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Salicylic-acid–induced recovery ability in salt-stressed *Hordeum vulgare* plants

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Abstract: The recovery ability of barley plants from salt stress conditions was assessed using foliar application of salicylic acid (SA) in a study conducted under controlled conditions. The barley plants (*Hordeum vulgare* L. 'Reyhan') were subjected to saline water with varying salinity levels (tap water 0.67 dS m^{-1} as control, 3, 6, 9, and 12 dS m^{-1}) from 14 to 42 days after sowing (DAS). Then the plants were subjected to recovery treatments for 4 weeks, from 42 to 70 DAS. The recovery treatments included: non-recovery (R_0), irrigation with tap water (R_1), and irrigation with tap water + 2 foliar applications of SA with a 1-week interval (R_2). The results showed that salt stress decreased shoot and root dry weight, leaf K^+ concentration, and photosynthesis rate, while it increased leaf Na^+ concentration and free proline, soluble protein, and chlorophyll contents. These reductions were related directly to stress intensity. Both recovery treatments increased shoot dry weight, Na^+ concentration, free proline, chlorophyll content, and photosynthetic rate. Compensation of root dry weight losses due to salt stress was observed only in R_1. However, for other measured traits recovery ability with R_2 was greater than with R_1. Overall, it appeared that although recovery treatments could not fully eliminate salt-induced damages, the recovery treatment with SA proved to be very effective in alleviating the adverse effects of salt stress on barley plants.

Key words: Recovery ability, sodium, potassium, free proline, soluble protein

1. Introduction

Crop production in arid and semi-arid regions is restricted by soil salinity and soil moisture deficiencies. Salinity in soil or irrigation water is the major limiting factor for crop growth in many regions of the world (Siddiqui et al., 2006; Ashraf et al., 2008; Kausar et al., 2013). Salt stress at any stage of crop growth can cause an irreversible loss in yield potential (Munns, 2002; Hameed et al., 2013) in many crops including barley. However, seed germination and seedling establishment are the periods when barley is most sensitive to salinity (Emam, 2011). Rapid and uniform field emergence is vital for achieving maximum yield and quality of annual crops (Siddiqui et al., 2006; Pirasteh-Anosheh et al., 2011) such as barley.

Under salinity conditions, exogenous application of plant growth regulators (PGRs) may overcome much of the internal PGR deficiency and mitigate salinity-induced inhibitory effects (Ashraf et al., 2008). Like other known plant growth regulators, salicylic acid (SA) is thought to play a major role in defence mechanisms against salinity stress (Deef, 2007; Mutlu et al., 2013). For example, foliar application of SA modulates activities of key intracellular antioxidant enzymes (superoxide dismutase and peroxidise) and consequently increases plant tolerance to environmental stresses (Pirasteh-Anousheh et al., 2012). In addition, SA foliar application alleviated the adverse effects of stresses, which were mainly ascribed to the enhanced synthesis/accumulation of free proline and soluble proteins. Proline is one of the most common osmolytes accumulating in crops in response to a variety of environmental stresses, such as salinity (Bates et al., 1973; Ashraf and Foolad, 2007; Nikolaeva et al., 2010). SA application is thought to be actively involved in enhanced synthesis of soluble proteins thereby improving plant adaptation to stresses (Pareek et al., 1997; Mutlu et al., 2013). Thus, increased SA-induced protein accumulation in salt-stressed plants may be attributed to increased tolerance to salinity stress. Previous studies have demonstrated that SA plays an important role in determining the tolerance of crops to salt stress (El-Tayeb, 2005; Hussein et al., 2007; Ashraf et al., 2010), especially at the seedling stage (Deef, 2007). Many researchers have shown that adverse effects of salt stress on plants could be alleviated by exogenous application of SA (Hussein et al., 2007; Noreen and Ashraf, 2008; Ashraf et al., 2010; Pirasteh-Anosheh and Emam, 2012; Pirasteh-Anosheh et al., 2012).

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Although application of SA to various plants grown on saline soil has been examined frequently, there is little information on the recovery of plants from salinity damage using SA. Such studies might be important for barley, as it is the fourth most important crop in the world, and its growth and yield are adversely affected by salinity stress. Recovery ability is the ability of a plant to regain normal status (fully or partially) after being exposed to stress conditions. This ability could be additive or subtractive based upon the nature of the trait; for example, enhancement in K^+ concentrations or reduction in Na^+ concentrations in plants after relief from salinity stress. A plant with greater recovery ability could produce a greater yield. Thus, the key objective of the present research was to examine whether SA could improve the recovery ability of barley plants grown under salinity conditions. In this study we evaluated the effect of recovery with SA on barley growth and some key physiobiochemical traits under different salinity regimes.

