Chlorophyll fluorescence in pepper seedlings after seed priming and induction of salt stress[1]

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ABSTRACT

Chlorophyll fluorescence analysis is a method that has been widely disseminated among researchers for ensuring fast, accurate and non-destructive results. Thus, this study aimed to assess the chlorophyll *a* fluorescence of pepper seedlings grown from seeds that were unprimed, hydroprimed and primed with 24-EpiBL at 10^{-8} M after induction of salt stress at three concentrations (0.0, -0.2 and -0.4 MPa). To carry out the experiment, the Seed Reporter *Camera Spectral & Color Imaging System* (PhenoVation Life Sciences, Wageningen, Netherlands) was used. Three lots of seeds of the pepper cultivar 'Biquinho' were used. Chlorophyll fluorescence in the seedlings was evaluated on the seventh day after the seeds were subjected to different levels of salt stress. The chlorophyll fluorescence images obtained were analyzed by the *CFTI - Analysis software* (version 4.5). Through the data obtained it was possible to verify that, in general, there was an increase in chlorophyll *a* fluorescence in seedlings grown from seeds primed with 24-EpiBL. In short, the chlorophyll fluorescence technique proved to be viable and efficient, in addition to being a practical tool that can be used to support the analysis of the quality of pepper seedlings, whether or not subjected to saline stress conditions.

Keywords: image analysis; 'biquinho' cultivar; seedreporter.

INTRODUCTION

The pepper crop is part of an important segment of the vegetable market. It is a species that is increasingly expanding, especially in the Northeast region of Brazil, given its economic importance and high profitability. The 'Biquinho' pepper cultivar is one of the most used by small producers who aim at high yield, acceptance by the consumer market and good financial return (Caixeta *et al.*, 2014).

Pepper seeds in Brazil are expensive when compared to those other vegetables, so obtaining uniform initial stands, by using seeds with high physiological potential, becomes an essential factor for success in production systems (Bisognin *et al.*, 2016). Seeds of pepper plants, whose growth is indeterminate, can be harvested while still immature, which consequently results in the harvest of seeds with distinct maturity levels (Abud *et al.*, 2013). This can directly interfere in the physiological quality of the lots, resulting in slow and irregular germination (Gagliardi & Marcos-Filho, 2011). This behavior can be further aggravated at sites where conditions are not so favorable to germination, such as saline soils (Pezo *et al.*, 2020).

Salinity is one of the main reasons for the reduction of crop yield in several regions of Brazil (Paiva *et al.*, 2020). Excess salts reduce water availability to plants, in addition to causing nutritional imbalance and ion toxicity, in such a

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way that it affects crop yield and, consequently, production quality (Pedrotti *et al.*, 2015).

Techniques that can assist in the detection of the physiological quality of seeds are important for a more accurate analysis in the choice of lots with higher production potential. One of the tests used in this choice is the evaluation of chlorophyll fluorescence of seedlings, which can be employed as a new marker to analyze the physiological potential of seeds (Silva *et al.*, 2021).

It is worth pointing out that the seed deterioration process comprises the breakdown of chlorophyll molecules, with consequent changes in fluorescent properties. In general, the images produced from chlorophyll fluorescence can be employed to properly distinguish seedlings with superior and inferior quality, in an agile and dynamic way (Galletti *et al.*, 2020; Silva *et al.*, 2021; Sudki *et al.*, 2021). The SeedReporter instrument makes it possible to evaluate the chlorophyll fluorescence and vigor of seedlings in stressful or non-stressful situations. In addition, it is possible to compare the differences in vigor observed in the seeds based on the performance of seedlings. Thus, this study aimed to evaluate the chlorophyll a fluorescence of pepper seedlings grown from unprimed, hydroprimed and conditioned seeds with 24-epibrassinolide after induction of salt stress.

MATERIAL AND METHODS

This study was conducted at the Radiobiology and Environment Laboratory of the Center for Nuclear Energy in Agriculture, University of São Paulo (CENA/USP), in Piracicaba, SP, Brazil, using the SeedReporter *Camera Spectral & Colour* Imaging System (PhenoVation Life Sciences, Wageningen, The Netherlands).

