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Toxic effect of elements on the germination and initial development of barley seeds (*Hordeum vulgare* L.)

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Abstract

Barley (*Hordeum vulgare*) is an agricultural vegetable from the Poaceae family used in food and beer production. The study aimed to evaluate the toxic effect of Aluminum (Al) and Copper (Cu) on germination and initial development in barley seeds cultivar KWS Irina. Different concentrations (0, 35, 85 and 125 mg L^{-1}) of aqueous solution of Al and Cu were produced from their chlorides. The toxicity experiment was carried out in a germination box maintained in a germination chamber with a 12-h photoperiod. After 15 days of germination, the seedlings were measured using a millimetric ruler (cm) where they were evaluated for plant length, root length, and fresh and dry mass of plant and root determined on a digital analytical scale (g). Barley seedlings cultivar KWS Irina demonstrated to be intolerant to concentrations of the toxic elements Al and Cu in all plant parameters analyzed, except for plant dry mass. Future studies should be carried out comparing the initial and reproductive development of this barley cultivar in terms of the presence and absence of toxic elements.

Keywords: toxicity, heavy metals, Poaceae family, barley, agricultural cultivar, toxicity tolerant plants.

Efeito tóxico de elemetos sobre a germinação e desenvolvimento inicial de sementes de cevada (*Hordeum vulgare* L.)

Resumo

Cevada (*Hordeum vulgare*) é um vegetal agrícola da família Poaceae utilizada na alimentação e na produção de cervejas. O estudo teve por objetivo, avaliar o efeito tóxico do Alumínio (Al) e Cobre (Cu) sobre a germinação e desenvolvimento inicial em sementes de cevada cultivar KWS Irina. Foram produzidas diferentes concentrações (0, 35, 85 e 125 mg L^{-1}) de solução aquosa de Al e Cu a partir de seus cloretos. O experimento de toxicidade foi realizado em caixa germinadora mantidas em câmara de germinação com fotoperíodo de 12 h. Após 15 dias de germinação, as plântulas foram mensuradas com auxílio de régua milimétrica (cm) onde fora avaliadas para comprimento de planta, comprimento de raiz, e para massa fresca e seca de planta e raiz determinada em balança analítica digital (g). Plântulas de cevada cultivar KWS Irina demonstraram ser intolerantes as concentrações dos elementos tóxicos Al e Cu em todos os parâmetros de planta analisadas, exceto para massa seca planta. Estudos futuros, deverão ser realizados comparando o desenvolvimento inicial e reprodutivo desse cultivar de cevada quanto a presença e ausência de elementos tóxicos.

Palavras-chave: toxicidade, metais pesados, família Poaceae, cevada, cultivar agrícola, plantas tolerantes a toxicidade.

1. Introduction

Hordeum vulgare L. (barley), belonging to the Poaceae family such as wheat, rice and corn, was one of the first vegetable species of agricultural interest to be domesticated by man (Ozyigit et al., 2021). This crop has wide adaptability to different climates and soils and is widely used in the production of beer and animal feed and stands out among the most produced grasses in the world (Galon et al., 2011; Cakir et al., 2021).

The annual production of barley grains increases as new cultivars are produced that are resistant to large variations in climate and different types of cultivated soils. In Brazil, H. vulgare is cultivated in the South region, where colder climates predominate, where this culture has seen a prosperous rise (Cunha et al., 2001). However, Embrapa Trigo developed barley cultivars with greater resistance to warmer climates found in the Central-West and Northern regions of Brazil (Mori; Minella, 2012).

In addition to resistance, we also have the tolerance factor, as acidic soils or those that have suffered environmental contamination predominate some metals that cause toxicity not only in barley, but in other major crops such as artichokes, beans, millet, corn, soybeans, sorghum, etc. (Milk; Zampieron, 2012; Stefanello; Goergen, 2019). In Brazilian Cerrado soils where liming is not carried out correctly, Aluminum (Al), Copper (Cu) and Boron (B) are some of the various metals that can cause toxicity in plants, including barley, where in addition to these, we also have problems due to metals due to environmental pollution (Gabriel et al., 2019; Ellwanger; Chies, 2023).

