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Influence of Salicylic Acid on Stress Tolerance During Seed Germination of *Triticum aestivum* and *Hordeum vulgare*

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Abstract: The relationship between compatible solutes (osmolytes) and antioxidants are the strategies that plants have developed to tolerate salt stress. Pre-treatment of wheat (*Triticum aestivum* L. cv) and barley (*Hordeum vulgare* L. cv) with Salicylic acid (SA) can be enhanced their tolerance to saline stress during germination. The alleviation of oxidative damage and increased resistance to salt stress induced by 150 mM NaCl treatments often correlate with a more efficient antioxidative defense systems and detoxification mechanisms. Pre-treatment of wheat and barley plants with salicylic acid (SA) enhanced antioxidant activities in concentration dependent manner and increased the stress tolerance of seedlings. Improved acclimation of SA-pre-treated plants to salt stress depended on the activation of the antioxidative and accumulation of ionic and non-ionic osmolytes. The present work deals with the possible role of the osmotic adaptation and oxidative defense mechanisms of wheat and barley plants with low concentrations of SA. During the germination period, a considerable increase was observed in proline levels (up to 185% in *T. aestivum* and about 128% in *H. vulgare*) in the seedlings subjected to saline stress (S), whereas in the SAS group, the proline increment was not significantly. Starting from the fourth day of germination, betaine levels in seedlings pretreated with Salicylic acid and then with water (SAW group) and in SAS showed a significant increase versus C and S seedlings, possibly because such a precursor promotes betaine biosynthesis. This could be responsible for the enhanced growth observed in SAS versus S seedlings, as well as for preventing the decrease in chlorophyll content in the SAS group. The accumulation of betaine seems to correlate with the greater tolerance of these seedlings against saline stress. A relationship between antioxidant glutathione and salt tolerance was observed in both plants, under the effect of Salicylic acid.

Key words: *Triticum aestivum* and *Hordeum vulgare* · osmotic adaptation · growth creature · salt stress · salicylic acid

INTRODUCTION

Because of increases in global population, world agriculture must produce a greater yield per unit area than ever before. However, worldwide one-half of all irrigated lands are seriously affected by salinity or water logging. Currently, more land is not being irrigated due to salinity problems than there is new land coming under irrigation [1]. It is believed that in the past soil salinity has contributed to the decline of several ancient civilizations. Irrigated agriculture takes on a special importance in this regard as it has a high yield per unit area and is less dependent on the uncertainties of weather. Furthermore, high-quality water needed for agriculture is becoming increasingly scarce due to changing environmental standards and rising demands from urban areas [2, 3].

The deleterious effects of salinity on plant growth are associated with (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress), or (4) a combination of these factors [4]. All of these cause adverse apheliotropic effects on plants. On the whole, it is accepted that the accumulation of compatible osmolytes in the cell can prevent the exit of water or increase its entry, providing the required turgidity for cell expansion [5]. Plant possesses a complex antioxidant defense system that is formed by hydrophilic and lipophilic compounds to alleviate and protect the plant cell against salt stress oxidative damage. Glutathione from the most soluble antioxidants implicated in the adaptation of plants to environmental stress, but it must be kept in reduced form [6]. The selected species are one sensitive (*Triticum aestivum*) and other more tolerant (*Hordeum vulgare*) [7, 8].

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Salicylic acid (SA), a plant Phenolic is now considered as a hormone-like endogenous regulator and its role in the defense mechanisms against biotic and a biotic stress has been well documented [9, 10]. It was found that inhibition of catalase, a H_2O_2 scavenging enzyme, by SA plays an essential role in the generation of reactive oxygen species [11]. By increasing H_2O_2 concentration of the tissues, moderate doses of SA may activate the antioxidative mechanisms. Application of exogenous SA enhanced the drought and salt stress resistance of plants [12, 13], but the results were contradictory and depended on the developmental phase of plants [14] or on the experimental conditions [15]. Both high salinity and drought give rise to ionic and osmotic effects combined with oxidative damage in tissues. Previous studies demonstrated that SA plays an important role in determining the sensitivity of plants to various a biotic stresses [16, 17], notably at the seedling stage [14]. The inclusion of SA at 0.5 mM in the germination medium was associated with increase germination percentage of tomato [18].

