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## **THE EFFECTS OF SEED INVIGORATING TREATMENTS ON WHEAT SEEDLING GROWTH AND ACTIVITY OF ENZYMATIC ANTIOXIDANTS**

### **SUMMARY**

Pre-sowing seed invigoration methods are promising approach to alleviate the adverse effects of abiotic stresses on seed germination and early seedling establishment. The current study was conducted to evaluate the impacts of pre-plant soaking of wheat (cv. Sardari) seeds in different concentration of salicylic acid and ascorbic acid (30, 60, 90, 120, 150, and 180 ppm) on seedling early growth and antioxidant activity. Results showed that the highest germination percentage were recorded for seed treated with dilute solutions of acid salicylic (30 and 60 ppm) and intermediate concentration of acid ascorbic (90 ppm). The mentioned concentration increased the germination rate by 80%, root length by 7 cm and shoot length by 5 cm when compared with control. Hormonal seed treatments through 60 and 90 ppm ascorbic acid induced an increased seedling dry weight. However, the highest seedling vigor was achieved by low concentration of salicylic acid. Hormonal priming with acid ascorbic had a more stimulating and increasing effect on the activity of the superoxide dismutase enzyme compared to salicylic acid. The highest activity of catalase and guaiacol peroxidase were recorded for seed primed with 90 ppm salicylic acid and 60 ppm ascorbic acid, respectively. It is concluded that hormonal seed treatments has increased seedling early growth and antioxidant activity and the best performance was related to 30 ppm salicylic acid and 60 and 90 ppm ascorbic acid. The results of the present study elucidated that hormonal seed treatments as a useful, efficient and easy invigorating approach should be seriously considered in wheat fields.

**Keywords:** Acid salicylic, germination rate, pre-sowing treatments, ROS scavenging, seed invigoration, seedling vigor index

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## INTRODUCTION

The semi-arid regions of West Asia have special climatic characteristics and most of the rainfall occurs during winter, and this has caused the main crop of these regions to be winter wheat, barley and some cool season food legumes (Ahmed *et al.*, 2022). Wheat is one of the most important crops in the world and a strategic crop in Iran. However, the most significant constraint of rainfed farming in mentioned region is the shortage of water during the planting and final stage of seed filling (Ghaffari 2010; Tavanpour and Ghaemi 2016). Most of the annual rainfall in the rainfed cold regions usually happens in autumn and winter (75% of the rains) at that time due to the severe drop in temperature, plant growth is stopped for three to four months and plant only receive 25% of the total annual rains during the active growth in spring (during the end of the tillering stage to seed maturity), this causes a significant exacerbation of drought and heat stress at the end of the growth season in many areas. Additionally, in rainfed farming, rapid and uniform emergence and swift establishment of seedlings is necessary to use the autumn rains and grow the plant sufficiently before the onset of severe cold (Arun *et al.*, 2022). Specifically tolerance to temperatures below freezing is though often of pivotal importance for the survival of wheat seedling across winter and it can be affect by vernalization requirement and cold acclimation condition. Therefore, it seems that in semi-arid Mediterranean-type environments, any type of crop management that can accelerate uniform emergence can reduce autumn break, increase rainfall use efficiency and improve acclimatization to cold (Flohr *et al.*, 2021). Some of the major constraints on wheat growth in semi-arid region include the timing and amount of autumn rains for sowing. In cropping systems, the autumn break is one of the most important events of any year. It is the first significant rainfall event of the winter growing season and signals the start of the growth period. On the other hand due to wide variation in the timing of the arrival of adequate autumn rains for sowing during different years and also the aggravation of this issue due to climate changes during the recent decades, it is difficult to choose a calendar date for sowing wheat seeds (Pook *et al.*, 2009). Based on the previous studies, it can be speculated that seed invigoration techniques can be good crop management's option under some limiting environmental conditions (Hussain *et al.*, 2019; Marthandan *et al.*, 2020; Johnson and Puthur 2021; Cruz *et al.*, 2021). Seed priming is an important cost-effective method which can improve the seed germination and is widely used to synchronize and accelerate the germination of individual seeds (Hussain *et al.*, 2017). Seed priming base include controlled hydration of seeds up to particular point where the preliminary and intracellular processes of germination begins; however, the radical protrusion does not take place (Singh *et al.*, 2018). It is generally acknowledged that seed priming can provide improved and uniform seedling emergence by reducing the seed hydration time during the germination, increase the gene expression and also activating the pre-germinative enzymes and metabolism and also enhances the antioxidant/DNA repair activities (Forti *et al.*, 2020). There are many reports on

seed priming toward improving seed germination, seedling emergence, stand establishment, crop growth, nodulation, and productivity in various cereal crop species (Khan *et al.*, 2011; Tabatabaei, 2013; Hussain *et al.*, 2017; Khan *et al.*, 2020). Seed priming induces antioxidant enzymes activity and can significantly minimize lipid peroxidation (Janmohammadi 2012; Khan *et al.*, 2020). Despite some studies, it still retains empirical features especially in the form of on-farm priming. It has been revealed that between cost-benefit management strategies exogenous application of hormone like material minimize loss of productivity and yield under unfavorable conditions (Janmohammadi *et al.*, 2012).

