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Effect of Salicylic Acid on Net Photosynthetic Rate, Chlorophyll Fluorescence, and Antioxidant Enzymes in *Vigna radiata* Plants Exposed to Temperature and Salinity Stresses

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ABSTRACT

Ten-days-old seedlings of mung bean (*Vigna radiata* cv. 'T-44') were exposed to salicylic acid (SA) and/or temperature and/or NaCl stresses. The treated seedlings were sampled at 18 days after sowing (DAS) to assess the change in growth pattern, photosynthetic attributes, quantum yield of PSII (Fv/Fm), activity of antioxidative enzymes i.e. peroxidase (POX), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and the activity of nitrate reductase and carbonic anhydrase. The plants exposed to temperature and/or saline stress exhibited a reduction in growth, photosynthesis, and the activity of nitrate reductase and carbonic anhydrase. However, treatment with SA both in the presence or absence of stresses significantly improved the values for the above mentioned parameters. Moreover, the activities of antioxidative enzymes and proline content increased in response to both SA and the stress(s). The interaction of salinity and temperature stress with SA treatment had an additive effect enhancing significantly the values by 107.3% (POX), 37.2% (CAT), 37.5% (SOD), 55.3% (GR) and 65.3% (proline content) over control. SA treatment significantly affected the membrane stability index (MSI) under stressed as well as unstressed conditions resulting in a significant enhancement, 27% more than the MSI of plants exposed to a combined temperature and salinity stress. It may therefore be concluded that SA plays an important role in signaling pathways of plants leading to resistance against temperature and/or salinity stresses.

Keywords: antioxidative enzymes, photosynthesis, quantum yield, salicylic acid, salinity, temperature, *Vigna radiata*

INTRODUCTION

Environmental stresses are the main factors that limit agricultural productivity worldwide. Stresses associated with temperature, salinity and drought, singly or in various combinations, are likely to further enhance the severity of problems to which plants will be exposed in the future. 30 years ago, approximately 20% of the world's cultivated land area and 50% of all irrigated lands was affected by salinity (Rhoades and Loveday 1990). Still, according to the FAO Land and Nutrition Management Service (2008), over 6% of the world's land is affected by either salinity or sodicity which accounts for more than 800 million ha of land. Plants are affected in several ways by increasing salt concentration. It causes osmotic stress, specific ion toxicity and nutrient deficiency thereby affecting a range of physiological process involved in cell metabolism (Munns 2002). An excessive amount of salt causes enzyme inhibition such as carbonic anhydrase (Ali *et al*. 2008), Rubisco, phosphenolpyruvate carboxylase (Soussi *et al*. 1998), antioxidant enzymes (Khan *et al*. 2009) and metabolic dysfunctions (Booth and Beardall 1991) such as degradation of photosynthetic pigments (Soussi *et al*. 1998), lipid peroxidation (Ashraf *et al*. 2010). Photosynthesis (Zobayed *et al*. 2005), protein synthesis and energy and lipid metabolism are the other major factors affected by soil salinity (Geissler *et al*. 2009).

Temperature stress is another major limiting factor in normal growth and productivity of plants. One of the common consequences of these two stress factors are the overproduction of reactive oxygen species (ROS), such as superoxide radical (O_2^-), hydroxyl radical (OH), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) that are extremely toxic to plants and can cause damage to nucleic acids, proteins, lipids and ultimately upsetting homeostasis (Mittler 2002; Schutzendubel and Polle 2002). However, plants have evolved specific protective mechanisms to mitigate and repair the damage initiated by ROS. The major ROS-scavenging mechanisms include enzymatic system (superoxide dismutase, catalase, peroxidase, glutathione reductase, ascorbate peroxidase) and non-enzymatic system (proline, tocopherols, glutathione, ascorbic acid etc.) to counter the oxidative stress and protect the plants from oxidative stress (Apel and Hirt 2004; Shevyakova *et al*. 2009) generated by salinity (Ali *et al*. 2007) and temperature stress (Almeselmani *et al*. 2006, 2009).

Salicylic acid (SA) is a common phenolic compound synthesized by plants and regarded as a plant hormone (Hayat and Ahmad 2007). It plays diverse physiological roles in plants that include inhibition of dry mass accumulation (Schettel and Balke 1983), stomatal movement (Hayat and Ahmad 2007), ion uptake and transport (Harper and Balke 1981), membrane permeability (Barkosky and Einhellig 1993), photosynthesis (Fariduddin *et al*. 2003; Wang *et al*. 2010) and enzyme activities (Hayat and Ahmad 2007; Palma *et al*. 2009). Moreover, SA provides protection against a number of abiotic stresses such as heat stress in mustard seedling (Dat *et al*. 1998) drought stress in tomato (Hayat *et al*. 2008) saline stress in *Brassica juncea* (Yusuf *et al*. 2008), and heavy metal stress in barley seedlings (Metwally *et al*. 2003). The role of SA under changing environments has been recently reviewed (Hayat *et al*. 2010).

Studies are available exploring the individual effects of temperature or salinity stress; however, very little is known about the interactive effects of temperature and salinity stress, since they are interrelated (as temperature stress results in salt accumulation due to excessive evaporation) and the roles played by exogenous SA on salinity- and/or temperature-induced changes in plants are not studied yet. Therefore, the present study was carried out to have a better understanding about these two stress factors individually as well as in combination under the influence of SA.

MATERIALS AND METHODS

Plant material

Authentic seeds of mung bean (*Vigna radita* L. Wilczek) cv. 'T-44' were obtained from the National Seed Corp. Ltd. New Delhi. The healthy seeds were surface sterilized with 5% sodium hypochlorite solution followed by repeated washing with deionised water and were sown in plastic pots (~15 cm in diameter), filled with sandy loam soil mixed with farmyard manure in a ratio of 9: 1. The pots were lined in a net house under natural environmental conditions and were irrigated with double distilled water (DDW) on alternate days.

Chemicals and reagents

All the chemicals and reagents used in the experiment were purchased from Merk Ltd. (India).

