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Salicylic acid exogenous application enhances durum wheat growth under water stress conditions

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Abstract

Drought is one of the major abiotic stresses affecting agricultural production. This stress has negative effects on the quality, production and productivity of durum wheat (*Triticum durum* Desf.), especially in arid and semi-arid areas. This work focused on i) the effect of seed priming by salicylic acid (SA) on durum wheat germination under drought stress conditions induced by polyethylene glycol (PEG6000). Four treatments were applied (T0: PEG- , AS$^-$; T1: PEG- , AS+; T2: PEG+, AS$^-$; T3: PEG+, AS+) and ii) the effect of SA foliar application on the physiological traits of durum wheat varieties conducted in rainfall condition in a semi-arid environment. Two treatments were applied (drought stress, drought stress + salicylic acid). The results showed that seed priming by SA improved the germination process and seedling growth of durum wheat under water deficit conditions. The highest values of germination percentage (76.66%), vigor index (398.12) and shoot length (5.37 cm) were recorded with SA seed priming. In field experiment, obtained results showed that the highest values of relative water content (68.50%), leaf area (28.97 cm^2) and membrane stability index (86.50%) were recorded with SA foliar application. Thus, the use of SA could be recommended under water stress conditions, as a new approach in integrated drought management.

Keywords: Durum wheat; drought; seed priming; salicylic acid

1. Introduction

Agriculture is threatened by climate change and extreme weather events [1]. North Africa, including Tunisia, is considered a 'hotspot' region [2]. Tunisia is particularly exposed to water stress [3], which limits plant growth and yield. By 2030, it is expected that average annual temperature will increase by 1.1°C, resulting in a sharp annual decline in rainfall and water resources [4]. This will have a severe impact on rainfed durum wheat, a yield decrease by almost 30% [2]. In semi-arid regions, improving cereal crop yields under water stress is a main objective in research programs aimed [5].

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Recent studies have revealed the effectiveness of using new strategies to mitigate the impact of water stress, such as the exogenous application of growth regulators, chemicals and synthetic hormones like salicylic acid (SA). It is considered to be a phytohormone. It belongs to an extremely diverse group of phenolic compounds and it is abundant in the bark and leaves of plants. SA plays an important role in inducing plant defense responses against unfavorable environmental conditions [6]. It has an important effect on a wide range of physiological processes, from seed germination to flowering and fruit ripening. The use of SA as seed or foliar treatment improved the response of wheat plants grown under water stress [7].This acid increased proline accumulation through an increase in abscisic acid content [8]. An improvement in catalase activity in wheat was observed with the application of SA under water deficit conditions [8].

SA seed priming stimulated germination and growth of wheat plants under water stress [9]. Foliar spraying of SA improved plant growth characteristics, exerting a stimulatory impact by directly interfering with the enzymatic activities that promote growth. Under water deficit conditions, SA improved growth attributes, such as shoot and root length, as well as fresh and dry weights of wheat. Spraying 6 mM SA improved shoot length by 11.35% and 20.35% and root length by 7.32% and 3.13% respectively under unstressed and water-stressed conditions [10].

Thus, the present study was conducted to assess the effect of salicylic acid seed priming on germination traits and to evaluate the effect of its foliar application on physiological performance of five durum wheat varieties grown under rainfed conditions in a semi-arid environment.

2. Material and methods

2.1. Plant material

Plant material consists of five (05) durum wheat varieties: Karim, Salim, Maâli, Dhahbi and INRAT 100. Mophological characteristics and origins of these varieties are presented in table 1.

