



Genotypic differences for salt tolerance in bael (*Aegle marmelos*) cultivars

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ABSTRACT

Salinity induced changes in physiological relations and the concomitant effects on plant growth were recorded in four bael (*Aegle marmelos* Correa) cultivars, viz. NB-5, NB-9, CB-1 and CB-2. Plants raised in normal soil (EC_e 1.3 dS/m) were irrigated with tap (EC_{iw} 0.5 dS/m) and saline (EC_{iw} 3 and 6 dS/m) waters. Data were recorded for growth, physiological parameters and mineral nutrition 180 days after imposing the salt treatments. NB-5 outperformed other cultivars under saline conditions by maintaining higher leaf chlorophyll and proline levels, retaining Na^+ ions in stem and root tissues and by preferentially accumulating K^+ and Ca^{2+} ions to overcome the toxic effects of Na^+ . Break down of salt tolerance in other cultivars at 6 dS/m salinity can be explained by build up of Na^+ to the toxic levels and an accompanying decrease in leaf and stem K^+ concentrations. Based on these findings, bael cultivar NB-5 appears to be suitable for commercial cultivation in salt-affected soils.

Key words: Bael cultivars, Genotypic differences, Mineral partitioning, Physiological relations, Salinity

Salinity is an encompassing term used to refer to saline and sodic soils and the marginal quality water unsuitable for irrigation. Globally, salinity adversely affects the productivity of nearly 20% of the irrigated lands. In India, approximately 6.73 million ha area suffers from salinity and sodicity stresses to varying extents. Despite the availability of potential reclamation technologies, continued salinization of new agricultural lands is a cause for concern. In several irrigated areas of world, waterlogging and excess salt accumulation have gradually emerged as the serious environmental constraints and are likely to worsen if left unaddressed. Conventionally, salt-affected soils are reclaimed by the application of chemical amendments (e.g. gypsum) and leaching with the fresh water. Of late, however, growing shortages of gypsum and fresh water are hindering the soil reclamation projects in India and other salt-affected regions of the world (Sharma and Singh 2015, Sharma and Singh 2017). Available evidence suggests that use of salt tolerant cultivars could be a viable means of productivity enhancement in saline areas. Although most of the fruit crops are adversely affected under saline conditions, marked inter-varietal differences have been recorded in several species implying that screening of available germplasm is necessary to identify the promising salt tolerant genotypes for commercial cultivation.

Bael (*Aegle marmelos* Correa; family Rutaceae) is an under-utilized fruit of Indian origin valued for its nutritive and medicinal properties. In the past few decades, development of improved cultivars has spurred interest in the commercial cultivation of bael in different parts of country. Conclusive evidence on threshold salt tolerance of improved bael cultivars may benefit the prospective growers in making informed decisions on varietal selection for salt affected soils (Singh *et al.* 2015). In spite of reportedly hardy nature of bael tree in terms of tolerance to salinity, high pH, drought and other stresses, little is known about the response of commercial cultivars to the excess salts in the growing medium. Wide variations have been noted for soil saturation extract salinity (EC_e) tolerance in bael seedlings ranging from 3.5 dS/m (Toan 2012) to 10.5 dS/m (Shukla and Singh 1996b); apparently due to differences in experimental conditions. In contrast, improved cultivars often show moderate levels of salt tolerance (EC_e 6-7 dS/m) (Singh *et al.* 2015, Singh *et al.* 2016). These observations suggest inherent genetic differences for salt tolerance in bael. Consistent with these facts, a study was conducted to understand the effects of saline irrigation on plant growth, physiological activities and mineral uptake in bael cultivars NB-5, NB-9, CB-1 and CB-2 which are increasingly being adopted for commercial cultivation in arid salt affected parts of India.

MATERIALS AND METHODS

This study was conducted during 2014-2015 with one-year old plants of four bael cultivars, namely, Narendra Bael-5 (NB-5), Narendra Bael-9 (NB-9), CISH Bael-1