Use of plant growth regulators and hormones like substances as priming agents has been found to increase plant growth and significantly enhance plant mechanisms to cope with abiotic stress. Although few studies have employed hormones substances under semi-arid condition, these studies are yielding promising results (Farooq *et al.*, 2013; Imran *et al.*, 2013; Shah *et al.*, 2019). Salicylic acid (SA) is a phenolic compound which acts as an imperative signal molecule, since it regulates metabolic and physiological processes in plant (Sharma *et al.*, 2020). In addition to positive effects of increased biosynthesis of SA under some conditions, it seems that exogenous SA application can significantly improve plant abiotic stress-tolerance (Zafar *et al.*, 2023). Boukari *et al.* (2019) showed that the pretreatment of alfalfa seeds with SA under salinity stress improved plant growth, water content, content of photosynthetic pigments. Also improved scavenging of reactive oxygen species (ROS) capacity through the SA application during seed pre-sowing process has been reported in the literature (Janmohammadi, 2012; Bortolin *et al.*, 2020).

Ascorbic acid (ASC) performs as a co-factor for some critical enzymes and preserves the process of phytohormone intermediating signaling and numerous biological processes (Farooq *et al.*, 2013). ASC also controls plant growth through affecting cell division and cell expansion, modulates plant sense, and is involved in photosynthesis, hormone biosynthesis, and regeneration of antioxidants. Shah *et al.* (2019) reported that ASC priming significantly increased chlorophyll content and grain yield in late planted winter wheat and also up-regulation of diverse enzymatic antioxidants such as super oxide dismutase, peroxidase, and catalase. However, little is known about the use of hormone like substance to improve seed germination in *semi-arid* Mediterranean-type environments with long autumn break. The aim of this study was to investigate the potential role of salicylic acid and ascorbic acid in pre-sowing seed treatment on seedling early growth and antioxidant activity of winter wheat.

## MATERIAL AND METHODS

The experiments were conducted at the Research Laboratory of Crop Production, College of Agriculture, University of Maragheh, Iran. The natural deteriorated seeds (two years of storage) of winter wheat (*Triticum aestivum* L.) cultivar, Sardari, were obtained from Dryland Agricultural Research Institute (DARI). Sardari is a winter-type with mean plant height of 100 cm and is well

adapted to semi-arid region with cold winter. Sardari also is resistant to shattering is a predominant dryland landrace in Iran (Roostaei *et al.*, 2018). The experiment was carried out using a completely randomized factorial design with four replicates and was developed in a petri dish system under a controlled condition of germinators. Pre-sowing treatments were including control (non-primed seeds) and hormone priming through different concentration of salicylic acid and ascorbic acid (30, 60, 90, 120, 150, and 180 ppm). Prior to seed pre-soaking treatments, healthy, unbroken, and spotless seeds were sorted out manually, afterward; the selected seeds were surface sterilized in 0.5% sodium hypochlorite (v/v) for 10 min and washed with water to prevent the growth of microbial contaminants present on the seed surface. The excess water was removed from the seeds with absorbent paper, and the seeds were air-dried to reach the initial weight, under natural conditions. Wheat seeds were primed in the dark at 25°C and relative humidity of 50–70% for 8 h with constant gentle shaking in a mechanical shaker. Seed germination was tested on filter papers placed in Petri dishes and moistened with sufficient distilled water. Twenty-five seeds were placed in each dish and incubated in the dark at  $25 \pm 1^\circ\text{C}$  and germinated seed were recorded daily for nine days.

Seedling growth parameters and final germination percentage (GP) were recorded according to AOSA (1983);  $GP = \frac{\text{total seeds germinated at end of trial}}{\text{number of initial seeds used}} \times 100$ . Germination rate index (GRI) calculated as  $= \frac{G1}{1} + \frac{G2}{2} + \dots + \frac{Gi}{i}$ ; where G1 is the germination percentage on day 1, G2 is the germination percentage at day 2; and so on. The methodology of GRI followed Farooq *et al.* (2013). Seed vigor index calculated by multiplying germination (%) and seedling length on the ninth day from the beginning of germination. The RWC (fresh weight - dry weight) / (turgid weight - dry weight)  $\times 100$  (Pieczyński *et al.*, 2013) was evaluated at the end of germination. Primed and unprimed seeds were planted under natural field condition (21 November) for assessment of mean emergence time. Mean emergence time (MET) was evaluated using  $MET = \frac{\sum Dn}{\sum n}$ , where n stands for the number of seeds that emerged on day D and D is the number of days counted from the start of emergence (Ruttanaruangboworn *et al.*, 2017).

For assessment of antioxidant enzymes activity shoots of wheat seedlings were collected at the 9<sup>th</sup> day of germination, and directly placed into liquid nitrogen, then stored at  $-80^\circ\text{C}$  until used. Approximately 500 mg of shoot sample were homogenized in an extraction buffer containing 100 mM potassium phosphate buffer (pH 7.5), 1 mM ethylene diaminetetraacetic acid (EDTA), 3 mM DL-dithiothreitol and 5% (w/v) insoluble polyvinylpyrrolidone (Boaretto *et al.*, 2014). Superoxide dismutase (SOD) activity inhibits photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. The monitoring of this inhibition is used to assay SOD activity (Giannopolitis and Ries 1977). Catalase (CAT) activity was measured in a spectrophotometer (240 nm) according to Cia *et al.* (2012), through monitoring the degradation of H<sub>2</sub>O<sub>2</sub> at 240 nm over 1 min, in a reaction mixture containing 1 mL of 100 mM

potassium phosphate buffer (pH 7.5) and 25  $\mu\text{L}$   $\text{H}_2\text{O}_2$  (30% solution). GPX activity (Guaiacol peroxidase) was measured by following the  $\text{H}_2\text{O}_2$  dependent oxidation of guaiacol at 470 nm, using an extinction coefficient of  $26.6 \text{ mM}^{-1} \text{ cm}^{-1}$  (Tayefi-Nasrabadi *et al.*, 2011). Ascorbate peroxidase (APX) assay was determined by reaction medium containing 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate and 0.1 mM  $\text{H}_2\text{O}_2$  (Alves *et al.*, 2018). The activity was determined by monitoring the rate of ascorbate oxidation at 290 nm at 30 °C, and values expressed as  $\mu\text{mol ascorbate min}^{-1} \text{ mg}^{-1}$  protein. Guaiacol peroxidase (EC 1.11.1.7) activity was measured using a reaction medium containing 50 mM phosphate buffer (pH 7), 9 mM guaiacol, and 19 mM  $\text{H}_2\text{O}_2$  (Lin and Kao 1999). The analysis was conducted in SPSS and mean comparison was performed by LSD test.