For this experiment, three lots of seeds of the pepper cultivar ‘Biquinho Amarela’ were divided as follows: unprimed, hydroprimed and primed with 10^{-8} M of 24-EpiBL (Silva *et al.*, 2015). The seeds were conditioned until reaching phase II of the three-phase absorption pattern. The time for the seeds to obtain the three-phase absorption pattern was predicted based on preliminary assessments of the water absorption rate of the seeds (Bewley & Black, 1994).

These seeds were then subjected to different levels of salt stress (0.0, -0.2 and -0.4 MPa). The saline solutions were qualified using sodium chloride (NaCl) and distilled water, adjusting the osmotic potentials through Van’t Hoff research. Four replicates of 25 seeds per lot were put to germinate on blotting paper, previously moistened with a volume of 0.2% potassium nitrate (KNO_3) solution, equivalent to 2.5 times the weight of the dry substrate (MAPA, 2009), and placed inside transparent acrylic boxes (11.0 x 11.0 x 3.5 cm). The boxes were covered with plastic bags to prevent water loss and kept in a Biological Oxygen Demand (B.O.D.)-type germination chamber regulated at 30 °C, with 8-h photoperiod. Seven days after sowing, the seedlings were transferred onto black cardboard to capture chlorophyll fluorescence images inside the SeedReporter cabinet using 620 nm excitation LEDs. A 730 nm optical filter and a CCD camera were used to detect the signs of chlorophyll fluorescence. The images obtained were analyzed by the *CFTI - Analysis* software (version 4.5), which extracted the mean values of the average chlorophyll *a* fluorescence.

Statistical analysis

The experiment was set up in a completely randomized design (CRD) with four replicates for each lot. The results were subjected to analysis of variance, in a 3 x 3 factorial scheme: CONT (control), HYDRO (hydropriming) and 24-EPIBL (priming with 24-EpiBL at concentration of 10^{-8} M) *versus* three lots of pepper seeds. All treatments were subjected to different salinity levels (0.0, -0.2 and -0.4 MPa) and the means were compared by Tukey test ($p < 0.05$).

RESULTS AND DISCUSSION

In the present study, the different salinity levels considerably affected the chlorophyll *a* fluorescence, and seedlings grown from seeds subjected to the salinity level of 0.0 MPa (control) had a greater increase in chlorophyll *a* when the seeds were previously primed with 24-EpiBL or hydroprimed (Lot 3) (Figure 1).

Chlorophyll reflects the photosynthetic capacity of the plant, so it plays a considerable role in the capture and transmission of light energy in photosynthesis and is one of the most important indicators of salt tolerance (Zhao *et al.,* 2019). Salvatori *et al.* (2014) highlight that chlorophyll fluorescence analysis is an excellent tool to indicate the functionality of the photosystem in plants under stress. This test is sensitive to conditions of salt stress, and any possible damage to the photosynthetic machinery can be observed using this technique (Azevedo-Neto *et al.*, 2011).

The elements that participate in the photosynthetic

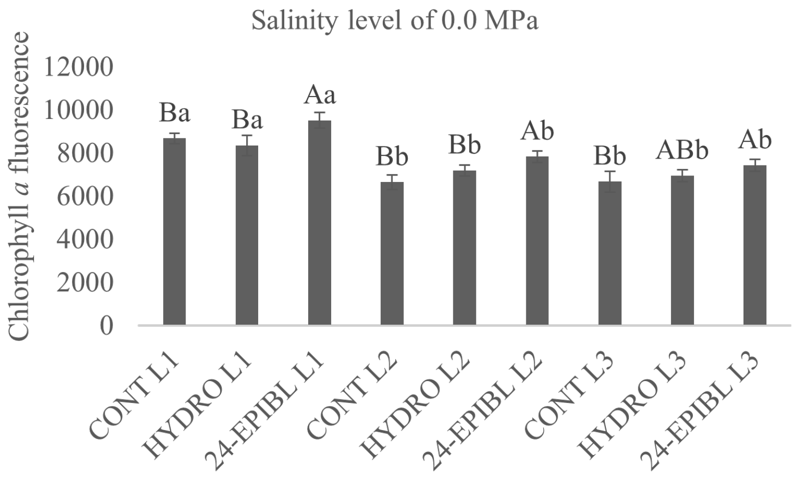

Figure 1: Chlorophyll a (Chl) fluorescence in seedlings grown from three lots of seeds (L1, L2 and L3) of the pepper cultivar 'Biquinho' subjected to three treatments: CONT (control), HYDRO (hydropriming) and 24-EPIBL (priming with 24-EpiBL at 10^{-8} M), under the salinity level of 0.0 MPa. Uppercase letters refer to the comparison between treatments (Control, Hydropriming and Priming with 24-EpiBL). Lowercase letters represent the comparison between lots. Equal uppercase or lowercase letters do not differ from each other (Tukey test, $p < 0.05$).