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(CB-1) and CISH Bael-2 (CB-2) grafted on local seedling rootstock. The plants procured from ICAR-CISH, Lucknow, India were raised in experimental columns containing about 76 kg soil ($EC_e \sim 1.3$ dS/m) at ICAR-CSSRI Experimental Farm, Karnal ($29^{\circ}43'N$, $76^{\circ}58'E$; 245 m above the mean sea level). Plants were initially irrigated with normal water (EC_{iw} 0.5 dS/m) for better establishment. Salt treatments {control (normal water; EC_{iw} 0.5 dS/m), marginally saline water (MSW; EC_{iw} 3.0 dS/m) and saline water (SW; EC_{iw} 6.0 dS/m)} were imposed after six months of plant establishment. Measured quantities of NaCl, $CaCl_2 \cdot 2H_2O$ and $MgSO_4 \cdot 7H_2O$ salts (0.8237 g, 0.7776 g and 0.9736 g, respectively, per litre of water for 3.0 dS/m EC_{iw} ; and 1.2764g, 1.8662 g and 2.3366 g, respectively, per litre of water for 6.0 dS/m EC_{iw}) were dissolved in water to obtain the saline solutions having a constant sodium adsorption ratio of 5 and Ca: Mg ratio of 2: 1. Plants were weekly irrigated and saline irrigations were withheld at the onset of salt stress symptoms after 180 days of salt treatment. Plants were uprooted and washed with the tap water followed by two washings with the distilled water to remove the dust and salt particles. Stem length and inter-nodal length were determined using the measuring tape while stem girth (SG) was measured using the Vernier scale. Shoot fresh weight and root fresh weight were recorded after harvest. Plants were separated into shoots and roots, and dried to a constant weight at 60° in an electric oven to measure shoot dry weight and root dry weight. Membrane injury index and relative water content in leaves were estimated using the methods of Blum and Ebercon (1981) and Barrs and Wheatherly (1962), respectively. Leaf pigments (a, b, total chlorophyll and carotenoids) were estimated using the method of Hiscox and Israelstam (1979) using dimethyl sulfoxide (DMSO). Fully expanded leaves from the middle layer of plants were gently detached, washed and weighed. Two hundred mg leaf sample was then kept into a test tube containing 5 ml of DMSO. Test tube was then placed into oven at $60^{\circ}C$ for about 4 h to facilitate the extraction of pigments. After keeping the test tubes at room temperature for 2 hr, readings were taken at 645 and 665 nm using the spectrophotometer. DMSO was used as blank. Calculations for different pigments were made according to the following formulae:

$$\begin{aligned} \text{Chl 'a' } (\mu\text{g/ml}) & - 12.19 A_{665} - 3.45 A_{645} \\ \text{Chl 'b' } (\mu\text{g/ml}) & - 21.99 A_{645} - 5.32 A_{665} \\ \text{Total chlorophyll} & - \text{Chl 'a' + Chl 'b'} \\ \text{Carotenoids} & - (1000 A_{480} - 2.86 \text{ Chl a} - 129.9 \\ & \text{Chl b})/2.21 \end{aligned}$$

Proline content was estimated using the method of Bates (1973). Three hundred mg fresh leaves were homogenized in 5 ml of 3% sulphosalicylic acid and then centrifuged at 5000 rpm for 15 minutes to obtain the supernatant. Subsequently, 2 ml of supernatant, acid ninhydrin and acetic acid each was mixed and the mixture was kept in boiling water bath for 1 h at $100^{\circ}C$. Thereafter, reaction was terminated by keeping tubes in ice-bath. Then 4.0 ml of toluene was added.

After vigorous shaking, the upper organic phase was taken after attainment of room temperature and absorbance was recorded at 520 nm by using toluene as blank. A standard curve was prepared by using graded concentration of proline in 3% sulphosalicylic acid. Proline content was expressed as $\mu\text{g/g}$ fresh weight. Leaves were dried at $60^{\circ}C$ for 48 h, weighed and crushed in a hammer mill and stored at the room temperature. Approximately 50 mg of dried and powdered leaf material was extracted with 1 M HNO_3 at $100^{\circ}C$. Na^+ and K^+ contents were determined through flame photometry while Ca^{2+} was determined through atomic absorption spectrometry. The experiment was laid out in a randomized complete block design with three replications. Data were analyzed using Indian NARS Statistical Computing Portal (<http://stat.iasri.res.in/sscnarsportal>). For comparison of means, Duncan's test was used at 5% level of significance.