## RESULTS AND DISCUSSION

Seed priming with salicylic acid (SA) and ascorbic acid (ASC) had significant ( $P \leq 0.05$ ) effects on germination percentage (GP), germination rate (GR) and seedling growth parameters of wheat as shown in Table 1.

Table 1. Comparison of means of germination and seedling growth characteristics of wheat (*Triticum aestivum* L.) as affected by different concentration of salicylic acid and ascorbic acid.

	GP	GR	RL	SL	SFW	SDW	RWC	MET	CAT	GPX
C	79.33 <sup>g</sup>	66.33 <sup>h</sup>	6.26 <sup>g</sup>	6.48 <sup>f</sup>	195.00 <sup>f</sup>	28.66 <sup>d</sup>	89.33 <sup>c</sup>	9.66 <sup>a</sup>	29.33 <sup>g</sup>	204.00 <sup>h</sup>
SA30	98.00 <sup>a</sup>	137.00 <sup>a</sup>	13.00 <sup>ab</sup>	10.43 <sup>a</sup>	286.33 <sup>b</sup>	48.00 <sup>b</sup>	94.00 <sup>a</sup>	5.33 <sup>de</sup>	44.66 <sup>b</sup>	255.33 <sup>bc</sup>
SA60	90.33 <sup>bc</sup>	100.33 <sup>d</sup>	10.11 <sup>c</sup>	9.03 <sup>b</sup>	225.33 <sup>c</sup>	41.32 <sup>bc</sup>	93.00 <sup>a</sup>	7.00 <sup>cd</sup>	37.66 <sup>de</sup>	238.00 <sup>def</sup>
SA90	88.00 <sup>cd</sup>	90.66 <sup>f</sup>	9.30 <sup>cd</sup>	8.80 <sup>b</sup>	233.33 <sup>de</sup>	38.00 <sup>c</sup>	90.66 <sup>bc</sup>	6.66 <sup>d</sup>	51.00 <sup>a</sup>	275.66 <sup>a</sup>
SA120	85.33 <sup>def</sup>	90.66 <sup>f</sup>	8.66 <sup>def</sup>	7.70 <sup>de</sup>	233.66 <sup>de</sup>	43.66 <sup>bc</sup>	92.00 <sup>abc</sup>	8.66 <sup>b</sup>	40.00 <sup>bcd</sup>	249.66 <sup>cd</sup>
SA150	85.66 <sup>de</sup>	78.66 <sup>g</sup>	9.56 <sup>cd</sup>	7.96 <sup>cd</sup>	231.33 <sup>de</sup>	42.66 <sup>bc</sup>	92.00 <sup>abc</sup>	8.00 <sup>bc</sup>	35.33 <sup>ef</sup>	231.33 <sup>efg</sup>
SA180	83.33 <sup>ef</sup>	82.33 <sup>fg</sup>	9.16 <sup>cde</sup>	7.80 <sup>de</sup>	206.33 <sup>f</sup>	34.33 <sup>cd</sup>	92.33 <sup>abc</sup>	7.66 <sup>c</sup>	30.33 <sup>fg</sup>	238.00 <sup>def</sup>
ASC30	83.33 <sup>ef</sup>	99.00 <sup>de</sup>	7.77 <sup>f</sup>	8.70 <sup>bc</sup>	250.33 <sup>c</sup>	41.66 <sup>bc</sup>	90.33 <sup>bc</sup>	5.66 <sup>de</sup>	44.33 <sup>bc</sup>	243.00 <sup>cde</sup>
ASC 60	96.00 <sup>a</sup>	100.33 <sup>d</sup>	11.78 <sup>b</sup>	10.78 <sup>a</sup>	288.66 <sup>b</sup>	57.00 <sup>a</sup>	92.66 <sup>ab</sup>	5.00 <sup>e</sup>	52.33 <sup>a</sup>	265.00 <sup>ab</sup>
ASC 90	92.00 <sup>b</sup>	90.66 <sup>f</sup>	13.60 <sup>a</sup>	10.93 <sup>a</sup>	313.00 <sup>a</sup>	65.00 <sup>a</sup>	94.00 <sup>a</sup>	5.33 <sup>de</sup>	45.33 <sup>b</sup>	273.66 <sup>a</sup>
ASC 120	84.33 <sup>def</sup>	91.00 <sup>ef</sup>	8.70 <sup>def</sup>	7.80 <sup>de</sup>	242.33 <sup>cd</sup>	36.66 <sup>cd</sup>	90.00 <sup>bc</sup>	6.66 <sup>d</sup>	40.33 <sup>bcd</sup>	246.33 <sup>cd</sup>
ASC 150	78.66 <sup>g</sup>	81.33 <sup>g</sup>	7.91 <sup>ef</sup>	7.02 <sup>ef</sup>	254.00 <sup>c</sup>	42.33 <sup>bc</sup>	91.66 <sup>abc</sup>	7.33 <sup>c</sup>	41.66 <sup>bcd</sup>	224.66 <sup>fg</sup>
ASC 180	81.66 <sup>fg</sup>	83.33 <sup>fg</sup>	8.53 <sup>def</sup>	7.64 <sup>de</sup>	208.33 <sup>f</sup>	43.66 <sup>bc</sup>	89.33 <sup>c</sup>	8.00 <sup>bc</sup>	39.00 <sup>cde</sup>	218.00 <sup>gh</sup>
LSD	3.79	8.07	1.29	0.826	15.85	8.55	3.42	0.36	5.33	14.70

