil moisture during germination.*

***Keywords*— amino acid, cold test, germination test, soybean, seed vigor.**

I. INTRODUCTION

Soybean is highlighted as the sixth most produced staple crop in the world and occupies the fourth largest area of cultivation (FAO, 2025). Brazil stands out as the World's largest producer of soybeans (USDA-FAS, 2025), with a production in the harvest season of 2024/2025 reaching approximately 164 million metric tons (IBGE, 2025). Nevertheless, the country faces big challenges and risks regarding the production of this crop (Da-Silva *et al.*, 2020).

Seed quality is one of the many factors that considerably affect soybean establishment and yield. Although, seeds bring with themselves high genetic potential, this potential does not guarantee uniform establishment and initial soybean stand quality in commercial fields (Mariano-da-Silva *et al.*, 2025; Mógor *et al.*, 2008). Hence, this has led to the emergence of various agricultural inputs designed to be applied directly to seeds with the aim of improving crop establishment, as it is the case of the use of the essential amino acid L-tryptophan. These inputs have been associated with greater root development and stress resistance (Pessoa, 2021).

L-tryptophan is one of the 20 essential amino acids required for protein biosynthesis. This amino acid contains an amino group (basic), a carboxyl group (acidic), and a side chain containing an indole structure, making it an aromatic and nonpolar amino acid (Mariano-da-Silva, 2023). Plants synthesize tryptophan either from shikimic acid or anthranilic acid, and when degraded, it is responsible for producing auxin (Taiz *et al.*, 2021).

Ahemad & Kibret (2014) demonstrated that auxin levels increase in plant tissues following an exogenous application of L-tryptophan. It is well known that L-tryptophan is the main precursor in auxin biosynthesis pathways, a phytohormone that plays a fundamental role in cell elongation (Taiz *et al.*, 2021). In association with gibberellin, jasmonic acid, salicylic acid, and abscisic acid, auxin positively influences the effect of PIN proteins, which regulate radicle protrusion (Mukherjee *et al*., 2014; Wan *et al*., 2018). On the other hand, the interaction between auxin and jasmonic acid promotes plant tolerance to abiotic stresses, such as drought, and biotic stresses caused by fungi or insects (Hussain, 2024; Wan *et al*., 2018).

Vigor tests (stress conditions: water deficit, salinity, flooding, cold, controlled deterioration, and accelerated aging) were developed to identify differences in the physiological potential of seed batches (Krzyzanowski *et al*., 2020). The cold test is probably the most studied vigor test, assessing the ability of seeds to germinate in cold and moist soils (Cicero *et al*., 2020). Therefore, this test is expected to verify the protective effect of L-tryptophan.

These cold and humid conditions during sowing are commonly found in Southern states of Brazil, where, according to the Ordinance 886 of the Ministry of Agriculture and Livestock (MAPA, 2023), soybean sowing can begin as early as September 11 (Paraná State), October 01 (Rio Grande do Sul State), and October 02 (Santa Catarina State).

The objective of this study was to evaluate the effects of L-tryptophan on the germination process and seedling development of soybeans subjected to the cold test, using the parameters of germination, root length, shoot length, and dry matter.

II. MATERIAL AND METHODS

2.1 Experimental settings

The experiment was conducted at the Seed and Grain Laboratory located at the Federal University of Fronteira Sul, campus Chapecó. The soybean seeds used during the experiment were produced during the 2023/2024 harvest season by Limagrain Brasil S/A. These seeds are from the cultivar 60163IPRO (category S1) and present a germination potential of 80% and a purity of 99%.

2.2 Experimental design

The trial was conducted using a completely randomized factorial design with two factors and four replications (Ares & Granato, 2014; Pimentel-Gomes, 2000). The first factor consisted of two sub-factors: the presence (CT) or absence of the cold test (ACT). The second factor consisted of four different sub-factors: 0, 0.02, 0.04, and 0.08 g of tryptophan per kg^{-1} of seed.