Table 1 Characteristics of studied durum wheat varieties

Varieties	Origin	Morphological characteristics
Karim	Variety selected by INRAT from the CIMMYT cross CM9799, registered in 1980.	Spike: yellowish white Straw: hollow profile with fairly thick walls
Salim	Variety registered in 2009	Ear: blackish barbed square Straw: strong and moderately lodging resistant Very productive variety, more resistant to Septoria and rust (brown and yellow).
Maâli	Variety resulting from the crossing: D92-27, carried out at the INRAT and registered in 2007.	Ear: elongated white with parallel edges. Straw: hollow section with thin walls along the entire length.
Dhahbi	Variety registered in 2018 and selected by INRAT	Straw: yellowish- white with black barbs. A variety with large grains (weight of 1000 grains between 45 and 59g) and a high specific weight. High gluten content and good semolina color.
INRAT 100	Variety selected by INRAT at CIMMYT and registered in 2017	Medium size with a high specific weight and good quality.

(Boeuf, [11], Deghaïs et al., [12], Ben Youssef, [13])

2.2. Experiment 1: Effect of salicylic acid on durum wheat germination under water deficit condition

This experiment was conducted at the agronomy laboratory of the Higher Agricultural School of Kef using three durum wheat varieties: Karim, Salim and Maâli.

2.2.1. Experimental Design and Treatments

Water deficit was induced by polyethylene glycol (PEG 6000), a stable agent that is soluble in water and it is non-toxic even at high concentrations [14]. The polyethylene glycol solution, has an osmotic potential of -3.02 bar, was prepared by dissolving 125 g of PEG 6000 (molecular weight 6000 g mol-1, purity> 99.0%; Sigma-Aldrich solutions, St. Louis, USA) in one liter of distilled water.

Seeds of different varieties were disinfected with sodium hypochlorite (10%) for 20 min, rinsed three times with distilled water. Then, different seed lots of each variety were soaked in a 0.7 mM salicylic acid solution (0.0483 g / 0.5 L) for 24 h.

Seeds of each variety were placed in 90 mm diameter Petri dishes (Sterilin Ltd., Cambridge, UK), lined with two layers of filter paper. Each dish contained 10 seeds for each variety and each treatment.

A completely randomized design, with 3 replications for each treatment was performed. Petri dishes were incubated for 10 days in the dark in a growth chamber at 50% relative humidity and an average temperature of 22 ± 2°C day and night [15] during 10 days. The germinated grains were counted every day. Every 2 days, 5 ml of each PEG solution, or distilled water was added to the Petri dishes as follows:

- Treatment 0: without water deficit and without addition of AS (PEG^- ,SA^-)
- Treatment 1: without water deficit and with the addition of AS (PEG^-,SA^+)
- Treatment 2: under water deficit and without addition of SA (PEG^+,SA^-)
- Treatment 3: under water deficit with the addition of SA (PEG^+,SA^+)

2.2.2. Measured Parameters

Four parameters were measured:

- Germination percentage (GP):

This is expressed as the ratio of the number of germinated grains to the total number of grains.

$$\text{Germination percentage} = (GP, \%) = (n/N) \times 100$$

n: total number of germinated seeds.
N: total number of observed seeds

- Vigor index (VI)

The vigor index is a dynamic indicator used to measure the germination power of seeds.

$$SVI = GP \times SL, \text{ where SL (seedling length) is the average of } SL + RL$$

- Shoot length (SL, cm)
- Root length (RL, cm)

2.3. Experiment 2: Effect of salicylic acid foliar application on durum wheat physiological performance

2.3.1. Experimental site and design

The experiment was carried out during the 2021-2022 growing season at the Kef station of the National Institute for Agronomic Research (INRAT), at 8.7° longitude and 36.17° latitude. The bioclimatic zone is semiarid, with cold winters and extremely irregular rainfall throughout the year. Average rainfall and average monthly temperatures are shown in Figure 1.

A field experiment, in a split-split plot design with three replications was conducted. The main factor was: salicylic acid treatment (with and without SA) and the sub-factor was: seven durum wheat varieties which were randomly distributed in each block. Foliar treatments of SA were carried out at the early tillering and anthesis stages at a rate of 100mg/l. The row spacing was 0.5 m and the length of each row was 2.5 m. The seed was sown manually on 30/11/2021, at 25 grains /2.5 m. density.