Treatment pattern and experimental design

On the 10th day after sowing (DAS), seedlings were divided into eight groups, each comprised of 5 replicates. Group I was supplemented with DDW and served as the untreated control for all the other groups. On the basis of our earlier findings Group II plants were supplemented with 10^{-5} M SA (Fariduddin *et al*. 2003). Group III plants were exposed to 40°C in a growth chamber for 24 hr. Group IV plants were supplemented with 100 mM NaCl (Hayat *et al*. 2006, 2007). Group V plants were supplemented with 100 mM NaCl and were also exposed to 40°C for 24 hr. Group VI plants were exposed to 40°C for 24 hr and were supplemented with 50 ml of 10^{-5} M SA. Group VII plants received 100 mM NaCl as well as 10^{-5} M SA. Group VIII plants were exposed to 40°C for 24 hr and were also supplemented with 50 ml of 100 mM NaCl as well as 10^{-5} M SA.

The plants were then allowed to recover for 7 days. The experiment was laid out in a completely randomized design. The plants were sampled 18 DAS to make various observations.

Plant growth analysis

The plants were removed from the pots along with soil and were dipped in a bucket filled with water. The plants were moved smoothly to remove the adhering soil particles and the length of roots and shoots was measured by using a meter scale. The plants were weighed to record the fresh mass. The plants were then placed in an oven at 80°C for 24 h. These dried plants were weighed to record the dry mass. The average leaf area plant^{-1} was measured manually by using a graph sheet, where the squares covered by the leaf were counted to note the leaf area. The three leaves were randomly picked from each plant to calculate average leaf area, which was multiplied with the number of leaves plant^{-1}.

Estimation of membrane stability index (MSI) and electrolyte leakage

Membrane stability index (MSI) was estimated by adding 200 mg leaf material to 10 ml of DDW in two sets. One set was heated at 40°C for 30 min in a water bath and the electrical conductivity bridge C_1 was measured with a conductivity meter (METZER –M, METZER Optical Instrument Pvt. Ltd., Hyderabad). The second set was boiled at 100°C in a water bath for 10 min and the electrical conductivity bridge C_2 was also measured with a conductivity meter. MSI was calculated using the formula (Sairam 1994):

$$MSI = [1 - (C_1/C_2)] \times 100$$

The total inorganic ions leaked out from the leaves were measured as described by Sullivan and Ross (1979). 20 leaf discs were placed in a boiling tube containing 10 ml of deionized water and were heated at 45°C and electrical conductivity was measured as (EC_a). Tubes were heated at 55°C for 30 min in a water bath and electrical conductivity was measured (EC_b). Immediately afterwards the controls were boiled at 100°C for 10 min and the electrical conductivity was again recorded (EC_c). Electrolyte leakage was calculated using the formula:

$$\text{Electrolyte leakage (\%)} = \frac{EC_b - EC_a}{EC_c} \times 100$$

Gas exchange and chlorophyll fluorescence measurement

Gas exchange parameters were determined on fully expanded leaves by using an infrared gas analyzer (IRGA) portable photosynthesis system (LI-COR 6400, LI-COR, Lincoln, NE, USA) on a clear sunny day during 11:00-13:00 to measure the net photosynthetic rate (P_N), internal CO_2 concentration (C_i), stomatal conductance (g_s), water use efficiency (WUE) and transpiration rate (E).

Chlorophyll (Chl) fluorescence parameters were measured with the leaf chamber fluorometer (LI-COR 6400) in the same leaf that was used for gas exchange measurements.

Determination of carboxylation efficiency and relative water content

The formula used by Tiwari *et al*. (1998) was adopted for this purpose where the observed values were added to compute the rate of the carboxylation reaction:

$$\text{Carboxylation efficiency (CE) (mol m}^{-2}\text{s}^{-1}\text{)} = \frac{\text{Net photosynthetic rate}}{\text{Internal } CO_2}$$

The relative water content (RWC) was determined in fresh leaf discs of 2 cm^2 diameter, excluding the midrib. Discs were weighed quickly and immediately floated on DDW in Petri dishes to saturate them with water for the next 24 h in the dark. The adhering water of the discs was blotted and the turgor mass was noted. The dry mass of the discs was recorded after dehydrating them at 70°C for 48 h. RWC was calculated by placing the values into the following formula (Hayat *et al*. 2007):

$$RWC = \frac{\text{Fresh mass - dry mass}}{\text{Turgor mass - dry mass}} \times 100$$

SPAD chlorophyll value and leaf water potential (Ψ)

Chl in the fresh leaf samples was measured by using Minolta SPAD Chl meter (SPAD-502, Konica Minolta Sensing Inc. Japan). Leaf water potential of the fresh leaf sample was measured by the PSYPRO water potential system (WESCOR Inc., Logan, USA).

Estimation of enzyme activity

The activity of nitrate reductase (NR, E.C. 1.6.6.1) was measured following the method adopted by Jaworski (1971). The fresh leaf samples were cut into small pieces and transferred to plastic vials containing phosphate buffer (pH 7.5) followed by the addition of potassium nitrate and isopropanol solutions. The reaction mixture was incubated at 30°C for 2hr followed with the addition of *N*-1-naphthyletylenediamine dihydrochloride and sulphanilamide. The absorbance of the colour was read at 540 nm and was compared with that of the calibration curve. The activity of NR (nmol NO_2 g^{-1} h^{-1}) was computed on a fresh mass basis.

The activity of carbonic anhydrase (CA, E.C. 4.2.1.1) was determined following the procedure described by Dwivedi and Randhawa (1974). The leaf (third leaf from top) from each samples were cut into small pieces and suspended in cystein hydrochloride solution. The samples were incubated at 4°C for 20 min. The pieces were blotted and transferred to test tubes containing phosphate buffer (pH 6.8) followed by the addition of alkaline bicarbonate solution and bromothymol blue indicator. The test tube was incubated at 5°C for 20 min. The reaction mixture was titrated against 0.05 N HCl after the addition of 0.2 ml of methyl red indicator. The results were expressed as mol (CO_2) kg^{-1} leaf fresh mass s^{-1}.

Leaf tissue (0.5 g) was homogenized in 5 ml of 50 mmol phosphate buffer (pH 7.0) containing 1% insoluble polyvinylpyrolidine (lower MW = 40,000) The homogenate was centrifuged at 15,000 rpm for 10 min and the supernatant was used as the

source of enzyme. The extraction was carried out at 4°C.