C: No-priming (intact seeds), SA30-18: salicylic acid concentration (ppm), ASC: ascorbic acid, GP: germination percentage (%), GR: germination rate index, RL: root length of seedling (cm), SL: shoot length of seedling (cm), SFW: seedling fresh weight (mg), SDW: seedling dry weight (mg), RWC: relative water content of seedling (%), MET: mean emergence time (days), CAT: catalase activity ( $\text{Units mg}^{-1} \text{ protein}$ ) and GPX: guaiacol peroxidase ( $\text{Units mg}^{-1} \text{ protein}$ ). Data with the same letter are not significantly different at  $p < 0.05$  (LSD: Least Significant Difference).

However, the effect of hormone-like compounds was largely dependent on their concentrations. Assessment of GP showed that although all hormone

priming treatments were able to increase this parameter compared to the control, the highest increase was recorded in very low concentrations of SA (18%) and medium concentrations of ASC (14%). The results of the germination rate showed that despite the increase in the germination rate of primed seeds by both hormones, the rate of increase was much prominent by low concentrations of salicylic acid (30 and 60 ppm) that the germination rate was about twice as compared to the control. However, the germination rate in the seeds primed with ascorbic acid was on average about 20% higher than the no-primed seeds (Table 1). Mean comparison of the seedling root length showed that the effects of ascorbic acid and salicylic acid on this parameter were similar to each other, altogether the hormonal priming increased the root length by about 60% compared to the no-primed seeds. The longest roots were recorded in the seeds treated with 30 ppm salicylic acid or 90 ppm ascorbic acid. Affectability of shoot length from seed priming treatment was lower than root length as priming treatments only increased this component by 30% compared to the control conditions. The longest shoot recorded for seed primed with 30 and 60 ppm SA or 60 and 90 ppm ASC.

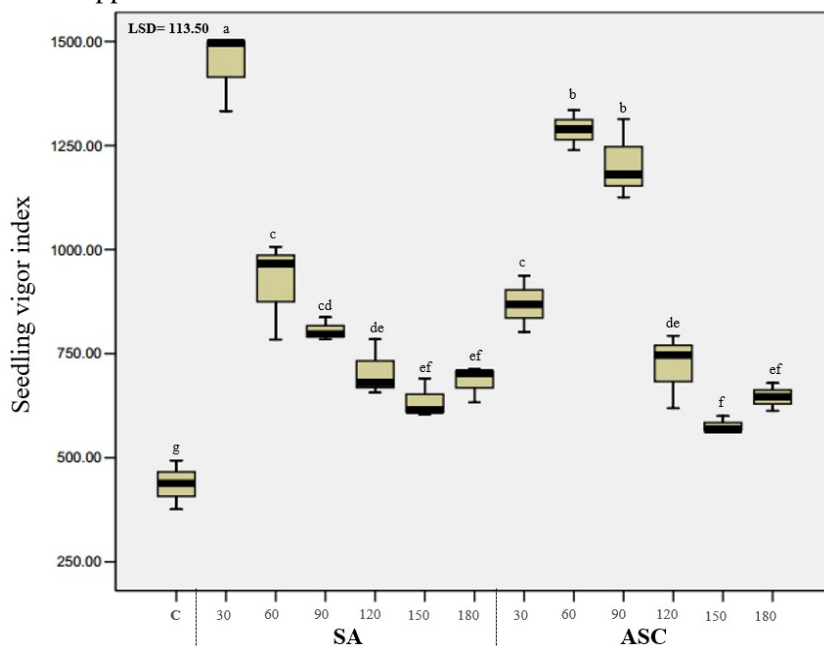


Fig. 1. Effects of wheat seeds pre-sowing treatment in different concentration of hormone-like substances on seedling vigor index. SA: C: Control or non-primed; SA: salicylic acid, ASC: ascorbic acid. All values represent means  $\pm$  standard deviations (SD) of four replicates. Bars showing the same letters are not significantly different at  $P \leq 0.05$  as determined by LSD test.

Assessment of seedling vigor index also showed that the highest vigor recorded for seed treated with 30 ppm of SA that followed by seeds treated with 60 and 90 ppm of ASC (Figure 1). The aforementioned priming conditions improved the vigor index by 2.5-3 times when compared with no-primed seeds. However, other priming treatments also had a positive effect on this index and increased this index by 50-75% compared to the control. The lowest positive effect of priming treatments on vigor index was observed in high concentrations of ascorbic and salicylic acid. Evaluation of seedling fresh weight showed that the heaviest seedlings were obtained from seeds primed with 90 ppm Asc. So that their weight was about one and a half times more than the weight of seedlings in intact seeds. Although both hormones used in priming increased the dry weight of seedlings compared to the control, the improving effect of ascorbic acid on seedling dry weight was more evident than salicylic acid. Seed priming with salicylic acid and ascorbic acid increased the dry weight of seedlings by 44% and 66%, respectively, compared to control. The highest dry weight of seedlings was recorded for seeds treated with 60 and 90 ppm ASC. Relative water content (RWC) of seedling affected by priming treatment ( $P \leq 0.05$ ). The positive effect of salicylic acid on RWC was more evident than ascorbic acid and the highest RWC was recorded for seed primed with 30 and 60 ppm SA or 90 ppm ASC. Between the seedling development characteristics Mean emergence time (MET) can reflect a lot of information. MGT is a measure of the rate and time-spread of germination and emergence, focusing on the day at which most seedling have emergence.