2. Materials and methods

This study was carried out as a factorial experiment based on completely randomised design (CRD) with 6 replicates at the research greenhouse of Shiraz University, Iran, in 2012. Treatments comprised 5 salt stress levels and 3 recovery types (5 × 3). Saline water treatments were: tap water (0.67 dS m^{-1}, as control), 3 (3ST), 6 (6ST), 9 (9ST), and 12 (12ST) dS m^{-1}, and recovery treatments were non-recovery, recovery with water, and with 2 foliar applications of salicylic acid.

The pots (5 L) were filled with Daneshkadeh series soil (soil classification: fine, mixed, mesic, Cacixerollic Xerochrepts) at a 2:1 ratio of soil:sand. The physicochemical properties of the soil used for experimentation are given in Table 1. The viable seeds of the barley 'Reyhan', which is widely grown in this region, were sown in April 2012 at a depth of 3–4 cm in pots. Fifteen seeds were sown in each pot, and the seedlings were thinned to 8 soon after emergence, which occurred 4 days after sowing (DAS). Each pot was considered an experimental unit. Minimum and maximum temperatures in the greenhouse were 14 and 28 °C, respectively, and relative humidity 55%–60%. The plants were exposed to 14 h illumination every day.

Salinity treatments (ST) were applied at 14 DAS using a 2:1 weight ratio of NaCl: $CaCl_2$ solutions and controlled by a portable EC-meter. The salinity levels in each pot were developed by the application of saline water at 2 subsequent irrigations. The pots were irrigated to attain field capacity (FC) every week. The plants, except in control treatments, experienced salinity stress from 14 to 42 DAS (salt period); thereafter, the plants were subjected to recovery treatments (freed from salt treatment) for 4 weeks (recovery period). The recovery period lasted from 42 to 70 DAS. The recovery treatments included: non-recovery, irrigation with tap water, and irrigation with tap water + 2 foliar applications of SA solution with a 1-week interval. SA foliar application was done using 1 mM concentration at 42 and 49 DAS based on 300 L ha^{-1}.

Samplings were done at 3 stages: 14 DAS (early emergence, before the application of salt), 28 and 42 DAS (end of salt period), and 56 and 70 DAS (end of recovery period). The parameters measured in this study were shoot dry weight (SDW), root dry weight (RDW), leaf sodium (Na^+), leaf potassium (K^+), leaf free proline (FP) content, leaf total soluble protein (TSP), chlorophyll content index (CCI), and photosynthetic rate (Pn). For SDW and RDW determination, 3 plants were randomly selected from each pot, and their average dry weight was considered the mean for that treatment. The plants were separated into roots and shoots for the determination of their dry weight, which was measured after oven-drying the samples at 70 °C for 48 h. For determining FP and TSP, leaves of 3 randomly selected plants from each pot were individually harvested at each stage, immediately frozen in liquid nitrogen, freeze-dried, and stored at −80 °C for future extraction. The frozen leaves were ground to fine powder in liquid nitrogen using a pestle and mortar. The FP was determined using a spectrophotometer (Biochrom Ltd., Biowave S2100, Cambridge, UK) according to the acid-ninhydrin method (Bates et al., 1973). TSP was measured following Bradford (1976) using bovine serum albumin (BSA) as a standard. Na^+ and K^+ concentrations in plant tissues were measured using a flame photometer. CCI and Pn were measured using a SPAD chlorophyll-meter (Opti-Sciences X, USA) and portable photosynthesis system (IGRA model LCA4-ADC, Hoddeson, UK), respectively. The SPAD chlorophyll-meter is a hand-held spectrometer that measures light (650 nm) absorbed by single leaves and gives a non-destructive estimate of plant chlorophyll. The IGRA operates in a closed system mode; the leaves are enclosed in a chamber with no exchange of air with the outside. Photosynthetic rate is calculated from the rates of

Table 1. Some physicochemical properties of the experimental soil.

EC (dS m^{-1})	pH	OM (%)	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	Texture
0.60	7.09	1.124	0.15	12.0	720	Silty loam

change in CO_2 concentrations. The following formula was devised to quantify the recovery ability (RA):

$$RA = \left| \frac{R-S}{C-S} \right|,$$

where *R*, *C*, and *S* are values for the measured characters under recovery, control (without stress), and stress conditions, respectively. RA can be greater than and equal to zero; if RA > 0 it means that the recovery is partially achieved, if RA = 1 it means that recovery is fully achieved. If RA > 1, the stressed plant performed better than the control due to specific treatments during recovery.

The data for each variable were subjected to analysis of variance (ANOVA) SAS v. 9.1 software. Significant differences between treatment means were determined using the least significant difference (LSD) test at $P \leq 0.05$.

