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# Alleviating Impact of PEG Induced Drought Stress on Maize Seed Germination and Vigour with Effective Seed Priming Agents

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## ABSTRACT

**Background:** Drought stress poses a significant challenge to agricultural productivity, particularly in regions with limited water availability. Maize, a staple crop worldwide, is highly susceptible to drought stress, leading to reduced seed germination, stunted growth and ultimately diminished yields. Addressing this issue, in this study various phytohormones and polyamines were used as priming agents for seeds to evaluate its performance to mitigate drought stress in maize seed germination and vigour. It plays crucial roles in regulating plant growth and development, as well as responses to various environmental stresses, including drought. These hormones modulate physiological and biochemical processes, leading to adaptive changes that helps maize plants to cope with water scarcity.

**Methods:** The experiment was conducted by exposing maize seeds primed with different phytohormones and polyamines in optimum along with non primed seeds (control) to various levels of PEG concentration ranging from -0.2 MPa to -1.0 MPa for creating drought stress. Primed seeds subjected to a germination test. The seedling growth parameters and seed vigour were evaluated to analyse better performing seed priming treatment to mitigate drought stress.

**Result:** From the experiment it is concluded that increase in concentration of PEG (from -0.2 Mpa to -1.0 MPa) decreased the seed germination and seed vigour traits in maize. Among seed priming treatments, seeds primed with melatonin 150 µM showed greater performance seedling vigour and its related traits even under water deficit stress condition. In conclusion, seed priming with melatonin exhibited superior efficacy in mitigating drought stress in maize. This finding highlights the potential of melatonin as a promising seed priming agent for enhancing drought tolerance in maize seeds. As seed priming is simple, easy and cost effective method, farmers can use this technology as one of their practice to eliminate the effect of drought stress in maize crop.

**Key words:** Climate change, Drought stress, Maize seed germination and vigour, Phytohormones and polyamines, Seed priming.

## INTRODUCTION

Maize is predicted to become the crop with the greatest production globally, by 2025 and by 2050, demand for maize will double in the developing world (Partheeban *et al*., 2017). Maize, being a crucial staple crop globally, is particularly vulnerable to drought stress during its critical growth phases, leading to substantial reductions in productivity. Water deficit stress called drought stress affects almost every developmental stage of the plant. However, damaging effects of this stress was more noted when it coincided with various growth stages such as seed germination; seedling shoot and root growth stage and flowering (Rauf 2007; Khayatnezhad *et al*., 2010). The adverse effect of water shortage on seed germination and seedling growth has been well reported in different crops (Mostafavi *et al*., 2011; Khodarahmpour, 2011).

A lack of moisture during germination and subsequent growth stages can result in decreased production and delayed maturity time, as reported in previous studies in some of crops (Berg *et al*., 2014). The impact of drought stress on seed germination and seedling growth is influenced by both the intensity of the stress and the genetic makeup of the plant. In order to identify drought-tolerant trait across various plant species, an increasing number of studies have utilized *in vitro* screening methods (Kochev 2003, Turkan *et al*., 2005; Zarei *et al,* 2007; Hubbard *et al*., 2012; Merida *et al*., 2019; Pereira *et al*., 2002; Erayman *et al*., 2006). Previous studies have also demonstrated that drought tolerance observed during *in vitro* germination assays is highly correlated with field conditions (Brown *et al*., 1989;

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Khajeh-Hosseini). The osmolyte induced drought stress is one of the simulation methods which recreate drought conditions by applying osmotic stress, which increases the medium's osmotic pressure compared to plant tissues. One commonly used method for in vitro screening of drought tolerance in plants involves inducing stress using osmotic agents like PEG-6000 as it has a high molecular weight, inert and non-toxic and simulates drought conditions without any harmful effects on cells (Emmerich, 1990; Jiang *et al*., 1995; Tarkow *et al*., 1996).