Mean germination time decreased significantly ( $p \leq 0.01$ ) for seedlings grown from primed seed low concentration ASC and SA. The fastest seedling emergence occurred in seeds primed with 30 and 60 ppm ASC. Seed priming with low concentration of ASC and SA decreased the MGT by 45% and 35% compared to no-primed seeds. Seed priming treatments had a significant effects on the performance of enzymatic antioxidant of wheat seedlings. Although the effect of different concentrations on the activity of antioxidant enzymes was statistically significant, these effects were somewhat different from germination indices. The highest activity of APX were recorded for seedling grown from seed primed with low concentration of SA or 120 ppm ASC (Figure 2). Other priming treatments also increased the activity of APX. However, there was a significant difference between the effects of the previously mentioned treatments and other priming treatments.

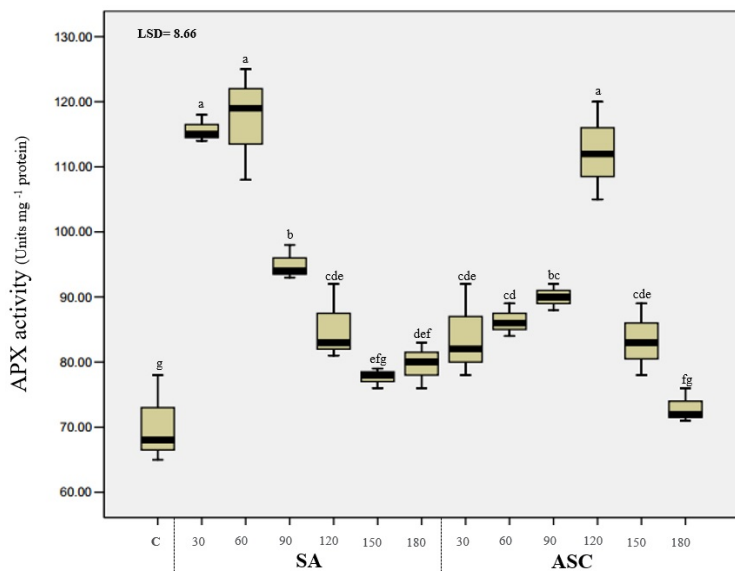


Fig. 2. The effect of hormonal seed priming on Ascorbate peroxidase activity in wheat seedling. SA: C: Control or non-primed; SA: salicylic acid, ASC: ascorbic acid. In each hormone, the numbers indicate the concentrations used for priming (ppm). All values represent means  $\pm$  standard deviations (SD) of four replicates. Bars showing the same letters are not significantly different at  $P \leq 0.05$  as determined by LSD test.

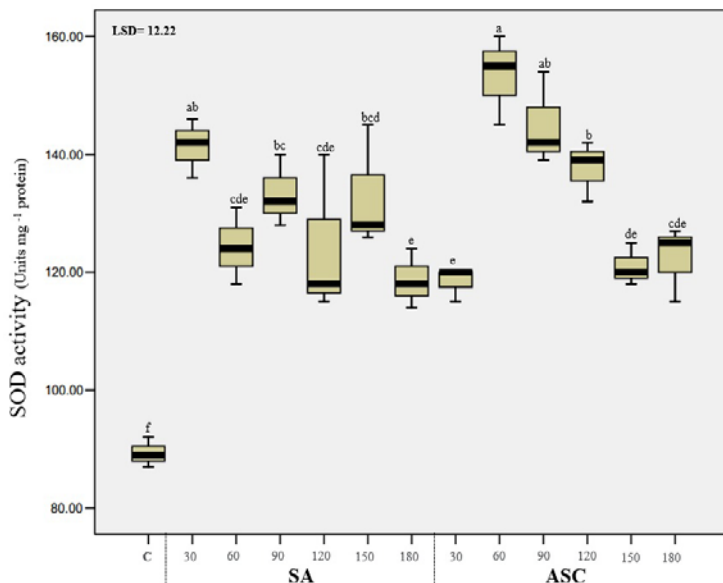


Fig. 3. Effect of seed pre-sowing treatment in different concentration of hormone like substance (ppm) on activity of superoxide dismutase in wheat seedling. SA: salicylic acid, ASC: ascorbic acid. All values represent means  $\pm$  standard deviations (SD) of four replicates. Bars showing the same letters are not significantly different at  $P \leq 0.05$  as determined by LSD test.



Furthermore evaluation of CAT activity showed that the highest level of CAT activity was related at the concentration of 120 ppm SA and 60 ppm ASC which was 75% higher than the no-primed seeds. Likewise seed priming had positive effect on activity of guaiacol peroxidase the highest GPX activity was observed in seeds treated with 90 ppm SA or Asc which was about 14% higher than the no-primed seeds. (Table 1). Assessment of SOD indicated that all priming improved the SOD activity 30-60% compared to no-primed seeds. The highest SOD activity recorded for seedling grown with seeds primed with 60 ppm ASC (Figure 3).

