

EFFECT OF EXOGENOUS PLANT HORMONES ON GERMINATION AND RESISTANCE OF DURUM WHEAT (*TRITICUM DURUM* DESF. VAR. *VITRON*) TO DROUGHT STRESS

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Abstract

Description of the subject: Cereals crops are subjected to drought at different growth stages and cause considerable yield loss, thus threatening global food security. Hormonal seed priming can markedly improve the germination and the growth on wheat under abiotic stress.

Objective: The aim of our work is to study the effect of exogenous phytohormones on the germination and growth of durum wheat (*Triticum durum* Desf. Var. Vitron) under (PEG-6000) induced drought stress with the concentration of 12,5% (w/v) as to generate osmotic potential of 216 KPa.

Methods: Exogenous Gibberellic Acid (GA3), Salicylic Acid (SA) and Auxin (IAA), were applied by priming technique. Seven treatments were used as follow; Control, GA3 (0,1mM), GA3 (0,25mM), SA (0,1mM), SA (0,25mM), IAA (0,1mM) and IAA (0,25mM), to investigate the effect of priming on seed germination rate, germination precocity, electrolyte leakage, relative water content and proline.

Results: Results show that exogenous plant hormones improve germination parameters of (*T. durum*) under PEG stress. Exogenous GA3, SA and IAA positively enhanced the growth profile of seedlings by improving proline and relative water content and decreasing electrolyte leakage under drought.

Conclusion: The performance of primed seed with plant hormones was found better than control under PEG-induced drought, and more investigations are required to explore the possible effects of these phytohormones on plants growth and productivity under drought stress.

Keywords: Plant hormones, priming, germination, *Triticum durum* Desf., PEG-6000, drought stress.

EFFET DES PHYTOHORMONES EXOGENE SUR LA GERMINATION ET LA RESISTANCE DU BLE DUR (*TRITICUM DURUM* DESF. VAR. *VITRON*) AU STRESS HYDRIQUE

Résumé

Description du sujet : Les cultures sont soumises à la sécheresse à différents stades de croissance et entraînent des pertes de rendement considérables, menaçant ainsi la sécurité alimentaire mondiale. L'amorçage hormonal des graines peut améliorer considérablement la germination et la croissance du blé soumis à un stress abiotique.

Objectifs : L'objectif de notre travail est d'étudier l'effet des phytohormones exogènes sur la germination et la croissance du blé dur (*Triticum durum* Desf. Var. Vitron) sous stress hydrique induit par le PEG-6000 avec la concentration de 12,5% (m/v) afin de générer un potentiel osmotique de 216 KPa.

Méthodes : L'acide gibbérellique (AG3), l'acide salicylique (AS) et l'auxine (IAA) exogènes ont été appliqués par technique d'amorçage. Sept traitements ont été utilisés comme suit : Témoin, AG3 (0,1mM), AG3 (0,25mM), AS (0,1mM), AS (0,25mM), IAA (0,1mM) et IAA (0,25mM), pour étudier l'effet de l'amorçage sur le taux de germination des graines, la précocité de germination, la fuite d'électrolytes, la teneur relative en eau et la proline.

Résultats : Les résultats montrent que les hormones végétales exogènes améliorent les paramètres de germination de (*T. durum*) sous stress induit par le PEG. L'AG3, l'AS et l'IAA exogènes ont amélioré la croissance des plantules en améliorant la teneur en proline et la teneur relative en eau et en réduisant les fuites d'électrolytes en condition du stress hydrique.

Conclusion : La performance des semences amorcées par des hormones végétales s'est révélée meilleure que le témoin (non traités) contre la sécheresse provoquée par le PEG, et davantage études sont nécessaires pour explorer les effets possibles de ces phytohormones sur la croissance et la productivité des plantes sous stress hydrique.

Mots clés : Phytohormones, priming, germination, *Triticum durum* Desf., PEG (6000), stress hydrique.