machinery are termed as photosynthetic pigments, photosystems, electron transport systems, gas exchange processes and enzymes involved in carbon metabolism. These constituents are determinant for photosynthetic efficiency and are heavily damaged by salt stress conditions. As an example, when a large amount of salt is deposited in the plant cell for an interval of time, the permeability of the membrane and the function of the thylakoids in the chloroplasts are compromised. In these situations there will be reductions in the activity of photosystems and in chlorophyll fluorescence (Wang *et al.*, 2009; Tsai *et al.*, 2019).

Figure 2 shows seedlings representing the salinity level of 0.0 MPa (control), whose results were similar to those obtained for seedlings grown from hydroprimed seeds. Among the samples, heterogeneity due to differences in vigor was observed even in those belonging to the same treatment. This difference is commonly observed in seeds belonging to the same lot (Rodrigues *et al.*, 2020). Seedlings originated from seeds primed with 24-EpiBL, besides having a noticeable longer length, also had higher chlorophyll fluorescence.

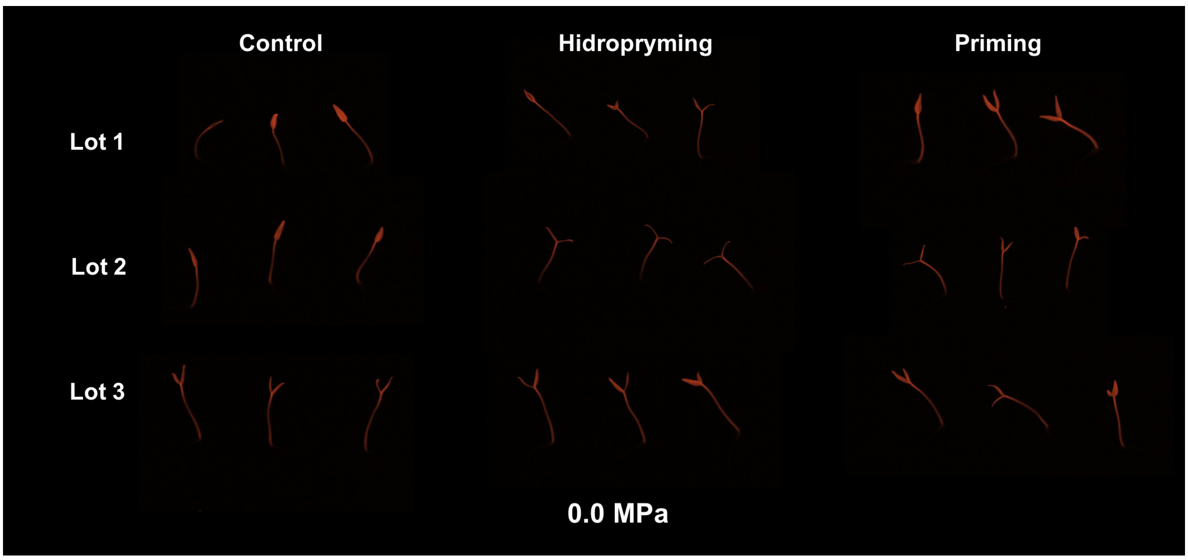

Figure 2: Images of chlorophyll fluorescence of pepper seedlings for the salinity level of 0.0 MPa in treatments control, hidroprying and priming, submitted to lots 1, 2 and 3.

Figure 3 shows the color scale for interpreting the images. Colors closer to black indicate that the seedlings will have lower chlorophyll fluorescence, that is, lower development and possibly lower vigor. Conversely, lighter colors up to white indicate higher chlorophyll fluorescence.

Results similar to the previously presented data were also observed for the salinity level of -0.2 MPa, at which there were significant effects on the increase of chlorophyll *a* fluorescence in seedlings grown from seeds primed with 24-EpiBL (Figure 4). For the other treatments, control and hydropriming, the salinity levels caused a decrease in the studied variable.