Toxic metals are also known as “heavy metals” where they are easily absorbed by plants, mainly by the roots and aerial parts. Toxic metals compete with the elements that constitute the macro and micronutrients that the plant needs for its full development (Augusto et al., 2014). Among other toxic elements in addition to Al, Cu and B, we mention Cadmium (Cd), Lead (Pb), Chromium (Cr), Barium (Ba), Strontium (Sr) and Mercury (Hg), although this list of toxic metals be extensive. The content of these elements varies and can be found in contaminated soils, close to natural reserves (deposits) or transported by the wind in anthropic processes such as fires and sludge wastewater used in fertigation (Ramalho; Sobrinho, 2001; Gonçalves et al., 2009).

In a study carried out with barley by Ozyigit et al. (2021) researchers revealed that barley plants can manage stress in some plant parameters under low stress conditions with Cd, however, higher concentrations negatively affected it, although they observed that the plants showed vitality throughout the experiment. In addition to Cd, elements such as Cu and Pb were evaluated in studies in Ireland where researchers observed that Pb had a high concentration in the roots of other monocotyledons and the highest Cu content in shoots of various vegetables. Still in the discussion of this study, these two elements are indicators of industrial and domestic contamination in the marsh region along the Suir estuary where the research was carried out by Fitzgerald et al. (2003). In another study by Chatterjee & Chatterjee (2000) evaluating dicot stress involving Cr, Cobalt (Co) and Cu in cauliflower plants (*Brassica oleracea* L., *var. Bottrytis cv.* Maghi), these collaborators demonstrated that these toxic ions influenced by reducing the concentration of chlorophyll through the inhibition of electron transport in the photosynthesis of this plant.

Some of these elements can also be radioactive and can cause serious physiological problems in plants, such as a low germination rate, reduction in the fresh and dry mass of roots, shoots and straw, and can also cause chlorosis in young leaves; they prevent the absorption of other elements essential to plants, in addition to being deposited in significant amounts in seeds that are used in human and animal food (Athar; Ahmad, 2002; Naranjo-Jiménez; Wingching-Jones, 2023).

Considering those different concentrations (low, moderate or high) of Al, Cd, Cu, Cr and Pb negatively affect the germination and development of vegetables, it is necessary to know the effects of these toxic elements on the germination and initial vegetative development of barley. Therefore, the objective of this study was to verify the tolerance of barley seeds (*Hordeum vulgare* L.) cultivar KWS Irina in germination and initial development under different concentrations of Al and Cu in an *in vitro* test.

2. Materials and Methods

2.1. Reagents and equipment

Aluminum Chloride P.A – ACS (Dinâmica, Brazil), Copper Chloride P.A – ACS (Neon, Brazil). Germination chamber with photoperiod (Solid Steel, Mod. Bio SSGF-342, Brazil).

2.2. Experimental local

The study was developed at the Technological Chemistry Laboratory, of the Agrochemistry Department of the

Goiano Federal Institute, Rio Verde, Goiás, Brazil, in November 2023.

2.3. Preparation and concentrations of Al and Cu

Aqueous solutions containing the metals Al^{3+} and Cu^{2+} in the form of chlorides were produced with distilled water in the corresponding concentrations (Table 1).

Table 1. Metals and concentrations corresponding to zero (control); 35, 85 and 125 mg L^{-1}.

Solutions	**Concentrations in mg L^{-1}**			
	Control*	**35**	**85**	**125**
$AlCl_2.6H_2O^{(1)}$	-	0,0175	0,0425	0,0625
$CuCl_2.2H_2O^{(2)}$	-	0,0175	0,0425	0,0625

Note: *Control is the zero level that corresponds to the control, where only distilled water was used. Source: Authors, 2023.

2.4. Germination

H. vulgare seeds were collected in production area in Paraná State, Brazil, 2022/2023 harvest with a germination rate of 98.7%, cultivar KWS Irina. The seeds were kept in refrigerator at 8 ºC until analysis. The germination test was carried out in transparent acrylic germination box (Gerbox). Three sheets of germitest paper were moistened with water or a metal solution equivalent to 2.5 times the mass of the non-hydrated paper. The germination boxes were kept in a B.O.D. germinator, with a 12-hour photoperiod and a temperature of 21 °C for 6 days. Four replications were performed with 25 seeds. The germination rate result was expressed as a percentage (%) (Alves et al., 2015) adapted.