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INTRODUCTION

Water is one of the major limiting factors for the agricultural production in arid and semiarid areas. Drought is one of the principal abiotic stresses that negatively influence the growth of plant and yield [1]. It occurs when the loss of water by the plant exceeds the capacity of water absorption by the roots from the soil for long enough to cause irreversible damages to the plant [2] and [3]. Drought conditions create osmotic stress in organisms, which eventually cause desiccation and resistance to water uptake in plants [4]. Hence, improved tolerance to drought has been an important goal in crop improvement programs [5]. Drought tolerance is a complex trait affected with many genes and mostly conditioned by many component responses, which may interact and may be different with respect to types, intensity and duration of water deficit [6]. This stress may occur in nearly all climatic regions. Plants had to evolve different mechanisms for sensing and responding to drought [7]. Their interactions with the environment as well as their growth and development are regulated by plant hormones [8]. Exogenous application of plant growth regulators is a strategy to increase yield, improve crop quality and regulate uptake and accumulation of mineral nutrients in plants [9]. Participation of salicylic acid (SA) in drought responses is indicated by elevation of this hormone after water withdrawal as well as by positive effects of exogenous SA application on plant tolerance [10]. Auxins can influence plant adaptation to adverse environmental conditions by control of plant growth [11]. Thus, a complex cross-talk among different phytohormones is underlying drought stress responses. Gibberellic acid (GA3) is an important plant growth regulator that affects plant growth and development by inducing metabolic activities and regulating nitrogen utilisation [12]. Exogenously provided GA restored the normal growth and water deficit sensitivity in the plants and many studies indicated that GAs plays important roles in plant responses to drought stress [13]. The current experiment was performed to evaluate the capability of exogenous GA, ABA and IAA in response to germination, initial growth, oxidative damage and defence system of durum wheat (*Triticum durum* Desf. Var. *Vitron*) under PEG induced drought stress.

MATERIAL AND METHODS

1. Plant material

Our work was conducted on seeds of durum wheat (*Triticum durum* Desf.), variety *Vitron*, where the seeds come from the Technical Institute of Field Crops (ITGC El Harrach), the seeds have a specific purity and a germination faculty of 98%. The variety *Vitron* is characterized by its resistance to cold and by its sensitivity to drought and water stress.

2. Experimental design

2.1. Seed pre-treatment (priming)

The application of the different treatments is carried out by the priming technique. Which consists in soaking the durum wheat seeds in the prepared hormonal solutions at different concentrations during 6h. The seeds are then rinsed and rehydrated on sterile filter paper at 25°C, using a ventilated oven; until they reach the initial moisture, which means the moisture they had before, they were soaked.

2.2. Treatments used

In our experiment, we used three plant hormones with two concentrations for each, as germination activators. For this purpose, we placed the durum wheat seeds into seven seed lots. The first lot T0 (Control) did not had any treatment, the second and third lot T1 and T2 had a pretreatment with Gibberellic Acid (GA3), the fourth and fifth lot received a pretreatment with Salicylic Acid (SA) and the last lots T6 and T7 received a hormopriming by Auxin (IAA). It should be noted that all seed lots were imbibed for 6h.

Table1: Description of the treatments used

Treatment	Description	Concentration	Soaking time
T ₀	Test	/	/
T ₁	GA3	0,1mM	6h
T ₂	GA3	0,25mM	6h
T ₃	SA	0,1mM	6h
T ₄	SA	0,25mM	6h
T ₅	IAA	0,1mM	6h
T ₆	IAA	0,25mM	6h

2.3. Germination

Durum wheat seeds were disinfected with sodium hypochlorite at 2.5% for 10min, and then rinsed with distilled water to remove any traces of chlorine before germination. After finishing the various pretreatments using the priming method, seeds are placed in 90mm Petri dishes lined with two layers of sterile filter paper and baked at 25°C. Germination is

spotted by the emergence of the radicle out of the seed coats, which is at least 2mm long. [6].

2.4. Measured parameters

- Germination percentages (G%) were calculated as total number of germinated seeds by total number of seed used into 100.
- Precocity of germination: The precocity of seeding which corresponds to the rate of seeds germinated from the 1st day.
- Electrolyte leakage was estimated as described previously [14]. Firstly, the electrical conductivity (ECa) of the 20 leaf disks submerged was measured. Secondly, the leaf disks were put in test tubes and incubated at 55°C for 25 min, and the electrical conductivity (ECb) was measured. Finally, the test tubes were boiled at 100°C for 10 min, and the electrical conductivity (ECc) was determined. Electrolyte leakage was calculated using the following formula: electrolyte leakage (%) = (ECb – ECa)/ECc × 100.
- The relative leaf water content is the ratio of the water content of the sample at the time of harvest to the maximum water content when the cells are fully turgid. It is calculated as $RWC \% = (P_i - P_s) / (P_{pt} - P_s) \times 100$ were P_i : initial fresh weight; P_{pt} : fully turgid weight; P_s : dry weight.
- Proline has been determined by the Troll and Lindsey method [15]. A standard range is made using a pure proline solution and optical density is read at 515 nm with a spectrophotometer.

