J Plant Growth Regul (2011) 30:425–435
DOI 10.1007/s00344-011-9205-0

Role of Salicylic Acid in Promoting Salt Stress Tolerance and Enhanced Artemisinin Production in *Artemisia annua* L.

Tariq Aftab · M. Masroor A. Khan · Jaime A. Teixeira da Silva · Mohd. Idrees · M. Naeem · Moinuddin

Received: 1 October 2010 / Accepted: 6 January 2011 / Published online: 24 March 2011

Abstract In the present investigation, the role of salicylic acid (SA) in inducing salinity tolerance was studied in *Artemisia annua* L., which is a major source of the antimalarial drug artemisinin. SA, when applied at 1.00 mM, provided considerable protection against salt stress imposed by adding 50, 100, or 200 mM NaCl to soil. Salt stress negatively affected plant growth as assessed by length and dry weight of shoots and roots. Salinity also reduced the values of photosynthetic attributes and total chlorophyll content and inhibited the activities of nitrate reductase and carbonic anhydrase. Furthermore, salt stress significantly increased electrolyte leakage and proline content. Salt stress also induced oxidative stress as indicated by the elevated levels of lipid peroxidation compared to the control. A foliar spray of SA at 1.00 mM promoted the growth of plants, independent of salinity level. The activity of antioxidant enzymes, namely, catalase, peroxidase, and superoxide dismutase, was upregulated by salt stress and was further enhanced by SA treatment. Artemisinin content increased at 50 and 100 mM NaCl but decreased at 200 mM NaCl. The application of SA further enhanced artemisinin content when applied with 50 and 100 mM NaCl by 18.3 and 52.4%, respectively. These results indicate that moderate saline conditions can be exploited to obtain higher artemisinin content in *A. annua* plants, whereas the application of SA can be used to protect plant growth and induce its antioxidant defense system under salt stress.

Keywords *Artemisia annua* L. · Artemisinin · Antioxidant enzymes · Oxidative stress · Photosynthesis · Salicylic acid

T. Aftab (✉) · M. M. A. Khan · Mohd. Idrees · M. Naeem · Moinuddin
Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh 202 002, India
e-mail: tarik.alig@gmail.com

J. A. T. da Silva
Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki-cho, Ikenobe 2393, Kagawa-ken 761-0795, Japan

Introduction

Salinity is one of the environmental factors limiting soil fertility and plant production (Silveira and others 2001; More and others 2004; Khan and Panda 2008). Plants exposed to salt stress adapt their metabolism to cope with a changing soil environment. Under stress conditions such as salinity, drought, and low and high temperature, plants produce reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radical, and singlet oxygen, which can damage mitochondria and chloroplasts by disrupting cellular structures. ROS are harmful to plant growth and productivity. They deteriorate membrane function (Mishra and Choudhuri 1999). Under saline conditions, ROS also limit NO_3^- uptake and its reduction, thus affecting nitrogen assimilation and, thereby, plant growth (Silveira and others 2001). To scavenge ROS, plants synthesize different types of antioxidant compounds or activate key antioxidant enzymes (Mittler 2002). Hence, the alleviation of oxidative damage and increased resistance to salinity and other environment stresses are often correlated with an efficient antioxidative system (Jaleel and others 2007). Salicylic acid (SA) is a signal molecule or chemical messenger and its role in the defense mechanism is well established in plants (Gunes and others 2005; Gautam and Singh 2009; Syeed and Khan 2010). It plays a significant

role in salt tolerance in many crops (Singh and Usha 2003; El-Tayeb 2005; Stevens and others 2006; Gautam and Singh 2009). Exogenous application of SA has been shown to influence a range of diverse processes in plants, including seed germination, stomatal closure, ion uptake and transport, membrane permeability, photosynthesis, and plant growth rate (Barkosky and Einhellig 1993; Khan and others 2003; Gunes and others 2005; Aftab and others 2010a). Concerning secondary metabolites, SA (0.5–2.00 mM) has been reported to increase artemisinin content in *Artemisia annua* L. (Pu and others 2009; Guo and others 2010; Aftab and others 2010a).

Artemisinin, an efficient antimalarial drug, is effective against multidrug-resistant strains of the malaria parasite *Plasmodium falciparum* (Kremsner and Krishna 2004). It can be extracted from the leafy tissues and flowers of *Artemisia annua* plant (Duke and Paul 1993). However, the concentration of artemisinin in the plant is very low, ranging from 0.01 to 0.8% (Van Agtmael and others 1999). Because the chemical synthesis of artemisinin is complicated and not economically feasible in view of the poor yield of the drug, the intact plant remains the only viable source of artemisinin production and, therefore, enhanced production of artemisinin content in the plant is highly desirable (Abdin and others 2003; Aftab and others 2010a, b, c). Although there are some reports on the response of *A. annua* to salinity, few reports address the alleviation of the damaging effects of salt stress on growth and productivity of the plant. Prasad and others (1998) found that artemisinin yield increased significantly as salinity stress increased up to 6.0 dS/m, but further increases in salinity decreased the yield significantly. However, artemisinin content in vegetative tissues was not influenced by salinity stress. Qureshi and others (2005), Qian and others (2007), and Aftab and others (2010d) reported that even though artemisinin yield was negatively influenced by salt stress, the artemisinin content increased. The present research was undertaken to determine the salt stress-mediated changes in the growth, photosynthesis, lipid peroxidation, antioxidant enzymes, and artemisinin content of *A. annua* and to explore the role of SA in salt stress mitigation in this regard.

Materials and Methods

Plant Material, Growth Conditions, Salinity, and SA Treatments

The pot experiment was conducted on *A. annua* under natural conditions in a net house using three concentrations of NaCl: 50, 100, and 200 mM. Foliar application of SA at 1.00 mM concentration was carried out with and without salt stress. Seeds of *A. annua* were initially surface sterilized with 95% ethyl alcohol for 5 min and then washed thoroughly with double-distilled water (DDW) before sowing. Each experimental pot (25 cm diameter × 25 cm height) was filled with 5.0 kg of a homogeneous mixture of soil and farmyard manure (4:1) prior to sowing seeds. Physical and chemical characteristics of the soil were as follows: texture of sandy loam; pH (1:2) 8.0; electrical conductivity (EC) (1:2) 0.48 m mhos/cm; available N, P, and K of 97.46, 10.21, and 147.0 mg kg^{-1} soil. Seeds were sown at a depth of 2 cm. The salinity treatment was applied when plants attained three to four true leaf stage. To avoid osmotic shock, the NaCl concentration was increased gradually using 25 mM of NaCl daily until a final concentration of 50, 100, and 200 mM was maintained. A foliar spray of SA was applied three times (at 1-week intervals) to the foliage (that is, entire aerial part) on nonstressed as well as stressed plants starting from the fifth day of attaining the final salinity treatment. Each pot, containing a single plant, was considered as one replicate and there were five such replicates of each treatment. The experiment was conducted according to a completely randomized block design and sampling for various parameters was carried out at 150 days after sowing (DAS).