2.5. Al and Cu toxicity assay in H. vulgare

At 15 days after seedling emergence, the following parameters were evaluated: plant lenght (PL) and root lenght (RL) with the aid of a millimetric ruler, the results were expressed in centimeters (cm). The fresh plant matter (FPM) and fresh root matter (FRM) were obtained on a digital analytical balance with a precision of 0.0001 g, the results were expressed in grams (g). Then, the seedlings were fragmented (aerial and root) with the aid of a scalpel blade and then placed in glass Petri dishes. Soon after, they were transferred to an oven with a controlled temperature of 65 °C until constant mass was obtained. Subsequently, the fragmented seedlings had their masses determined in plant dry matter (PDM) and root dry matter (RDM) determined on a digital analytical balance, with results expressed in (g) as described by Stefanello and Goergen (2019).

2.6. Statistical analysis

The experimental design adopted was completely randomized, where treatments consisted of different concentrations of metals (Al^{3+} and Cu^{2+}) and a high vigor barley cultivar. The results were submitted to analysis of variance (ANOVA) using the F test and, when there was a significant effect, regression analysis was performed using the Sisvar program (Ferreira, 2019).

3. Results

3.1. Germination rate

The germination rate was 98.9%.

3.2. Toxic effect of Al on Hordeum vulgare

The analysis of variance indicated significant differences depending on the treatments for all variables PL, RL, FPM, FRM, PDM and RDM for Al metal on germinated barley seeds (Table 2).

Table 2. Summary of the analysis of variance for the variables: plant lenght (PL), root length (RL), fresh plant matter (FPM), fresh root matter (FRM), plant dry matter (PDM) and root dry matter (RDM) in relation to Aluminum (Al) absorption by barley seedlings.

SV	**PL**	**RL**	**FPM**	**FRM**	**PDM**	**RDM**
Doses Al	21.37*	11,55*	0.00*	0.00*	0.00*	0.00*
Residue	2.47	1.82	0.00	0.00	0.00	0.00
CV %	18.32	29,79	20.82	23.49	18.81	18.48

Note: Significant at 5% probability by the F test. CV = coefficient of variation. Source: Authors, 2023.

In relation to PL (Figure 1A), significant differences were observed between Al treatments in barley seedlings with values varying between 11.1 (control) and 6.1 cm (125 mg L^{-1}) with linear regression. The RL demonstrated a polynomial regression with a variation between 6.5 (control) and 4.1 cm (125 mg L^{-1}) (Figure 1B).

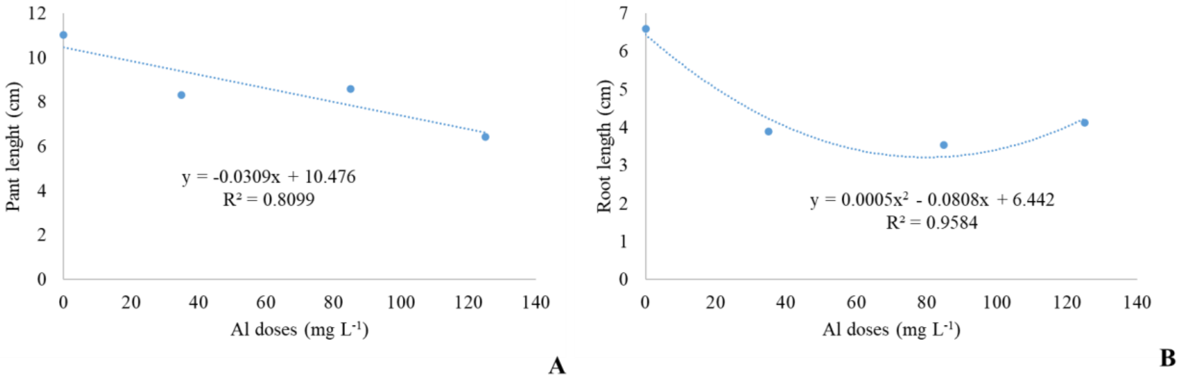

Figure 1. Length of the aerial part (**A**) and root (**B**) of *Hordeum vulgare* seedlings in different concentrations of Aluminum (mg L^{-1}). Source: Authors, 2023.

For FPM (Figure 2A), it showed linear regression with averages between 0.08 g (control) and 0.05 g (125 mg L^{-1}), with significant differences between Al treatments on seedlings. And for FMRP (Figure 2B) there was also a difference between the concentrations with an increase only in the 35 mg L^{-1} concentration, the control and the 125 mg L^{-1} concentration showed no difference between the means with 0.02 g, with a polynomial regression.