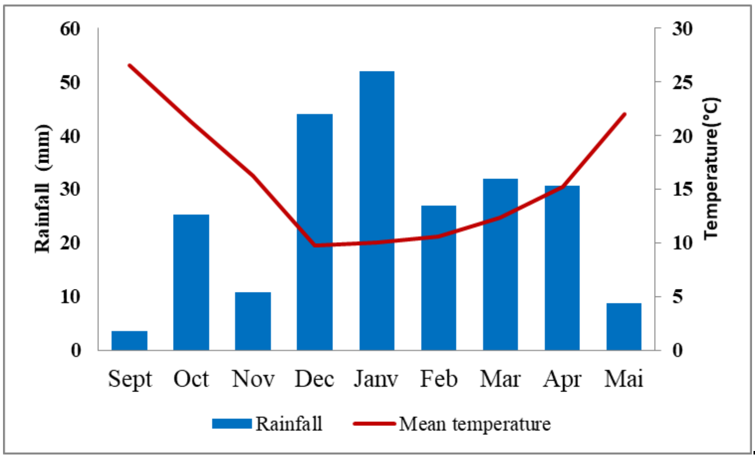

Figure 1 Average rainfall and average monthly temperatures recorded during the 2021/2022 growing season

Nitrogen was applied in the form of ammonium nitrate (33.5%), divided into 3 applications: at the 3-leaf stage (30% of the total quantity), at the tillering stage (40% of the total quantity) and at the 2-node stage (30% of the total quantity). Weed control was carried out manually and chemically with the herbicide ZOOM (65.9% Dicamba + 4.1% Triasulfuron), applied at the tillering stage to control grass weeds.

2.3.2. Measured parameters

- Leaf area (LA)

The average leaf area is determined from a sample of three seedlings per treatment using the following formula (Zhao et al., 2013)[16]:

Flag leaf area (cm^2) = flag leaf width x flag leaf length x 0.74

- Relative water content (RWC)

The relative water content of leaves was determined by the method described by Barrs and Weatherley, [17]. According to this method, the leaves are cut at the base of the leaf, they are weighed immediately to obtain their fresh weight (FW).These leaves are then put in test tubes filled with distilled water and placed in the dark in a cool place, after24h the leaves are removed, passed through blotting paper to absorb water from the surface, weighed again to obtain the turgid weight (TW).

Finally, the samples were placed in an oven regulated at 80°C for 48 h and weighed to obtain their dry weight (DW). The relative water content was calculated using the following formula [18]:

$$RWC = ((FW - DW)/(TW - DW)) \times 100$$

- Membrane stability index (MSI)

The membrane stability index was determined according to the method of Premachandra et al.[19] modified by Sairam [20]. Leaf discs (100mg) were carefully washed in tap water, then washed in bi-distilled water. The discs were then heated in 10 ml of bi-distilled water at 40°C for 30 min.

The electrical conductivity (C1) was recorded using a conductivity meter.

The same samples were then placed in a boiling water bath (100°c) for 10 min and their electrical conductivity was recorded (C2).

$$MSI = [1- (C1/C2)]* 100$$

2.4. Statistical analysis

Collected data were subjected to variance (ANOVA) analysis using SPSS (version 20.0). Means were compared using Duncan's test at P = .05, P < .01 and P < .001.

3. Results and discussion

3.1. Trial 1: Effect of salicylic acid on durum wheat germination under water stress condition

The results of variance analysis for studied traits under osmotic stress conditions indicated a highly significant (p<0.01 and p< 0.001) effect of drought stress treatments and treatments × varieties interaction and the presence of a considerable genotypic variation (p< 0.001) for VI and SL (table 2).