Determination of Growth and Yield Parameters

The plants from each treatment were carefully uprooted and shoot height was recorded. Plants were washed with tap water to remove adhering foreign particles. Plant roots were removed and the individual shoot fresh weight was recorded. The shoots were dried at 80°C for 48 h and their dry weights were then recorded. Total leaves of the plants were weighed to determine leaf yield.

Determination of Photosynthetic Parameters and Pigments

Net photosynthetic rate (P_N) was measured on sunny days at 11:00 a.m. using fully expanded leaves with the help of an IRGA (infrared gas analyzer, LI-COR 6400 Portable Photosynthesis System, LI-COR, Lincoln, NE, USA). Before carrying out the measurements, the IRGA was calibrated and zero was adjusted approximately every 30 min during the measurement period. Each leaf was enclosed in a gas exchange chamber for 60 s. All the attributes measured by IRGA were recorded three times for each treatment.

Total chlorophyll content in the fresh leaves was estimated using the method of Lichtenthaler and Buschmann (2001). For chlorophyll extraction, the fresh tissue from interveinal leaf area (5 mg) was ground using a mortar and pestle containing 80% acetone. The absorbance of the

solution (chlorophyll extract) was recorded at 662 and 645 nm using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan).

Assay of Nitrate Reductase and Carbonic Anhydrase Activity

Nitrate reductase (NR; E.C. 1.6.6.1) activity in the leaf was determined using the intact tissue assay method of Jaworski (1971). Chopped leaf pieces (200 mg) were incubated for 2 h at 30°C in a 5.5-ml reaction mixture, which contained 2.5 ml of 0.1 M phosphate buffer, 0.5 ml of 0.2 M potassium nitrate, and 2.5 ml of 5% isopropanol. The nitrite that formed subsequently was calorimetrically determined at 540 nm after azocoupling with sulfanilamide and naphthylene diamine dihydrochloride. NR activity was expressed as nM NO_2 g^{-1} FW h^{-1}.

Carbonic anhydrase (CA; E.C. 4.2.1.1) activity was measured in fresh leaves using the method described by Dwivedi and Randhawa (1974). Two hundred milligrams of fresh leaf pieces were incubated in Petri dishes containing 10 ml of 0.2 M cysteine hydrochloride solution for 20 min at 4°C. Four milliliters of 0.2 M sodium bicarbonate solution and 0.2 ml of 0.022% bromothymol blue were added to each test tube. The reaction mixture was titrated against 0.05 N HCl using methyl red as indicator. The enzyme was expressed as μM CO_2 kg^{-1} leaf FW s^{-1}.

Determination of Electrolyte Leakage and Proline Content

To assess membrane permeability, electrolyte leakage was determined using the method of Lutts and others (1995). Leaf samples were washed three times with DDW to remove surface contaminants. Young leaf discs were placed in a vial containing 10 ml of DDW which was closed and incubated on a rotatory shaker for 24 h. Subsequently, the electrical conductivity (EC_1) of the solution was determined. Samples were then autoclaved at 120°C for 20 min and the final electrical conductivity (EC_2) was noted after cooling the solution at room temperature. Electrolyte leakage was calculated as

$$\text{Electrolyte leakage}\ (\%) = (EC_1/EC_2) \times 100$$

The proline content was estimated using the method of Bates and others (1973). The leaf material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000 rpm. The supernatant was used to estimate the proline content. The reaction mixture, consisting of 2 ml of acid ninhydrin and 2 ml of glacial acetic acid, was boiled at 100°C for 1 h. After terminating the reaction in an ice bath, the proline was extracted with 4 ml of toluene and its absorbance was recorded at 520 nm.

Lipid Peroxidation Rate (TBARS Content)

Oxidative damage to leaf lipids was determined by estimating the content of total 2-thiobarbituric acid reactive substances (TBARS) expressed as malondialdehyde (MDA) equivalents. TBARS content was estimated using the method of Cakmak and Horst (1991). TBARS were extracted from 0.5 g of chopped fresh leaves ground with 5 ml of 0.1% (w/v) trichloroacetic acid (TCA). After centrifugation at 12,000*g* for 5 min, a 1-ml aliquot from the supernatant was added to 4 ml of 0.5% (w/v) tertiary butyl alcohol (TBA) prepared in 20% (w/v) TCA. Samples were incubated at 90°C for 30 min. Thereafter, the reaction was stopped in an ice bath. Samples were centrifuged at 10,000*g* for 5 min, and absorbance of the supernatant was recorded at 532 nm using a spectrophotometer. The values were corrected for nonspecific turbidity by subtracting the readings obtained at an absorbance of 600 nm. TBARS content was expressed as nmol g^{-1} FW.

Antioxidant Enzymes Assay

A crude enzyme extract was prepared by homogenizing 500 mg of leaf tissue in extraction buffer containing 0.5% Triton X-100 and 1% polyvinylpyrrolidone in 100 mM potassium phosphate buffer (pH 7.0) using a chilled mortar and pestle. The homogenate was centrifuged at 15,000*g* for 20 min at 4°C. The supernatant was used for the enzymatic assays.

Catalase (CAT; E.C. 1.11.1.6) activity was measured according to the method of Chandlee and Scandalios (1984) with a slight modification. The assay mixture contained 2.6 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.4 ml of 15 mM H_2O_2, and 0.04 ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. Enzyme activity was expressed in U mg^{-1} protein (U = 1 mM of H_2O_2 reduced min^{-1} mg^{-1} protein).

Peroxidase (POX; E.C. 1.11.1.6) activity was assayed using the method of Kumar and Khan (1982). The POX assay mixture contained 2 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H_2O_2, and 0.5 ml of enzyme extract. The reaction mixture was incubated for 5 min at 25°C. The reaction was terminated by adding 1 ml of 2.5 N H_2SO_4. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a reagent blank prepared by adding the extract after the addition of 2.5 N H_2SO_4 at zero time. The activity was expressed in U mg^{-1} protein. One unit of the enzyme activity corresponded to the amount of

enzyme that caused an increase in the absorbance of 0.1 min^{-1} mg^{-1} protein.

Superoxide dismutase (SOD; E.C. 1.15.1.1) activity was assayed as described by Beauchamp and Fridovich (1971). The reaction mixture contained 1.17×10^{-6} M riboflavin, 0.1 M methionine, 2×10^{-5} M KCN, and 5.6×10^{-5} M nitroblue tetrazolium salt (NBT) dissolved in 3 ml of 0.05 M sodium phosphate buffer (pH 7.8). Then 3 ml of the reaction medium was added to 1 ml of enzyme extract. The mixtures were illuminated in glass test tubes by two sets of Philips 40-W fluorescent tubes in a single row. Illumination was used to initiate the reaction at 30°C for 1 h. Identical solutions that were kept in the dark served as blanks. The absorbance was read at 560 nm in the spectrophotometer against a blank. SOD activity was expressed as U mg^{-1} protein. One unit is defined as the amount of change in the absorbance by 0.1 h^{-1} mg^{-1} protein.