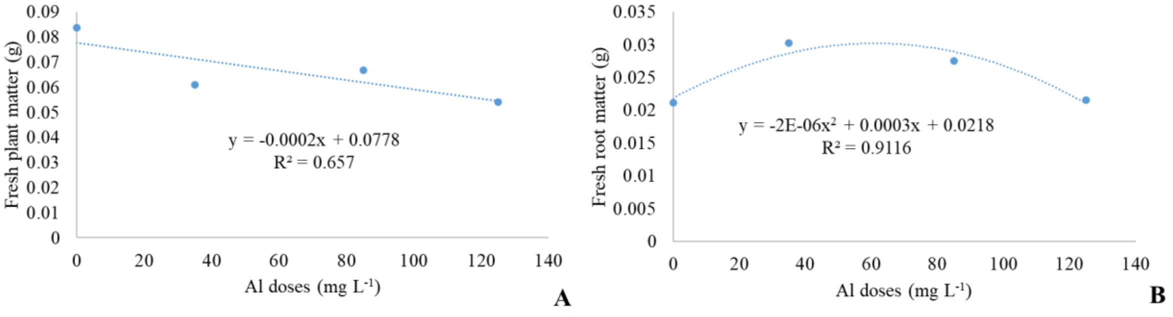

Figure 2. Fresh plant matter (**A**) and fresh root matter (**B**) of *Hordeum vulgare* seedlings at different concentrations of Aluminum (mg L^{-1}). Source: Authors, 2023.

The PDM of barley seedlings showed a reduction with linear regression varying between 0.008 (control) and 0.006 (125 mg L^{-1}) (Figure 3A). The same was observed for RDM (Figure 3B) with polynomial regression with a variation between 0.007 (control) and 0.005 (125 mg L^{-1}).

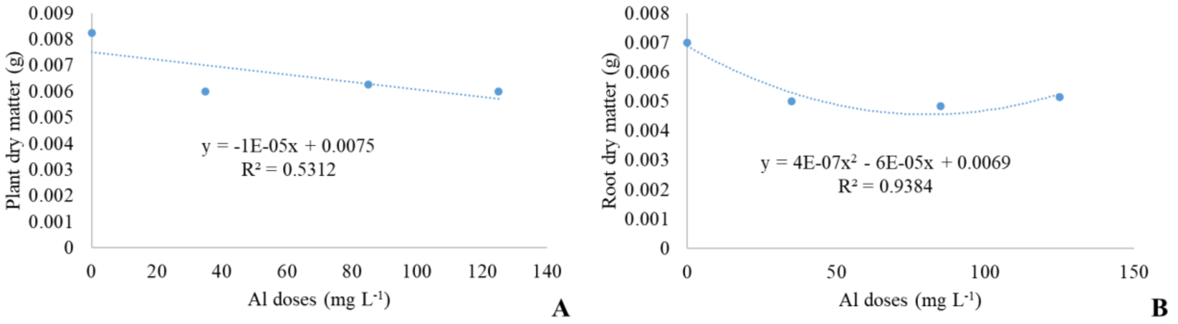

Figure 3. Plant dry matter (**A**) and root dry matter (**B**) of *Hordeum vulgare* seedlings at different concentrations of Aluminum (mg L^{-1}). Source: Authors, 2023.

3.3. Toxic effect of Cu on Hordeum vulgare

The analysis of variance indicated significant differences depending on the treatments for the variables PL, RL, FPM, PDM and RDM for the Cu element on barley seeds (Table 3). There was no significant difference in the FRM variable using the F test.

Table 3. Summary of the analysis of variance for the variables: plant length (PL), root length (RL), fresh plant matter (FPM), fresh root matter (FRM), plant dry matter (PDM) and root dry matter (RDM) in relation to Copper (Cu) absorption by barley seedlings.

SV	**PL**	**RL**	**FPM**	**FRM**	**PDM**	**RDM**
Doses Cu	67.02*	25.78*	0.00*	0.00^{ns}	0.00*	0.00*
Residue	2.45	1.03	0.00	0.00	0.00	0.00
CV %	19.90	23.59	17.35	40.38	19.30	37.59

Note: ^{ns} Not significant. Significant at 5% probability by the F test. CV = coefficient of variation. Source: Authors, 2023.