Due to the increasing occurrence of drought stress, several strategies have been developed to minimize its impact on maize production. In this context, this study recorded the response of maize seeds primed with different phytohormones and polyamines, as well as non-primed seeds and hydroprimed seeds, to simulated drought stress condition. The effects of stress were assessed based on various traits related to seed germination and seedling growth potential and significant differences were observed among treated seeds.

## MATERIALS AND METHODS

The present research work was carried out at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. The experiment was conducted with four replications in a completely randomized block design. Maize genotype COH(M) 8 seeds were selected to conduct this experiment.

The experiment was conducted by exposing maize seeds primed with different phytohormones and polyamines *viz*., melatonin 150 µM, serotonin 100 µM, spermine 50 µM, spermidine 50 µM, salicylic acid 100 ppm, brassinolides 0.5 ppm and distilled water (Hydropriming), along with non primed seeds (control) to various levels of PEG concentration ranging from -0.2 MPa to -1.0 MPa for creating drought stress. Priming agents were directly dissolved in double distilled water (except those which will dissolve in ethanol) and seeds were soaked in the chemical solutions for 12 hours in a 1:1 (w/v) ratio and the temperature was maintained between 10° to 15°C. After priming, the seeds were removed from the solutions, rinsed in water, re-dried to the original moisture content and then subjected to a germination test as outlined by (ISTA 2019). The unprimed seeds were used as a control. The study was performed using roll towel method. The seeds were selected for size homogeneity, surface sterilized for 5 min in 1% (v/v) sodium hypochlorite and then rinsed twice in distilled water. 100 seeds of per replication was placed in the germination paper with corresponding PEG concentration (control, - 0.2 MPa, -0.4 MPa, -0.6 MPa, -0.8 MPa, -1.0 MPa) and kept in an incubator (35-40% relative humidity) at 25°C. Respective PEG solution was applied on daily basis after draining out the previously applied solution. Number of seeds germinated was manually counted on each day up to 7 days and the seed germination characters was considered based on the emergence of radicle and plumule (2 mm). After seven days, germination percentage and seedling vigour index was measured by following the protocol of International Seed Testing Association (ISTA, 1996).

The promptness index (PI) and germination stress tolerance index (GSI) were calculated using the following formulae given by Ashraf *et al*. (1990). By the end of the 7$^{th}$ day, plant germination %, shoot height stress index (SHSI) (%), root length stress index (RLSI) (%) and seed vigour were also measured.

**Evaluation of morphological parameter of primed seeds with phytohormones and polyamines under different concentration of drought stress condition**

**Promptness index**

PI (%) = nd2 (1.00) + nd4 (0.75) + nd6 (0.5) + nd8 (0.25)

Where,

PI = Promptness index.

nd = Number of seeds germinated on the day of observation (Partheeban *et al*., 2017; Shah *et al*., 2020; George 1967).

**Germination (%)**

According to the seed testing rules of (ISTA 1996), the germination tests were conducted in the germination room with 25±2! and 95% relative humidity by using the roll towel method. After the final count (*i.e*,7 days after sowing) the normal seedling count was taken for each replication and the germination percentage was calculated.

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds sown}} \times 100$$

**Germination stress tolerance index**

$$\text{GSTI (\%)} = \frac{\text{PI of stressed seeds}}{\text{PI control seeds}} \times 100$$

(Ashraf *et al*., 1996; Shah *et al*., 2020)

**Shoot length stress index (%)**

$$\text{SLSI (\%)} = \frac{\text{Shoot length of stressed plant}}{\text{Shoot length control plants}} \times 100$$

**Root length stress index (RLSI) (%)**

$$\text{RLSI (\%)} = \frac{\text{Root length stressed plant}}{\text{Root length of control plants}} \times 100$$

**Vigour index**

The vigour index (VI) was calculated by using the method given by Abdul-Baki and Anderson (1973) and the values were expressed in the whole number.

Vigour index = Germination (%) × [Root length (cm) + Shoot length (cm)]

**Statistical analysis**

The analysis of variance was carried out and comparison was done by Duncan's multiple range test (DMRT). The mean difference is significant at the P-values <0.05. Statistical analysis was performed using the SPSS 16.0 software (SPSS Inc., Chicago, USA).