3. Results

Shoot dry weight was significantly affected by salinity, recovery, and their interaction (Table 2). Shoot dry weight at non-saline and 3ST was higher in the non-recovery treatment. Shoot dry weight measured 4 and 6 weeks after planting showed no significant differences between non-saline control and salinity treatment (3ST). However, significant differences between these 2 treatments were observed with time. The increase in SDW from 14 DAS up to 70 DAS at 6 and 9ST was negligible, and no change was observed in SDW at the highest salinity (12ST) with time (Figure 1a). The recovery treatments enhanced SDW measured at 42 DAS at all salinity treatments, and such enhancement was markedly low at higher saline conditions (Figure 1b). The recovery due to the application of SA was associated with higher dry weight compared to dry weight by water recovery (Table 3). The second (6ST) and third (9ST) salinity treatments did not have significant differences at the end of the experiment in terms of SDW. Shoot dry weight at 12ST increased significantly (20%) at recovery with SA in comparison to water (Figure 1c; Table 3).

The effects of salinity ($P \leq 0.01$), recovery ($P \leq 0.05$), and their interaction ($P \leq 0.01$) on RDW were significant (Table 2). Under non-recovery conditions, RDW showed a clear increasing trend in 3ST (68.2%) as well as control (non-saline) treatments (51.2%), and in 6, 9, and 12ST no clear-cut increase in RDW was observed (Figure 2a). Root dry weights at 6, 9, and 12ST were significantly lower than those at control and 3ST (Figure 2a). Until 42 DAS, the trend in recovery with water was similar to that in the plants receiving no recovery treatments (Figures 2a and b). However, after 42 DAS, the recovery treatments applied to plants at 6, 9, and 12ST mitigated to some extent salinity-induced reductions (14.6%, 13.3%, and 14.05%, respectively) in RDW (Figure 2b). SA-induced recovery from salt-induced reduction in RDW was significantly lower than in fresh water recovery (Figure 2c).

Leaf Na^+ concentration was significantly affected by salinity ($P \leq 0.01$), recovery ($P \leq 0.05$), and the interaction between them ($P \leq 0.01$) (Table 2). Under non-recovery treatments, leaf Na^+ concentrations in barley plants grown in non-saline treatments did not alter throughout the experiment (Figure 3a), but they showed the additive trend with an increase in external salt level. The recovery treatment with fresh water reduced leaf Na^+ concentrations in barley plants at 3 and 6ST (11.9% and 15.7%, respectively); however, in 9 and 12ST, the leaf Na^+ concentrations showed a steady state situation from 42 to 70 DAS (Figure 3b). SA-treated plants had lower leaf Na^+ concentrations than those under non-recovery and

Table 2. Variance analysis of the effects of salt stress and recovery on some barley characteristics.

		Mean squares							
SOV	df	SDW (g)	RDW (g)	Na^+ (mg/g)	K^+ (mg/g)	FP (µmol/g)	TSP (mg/g)	CCI (SPAD)	Pn (µmol CO_2 m^{-2} s^{-1})
Salt (S)	5	37.981**	112.382**	0.982**	13.342**	2.302**	1.231**	7.135**	32.870**
Recovery (R)	2	14.033*	98.871*	0.892*	0.231ns	0.998**	0.023ns	9.028**	26.381**
S × R	10	15.342*	128.091**	1.002**	0.023ns	0.001ns	0.002ns	0.034ns	2.103*
CV		12.38	11.98	7.87	8.76	5.65	9.82	14.65	13.23

SDW: shoot dry weight; RDW: root dry weight; Na^+: sodium concentration; K^+: potassium concentration; FP: free proline content; TSP: total soluble proteins; CCI: chlorophyll content index; Pn: photosynthetic rate; SOV: source of variation; df: degree of freedom; CV: coefficient of variance. ns: non-significant, * and ** significant at 5% and 1% probability level, respectively.