Table 2 Variance analysis (mean square and F test) for germination percentage (GP), vigor index (VI), shoot length (SL) and root length (RL) of studied durum wheat varieties under different applied treatments

Source of variation	ddl	GP	VI	SL	RL
Treatments	3	647.22**	1055637.39***	83.23***	177.08***
Varieties	2	6669.44***	740912.33***	21.27***	31.75***
Treatments × Varieties	6	91.66ns	56935.51***	9.78***	3.55ns

ddl: degree of freedom; ns: not significant; *: significant at p<0.05; ** significant at p<0.01; ***significant at p<0.001.

3.1.1. Germination percentage

The obtained results showed that the lowest germination percentage (66.6%) was recorded under water deficit without AS addition (Figure 2, A). Similar results were obtained by Kizilgeci, et al. [21] in wheat. However, osmo-priming by SA improved GP by 2.6% and 15% under no stress and stress conditions, respectively. The highest PG (85.5%) was obtained using AS. These results are consistent with those obtained by Marcińska et al. [7] in wheat. Germination is an important growth stage in seedling establishment and had a crucial role in crop production. The germination process consists in two phases: imbibition which depends on seeds physical characteristics and a heterotrophic growth phase between imbibition's and emergence [22]. Germination percentage increases after SA seed priming. The positive effect of SA is related to partial inhibition of the production of active oxygen species. An artificial enhance of antioxidant like SA in cellular level is advantageous to improve seed germination under stress condition [23].

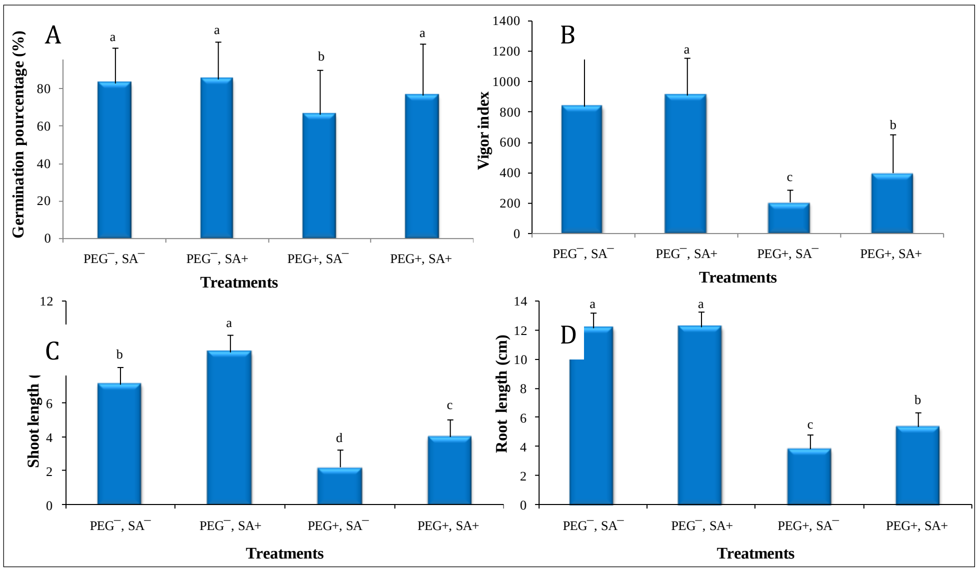

Figure 2 Effect of seed priming with salicylic acid on the germination percentage (A), vigor index (B), shoot length (C) and root length (D) of studied durum wheat varieties

3.1.2. Vigor index

Figure 2 B shows that the osmo-priming treatment using SA had a positive effect on the vigor index of durum wheat seedlings. The highest values (913.19 and 840.46) were recorded with SA seed priming under both stressed and unstressed conditions. The SA treatments effectively improved the negative effects of drought stress through improving photosynthetic performance, maintenance of membrane permeability and enhancing the activity of antioxidant enzymes[24].

