

EXOGENOUS ACETYL SALICYLIC ACID (ASPIRIN) EFFECTS ON STRESS TOLERANCE DURING SEED GERMINATION OF *TRITICUM DURUM* DESF.

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Abstract: The effects of the exogenous application of salicylic acid (SA) on the germinating response of *durum* wheat seeds (*Triticum durum*, Desf.) were studied under salt stress conditions. In this study, an assay was conducted on a group (SA) composed of seeds treated with 0.04 mM SA, a second group (NaCl) treated with 120 mM NaCl and another group (SA / NaCl), pretreated by SA and submitted at a salt stress. In the control group (C) we use only distilled water. The measured parameters are the Germinating Capacity (GC), the Daily Average of Germination (ADG), the Kinetic of Germination (KG), the Germination Rate (t50), the Mobilization of Reserves during germination, represented by the Residual Dry Matter (RDM), the Proline and Glutathione (GSH) content. The results show that GC is not affected by the addition of (SA) and in the group pretreated by SA and subjected to salt stress (SA / NaCl). At the same time, the application of NaCl (120 mM) greatly reduces GC. NaCl decreases the germination rate and inhibits the process of mobilization of reserves while decreasing ADG compared to SA and SA / NaCl group. A significant increase in the level of proline is recorded (300%) in the group (NaCl) contrary to the rates obtained after pretreatment with SA and which are similar to those obtained in the group C. Finally, the variations observed with GSH in the presence of SA, highlight an important link between the role of this compound as an antioxidant and the tolerance of seeds to salt stress (NaCl).

Keywords: Salicylic acid, Salt stress, *Triticum durum*, Proline, Glutathione.

INTRODUCTION

The increase in the world's population requires ever greater production of agricultural products such as wheat. Among the most important factors affecting wheat production is salinity. Indeed, this phenomenon is a limiting environmental factor for agricultural production and is directly related to its intensity (Wilson, and Zeng, 2005). At the same time, the water needed for agriculture is scarce because of the ever-increasing demand of the urban population (Zeng, 2004, Walia, Wilson and Close, 2005). Thus, salinity is a limiting factor in crop production (Ghassemi et al., 1995, Silveira et al., 2001, More et al., 2004, Khan and Panda 2008), and these adverse effects on plant growth constitute an important axis of major research, they express themselves through a certain number of parameters such as the osmotic potential, the nutritional balance, or the stress generated by certain ions (salt stress). In this case, the ions tend to accumulate in the soil and then pass inside the cells (Hasegawa et al., 2000), where depending on their level of accumulation, they can reach toxic levels responsible for metabolic disturbances (Munns, 2002). At the same time, Salicylic Acid (SA) plays an important role in plant protection during abiotic stress, as is the case in wheat (Shakirova and Bezrukova 1997, Sakhabutdinova et al., 2003) and various stresses (Singh and Usha, 2003, Gunes et al., 2007, Dat et al., 1998, Rao and Davis, 1999, Bouchelaghem et al., 2011, Alayat et al., 2014). In tomato, the addition of 0.5 mM SA stimulates the germination percentage

(Rao and Davis, 1999). SA is a chemical messenger whose role in defense mechanisms is well understood (Klessing and Malamy, 1994). The present work investigates the effect of pretreatment by low concentrations of SA on the biochemical changes observed in *T. durum*, such as GC, GS, ADG, KG, GR, RDM, content of Proline and Glutathione under salt stress.

MATERIALS AND METHODS

Seed cultivation and treatments: Wheat seeds are provided from the OAIC (Algerian Inter-professional Cereals Office), Annaba, Algeria. The wheat seeds (*Triticum durum* Desf) are first washed with distilled water and then disinfected by passing in sodium hypochlorite solution (1%) for 10 min. The sterilized seeds thus are germinated in Petri dishes (10 cm in diameter, with 10 seeds per dish) and placed in a controlled climate chamber set at a temperature of 25°C and a relative humidity of 85% for 48 hours. The seeds are sprayed with 20 ml of solutions whose composition is as follows: (C): Water-treated controls, (NaCl): treated with 120 mM NaCl, (SA): treated with 0.04 mM Salicylic Acid, (SA/NaCl): treated with the 0.04 mM Salicylic Acid (SA) + 120 mM NaCl. Germination is considered positive when the radicle reaches a length of 5 mm (Kaur et al., 1998).