POX (E.C. 1.11.1.7) and CAT (E.C. 1.11.1.6) activities were assayed following the procedure described by Chance and Maehly (1956). CAT activity was estimated by titrating the reaction mixture consisting of phosphate buffer (pH 6.8) 0.1 M H_2O_2, enzyme extract and 2% H_2SO_4 against potassium permanganate.

The reaction mixture for POX consisted of pyrogallol phosphate buffer (pH 6.8), 1% H_2O_2 and enzyme extract. Change in absorbance, due to catalytic conversion of pyrogallol to perpurogallin, was noted at an interval of 20 s for 2 min at 420 nm. A control set was prepared by using distilled water instead of enzyme extract.

The activity of SOD (E.C. 1.15.1.1) was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) using the method of Beauchamp and Fridovich (1971). The reaction mixture containing 50 mmol phosphate buffer (pH 7.8), 13 mmol methionine, 74 mmol NBT, 2 mmol riboflavin, 0.1 mmol EDTA and 0.5 ml enzyme extract and was placed under a 15 W fluorescent lamp. The reaction was started by switching on the light and was allowed to run for 10 min. The reaction was stopped by switching off the light. 50% inhibition by light was considered as one enzyme unit.

The activity of glutathione reductase (GR) was assayed as per the method of Smith *et al*. (1988). The reaction mixture contained, 66.67 mM potassium phosphate buffer (pH 7.5), 0.33 mM EDTA, 0.5 mM 5,5-dithiobis-(2-nitrobenzoic acid) in 0.01 M potassium phosphate buffer (pH 7.5), 66.67 mM NADPH, 66.67 mM oxidized glutathione and 0.1 ml enzyme extract. The reaction was started by adding oxidized glutathione and the increase in absorbance at 412 nm was recorded spectrophotometrically (Spectronic-20, Milton Roy, USA).

Determination of lipid peroxidation, H_2O_2 and proline content

Lipid peroxidation rates were estimated by measuring the malondialdehyde (MDA) equivalent according to Hodges *et al*. (1999). 0.5 g of leaf sample was homogenized in a mortar pestle with 80% ethanol. The homogenate was centrifuged at 3000 × *g* for 10 min at 4°C. The pellet was extracted twice with the same solvent. The supernatant was added to a test tube with an equal volume of the solution that was composed of 20% trichloroacetic acid (TCA), 0.01% butylated hydroxy toluene and 0.65% thiobarbutyric acid. Samples were heated at 95°C for 25 min and cooled to room temperature. Absorbance was recorded at 440, 532 and 600 nm. Lipid peroxidation equivalent (n mol MDA ml^{-1}) was calculated by using the formula given by Hodges *et al*. (1999):

$$[A_{65}(532 + TBA)–(Abs_{600} + TBA)–(Abs_{532\ -\ TBA} – Abs_{560\text{-}TBA})] = A$$

$$[Abs_{440} + TBA = Abs_{600+TBA)} – 0.0571] = B$$

$$\text{MDA equivalents (n mol } m^{-1}) = (A\text{-}B/157000)10^6$$

The content of H_2O_2 was determined by monitoring A_{410} of titanium peroxidase complex according to the method described by Patterson *et al*. (1984).

The proline content in fresh leaf samples were determined by adopting the method of Bates *et al*. (1973). To the leaf extract in sulphosalicylic acid, an equal volume of glacial acetic acid and ninhydrin solutions were added. The sample was heated at 100°C, to which 5 ml of toluene was added. The absorbance of toluene layer was read at 528 nm on a spectrophotometer.

Statistical analysis

The experiment was conducted according to a simple randomized block design. A total of 5 replicates for each treatment were taken. Treatment means were compared by analysis of variance using SPSS version 10 (SPSS, Chicago, IL, USA). Least significance difference (LSD) was calculated at the 5% probability level. Treatment means were separated using Duncan's multiple range test.

RESULTS

The stress generated by temperature and/or salinity resulted in a significant decrease in all the growth characteristics: shoot and root length, plant fresh and dry mass, leaf area (**Fig. 1**), relative water content (**Fig. 2**) and leaf water potential (**Fig. 5**). The combined effects of these two stresses were found to be more toxic than their individual effects. However, treatment with SA significantly improved these attributes both in the presence as well as in absence of temperature and/or salinity stresses. The response generated by SA was higher in the stress-free conditions compared to that under salinity and/or temperature stressed condition showing a significant increase of 22.0, 30.0, 19.7, 22.7, 6.2 and 13.9% in the above mentioned parameters respectively, compared to the control.

The SPAD Chl value (**Fig. 3**) and all the photosynthetic attributes P_N, g_s, C_i, WUE (**Figs. 3, 4**) and carboxylation efficiency (**Fig. 3**), including maximum quantum yield of PS II (Fv/Fm) (**Fig. 3**), declined significantly following exposure to either temperature or salinity, alone or combined. However, subsequent treatment of these stressed plants with SA significantly improved these attributes. The time at which SA applied afterwards is decisive for plants appropriate response. The upexpressed assembly of SA based receptors and molecules downstream is optimum following to stress regime, which gradually declines with time laps. The follow-up treatment effectively quenches the receptors and appropriate response subsequent to SA application to recover prevailing stress. SA treatment alleviated the toxicity generated by temperature stress alone as indicated by the increased lipid peroxidation, H_2O_2 and proline content and revealed the values for these parameters were almost comparable with that of the control. SA treatment to unstressed plants resulted in maximum and significant increase of 36.3% (SPAD chlorophyll value), 38.4% (P_N), 21.4% (g_s), 11.2% (C_i), 31.5% (WUE), 23.5% (E) and 25.0% (carboxylation efficiency) more than the control.

The activity of antioxidative enzymes (POX, CAT, SOD and GR) and metabolite (proline content) (**Figs. 4, 5**) exhibited an increasing trend in response to temperature and/or saline stress; moreover, treatment with SA both in the presence or absence of stresses had an additive effect on the enzymatic activity and on proline content. The interaction of SA with the combined effect of temperature and salinity was most effective and enhanced the values of POX, CAT, SOD, GR and that of proline by 107.3, 37.2, 37.5, 55.3 and 65.3% respectively, more than the control (**Figs. 4, 5**).