3.1.3. Shoot length

PEG-induced water deficit has a negative effect on shoot length (SL), lowest value (2.24 cm) was recorded under osmotic stress without adding SA (figure 2, C). This may be the result of reduced protoplast swelling and dehydration, which are linked to reduced cell expansion and prevention of mitosis [25]. Seed priming by SA improved SL. Khamech et al. [26] reported that SA treatment was able to improve germination, plant development and biomass accumulation under water stress conditions in wheat varieties

3.1.4. Root length

Water deficit significantly reduces the length of the root system (RL) (figure 2, D). Seed priming with SA increased RL by 0.04% and 39.11% respectively under stress and no stress conditions. The results obtained by El Bahay, [27] showed that SA increased the length of wheat roots under water deficit. Amin et al. [28] reported that SA stimulates root growth, which may be due to the increase in hydraulic conductivity as a result of the increase in cell wall thickness. The increased root system facilitates plant adaptation to water stress. Jaleel et al. [29] elucidate that this could help plants in selective ion uptake, transportation and maintaining plant water status.

3.2. Trial 2: Effect of foliar application of salicylic acid on the physiological performance of durum wheat in semi-arid regions

The aim of this field trial was to study the effect of salicylic acid foliar application on durum wheat varieties response to water stress in a semi-arid environment. Variance analysis revealed a significant effect ($p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$) of treatments and varieties on all measured parameters (Table 3).

Table 3 Variance analysis (mean square and F test) of physiological parameters: relative water content (RWC, %), leaf area (LA, cm^2) and membrane stability index (MSI, %) of studied durum wheat varieties under the different applied treatments

Source of variation	**ddl**	**RWC (%)**	**LA (cm^2)**	**MSI (%)**
Treatments	1	522.02*	59.68**	375.067**
Varieties	4	366.64**	139.04 ***	14.271 ns
Treatments × Varieties	4	494.89***	13.43 ns	108.20ns

ddl: degree of freedom; ns: not significant; *: significant at $p<0.05$; ** significant at $p<0.01$; ***significant at$p<0.001$.

3.2.1. Leaf area

Figure 3 A shows that the addition of salicylic acid improved leaf area in all varieties. The highest values (28.97cm^2 and 25.92 cm^2) were obtained by SA foliar application in Dhahbi and Maâli varieties respectively. According to Zhu et al. [30], leaf area is a very important trait as it is involved in the photosynthesis processes, plant transpiration and source-sink translocation. The positive effect of SA on leaf area can contribute to high grain yields. SA is an endogenous hormone, it adjusts physiological and biochemical processes, reduces reactive oxygen species generation and regulates defense system in order to maintain osmotic adjustment during stressful climate [31].