RESULTS AND DISCUSSION

Visible symptoms of salt injury

Irrigation with marginally saline water (EC_{iw} 3 dS/m) did not cause any injury in NB-5 and CB-1 cultivars as evidenced by the healthy appearance of leaves throughout the experiment. Although NB-9 and CB-2 plants showed partial yellowing and leaf abscission, they tended to recover after saline treatments were withheld. In contrast, tested cultivars showed marked differences with each other when irrigated with saline water (6 dS/m). Salt effects initially appeared as the yellowing of leaves followed by scorching and chlorosis of the leaf edges in all the cultivars except NB-5. With the increase in the duration of salt treatment, chlorotic spots gradually enlarged in size, turned necrotic and the affected leaves dropped off giving a wilted and sparse look to NB-9 and CB-2 cultivars (Fig 1).

Plant growth

Saline irrigation adversely affected stem and root growth in all the cultivars (Table 1). With the application of marginally saline water, stem length marginally decreased ($\sim 6\%$) in NB-5, CB-1 and CB-2 each and by $\sim 18.0\%$ in NB-9 than control. However, stem length declined by 9.7%, 20.8%, 11.4% and 19.0% in NB-5, NB-9, CB-1 and CB-2 cultivars, respectively, under saline water treatment (6 dS/m) compared to the respective control. Interestingly, saline irrigations did not cause any significant decrease in stem girth in all the cultivars. Genotypic differences were also apparent with regard to salt induced reductions in inter-nodal length. While irrigation with marginally saline water (3 dS/m) caused only nominal decreases in inter-nodal length (3-8%) in different cultivars, saline water application led to relatively greater decreases with NB-5, NB-9, CB-1 and CB-2 cultivars producing 16.4%, 11.1%, 13.9% and 16.8% shorter inter-nodes, respectively, than salt-free plants. Salt treated plants showed considerably less fresh and dry weights of shoots and roots than control. With the use of marginally saline water, both NB-5 and CB-1 plants showed only meagre differences ($\sim 10.0\%$) but those of NB-9 and



Fig 1 Visible symptoms of salt injury in saline irrigated (EC_{iw} 6 dS/m) bael cultivars (From top left- NB-5, NB-9, CB-2 and CB-1; in clockwise direction)

Table 1 Effect of saline irrigation of on plant growth in bael cultivars

Cultivar	EC_{iw} (dS/m)	SL (cm)	SG (cm)	IL (cm)	SFW (g)	SDW (g)	RFW (g)	RDW (g)
NB-5	0.5	98.2cd	3.53ab	4.46bc	1343.7c	653b	628d	397.7c
	3	92.3de	3.47bc	4.32cd	1203.3d	579.3c	530.7g	324.3ef
	6	88.7e	3.43bc	3.73g	1010f	463.7e	490.7h	287.7g
NB-9	0.5	112.7a	3.57ab	4.53ab	1139.3e	568c	598e	403c
	3	92de	3.4bc	4.23de	791.6h	347g	479.7h	308f
	6	89.3e	3.4bc	4.03f	511j	302h	347i	246.7h
CB-1	0.5	111a	3.8ad	4.5ab	1485a	694.7a	812a	427.3b
	3	104.3bc	3.57ab	4.13ef	1345c	527.7d	716c	359d
	6	98.3cd	3.53ab	3.87g	856.6g	404.3f	475.3h	251.3h
CB-2	0.5	110ab	3.8ad	4.6a	1418.3b	651.7b	741.3b	449a
	3	102.7c	3.63ab	4.3d	1024.7f	566.3c	553.3f	326e
	6	89e	3.4bc	3.83g	567.3i	309.7h	317j	218.3i

Means with at least one letter common in a column are not significantly different using Duncan's Test at 5% level of significance. SL= Stem length; ST= Stem thickness; IL= Inter-nodal length; SFW= Shoot fresh weight; SDW= Shoot dry weight; RFW= Root fresh weight; RDW= Root dry weight.

CB-2 registered much larger reductions (27.0-30.0%) in shoot fresh weight vis-à-vis control. In contrast, application of saline water led to steep fall in shoot fresh weight with NB-5, NB-9, CB-1 and CB-2 showing nearly 25%, 30%, 42% and 60% reductions than control. A similar trend was noted for shoot dry weight with NB-5 showing the lowest (~29%) and CB-2 the highest (~52%) decreases compared to control when treated with saline water (6 dS/m). Root fresh and dry weights also consistently decreased in the salinized plants. Application of marginally saline water led to marginal reductions in root fresh weight (12-15%) in NB-5 and CB-1, and moderate reductions (20-25%) in NB-9 and CB-2. In marginally saline (3 dS/m) and saline water (6 dS/m) treatments, reductions in RDW compared to control were the minimum (18.5% and 27.6%, respectively) in NB-5 and the maximum (27.4% and 51.4%, respectively) in CB-2 cultivars.