## RESULTS AND DISCUSSION

Findings from this experiment suggest that drought stress had a negative impact on various traits associated with seed germination and seedling growth. It appears that all the treated seeds primed with phytohormones and polyamine and hydro primed seeds were also affected by drought stress, but there was significant variation among them in terms of how they responded to the stress. Our results clearly demonstrate that drought stress poses challenges to the processes of seed germination and seedling growth. The study findings indicate that the application of various concentrations of PEG 6000, ranging from -0.2 MPa to -1.0 MPa compared to ambient conditions, had a notable impact on the germination of maize seeds treated with different phytohormones and polyamines. Through analysis of variance and mean comparison, it was determined that significant variations existed among the primed seeds and their interactions subjected to different levels of drought stress.

Promptness Index and germination percentage of maize seeds was adversely affected due to the application of different levels (-0.2MPa, -0.4 MPa, 0.6 MPa, 0.8 MPa and -1.0 MPa) of PEG 6000 on all the treatments. The results illustrate that there was a decrease in promptness index and germination percentage of all the treatments with increase in the stress levels (Table 1 and Fig 1). Seeds Primed with melatonin 150 µM (10.15%) @ -0.4 MPa and performed better and showed tolerance against PEG induced water deficit stress even at higher level of osmotic stress condition. The application of PEG 6000 to induce water stress revealed a clear pattern: as the concentration of stress increased, the Germination Stress Tolerance Index (GSTI) decreased. The GSTI values of seeds treated with melatonin 150 µM, ranging from 7.79% to 1.63% from 0.2 MPa to 1.0 MPa compared to ambient condition indicated significant variations among other treatments compared to the control group. These results are illustrated in Fig 2. The findings demonstrate that higher levels of water stress, as induced by increasing concentrations of PEG-6000, had a negative impact on the GSTI. This suggests that the ability of melatonin to tolerate and overcome the germination stress was compromised under more severe stress conditions.

The shoot length stress index was observed from 27.5% to 2.49% in case of seeds primed with melatonin 150 µM and in case of serotonin 100 µM it is ranging from 28.9% to 0.86% was observed (Fig 3). The decrease in the shoot length stress index was reduced by priming the seeds with melatonin 150 µM and serotonin 100 µM. Similarly, the root

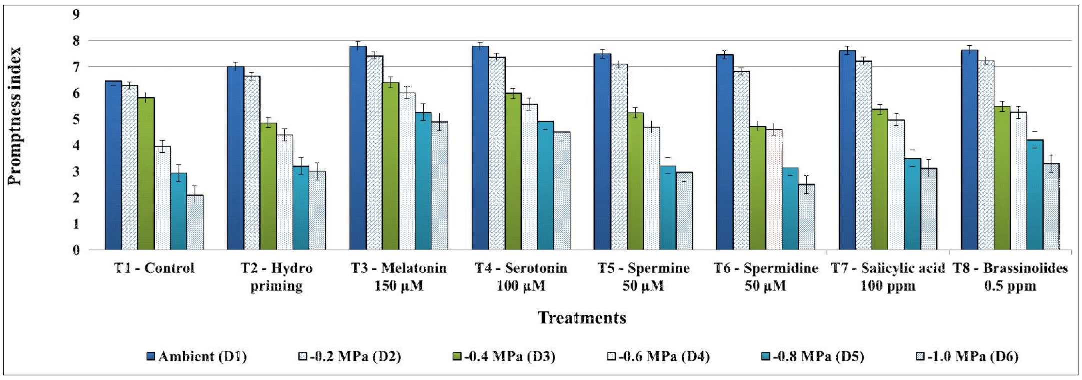


**Fig 1:** Effect of various concentrations of PEG levels on promptness index of maize seeds primed with phytohormones and polyamines.

