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Salicylic acid alleviates salinity-caused damage to foliar functions, plant growth and antioxidant system in Ethiopian mustard (*Brassica carinata* A. Br.)

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Abstract

Background: Soil salinity is considered as one of the major environmental factors that has reduced plant productivity worldwide. This study investigates the impact of salinity on plant growth attributes, biochemical and physiological leaf characteristics in two cultivars (Adet and Merawi) of *Brassica carinata* and also explores the role of salicylic acid (SA) in mitigating the effect of salt stress.

Methods: Four-week-old cultivars were treated with NaCl (50, 100 and 150 mM) and SA (0.5 mM) and watered regularly with 100% field capacity. Thus, they were grown under eight different treatments (*T*1 = no NaCl, no SA; *T*2 = 0 mM NaCl with 0.5 mM SA; *T*3 = 50 mM NaCl without SA; *T*4 = 50 mM NaCl with 0.5 mM SA; *T*5 = 100 mM NaCl without SA; *T*6 = 100 mM NaCl with 0.5 mM SA; *T*7 = 150 mM NaCl without SA; and *T*8 = 150 mM NaCl with 0.5 mM SA). Nine-week-old cultivars were sampled for analyzing the growth attributes, plant water status, nitrate reductase activity, proline accumulation, photosynthetic traits, lipid peroxidation level and activity of antioxidant enzymes.

Results: Salinity treatments hampered the overall plant growth performance in a dose-dependent manner. Salinity also reduced photosynthetic efficiency by inhibiting chlorophyll synthesis, nitrate reductase activity, chlorophyll fluorescence, stomatal conductance, net photosynthetic and transpiration rates and plant water status. On the other hand, SA application alleviated the adverse effects of salinity and improved the performance of the studied parameters in both the cultivars. Higher dose of salinity increased proline production, but SA application mitigates this impact in both the cultivars studied. The activity of antioxidant enzymes increased under salt stress in a dose-dependent manner. SA treatment to normal or salinity-stressed plants increased the enzymes activity, showing that SA has a crucial role in modulating the cell redox balance and protecting the plants from oxidative damage. SA significantly reduced the salinity-caused effects on the overall performance of plants and their antioxidant systems in both the cultivars. Of the two cultivars, Adet was more tolerant to salinity than Merawi.

Conclusions: Foliar application of SA improved the performance of Ethiopian mustard cultivars and mitigated the damage caused by salt stress.

Keywords: Salicylic acid, Salinity stress, Morpho-physiological attributes, Biochemical changes, Reactive oxygen species, Enzymatic activities, Stress tolerance

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Background

According to an estimate [1], productivity of food crops needs to be increased by 70% for an extra 2.3 billion individuals up to 2050. Like other abiotic stresses, soil salinity has a remarkable impact on growth, yield and distribution of plants worldwide. Globally, saline soils constitute approximately 10% of the land surface (950 Mha) and 50% of the whole irrigated land (230 Mha) [2], and the influence of salinity is spreading consistently. Maintenance of plant productivity on the saline land will be one of the greatest challenges in the coming years. Salinity stress normally causes reduction in plant growth, which is interceded by an array of physiological, biochemical and molecular changes [2–6]. Salinity hampers the uptake of water, while the consequent high accumulation of Na^+ and Cl^- together with a decline of K^+ leads to a nutrient imbalance [7]. In general, high salinity causes stomatal closure and lowers the level of green pigments and photosynthesis. Further, it increases the generation of reactive oxygen species (ROS), viz. hydrogen peroxide, superoxide, hydroxyl radical and singlet oxygen, which damage the cellular machinery [8–12]. The ROS production leads to chlorophyll deprivation and membrane lipid peroxidation (measured as malondialdehyde content), reducing the membrane fluidity and selectivity [13, 14]. The most affected parameters include the net photosynthetic rate (P_N) and water use efficiency (WUE), which ultimately restrict the plant growth rate [3, 15, 16]. The decline in photosynthesis under salinity stress may be due to inhibition of photosystem II complex and a loss of chlorophyll pigments [17]. These alterations are reflected by chlorophyll fluorescence (F_v/F_m), which is conveniently used for detecting and quantifying the plant tolerance to stressful conditions [16, 18–21].

Plants have exhibited an array of enzymatic and non-enzymatic defense mechanisms to protect cells from oxidative damage [22–25]. Plant growth regulators such as auxins, brassinosteroid, jasmonates and strigolactones play a vital role in connection with the signaling network and the developmental and adaptive phenomena in plants growing under stress [3, 26, 27]. Salicylic acid (SA), a phenolic growth regulator, fortifies the plant defense against a variety of stresses [28–31]. Hao et al. [32] reported the SA-induced expression of 59 proteins involved in a variety of cell responses and metabolic processes in *Cucumis sativus*. SA application stimulated salt tolerance via enhancing the photosynthetic rate and carbohydrate metabolism in maize [33] and induced the NR activity, ATP sulfurylase and antioxidant enzymes in mungbean [34]. In *Arabidopsis*, SA application suppressed the adverse impact of salt stress by reducing the K^+ leakage in root tissues and increasing the activity of H^+-ATPase [35], which strengthens the Na^+/H^+ exchanger at the plasma membrane and reduces the Na^+ accumulation in cytosol [65]. SA treatment also reduces lipid peroxidation and may interact with other plant hormones to enhance plant resistance and/or tolerance to salt stress [29, 31, 36, 37].

Soil salinity is common in the Rift Valley, Awash Valley and the lowland areas of Ethiopia [38]. It may become more severe in the following years due to lack of appropriate management practices and a growing interest toward a heavy-irrigation agriculture. In the highlands of Ethiopia, *Brassica carinata* (Ethiopian mustard) is the third most important oil crop after *Guizotia abyssinica* (noug) and *Linum usitatissimum* (linseed) [39, 40]. Among the oil crops of the common ecological niche, *B. carinata* gives the highest yield. Its leaves are used as vegetable, whereas seeds are used in preparing 'Injera', a local food item. The high erucic-acid content of seeds renders them useful for the biodiesel, biopolymers, lubricants, and soap and surfactants industries [41]. The plant displays inter-cultivar/line variation for tolerance to salinity. Twenty-five strains of *B. carinata* have been examined for salt tolerance at the germination and seedling stages [42], but the role of SA with reference to salt-stress effects in this species has not been investigated so far. The present study attempts to determine the salinity-caused changes in plant growth, leaf water status, photosynthetic efficiency and the enzymatic antioxidant system and then to evaluate the interactive effect of SA application with reference to these parameters in the Adet and Merawi cultivars of *B. carinata.*

Methods

Experimental set-up

The experiments were conducted at University of Gondar, located at 12°35′14.19″N, 37°26′29.53″E, at 2143 m above mean sea level. The annual average of the maximum and minimum temperature at Gondar lies ~27 and ~16 °C, respectively, whereas mean relative humidity (RH) and yearly precipitation are ~56% and 1161 mm, respectively. During the experimental period (March–April), RH was 50%, the maximum and the minimum daily temperature remained 29 ± 1 and 18 ± 1 °C, respectively, and no rainfall was observed during experimentation.