One of the effects of salinity on seedlings refers to the direct interference in Photosystem II, making it impossible to break down the water molecule to acquire the electrons needed in the photochemical process, in addition to reducing the contents of photosynthetic pigments. The decrease in chlorophyll content can be attributed to increased degradation and inhibited synthesis of the pigment (Hanchi *et al.*, 2014; Parihar *et al.*, 2015).

Chlorophyll loss is usually accompanied by the inactivation of photochemical reactions, especially those mediated by PSII in plants exposed to salt stress. Increase in the activities of enzymes, such as chlorophyllase, hydroxylase and dioxygenase, expedites the catabolism of chlorophyll (Maimaiti *et al.*, 2014), and the consequence is degradation of the molecules of this pigment. In addition to all these effects, the excess of salts in the absorption substrate causes deleterious effects on the seedlings, leading to reductions in their development (Silva *et al.*, 2016). Figure 5 shows the images related to the salinity level of -0.2 MPa.

Seedlings whose seeds were previously primed with 24-EpiBL, despite being subjected to a stressful condition, showed greater development compared to those of the other treatments, highlighting that the plant regulator used was able to mitigate the harmful effects of salt stress on the pepper crop (Houimli *et al.*, 2008; Houimli *et al.*, 2010; Samira *et al.*, 2012; Abbas *et al.*, 2013).

In the control treatment, for example, greater degradation was observed in the seedlings. Seedlings that develop in saline media usually have their photosynthetic activity compromised and, therefore, lower levels of chlorophyll (Silva *et al.*, 2016). Phytohormones, in turn, have an essential function in the metabolism of plants, primarily regulating the main metabolic pathways to maintain growth under adverse conditions (Fahad *et al.*, 2015).

In tomato seeds primed with BRs it was possible to observe this effect, because salinity reduced the growth and photosynthesis of seedlings; however, the action of priming significantly mitigated the decline (Ahanger *et al.*, 2020). According to Zhao *et al.* (2019), the presence of NaCl during seedling development inhibits the differentiation of plant tissues and organs, reduces the incidence of leaf primordia, and culminates in premature death of seedlings.

The salt stress condition can hinder the synthesis of 5-aminolevulinic acid, a precursor molecule of chlorophyll, and also elevate the activity of the chlorophyllase enzyme, which damages chlorophyll (Shin *et al.*, 2020). These consequences may have been observed due to the decrease in osmotic potential, preventing the seedlings from absorbing water, since the need for water consumption to produce a unit of dry matter depends on several factors, including water availability (Hussain *et al.*, 2016; Sousa, 2019).

Mendes *et al.* (2011) observed a similar result in chlorophyll *a* levels when pineapple plants were exposed to salt stress, whose effect was attributed to a response of increase in the chloroplast, indicating the activation of a protection mechanism of the photosynthetic machinery.

It is important to highlight that salt tolerance is associated with tolerance to osmotic and ionic stress, in addition to the elimination of reactive oxygen and changes in metabolic pathways and tissue morphology. At the molecular level, it can be observed that salt stress is related to mitogen-activated protein kinase signal transduction (Zhao *et al.*, 2019). Three main mechanisms of salinity tolerance have been proposed by Munns & Tester (2008), namely: ion exclusion, tissue tolerance, and maintenance of growth and water absorption. Other physiological components, such as initial seedling growth, may also contribute to salinity tolerance (Petrović *et al.*, 2016). For the salinity

Figure 3: Scale for interpreting chlorophyll a fluorescence images.