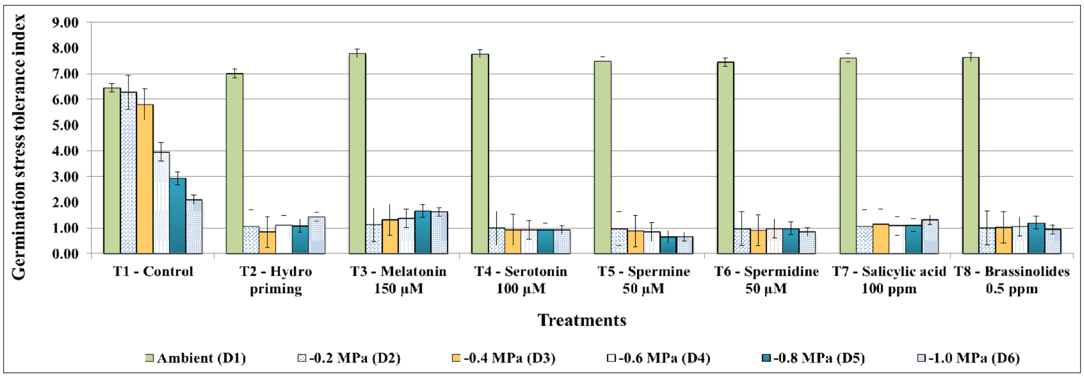


**Fig 2:** Effect of various concentrations of PEG levels on germination stress tolerance index of maize seeds primed with phytohormones and polyamines.

length stress index also shows their highest range in ambient condition which started to reduce by increasing the PEG induced osmotic stress levels. The root length stress index in this treatment found to decrease from 30.5% to 2.77% in seeds treated with melatonin 150 µM. Thus, the decrease in root length stress index can be minimized by seed priming with melatonin 150 µM (Fig 4). Seed vigour index of 2695 was observed at ambient condition which is followed by 2444 at -0.2 Mpa, 2100 at -0.4 MPa, 1414 at -0.6 MPa, 1078 at -0.8 MPa and 845-1.0 MPa in case of melatonin 150 µM primed seeds. The decrease in vigour index in higher stress levels were abridged by enhancing the seeds with melatonin priming (Table 2).

Drought events have become more frequent and severe due to climate change, leading to significant ecological and socio-economic impacts (Smith *et al.*, 2019). Rising temperatures and changing precipitation patterns exacerbate drought conditions, affecting agriculture, water resources and ecosystems (IPCC, 2021). Urgent measures are required to mitigate the effects of drought and address the underlying causes of climate change to ensure a sustainable future (UNCCD, 2021). Understanding the variation in response to drought stress among different seed priming treatments can be valuable for researchers. It opens up possibilities for identifying and selecting suitable priming agent with improved drought tolerance, which could contribute to the development of more resilient crops in the face of climate change and water scarcity.

The results from this study showed that the concentration of PEG 6000 played a crucial role in influencing the germination process and its related traits of maize seeds when combined with phytohormones and polyamines. The observed significant differences between the primed seeds exposed to different levels of drought stress further emphasize the importance of considering the specific conditions and treatments applied to enhance seed germination. In this research, the study focused on utilizing seed germination and seedling growth potential as indicators to assess effect of different seed priming treatments associated with drought tolerance and to identify suitable priming agent for maize with the highest tolerance to drought stress conditions. The results demonstrated the negative impacts of drought on the maize seed germination, with the severity of these impacts generally corresponding to the intensity and levels of the stress. These findings align with previous research and provide additional support for the effectiveness of using PEG as a substance to simulate drought conditions in laboratory settings in soyabean germplasm (Pavli, 2020).