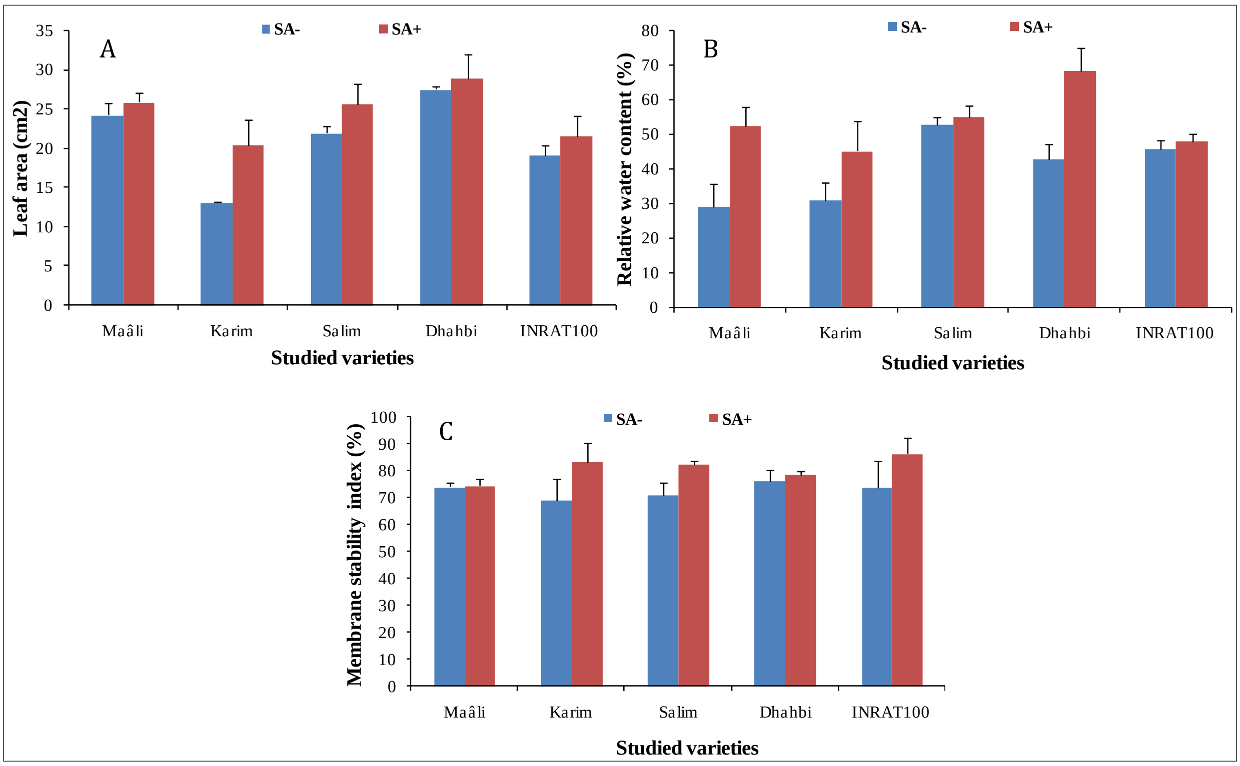

Figure 3 Effect of salicylic acid foliar application in leaf area (A), relative water content (B) and membrane stability index (C) of studied durum wheat varieties

3.2.2. Relative water content

The lowest relative water content (RWC) (29.08%) was recorded under rainfed conditions. According to Sairam et al. [32], RWC decreased significantly in wheat plants under water-stress conditions. The RWC drops as transpiration rate exceed absorption which decrease in cell turgor [33].Thought obtained results showed that the addition of SA improved this parameter. The highest value (68.5%) was obtained in the presence of SA in Dhahbi variety. Results were in agreement with the findings of Kabiri et al. [34]. The increase of RWC is related to the role of SA in compatible osmolytes accumulation in stressed plants.

3.2.3. Membrane stability index

Membrane stability is the main component of water stress tolerance [35]. Drought stress had a negative impact on MSI of all durum wheat varieties grown under rainfed conditions in a semi-arid environment (Figure 2C). Drought stress disturbs cell membrane stability by the accumulation of ions, thus ion leakage and leaf wilting started [33]. However, foliar application of SA improved MSI. Similar results were obtained by Ahmad et al. [36] for bread wheat varieties subjected to water deficit. SA exogenous application increases Ca^{+2} accumulation which can keep membrane stability [37].

4. Conclusion

Water scarcity is a real threat to food security and the sustainability of agricultural production. In Tunisia, water deficit is one of the main factors responsible for low and variable durum wheat yields. The study shows that osmo-priming with salicylic acid had positive effects on all germination traits and plant growth under PEG-induced water deficit conditions. In addition, foliar application of salicylic acid improved physiological performance: relative water content, leaf area and membrane stability index in all durum wheat varieties. These results suggest that salicylic acid could be used as an effective growth regulator for plants under drought stress. However, doubts remain as to the appropriate dose and the most effective method of SA application (e.g. seed imbibition, foliar application or incorporation into the soil).

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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