Reductions in plant growth are commonly seen under saline conditions. Decreased availability of photoassimilates suppresses the plant growth by arresting the shoot elongation and leaf emergence while older leaves continue to abscise giving the plants a stunted and sparse look. Nonetheless, marked genotypic differences exist even in highly salt sensitive crops like citrus where different scion and rootstock cultivars greatly vary with each other in salt-induced growth reduction (Singh *et al.* 2015). Salt stressed citrus genotypes Cleopatra and Shekwasha showed minimal chlorosis and leaf drop symptoms, while Fuzhu, Willowleaf, and King of Siam were severely affected due to excessive salt translocation from roots to leaves (Yahmed *et al.* 2015). Excess salts hamper the plant growth initially by reducing the water uptake (i.e. osmotic stress) and subsequently by causing the salt-specific (e.g. Na⁺) injuries. Salinized bael plants show excessive uptake of Na⁺ ions and reduced tissue concentrations of K⁺ and Ca²⁺ resulting in metabolic

impairments, decrease in photosynthetic assimilation and the eventual appearance of injury symptoms such as yellowing and scorching of the leaves, development of necrotic spots and, in severe cases, defoliation (Shukla and Singh 1996a, Singh *et al.* 2015). At 6.5 dS/m salinity, only about one thirds of the leaves showed salt injury symptoms in bael cultivar NB-5 while nearly two thirds of the leaves were severely affected in NB-9 and CB-2 plants (Singh *et al.* 2016).

Physiological parameters

Irrespective of the cultivar, membrane injury index (MII) invariably increased with the increase in salinity. Nonetheless, MII was much lower in NB-5 cultivar; especially when irrigated with marginally saline water. Cultivar NB-9 was the most severely affected showing nearly three- and fourfold higher MII values when irrigated with marginally saline and saline waters, respectively, than non-salinized plants. Relative water content (RWC) in the leaves dropped with the addition of salts into irrigation water. The lowest (6.7%) and the highest (34.0%) decreases in RWC were noted in marginally saline water irrigated NB-5 and CB-2 cultivars, respectively, compared to control. A similar trend was recorded in saline water treated plants with NB-5 showing only 15.3% decrease than control while it ranged from 35.8-47.6% in other cultivars. At 6 dS/m salinity, total leaf chlorophyll content was not affected in NB-5 while significant reductions were noted in other cultivars. In contrast, total chlorophyll dropped by 16.8% in NB-5, 59.1% in NB-9, 62.0% in CB-1 and 75.1% in CB-2 irrigated with saline water. Decrease in leaf carotenoids in salinized plants showed a similar trend, albeit less pronounced, to that of total chlorophyll. Reductions in leaf carotenoid contents were comparatively smaller compared to that for chlorophyll when marginally saline water was used. Irrigation with saline water, in contrast, caused marginal (18.1% in

Table 2 Effects of salinity on leaf membrane damage, relative water content, photosynthetic pigments and proline concentration in salt stressed bael cultivars

Cultivar	EC _{iw} (dS/m)	MII	RWC (%)	Photosynthetic pigments and proline				
				Chl. a	Chl. b	Total chl. (mg/g FW)	Carotenoids	Proline
NB-5	0.5	11.29j	80.87a	2.06c	0.62b	2.68c	3.64b	4.23g
	3	14.68h	75.43b	2.02c	0.55c	2.58c	3.32cd	7.68e
	6	21.19g	68.55c	1.83d	0.40ef	2.23d	2.98e	8.35cd
NB-9	0.5	12.95hij	81.88a	2.64a	0.81a	3.45a	4.01a	7.86de
	3	28.71e	61.03d	1.76de	0.49d	2.25d	3.20cde	8.57c
	6	50.42b	48.65f	1.12g	0.29gh	1.41f	2.95e	11.07a
CB-1	0.5	11.68ij	81.21a	2.31b	0.78a	3.08b	3.38bc	7.47e
	3	24.26f	65.50c	1.05gh	0.35fg	1.40f	3.35bcd	9.17b
	6	43.13c	52.12ef	0.93h	0.24hi	1.17g	2.32f	11.25a
CB-2	0.5	13.56hi	80.92a	1.68e	0.49cd	2.17d	3.45bc	6.11f
	3	38.35d	53.35e	1.44f	0.42e	1.86e	3.07de	7.38e
	6	53.45a	42.43g	0.33i	0.20i	0.54h	0.94g	9.21b

Means with at least one letter common in a column are not significantly different using Duncan's Test at 5% level of significance. MII= Membrane Injury Index; RWC= Relative Water Content, Chl.- Chlorophyll.