Seeds of *Brassica carinata* (A. Br.) cultivars Adet and Merawi (Fig. 1a, b) procured from Gondar Agricultural Research Centre were sterilized with ethanol (80%) for around 15 min, rinsed with distilled water, and then sown in plastic trays containing soil (75%) and farmyard manure (FYM 25%). After 2 weeks of germination, uniform-sized seedlings of each cultivar were shifted in plastic pots (25 cm width × 26 cm height) filled with 8.5 kg media (comprising of soil and FYM in 3:1 ratio) and sown at a depth of ~2 cm. Each plastic pot contained

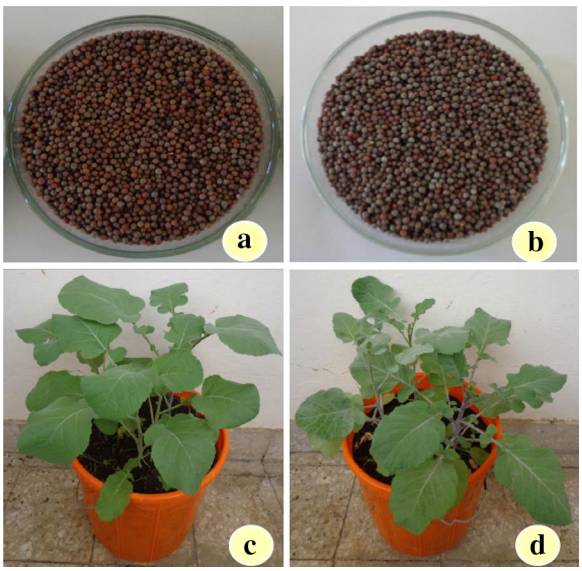

Fig. 1 Seeds of *Brassica carinata* (**a**) Adet (**b**) Merawi; and the respective potted plants (**c**, **d**)

three seedlings (Fig. 1c, d). The potted seedlings were irrigated with tap water daily at 100% field capacity (FC) up to 2 weeks, which was considered as the acclimatization period. The sandy loam soil (62.56% sand, 14.88% clay and 22.56% silt) had a pH of 7.23 and an EC of 0.69 ms cm^{-1}. After 4 weeks, a randomized design was adopted with 5 replications per treatment and 3 plants per replication for both the cultivars.

Salt treatment and foliar spray of salicylic acid

From the fifth week (after germination), the soil of each pot was treated with NaCl concentrations (0, 50, 100 and 150 mM) and watered regularly with 100% field capacity. Additionally, salicylic acid (0.5 mM) procured from SD Fine Chem Limited, Mumbai, India, was applied to the aerial plant parts four times (at one-week interval) starting from the fifth week after germination up to the eighth week. The pot soil was covered by a plastic sheet during the SA spray in order to avoid the access of SA solution to the root system. Thus, plants of each cultivar were grown under eight different treatments ($T1$ = no NaCl, no SA; $T2$ = 0 mM NaCl with 0.5 mM SA; $T3$ = 50 mM NaCl without SA; $T4$ = 50 mM NaCl with 0.5 mM SA; $T5$ = 100 mM NaCl without SA; $T6$ = 100 mM NaCl with 0.5 mM SA; $T7$ = 150 mM NaCl without SA; and $T8$ = 150 mM NaCl with 0.5 mM SA). NaCl dissolved in tap water was supplied (300 ml per pot) to the seedlings of $T3$–$T8$ on every second day, from the fifth week onward, while only tap water was used for $T1$ and $T2$. Sampling was done by uprooting the plants gently when they were 9 weeks old.

Measurement of growth variables

Plant growth attributes and some of the characteristic foliar features of both cultivars were recorded for each treatment, i.e., $T1$ to $T8$. The length of root and stem was measured in cm and opened leaves were counted. Ground-line basal diameter (mm) of the stem was measured with an electronic digital calliper. In addition, the length, width (each in mm) and area of leaf (mm^2) were measured using Leaf Area Meter (AM 300, ADC Bio Scientific Limited, UK). The roots, stems and leaves of plants from each treatment were separated to measure their total dry mass with the help of electronic digital balance (CY510, Citizen Scale, Poland). Five replications were used for each parameter.

Measurement of chlorophyll concentration

Chlorophyll concentration in leaf tissues was estimated following the method of Hiscox and Israelstam [43]. Test tubes containing 0.5 g of green-leaf tissue in 7 ml of dimethyl sulfoxide (DMSO) were kept in oven at 65 °C for 1 h, and DMSO was added to this chlorophyll extract obtained from each sample of each cultivar in order to make a volume of 10 ml. Of this, 3 ml extract was transferred to polystyrene cuvettes and optical density (OD) recorded at 480, 510, 645 and 663 nm, using a $T60$ UV–Vis spectrophotometer (PG Instruments Limited, England). DMSO was used for the blank. The chlorophyll and carotenoid contents were obtained using the formulae given by Duxbury and Yentsch [44] and MaClachlan and Zalick [45], respectively.

Measurement of relative water content

Water status of leaves of both the cultivars was estimated for each treatment by measuring the relative water content (RWC) of fully developed leaves, which were weighed to obtain the fresh weight (FW) and then kept in distilled water for overnight at about 5 °C in the dark, before obtaining their turgid weight (TW). It was then oven-dried (80 °C) for 12 h and weighed again to obtain the dry weight (DW). RWC was calculated as:

$$\mathrm{RWC} = \{(\mathrm{FW} - \mathrm{DW}) \div (\mathrm{TW} - \mathrm{DW})\} \times 100$$

Measurement of foliar functions

Chlorophyll fluorescence and gas exchange measurements were recorded in the morning (10–11 a.m.) from the first, second and third leaves of each cultivar, and means of the values were obtained. Chlorophyll fluorescence was recorded for each treatment by using a portable Multi-Mode OS5P Chlorophyll Fluorometer (Opti-Sciences, Inc., USA). Prior to fluorescence measurements, the upper surface of the leaf was pre-darkened for 30 min by using leaf clips to secure a complete

relaxation of all the reaction centers. The basal non-variable chlorophyll fluorescence (F_o), maximal fluorescence induction (F_m), and variable fluorescence (F_v) were determined to calculate the maximum quantum yield of PSII efficiency (F_v/F_m) by using the formula $F_v/F_m = (F_m - F_o)/F_m$. Moreover, stomatal conductance (g_s), net photosynthetic rate (P_n) and transpiration rate (E) were recorded from fully expanded attached leaves with the help of a portable leaf gas exchange system (ADC BioScientific Limited, U.K.). All these measurements were taken on 15 plants from each treatment and with 3 replicates for each plant. Water use efficiency (WUE) in the photosynthesizing leaf was worked out as the ratio of photosynthesis to water loss in transpiration, i.e., $WUE = P_n/E$.

Measurement of nitrate reductase activity and the proline and TBARS contents

Nitrate reductase (NR; E.C. 1.6.6.1) activity in the intact leaf tissue was estimated for each treatment by the method of Jaworski [46] and expressed in $nMNO_2\ g^{-1}$ FW h^{-1}. Leaf proline content for each treatment was estimated following the method of Bates et al. [47] and expressed in $\mu g\ g^{-1}$ FW. The content of total 2-thiobarbituric acid reactive substances (TBARS) was determined by using the method of Cakmak and Horst [48], which involves the use of trichloroacetic acid (TCA) and thiobarbituric acid (TBA), and expressed in $nmol\ g^{-1}$ FW.