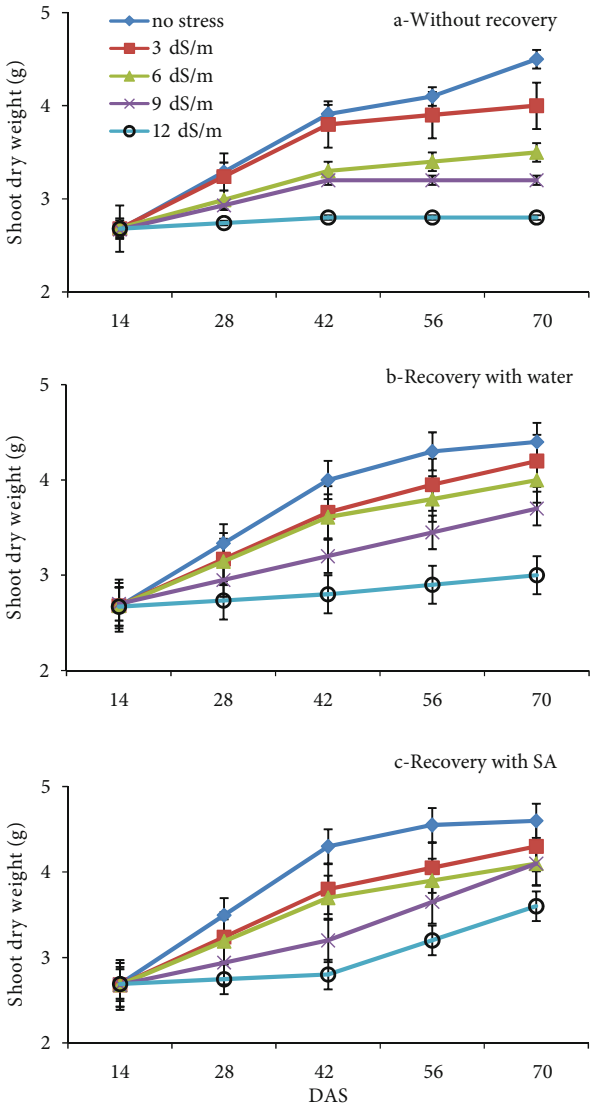

Figure 1. Shoot dry weight (±SE) recovery under different salinity levels using water and salicylic acid (SA).

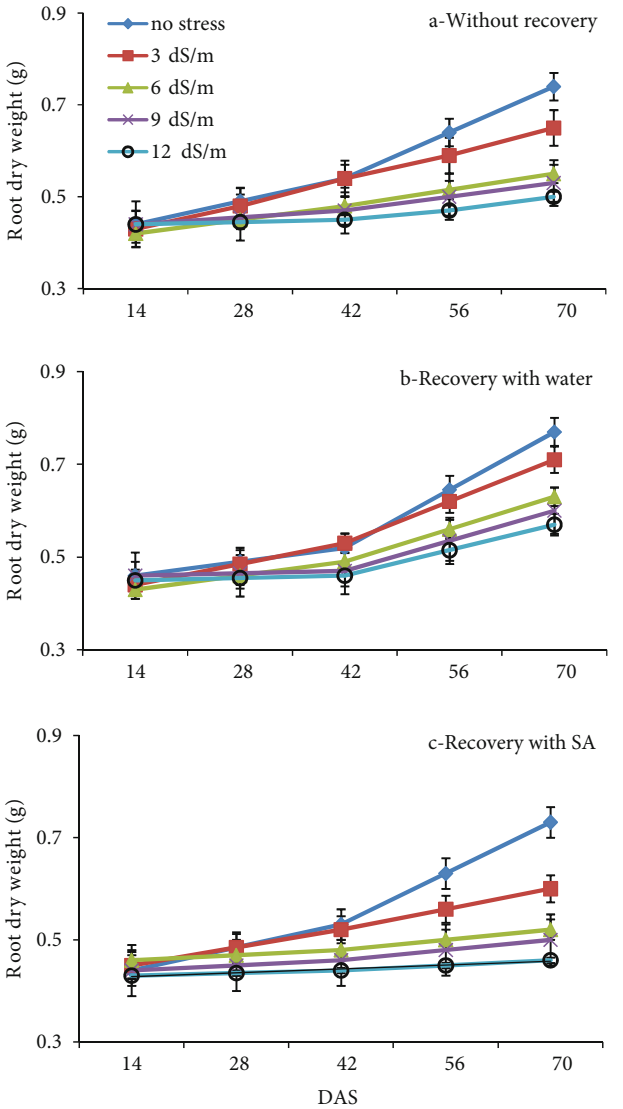

Figure 2. Root dry weight (±SE) recovery under different salinity levels using water and salicylic acid (SA).

Table 3. Comparison of the recovery abilities of salicylic acid (SA) and water in terms of some barley traits under different salinity regimes.

Salinity (dS m^{-1})	SDW		Na^+		Pn	
	Water	SA	Water	SA	Water	SA
3	0.40c	0.60ab	0.83b	1.19a	0.56ab	0.60a
6	0.50b	0.60ab	0.56bc	0.64c	0.24d	0.56ab
9	0.38c	0.69a	0.10e	0.44c	0.13e	0.54b
12	0.12d	0.47bc	0.14e	0.36d	0.10e	0.42c
Average	0.35B	0.59A	0.41B	0.66A	0.26B	0.53A

Means with same letters within each variable do not differ significantly at $P < 0.01$.
SDW: shoot dry weight; Na^+: sodium concentration; Pn: photosynthetic rate.