NB-5), moderate (26.4-31.4% in NB-9 and CB-1) and large (72.8%) reductions in leaf carotenoid contents compared to the respective control plants. Notwithstanding the high leaf proline levels in salt-free plants, all the cultivars except NB-5 showed a relatively inefficient proline upregulation pattern at both the salinity levels. Leaf proline concentration increased by 57.9%, 8.6%, 20.4% and 18.8% in NB-5, NB-9, CB-1 and CB-2, respectively, when irrigated with marginally saline water. With further increase in salinity, NB-5 showed twofold higher leaf proline levels while it was only 1.5 times higher than control in rest of the cultivars.

In salinized plants, osmotic stress triggers the accumulation of reactive oxygen species such as hydroxyl radicals (OH) and superoxide anions (O₂⁻) to excessive levels resulting in oxidative damage to the membrane lipids and proteins and the eventual disintegration of cell membranes, higher cell permeability and solute efflux. Genotypic differences for salt induced membrane injury in citrus (Singh *et al.* 2014) and bael (Singh *et al.* 2016) can be ascribed to the differences in the lipid composition of plasma membranes as certain lipid classes may have relatively better stability under saline conditions. Relative water content of a leaf indicates its hydration status, i.e. actual water content compared to the maximal water holding capacity at full turgidity (Mullan and Pietragalla 2012). It is commonly used to measure cellular water deficit in salt stressed plants. Salt induced reductions in leaf relative water content in the tested bael cultivars seem to be caused by salinity induced water deficit. Genotypes maintaining turgid leaves under stress conditions have a physiological advantage in terms of better stomatal regulation and higher photosynthetic efficiency (Mullan and Pietragalla 2012). Salinized plants tend to have lesser leaf chlorophyll than control plants. Salt treatment reduced total leaf chlorophyll concentration by 74% and 20% in citrus rootstocks Alemow and Cleopatra mandarin, respectively, suggesting that chlorophyll is differentially

affected in genotypes differing in salt exclusion. Besides detrimental effects on chlorophyll structure and chlorophyll-protein complexes, salt ions also suppress specific enzymes involved in the biosynthesis of photosynthetic pigments. Furthermore, Na⁺ has an antagonistic effect on Mg²⁺ uptake required for chlorophyll biosynthesis (Navarro *et al.* 2014). Leaf carotenoids significantly decreased in salinized plants of mulberry genotypes M-5 and S-30 but increased in BC2-59 up to 12 dS/m salinity. BC2-59 also showed lesser reductions in leaf chlorophyll levels (Agastian *et al.* 2000). In this study, leaf proline concentration increased with increasing salinity in all the cultivars but to a greater extent in NB-5 as compared to the proline levels in control plants. Among citrus rootstocks, salt tolerant Attani-1 showed relatively higher increase in leaf proline concentration at 50 mM salinity than salt sensitive Attani-2 and *Jatti Khatti* (Singh *et al.* 2014).

Ion partitioning

Data on mineral distribution in different plant parts (Table 3) indicated that cultivar NB-5 tended to retain Na⁺ ions in lower parts at both the salinity levels. At low salinity, Na⁺ concentrations in stems and roots of NB-5 plants were about 70% higher than in leaves resulting in restricted translocation of Na⁺ to foliage. At 6 dS/m salinity, although stem Na⁺ levels were twofold higher compared to leaves; roots contained only 45% more Na⁺ ions indicating decrease in Na⁺ retention in roots under elevated salinity. Cultivar CB-1 also showed a similar, albeit weaker, response with regard to Na⁺ partitioning. Although it maintained low leaf and high root Na⁺ levels at low salinity, high salinity induced breakdown of root Na⁺ retention led to excessive Na⁺ accumulation in both leaves and stems of salinized plants. In comparison to control, salt treated NB-9 and CB-2 plants invariably had more Na⁺ in leaves than in stem and root tissues. Normal water irrigated plants of NB-5 cultivar

Table 3 Mineral partitioning in different plant parts in salinized bael cultivars