Measurement of antioxidant enzymes

Leaf material (500 mg) from each cultivars and treatment was homogenized in the extraction buffer {0.5% Triton X-100 [$C_{14}H_{22}O(C_2H_4O)_n$] and 1% polyvinylpyrrolidone [$(C_6H_9NO)_n$] in 100 mM potassium phosphate buffer, pH 7.0}. Chilled mortar and pestle were used for preparing the crude extraction. The homogenate was then centrifuged at 15,000 rpm (20 min) at 4 °C, and the supernatant was used for antioxidant enzymes assays. Activity of catalase (CAT; E.C. 1.11.1.6) and superoxide dismutase (SOD; E.C. 1.15.1.1) was measured following a slightly modified method of Chandlee and Scandalios [49] and that of Beauchamp and Fridovich [50], respectively, and expressed in U mg^{-1} protein. Activity of peroxidase (POX; E.C. 1.11.1.7) was measured using guaiacol as the substrate, as described by Kumar and Khan [51] and modified by Husen [52], and expressed in U mg^{-1} protein.

Statistical analysis

Analysis of data was done by using the Statistical Package for Social Sciences (SPSS, Version 16.0) software (SPSS Inc., Illinois, USA). Two-way analysis of variance was performed to obtain the significant difference among treatments and cultivars. Significance of difference ($P < 0.05$) among the mean values was worked out by the Duncan test.

Results

The data regarding the effect of salinity (0, 50, 100 and 150 mM) and salicylic acid (SA) treatments (0 and 0.5 mM) on Adet and Merawi cultivars with reference to various growth attributes are given in Table 1. Salt stress markedly suppressed plant growth in a dose-dependent manner for both the cultivars at $P < 0.05$ level. High salt concentration (150 mM) showed a comparable effect in Adet and Merawi, as it reduced the root length by 47 and 49%, shoot length by 35 and 38%, number of leaves by 20 and 29%, leaf width by 17 and 19%, leaf length by 19 and 20% and leaf area by 28 and 27%, respectively. The roots of cv Adet were longer than those of cv Merawi under the control as well as saline conditions. The decrease in growth attributes was lesser in Adet than in Merawi. Further, all growth attributes of Adet cultivar except for the root length, basal diameter and leaf length were significantly enhanced by the foliar application of 0.5 mM SA. In the case of Merawi, all growth attributes except for the basal diameter and leaf length were significantly ($P < 0.05$ level) increased on application of 0.5 mM SA, compared to the control. Further, SA application on salt-stressed condition alleviated the effect of salinity and improved all the growth traits significantly ($P < 0.05$ level), compared to controls for both the cultivars.

There was a higher biomass production in roots, stem, leaves and the whole plant of the controls, compared to plants treated with salt or SA. Of the various salt concentrations, the highest dose (150 mM) was most effective in reducing the plant biomass production for both the cultivars. In comparison with the controls, the reduction in roots, stem, leaves and whole plant biomass production at 150 mM was up to 54, 46, 41 and 45%, respectively, in cv Adet and up to 55, 49, 42 and 46%, respectively, in cv Merawi. On the whole, Adet was better than Merawi in terms of biomass production. In both cultivars, foliar application of 0.5 mM SA significantly increased (at $P < 0.05$) the biomass production in all plant parts except roots, compared to the control. It also alleviated the effect of salinity significantly, and enhanced the biomass production of all plant parts in the salt-stressed plants of both the cultivars (Table 2).

Compared with the control, the quantity of leaf pigments (chlorophyll and carotenoids) declined with increase in the salinity level. At the highest concentration (150 mM) used, chlorophylls *a* and *b*, total chlorophyll and carotenoids declined by 57, 49, 48 and 50%, respectively in cv Adet, and by 55, 49, 53 and 58%, respectively, in cv Meravi, as compared to the control.

Table 1 Effect of salicylic acid treatments on growth attributes of *B. carinata* cultivars grown under salt stress

Cultivars	Treatments	Root length (cm)	Shoot length (cm)	Stem basal diameter (mm)	Number of leaves	Leaf area (mm^2)	Leaf width (mm)	Leaf length (mm)
Adet	Control	14.47 ± 0.68a	34.01 ± 2.01b	4.48 ± 0.23a	9.70 ± 0.44b	3482.13 ± 120.39b	54.41 ± 1.16b	114.50 ± 5.65a
	0 mM NaCl, with 0.5 mM SA	15.21 ± 0.74a (5.11)	46.82 ± 2.04a (37.67)	4.56 ± 0.21a (1.79)	12.51 ± 0.49a (28.97)	3773.52 ± 196.37a (8.37)	59.87 ± 1.23a (10.03)	121.43 ± 7.21a (6.05)
	50 mM NaCl without SA	12.94 ± 0.76b (10.57)	32.69 ± 1.98bc (3.88)	4.44 ± 0.13a (0.89)	9.20 ± 0.47b (5.15)	3201.51 ± 129.40b (8.06)	51.85 ± 0.98c (4.71)	108.93 ± 6.65a (4.86)
	50 mM NaCl with 0.5 mM SA	13.79 ± 0.68b (4.70)	42.83 ± 3.16a (25.93)	4.47 ± 0.19a (0.22)	11.24 ± 0.49a (15.88)	3473.95 ± 123.37a (0.23)	53.85 ± 1.05b (1.03)	112.78 ± 5.57a (1.50)
	100 mM NaCl without SA	10.86 ± 0.72bc (24.95)	29.11 ± 3.01c (14.41)	4.42 ± 0.17a (1.34)	8.77 ± 0.49b (9.59)	2868.69 ± 110.45c (17.62)	49.14 ± 1.05c (9.69)	100.45 ± 3.07ab (12.27)
	100 mM NaCl with 0.5 mM SA	12.83 ± 0.69c (11.33)	38.74 ± 2.44b (13.91)	4.43 ± 0.20a (1.12)	9.83 ± 0.45b (1.34)	3081.26 ± 112.04bc (11.51)	52.20 ± 1.05b (4.06)	110.74 ± 4.12a (3.28)
	150 mM NaCl without SA	7.59 ± 0.73d (47.55)	22.07 ± 2.36d (35.11)	4.20 ± 0.18ab (6.25)	7.68 ± 0.41c (20.82)	2497.72 ± 125.30de (28.27)	44.66 ± 1.17d (17.92)	92.75 ± 5.98b (19.00)
	150 mM NaCl with 0.5 mM SA	9.46 ± 0.70cd (34.62)	26.73 ± 1.58c (21.41)	4.28 ± 0.17a (4.46)	8.14 ± 0.37b (16.08)	2784.54 ± 110.82c (20.03)	48.64 ± 0.83c (10.60)	97.93 ± 5.07b (14.47)
Merawi	Control	12.16 ± 0.70b	33.50 ± 1.72b	4.38 ± 0.21a	9.33 ± 0.48b	3270.02 ± 133.89b	54.04 ± 1.01b	113.70 ± 5.87a
	0 mM NaCl, with 0.5 mM SA	13.61 ± 0.72a (11.92)	42.17 ± 2.41a (25.88)	4.41 ± 0.18a (0.68)	11.87 ± 0.52a (27.22)	3501.49 ± 111.45ab (7.08)	57.27 ± 1.31a (5.98)	120.73 ± 6.56a (6.18)
	50 mM NaCl without SA	10.88 ± 0.78b (10. 53)	31.05 ± 1.56c (7.31)	4.34 ± 0.16a (0.91)	8.29 ± 0.51b (11.15)	3016.92 ± 120.32c (7.74)	51.64 ± 1.12c (4.44)	106.53 ± 4.94a (6.31)
	50 mM NaCl with 0.5 mM SA	12.93 ± 0.86b (6.33)	38.64 ± 2.91b (15.34)	4.39 ± 0.18a (0.23)	10.72 ± 0.56a (14.90)	3184.06 ± 113.09bc (2.63)	53.60 ± 1.04b (0.81)	112.64 ± 4.85a (0.93)
	100 mM NaCl without SA	9.01 ± 0.62c (25.90)	27.68 ± 2.70c (17.31)	4.31 ± 0.15a (1.60)	7.12 ± 0.47c (23.69)	2663.94 ± 127.49d (18.53)	48.42 ± 0.94c (10.40)	97.04 ± 4.11b (14.65)
	100 mM NaCl with 0.5 mM SA	10.92 ± 0.79bc (10.20)	33.42 ± 1.76b (0.24)	4.32 ± 0.22a (1.37)	8.27 ± 0.42b (11.36)	2805.82 ± 115.19cd (14.20)	51.94 ± 1.07b (3.89)	105.74 ± 4.54a (7.00)
	150 mM NaCl without SA	6.10 ± 0.82e (49.84)	20.62 ± 3.00d (38.45)	3.87 ± 0.14b (11.64)	6.61 ± 0.40d (29.15)	2311.73 ± 118.83e (29.31)	43.73 ± 1.08d (19.08)	90.67 ± 4.01c (20.26)
	150 mM NaCl with 0.5 mM SA	8.52 ± 0.69d (29.93)	24.76 ± 1.84cd (26.19)	3.98 ± 0.12b (9.13)	7.24 ± 0.39c (22.40)	2596.83 ± 115.39de (20.59)	45.92 ± 0.94d (15.03)	96.07 ± 4.43b (15.51)