The aim of present work was to reveal whether wheat and barley plants pre-treated with low concentrations of SA could tolerant salt stress.

MATERIALS AND METHODS

Seeds culture and treatments: Surface sterilized seeds of wheat (*Triticum aestivum* L. cv) and barley (*Hordeum vulgare* L. cv) were placed in Petri dishes with distilled water in a controlled environmental chamber for 24 h in a glasshouse at Faculty of Science, Taif University and S.A. during October 2006. Soaked seeds were then distributed in plastic trays (15x15 cm) with three layers of filter paper moistened with the solutions indicated for each treatment, as follows:

Control in water (C). -In water for 6 h and then treated with 150 mM NaCl (S). - Treated with 0.05 mM salicylic acid for 6 h and then in water (SAW). - Treated with 0.05 mM Salicylic acid for 6 h and then subjected to 150 mM NaCl (SAS). Each treatment consisted of 10 plastic trays each containing 20 seeds. Samples were collected randomly at 2, 4, 6 and 8 days and the length of seedlings were measured.

Growth measurement: Plants growth was estimated by measuring accumulation of root and shoot weight (after drying the plants material at 70°C for 48-72 h.) Relative water content was also measured and expressed as a percentage according to the following equation:

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

Recovery of seedlings: At the end of the experiment described above (day 8), seedlings were transferred to 15 cm plastic pots containing vermiculite, maintained in the same environmental condition and watered daily with a half nutrient solution [19] for 10 days.

Analyses: Chlorophyll content: was determined, at 6 and 8 days after germination and in recovered plants. It was extracted by homogenizing 0.5 g fresh weight of green tissues (leaf plus stem) of the seedlings in 10 ml of 95% ethanol. After centrifugation for 10 min at 5000 rpm, the chlorophyll content was analyzed spectrophotometrically on the ethanolic supernatant at 654 nm as described by Wintermans and De Mots [20].

***Proline*:** 0.5 g FW was homogenized in 5 ml of 3% sulphosalicylic acid. After centrifugation for 10 min at 5000 rpm, proline was estimated spectrophotometrically at 520 nm using the ninhydrin method [21]. Purified proline was used for standardization.

***Betaine assay*:** 0.5 g FW were homogenized with 5 ml of methanol and extracts were phase-separated with chloroform and water as described previously [22]. After evaporating the aqueous phase to dryness in an air stream of distilled water and 0.3 ml of slurry of dowex -50 ion exchange resin in the H^+ form were added [23]. Betaine was determined according to the per iodide method [24].

***Glutathione determination*:** none-protein thiols were extracted by homogenizing 0.3 g FW in 1.5 ml of 0.1N HCl. After centrifugation at 15000 rpm for 30 min at 4°C, the supernatants were used for analysis. Total glutathione was determined in the homogenates by spectrophotometry at 412 nm [25].

Statistical analysis: Results indicated as mean values±SEM. Differences between control and treated seeds were analyzed by one-way ANOVA, taking $P<0.05$ a significant, according to Tukey's Multiple Range Test (MRT).

RESULTS AND DISCUSSION

Influence of SA on stress tolerance during seed germination: Previous studies demonstrated that SA plays an important role in determining the sensitivity of plants to various a biotic stresses [16, 17], notably at the seedling stage [14]. To further document the influence

Table 1: Effect of salicylic acid pre-treatment on *T. aestivum* and *H. vulgare* relative water content (%). Values are the means ±SEM of three replicated measurements

	Time in days							
	2		4		6		8	
Group	*T. aes.*	*H. vul.*	*T. aes*	*H. vul.*	*T. aes.*	*H. vul.*	*T. aes.*	*H. vul.*
C	35±3	30±2	46±3	39±2	52±4	47±2	69±4	66±3
S	26±2***	29±1	31±3**	35±2**	36±3***	40±2*	42±2***	58±2**
SAW	41±2*	33±2*	52±2*	44±3**	70±3**	60±3***	83±3*	78±2***
SAS	38±2	30±2	42±2	38±2	58±3*	46±3	71±3	65±3