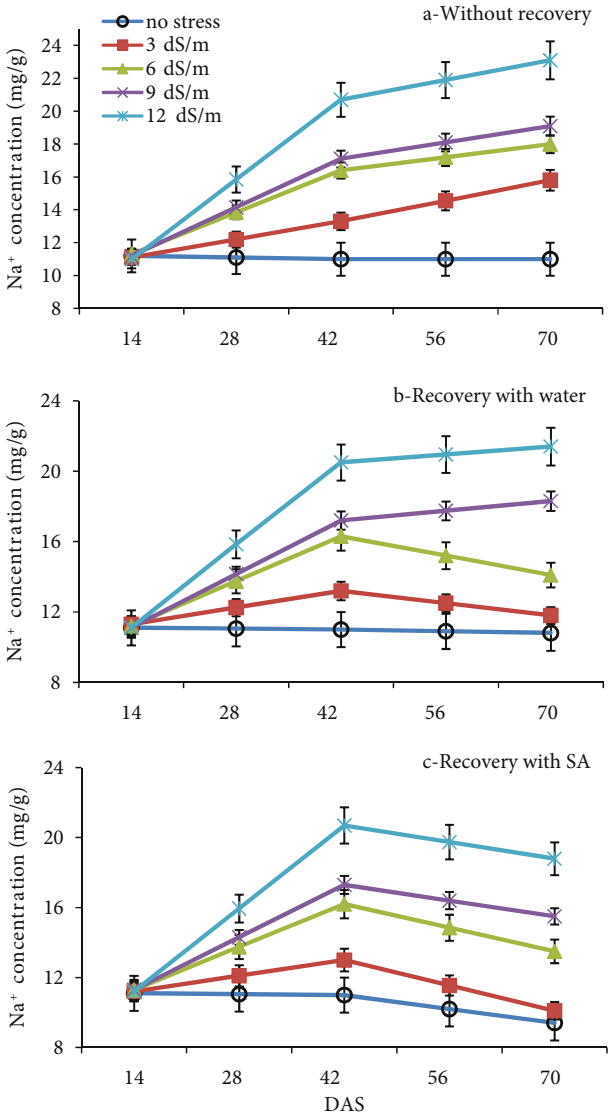

Figure 3. Na^+ concentration (±SE) recovery under different salinity levels using water and salicylic acid (SA).

recovery treatment with fresh water (Tables 3). The SA application reduced leaf Na^+ concentrations in all plants experiencing varying salinity treatments; however, the rate of reduction in Na^+ concentration was related to the salinity level of the growth medium so that in 3ST the leaf Na^+ concentration decreased (18.1%) so drastically that it was not different than in plants experiencing no salinity treatment (i.e., control) (Figure 3c).

Leaf K^+ concentrations did not differ significantly under salinity treatments until 14 DAS. After 14 DAS, leaf K^+ concentrations increased significantly in plants treated with varying salinity treatments (Table 4). Increases in K^+ concentrations at 42 DAS compared to 14 DAS were 30.7, 23.7, 22.1, 13.5, and 7.4% in control and 3, 6, 9, and 12ST, respectively. At 70 DAS, K^+ concentrations increased in control (12.9%) and 3ST (2.5%); however, it was significant only in the control treatment. Among other salinity levels, K^+ concentrations increased significantly so that there were 5.5, 17.2, and 34.4% reductions in K^+ concentrations under 6, 9, and 12ST, respectively (Table 4).

The effect of salinity ($P \leq 0.01$) and recovery ($P \leq 0.01$) on leaf free proline content was significant; however, their interaction was non-significant (Table 2). Until 42 DAS, proline content increased significantly in non-recovery and recovery treatments with water or SA. There were no significant differences in proline content among the recovery treatments in 14- and 42-day-old plants (Table 4). In non-recovery treatment, plants at 70 DAS showed no significant change in free proline, while the recovery treatment with water or SA caused a significant increase in proline content in plants at 70 DAS compared to those at 42 DAS (Table 4). The increases in proline content due to recovery treatment with water or SA were 9.3 and 24.6%, respectively. At 14 DAS there was no significant difference among the effects of salinity treatments in terms of free proline content. However, in plants at 42 DAS, proline content increased in all salinity treatments. Such increases in proline content were closely associated with the stress severity (Table 4).

Total soluble proteins (TSP) were significantly influenced by salinity (Table 2). There was no significant difference in TSP in 14-day-old plants subjected to varying salinity regimes. At 42 DAS, TSP increased in all salinity treatments, especially 6, 9, and 12ST. The highest and lowest TSP was found in 12ST (149.84 mg/g DM) and control (124.00 mg/g DM), respectively. At 70 DAS, the highest and lowest TSP were also observed at 12ST (167.44 mg/g DM) and control (136.20 mg/g DM), respectively. No significant difference was found between TSP at control and 3ST throughout the experiment (Table 4).

The effect of salinity ($P \leq 0.01$) and recovery ($P \leq 0.01$) on CCI was significant (Table 2). There was no significant difference between the recovery and non-recovery treatments until 42 DAS. However, CCI differed significantly in plants grown under varying saline regimes at 70 DAS. The highest CCI (15.1) and lowest (13.0) CCI at this time were found in recovery treatment with SA and non-recovery treatment, respectively (Table 4). There was no significant difference among the salinity treatments in terms of CCI until 14 DAS. From 14 to 42 DAS, CCI increased in all salinity treatments. Nevertheless, this increase was greatest at the highest salinity regime. The highest CCI (14.5) and lowest (10.2) CCI were found in 12ST and control, respectively (Table 4).