Each value represents the mean ± SE of five replicates. Numbers followed by different letters indicate significant differences ($P < 0.05$) according to the Duncan test

Values within parenthesis are percent variation as obtained from the control plants of respective cultivars

The chlorophyll b and carotenoid contents were significantly higher in Adet than in Merawi. Compared with the control, SA application (0.5 mM) to cv Adet caused a significant increase of 5, 10 and 21% in the chlorophyll *b*, total chlorophyll and carotenoids contents, respectively, while chlorophyll *a* content increased only nonsignificantly. In cv Merawi, however, only the total chlorophyll increased significantly (by about 7%) over the control. Furthermore, foliar application of SA on salt-stressed plants markedly reduced the damage caused by salinity to chlorophylls *a* and *b*, total chlorophyll and carotenoids; thus, *T*8 showed a significant ($P < 0.05$ level) improvement over *T*7 in both the cultivars (Table 3).

Salt-stress treatments reduced the physiological efficiency of leaves in both the cultivars in comparison with the respective controls. The degree of reduction of RWC was high (around 26%) for both the cultivars at 150 mM salt treatment. However, foliar application of SA (0.5 mM) on salt-stressed plants significantly reduced the salinity-induced loss in both the cultivars (Table 4). Furthermore, in general, chlorophyll fluorescence (F_v/F_m), stomatal conductance (g_s), net photosynthetic rate (P_n), transpiration rate (E) and water use efficiency

Table 2 Effect of salicylic acid treatments on the biomass of *B. carinata* cultivars grown under salt stress

Cultivars	Treatments	Root (g)	Stem (g)	Leaves (g)	Whole plant (g)
Adet	Control	0.42 ± 0.014a	1.33 ± 0.043b	1.24 ± 0.037b	2.99 ± 0.13b
	0 mM NaCl, with 0.5 mM SA	0.44 ± 0.018a (4.76)	1.63 ± 0.045a (22.56)	1.51 ± 0.032a (21.77)	3.58 ± 0.2a (19.73)
	50 mM NaCl without SA	0.35 ± 0.013b (16.67)	1.14 ± 0.040c (14.29)	1.12 ± 0.027c (9.68)	2.61 ± 0.1d (12.71)
	50 mM NaCl with 0.5 mM SA	0.36 ± 0.011b (14.29)	1.42 ± 0.059b (6.77)	1.23 ± 0.026bc (0.81)	3.01 ± 0.15b (0.67)
	100 mM NaCl without SA	0.29 ± 0.009d (30.95)	0.96 ± 0.032c (27.82)	0.98 ± 0.029de (20.97)	2.23 ± 0.12e (25.42)
	100 mM NaCl with 0.5 mM SA	0.31 ± 0.012d (26.19)	1.22 ± 0.059d (8.27)	1.18 ± 0.030c (4.84)	2.71 ± 0.1c (9.36)
	150 mM NaCl without SA	0.19 ± 0.015g (54.76)	0.71 ± 0.051g (46.62)	0.73 ± 0.041g (41.13)	1.63 ± 0.09g (45.48)
	150 mM NaCl with 0.5 mM SA	0.25 ± 0.016ef (40.48)	0.94 ± 0.040cd (29.32)	1.01 ± 0.036de (18.55)	2.20 ± 0.14b (26.42)
Merawi	Control	0.40 ± 0.016a	1.30 ± 0.047b	1.18 ± 0.032c	2.88 ± 0.12b
	0 mM NaCl, with 0.5 mM SA	0.43 ± 0.012a (7.50)	1.61 ± 0.049a (23.85)	1.40 ± 0.028a (18.64)	3.44 ± 0.15a (19.44)
	50 mM NaCl without SA	0.32 ± 0.014c (20.00)	1.06 ± 0.037d (18.46)	1.04 ± 0.022d (11.86)	2.42 ± 0.08c (15.97)
	50 mM NaCl with 0.5 mM SA	0.34 ± 0.016bc (15.00)	1.34 ± 0.040b (3.08)	1.21 ± 0.037bc (2.54)	2.89 ± 0.14b (0.35)
	100 mM NaCl without SA	0.27 ± 0.010e (32.00)	0.81 ± 0.046e (37.69)	0.93 ± 0.032e (21.19)	2.07 ± 0.09e (28.13)
	100 mM NaCl with 0.5 mM SA	0.30 ± 0.009d (25.00)	1.10 ± 0.056cd (15.38)	1.10 ± 0.032c (6.78)	2.50 ± 0.12c (13.19)
	150 mM NaCl without SA	0.18 ± 0.013g (55.00)	0.66 ± 0.043g (49. 23)	0.68 ± 0.038g (42.37)	1.54 ± 0.07g (46.53)
	150 mM NaCl with 0.5 mM SA	0.23 ± 0.014e (42.50)	0.80 ± 0.047f (38.46)	0.84 ± 0.040f (28.81)	1.87 ± 0.1e (35.07)

Each value represents the mean ± SE of five replicates. Numbers followed by different letters indicate significant differences ($P < 0.05$) according to the Duncan test. Values within parenthesis are percent variation as obtained from the control plants of respective cultivars

(WUE) were reduced in salinity-affected plants. In both cultivars, the degree of reduction in F_v/F_m, g_s, P_n, E and WUE was increased with increase in the level of salinity. At 150 mM, cv Adet exhibited a decrease of 23% in F_v/F_m, 27% in g_s, 38% in P_n, 30% in E and 11% in WUE in comparison with the control, whereas these parameters declined by 24, 24, 42, 27 and 21%, respectively, in cv Merawi. The values of all these parameters except g_s were higher in Adet than in Merawi cultivar. SA application increased P_n, compared to the control plants, and also alleviated significantly the salinity-induced reduction in F_v/F_m, g_s, P_n, E and WUE in both the cultivars (Table 4).