Table 2: Effect of salicylic acid pre-treatment on the length (root plus shoot, cm) during seedlings development of *T. aestivum* and *H. vulgare*. Values are the means ± SEM of three replicated measurements

	Time in days							
	2		4		6		8	
Group	*T. aes.*	*H. vul.*	*T. aes*	*H. vul.*	*T. aes.*	*H. vul.*	*T. aes.*	*H. vul.*
C	1.8±.2	2.3±.2	9.3±.3	8.4±.4	25.5±.5	21.7±.6	36.1±.4	28.6±.6
S	.6±.1***	1.8±.1	2.6±.1****	6.1±.3	8.8±.2***	11.6±.4**	11.8±.2****	18.5±.4***
SAW	2.1±.1**	2.5±.2	10.8±.3	9.2±.4*	32.6±.6*	25.4±.5**	42.7±.6*	30.6±..4*
SAS	1.4±.1**	2.1±.1	6.1±.2*	7.5±.4	21.2±.4**	18.5±.5*	34.6±.5**	25.4±.4*

C=Control S=Treated with 150mM NaCl after 6 h in water SAW =Treated with 0.05 mM SA and then in water SAS= Treated with 0.05 mM SA and then in 150 mM NaCl *=Significant at P<0.5 **=Significant at P<0.1 ***=Significant at P<0.05

of this molecule at the level of seed germination, wheat (*Triticum aestivum* L. cv) and barley (*Hordeum vulgare* L. cv) seeds were germinated under salt stress conditions with or without SA addition. To determine the growth of *T. aestivum* and *H. vulgare* seedlings starting from the second day of the experiment, their length and relative water content (RWC) as well as dry weight of roots and shoots were measured. RWC was significantly lower in the S groups from the second day as compared with the C group. This decrease persisted up to the eight day (Table 1). The decrease was more in *T. aestivum* (39.1%) than in *H. vulgare* (12.12%) in S group compared with the C group at eight day. The pre-treatment with SA increase the water content of the seedlings with or without stress. Szepesi1 *et al*. [18] showed that SA pre-treatments reduced K^+ contents of leaves under salt stress and increased water potential as well as water content.

The length of S seedlings was significantly lower than that of the control over the whole experiment in *T. aestivum*, reaching a length more than 3-fold less at the eight day of treatment (Table 2). In *H. vulgare* the salinity was less effect over the whole experiment i.e. the less at the eighth day of treatment reached to 36% (less than one fold). Stress induced root necrosis (in *T. aestivum*) but secondary roots failed to appear and therefore root length was more affected than shoot length under salt stress conditions. The protective effect of salicylic acid pre-treatment against saline stress was shown by the greater length and RWC of SAS versus S seedlings (Tables 1, 2). On the eight day, mean SAS length was more than thrice as much as S and was accompanied by the appearance of secondary roots in the former seedlings (*T. aestivum*). In *H. vulgare* SAS reached to nearest as C group. SAW pre-treatment increase the seedlings length and RWC than control, so it has activation the growth measurements in the two plants (Tables 1, 2).

Increase in salinity from 0 to 150 mM NaCl significantly decrease root and shoot biomass of *T. aestivum* by 55% and 72% respectively meanwhile the decrease were 41% and 38% at *H. vulgare* (Table 3). Seedlings pre-treatment with SA prevented the decrease in biomass caused by saline stress. Moreover seedlings pre-treatment with SAW have higher biomass than C seedlings in both plants. Under the influence of salt stress the osmotic potential greatly decreased and the SA pre-treatments moderated it. The increased water potential values in SA pre-treated samples under ionic and non-ionic osmotic stress suggest that accumulation of inorganic or organic osmolytes makes the surplus of

Table 3: Effect of salicylic acid pre-treatment on root, shoot dry weights and root/shoot ratio of *T. aestivum* and *H. vulgare* after 8 days (g/seedling). Values are the means ±SEM of three replicated measurements