Determination of Endogenous ROS Production

The content of H_2O_2 in the leaves was determined according to the method of Mukherjee and Choudhuri (1983). Fresh leaves (0.5 g) were homogenized using a cold mortar and pestle in precooled acetone (5 ml) and the homogenate was centrifuged at 12,000*g* for 5 min. One milliliter of the supernatant was mixed with 0.1 ml of 5% $Ti(SO_4)_2$ and 0.2 ml of 19% ammonia. After a precipitate was formed, the reaction mixture was centrifuged at 12,000*g* for 5 min. The resulting pellet was dissolved in 3 ml of 2 M H_2SO_4 and the absorbance was read at 415 nm using a spectrophotometer. The H_2O_2 concentration was calculated according to a standard curve of H_2O_2 that was prepared by using H_2O_2 ranging from 0 to 10 µM.

Singlet oxygen (O_2^-) production was measured as described by Able and others (1998) with some modifications. The reduction of XTT [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carbo-xanilide] in the presence of O_2^- was monitored. The fresh leaves (0.5 g) were homogenized with 1 ml of 50 mM Tris–HCl buffer (pH 7.5) and centrifuged at 12,000*g* for 15 min. The reaction mixture contained 50 mM Tris–HCl buffer (pH 7.5), 100 µl of supernatant, and 0.5 mM XTT. The reduction of XTT was determined by measuring the absorbance at 470 nm using a spectrophotometer. The quantity of O_2^- was determined using the molar extinction coefficient (ε) of 2.16×10^4 M^{-1} cm^{-1}.

Artemisinin Extraction and Estimation

Dry leaf material (1 g) was used for the estimation of artemisinin that was modified to a compound Q_{260} and quantified using a high-performance liquid chromatography (HPLC) method (Zhao and Zeng 1986). A standard curve was prepared using 1 mg of standard artemisinin (Sigma-Aldrich, St. Louis, MO, USA) dissolved in 1 ml of HPLC-grade methanol to make the stock solution. Artemisinin was extracted with 20 ml of petroleum ether using a shaker maintained at 70 rpm for 24 h. After 24 h, solvent was decanted and pooled and 20 ml of petroleum ether was again added; this step was repeated three times. Petroleum ether fractions were pooled and concentrated under reduced pressure and residues defatted with CH_3CN (10 ml × 3). Precipitated fat was filtered and the filtrate concentrated under reduced pressure. Residues were dissolved in 1 ml of methanol. A 100-µl aliquot of each sample of each treatment was added to 4 ml of 0.3% NaOH. The samples were incubated in a shaking water bath maintained at 50°C for 30 min and then cooled and neutralized with glacial acetic acid (0.1 M in 20% MeOH). The pH (6.8) of the solution was maintained. Derivatized artemisinin was analyzed and quantified through a reverse phase column (C18, 5 µm, 4.6 mm, 250 mm) using a premix of methanol:10 mM K-phosphate buffer (pH 6.5) in a 60:40 ratio having the mobile phase at a constant flow rate of 1 ml/min, with the detector set at 260 nm. Artemisinin was quantified against the standard curve of artemisinin.

Statistical Analysis

Each pot was treated as one replicate and all the treatments were replicated five times. The experiment was conducted according to a simple randomized complete block design. Data were analyzed statistically using SPSS statistical software v. 17 (SPSS Inc., Chicago, IL, USA). Mean values were compared statistically by Duncan's multiple-range test (DMRT) at $p < 0.05$.

Results and Discussion

Various NaCl treatments lowered the values of growth attributes in the present study (Table 1). Maximum growth inhibition was caused by 200 mM of NaCl (Table 1). The salt-induced reduction in growth parameters in *A. annua* plants is in agreement with the reports published earlier regarding different crops (Prasad and others 1998; Qureshi and others 2005; Qian and others 2007; Athar and others 2008; Siddiqui and others 2008; Khan and others 2010; Aftab and others 2010d). Salinity has been shown to reduce the imbibition of water by roots because of lowered osmotic potentials of the substrate, and to cause changes in metabolic activities leading to the reduction in plant growth (Meneguzzo and others 1999; Jaleel and others 2007). The overall reduction in the growth of *A. annua* plants might be ascribed to the ill effect of salt stress on the various physiological processes such as photosynthesis, nutrient

Table 1 Effect of salicylic acid (SA) and different concentrations of salinity (NaCl) and SA on growth and yield attributes of *Artemisia annua* L.

Treatments	Shoot length per plant (cm)	Root length per plant (cm)	Shoot dry weight per plant (g)	Leaf dry weight per plant (g)
Control	85.6 ± 3.21^{bc}	29.7 ± 0.94^{c}	114.7 ± 4.35^{b}	28.8 ± 1.03^{b}
1.00 mM SA	96.2 ± 4.87^{a}	36.2 ± 1.13^{a}	129.8 ± 5.57^{a}	32.4 ± 1.45^{a}
50 mM NaCl	77.1 ± 3.23^{d}	26.3 ± 0.81^{d}	95.6 ± 3.17^{d}	25.1 ± 0.92^{c}
100 mM NaCl	65.7 ± 2.21^{e}	22.9 ± 0.98^{e}	82.3 ± 3.15^{e}	21.2 ± 0.84^{d}
200 mM NaCl	53.5 ± 1.14^{f}	15.7 ± 0.67^{f}	65.8 ± 2.31 ^{g}	16.4 ± 0.59^{f}
50 mM NaCl + SA	88.3 ± 3.65^{b}	31.6 ± 1.31^{b}	109.2 ± 4.08^{c}	27.1 ± 1.14^{bc}
100 mM NaCl + SA	83.1 ± 4.11^{c}	27.7 ± 0.86 ^{cd}	93.4 ± 3.89^{d}	24.3 ± 0.81^{c}
200 mM NaCl + SA	74.3 ± 3.86^{de}	21.3 ± 0.79^{e}	74.3 ± 3.23^{f}	18.4 ± 0.65^{e}

Means within a column followed by the same letter are not significantly different ($p \leq 0.05$). The data shown are mean of five replicates ± SE

homeostasis, accumulation of compatible solutes, and activities of antioxidant enzymes. SA, when applied at 1.00 mM, improved the vegetative growth of plants, thereby increasing the dry matter of plants in both saline and nonsaline conditions. However, the positive effect of SA was more pronounced in plants treated with 50 and 100 mM NaCl as compared to 200 mM NaCl (Table 1). These results are in agreement with those of El-Tayeb (2005) and Gautam and Singh (2009), who reported that foliar application of SA at 1.00 and 0.50 mM improved the growth parameters under salt stress in barley and wheat, respectively. An increase in growth parameters of salt-affected plants in response to SA might be related to the protective role of SA on membranes that might increase the tolerance of plants to salt stress (Wang and Li 2006; Aftab and others 2010a). Furthermore, an SA-mediated increase in dry matter of *A. annua* seedlings under NaCl stress could be due to the SA-induced antioxidant function and metabolic activity in plants (Gunes and others 2007).