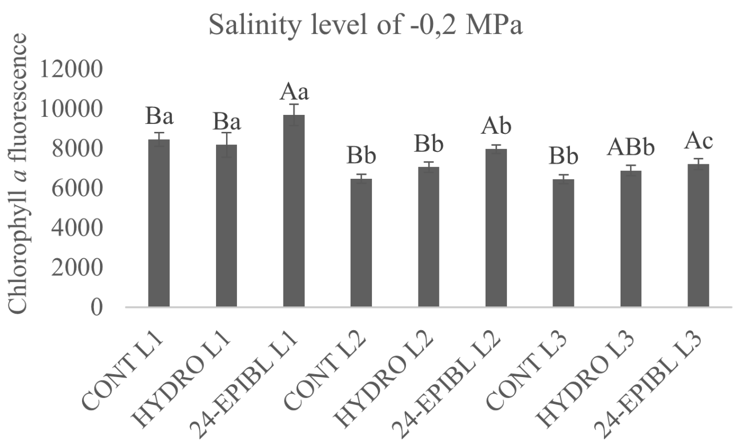

Figure 4: Chlorophyll a (Chl) fluorescence in seedlings grown from three lots of seeds (L1, L2 and L3) of the pepper cultivar 'Biquinho' subjected to three treatments: CONT (control), HYDRO (hydropriming) and 24-EPIBL (priming with 24-EpiBL at 10^{-8} M), under the salinity level of -0.2 MPa. Uppercase letters refer to the comparison between treatments (Control, Hydropriming and Priming with 24-EpiBL). Lowercase letters represent the comparison between lots. Equal uppercase or lowercase letters do not differ from each other (Tukey test, $p < 0.05$).

level of -0.4 MPa (Figure 6), an even more promising result was found when compared to the other levels because, as the salinity level increased, chlorophyll *a* fluorescence decreased; however, seedlings grown from seeds primed with 24-EpiBL managed to maintain the integrity of their photosynthetic machinery.

Figure 7 presents images of seedlings originated from seeds subjected to the salinity level of -0.4 MPa; the differences between the seedlings of each treatment are easily observed, clearly showing that the development of seedlings of the control treatment was lower when compared to those of the other treatments. In canola plants, it was also possible to identify that chlorophyll fluorescence was able to distinguish genotypes highly tolerant to salt stress (Athar *et al.*, 2015). Similar data were obtained in eggplant plants, for which chlorophyll fluorescence can be used as a diagnostic tool to identify salt-tolerant cultivars (Hanchi *et al.*, 2014).

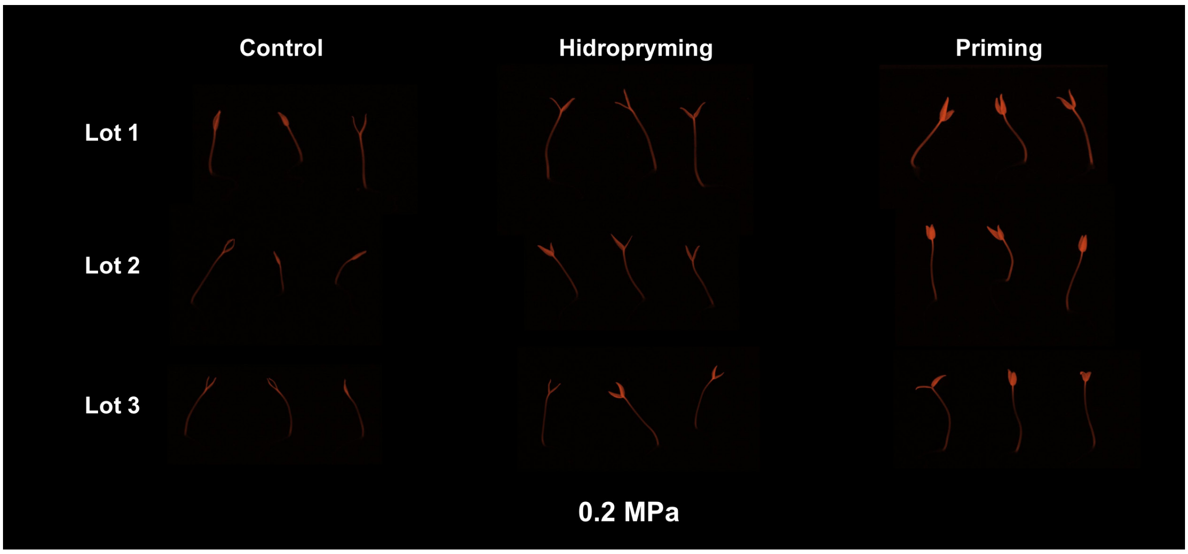

Figure 5: Images of chlorophyll fluorescence of pepper seedlings for the salinity level of -0.2 MPa in treatments control, hidroprying and priming, submitted to lots 1, 2 and 3.