NR activity was reduced under salinity stress; the degree of reduction increasing with the increase in salinity level. Thus, at 150 mM salt concentration, it declined by ~ 19% in both the cultivars. On the contrary, compared to the control, it increased by 6% in Adet and by a non-significant 2% in Merawi due to SA application. In general, Adet showed a higher NR activity than Merawi. In both these cultivars, SA application significantly ($P < 0.05$ level) reduced the salinity-induced decline of NR activity in the salt-stressed plants (Fig. 2a).

The contents of proline and TBARS increased significantly under salinity stress in both the cultivars in a dose-dependent manner (Fig. 2b, c). Thus, compared to the control plants, the proline and TBARS contents increased by 16 and 57%, respectively, in cv Adet, and up to 15 and 53%, respectively, in cv Merawi under 150 mM salt concentration. The level of increase of both proline and TBARS was higher in Adet than in Merawi. These salinity-induced increases were reduced greatly by SA application ($P < 0.05$), in both the cultivars (Fig. 2b, c).

The activity of antioxidant enzymes, viz. superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) increased with increasing salt stress in both the cultivars and went up to 29, 27 and 179%, respectively, in cv Adet, and up to 32, 25 and 194% in cv Merawi, respectively, with 150 mM salt treatment, in comparison with the

Table 3 Effect of salicylic acid treatments on leaf pigments of *B. carinata* cultivars grown under salt stress

Cultivars	Treatments	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)
Adet	Control	6.89 ± 0.57a	4.93 ± 0.12a	12.17 ± 0.17a	3.14 ± 0.06b
	0 mM NaCl, with 0.5 mM SA	7.13 ± 0.68a (3.48)	5.21 ± 0.08c (5.68)	13.42 ± 0.30b (10.27)	3.81 ± 0.08a (21.34)
	50 mM NaCl without SA	5.17 ± 0.47b (24.96)	4.16 ± 0.21b (15.62)	10.27 ± 0.15c (15.61)	2.95 ± 0.07b (6.05)
	50 mM NaCl with 0.5 mM SA	6.79 ± 0.58a (1.45)	4.87 ± 0.14a (1.22)	12.08 ± 0.15a (0.74)	3.07 ± 0.05b (2.23)
	100 mM NaCl without SA	4.71 ± 0.43bc (31.64)	3.47 ± 0.15d (29.61)	9.32 ± 0.19d (23.42)	2.41 ± 0.04d (23.25)
	100 mM NaCl with 0.5 mM SA	5.28 ± 0.56b (23.37)	4.63 ± 0.19a (6.09)	10.28 ± 0.28c (15.53)	3.04 ± 0.05b (3.18)
	150 mM NaCl without SA	2.96 ± 0.47e (57.04)	2.47 ± 0.21f (49.90)	6.21 ± 0.26g (48.97)	1.57 ± 0.08f (50.00)
	150 mM NaCl with 0.5 mM SA	4.03 ± 0.45c (41.51)	3.26 ± 0.14d (33.87)	8.02 ± 0.22d (34.10)	2.26 ± 0.04d (28.03)
Merawi	Control	6.43 ± 0.54a	4.12 ± 0.13b	13.04 ± 0.15a	3.02 ± 0.04b
	0 mM NaCl, with 0.5 mM SA	7.02 ± 0.53a (9.18)	4.97 ± 0.18b (20.63)	12.84 ± 0.28b (1.53)	3.25 ± 0.09b (7.62)
	50 mM NaCl without SA	5.04 ± 0.44b (21.62)	4.03 ± 0.13b (2.18)	10.13 ± 0.24c (14.88)	2.88 ± 0.06c (4.64)
	50 mM NaCl with 0.5 mM SA	6.37 ± 0.42a (0.93)	4.10 ± 0.12b (0.49)	11.07 ± 0.18a (15.11)	3.00 ± 0.04b (0.66)
	100 mM NaCl without SA	4.52 ± 0.38bc (29.70)	3.22 ± 0.13e (21.84)	9.17 ± 0.13d (29.68)	2.35 ± 0.06d (22.19)
	100 mM NaCl with 0.5 mM SA	5.11 ± 0.45bc (20.53)	4.17 ± 0.21b (1.21)	10.02 ± 0.22c (23.16)	2.97 ± 0.07b (1.66)
	150 mM NaCl without SA	2.85 ± 0.38e (55.68)	2.09 ± 0.16g (49.27)	6.08 ± 0.24g (53.37)	1.24 ± 0.07g (58.94)
	150 mM NaCl with 0.5 mM SA	3.99 ± 0.32c (37.95)	3.00 ± 0.16e (27.18)	8.05 ± 0.20f (38.27)	2.07 ± 0.05e (31.46)

Each value represents the mean ± SE of five replicates. Numbers followed by different letters indicate significant differences ($P < 0.05$) according to the Duncan test. Values within parenthesis are percent variation as obtained from the control plants of respective cultivars

control. The CAT and POX activity was higher in Adet, while SOD went ahead in Merawi. Moreover, SA application significantly decreased the salinity-induced increase in the activity of SOD, CAT and POX enzymes in both the cultivars, as shown in Fig. 3.

Discussion

The present study indicates that the salinity-induced loss of the growth and biomass of *B. carinata* is relatively stronger in cultivar Merawi than in Adet. The saline environment in the soil influences water imbibition by roots due to low osmotic potential of the substrate, besides hampering the phenomena of photosynthesis, protein synthesis, nutrient homeostasis, compatible solutes accumulation and the antioxidant defense mechanisms [5, 8, 25, 53]. The salinity-caused decline in growth and biomass of *B. carinata* cultivars might be due to reduced leaf area, imbalance in plant water status and low production of photoassimilates [3–5, 54]. The beneficial role of various plant hormones, including salicylic acid (SA), in signaling network, and in the developmental and adaptation processes of plant species against the biotic and abiotic stresses has long been known. SA application significantly improved the growth attributes in *B. carinata*, as observed earlier in maize [33], barley [55], mungbean [56] and mustard [57].

The salt-induced decline in the chlorophylls *a* and *b*, total chlorophyll and carotenoids contents in *B. carinata* is likely to be due to the oxidation of chlorophyll and other chloroplast pigments and the instability of pigment-protein complex under the influence of salinity [58]. The positive influence of SA application substantiates the early findings with certain crops including soybean [59], maize [33] and sunflower [60]. This could possibly involve stimulation of Rubisco activity and leaf pigment biosynthesis.