Salt stress reduced the net photosynthetic rate, stomatal conductance, and internal CO_2 concentration, with maximum reduction observed in plants grown with 200 mM of NaCl, which caused damage to the leaf tissues (Table 2). A decline in plant productivity under salt stress is often associated with reduction in photosynthetic capacity via the generation of ROS (Ashraf 2004; Noreen and others 2010). In fact, salt stress hinders photosynthesis at multiple levels, such as pigments, stomatal functioning, gaseous exchange, structure and function of thylakoid membrane, electron transport, and enzyme activities, by hampering the oxidative stress mitigation mechanisms and cellular metabolism of plants (Sudhir and Murthy 2004). Excessive salt concentrations might cause the closure of stomata, thereby decreasing the partial CO_2 pressure and internal CO_2 concentration and consequently resulting in a decreased net photosynthetic rate (Bethkey and Drew 1992). SA application significantly improved net photosynthesis; this could be attributed to the role of SA in improving the functional state of the photosynthetic machinery in plants either by the mobilization of internal tissue nitrate or by chlorophyll biosynthesis (Shi and others 2006). Furthermore, SA has also been reported to enhance photosynthetic capacity in maize through stimulation of Rubisco activity (Khodary 2004). The SA-enhanced photosynthetic rate under salt stress found in our study is in agreement with that of some earlier studies on wheat and maize (Singh and Usha 2003; Khodary 2004). Chlorophyll content was also reduced in salt-stressed plants, the most toxic effect being noted at 200 mM NaCl (Table 2). The decrease in chlorophyll content in salt-affected *A. annua* plants might be attributed to the possible oxidation of chlorophyll and other chloroplast pigments coupled with instability of the pigment protein complex under salt stress (Stepien and Klobus 2006).

Salinity stress decreased the activity of NR and CA measured in the leaves of salt-treated *A. annua* plants. At 200 mM NaCl, the activities of both enzymes were minimal compared to those of the control and other salt levels (Table 3). When SA was applied to the plants, NR and CA activities in the leaves significantly increased. Moreover, SA almost nullified the effects of stress caused by 50 and 100 mM NaCl and improved the growth to some extent at 200 mM of NaCl (Table 3). These results coincide with the findings of Nathawat and others (2005), Siddiqui and others (2008), and Aftab and others (2010d), who reported a decreased N uptake and diminished activity of nitrogen-assimilation enzymes under salinity stress. The increase in NR activity in *A. Annua* leaves might be attributed to the role of SA in stabilizing the plasma membrane, as evidenced by Agarwal and others (2005) who used the membrane stability index in wheat under salt stress. Membrane stability might, in turn, facilitate the improved uptake of nutrients, including that of nitrate, which acts as an inducer of NR (Campbell 1999). The enhancement of

Table 2 Effect of salicylic acid (SA) and different concentrations of salinity (NaCl) and SA on net photosynthetic rate, stomatal conductance, internal CO_2, and total chlorophyll content of *Artemisia annua* L

Treatments	Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	Stomatal conductance (mol m^{-2} s^{-1})	Internal CO_2 (μmol m^{-2} s^{-1})	Total chlorophyll content (mg g^{-1} FW)
Control	17.8 ± 0.14^{bc}	0.38 ± 0.002^{bc}	309.1 ± 3.51^{b}	1.3 ± 0.009^{b}
1.00 mM SA	19.2 ± 0.18^{a}	0.39 ± 0.003^{a}	317.0 ± 2.73^{a}	1.4 ± 0.009^{a}
50 mM NaCl	16.1 ± 0.13^{d}	0.36 ± 0.002^{c}	291.2 ± 2.87^{bc}	1.2 ± 0.008^{d}
100 mM NaCl	14.1 ± 0.15^{e}	0.31 ± 0.002^{d}	265.1 ± 2.15^{d}	0.9 ± 0.007^{e}
200 mM NaCl	9.4 ± 0.14^{f}	0.24 ± 0.002^{e}	232.6 ± 2.31^{e}	1.0 ± 0.008^{f}
50 mM NaCl + SA	18.1 ± 0.13^{b}	0.39 ± 0.003^{b}	311.4 ± 3.08^{b}	1.3 ± 0.009^{bc}
100 mM NaCl + SA	17.1 ± 0.11^{c}	0.38 ± 0.004^{b}	302.3 ± 3.82^{b}	1.2 ± 0.008^{c}
200 mM NaCl + SA	15.3 ± 0.16^{de}	0.36 ± 0.003^{c}	284.5 ± 3.13^{c}	0.9 ± 0.006^{ef}

Means within a column followed by the same letter are not significantly different ($p \leq 0.05$). The data shown are mean of five replicates ± SE

Table 3 Effect of salicylic acid (SA) and different concentrations of salinity (NaCl) and SA on NR activity, CA activity, electrolyte leakage, and proline content of *Artemisia annua* L

Treatments	NR activity (nmol NO_2^- g^{-1} FW h^{-1})	CA activity (μmol CO_2 kg^{-1} FW s^{-1})	Electrolyte leakage (%)	Proline content (mg g^{-1} FW)
Control	325.2 ± 4.77^{b}	231.8 ± 3.71^{b}	9.3 ± 0.15^{e}	9.8 ± 0.45^{e}
1.00 mM SA	341.6 ± 5.14^{a}	245.7 ± 4.93^{a}	8.5 ± 0.12^{f}	8.1 ± 0.61^{f}
50 mM NaCl	314.3 ± 4.92^{c}	224.5 ± 4.27^{c}	13.3 ± 0.18^{d}	10.5 ± 0.72^{de}
100 mM NaCl	289.4 ± 4.48^{d}	207.4 ± 3.78^{d}	16.2 ± 0.22^{b}	13.3 ± 0.83^{b}
200 mM NaCl	211.3 ± 3.74^{f}	152.5 ± 3.20^{f}	19.2 ± 0.25^{a}	17.5 ± 0.91^{a}
50 mM NaCl + SA	331.5 ± 4.92^{b}	230.7 ± 4.27^{b}	13.8 ± 0.18^{cd}	11.2 ± 0.53^{d}
100 mM NaCl + SA	322.6 ± 4.48^{bc}	221.2 ± 3.78^{c}	15.1 ± 0.22^{c}	12.3 ± 0.66^{c}
200 mM NaCl + SA	267.1 ± 3.74^{e}	182.3 ± 3.20^{e}	15.7 ± 0.25^{bc}	13.7 ± 0.73^{b}

NR nitrate reductace, *CA* carbonic anhydrase

Means within a column followed by the same letter are not significantly different ($p \leq 0.05$). The data shown are mean of five replicates ± SE

NR activity by SA treatment might have increased the overall growth and yield of SA-treated *A. annua* plants under salt stress (Table 1).