The measured parameters are:

- Germination Capacity (GC): expressed as the ratio of the number of germinated seeds to the total number of seeds per Petri dish.

- Average Daily of Germination (ADG): Expressed by the ratio of the Percentage of Final Germination on the corresponding number of days (Osborne et al., 1993).

- Kinetics of Germination (KG): Expressed by monitoring germination at times. The number of germinated seeds is noted at 12; 24; 36; 48; 60; 72; 84 and 96 hours after the start of the experiment.

- The germination rate t_{50} , (GR): This expressed the germination energy responsible for the depletion of seed reserves. The germination speed is estimated by the mean time (t_{50}) which represents the germination of 50% of the total number of seeds (Lang, 1965).

- Mobilization of Reserves during Germination (RDM): it corresponds to the nutritional contribution during the growth of the pellet during the early stages of its development. It is estimated by the content of the Residual Dry Matter (RDM) of the seed after a germination of 4 days.

- Proline: The determination of proline is carried out by homogenizing 0.5 g of fresh tissue in 5 ml of 3% solution of sulfosalicylic acid. The homogenate is centrifuged for 10 min x 5000 rpm. The proline content is determined by the change in absorbance at 520 nm (Khalid et al., 2001). The standard curve is made with pure proline.

- Glutathione (GSH): The assay is carried out following extraction of the non-protein thiols by homogenizing 0.3 g of tissues in 1.5 ml of 0.1 N HCl solution. The mixture is then centrifuged for 30 min x 1500 rpm. The supernatant is used for the determination of glutathione by spectrophotometry at 412 nm. (Mwale et al., 2003).

RESULTS

Effects of SA on the Germinative Capacity and Average Daily Germination of wheat seeds subjected to salt stress:

It is found (Figure 1) that the germination of seeds treated with NaCl (120 mM) is strongly affected, in fact, there is a reduction of the GC of nearly 70% compared to control seeds. The SA treatment increases the GC of the seeds which slightly exceeds the rate observed in the control seeds. In seeds under stress conditions (120 mM NaCl), pretreatment with SA prevents the inhibition of GC observed following salt stress. At the same time, the figure shows that the ADG of seeds under salt stress is the lowest (9%) (compared to controls). SA treatment causes stimulation of ADG whose values match those obtained in control seeds. Pretreatment by SA of seeds under salt stress stopped the effect previously observed in the presence of NaCl.