Relative water content (RWC) of leaf indicates the physiological water status of plants. In our study, the

Table 4 Effect of salicylic acid treatments on functional attributes of leaves of *B. carinata* cultivars grown under salt stress

Cultivars	Treatments	Relative water content (%)	Chlorophyll fluorescence (Fv/Fm)	Stomatal conductance (mol $m^{-2}s^{-1}$)	Net photosynthetic rate (μ mol CO_2 m^{-2} s^{-1})	Transpiration rate (m mol $m^{-2}s^{-1}$)	Water use efficiency
Adet	Control	72.69 ± 1.58a	0.821 ± 0.007a	0.196 ± 0.006a	15.77 ± 0.59b	5.20 ± 0.18a	3.03 ± 0.29a
	0 mM NaCl, with 0.5 mM SA	73.86 ± 3.17a (1.61)	0.834 ± 0.004a (1.58)	0.198 ± 0.004a (1.02)	19.21 ± 0.56a (21.81)	5.38 ± 0.19a (3.46)	3.57 ± 0.25a (17.82)
	50 mM NaCl without SA	71.40 ± 2.18a (1.77)	0.781 ± 0.013b (4.87)	0.182 ± 0.002b (7.14)	14.72 ± 0.56bc (6.66)	4.97 ± 0.14a (4.42)	2.96 ± 0.27b (2.31)
	50 mM NaCl with 0.5 mM SA	72.06 ± 2.77a (0.86)	0.815 ± 0.011a (0.73)	0.194 ± 0.004a (1.02)	17.13 ± 0.59a (8.62)	5.03 ± 0.13a (3.27)	3.41 ± 0.24a (12.54)
	100 mM NaCl without SA	68.72 ± 2.62a (5.46)	0.738 ± 0.007c (10.11)	0.161 ± 0.004c (17.86)	11.87 ± 0.73d (24.73)	4.46 ± 0.17b (14.23)	2.66 ± 0.19b (12.21)
	100 mM NaCl with 0.5 mM SA	72.84 ± 2.12a (0.21)	0.764 ± 0.016b (6.94)	0.167 ± 0.005c (14.80)	15.07 ± 0.61c (4.44)	4.56 ± 0.17ab (12.31)	3.30 ± 0.17a (8.91)
	150 mM NaCl without SA	52.83 ± 2.17c (27.32)	0.628 ± 0.009d (23.51)	0.143 ± 0.003e (27.04)	9.74 ± 0.74e (38.24)	3.64 ± 0.14e (30.00)	2.68 ± 0.19b (11.55)
	150 mM NaCl with 0.5 mM SA	65.49 ± 1.98ab (9.91)	0.741 ± 0.007b (9.74)	0.163 ± 0.007cd (16.84)	11.74 ± 0.82d (25.55)	4.09 ± 0.14c (21.35)	2.87 ± 0.14b (5.28)
Merawi	Control	70.28 ± 1.74a	0.813 ± 0.008a	0.185 ± 0.005a	15.39 ± 0.52b	4.81 ± 0.20a	3.20 ± 0.24a
	0 mM NaCl, with 0.5 mM SA	71.94 ± 2.64a (2.36)	0.827 ± 0.006a (1.72)	0.187 ± 0.006a (1.08)	18.25 ± 0.63a (18.58)	4.97 ± 0.23a (3.33)	3.67 ± 0.23a (14.69)
	50 mM NaCl without SA	69.03 ± 2.17a (1.78)	0.771 ± 0.011b (5.17)	0.167 ± 0.004c (9.73)	14.08 ± 0.57c (8.51)	4.61 ± 0.12ab (4.16)	3.05 ± 0.22b (4.69)
	50 mM NaCl with 0.5vmM SA	71.34 ± 2.04a (1.51)	0.802 ± 0.013a (1.35)	0.184 ± 0.005a (0.54)	15.68 ± 0.57b (1.88)	4.72 ± 0.19ab (1.87)	3.32 ± 0.21ab (3.75)
	100 mM NaCl without SA	66.13 ± 1.83ab (5.90)	0.726 ± 0.011c (10.70)	0.152 ± 0.003d (17.84)	11.23 ± 0.62d (27.03)	4.02 ± 0.18c (16.42)	2.79 ± 0.21b (12.81)
	100 mM NaCl with 0.5 mM SA	70.28 ± 1.74a (0.00)	0.758 ± 0.013b (6.77)	0.161 ± 0.004c (12.97)	14.71 ± 0.57c (4.42)	4.16 ± 0.19c (13.51)	3.54 ± 0.19a (10.63)
	150 mM NaCl without SA	51.52 ± 2.08c (26.69)	0.614 ± 0.007d (24.48)	0.139 ± 0.002e (24.86)	8.85 ± 0.63e (42.50)	3.51 ± 0.12e (27.03)	2.52 ± 0.17c (21.25)
	150 mM NaCl with 0.5 mM SA	63.48 ± 1.53b (9.68)	0.738 ± 0.008b (9.23)	0.151 ± 0.006d (18.38)	11.03 ± 0.80d (28.33)	3.79 ± 0.13de (21.21)	2.91 ± 0.13b (9.06)

Each value represents the mean ± SE of five replicates. Numbers followed by different letters indicate significant differences ($P < 0.05$) according to the Duncan test. Values within parenthesis are percent variation as obtained from the control plants of respective cultivars

decreased RWC in both the cultivars under increased salinity is indicative of a loss of cell turgor that leads to a limited water availability for the cell extension and expansion. Moreover, the increase in leaf RWC in response to SA application could possibly be an adaptive symptom to improve the degree of moistness and sustain the water balance in plant tissues under the salinity-induced osmotic stress [61, 62].

As the plant growth is intimately linked to the rate of photosynthesis, any decline in growth due to salt stress is attributable to the suppression of photosynthesis [3, 56, 63, 64]. The dose-dependent salinity-induced reductions in g_s, P_n, E and WUE differ considerably between the cultivars, possibly due to their differential optimum requirement of photoassimilates for healthy growth, whereas the alleviative effect of SA might be due to its positive role in chlorophyll biosynthesis and/or nitrate mobilization in the tissue [65]. SA also stimulates Rubisco activity [33]. Ashraf et al. [66] found the influence of SA on photosynthesis to be concentration-dependent; low concentrations (less than 10 μM) mitigated the salinity-induced decline in photosynthesis in various plant species. In *Brassica juncea* also, SA alleviated the adverse effects of salinity and improved P_n and plant growth by enhancing the enzymes action in ascorbate–glutathione pathway, thus suggesting its role in maintaining the redox balance under salt stress [57]. *Arabidopsis* mutant with high endogenous SA concentration (*siz1*) exhibited reduced stomatal apertures and increased salt tolerance [67]. Our observations, showing a dose-dependent decline under salt stress and a rise due to SA treatment, find support from some early reports on tomato [64], *Torreya grandis* [61] and *Vicia faba* [16]. The decline of E and WUE under salt stress