Photosynthetic rate was significantly affected by salinity stress ($P \leq 0.01$), recovery ($P \leq 0.01$), and their interaction ($P \leq 0.05$) (Table 2). In non-recovery treatment, Pn increased in plants growing under control

Table 4. Effect of recovery and salinity treatments on soluble proteins, chlorophyll content, K^+ concentration, and free proline content in barley plants.

Treatments		TSP (mg/g DW)			CCI (SPAD)			K^+ (mg/g DW)			FP (µmol/g DW)		
		14DAS	42DAS	70DAS	14DAS	42DAS	70DAS	14DAS	42DAS	70DAS	14DAS	42DAS	70DAS
Recovery	Non-recovery	122.20^{a}	138.56^{a}	150.58^{a}	9.3^{a}	12.2^{a}	13.0^{c}	25.0^{a}	30.3^{a}	28.6^{a}	1.19^{a}	3.10^{a}	3.14^{bc}
	Water	122.92^{a}	138.30^{a}	150.48^{a}	9.2^{a}	12.2^{a}	14.6^{b}	25.3^{a}	29.7^{a}	28.4^{a}	1.20^{a}	3.07^{a}	3.28^{b}
	Salicylic acid	120.24^{a}	139.40^{a}	150.94^{a}	9.3^{a}	12.3^{a}	15.1^{a}	25.3^{a}	30.4^{a}	28.8^{a}	1.20^{a}	3.07^{a}	3.74^{a}
Salinity (dS m^{-2})	Non-stress	120.00^{a}	124.00^{c}	136.20^{c}	9.3^{a}	10.2^{de}	11.1^{e}	25.4^{a}	33.2^{a}	37.5^{a}	1.23^{a}	1.40^{c}	1.40^{d}
	3	122.30^{a}	125.97^{c}	134.94^{c}	9.3^{a}	10.8^{d}	11.9^{d}	25.3^{a}	31.3^{ab}	32.1^{b}	1.21^{a}	1.45^{c}	1.42^{d}
	6	122.77^{a}	144.34^{b}	156.37^{b}	9.2^{a}	11.6^{c}	13.0^{c}	24.8^{a}	30.3^{b}	28.7^{c}	1.19^{a}	3.51^{b}	2.82^{bc}
	9	121.00^{a}	149.14^{a}	158.40^{b}	9.4^{a}	14.0^{ab}	16.2^{b}	25.1^{a}	28.5^{c}	24.3^{d}	1.19^{a}	3.82^{b}	3.27^{b}
	12	122.87^{a}	149.84^{a}	167.44^{a}	9.1^{a}	14.5^{a}	19.4^{a}	25.4^{a}	27.3^{c}	20.3^{e}	1.17^{a}	5.23^{a}	4.58^{a}

Means with same letters in each column within each variable do not differ significantly at $P < 0.01$.
TSP: total soluble protein; CCI: chlorophyll content index; K^+: potassium concentration; FP: free proline content; DAS: days after sowing.

and 3ST (47.8 and 24.2%, respectively) throughout the experiment, i.e., from 14 to 70 DAS. Pn in plants at 6ST was unchanged, while it decreased significantly at 9 and 12ST (7.2 and 16.0%, respectively) (Figure 4a). The recovery treatment with water substantially alleviated reductions in Pn occurring at 3ST. In plants at 70 DAS, the recovery with fresh water also increased Pn significantly at 3 and 6ST. These recovery abilities were significantly associated with stress severity. Pn increased significantly in plants growing under 9 and 12 dS/m in the recovery treatment with water (Figures 4a and b). Although Pn increased in plants receiving SA as a recovery treatment, the increasing effect of SA was not as alarming as in the water treatment (Figure 4c and Table 3).