For PL (Figure 4A), significant differences of Cu treatments were observed in barley seedlings. The average values were 11.01 cm (control) and 3.41 cm (125 mg L^{-1}) with a linear regression. The same was observed for RL with a linear regression with values between 6.58 cm (control) and 2.16 cm (125 mg L^{-1}) (Figure 4B).

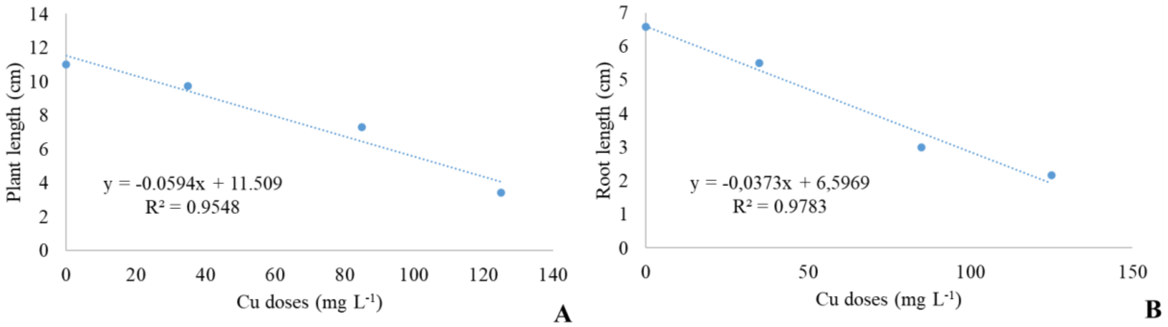

Figure 4. Plant length (A) and root length (B) of *Hordeum vulgare* seedlings in different concentrations of Copper (mg L^{-1}). Source: Authors, 2023.

The FPM showed a significant difference in different Cu treatments in barley seedlings. The control presented a value of 0.08 g and for 125 mg L^{-1} with 0.03 g, it is possible to observed that the results presented a linear regression (Figure 5).

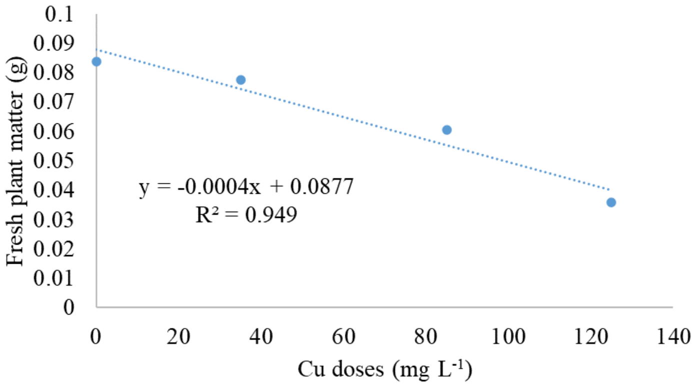

Figure 5. Fresh plant matter of *Hordeum vulgare* seedlings at different concentrations of Copper (mg L^{-1}). Source: Authors, 2023.

For PDM and RDM (Figure 6 A and B), both presented linear regression with results between 0.008 (control) and 0.003 (125 mg L^{-1}) and between 0.007 g (control) and 0.004 g (125 mg L^{-1}) , respectively. The Cu element demonstrated a reduction in the dry mass of barley seedlings, which negatively influences the straw.

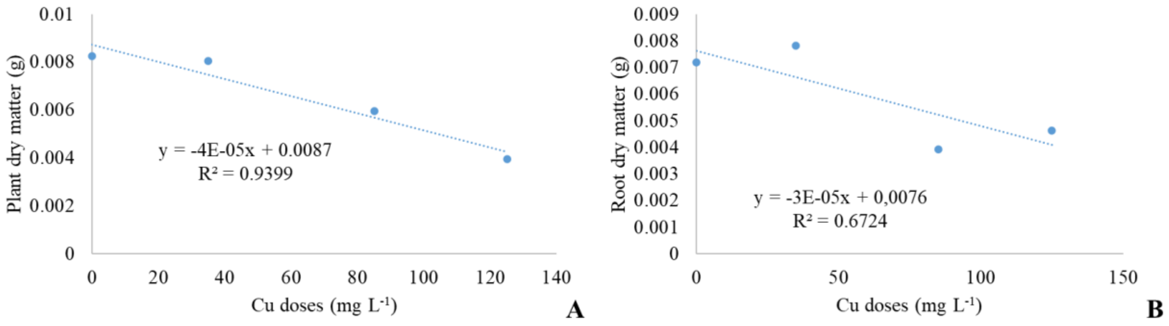

Figure 6. Plant aerial matter (**A**) and root dry matter (**B**) of *Hordeum vulgare* at different concentrations of Copper (mg L^{-1}). Source: Authors, 2023.