NaCl-fed plants also showed lower CA activity, which could be due to low activation of Rubisco that might be concomitant with reduced net photosynthetic rate, carbon metabolism, leaf chlorophyll content, and photosynthetic efficiency, as suggested by Seeman and Critchley (1985). The activity of CA was improved by SA treatment in both salt-stressed and stress-free plants (Table 3). These results are consistent with those of Fariduddin and others (2003) and Aftab and others (2010a) regarding mustard and *A. annua*, respectively. The role of SA in the enhanced activation of Rubisco and PEP carboxylase under water stress has been reported previously (Singh and Usha 2003; Mateo and others 2006). The enhanced activities of the photosynthetic enzymes in SA-treated plants might have ultimately resulted in an improved photosynthetic rate and plant biomass in both stressed and unstressed conditions (Tables 1 and 2).

The toxic effect of salinity on electrolyte leakage and proline accumulation was proportional to the salt concentration applied. The highest concentration (200 mM NaCl) decreased both parameters to the maximum extent (Table 3). Electrolyte leakage indicates cell membrane injury when plants are subject to salinity stress. Hence, maintenance of the integrity of cellular membranes under salt stress is considered an integral part of the mechanism of salinity tolerance (Stevens and others 2006). Salt stress has also been reported to significantly increase electrolyte leakage in linseed (Khan and others 2007) and mustard (Siddiqui and others 2008). In this investigation, the follow-up treatment of SA completely overcame the effects of salinity in plants grown with 50 or 100 mM NaCl and partially in plants treated with 200 mM NaCl (Table 3). These results are in agreement with those of Stevens and others (2006) regarding tomato and Yildirim and others (2008) about cucumber. Both studies suggested the SA facilitated maintenance of membrane functions through induction of antioxidant mechanisms and elevated ion

uptake, thereby protecting the plants against the oxidative damage (El-Tayeb 2005). In the present study, the increase in proline content in the NaCl-treated plants was noted, with the highest level being attained with 200 mM of NaCl (Table 3). However, SA application reduced the proline content. Because proline is often considered a compatible solute involved in osmotic adjustment under stress (Azooz and others 2004), reduction in the level of proline in SA-treated plants indicates the stress amelioration role of SA that might be responsible for maintenance of plant growth under stress.

Oxidative stress was assessed by measuring the lipid peroxidation rate (TBARS content) when *A. annua* plants were exposed to different NaCl concentrations. The TBARS content and activity of antioxidant enzymes were significantly enhanced in plants subjected to salt stress. The highest TBARS content was observed when plants were supplied with 200 mM of NaCl (Table 4). Mittler (2002) suggested that membrane damage might be due to high H_2O_2 levels, which could accelerate the formation of hydroxyl radicals and, thus, induce lipid peroxidation. Furthermore, in NaCl-treated plants, the cause of oxidative stress might be decreased stomatal conductance in response to an osmotic imbalance and reduced leaf water potential. Similar to that in the present study, other researchers have also found an increase in TBARS content under salt stress in *A. annua*, *Brassica rapa*, and *Catharanthus roseus* (Qureshi and others 2005; Jaleel and others 2007; Noreen and others 2010; Aftab and others 2010d, e, 2011). In this study, the oxidative damage was ameliorated by the application of SA at 1.00 mM. This was also observed by Gunes and others (2007) in maize.

There was a higher level of CAT activity in NaCl-stressed plants, with the most toxic effect caused by 200 mM NaCl (Table 4). POX and SOD activities also increased in salt-treated plants, with the highest levels occurring at 200 mM NaCl. Interestingly, SA further enhanced the activities of the antioxidant enzymes CAT, POX, and SOD in salt-affected plants, with the highest activities of POX and SOD observed at 200 mM

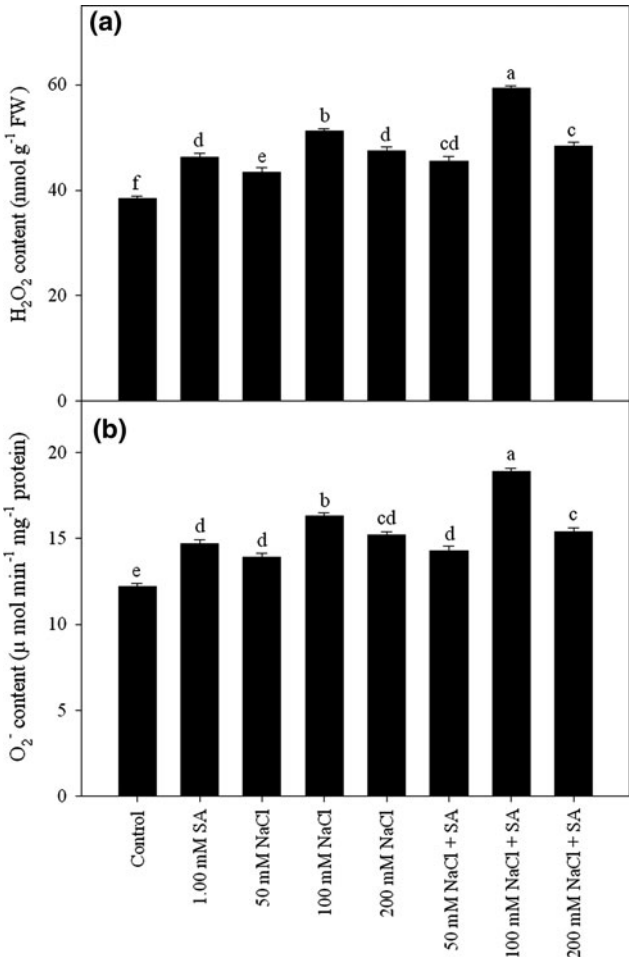

Fig. 1 Effect of SA and different concentrations of salinity on H_2O_2 (**a**) and O_2^- (**b**) contents of *Artemisia annua* L. Bars showing the same letter are not significantly different at $p \leq 0.05$ as determined by Duncan's multiple range test. Error bars (⊤) show SE

Table 4 Effect of salicylic acid (SA) and different concentrations of salinity (NaCl) and SA on TBARS content, CAT, POX, and SOD activities of *Artemisia annua* L

Treatments	TBARS content (nmol g^{-1} FW)	CAT activity (U mg^{-1} protein)	POX activity (U mg^{-1} protein)	SOD activity (U mg^{-1} protein)
Control	6.3 ± 0.19^{e}	9.6 ± 0.21^{g}	45.2 ± 0.95^{f}	1.3 ± 0.05^{g}
1.00 mM SA	5.2 ± 0.28^{f}	15.5 ± 0.63^{e}	61.3 ± 1.26^{d}	1.6 ± 0.05^{f}
50 mM NaCl	7.5 ± 0.32^{cd}	13.6 ± 0.43^{f}	51.4 ± 1.34^{e}	1.9 ± 0.06^{e}
100 mM NaCl	9.4 ± 0.44^{b}	14.6 ± 0.55^{ef}	59.8 ± 0.99^{d}	2.4 ± 0.07^{d}
200 mM NaCl	12.5 ± 0.84^{a}	18.3 ± 0.61^{d}	72.5 ± 1.17^{bc}	2.8 ± 0.08^{c}
50 mM NaCl + SA	6.9 ± 0.22^{d}	21.6 ± 0.83^{c}	62.4 ± 1.34^{c}	2.1 ± 0.06^{d}
100 mM NaCl + SA	7.2 ± 0.38^{d}	18.3 ± 0.74^{b}	67.4 ± 0.97^{b}	2.5 ± 0.07^{b}
200 mM NaCl + SA	8.3 ± 0.34^{c}	16.3 ± 0.71^{a}	81.3 ± 1.76^{a}	3.1 ± 0.09^{a}