2.5. Application of drought stress

The study of seed and seedling response to water stress is carried out by soaking 28 Petri dishes containing seeds treated with the

different hormone solutions plus the control, with a 12.5% polyethylene glycol-6000 (PEG 6000) solution. Because polyethylene glycol 6000 has a high molecular weight and cannot pass the cell wall, it has been used to determine drought resistance at the germination stage and to establish varied amounts of water potential in germination studies.

3. Statistical analysis

One-way ANOVA was applied to analyse the difference between treatments using the STATGRAPHICS-CENTURION XVI software (version 16.1.18). Different letters were used to portray the significant difference among treatments by Fisher's test (LSD) at 95% of confidence interval.

RESULTS

1. Germination rate (%)

Figure 1 shows the effect of priming on germination percentage of durum wheat (*Triticum durum* Desf.) seeds under water stress. The statistical analysis shows a significant difference ($p < 0.01$) of the treatment factor on seed germination rate. The results obtained show that the pretreatment with auxin at a concentration of 0.1mM (T5) marked the best values of germination rate with 98% of seeds germinated, however this value decreased to 93% when the concentration of this hormone increases to 0.25mM (T6). While, the lowest values of this parameter were recorded in seeds treated with concentrated AG3 of 0.25mM (T2) and 0.1mM (T1) with germination rates of 87 and 85% respectively. While, SA treated seeds (T3 and T4) and control seeds (T0) show the same germination percentage with (93%).

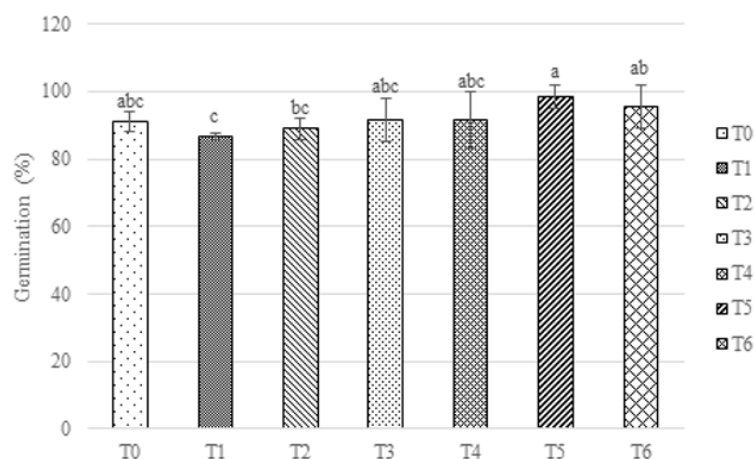


Figure 1: Variation in germination rates of *Triticum durum* Desf. seeds under drought

2. Germination precocity (%)

The results presented in Figure 2 show that seed pre-treatment improves the ability of seeds to germinate in a medium with high osmotic potential by increasing germination precocity. Statistical analysis indicated a significant difference ($p < 0.01$) of priming on germination precocity of durum wheat seeds. The highest values of germination precocity were observed in seeds treated with AG3 at 0.1mM (T1) and

IAA at 0.25mM (T6) with a rate of 76.66% of germinated seeds. While treatments T3, T4 and T5 did not show a statistically significant difference between them, with mean values of 68.33%, 63.33% and 68.33% respectively. However, the lowest germination precocity was recorded in treatments T0 (control) and T2 (0.25 mM AG3) with the rates of 40% and 55.55% of germinated seeds respectively.

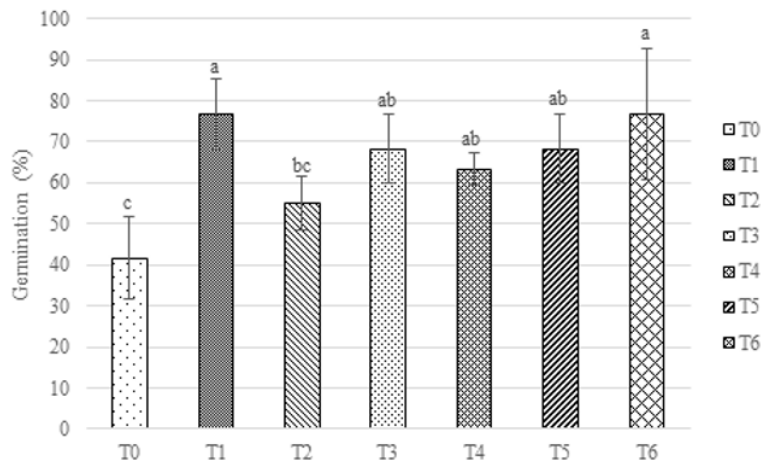


Figure 2 : Variation in germination precocity of *Triticum durum* Desf. under drought.