The activity of NR and CA decreased significantly after temperature and/or NaCl treatment (**Fig. 5**). However, SA treatment of the plants grown under stress-free conditions significantly enhanced the activity of these enzymes by 4.4 and 25.4%, respectively more than the control. Further, treatment of temperature-stressed plants with SA completely alleviated the adverse effects of high temperature; the values of NR and CA were statistically equal to those of the control. The treatment of plants exposed to NaCl stress alone or in combination with temperature stress resulted in partial or complete alleviation of the ill effects produced by the activity of these two enzymes (**Fig. 5**). SA treatment significantly neutralized the damage caused by these two stresses alone or in combination, although it was more effective in plants exposed to temperature stress alone and almost at the same level as the values of the control.

A completely different trend of response was observed in electrolyte leakage, H_2O_2 content, lipid peroxidation and MSI, where plants exposed to SA both in the presence or absence of temperature and/or NaCl stress (**Figs. 1, 2**). Electrolyte leakage, H_2O_2 content, and lipid peroxidation increased significantly in plants exposed to either stress factor and maximum values for these parameters were recorded in plants receiving a combination of these two stresses. The application of SA to the plants exposed to a combined effect of salinity and high temperature stress significantly decreased the values for electrolyte leakage, H_2O_2

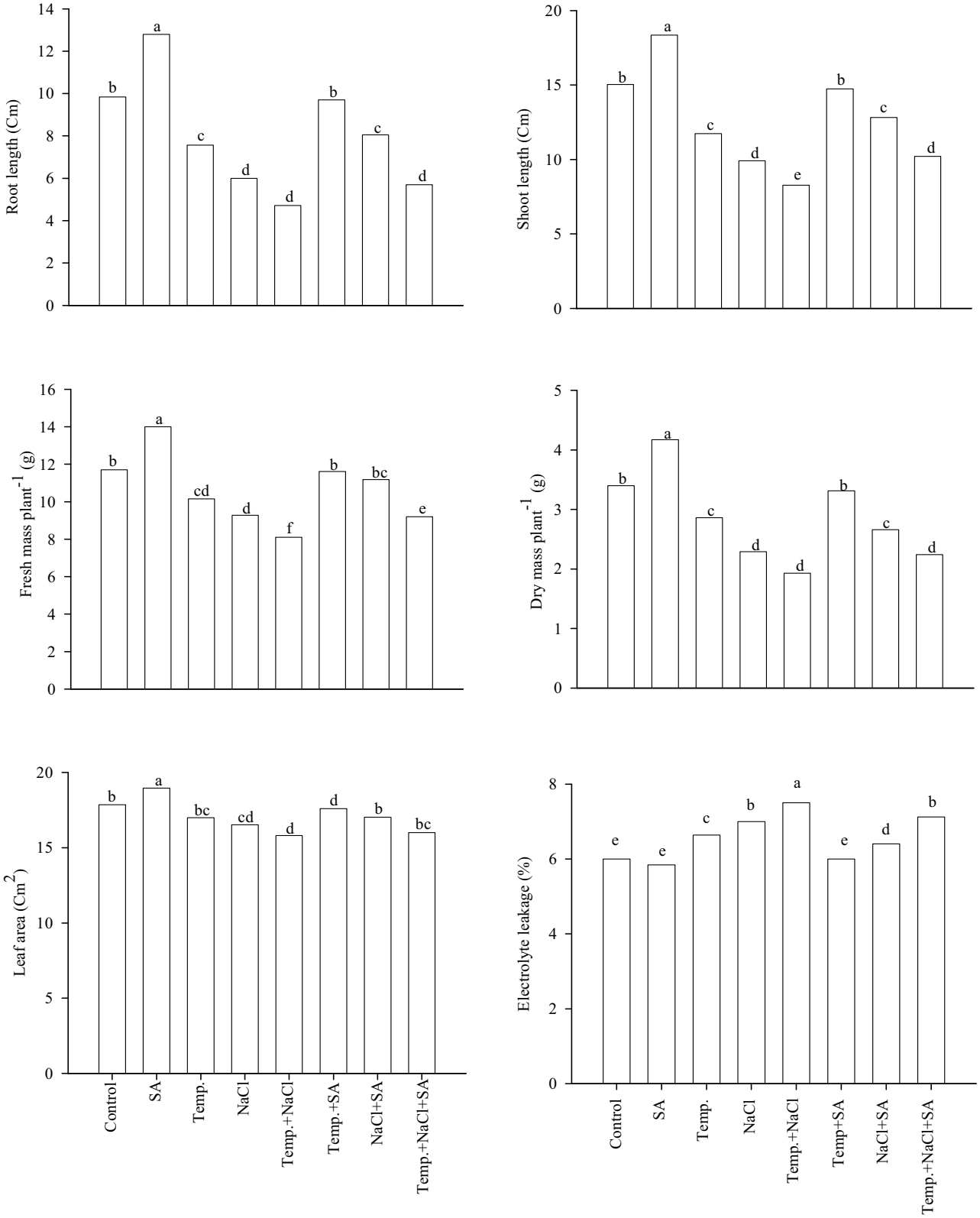

Fig. 1 Effect of salicylic acid (SA) (10^{-5}M) on root length, shoot length, fresh mass plant^{-1}, dry mass plant^{-1}, leaf area and electrolyte leakage in mung bean (*Vigna radiata* L. Wilczek) cv. 'T-44' exposed to high temperature (40°C) and/or NaCl (100 mM) stress.

content and lipid peroxidation by 5, 3 and 4%, respectively compared to plants exposed to the combined effect of salinity and temperature stress.

Foliar application of SA to unstressed plants significantly increased the value of MSI by 22.7% more than the control. However, the exposure of plants to salinity and/or temperature stress resulted in a significant reduction in MSI; maximum reduction of 41% more than the control was observed in plants exposed to a combined effect of high temperature and salinity stress. Further, spraying plants (exposed to temperature and salinity stress) with SA enhanced significantly the value of MSI by 27% more than plants exposed to a combined effect of salinity and temperature stress (**Fig. 2**).