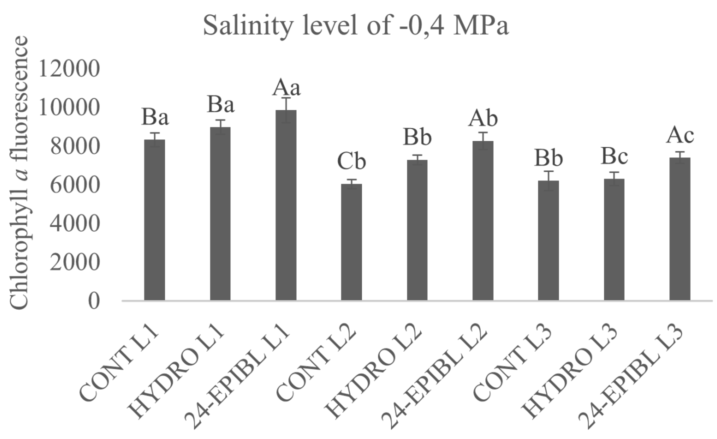

Figure 6: Chlorophyll a (Chl) fluorescence of seedlings grown from three lots of seeds (L1, L2 and L3) of the pepper cultivar 'Biquinho' subjected to three treatments: CONT (control), HYDRO (hydropriming) and 24-EPIBL (priming with 24-EpiBL at 10^{-8} M), under the salinity level of -0.4 MPa. Uppercase letters refer to the comparison between treatments (Control, Hydropriming and Priming with 24-EpiBL). Lowercase letters represent the comparison between lots. Equal uppercase or lowercase letters do not differ from each other

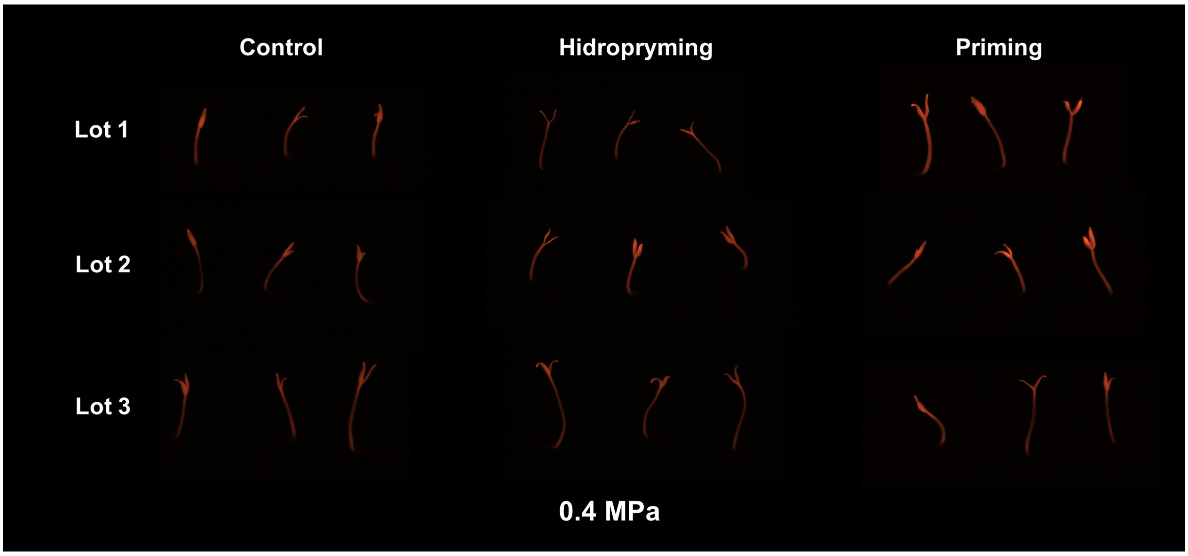

Figure 7: Images of chlorophyll fluorescence of pepper seedlings for the salinity level of -0.4 MPa in treatments control, hidroprying and priming, submitted to lots 1, 2 and 3.

From a practical point of view, chlorophyll fluorescence can provide fast and practical results in determining the physiological quality of seed lots subjected to stress conditions. Pepper seedlings can be used to distinguish lots with higher or lower vigor. This evaluation ensures effective, accurate and reliable results. This technique is a viable option to observe changes in seedling development caused by salt stress.

CONCLUSION

The chlorophyll fluorescence evaluation technique presents viable and efficient results, besides being a practice with tools that can be used as support in the analysis of the quality of pepper seedlings subjected to salt stress conditions. In general, seedlings whose seeds were primed with 24-EpiBL showed a greater increase in chlorophyll fluorescence even under a high level of salt stress.

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