3. Electrolyte leakage (EL%)

The results of the statistical analysis indicate that seed priming leads to an improvement of the cell membrane structure of the seedlings under water stress through the inhibition of electrolyte leakage. Under water stress condition, the results in Figure 3 show that the percentage of electrolyte leakage is higher in control seedlings (T0) with a rate of 77.22%,

followed by seedlings from seeds pre-treated with IAA and AS at concentrations of 0.25mM (T6) and 0.1mM (T5) which give slightly lower leakage with rates of 74.61% and 70.27% respectively. On the other hand, the lowest electrolyte leakage rates were obtained in plants from seeds pre-treated with AG3 at 0.1mM (T1) and AS at 0.25mM (T4) with the percentages of 50.15% and 53.75% respectively.

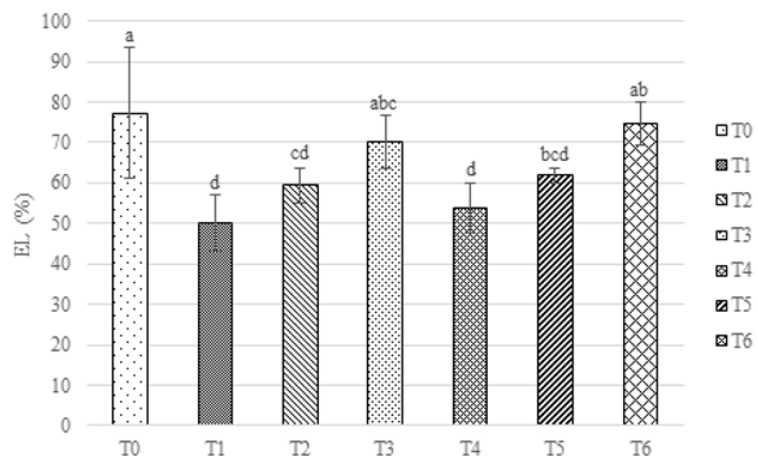


Figure 3: Variation in (EL) means of *Triticum durum* Desf. plants under drought (3 weeks after sowing).

4. Relative water content (RWC %)

The relative water content of the leaves is the ratio of the water content of the sample at extraction to the maximum water content when

the cells are fully turgid. The results in Figure 4 show that seed pre-treatment positively affects the water status of wheat (*Triticum durum* Desf.) plants under water stress.

The analysis of variance revealed a highly significant difference ($p < 0.01$) between the different treatments on the relative leaf water content. Under water deficit, seedlings from seeds pre-treated with exogenous hormones of different concentrations showed significant

relative water contents ranging from 61 to 69%. On the other hand, water stress induced a low water status in plants from untreated seeds (T0) which recorded the lowest water content (57.33%).

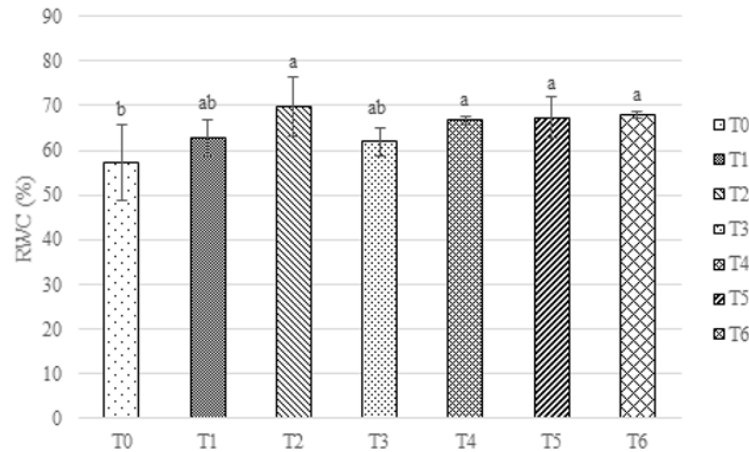


Figure 4: Variation in relative water content mean of *Triticum durum* Desf. plants under drought (3 weeks after sowing).