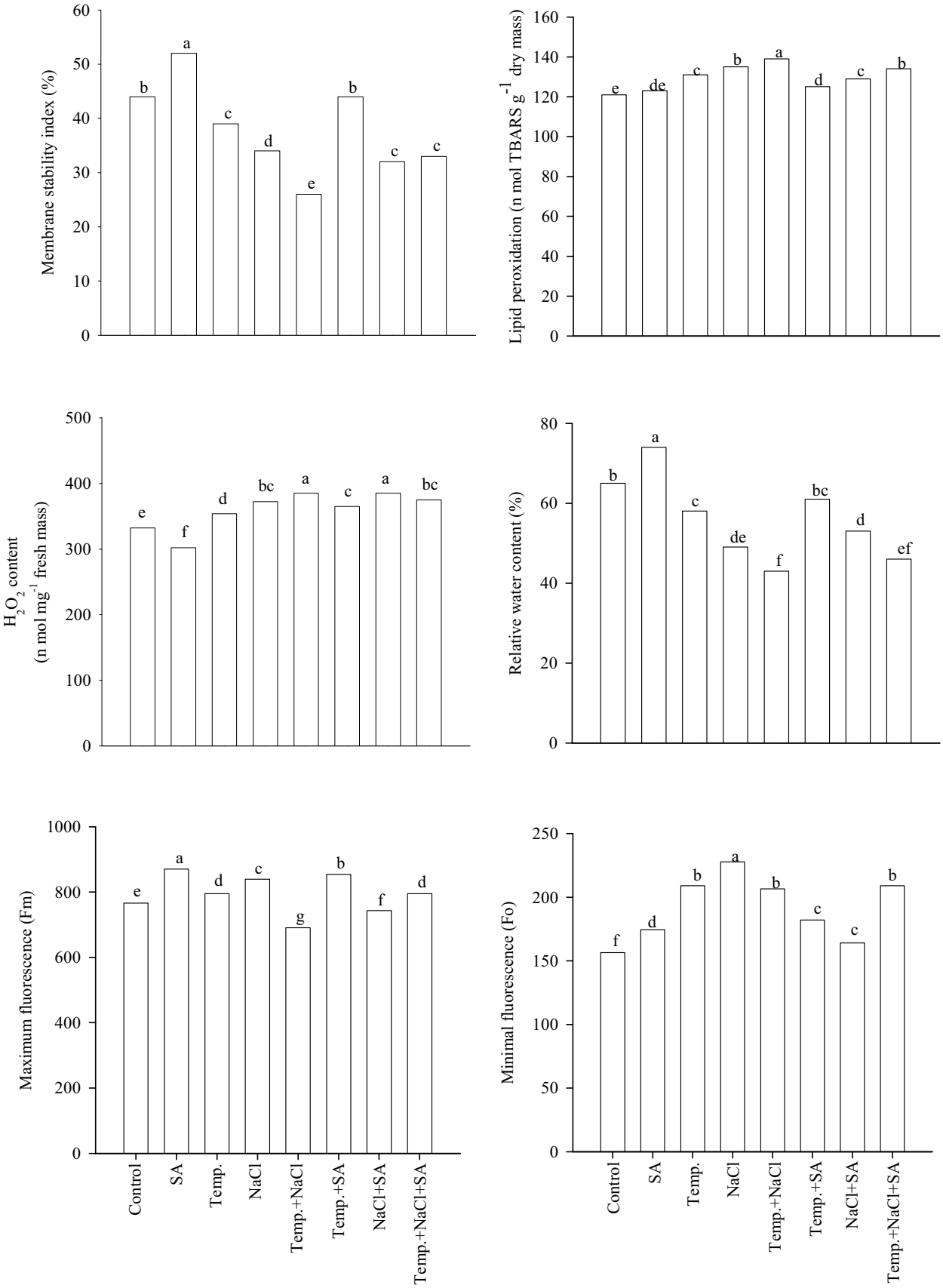

Fig. 2 Effect of salicylic acid (SA) (10^{-5}M) on membrane stability index (MSI), lipid peroxidation, H_2O_2 and relative water content (RWC), maximum fluorescence (Fm) and minimal fluorescence (Fo) in mung bean (*Vigna radiata* L. Wilczek) cv. 'T-44' exposed to high temperature (40°C) and/or NaCl (100 mM) stress.

DISCUSSION

It is an established fact that free-radical induced peroxidation of membrane lipids is the reflection of stress induced damage at the cellular level (Jain *et al*. 2001). Therefore, relative electrolyte leakage, lipid peroxidation and MSI have been widely used as criterion to assess the magnitude of injury, caused by any stress factor (heat, salinity, drought or all) in various crops (Khan *et al*. 2009; Palma *et al*. 2009). In the present investigation, electrolyte leakage and lipid peroxidation appears due to temperature and/or saline stress. This supports the belief that the increase in electro-