2.3 Treatment with L-tryptophan

The soybean seeds used in the trial were divided into eight batches of 200 g each and stored in transparent plastic bags. L-tryptophan was previously diluted in water in Petri dishes, generating the four concentrations previously mentioned. Once the solution was added to the plastic bags, air was injected, and the bags were vigorously shaken until the distribution of the treatments was considered homogeneous. The batches were then placed to dry in the shade at an approximate temperature of 25 °C for 24 h (Marcos-Filho, 2015).

2.4 Cold and germination test

Each batch was divided into two sub-batches, where one sub-batch was allocated to the conventional germination test and the other to the cold test. Seeds designated for the cold test were sown on Germitest® paper substrate previously moistened with a volume of water corresponding to 3.0 times the dry weight of the paper. The rolls were then placed inside plastic bags, sealed with adhesive tape, and stored in a BOD chamber at 10 ± 1 °C for five days (Cicero *et al*., 2020; Sá *et al*., 2011). After this period, the bags were opened, and the germination test was conducted. Seeds designated for the conventional germination test were sown on Germitest® paper substrate, previously moistened with a volume of water equivalent to 2.5 times the dry weight of the paper. The paper rolls were maintained in a germinator at a constant temperature of 25 ± 1 °C (Brasil, 2025). Germination evaluations were performed on the fifth and eighth days after sowing, and the data were converted into percentage of normal seedlings (Brasil, 2025; Sá *et al*., 2011).

2.5 Root and shoot length

With the aid of a millimeter ruler, the length of the primary root and shoot length were measured, and the average results were expressed in cm per seedling (Brasil, 2025; Sá *et al*., 2011).

2.6 Dry matter

The determination of dry matter was performed on normal seedlings, quantifying the shoot and root portions. After weighing, the seedlings were placed in paper bags and dried in an oven with forced air circulation at a constant temperature of 80 ± 2 °C for 24 hours (Sá *et al*., 2011). After drying, the samples were weighed again after several hours at room temperature, and the dry matter content was determined and expressed in grams per seedling.

2.7 Statistical analysis

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 variables (F-test at 95%). 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 (Pimentel-Gomes, 2000; Zimmermann, 2004).

III. RESULTS AND DISCUSSION

According to the analysis of variance (F-test), a significant interaction was observed between the CT/ACT factors and L-tryptophan doses for all tested variables, including the percentage of normal seedlings, percentage of abnormal seedlings, root length, shoot length, and dry matter content, indicating a dependency between these factors. By further analyzing the interaction effect through an additional analysis of variance (F-test), where the levels of the L-tryptophan dose factor were compared within each CT/ACT factor level (and vice versa), a significant effect was observed for the CT and ACT factors within all tested L-tryptophan doses for all variables (Table 1). A significant effect was also observed for the L-tryptophan dose factor within each CT/ACT condition, specifically at the doses of 0, 0.02, 0.04, and 0.08 g kg^{-1} of seed for all analyzed variables. This effect can be observed through the estimates of the second-order polynomial equations presented in Fig. 01 (A, B, C, D, E and F).

The results obtained demonstrated that increasing the L-tryptophan dose led to a higher percentage of germinated seeds, both in those subjected to cold treatment (CT) and those without it (ACT), indicating that L-tryptophan positively modulated seed germination. Additionally, L-tryptophan treatment reduced the percentage of hard and dead seeds compared to the control. However, this effect was accompanied by an increase in abnormal seedlings in the cold-treated group.

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 cold test and treated with different doses of L-tryptophan.

Variable	**Treatment**	**L-tryptophan concentration (g kg^{-1} of seeds)**			
		0.0	**0.02**	**0.04**	**0.08**
Normal seedlings (%)	**ACT**	**77.3 a**	**81.4 a**	**85.3 a**	**85.8 a**
	CT	**60.3 b**	**74.0 b**	**75.0 b**	**76.0 b**
		CV = 2.741%			
Abnormal seedlings (%)	**ACT**	**19.8 a**	**10.0 b**	**12.5 b**	**12.5 b**
	CT	**16.5 b**	**17.8 a**	**16.8 a**	**16.8 a**
		CV = 20.425%			
Ungerminated seeds (dead and hard seeds) (%)	**ACT**	**3.0 b**	**8.6 ns**	**2.3 b**	**1.8 b**
	CT	**23.3 a**	**8.3 ns**	**8.4 a**	**7.3 a**
		CV = 20.233%			
Root length (cm seedling^{-1})	**ACT**	**8.05 a**	**11.07 a**	**11.18 a**	**11.88 a**
	CT	**6.63 b**	**8.19 b**	**8.10 b**	**8.51 b**
		CV = 5.532%			
Shoot length (cm seedling^{-1})	**ACT**	**0.93 a**	**1.33 a**	**1.47 a**	**1.68 a**
	CT	**0.40 b**	**0.57 b**	**0.54 b**	**1.03 b**
		CV = 12,068%			
Dry matter (g seedling^{-1})	**ACT**	**0.3138 ns**	**0.8293 a**	**0.7793 a**	**0.7302 a**
	CT	**0.3163 ns**	**0.2927 b**	**0.5643 b**	**0.5918 b**
		CV = 10.200%			