Means within a column followed by the same letter are not significantly different ($p \leq 0.05$). The data shown are mean of five replicates ± SE

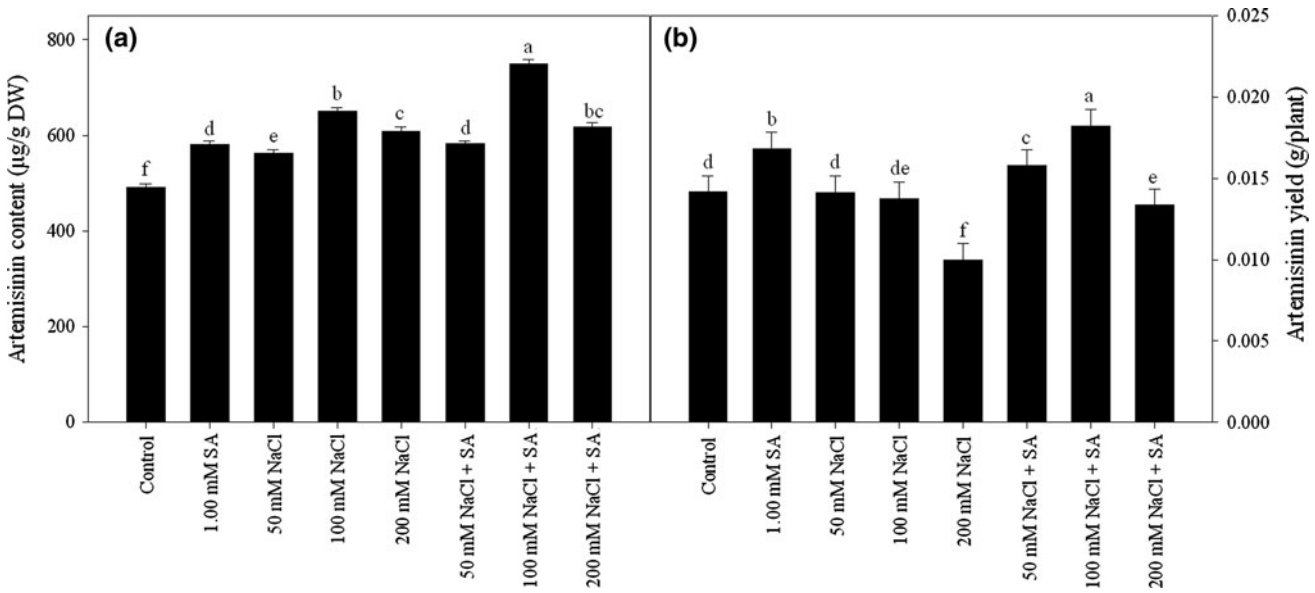

Fig. 2 Effect of SA and different concentrations of salinity on artemisinin content (**a**) and artemisinin yield (**b**) of *Artemisia annua* L. Bars showing the same letter are not significantly different at $p \leq 0.05$ as determined by Duncan's multiple range test. Error bars (⊤) show SE

NaCl + 1.00 mM SA. CAT activity was the highest at 50 mM NaCl + 1.00 mM SA. The control had the lowest values for all antioxidant enzymes measured. Under salt stress, plants are overloaded with ROS, which inhibit several plant processes and cause damage to plants in different ways. Thus, to maintain metabolic functions under stress, scavenging of ROS is essential. ROS scavenging depends on detoxification mechanisms provided by antioxidant enzymes. The increase in the activities of CAT, POX, and SOD in NaCl-treated *A. annua* plants has also been documented by various workers investigating salinity stress in several plant species (Jaleel and others 2007; Koca and others 2007; Aftab and others 2010b; Idrees and others 2011). In this regard, CAT and POX appear to play an essential protective role in scavenging ROS in coordination with SOD (Jaleel and others 2009). SOD initiates detoxification of singlet oxygen by producing H_2O_2, which is eliminated by its conversion to H_2O in subsequent reactions. SA-induced CAT, POX, and SOD activities in salt-treated *A. annua* plants indicates that SA can play a critical role in modulating the cell redox balance, thereby protecting plants against oxidative damage (Yang and others 2004).

The content of endogenous H_2O_2 and O_2^- was measured to determine the internal ROS status induced in *A. annua* plants upon exposure to salinity treatments. The contents of both H_2O_2 and O_2^- were higher in plants treated with various concentrations of NaCl, indicating an increase in ROS level of the cells in salt-treated plants. The application of SA further induced the generation of ROS. Maximum ROS generation occurred with 100 mM NaCl + 1.00 mM SA, decreasing slightly when a higher concentration of salt was applied to the soil (Fig. 1a, b). Enhanced ROS production in *A. annua* plants has also been detected in response to environmental stresses other than salt stress (Qureshi and others 2005; Ferreira 2007; Guo and others 2010).

In our previous study, we also showed that mild boron or salt (NaCl) stress might increase H_2O_2 production in *A. annua* plants (Aftab and others 2010c, d, e, 2011). Among the antioxidant enzymes, CAT, POX, and SOD are well-known ROS scavengers. When the ROS contents exceed their threshold level under stress, they are scavenged by these enzymes. SA treatment increased CAT and POX activities to scavenge the elevated level of ROS in this study.

Artemisinin content and yield increased in *A. annua* plants when 50 and 100 mM NaCl were applied, compared to untreated plants; although 200 mM NaCl caused a decrease in artemisinin content (Fig. 2a, b). In defense of our results, Wallaart and others (2000), Ferreira (2007), Pu and others (2009), and Guo and others (2010) revealed a direct relationship between ROS and artemisinin content. In our previous studies also, artemisinin content was significantly enhanced upon exposure to boron stress (0.50 and 1.00 mM) and salt stress (50 and 100 mM), but it decreased when higher concentrations of either stressor were applied (Aftab and others 2010c, d). From the current research, it is clear that low levels of salinity (50 and 100 mM) might promote artemisinin biosynthesis (Fig. 2). According to Pu and others (2009), SA regulates gene transcription in the artemisinin biosynthetic pathway and thereby increases artemisinin concentration in *A. annua*

leaves. Our results are in agreement with the observations made by Wallaart and others (2000), Ferreira (2007), Pu and others (2009), and Guo and others (2010), who advocated that relatively high levels of ROS were produced in response to various stresses that, in turn, resulted in enhanced conversion of dihydroartemisinic acid to artemisinin.