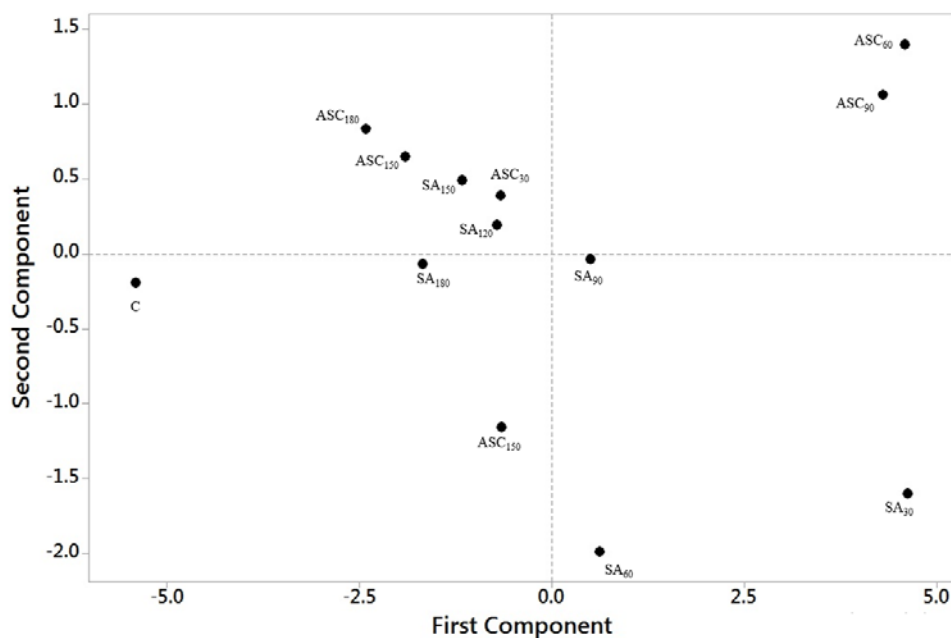


Fig. 4. Principal component analysis (PCA) based on combined treatments (hormonal substance and concentration) which measured for evaluated *seedling growth parameters*.

Figure 4 displays a biplot in the dimension of the first and second PCs. On the plot, two main groups of combined treatments were separated so that for factor 1 there were best seed priming treatment i.e. low concentration of SA and low to medium concentration of ASC. Besides, factor 2 separated control and high concentration of SA which exhibited very low performance for most of the evaluated germination parameters and activity of antioxidant enzymes. The cosine of the angle between two traits indicates their correlation situation, the smaller the angle between two traits show the more significant and positive correlation. In Figure 5, the most prominent relation is the strong positive association between germination percentage, germination rate, seedling vigor index, activity of SOD, activity of GPX and seedling length as indicated by the small obtuse angles between their vectors ( $r = \cos 0 = +1$ ).

We were able to confirm our initial hypothesis, since pre-sowing seed treatment with hormone like substance increased the majority of studied parameters. Since the seeds were stored for more than one year in storage conditions, the obtained results showed the positive effect of hormone priming treatments on improving the germination parameters of natural deteriorated seeds. As long storage period leads to a natural deterioration of seeds and the most imperative causes for such deterioration is the increased activity of the analytical enzymes e.g. amylase, phospholipases, proteases and phytase which reduces viability and vigor of seeds (Cheyed, 2020). Considering that most of the farmers use their own seeds for planting and their storage conditions are often traditional and not favorable, hormonal priming treatments will be very important in improving the germination deteriorated seeds. The finding showed that hormone priming treatment probably improve seed germination through the increase or optimizing of the recovery process of antioxidants. Our finding showed that activity of enzymatic enzymes increased by low and medium concentration of SA and ASC. Furthermore, the used hormone-like substances themselves have antioxidant properties and can play a role in scavenging of reactive oxygen species (ROS). ASC is a small water soluble antioxidant molecule that participates as a primary substrate in the cyclic detoxification and ROS neutralization pathway, such as superoxide and singlet oxygen (Bilska *et al.*, 2019). ROS levels are determined by a tightly controlled balance between production and breakdown. In general, ROS are produced unintentionally even under favorable developmental conditions (Mhamdi and Van Breusegem 2018), and if their concentration increases, they can cause the lipid peroxidation and some other injuries. In this regard, it is necessary to control the amount of ROS by increasing the activity or amount of antioxidant enzymes to survival of the seedling. This finding also corroborates the ideas of Bortolin *et al.* (2020), who suggested that seed priming with SA increased antioxidant activity of enzymes such as superoxide dismutase (SOD) and ascorbate peroxidase (APX) in *Trifolium* species. However, our results showed that the response of antioxidant enzymes to hormone treatments is highly dependent on hormone concentrations. Our findings indicated that seed priming with low concentration of ASC and SA resulted in vigorous, rapid, as well as uniform emergence under field condition. Average emergence time is considered as an important index in evaluating the effect of seed priming treatments on seedling strengthening in real conditions under soil physical limitations (De Ron *et al.*, 2016). Seedling growth primarily relies on inherent vigor and some environmental factors such as light, temperature, water and nutrient availability. It seems that seed soaking in low concentrations of ASC and SA by activation and facilitation of germination

process can result in unique and fast growth of both root and shoot in seedling. The formerly mentioned cases accelerate the access of seedlings to environmental factors such as soil moisture, nutrients and light.

Seed soaking in low concentration of SA and low to medium concentrations of ASC gave the best growth performance of seedling, suggesting that these are ideal hormone priming for wheat under current experiment condition. The results of fresh and dry weight of seedlings indicated that although SA treatments could increase the fresh weight significantly, the highest dry weight recorded for ASC treatments. These results suggest that seedling grown from SA primed seeds had succulent growth and most of their fresh weight was based on water. This can be partly caused by osmotic regulation and better water absorption. The lowest amount of improving effect of priming treatments on germination parameters and seedling growth was observed in high concentrations of ascorbic and salicylic acid, which can be attributed to poisoning effects of high concentration or activation of other signaling pathways that were unrelated to the initiation of germination processes. The positive effects of seed priming with low concentration of hormones on germination and seedling growth can be mainly attributed to higher starch metabolism and better reserve mobilization, increased synthesis of RNA and DNA, enhanced respiration rates, higher synthesis and accumulation of metabolites, maintenance of membrane stability, and higher activities/levels of antioxidants.