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.
ns – not significant according to the F-test (5%);
ACT – absence of the cold test;
CT – presence of the cold test;
CV – Coefficient of variance.

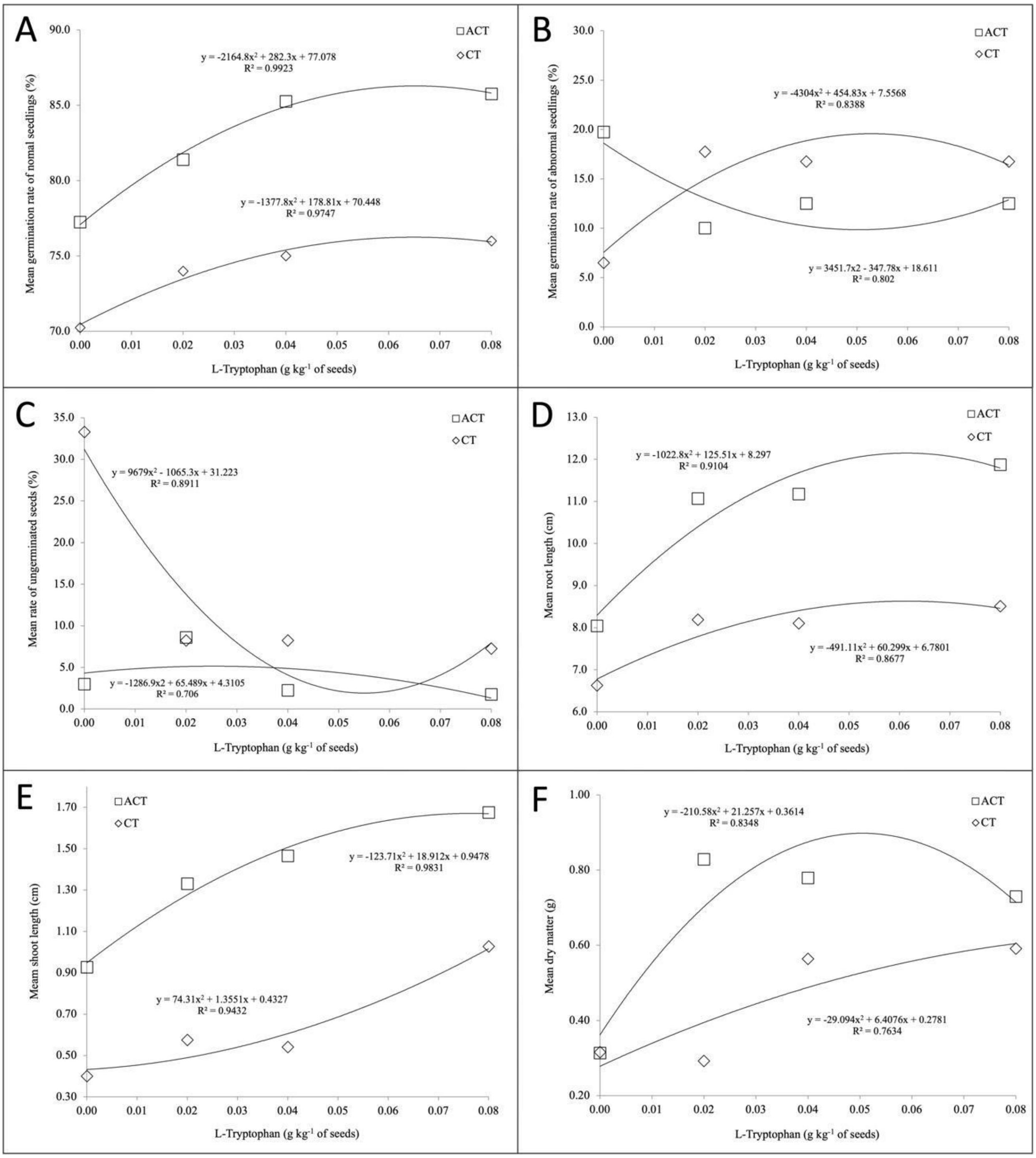

Fig. 1. Second-order polynomial equations referring to (A) mean germination rate of normal seedlings, (B) mean germination rate of abnormal seedlings, (C) ungerminated seeds, (D) mean root length of seedlings, (E) shoot length of seedlings and (F) dry matter of seedlings, of soybean (Glycine max L), with (CT) and without (ACT) the application of the cold test and treated with different doses of L-tryptophan.

As the treatments combining CT and L-tryptophan resulted in more than 70% normal seedlings, it can be inferred that L-tryptophan effectively protected the seeds from the stress induced by the test (Cicero *et al*., 2020).

This trend has already been reported by several authors. Korkmaz *et al*. (2020) demonstrated that pepper (*Capsicum annuum* L.) seeds treated with L-tryptophan showed a significant improvement in germination rates under salt stress. Hussain *et al*. (2024), evaluating the effects of L-tryptophan on sunflower (*Helianthus annuus* L.) seed germination under subtoxic doses of cadmium, reported that the amino acid reduced the metal's adverse effects on seed germination rates. Queiroz *et al*. (2023) demonstrated that the exogenous application of L-tryptophan in soybean (*Glycine max* L.) seeds significantly increased germination rates, concluding that the amino acid can serve as an important inducer of seed germination and vigor. Hanci *et al*. (2019) tested the effects of various doses of a combination of L-tryptophan and melatonin on the germination of onion (*Allium cepa* L. cv. Valenciana) seeds under low temperatures, observing significant increases in germination rates at 7 ºC. Similarly, Abdelkader *et al*. (2023) reported a 10% increase in germination rates compared to the control in onion (*A. cepa* L. cv. Gaza) seeds treated with L-tryptophan.