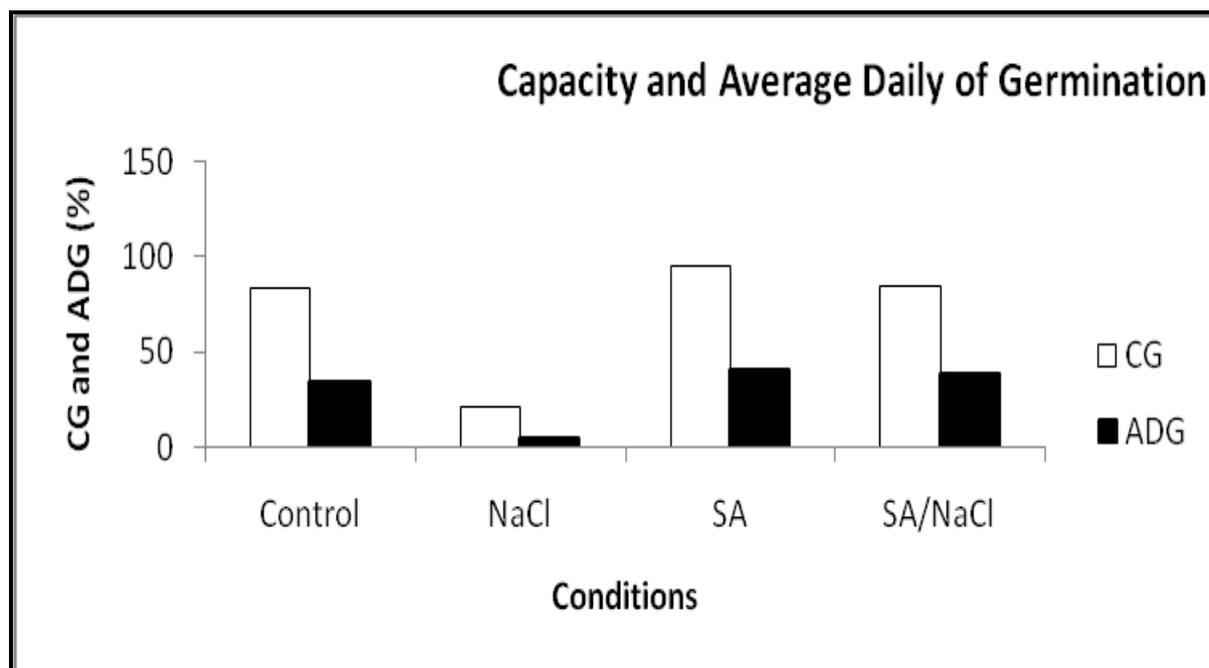


Fig. 1. Effect of salicylic acid pre-treatment on the Capacity and Average Daily Germination (GC and ADG) during seedlings development of *T. durum*.

Control: without treatment, NaCl: Treated with 120mM NaCl, SA: Treated with 0.04 mM SA and SA/NaCl: Treated with 0.04 mM SA and then in 120 mM NaCl.

2. Effects of SA on kinetic of germination of wheat seeds subjected to salt stress: In Figure 2, we have

shown the effects of SA on the Kinetic of Germination of wheat seeds subjected to salt stress.