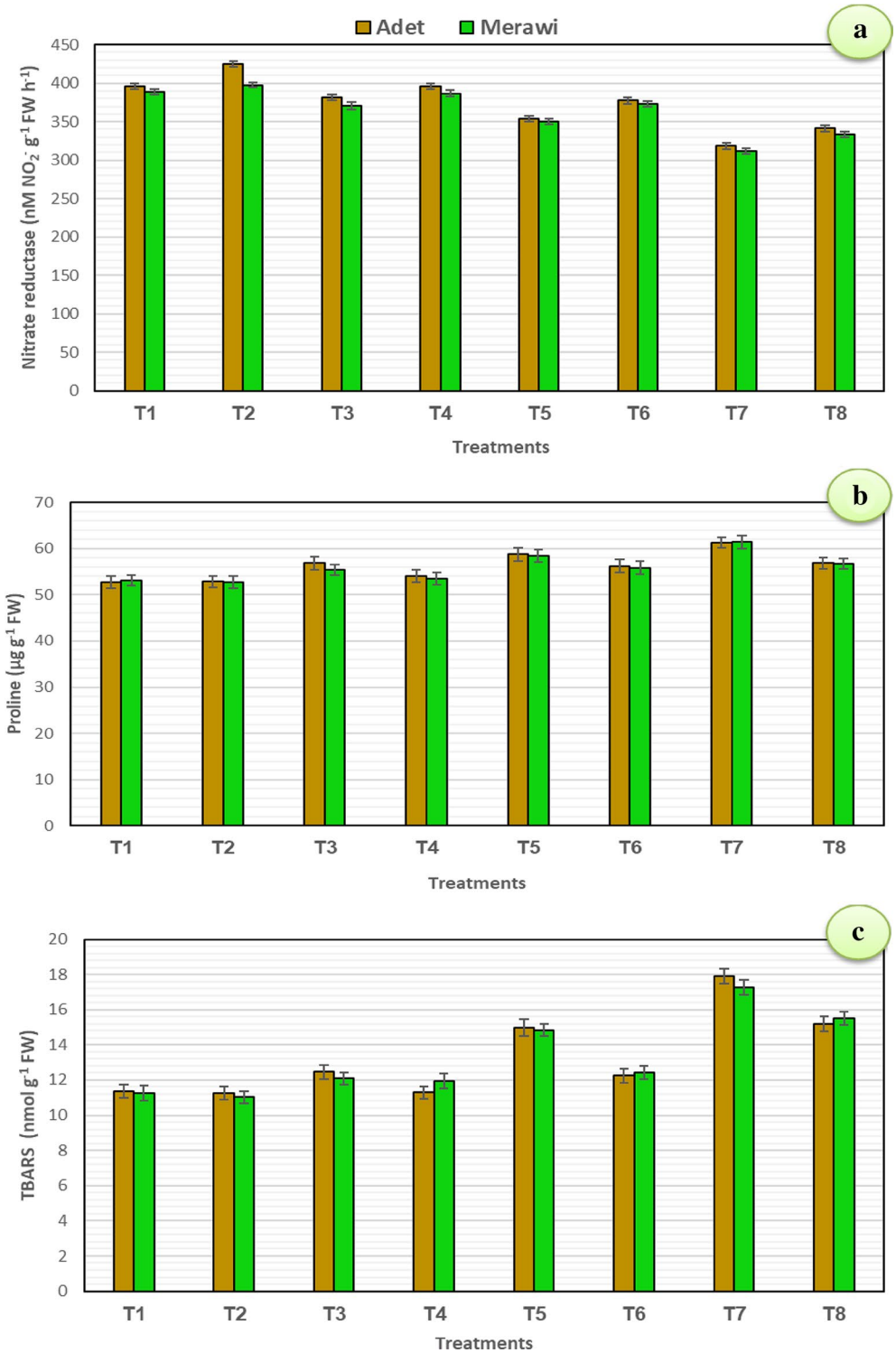

Fig. 2 Effect of salicylic acid treatments on the **a** nitrate reductase, **b** proline and **c** lipid peroxidation levels in the leaves of *B. carinata* cultivars grown under salt stress (where *T*1 = control; *T*2 = 0 mM NaCl with 0.5 mM SA; *T*3 = 50 mM NaCl without SA; *T*4 = 50 mM NaCl with 0.5 mM SA; *T*5 = 100 mM NaCl without SA; *T*6 = 100 mM NaCl with 0.5 mM SA; *T*7 = 150 mM NaCl without SA; and *T*8 = 150 mM NaCl with 0.5 mM SA). Each value represents the mean ± SE of five replicates

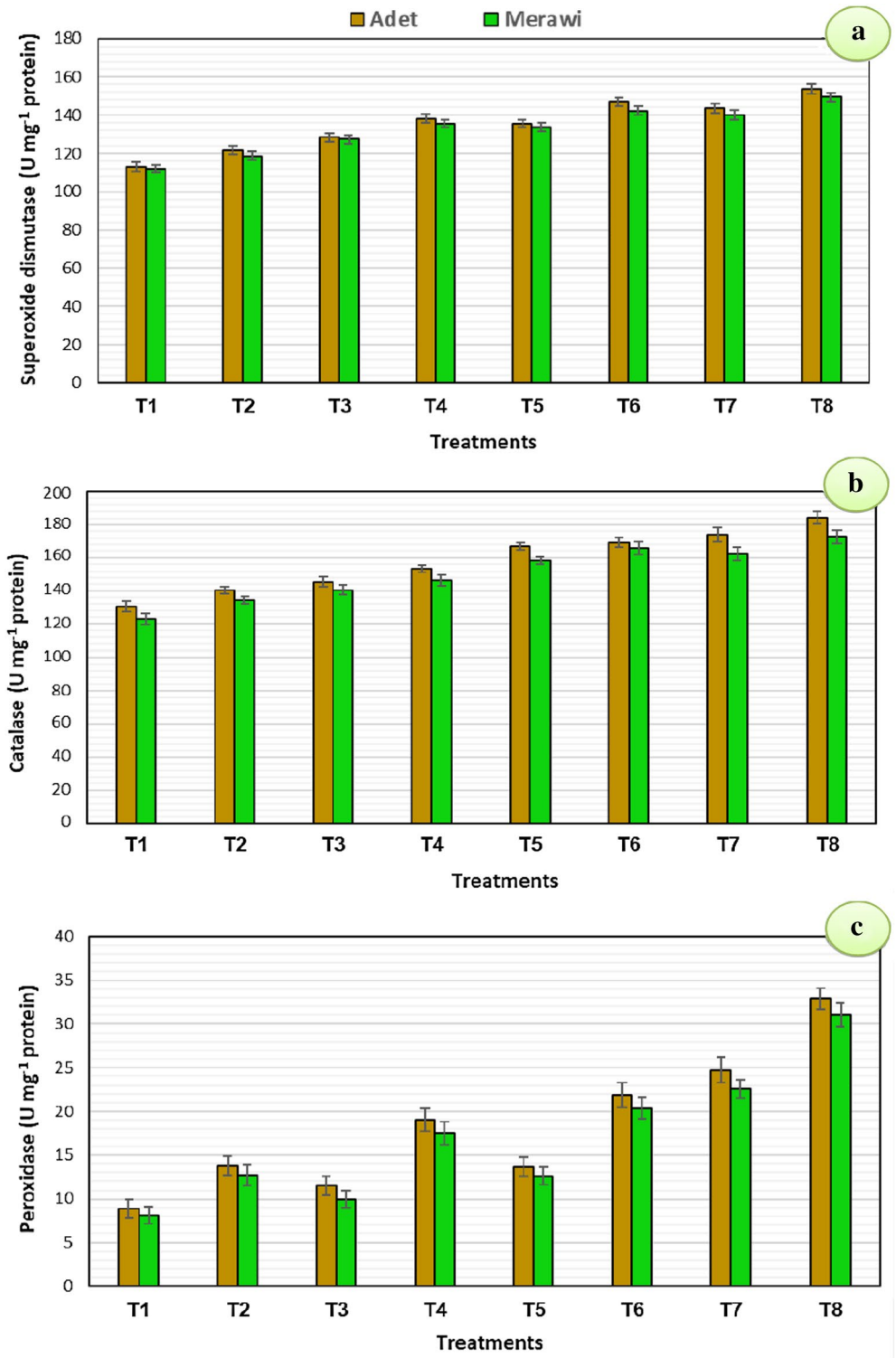

Fig. 3 Effect of salicylic acid treatments on antioxidant enzymes, viz. **a** superoxide dismutase, **b** catalase and **c** peroxidase in the leaves of *B. carinata* cultivars grown under salt stress (where *T*1 = control; *T*2 = 0 mM NaCl with 0.5 mM SA; *T*3 = 50 mM NaCl without SA; *T*4 = 50 mM NaCl with 0.5 mM SA; *T*5 = 100 mM NaCl without SA; *T*6 = 100 mM NaCl with 0.5 mM SA; *T*7 = 150 mM NaCl without SA; and *T*8 = 150 mM NaCl with 0.5 mM SA). Each value represents the mean ± SE of five replicates