5. Proline content ($\mu\text{g}/\text{gFW}$)

The results shown in figure 5 show that seed priming has a positive effect on the accumulation of proline, which is considered a stress metabolite in higher plants. Analysis of variance of the results revealed a highly significant difference ($p < 0.01$) of priming on proline accumulation in durum wheat (*Triticum durum* Desf.) leaves. The lowest proline content was recorded in plants from seeds pre-treated with AS at 0.1mM (T3) with a value of

0.038 $\mu\text{g}/\text{gMF}$, followed by seeds primed with AS at 0.25mM (T4) and IAA at 0.1mM (T5) with a value of 0.046 $\mu\text{g}/\text{gMF}$ for both treatments. On the other hand, under water stress, proline accumulation is higher in seedlings of T2 and T6 with a content of 0.089 and 0.081 $\mu\text{g}/\text{g MF}$ respectively, this accumulation is slightly lower in the control (T0) followed by seedlings pretreated with AG3 at 0.1g/l (T1) with values of 0.079 $\mu\text{g}/\text{g MF}$ and 0.075 $\mu\text{g}/\text{g MF}$ respectively.

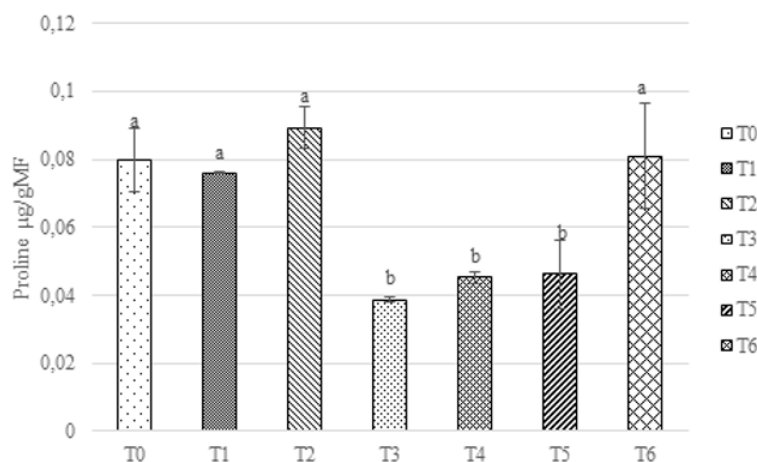


Figure 5: Variation in proline content means of *Triticum durum* Desf. plants under drought stress (3 weeks after sowing).

DISCUSSION

The results of the current study reveal that the priming with exogenous GA, ABA and IAA positively influenced the germination seeds, morphology and growth of durum wheat variety *Vitron* under drought stressed conditions. Drought damages plants during germination, reducing their qualitative and quantitative performance and creating weakness and heterogeneity in plant development in farmers' fields [16]. Germinating seeds and seedlings are exposed at critical stages of plant development to various abiotic stresses that pose serious risks to the plants growing near the soil surface. The stresses include drought, salinity and cold [17]. Thus, Seed germination is adversely affected by moisture stress.

To minimize the negative effects of abiotic stresses during germination and initial growth of seedlings, seeds have been treated with phytohormones. Among the most used are those with gibberellic acid [18], auxin [19] and salicylic acid [20]. In this research, low water potential decreased germination percentage, mean germination time under drought stress. Due to the reduction in osmotic pressure under low water potential, the imbibition process is disturbed, and the alpha-amylase enzyme activity is inhibited [21]. This study finds that seeds pre-treatment with exogenous phytohormones enhances the germination of durum wheat seeds under low water potential condition. Similar results were also found by Guirra *et al.* [22] and Gnawali & Subedi [23]. Priming with hormones reduces germination time and speeds up the seed germination process [24]. Therefore, this is the reason primed seeds are better compared to non-primed seeds. Furthermore, it increases antioxidant enzymes such as glutathione in primed seeds, and this enzyme may reduce lipid peroxidation activity during germination, leading to increased germination [25]. Furthermore, Guirra *et al.* [22] reported that seeds treated with plant regulators obtained a higher germination speed index compared to the control under all types of water statute. Our results showed that the growth of durum wheat seedlings was inhibited significantly under drought stress. It was thought that stress induced by PEG caused a water balance disorder, which led to serious decrease for osmotic adjustment. In addition, cell membranes are one of the first targets of many plant stresses and it is generally accepted that the maintenance of their integrity and stability under water stress conditions is a

major component of drought tolerance in plants [26]. Usually, electrolyte leakage and MDA level are regarded as biochemical indicators of osmotic and cellular damage in plant tissues [27]. Furthermore, drought stress had a significant effect on cell membrane permeability. These findings are consistent with Mozdzen *et al.* [28], where the authors reported higher electrolyte leakage in maize under drought stress. The increase in electrolyte leakage under drought stress occurs mainly due to the membrane damage under drought induced oxidative stress [29].