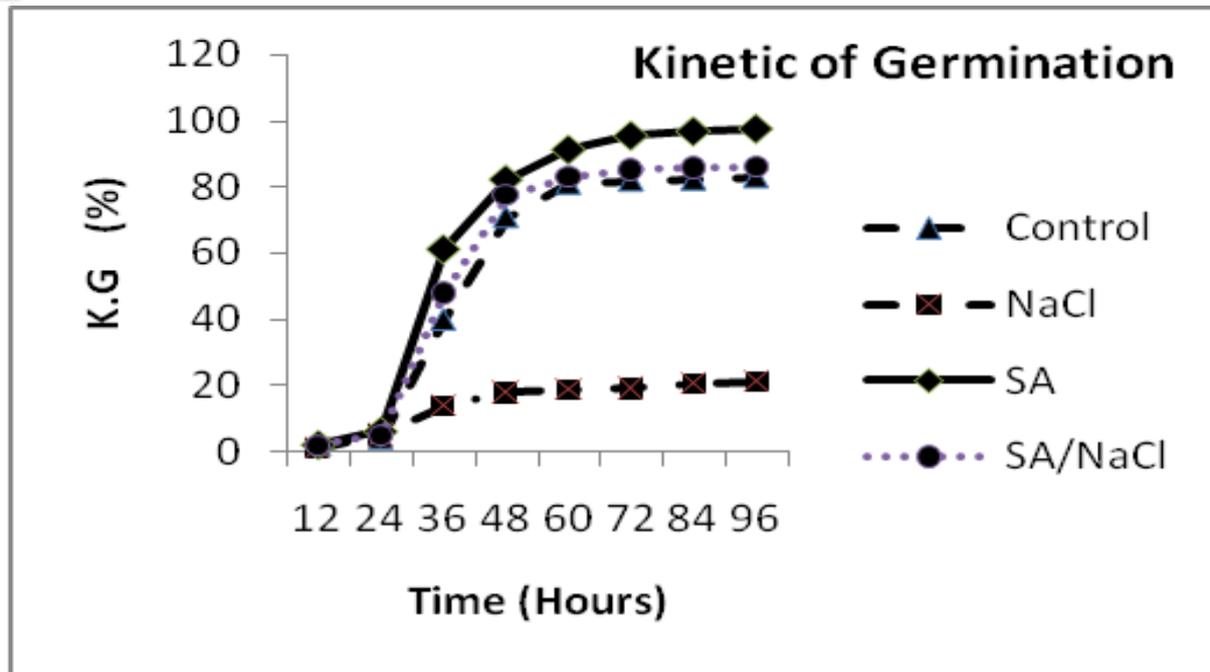


Fig. 2. Effect of salicylic acid pre-treatment on the Kinetic of Seed Germination (KG) of *T. durum*. Control: without treatment, NaCl: Treated with 120mM NaCl, SA: Treated with 0.04 mM SA and SA/NaCl: Treated with 0.04 mM SA and then in 120 mM NaCl.

Germination Rate Evolution of wheat seeds over time and according to the different conditions makes it possible to distinguish first a slow primary step during which the first wheat seeds will appear. This stage is prolonged (up to 96 h) with a very low germination rate for seeds under salt stress (NaCl). However, this phase is short in the control seeds (approximately 24 h, witnesses). A second linear step is then observed for the control seeds, those treated with SA and the seeds pretreated with SA and subjected to salt stress (SA / NaCl).

Finally, we observe a terminal stage with a maximum phase expressing the final germination rate (the highest). Thus, it is found that the germination of control seeds, those treated by SA and those pretreated by SA and subjected to salt stress (NaCl) is the fastest and that corresponding to the seeds subjected only to salt stress is the most important slow.

3. Effects of SA on the germination rate of wheat seeds subjected to salt stress: In Table 1, we have shown the effects of SA on the Rate of Germination of wheat seeds subjected to salt stress.

Table 1.

Effect of salicylic acid pre-treatment on the Rate of Seeds Germination (t50 in days) of *T. durum*

Conditions	Day 3	Day 5	Day 7	Day 9
Control	1,8	4,5	7,3	8,8
SA	2,4	6,8	9,9	14,2
NaCl	0,4	1,1	3,2	4,2
SA/NaCl	2	5,4	8,2	9,1

Control: without treatment, NaCl: Treated with 120mM NaCl, SA: Treated with 0.04 mM SA and SA/NaCl: Treated with 0.04 mM SA and then in 120 mM NaCl.

This parameter is expressed by the t50 which means the mean time corresponding to the germination of 50% of the total seeds. In this table, it can be seen that the recorded values of t50 are identical for control seeds and those treated with SA. A slight decrease is obtained in SA / NaCl seeds on day 3. The same evolution is observed for days 5, 7 and 9 with,

however, larger deviations as a function of time. So if the salt stress slows the t50, contrary the pretreatment by SA tends to stimulate it.

4. Effects of SA on the mobilization of reserves of wheat seeds subjected to salt stress: The effects of SA on the evolution of the mobilization of salt-stressed wheat seeds reserves are reported in Table 2.

Table 2.

Effect of salicylic acid pre-treatment on the Residual Dry Matter of Seeds (mg) of *T. durum*

Conditions	Day 3	Day 5	Day 7	Day 9
Control	17,3	19	22,6	25
SA	15,3	16,1	17,9	18,6
NaCl	101	115	125	131

SA/NaCl	56	61	64	72
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Control: Without treatment, NaCl: Treated with 120mM NaCl, SA: Treated with 0.04 mM SA and SA/NaCl: Treated with 0.04 mM SA and then in 120 mM NaCl.

This stage constitutes a primordial phase in the germinating process. Table 2 shows the effects of SA on wheat seeds subjected to salt stress and expressed as variations in Residual Dry Matter mass (RDM). It is found that the RDM is low and stable in control seeds and those treated by SA as a function of time. RDM increases considerably in the presence of NaCl, which reflects a reducing effect on the mobilization of reserves. This is observed for all the experiment times.