	T. aestivum			*H. vulgare*		
Group	Root	Shoot	Root/Shoot	Root	Shoot	Root/Shoot
C	0.022	0.096	0.229	0.017	0.055	0.309
S	0.010	0.027	0.370	0.010	0.034	0.294
SAW	0.034	0.142	0.239	0.021	0.086	0.244
SAS	0.024	0.099	0.242	0.020	0.061	0.328

Table 4: Effect of salicylic acid pre-treatment on the chlorophyll content (μg g^{-1} DW) during seedlings development of *T. aestivum* and *H. vulgare*. Values are the means ± SEM of three replicated measurements

	Time in days							
	2		4		6		8	
Group	*T. aes.*	*H. vul.*	*T. aes*	*H. vul.*	*T. aes.*	*H. vul.*	*T. aes.*	*H. vul.*
C	87±8	57±3	220±15	190±8	440±20	320±8	650±23	560±19
S	40±6***	46±4*	100±8***	105±8***	160±9***	180±5*	280±8***	280±15**
SAW	106±9*	74±6*	340±18*	260±13	620±20	430±6*	840±22	690±17
SAS	80±7*	50±4	205±12*	195±10*	420±12*	360±7	580±10	550±13*

C=Control S=Treated with 150mM NaCl after 6 h in water SAW =Treated with 0.05 mM SA and then in water SAS= Treated with 0.05 mM SA and then in 150 mM NaCl *=Significant at P<0.5 **=Significant at P<0.1 ***=Significant at P<0.05

water uptake possible as it can also be seen from the increased relative water contents of tissues [18]. SA pre-treatment regulate the nascent polypeptide-associated complex (NAC) during seeds germination. NAC is a heterodimeric complex that can reversibly bind to eukaryotic ribosome's [26]. It is presumed to prevent ribosome-associated nascent polypeptide from inappropriate interaction with proteins in the cytosol [27]. A recent study demonstrated that the NAC was down-regulated in rice (*Oryza sativa*) roots submitted to a salt stress in the presence of 150 mM NaCl [28]. Also, a proteomic study of sugar beet (*Beta vulgaris*) leaves identified the α-chain NAC as being down-regulated in response to drought [29]. This data also agrees with the proteomic results of Campo *et al.* [30] showing the induction in fungal-infected maize embryos of several proteins involved in initiation of protein synthesis or proteins that participate in the protein folding process.

Total chlorophyll was determined in green tissues in *T. aestivum* and *H. vulgare*. In S seedlings were lower than those of C seedlings of both plants (Table 4). On the eight day it was observed that the C seedlings had more than two fold that of S in *T. aestivum*, while about 1.5 fold that of *H. vulgare*. So *T. aestivum more* effective than *H. vulgare.* Seedlings pre-treatment with SA prevented the decrease in chlorophyll caused by saline stress. Since SA improved the photosynthetic performance of plants under stress conditions [31] and chlorophyll a fluorescence could give insight into the ability of a plant to tolerate environmental stresses. This can be partially overcome if plants are pre-treated with SA. Since under non-photo respiratory conditions the effective quantum yield of PSII provides useful information concerning photosynthetic performance of C3 plants, these results suggests that SA pre-treatment may improve the gross rate of carbon assimilation during osmotic stress. In the presence of SA, leaves accumulated different compatible osmolytes, such as sugars, sugar alcohol and proline. SA pre-treatment decreased the CAT activity both in the roots and leaves, but the activity of other enzymes associated with the antioxidative defense, superoxide dismutases (SOD), peroxides (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) exhibited different changes. As a general rule, the activity of these enzymes (CAT, SOD, POX and APX) decreased compared to the control in the leaves of tomato plants [18].

Pre-treatment of *T. aestivum* seeds with salicylic acid led to enhanced seedling tolerance to condition of saline stress during germination, as evidenced by the greater growth of SAS versus S seedlings evaluated through such parameters as length, RWC, root and shoot biomass as well as chlorophyll content (Table 1-4).