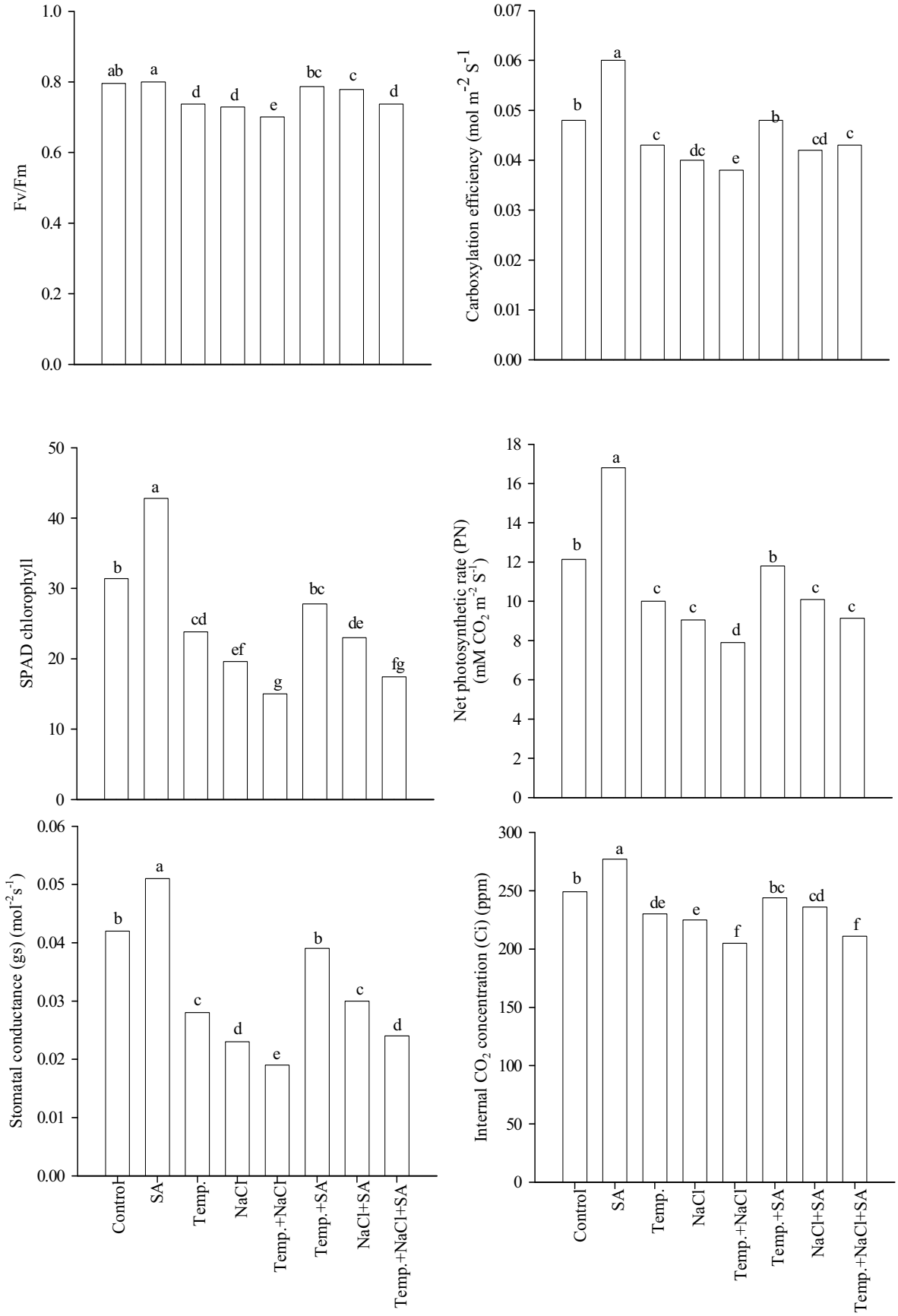

Fig. 3 Effect of salicylic acid (SA) (10^{-5} M) on maximal quantum yield (Fv/Fm), carboxylation efficiency (CE), SPAD chlorophyll content, net photosynthetic rate (P_N), stomatal conductance (g_s), internal CO_2 concentration (C_i) in mung bean (*Vigna radiata* L. Wilczek) cv. 'T-44' exposed to high temperature (40°C) and/or NaCl (100 mM) stress.

lyte leakage and lipid peroxidation is the result of plasmalemma injury, caused by ROS production. These results are consistent with previous reports for *Phalaenopsis* (Ali *et al*. 2005), turfgrass (Xu *et al*. 2006), *Lilium longiflorum* (Yin *et al*. 2008) when subjected to heat stress and in two beet species (Bor *et al*. 2003) and barley (El-Tayeb 2005) when exposed to saline stress. Furthermore, an increase in H_2O_2 content and a decrease in MSI (**Fig. 2**) in the present study