Moreover, exogenous phytohormones application showed a significant increase in membrane stability, and resultantly decreased the electrolyte leakage under drought stress. Similar findings were also reported by Tong *et al.* [30] and Zhao *et al.* [31], where hormoprime application decreased the electrolyte leakage under stress conditions. Plazek *et al.* [17], reported that the impact of seed treatments on ion leakage from cells, which is commonly used as an indicator of the degree of cell membrane permeability, as well as dehydrogenase and amylolytic activities in seeds.

Leaf relative water content (RWC), widely used as an index of tissue water status was significantly decreased under water stress conditions [32 and 33]. Meher *et al.* [16], reported that severe stress clearly affect the relative water content as compare to the control of same age group plant the significant differences in RWC was observed as compare to control and stressed of leaf and root. Plant growth regulators application significantly improved the RWC [34] and enhanced crop water use under drought stress conditions. Astaneh *et al.* [35], founded that stress treatment caused a significant decrease in the leaf RWC and increase in electrolyte leakage compared with control and recovered conditions According to mean comparisons, drought stress led to 13% reduction in RWC and 21% increase in ion leakage compared to normal irrigation, respectively. Awen *et al.* [36], indicated that exogenously applied plant hormones positively increased the leaf RWC and maintained the level of proline in pearl millet seedlings under PEG-induced drought stress. He *et al.* [37], reported that reduced RWC in leaves due to a decrease in water use efficiency of the roots in soybean plants under drought stress.

Therefore, exogenous hormones significantly increased the leaf RWC under drought stress, which could be due to closure of stomata and accumulation of ABA in the leaves to reduce water loss via evapotranspiration [37]. In addition, the results obtained by Gnawali & Subedi [23], showed that at the same level of water potential, GA3 priming has increased relative water content, an indicator of drought tolerance through osmoregulation with higher relative water content under different drought stresses.

An important antioxidant metabolite is proline, which accumulates in large quantities in plant cells in response to exposure to abiotic stress effects, especially water deficit [38]. Apart from contributing to cellular osmotic adjustment as a compatible solute, proline also decreases the level of various ROS forms in intact plant systems [39]. In many plant species, proline accumulation is considered as indicator of stress tolerance [40]. This amino acid can play several roles in stress tolerance by maintaining stable osmotic balance, protecting membrane enzymes and lipids from degradation, and acting as a free radical scavenger in the antioxidative defense process, and providing an alternative energy source for post-stress recovery mechanisms [41]. Singhal *et al.* [42], reported that Proline is the essential biochemical parameter indicative of plant stress status. Proline is synthesizing during drought stress. Generally, it was observed that proline content was higher in later stages than initial growth stages, and drought conditions having higher proline content compared to the control ones [43]. Proline has a vital role in cellular homeostasis, redox reaction, and maintaining the cell's energy status. The results of Singhal *et al.* [43], found that proline content elevated during the water stress condition, and the combined solute primed set showed the higher proline content. Therefore, it can be possible that priming treatment improves stress tolerance by elevating the proline content [42]. However, proline accumulation under drought stress is used as a marker of drought tolerance [43]. Our results was confirmed by the study of Wang *et al.* [44], which founded that priming promoted the accumulation of sucrose and proline in leaf of wheat, compared with non-primed plants in both cultivars. While the contents of sucrose and proline in roots showed no significant difference between the primed and the non-primed plants.

CONCLUSION

These results lead to the conclusion that drought stress harm durum wheat germination and development. Seed priming with exogenous plant growth regulatory plays a significant part in alleviating the damaging impact that drought stress produces. Seed priming is pre-hardening technique used in numerous crops to improve the seed germination, uniform seedling emergence and better crop stand. Therefore, this research demonstrated the effectiveness of applying exogenous hormones to reduce the adverse effects of drought stress on germination and early growth of seedlings under laboratory conditions. This study was following to find characters of resistant under drought stress and data from this study can be used to model plant hormones concentrations for wheat resistance to droughts. Additionally, it can be utilized for future research on resilient plants.

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