Conclusion

In summary, this study revealed that salt stress had inhibitory effects on the growth of *A. annua* plants. Salinity stress also promoted lipid peroxidation and production of antioxidant enzymes in the leaves of *A. annua*. However, a comparatively low level of salinity (50 and 100 mM NaCl) stimulated artemisinin biosynthesis in plants. When SA was applied exogenously, it ameliorated the toxic effects of salt, improved the growth attributes, lowered lipid peroxidation, and promoted the production of antioxidant enzymes. Most importantly, artemisinin content and yield were higher in SA-treated plants under salt stress. This SA-induced increase in artemisinin content could be attributed to the upregulation of some genes associated with enhanced salt stress tolerance. Therefore, it might be concluded that SA had stimulatory effects on artemisinin biosynthesis under salt stress.

Acknowledgments The authors thank Prof. J. F. S. Ferreira of the U.S. Department of Agriculture—Agriculture Research Service, West Virginia, for his valuable suggestions on cultivation of *A. annua* plants in India. Financial support to the first author in the form of a Research Assistantship by the Council of Science and Technology, UP, Lucknow (CST/D-3539) is also gratefully acknowledged.

References

Abdin MZ, Israr M, Rehman RU, Jain SK (2003) Artemisinin, a novel antimalarial drug: biochemical and molecular approaches for enhanced production. Planta Med 69:1–11

Able AJ, Guest DI, Sutherland MW (1998) Use of a new tetrazolium based assay to study the production of superoxide radicals by tobacco cell cultures challenged with avirulent zoospores of *Phytophthora parasitica* var *nicotianae*. Plant Physiol 117: 491–499

Aftab T, Khan MMA, Idrees M, Naeem M, Moinuddin (2010a) Salicylic acid acts as potent enhancer of growth, photosynthesis and artemisinin production in *Artemisia annua* L. J Crop Sci Biotech 13:183–188

Aftab T, Khan MMA, Idrees M, Naeem M, Singh M, Ram M (2010b) Stimulation of crop productivity, photosynthesis and artemisinin production in *Artemisia annua* L. by triacontanol and gibberellic acid application. J Plant Interact 5:273–281

Aftab T, Khan MMA, Idrees M, Naeem M, Ram M (2010c) Boron induced oxidative stress, antioxidant defense response and changes in artemisinin content in *Artemisia annua* L. J Agron Crop Sci 196:423–430

Aftab T, Khan MMA, Idrees M, Naeem M, Hashmi N, Moinuddin (2010d) Effect of salt stress on growth, membrane damage, antioxidant metabolism and artemisinin accumulation in *Artemisia annua* L. Plant Stress 4:36–43

Aftab T, Khan MMA, Idrees M, Naeem M, Moinuddin (2010e) Effects of aluminium exposures on growth, photosynthetic efficiency, lipid peroxidation, antioxidant enzymes and artemisinin content of *Artemisia annua* L. J Phytol 2:23–37

Aftab T, Khan MMA, Idrees M, Naeem M, Moinuddin, Hashmi N (2011) Methyl jasmonate counteracts boron toxicity by preventing oxidative stress and regulating antioxidant enzyme activities and artemisinin biosynthesis in *Artemisia annua* L. Protoplasma. doi:10.1007/s00709-010-0218-5

Agarwal S, Sairam RK, Srivastava G, Meena R (2005) Changes in antioxidant enzyme activity and oxidative stress by abscisic acid and salicylic acid in wheat genotypes. Biol Plant 49:541–550

Ashraf M (2004) Some important physiological selection criteria for salt tolerance in plants. Flora 199:361–376

Athar HR, Khan A, Ashraf M (2008) Exogenously applied ascorbic acid alleviates salt induced oxidative stress in wheat. Environ Exp Bot 63:224–231

Azooz MM, Shaddad MA, Abdel-Latef AA (2004) The accumulation and compartmentation of proline in relation to salt tolerance of three sorghum cultivars. Indian J Plant Physiol 9:1–8

Barkosky RR, Einhellig FA (1993) Effects of salicylic acid on plant water relationship. J Chem Ecol 1:237–247

Bates LS, Walden RP, Teare ID (1973) Rapid determination of free proline for water stress studies. Plant Soil 39:205–207

Beauchamp CO, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287

Bethkey PC, Drew MC (1992) Stomatal and non-stomatal components to inhibition of photosynthesis in leaves of *Capsium annum* during progressive exposure to NaCl salinity. Plant Physiol 99:219–226

Cakmak I, Horst J (1991) Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean (*Glycine max*). Physiol Plant 83:463–468

Campbell WH (1999) Nitrate reductase structure, function and regulation: bridging the gap between biochemistry and physiology. Annu Rev Plant Physiol Mol Biol 5:277–303

Chandlee JM, Scandalios JG (1984) Analysis of variants affecting the catalase development program in maize scutellum. Theor Appl Genet 69:71–77

Duke SO, Paul RN (1993) Development and fine structure of the glandular trichomes of *Artemisia annua* L. Int J Plant Sci 155:107–118

Dwivedi RS, Randhawa NS (1974) Evaluation of rapid test for hidden hunger of zinc in plants. Plant Soil 40:445–451

El-Tayeb MA (2005) Response of barley grains to the interactive effect of salinity and salicylic acid. Plant Growth Regul 45: 215–224

Fariduddin Q, Hayat S, Ahmad A (2003) Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity, and seed yield in *Brassica juncea*. Photosynthetica 41:281–284

Ferreira JFS (2007) Nutrient deficiency in the production of artemisinin, dihydroartemisinic acid and artemisinic acid in *Artemisia annua* L. J Agric Food Chem 55:1686–1694

Gautam S, Singh PK (2009) Salicylic acid-induced salinity tolerance in corn grown under NaCl stress. Acta Physiol Plant 31:1185–1190

Gunes A, Inal A, Alpaslan M, Cicek N, Guneri E, Eraslan F, Guzelordu T (2005) Effects of exogenously applied salicylic acid on the induction of multiple stress tolerance and mineral nutrition in maize (*Zea mays* L.). Arch Agron Soil Sci 51:687–695

Gunes A, Inal A, Alpaslan M, Eraslan F, Bagci EG, Cicek N (2007) Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. J Plant Physiol 164:728–736

Guo XX, Yang XQ, Yang RY, Zeng QP (2010) Salicylic acid and methyl jasmonate but not rose bengal enhance artemisinin production through invoking burst of endogenous singlet oxygen. Plant Sci 178:390–397

Idrees M, Naeem M, Aftab T, Khan MMA, Moinuddin (2011) Salicylic acid mitigates salinity stress by improving antioxidant defence system and enhances vincristine and vinblastine alkaloids production in periwinkle [*Catharanthus roseus* (L.) G. Don]. Acta Physiol Plant. doi:10.1007/s11738-010-0631-6

Jaleel CA, Gopi R, Sankar B, Manivannan P, Kishorekumar A, Sridharan R, Panneerselvam R (2007) Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. S Afr J Bot 73:190–195