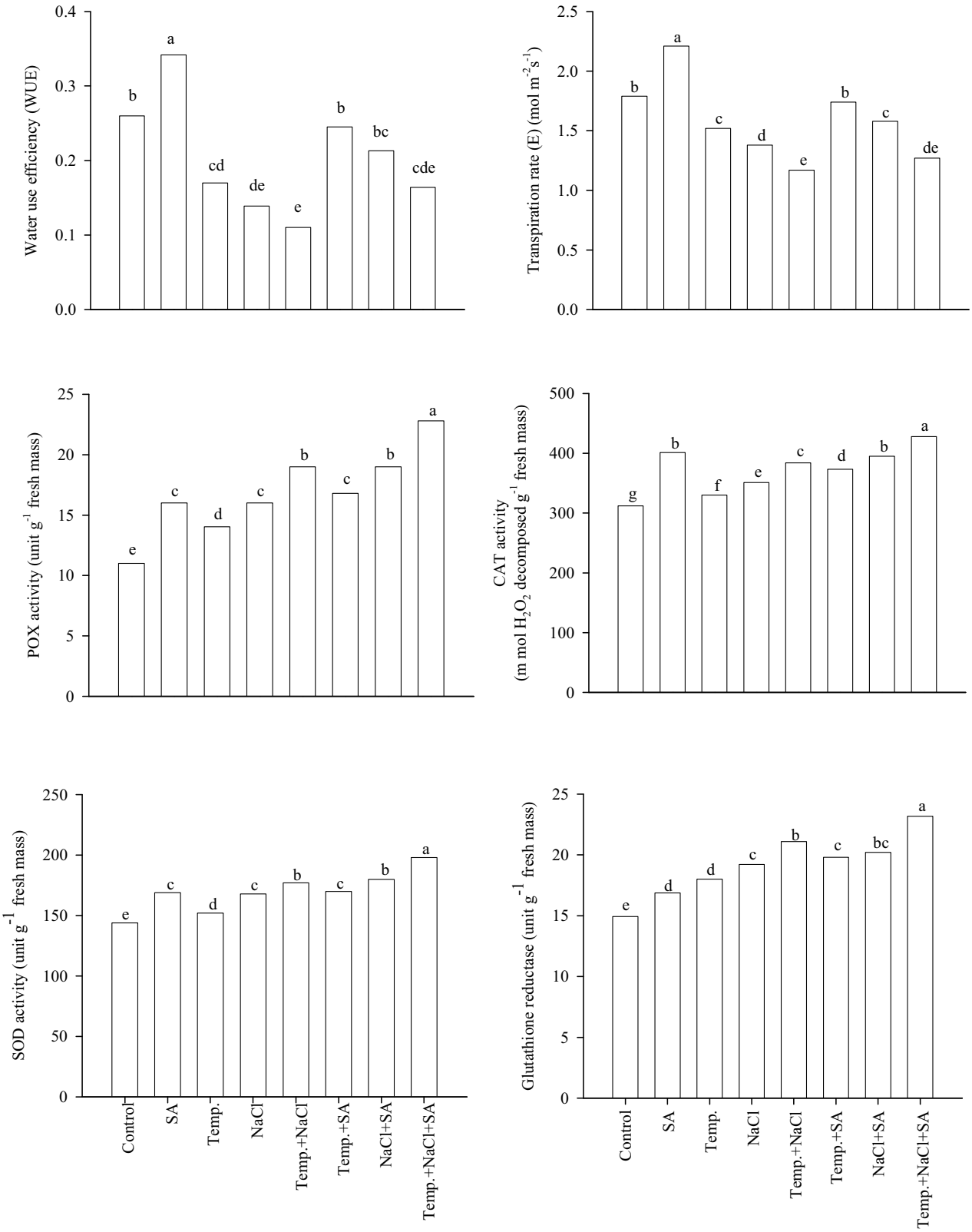

Fig. 4 Effect of salicylic acid (SA) (10^{-5} M) on water use efficiency (WUE), transpiration rate (E), peroxidase (POX) activity, catalase (CAT) activity, superoxide dismutase (SOD) activity and glutathione reductase (GR) activity in mung bean (*Vigna radiata* L. Wilczek) cv. 'T-44' exposed to high temperature (40°C) and/or NaCl (100 mM) stress.

provide additional support to the above observation. However, SA was found to significantly decrease electrolyte leakage, lipid peroxidation and H_2O_2 content and improve MSI both in unstressed as well as stressed plants. These results are in conformation with those of Agarwal *et al*. (2005) who showed that SA increased MSI in wheat. Similarly, El-Tayeb (2005) noted a significant decrease in electrolyte leakage and contents of MDA when treated with SA. Likewise, a loss in the RWC and leaf water potential is possibly the impact of these two stress factors on the permeability of the plasma membrane that affects the absorption of the ions and water (Aslam *et al*. 1984) and generates physiological drought (Hopkins 1995). A similar role of temperature stress has been reported earlier as it decreased the leaf water potential in *Lotus creticus* (Anon *et al*. 2004) and sugarcane (Wahid and Clouse 2007). A decrease in relative water content under saline stress has been reported in *Brassica juncea* (Ali *et al*. 2008; Yusuf *et al*. 2008). However, SA treatment alone or as a follow up treatment significantly improved the water status of the plants. Earlier studies

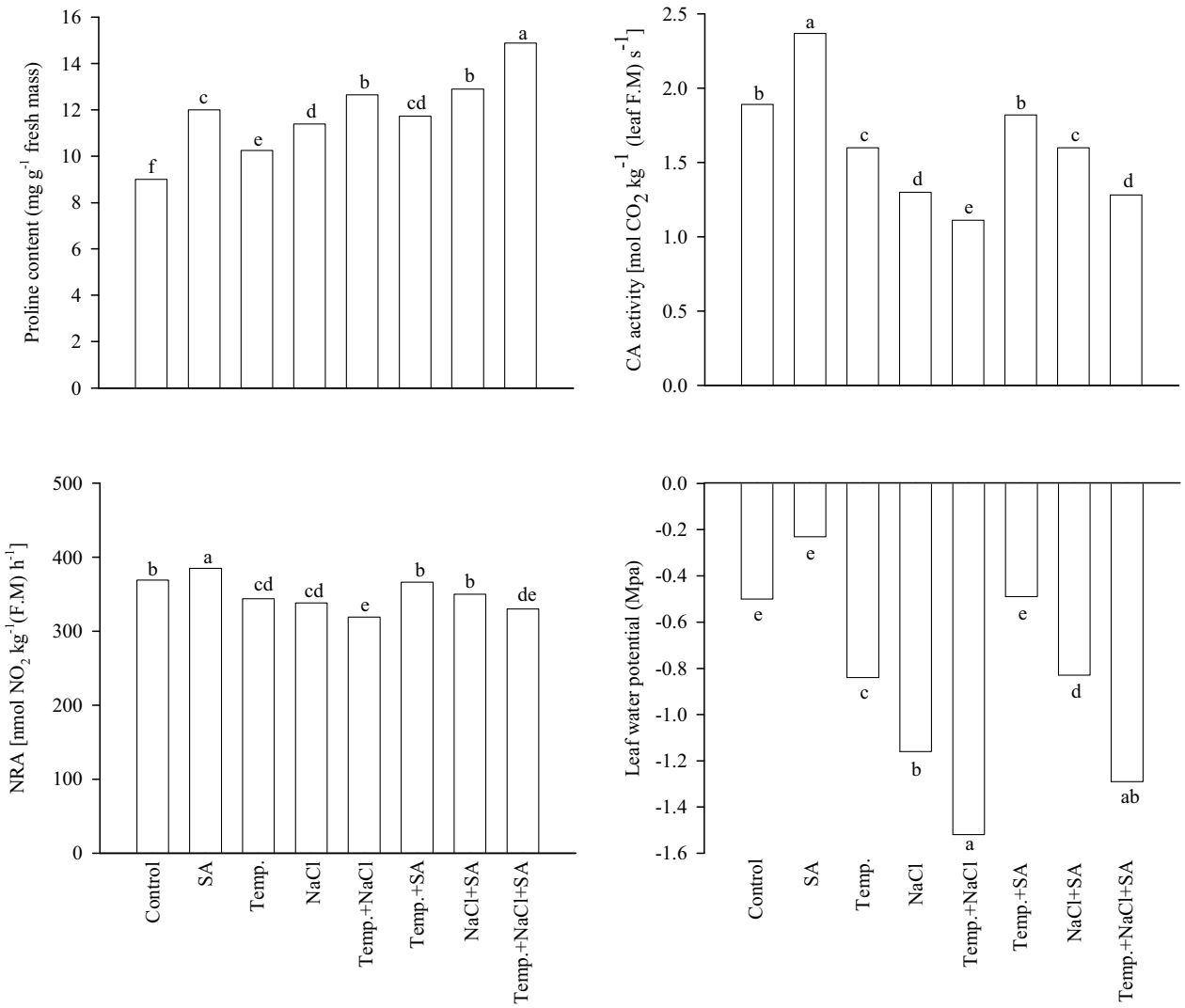

Fig. 5 Effect of salicylic acid (SA) (10^{-5} M) on proline content, carbonic anhydrase activity, nitrate reductase activity (NRA) and leaf water potential in mung bean (*Vigna radiata* L. Wilczek) cv. 'T-44' exposed to high temperature (40°C) and/or NaCl (100 mM) stress.

strongly favour the above role of SA. Acetyl salicylic acid and SA proved effective in inducing multiple stress tolerance in tomato and bean (Senaratna *et al*. 2000) and also in wheat and tomato against drought stress (Singh and Usha 2003; Hayat *et al*. 2008) and *Brassica juncea* against saline stress (Yusuf *et al*. 2008).