The variables root length and shoot length were also affected by the exogenous application of L-tryptophan. Although both parameters showed decreases when subjected to the cold stress test, it was observed that higher doses of L-tryptophan resulted in greater root and shoot length.

Similar results can be found in the literature. Hussain *et al*. (2024) demonstrated that L-tryptophan treatment mitigated the deleterious effects of cadmium on root and shoot growth in sunflower (*Helianthus annuus* L.) seedlings. Abdelkader *et al*. (2023) reported that the greatest root lengths were obtained when onion (*Allium cepa* L. cv. Gaza) seeds were treated with L-tryptophan. El-Sayed *et al*. (2019) showed that L-tryptophan alleviated the toxic effects of cadmium on plant height and leaf mass in eucalyptus (*Eucalyptus gomphocephala*).

Analyzing the results, it is evident that increasing L-tryptophan doses led to an increase in dry matter content, both in the CT and ACT treatments. As previously observed, L-tryptophan treatment enhanced shoot and root length parameters, directly influencing the dry matter content.

Other authors have also reported this trend. Sadak & Ramadan (2021) tested the effects of various L-tryptophan doses on the fresh and dry weight of the shoot in white lupin (*Lupinus termis* L.) under different water stress conditions, observing that the amino acid increased these parameters compared to the control. Sudadi & Suryono (2015) and Sanada & Agahara (2023) showed that exogenous L-tryptophan enhanced dry matter content in both shoots and roots of soybean (*Glycine max* L.).

IV. CONCLUSION

L-tryptophan exhibited a protective effect on soybean seeds subjected to the cold test, improving germination percentage, dry matter accumulation, root length, and shoot length. This suggests that L-tryptophan is a promising alternative for seed treatment, particularly for crops sown under low-temperature and soils with high moisture conditions.

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