## CONCLUSIONS

Our results revealed that the seed priming with hormone like substance positively affect germination and seedling growth stage. However, the effect of hormones in priming conditions strongly depends on the applied concentrations. Low concentration of salicylic acid (30 and 60 ppm) and medium concentration of ascorbic acid (60 and 90 ppm) had the most positive and promising impact. Mean emergence time decreased by seed soaking in low concentration of ASC and SA under natural field condition and in late sowing date. Our results revealed that hormonal seed priming in low concentration of SA and ASC can act as a low-cost and sustainable option. In summary these priming method can be a feasible solution to solve the problems caused by autumn break in semi-arid areas, and through that, it is possible to postpone planting dates until the beginning of autumn rains. Antioxidant activity has increased in seedlings deriving from primed seeds. Results from this study suggest that a part of growth improvement can be attributed to the stimulating the activity of antioxidant enzymes, still many molecular aspects remain unknown and require further studies.

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## REFERENCES

- Ahmed, M., Hayat, R., Ahmad, M., Ul-Hassan, M., Kheir, A.M., Ul-Hassan, F., Ur-Rehman, M.H., Shaheen, F.A., Raza, M.A. & Ahmad, S. (2022): Impact of climate change on dryland agricultural systems: a review of current status, potentials, and further work need. *International Journal of Plant Production*, 16(3), pp.341-363.
- Alves, R.C., Medeiros, A.S., Nicolau, M.C.M., Pizolato Neto, A., Oliveira, F.A., Lima, L.W., Tezoto, T. & Gratão, P.L. (2018): The partial root-zone saline irrigation system and antioxidant responses in tomato plants. *Plant Physiology and Biochemistry*, 127, 366–379.
- AOSA, (1983): Seed Vigour Testing Handbook. USA: Contribution, Association of Official Seed Analysis and SCST. - Available at: <https://www.analyzeseeds.com/product/seed-vigor-testing-handbook-2017>
- Arun, M. N., Hebbar, S. S., Senthivel, T., Nair, A. K., Padmavathi, G., Pandey, P. & Singh, A. (2022): Seed Priming: The Way Forward to Mitigate Abiotic Stress in Crops. In *Plant Stress Physiology-Perspectives in Agriculture*. IntechOpen
- Bilska, K., Wojciechowska, N., Alipour, S. & Kalemba, E. M. (2019): Ascorbic acid-The little-known antioxidant in woody plants. *Antioxidants*, 8(12), 645.
- Boaretto, L.F., Carvalho, G., Borgo, L., Creste, S., Landell, M.G.A., Mazzafera, P. Azevedo, R.A. (2014): Water stress reveals differential antioxidant responses of tolerant and nontolerant sugarcane genotypes. *Plant Physiology and Biochemistry*, 74, 165–175. <https://doi.org/10.1016/j.plaphy.2013.11.016>.
- Bortolin, G. S., Teixeira, S. B., de Mesquita Pinheiro, R., Ávila, G. E., Carlos, F. S., da Silva Pedroso, C. E. & Deuner, S. (2020): Seed priming with salicylic acid minimizes oxidative effects of aluminum on *Trifolium* seedlings. *Journal of Soil Science and Plant Nutrition*, 20(4), 2502-2511
- Boukari, N., Jelali, N., Renaud, J. B., Youssef, R. B., Abdelly, C. & Hannoufa, A. (2019): Salicylic acid seed priming improves tolerance to salinity, iron deficiency and their combined effect in two ecotypes of Alfalfa. *Environmental and Experimental Botany*, 167, 103820.
- Cheyed, S. H. (2020): Effect of storage method and period on vitality and vigour of seed wheat. *Indian Journal of Ecology*, 47(10), 27-31
- Cia, M.C., Guimarães, A.C.R., Medici, L.O., Chabregas, S.M. & Azevedo, R.A. (2012): Antioxidant response to water deficit by drought-tolerant and -sensitive sugarcane varieties. *Annals of Applied Biology*, 161, 313-324.
- De Ron, A. M., Rodiño, A. P., Santalla, M., González, A. M., Lema, M. J., Martín, I. & Kigel, J. (2016): Seedling emergence and phenotypic response of common bean germplasm to different temperatures under controlled conditions and in open field. *Frontiers in plant science*, 7, 1087.
- Farooq, M., Irfan, M., Aziz, T., Ahmad, I. & Cheema, S.A. (2013): Seed priming with ascorbic acid improves drought resistance of wheat. *Journal of Agronomy and Crop Science*, 199, 12–22.