Like the other parameters, the activity of NR enzyme decreased to a significant level under both stresses. It could be an expression of stress-induced inhibition of enzyme and/or metabolic dysfunction (Hopkins 1995) or restricting the uptake of nitrate (Aslam *et al*. 1984) which is not only the substrate for NR but also an inducer (Hernandez *et al*. 1996). However, the application of SA elevated the activity of this enzyme both in unstressed and stressed plants. Possibly, SA corrected and/or stabilized the damage caused by the stress and facilitated the uptake of nutrients (El-Tayeb and Ahmed 2010), including that of nitrate, which acts as an inducer of NR (Campbell 1999). The increase in the uptake of various nutrients including NO_3 and activation of NR under normal growth conditions is well established (Hayat *et al*. 2005) which strongly support our observations. Similarly SA, increased NR activity in tomato under drought stress (Hayat *et al*. 2008) and in *Brassica juncea* under salinity stress (Yusuf *et al*. 2008).

The present investigations reveal that temperature and/or salinity caused a significant reduction in the leaf gas exchange rate (net photosynthetic rate, stomatal conductance, internal CO_2 concentration, WUE, carboxylation efficiency) (**Figs. 3, 4**) and quantum yield of PSII (Fv/Fm) (**Fig. 3**). Abiotic stress reportly damages the photosynthetic machinery at multiple levels such as pigments, stomatal function and gaseous exchange, structure and function of thylakoid membrane, electron transport and the enzymes involved (Sudhir and Murthy 2004). Excess salts are known to cause the closure of stomata, thereby decreasing the partial CO_2 pressure (Bethkey and Drew 1992) as well as internal CO_2 pressure (Bethkey and Drew 1992) and consequently the activity of CA (**Fig. 5**) because its activity is largely regulated by the CO_2 concentration (Tiwari *et al*. 2005). A decrease in CA activity under temperature stress could have been the reason for inactivation of Rubisco (Crafts-Brandner and Salvucci 2002; Morales *et al*. 2003; Salvucci and Crafts-Brandner 2004a). Similarly, temperature stress is also reported to cause stomatal closure (Crafts-Brandner and Salvucci 2002) altered structure and fluidity of the thylakoid membrane (Mohanty *et al*. 2002) and diminishion of enzymatic activity of the calvin cycle such as sedoheptulose-1,7-bisphosphate (Nogues and Baker 2000; Lefebvre *et al*. 2005) and ribulose-1,5-carboxylase/oxygenase (Salvucci and Crafts-Brandner 2004b) that regulate the performance of the photosynthetic machinery. Moreover, the decrease in the quantum yield of PSII (F_v/F_m) in response to temperature and/or salinity noted in the present study are in consistent with those of Ogweno *et al*. (2008) who showed that temperature exposure caused a reduction in the F_v/F_m ratio of tomato. Similarly, Shahbaz *et al*. (2008) reported significant reduction in quantum yield of PSII of wheat cultivars on being subjected to saline medium. However, SA treatment significantly improved the photosynthesis and related attributes (**Figs. 3, 4**), which is well supported by our earlier

observations in *Brassica juncea*, under stress-free conditions (Fariduddin *et al*. 2003) and in tomato and mustard under drought and saline stresses (Hayat *et al*. 2008; Yusuf *et al*. 2008). In addition to this, the role of SA in the activation of Rubisco and PEP carboxylase under stress is also well documented (Singh and Usha 2003).

In spite of causing tissue dehydration, temperature also induces oxidative stress (Liu and Huang 2000) that results from an increased level of ROS in the cell (Schutzendubel and Polle 2002). These ROS may oxidize proteins, lipids and nucleic acids leading to mutation at the cellular level (Halliwell and Gutteridge 1999;di Toppi and Gabbrielli 1999). However, to protect themselves from the toxicity of oxidative stress, plants have endogenous system of enzymes (POX, CAT, SOD and GR) and non-enzymatic metabolites (ascorbate, gluthathione, proline and tocopherols). The exposure of the plants to temperature and/or saline stress significantly increases the activities of these enzymes and that of proline content to boost the resistant capacity of the plants (Rivero *et al*. 2004; Ali *et al*. 2007). Moreover, SA application further increased the activities of POX, CAT, SOD, GR and proline content that were more prominent in the stressed plants and were additive with SA. The use of advanced molecular approaches has revealed that number of detoxifying and antioxidant genes coding for protein enzymes that play crucial role in defense response (Holuigue *et al*. 2007). Moreover, SA activates the expression of these defense genes by triggering redox changes in components of the signal transduction pathway (Durrant and Dong 2004; Fobert and Despres 2005) that favours the protection of plants against the oxidative damage (Yang *et al*. 2004). A similar mechanism could have been operative in the stressed plants exposed to SA in the present study. SA application was also found to increase the activity of POX, CAT, SOD and GR in different plant species subjected to various abiotic stresses (Popova *et al*. 2003; Yusuf *et al*. 2008; Hayat *et al*. 2008).

CONCLUDING REMARKS

It is thus clear from the present investigation that the exposure of plants to high temperature and/or salinity resulted in serious physiological or metabolic perturbations manifested in the form of reduced growth, photosynthetic attributes and carboxylation efficiency in addition to the altered (increased) activity of antioxidative enzymes. An interesting aspect of this study is to observe the interactive effect of high temperature and salinity on the growth and physiological parameters of plants since the two stress types are interrelated, as temperature stress results in hyper accumulation of salts due to excessive evaporation and increased transpiration and as such to investigate the effect of exogenously applied salicylic acid on the salinity and/or high temperature induced changes in *Vigna radiata*.

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