4. Discussion

In our research for germination rate, our value was higher than the germination rate obtained by sowing and by Santos et al. (2018) of 95.7% for barley seeds, this guarantees that the logistical process presented quality and the seeds remained intact and without damage. The high germination rate improves initial growth, vigor, greater accumulation of dry matter, greater population of established plants and adequate distribution of plants in the planting area, where this set of benefits provided by germination results in greater productivity (Cichelero et al. , 2023).

We can observe that Al at all concentrations affected the initial development of barley seedlings for all parameters evaluated. Under conditions of Al extress, plant tolerance has been associated with the control of pH in the rhizosphere and the exclusion of Al by the plasma membrane of plant roots, as presented by Braccini et al. (2000) and Souza et al (2011). The same was observed for the Cu element, except for the PDM parameter, which did not demonstrate a significant difference between the other treatments. This demonstrates that the KWS Irina cultivar is not tolerant to these two toxic elements. The Cu at high levels in plants is involved in affecting plant growth and development (Guo et al., 2007). Wang et al. (2009) found in barley plants that the element Cu at different pHs negatively affected root elongation at different concentrations. In the study by Lock et al. (2007) researchers found that Ca^{2+}, Mg^{2+}, Na^{+} ions and pH do not show a connection between the toxicity of Cu^{2+} as there is no competition between the binding sites of this element with the Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} and H^{+} cations. Li et al. (2010) found 50% redaction under root elongation of barley seedlings testing different concentrations of Cu^{2+}.

These, among other toxic elements, tend to be deposited in the roots or shoots, however, this depends on the malleability of this metal and its binding site within the plant, and what role it will play in the production of special metabolites (Ahmad et al., 2023; Yang et al., 2024). Furthermore, the disturbance of plant metabolism in excess by Al and Cu causes a reduction in chlorophyll content, inhibiting plant growth and respiration. The toxic elements in the plant trigger the excessive production of reactive oxygen species (ROS), including superoxide radical ($O^{2.-}$), hydroxyl radical ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2).

Our results also corroborate other authors who evaluated the action of toxic elements on barley, such as the studies by Getelina & Soster (2015) where they observed that the element Silicon (Si) affected the length of the shoot with values between 13.4 and 11.8 cm (without silicon) and between 11.2 to 13.0 cm (with Si) when compared to the control 12.4 to 12.3 cm, and there was no significant difference for root length in the barley crop between 4 .5 and 4.1 cm. Still in this study, the responses of the cultivars regarding the presence of Si also showed a significant difference, the cultivars Ametista, Topázio and BRS Elis, the latter being the latter, where it showed a positive response regarding the increase in seedling height when Si was applied; and by Tsukamoto et al. (2006) where they found greater deposition in barley seedlings with different concentrations of Mn in the roots under stress conditions with low redistribution from roots to leaves.

5. Conclusions

Germinated seeds of barley cultivar KWS Irina demonstrated to be intolerant to different concentrations of the toxic elements Al and Cu (35, 85 and 125 mg L^{-1}) during initial development in the *in vitro* test. Al and Cu had a negative influence on all seedling parameters analyzed, except for seedling dry mass in the Cu element. Future work should be carried out evaluating barley plants up to the reproductive period so that a comparison can be made and understanding between the action throughout the development process of this agricultural plant species.

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7. Authors' Contributions

Hanyele Renata Leite: research development, carrying out the in vitro test, writing and publication. *Antonio Carlos Pereira de Menezes Filho*: research development, article writing and publication. *Leandro Carlos*: supplier of barley seeds and careful analysis. *Porshia Sharma*: responsible for translation. *Matheus Vinícius Abadia Ventura*: experimental monitoring and data analysis. *Carlos Frederico de Souza Castro*: responsible for the laboratory's funds. *Marconi Batista Teixeira*: responsible for the laboratory funds and co-supervisor of the second author. *Frederico Antônio Loureiro Soares*: advisor to the second author. *Daniel Noe Coaguila Nuñez*: advisor, study analysis, description and publication.

8. Conflicts of Interest

No conflicts of interest.

9. Ethics Approval

Not applicable.

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