It emphasize the importance of considering the concentration of PEG-6000 when assessing the germination stress tolerance of crop used. Further analysis and investigation are needed to identify the specific mechanisms that contribute to the observed variations in GSTI among treatments. These results of germinations stress tolerance index of maize seeds cope up with the study conducted by

**Table 1:** Germination % of maize seeds primed with phytohormones and polyamines under different level of PEG induced drought stress.

| Treatments | Ambient (D1) | -0.2 MPa ($D_2$) | -0.4 MPa (D3) | -0.6 MPa (D4) | -0.8 MPa (D5) | -1.0 MPa (D6) |
|---|---|---|---|---|---|---|
| T1 - Control | 90 (72)±0.18 $^{g}$ | 60 (51)±0.71$^{vw}$ | 52 (46)±0.80$^{vy}$ | 40 (39)±0.29$^{z2}$ | 20 (27)±0.18$^{z5}$ | 8 (16)±0.03$^{z6}$ |
| T2 - Hydro priming | 92 (74)±1.25 $^{ef}$ | 65 (54)±1.00$^{rs}$ | 59 (50) ±0.54$^{w}$ | 43 (41)±0.29$^{z1}$ | 37 (37)±0.09$^{z3}$ | 32 (34)±0.39$^{z4}$ |
| T3 - Melatonin 150 µM | 98 (82)±0.57 $^{a}$ | 94 (76)±0.70$^{cd}$ | 85 (67)±1.04$^{hi}$ | 79 (63)±0.59$^{k}$ | 70 (57)±1.12$^{no}$ | 64 (53)±0.37$^{rt}$ |
| T4 - Serotonin 100 µM | 97 (80)±0.10 $^{ab}$ | 91 (73)±1.18$^{fg}$ | 83 (66)±0.73$^{j}$ | 75 (60)±0.79$^{m}$ | 67 (55)±0.36$^{pq}$ | 62 (52)±0.15$^{tu}$ |
| T5 - Spermine 50 µM | 94 (76)±0.35 $^{cd}$ | 75 (60)±0.66 $^{lm}$ | 68 (56)±0.46$^{p}$ | 59 (50)±0.50$^{w}$ | 54 (47)±0.17$^{x}$ | 49 (44)±0.17$^{z}$ |
| T6 - Spermidine 50 µM | 93 (75)±0.22 $^{de}$ | 70 (57)±1.07$^{o}$ | 61 (51)±0.83$^{suv}$ | 55 (48)±0.90$^{x}$ | 48 (44)±0.31$^{stz}$ | 43 (41)±0.53$^{z1}$ |
| T7 - Salicylic acid 100 ppm | 95 (77)±0.55 $^{c}$ | 84 (66)±0.77$^{ij}$ | 72 (58)±0.07$^{n}$ | 67 (55)±0.93$^{pq}$ | 63 (53)±0.64$^{t}$ | 54 (47)±0.02$^{x}$ |
| T8 - Brassinolides 0.5 ppm | 96 (78)±1.31 $^{bc}$ | 86 (68)±0.97$^{h}$ | 77 (61)±0.50$^{l}$ | 71 (57)±0.60$^{no}$ | 65 (54)±1.08$^{qr}$ | 59 (50)±0.40$^{w}$ |

**Table 2:** Seedling vigour index of maize seeds primed with phytohormes and polyamines under different levels of PEG induced drought stress.