Table 5: Effect of salicylic acid pre-treatment on the proline content (µg g^{-1} DW) during seedlings development of *T. aestivum* and *H. vulgare*. Values are the means ± SEM of three replicated measurements

	Time in days							
	2		4		6		8	
Group	*T. aes.*	*H. vul.*	*T. aes*	*H. vul.*	*T. aes.*	*H. vul.*	*T. aes.*	*H. vul.*
C	2.8±.2	1.8±.1	5.4±.3	3.5±.2	6.2±.3	4.2±.2	5.2±.2	4.2±.2
S	3.2±.2*	2.2±.1*	8.5±.4***	4.2±.2*	11.5±.4***	9.2±.3**	14.8±.4***	9.6±.2***
SAW	2.6±.1	2±.1	4.2±.2*	3.7±.2	6.5±.3	4.8±.3	3.4±.1	4.5±.1
SAS	3±.2	2±.1	5.8±.3	3.6±.2	11.6±.4**	6.8±.3*	8.3±.3**	8±.2**

Table 6: Effect of salicylic acid pre-treatment on the betaine levels (µg g^{-1} DW) during seedlings development of *T. aestivum* and *H. vulgare*. Values are the means ± SEM of three replicated measurements

	Time in days							
	2		4		6		8	
Group	*T. aes.*	*H. vul.*	*T. aes*	*H. vul.*	*T. aes.*	*H. vul.*	*T. aes.*	*H. vul.*
C	2.3±.2	1.1±.1	7.6±.4	3.6±.2	12.4±1	8.3±.3	14.8±.6	5.4±.2
S	2.1±.2	1.6±.1*	6.5±.8*	5.4±.3**	11.2±1	7.3±..3*	13.4±.4	8.5±.5**
SAW	5.2±.4**	3.2±.1**	15.4±1**	9.4±.3***	19.6±2***	16.8±1***	23.5±1***	19.6±.4***
SAS	4.4±.2*	6.3±.2***	13.7±1*	12.2±.5**8	18.5±1***	14.1±.5***	20±.6**	14.6±.5***

C=Control S=Treated with 150mM NaCl after 6 h in water SAW =Treated with 0.05 mM SA and then in water SAS= Treated with 0.05 mM SA and then in 150 mM NaCl *=Significant at P<0.5, **=Significant at P<0.1, ***=Significant at P<0.05

H. vulgare is less sensitive plant than *T. aestivum*, so its adverse saline effect is less. Also pre-treatment with SA increase the tolerance of *H. vulgare* through the measure parameters. Szepesi1 *et al*. [18] show that SA substantially improved germination vigor under salt stress conditions at tomato plants. Sakhabutdinova *et al*. [32], showed that pre-sowing treatment with SA completely prevented salinity induced declines in the concentration of IAA and cytokines in seedlings and reduced accumulation of ABA, which might be a prerequisite for acceleration of growth resumption of wheat seedlings after withdrawal of stressor from the medium.

Levels of compatible osmolytes: In the S groups, proline levels were significantly increased starting from the second day and peaking at the eighth day of the experiment, with an increase of roughly 185% in *T. aestivum* and about 128% in *H. vulgare* above the controls; while in SAS seedlings, significantly smaller increments were observed (Table 5). In seedlings of both plants pre-treated with SA and subjected to saline stress (SAS group), the proline increment was much smaller (about 60%) than that of S group. Hare *et al*. [5] have contended that proline content increases when there is an injury to plant tissue. Possibly in seedlings pre-treated with SA and subjected to saline stress, where growth is greater and plant status better, damage is less and therefore proline levels are hardly increased with respected to the control seedlings.

When betaine content was determined, it was observed that control seedlings reached the maximal betaine level (23.5 um g^{-1} DW for *T. aestivum and* 19.6 um g^{-1} DW for *H. vulgare*) on the eight day after germination for the two plants. In seedlings subjected to saline stress there was little decrease in betaine content at *T. aestivum* seedlings, in contrast, there was an obvious increase at *H. vulgare* seedlings as compared to the control seedlings without treatments with SA. Starting from the fourth day of germination, pre-treatment with salicylic acid led to a significant increase in betaine levels in SAW and in SAS versus C and S seedlings in the two plants. Such an increase may be attributed to the fact that the addition of this precursor (salicylic acid) promotes betaine formation by stimulating its biosynthesis [33]. The protective role of betaine against saline stresses both in higher plants, in bacteria and in animals is widely recognized [34]. The significant increase of this osmolytes in plant tissue from seeds pre-treated with SA would help to explain the increase in tolerance to salinity. The