- Flohr, B. M., Ouzman, J., McBeath, T. M., Rebetzke, G. J., Kirkegaard, J. A. & Llewellyn, R. S. (2021): Redefining the link between rainfall and crop establishment in dryland cropping systems. *Agricultural Systems*, 190, 103105.
- Forti, C., Ottobriano, V., Bassolino, L., Toppino, L., Rotino, G. L., Pagano, A., Macovei, A. & Balestrazzi, A. (2020): Molecular dynamics of pre-germinative metabolism in primed eggplant (*Solanum melongena* L.) seeds. *Horticulture research*, 7, 32528699.
- Ghaffari, A. (2010): The role of Dryland Agricultural Research Institute in drought mitigation in Iran. In : López-Francos A. (comp.), López-Francos A. (collab.). Economics of drought and drought preparedness in a climate change context. Zaragoza : CIHEAM / FAO / ICARDA / GDAR / CEIGRAM / MARM, 2010. p. 273-278 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 95)
- Giannopolitis, C.N. & Ries, S.K. (1977): Superoxide dismutase: I. Occurrence in higher plants. *Plant Physiology*. 59, 309–314. <https://doi.org/10.1104/pp.59.2.309>
- Hussain, A., Rizwan, M., Ali, Q. & Ali, S. (2019): Seed priming with silicon nanoparticles improved the biomass and yield while reduced the oxidative stress and cadmium concentration in wheat grains. *Environmental Science and Pollution Research*, 26(8), 7579-7588
- Hussain, M., Farooq, M. & Lee, D. J. (2017): Evaluating the role of seed priming in improving drought tolerance of pigmented and non-pigmented rice. *Journal of Agronomy and Crop Science*, 203(4), 269-276.
- Imran, S., Afzal, I., Basra, S.M.A. & Saqib, M. (2013): Integrated seed priming with growth promoting substances enhances germination and seedling vigor of spring maize at low temperature. *International Journal of Agriculture and Biology*, 15, 1251–1257
- Janmohammadi, M. (2012): Alleviation the adverse effect of cadmium on seedling growth of greater burdock (*Aractium lappal* L.) through pre-sowing treatments. *Agriculture and Forestry*, 56(1-4), 55-70.
- Johnson, R. & Puthur, J. T. (2021): Seed priming as a cost effective technique for developing plants with cross tolerance to salinity stress. *Plant Physiology and Biochemistry*, 162, 247-257.
- Khan, F., Hussain, S., Khan, S. & Geng, M. (2020): Seed priming improved antioxidant defense system and alleviated Ni-induced adversities in rice seedlings under N, P, or K deprivation. *Frontiers in Plant Science*, 11, 565647.
- Khan, M. B., Gurchani, M. A., Hussain, M., Freed, S. & Mahmood, K. (2011): Wheat seed enhancement by vitamin and hormonal priming. *Pakistan journal of Botany*, 43(3), 1495-1499.
- Lin, C. C. & Kao, C. H. (1999): NaCl induced changes in ionically bound peroxidase activity in roots of rice seedlings. *Plant and Soil*, 216(1), 147-153.
- Marthandan, V., Geetha, R., Kumutha, K., Renganathan, V. G., Karthikeyan, A. & Ramalingam, J. (2020): Seed priming: a feasible strategy to enhance drought tolerance in crop plants. *International Journal of Molecular Sciences*, 21(21), 8258.
- Mhamdi, A. & Van Breusegem, F. (2018): Reactive oxygen species in plant development. *Development*, 145(15), dev164376
- Pieczynski, M., Marczewski, W., Hennig, J., Dolata, J., Bielewicz, D., Piontek, P., Wyrzykowska, A., Krusiewicz, D., Strzelczyk-Zyta, D. & Konopka-Postupolska, D. (2013): Down-regulation of CBP80 gene expression as a strategy to engineer a drought-tolerant potato. *Plant Biotechnology Journal*, 11: 459–469.

- Pook, M., Lisson, S., Risbey, J., Ummenhofer, C. C., McIntosh, P. & Rebbeck, M. (2009): The autumn break for cropping in southeast Australia: trends, synoptic influences and impacts on wheat yield. *International Journal of Climatology: A Journal of the Royal Meteorological Society*, 29(13), 2012-2026.
- Roostaei, M., Kamali, M. J., Roohi, E. & Mohammadi, R. (2018): Evaluation of Sardari bread wheat ecotypes under the rainfed cold conditions of Iran. *The Journal of Agricultural Science*, 156(4), 504-514.
- Ruttanaruangboworn, A., Chanprasert, W., Tobunluepop, P. & Onwimol, D. (2017): Effect of seed priming with different concentrations of potassium nitrate on the pattern of seed imbibition and germination of rice (*Oryza sativa* L.). *Journal of Integrative Agriculture*, 16(3), 605-613
- Shah, T., Latif, S., Khan, H., Munsif, F. & Nie, L. (2019): Ascorbic acid priming enhances seed germination and seedling growth of winter wheat under low temperature due to late sowing in Pakistan. *Agronomy*, 9(11), 757
- Sharma, A., Sidhu, G.P.S., Araniti, F., Bali, A.S., Shahzad, B., Tripathi, D.K., Brestic, M., Skalicki, M. & Landi, M. (2020): The role of salicylic acid in plants exposed to heavy metals. *Molecules* 25(3):540. <https://doi.org/10.3390/molecules25030540>
- Singh, K., Gupta, N. & Dhingra, M. (2018): Effect of temperature regimes, seed priming and priming duration on germination and seedling growth on American cotton. *Journal of Environmental Biology*, 39:83-91.
- Tabatabaei, S.A. (2013): Effect of osmo-priming on germination and enzyme activity in barley (*Hordeum vulgare* L.) seeds under drought stress conditions. *Journal of Stress Physiology & Biochemistry*, 9 (4). 66-74.
- Tavanpour, N. & Ghaemi, A. A. (2016): Zoning of Fars Province in terms of rain-fed winter wheat cultivation based on precipitation and morphological factors. *Iranian Journal of Irrigation & Drainage*, 10(4), 544-555.
- Tayefi-Nasrabadi, H., Dehghan, G., Daeihassani, B., Movafegi, A. & Samadi, A. (2011): Some biochemical properties of guaiacol peroxidases as modified by salt stress in leaves of salt-tolerant and salt-sensitive safflower (*Carthamus tinctorius* L.) cultivars. *African Journal of Biotechnology*, 10(5), 751-763.
- Zafar, Z., Rasheed, F., Mushtaq, N., Khan, M.U., Mohsin, M., Irshad, M.A., Summer, M., Raza, Z. & Gailing, O. (2023): Exogenous application of salicylic acid improves physiological and biochemical attributes of *Morus alba* saplings under soil water deficit. *Forests*, 14(2), 236. Doi: 10.3390/f14020236