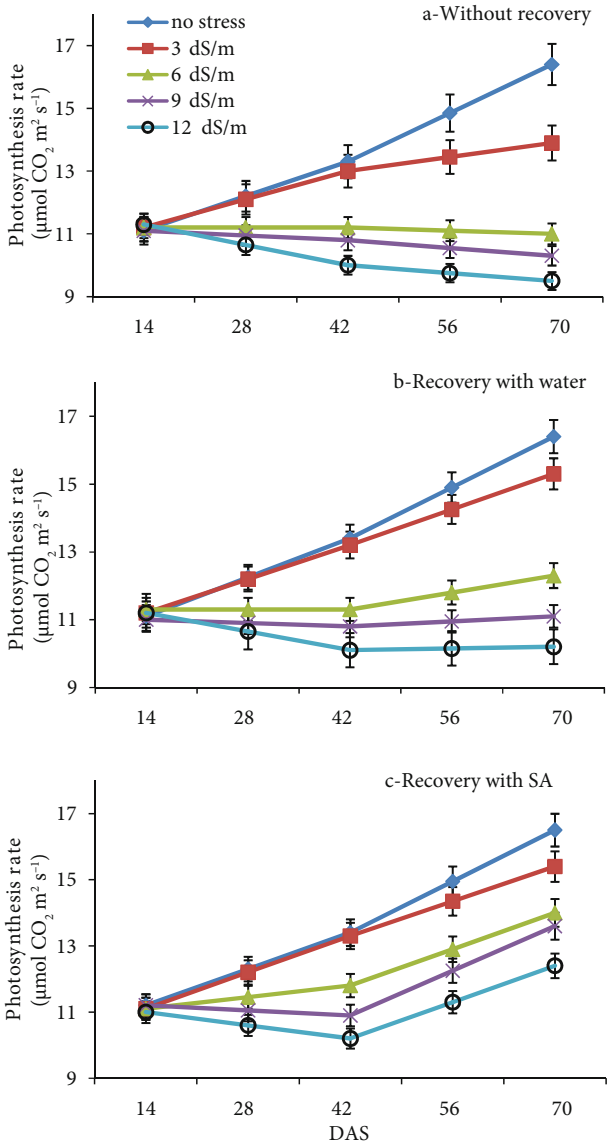

Figure 4. Photosynthesis rate (±SE) recovery under different salinity levels using water and salicylic acid (SA).

4. Discussion

From the results it is evident that recovery treatment with SA or water applied to barley plants experiencing varying levels of salinity caused a positive effect in terms of shoot biomass production. However, the recovery ability of SA was greater than that of water (Table 3). This suggests that SA, as a growth regulator, has an effective role in protecting plants against abiotic stresses (Hussein at al., 2007; Mutlu et al., 2013). For example, SA application has been reported to increase plant tolerance to stress conditions by offsetting dry weight reduction in different crops such as wheat (Shakirova et al., 2003; Noreen and Ashraf, 2008; Pirasteh-Anosheh and Emam, 2012), barley (El-Tayeb, 2005), and maize (Khodary, 2004). This might be due to the stimulatory effect of SA on shoot growth and allocation of more assimilates to the shoot (Noreen and Ashraf, 2008; Ashraf et al., 2010). However, in the present study SA application improved SDW during the recovery period, the period in which plants still experienced the adverse effects of salt stress on growth and metabolic processes. Carbohydrate accumulation during salt stress might have a positive role during the recovery period through carbohydrate remobilisation within the plant body (DeLacerda et al., 2005).

Application of water as a recovery treatment reduced Na^+ concentration in barley plants at lower salinity levels, while at higher salinity levels the Na^+ concentration showed a steady state situation. SA-treated, salt-stressed plants had higher ability to recover and reduce the salinity effects in terms of reducing tissue Na^+ concentrations (Table 3). Lower plant Na^+ concentration has been associated with salt tolerance in barley (Gorham et al., 1994), wheat (Kausar et al., 2013), and rice (Pareek et al., 1997). Salt tolerance in crop plants is generally associated with low uptake and accumulation of Na^+ (Ashraf and Harris, 2004; Ashraf et al., 2010; Hameed et al., 2013), a cation that has been shown to have adverse effects on crops due to its toxic effects (Ouerghi et al., 2000). Therefore, Na^+ concentration in plant tissues could be used as an important indicator for salinity tolerance (Volkmar et al., 1997; Tadayon and Emam, 2007). Foliar-applied SA reduced Na^+ concentrations in barley leaf tissue and, hence, improved salinity tolerance. Recovery from the negative effects of salinity on Na^+ concentrations were reported in barley (Ahmad and Wyn Jones, 1979), sorghum (DeLacerda et al., 2005), and other crops (Alarcon et al., 1993; Pardossi et al., 1998). This might be due to a negative balance of leaf xylem import and phloem export process after the alteration of leaf soluble salt during recovery (DeLacerda et al., 2005). It seems that a considerable amount of ions absorbed during stress may have been transferred to young leaves after recovery (Alarcon et al., 1993).

Although K^+ concentrations increased in plants subjected to all salinity treatments from 14 to 42 DAS, an increase in K^+ concentrations was observed in plants growing in control and 3ST only from 42 to 70 DAS. Salinity stress is known to alter the ion equilibrium in plant tissues (Tadayon and Emam, 2007; Kausar et al., 2013) and resultantly, some important ions could be effectively used as important selection criteria for salt tolerance. For example, K^+ concentration is thought to be an index of salinity tolerance in most crop species (Ashraf et al., 2008). Pakniyat et al. (2003) also noted that higher K^+ concentrations were associated with salt tolerance in barley.