Table 7: Effect of salicylic acid pre-treatment on the glutathione content (μg g^{-1} DW) during seedlings development of *T. aestivum* and *H. vulgare*. Values are the means ±SEM of three replicated measurements

	Time in days							
	2		4		6		8	
Group	*T. aes.*	*H. vul.*	*T. aes*	*H. vul.*	*T. aes.*	*H. vul.*	*T. aes.*	*H. vul.*
C	12.6±.2	18.8±1	14.4±1	19.5±2	11.2±2	17.2±2	10.2±1	16.4±2
S	7.2±.2*	12.2±1*	9.5±.4***	14.2±.2*	8.5±.4***	9.2±.3**	6±.4***	9.6±.2***
SAW	15.9±1	19.2±2	17±.2*	20.7±2	12.5±3	18±3	10.4±1	17.5±2
SAS	10.5±2***	27±3***	11±3***	36±.4***	20±4**	36±5****	28±5****	38±7***

C=Control S=Treated with 150mM NaCl after 6 h in water SAW =Treated with 0.05 mM SA and then in water SAS= Treated with 0.05 mM SA and then in 150 mM NaCl *=Significant at P<0.5, **=Significant at P<0.1, ***=Significant at P<0.05

accumulation recorded in seedlings starting from the fourth day could be responsible for the enhanced growth observed in SAS versus S seedlings, as well as for preventing the decrease in chlorophyll content in the BRS group. The results obtained in this work strongly suggest that *T. aestivum* accumulate more betaine than *H. vulgare* thus was attributed to the less sensitive of *H. vulgare* to salt stress, so its low needs to osmolyte.

Pre-treatment with salicylic acid showed an increase in osmolyte (proline and betaine) during germination, because its biosynthetic precursor, SA, could activate BADH, thus enhancing the accumulation of proline and betaine. The accumulation of these osmolyte seems to correlate with greater tolerance against stress. In a more general context, it could be said that the formation of a compatible osmolytes such proline and betaine, capable of stabilizing membranes and proteins, is responsible for the increase in tolerance against saline stress. On the other hand the inhibitory effect of betaine on proline accumulation would provide an alternative explanation for this finding, in agreement with the results obtained by Gibon *et al*. [35] and Larher *et al*. [36] with rape leaf discs.

Reduced glutathione content was constitutively lower in S group than in C group (Table 7). Glutathione levels were diminished in SAW and C groups in the experiment period as compared with shocked group. However, at SAS, the glutathione values showed an increase compared with C group. Adaptation to high NaCl levels involves increases in the antioxidant capacity of the cell to detoxify reactive oxygen species [37]. In concordance to Hernadez *et al*. [38], the profile observed in glutathione (GSH) content could indicate that this antioxidant soluble compound was involved in the salt tolerance. The increase in glutathione content due to SA treatment enhanced salt tolerance of both plants, may be totally or partly due to increased GSH synthesis and/or decrease rates of degradation [39]. Exogenous SA treatment leads to increased antioxidant capacity in barley (*Hordeum vulgare*) leaves [31] and stimulates peroxidase / catalase activities in plant cells [40] because of an enhanced accumulation of hydrogen peroxide under such conditions [41-43]. It is known that salt stress induces the generation of reactive oxygen species in plants [14, 44]. Thereby, it is possible that the presently observed induction of such enzymes by SA can provide an explanation for the improvement of *T. aestivum* and *H. vulgare* seed germination under salt stress brought about by this elicitor.

Plant recovery: At the third day of recovery, at *T. aestivum* both SAS and control plants had two pair of leaves; while only on the fifth day did the second pair appear in the S seedlings. On the sixth day of recovery, it was observed that root length of SAS plants was 38% greater than that of S group (Fig. 1). Seedlings pre-treated with SA and subjected to saline stress (SAS) had a greater recovery in chlorophyll levels (98% of the control) than those of the S group which reached 44% of the control value (Fig. 1).