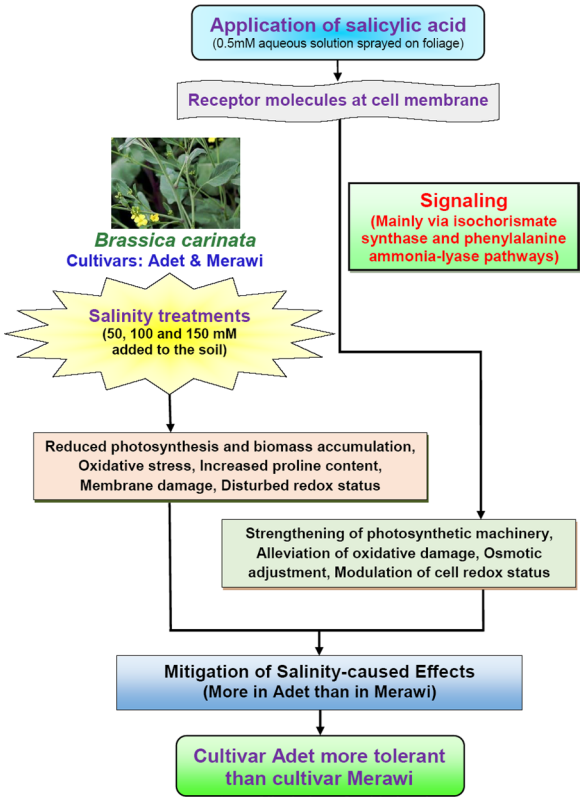

Fig. 4 A summarized depiction of the impact of salinity and the role/action of salicylic acid in stress mitigation

and their restoration by SA application in both cultivars also go in line with earlier investigation on vegetable crops [64, 68]. Salinity often alters water balance and thus reduces the WUE [3, 69], possibly due to inhibition of water absorption by roots and water translocation from roots to aerial plant parts.

The reduction in the photochemical efficiency of PSII (F_v/F_m) under stressful environment, duly linked with a decline of photosynthetic attributes, including leaf pigments and biomass production, has been used as an indicator for determining the seedling-stock quality [19, 70]. The dose-dependent decrease in F_v/F_m, as recorded in this study indicates that salinity affects the photochemistry of photosynthesis [3, 71]. Reduction in F_v/F_m ratio and a non-photochemical quenching coefficient (qN) under salt stress, and their restoration by SA treatment, were also observed in tomato plants [64]. Nevertheless, Asensi-Fabad and Munné-Bosch [72] have reported that under the salt-stress condition, the SA-deficient and SA-hyperaccumulating *Arabidopsis* mutants exhibited only insignificant difference in chlorophyll contents and the F_v/F_m ratio.

Nitrate reductase (NR) limits the reaction rate during nitrogen assimilation and hence is important for metabolic regulation and protein synthesis. NR activity was reduced by salinity stress in both the cultivars and increased by SA application due to mitigation of salinity-induced effects possibly by stabilizing the plasma membrane, as also observed in wheat [73]. This, in turn, could enhance the uptake of nutrients including nitrate, which induces NR [74].

In general, elevated levels of TBARS content, a product of lipid peroxidation, indicate the damage caused by free radicals to cell membranes that leads to oxidative stress. Our data depict a salt-concentration-dependent increase in the TBARS content in both the cultivars. High levels of H_2O_2 possibly damage the membrane, which expedites the generation of hydroxyl radicals and thus leads to lipid peroxidation [75]. SA application was ameliorative, possibly through improved membrane functioning, but the cultivar sensitivity to oxidative stress varied.

Proline detoxifies the excess ROS, improves the osmotic adjustment, lends protection to biological membranes and also stabilizes enzymes and proteins [22, 76]. The leaf proline content increased substantially with increase in salinity but SA application mitigates this impact in both the cultivars studied. Misra and Misra [77] have reported that the up-regulation of proline biosynthesis enzymes (viz. pyrroline-5-carboxylate reductase and γ-glutamyl kinase) and the down-regulation of proline oxidase activity led to an enhanced proline status, which helped in maintaining the cell turgor under salinity stress in *Rauwolfia serpentina*.

Activation of antioxidant enzymes is a vital strategy adopted by various plants to combat the ROS-induced oxidative damage and increase the stress tolerance. In our study, expression of antioxidant enzymes (SOD, CAT and POX) increased under salt stress in a dose-dependent manner. SA treatment to normal or salinity-stressed plants increased the enzymes activity, showing that SA can have a crucial role in modulating the cell redox balance and protecting the given plants from oxidative damage. The increased SOD activity facilitated the superoxide radical scavenging, which led to increased plant tolerance to oxidative stress. Increase in CAT and POX activity due to salinity as well as SA was also reported by Jini and Joseph [37]. SA pre-treatment mitigates the negative influences of salinity on photosynthesis and plant growth by strengthening the antioxidant system [56], whereas SA deficiency can facilitate the salinity-induced damage and suppress the antioxidant activities, as observed in *NahG* transgenic of *Arabidopsis* lines [78]. Li et al. [23] also observed the SA-induced enhanced salt tolerance in wheat through an improved transcript level of antioxidant genes such as *GPX1*, *GPX2*, *DHAR*, *GR*, *GST1*, *GST2*, *MDHAR* and *GS*, and a higher activity of the ascorbate (AsA)-GSH pathway enzymes.

Conclusion

Analysis of the data on growth features, photosynthetic efficiency and defense status of the two cultivars of *B. carinata* has brought out that growth performance of cv Adet was better than that of cv Merawi in terms of size as well as biomass of both root and shoot under salinity stress. Although differences in relative water content, chlorophyll fluorescence and stomatal conductance were nonsignificant, the chlorophyll contents, net photosynthetic rate and water use efficiency were markedly less affected by salinity in cv Adet. Likewise, although NR activity was almost similar and lipid peroxidation in terms of TBARS content was a little more in cv Adet, larger proline content and better modulation of antioxidant enzymes seemingly overcame the adverse impact of stress and displayed a better tolerance capacity and improved the growth of cv Adet, compared to cv Merawi. SA application mitigated the impact of salinity in both the cultivars studied, but was relatively more effective in cultivar Adet. A summarized impact of salinity and the role of SA in stress mitigation is presented in Fig. 4.

Authors' contributions

AH made major contribution to data collection, experimental work and drafted the manuscript. AH and MI wrote and reviewed the manuscript. SS and MKAA assisted in enzyme assays and statistical analyses, respectively. All authors read and approved the final manuscript.

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