Leaf free proline content increased in plants subjected to all 3 recovery treatments throughout the experiment, and at the end of the experiment the highest and lowest FP was obtained in non-recovery and recovery treatment with SA, respectively. The amino acid proline is known to occur widely in higher plants, and it usually accumulates in large quantities in response to environmental stresses (Ashraf and Foolad, 2007; Szabados and Savoure, 2009; Kausar et al., 2013). SA-induced increases in proline content under saline and drought stresses was shown by some other studies in barley (El-Tayeb, 2005; Bandurska and Stroinski, 2005), wheat (Pirasteh-Anosheh and Emam, 2012; Nayyar, 2003; Shakirova et al., 2003; Singh and Usha, 2003), and corn (Nayyar, 2003). Proline content is thought to be generally higher in salt tolerant genotypes/cultivars than in salt sensitive ones (Ashraf and Foolad, 2007; Pirasteh-Anosheh et al., 2011). Furthermore, a significant positive correlation has been reported between enhanced concentrations of intracellular proline and the ability of plants to survive under high salinity conditions (Ashraf and Foolad, 2007). Proline has also been shown to act as a molecular chaperone involved in protection of protein integrity and enhancement of the activities of different key enzymes (Szabados and Savoure, 2009). In the present study, although proline content increased in all plants subjected to different saline regimes, the recovery treatment with SA proved to be very effective in further increasing the proline levels in barley plants. It is possible that when the stress is over, a rapid breakdown of proline may make available a reasonable amount of reducing agents that can effectively up-regulate oxidative phosphorylation and, hence, synthesise ATP in mitochondria. The ATPs so generated can play a vital role in fast recovery of plants from salinity (Ashraf and Foolad, 2007). Singh and Gautam (2013) reported that SA-induced enhanced synthesis of proline improves plant tolerance against salinity stress. The enhanced proline content in SA-treated plants might be due to a reduction in dissolved proteins (El-Tayeb, 2005). Furthermore, enhanced proline accumulation in the presence of SA under salinity conditions implies the involvement of proline in the process of osmotic adjustment (Singh and Gautam, 2013). Proline accumulation occurs normally in the cytosol, where it contributes significantly to the cytoplasmic osmotic adjustment (Ashraf and Foolad, 2007).

Increase in TSP was also observed in plants subjected to all salinity treatments throughout the experiment. The increases in TSP in 12ST over control at 42 DAS and 70 DAS were 17.24% and 22.9%, respectively. Increase in protein content under stress conditions has been reported in barley (Tadayon and Emam, 2007), maize (Pirasteh-Anosheh et al., 2011), and wheat (Ranjbar et al., 2010). Protein synthesis could be a plant response to salinity (Volkmar et al., 1997; Ashraf and Harris, 2004). Increased total protein content in barley plants under salt stress conditions in the present study might be considered a plant mechanism for tolerance to saline stress. Proteins that accumulate in plants grown under saline conditions may provide a storage form of nitrogen that is reutilised during the recovery period (Ashraf and Harris, 2004). Thus, this trait could be considered a tolerance index in crop plants. In results similar to ours, higher TSP due to salt stress has been associated with salinity tolerance in crops such as barley (Tadayon and Emam, 2007), sunflower (Ashraf and Tufail, 1995), maize (Pirasteh-Anosheh et al., 2011), and rice (Pareek et al., 1997). In wheat, Ashraf and O'Leary (1999) reported that increased TSP due to salt stress was evident in all cultivars; however, this increase was higher in salt tolerant cultivars.

Chlorophyll content index in salt-stressed plants supplied with recovery treatment with water or SA was greater than CCI under non-recovery conditions. Increase in CCI has been reported with SA application in crops such as maize (Khodary, 2004), wheat (Nikolaeva et al., 2010; Pirasteh-Anosheh and Emam, 2012), and pea (Parida et al., 2008).

The recovery treatment with water or SA was found to be beneficial in alleviating the inhibitory effects of salt stress on Pn, although the effect of SA was more pronounced (Table 3). SA is known as an important plant growth regulator that can control stomatal closure, chlorophyll content (Khan et al., 2003; El-Tayeb, 2005; Hussein et al., 2007), Pn, and other physiological processes such as glycolysis, transpiration, uptake and transport of nutrients, membrane permeability, flowering and thermogenesis, and growth rate (Ashraf et al., 2010).

5. Conclusions

The difference in recovery ability with SA and water was low in plants that experienced lower salinity levels, and it was high in those that experienced higher salinity levels. Overall, the recovery with both SA and water significantly compensated for losses in barley plants due to salinity in

terms of SDW, leaf Na^+ concentration, free proline content, CCI, and Pn. On the other hand, recovery with fresh water had a positive effect only on RDW. SA was more effective than water for recovering barley plants, particularly from higher saline conditions. Further studies on recovery ability of SA in terms of other biochemical characteristics as well as grain yield and its components are necessary. In addition, identification of any possible relationship between cultivar recovery ability and final performance of the crop needs to be explored.

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