At *H. vulgare* the second leaf appear at second day in control as well as of SAW and SAS groups. At sixth day of recovery, it was observed that lateral roots length of SAS plants was 50% greater than that of S (Fig. 1), while shoot length of SAS plants was 75% greater than that of S. Seedlings of *H. vulgare* pre-treated with SA and subjected to saline stress (SAS) had a greater recovery in chlorophyll levels (95% of control) than those of the S group, which reached 60% of control value.

The obtained result shows that the adaptation to high NaCl (SAS) involves an increase in the antioxidant capacity (GSH) of the cell to detoxify reactive oxygen

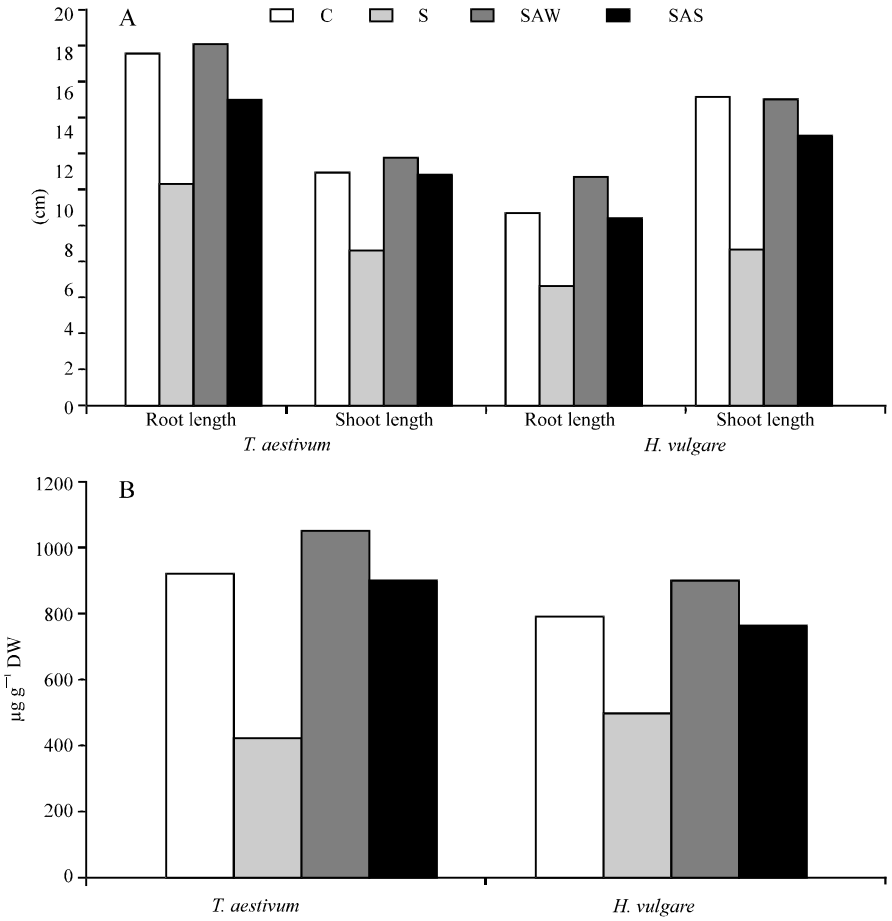

Fig. 1: Effect of recovery on root and shoot development (A) and total chlorophyll content (B) in *T. aestivum* and *H. vulgare* (watered daily with a half nutrient solution for 6 days)

species through both enzymatic and non enzymatic reactions [6]. To sum up, the present work demonstrates clearly that adaptation to high NaCl (salt tolerance) involves an increase in betaine and antioxidant (glutathione). In contrast salt stress produces increase in proline content [6].

Once the mechanism of SA action is better understood, new opportunities for agricultural biotechnology may become evident. Alongside unraveling the SA mode of action, other aspects such as uptake, transport and stability of SA as well as the development of SA analogues with high activity, should continue to be explored. It is only with this combined knowledge that unique mechanisms of stress resistance can lead to implementations, with predictable effects of SA application in the field, allowing for the full potential of SA to be harnessed in the future.

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