The pretreatment with SA gives average RDM values between 56 and 72mg and thus contributes to lifting the depressive effect observed in the presence of salt stress.

5. Effects of SA on the average proline and glutathione levels in wheat seeds subjected to salt stress:

Figure 3 shows the changes in the effects of SA on the average levels of proline and glutathione in wheat seeds subjected to salt stress.

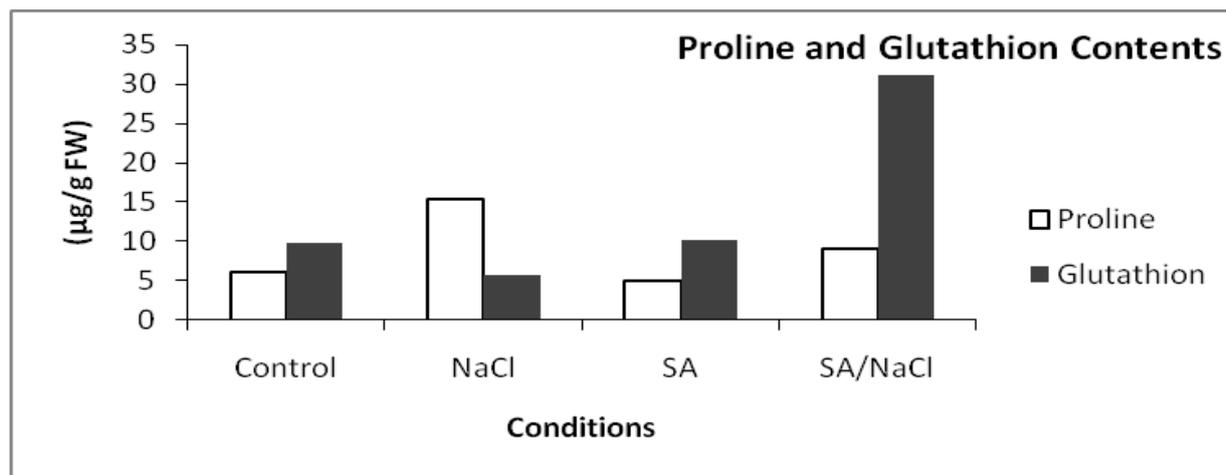


Fig. 3. Effect of salicylic acid pre-treatment on the proline content and the glutathione content (µg/g FW) during seedlings development of *T. durum* (Day 9).

Control: Without treatment, NaCl: Treated with 120mM NaCl, SA: Treated with 0.04 mM SA and SA/NaCl: Treated with 0.04 mM SA and then in 120 mM NaCl.

The seeds under salt stress (NaCl) have a proline level approximately 3 times higher than that of the control seeds. Treatment with SA slightly affects the level of proline which remains similar to that recorded in the control seeds. In pretreated SA seeds subjected to salt stress, there is an average (50%) increase in proline from 6.2 to 9µg / g FW. The reduced glutathione content is lower in seeds under salt stress (NaCl) than in control seeds (40%). SA treatment increases the glutathione content by up to 45% compared to stressed seeds (NaCl) and slightly compared to control seeds. However, in SA pre-treated seeds subjected to salt stress, the glutathione values obtained showed a significant increase compared to control seeds treated with SA (300%). The set of variations recorded with this parameter (GSH) demonstrates the implication of this product in the behavior of the wheat seed with respect to salt stress.