| Treatments | Ambient (D1) | -0.2 MPa ($D_2$) | -0.4 MPa (D3) | -0.6 MPa (D4) | -0.8 MPa (D5) | -1.0 MPa (D6) |
|---|---|---|---|---|---|---|
| T1 - Control | $1665\pm21.0^{k}$ | $942\pm10.90^{r}$ | $582\pm5.74^{x}$ | $380\pm5.56^{z}$ | $134\pm0.55^{z2}$ | $31\pm0.00^{z3}$ |
| T2 - Hydro priming | $1665\pm18.0^{j}$ | $1157\pm9.05^{o}$ | $915\pm5.91^{r}$ | $525\pm4.82^{y}$ | $285\pm2.23^{z1}$ | $170\pm2.83^{z2}$ |
| T3 - Melatonin 150 µM | $1665\pm19.3^{a}$ | $2444\pm36.58^{c}$ | $2100\pm15.72^{f}$ | $1414\pm3.85^{mn}$ | $1078\pm16.14^{p}$ | $845\pm13.51^{s}$ |
| T4 - Serotonin 100 µM | $1665\pm41.0^{b}$ | $2202\pm25.47^{e}$ | $1942\pm19.16^{h}$ | $1238\pm12.2^{l}$ | $851\pm3.76^{s}$ | $701\pm11.45^{uv}$ |
| T5 - Spermine 50 µM | $1665\pm19.2^{e}$ | $1628\pm5.54^{k}$ | $1278\pm19.57^{n}$ | $861\pm7.62^{s}$ | $529\pm6.30^{y}$ | $421\pm0.43^{z}$ |
| T6 - Spermidine 50 µM | $1665\pm2.1^{g}$ | $1421\pm19.34^{m}$ | $1049\pm12.49^{pq}$ | $743\pm9.86^{t}$ | $408\pm4.72^{z}$ | $318\pm3.35^{z1}$ |
| T7 - Salicylic acid 100 ppm | $1665\pm37.0^{d}$ | $1882\pm12.17^{i}$ | $1404\pm20.06^{m}$ | $1032\pm5.62^{q}$ | $662\pm5.86^{vw}$ | $508\pm3.97^{y}$ |
| T8 - Brassinolides 0.5 ppm | $1665\pm10.0^{c}$ | $1952\pm19.26^{h}$ | $1563\pm20.21^{l}$ | $1136\pm8.12^{o}$ | $728\pm3.72^{tu}$ | $643\pm3.50^{w}$ |

Shah *et al*. (2020), where they have screened drought tolerant high yielding chickpea genotypes based on physio-biochemical indices including germination stress tolerance index. Polyethylene glycol, which is utilized in this experiment, functions as an osmotic agent. It plays a significant role in regulating mineral elements, hormone and protein metabolism, as well as impacting signal transduction processes (Verslues *et al*., 1998). The process of water uptake by seeds is a physical phenomenon that triggers the activation of metabolic processes, resulting in the breaking of seed dormancy upon hydration. However, when polyethylene glycol (PEG) is used, it reduces the osmotic potential and limits the availability of water for the seeds, leading to reduced germination rates. Under drought conditions, the emergence of the radicle is primarily hindered due to a reduction in the water potential gradient between the external environment and the seed. Additionally, an increase in polyethylene glycol (PEG) concentration leads to a decrease in both root and shoot length. The decrease in shoot and root length may be attributed to hindered cell division and elongation processes, leading to a form of tuberization. This tuberization, coupled with lignification of the root system, enables the plant to enter a slowed-down state, awaiting favorable conditions. Thus, the results from this experiment show that decreased root and shoot length stress tolerance index which aligns with previous study by (Sagar *et al*., 2020) where they used Polyethylene Glycol (PEG) to induce drought stress on five rice genotypes at early seedling stage where they recorded root and shoot stress tolerance indices level under different stress concentrations.

Elevated concentrations of polyethylene glycol (PEG) during the growth of maize seedlings have been observed to inhibit their developmental traits and overall survival. Parameters such as seedling vigour index, shoot length, root length, germination percentage and dry weight consistently decreased when exposed to various stress levels. This decline in seedling vigor index became more pronounced with higher stress levels, which aligns with findings from Hellal *et al*. (2017), where they demonstrated the effect of PEG in inducing drought stress on barley seedling cultivars where the susceptible genotype recorded lower seedling vigour index than the tolerant one. In this study the different seed priming treatments exhibited significant variations in their response to PEG.