Jaleel CA, Riadh K, Gopi R, Manivannan P, Ines J, Al-Juburi HJ, Chang-Xing Z, Hong-Bo S, Panneerselvam R (2009) Antioxidant defense responses: physiological plasticity in higher plants under abiotic constraints. Acta Physiol Plant 31:427–436

Jaworski EG (1971) Nitrate reductase assay in intact plant tissue. Biochem Biophys Res Commun 43:1274–1279

Khan MH, Panda SK (2008) Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. Acta Physiol Plant 30:89–91

Khan W, Prithiviraj B, Smith D (2003) Photosynthetic response of corn and soybean to foliar application of salicylates. J Plant Physiol 160:485–492

Khan MN, Siddiqui MH, Mohammad F, Khan MMA, Naeem M (2007) Salinity induced changes in growth, enzyme activities, photosynthesis, proline accumulation and yield in linseed genotypes. World J Agri Sci 3:685–695

Khan MN, Siddiqui MH, Mohammad F, Naeem M, Khan MMA (2010) Calcium chloride and gibberellic acid protect linseed (*Linum usitatissimum* L.) from NaCl stress by inducing antioxidative defence system and osmoprotectant accumulation. Acta Physiol Plant 32:121–132

Khodary SEA (2004) Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt-stressed maize plants. J Agric Biol 6:5–8

Koca H, Bor M, Özdemir F, Türkan I (2007) The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environ Exp Bot 60:344–351

Kremsner PG, Krishna S (2004) Antimalarial combinations. Lancet 364:285–294

Kumar KB, Khan PA (1982) Peroxidase and polyphenol oxidase in excised ragi (*Eleusine coracana* cv. PR 202) leaves during senescence. Indian J Exp Bot 20:412–416

Lichtenthaler HK, Buschmann C (2001) Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. In: Current protocols in food analytical chemistry. Wiley, New York, pp F4.3.1–F4.3.8

Lutts S, Kinet JM, Bouharmont J (1995) Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. J Exp Bot 46:1843–1852

Mateo A, Funck D, Mühlenbock P, Kular B, Mullineaux PM, Karpinski S (2006) Controlled levels of salicylic acid are required for optimal photosynthesis and redox homeostasis. J Exp Bot 57:1795–1807

Meneguzzo S, Navari-Izzo F, Izzo R (1999) Antioxidative responses of shoots and roots of wheat to increasing NaCl concentrations. J Plant Physiol 155:274–280

Mishra A, Choudhuri MA (1999) Effects of salicylic acid on heavy metal-induced membrane deterioration mediated by lipoxygenase in rice. Biol Plant 42:409–415

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410

More SD, Hangarge DS, Raghavaiah CV, Joshi BM (2004) Performance of different safflower, *Carthamus tinctorious* L. genotypes with varied soil salinity levels. J Oilseeds Res 21:196–197

Mukherjee SP, Choudhuri MA (1983) Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Physiol Plant 58:166–170

Nathawat NS, Kuhad MS, Goswami CL, Patel AL, Kumar R (2005) Nitrogen-metabolizing enzymes: effect of nitrogen sources and saline irrigation. J Plant Nutr 28:1089–1101

Noreen Z, Ashraf M, Akram NA (2010) Salt-induced regulation of some key antioxidant enzymes and physio-biochemical phenomena in five diverse cultivars of turnip (*Brassica rapa* L.). J Agron Crop Sci 196:273–285

Prasad A, Kumar D, Anwar M, Singh DV, Jain DC (1998) Response of *Artemisia annua* L. to soil salinity. J Herbs Spices Med Plants 5:49–55

Pu GB, Ma DM, Chen JL, Ma LQ, Wang H, Li GF, Ye HC, Liu BY (2009) Salicylic acid activates artemisinin biosynthesis in *Artemisia annua* L. Plant Cell Rep 28:1127–1135

Qian Z, Gong K, Zhang L, Lv J, Jing F, Wang Y, Guan S, Wang G, Tang K (2007) A simple and efficient procedure to enhance artemisinin content in *Artemisia annua* L. by seeding to salinity stress. African J Biotech 6:1410–1413

Qureshi MI, Israr M, Abdin MZ, Iqbal M (2005) Responses of *Artemisia annua* L. to lead and salt-induced oxidative stress. Environ Exp Bot 53:185–193

Seeman JR, Critchley C (1985) Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. Planta 164:151–162

Shi Q, Bao Z, Zhu Z, Ying Q, Qian Q (2006) Effects of different treatments of salicylic acid on heat tolerance, chlorophyll fluorescence, and antioxidant enzyme activity in seedlings of *Cucumis sativa* L. Plant Growth Regul 48:127–135

Siddiqui MH, Khan MN, Mohammad F, Khan MMA (2008) Role of nitrogen and gibberellin (GA_3) in the regulation of enzyme activities and in osmoprotectant accumulation in *Brassica juncea* L. under salt stress. J Agron Crop Sci 194:214–224

Silveira JAG, Melo ARB, Viegas RA, Oliveira JTA (2001) Salinity induced effects on nitrogen assimilation related to growth in cowpea plants. Environ Exp Bot 46:171–179

Singh B, Usha K (2003) Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. Plant Growth Regul 39:137–141

Stepien P, Klobus G (2006) Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress. Biol Plant 50:610–616

Stevens J, Senaratna T, Sivasithamparam K (2006) Salicylic acid induces salinity tolerance in tomato (*Lycopersicon esculentum* cv. Roma): associated changes in gas exchange, water relations and membrane stabilization. Plant Growth Regul 49:77–83

Sudhir P, Murthy SDS (2004) Effects of salt stress on basic processes of photosynthesis. Photosynthetica 42:481–486

Syeed S, Khan NA (2010) Physiological aspects of salicylic acid-mediated salinity tolerance in plants. In: Anjum NA (ed) Plant nutrition and abiotic stress tolerance I. Plant stress 4(special issue 1), pp 39–46

Van Agtmael MA, Eggelte TA, van Boxtel CJ (1999) Artemisinin drugs in the treatment of malaria: from medicinal herb to registered medication. Trends Pharmacol Sci 20:199–204

Wallaart TE, Pras N, Beekman AC, Quax WJ (2000) Seasonal variation of artemisinin and its biosynthetic precursors in plants

of *Artemisia annua* of different geographical origin: proof for the existence of chemotypes. Planta Med 66:57–62

Wang LJ, Li SH (2006) Thermotolerance and related antioxidant enzyme activities induced by heat acclimation and salicylic acid in grape (*Vitis vinifera* L.) leaves. Plant Growth Regul 48:137–144

Yang Y, Qi M, Mei C (2004) Endogenous salicylic acid protects rice plants from oxidative damage caused by aging as well as biotic and abiotic stress. Plant J 40:909–919

Yildirim E, Turan M, Guvenc I (2008) Effect of foliar salicylic acid applications on growth chlorophyll and mineral content of cucumber (*Cucumis sativus* L.) grown under salt stress. J Plant Nutr 31:593–612

Zhao SS, Zeng MY (1986) Determination of qinghaosu in *Artemisia annua* L. by high performance liquid chromatography. Chin J Pharmacol Anal 6:3–5