DISCUSSION

Many studies carried out at the germination stage highlight the important role played by the SA during various biotic stresses (Borsani et al., 2001, Dat et al., 1998, Rao et al., 1999). During germination, the osmolarity of the medium controls the exit of the radicle while the mobilization of the reserves controls the growth of the seedling (Gomes et al., 1983). In this way, all the results reported in the literature indicated

the harmful effect of NaCl on the germination of many seed varieties (Wheat, Barley, Castor, Watermelon, Acacia, (Allagui et al., 1994, Ben Naceur, 2001, Raghavaiah et al., 2006, Askri et al., 2007, Hajlaoui et al., 2007, Ndour and Danthu, 1998, Jaouadi et al., 2010). Our work was carried out in order to follow the behavior of wheat seeds pretreated with low concentrations of SA during salt stress caused by treatment with NaCl. The effect of sodium chloride concentration (120 mM) on seed germination shows that germination capacity and germination rate is strongly affected by salt (from 83.2% for controls to 21.1% for seeds treated with 120mM NaCl). The studies of (Khalid et al., 2001, Mwale et al., 2003, Okçu et al., 2005) shows that the observed reduction in germination percentage is due to the effect of external osmotic pressure, which constitutes, on the one hand, an obstacle limiting the absorption of water and, on the other hand, an accumulation of Na⁺ and Cl⁻ in the embryo. This situation generates an inhibitory toxicity of the germinating process which causes the death of the embryo. Öner and Kirli, (2018) showed that Dry coleoptile and radicle weights and lengths decreased with increasing salt concentrations in *Triticum aestivum*. Results obtained by ALMANSOURI et al., (1999) and Ouhaddach et al., 2019, noted that stress imposition induced a decrease in relative growth rates, K concentrations, and leaf osmotic potential values, as

well as an increase in Na, proline and soluble sugar contents.

Pretreatment with SA increases GC, KG and t50. The decrease observed in the germination process accompanied by the variation of the ADG is directly related to the different regulatory processes activated by the seeds to regulate the excessive osmolarity of the medium: this result confirms the one reported in the work of Bliss et al., (1986). The values obtained in RDM can be explained in two ways: either an inhibition by the action of the salt on the enzymatic amylase activity (Gal et al., 2004, Gones et al., 1983), or by a direct action on the transport of energetic materials (starch) to the embryo. Pretreatment by SA in the presence of salt stress tends to increase the averages of the observed value of RDM. Szepesi et al. (2005) show that the addition of SA increases the water content and water potential in tomato. Sakhabutdinova et al., (2003) shows that SA reduces the accumulation of ABA which causes a stimulation of the germinating process. The proline contents (osmopolyte) obtained in the study can be explained by the fact that the pretreatment with SA does not seem to affect the seeds because of their similar status to that of the control seeds (absence of lesions and damages which generates the proline stimulation), (Hare et al., 1998). The variation of the glutathione content, particularly in SA-pretreated seeds subjected to salt stress, highlights the role played by this potent antioxidant during salt stress by its implication in triggering a detoxification mechanism that improves adaptation of seeds to stress through the increase of their antioxidant capacities and their resistance (detoxification) to the ROS generated during salt stress (Bellaire et al., 2000). Thus pretreatment with SA tends to stimulate an important synthesis of GSH which substantially reduces the importance of degradation caused by salt stress. Pirasteh-Anosheh et al., (2014) and recent work of Mohammadi et al., (2019) confirms that Salicylic-acid-induced recovery ability in salt-stressed wheat plants. Our results have shown, on the one hand, the osmotic and / or toxic action of NaCl and, on the other hand, the repairing action of SA. In this way, several other potentials aspects of SA, such as the structure, absorption, mode of action and mechanism, need to be explored.

CONCLUSION

The results obtained in our study indicate that SA strongly contributed to the protection of wheat seeds following salt stress. This is supported by perturbations observed in physiological and biochemical parameters (GC, ADG, KG, ADM, Proline, and GSH). Moreover, our results suggest that SA pretreatment could induce some mechanisms involved in the resistance and / or adaptive responses of seeds.

AUTHORS CONTRIBUTIONS

Part 1 – Stress tolerance, including conceptualization, methodology, data validation, data processing, preparation of the initial project, data collection, writing, and revision: Berrebbah H., Djebbar M. R.

Part 2 – Exogenous acetyl salicylic acid, including conceptualization, methodology, data validation, data processing, preparation of the initial project, data collection, writing, and revision: Djebbar A.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of personal or professional interest.

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