Further investigation into the specific mechanisms or genetic factors that underlie the different treatments in responses to drought stress would be worthwhile. This knowledge could aid in the development of targeted strategies for enhancing drought tolerance in crops and other plant species. These findings highlight the need for careful optimization of PEG 6000 concentrations and the selection of appropriate seed priming agent to enhance the germination potential of maize seeds under varying drought stress levels. Further investigation and exploration of the underlying mechanisms can provide valuable insights for

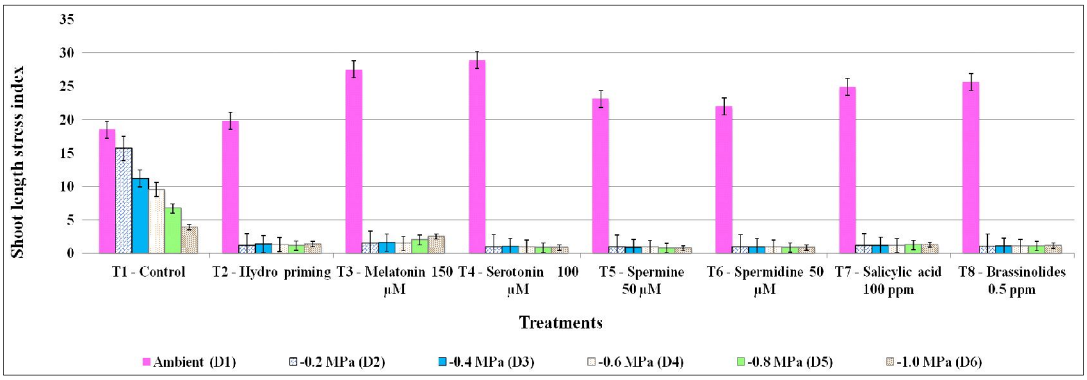


**Fig 3:** Effect of various concentrations of PEG levels on shoot length stress index of maize seeds primed with different phytohormones and polyamines.

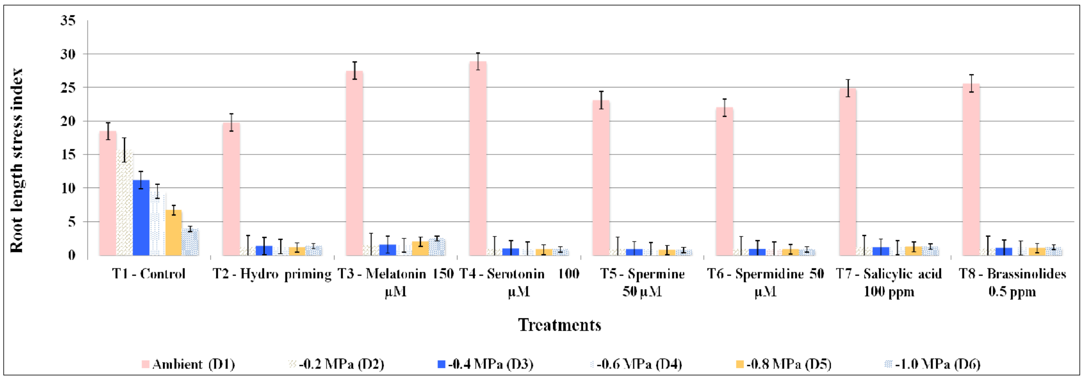


**Fig 4:** Effect of different PEG levels on root length stress index of maize seeds primed with different phytohormones and polyamines.

improving seed priming techniques and developing more drought-tolerant maize varieties.

## CONCLUSION

In conclusion, the experiment investigating the effect of seed priming with various phytohormones and polyamines demonstrated their potential in mitigating the adverse effects of PEG-induced drought stress on maize seedling growth parameters. The application of these treatments resulted in improved outcomes compared to the untreated control group. The priming treatments melatonin 150 µM positively influenced key growth parameters such as germination percentage, shoot length, root length and seed vigour index of maize seeds under drought stress condition. The use of phytohormones and polyamines acted as protective agents, enhancing the resilience and survival of maize seedlings under PEG induced drought stress. These findings suggest the promising role of seed priming techniques using phytohormones and polyamines as practical strategies for enhancing crop tolerance to water scarcity, ultimately contributing to the sustainable cultivation of maize and potentially other crops in drought-prone environments. Further studies are warranted to explore the underlying mechanisms and optimize the application protocols of these priming treatments for broader agricultural applications.

**Conflict of interest:** None.

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