Cultivar	EC _{iw} (dS/m)	Na ⁺ (% DW)			K ⁺ (% DW)			Na/K ratio			Ca ²⁺ (% DW)		
		Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
NB-5	0.5	0.44h	0.37h	0.88f	0.71f	0.69g	0.68a	0.63h	0.54fg	1.31g	2.63cd	3.18de	2.99bc
	3	0.74g	1.26d	1.25cd	1.07a	1.03ab	0.56b	0.69gh	1.22bc	2.23e	3.33a	3.72ab	2.52de
	6	1.2de	2.38a	1.75a	0.91bc	1.01bc	0.54bc	1.32ef	2.37a	3.22c	2.50de	3.12ef	2.40e
NB-9	0.5	0.77fg	0.5gh	1.39b	0.83d	0.98c	0.51de	0.93fgh	0.52g	2.74d	2.79bc	3.72b	3.25a
	3	1.4cd	0.63g	1.29bcd	0.94b	0.85de	0.53cd	1.48e	0.74ef	2.42e	2.90b	3.27d	3.02b
	6	2.2b	1.67c	0.87f	0.52h	0.76f	0.45g	4.20b	2.18a	1.94f	2.35ef	2.83g	2.43e
CB-1	0.5	0.8fg	0.61g	1.1e	0.6g	0.81ef	0.47fg	1.33ef	0.76ef	2.36e	2.58d	3.45c	3.13ab
	3	1.01ef	0.82f	1.8a	0.89c	0.9d	0.4h	1.13efg	0.91de	4.44a	2.24fg	3.26d	2.84c
	6	1.63c	1.93b	0.83f	0.81de	0.89d	0.25i	2.03d	2.16a	3.34c	1.81h	3.03f	2.67d
CB-2	0.5	0.91fg	0.60g	1.18de	0.9bc	0.76f	0.5e	1.01fgh	0.79e	2.37e	2.57d	3.85a	2.98bc
	3	2b	1.1e	1.83a	0.78e	1.07a	0.48ef	2.58c	1.03cd	3.81b	2.34ef	3.51c	2.54de
	6	2.67a	1.17de	1.33bc	0.52h	0.84de	0.42h	5.15a	1.38b	3.21c	2.15g	2.17h	2.10f

Means with at least one letter common in a column are not significantly different using Duncan's Test at 5% level of significance.

showed almost similar concentrations of K^+ in different plant parts. In contrast, saline irrigated NB-5 plants had 28-50% more K^+ in leaves, 46-49% more K^+ in stems but 15-18% less K^+ in roots compared to non-salinized plants. Salinized CB-1 plants also had 35-48% more K^+ in leaves and 11% more K^+ in stem tissues but exhibited lower root K^+ levels than control. These data indicated preferential K^+ uptake to overcome Na^+ induced toxic effects in both NB-5 and CB-1 cultivars. Yet, K^+ accumulation in stem and root tissue was relatively higher in NB-5 than CB-1. In NB-9 plants, leaf K marginally increased at 3 dS/m but declined by 37% in plants treated with saline water (6 dS/m). In CB-2 cultivar, salt stress caused significant decreases in leaf and root K^+ concentrations compared to control. Due to restricted uptake of Na^+ and preferential accumulation of K^+ , NB-5 plants maintained a considerably lower leaf Na^+ : K^+ ratio than other cultivars. Leaf Na^+ : K^+ ratio increased by about twofold in NB-5 compared to a fivefold increase in NB-9 and CB-2 cultivars as EC_{iw} increased from 0.5 to 6.0 dS/m. Leaf and stem Ca^{2+} levels in NB-5 plants increased by 27% and 17% at 3 dS/m, respectively, over control. At 6 dS/m salinity, however, leaf and stem Ca^{2+} concentrations dropped but were statistically at par with those of control plants. At low salinity, Ca^{2+} concentration remained unchanged in leaves of NB-9 plants but decreased by 13% and 7% in stem and root tissues, respectively. Further increase in salinity reduced leaf, stem and root Ca^{2+} levels by 13%, 24% and 25% than control. Salt treatment caused consistent decline in Ca^{2+} concentrations in different plant parts in both CB-1 and CB-2 cultivars. However, the magnitude of decrease was much more in CB-2 than in CB-1; especially under saline water treatment.

Under salt stress, excessive Na^+ alters the cytosolic K^+/Na^+ balance by inducing the passive uptake of Na^+ over K^+ . Because of their strongly similar hydrated ionic radii, ion channels often fail to discriminate between Na^+ and K^+ ions in saline soils (Blumwald 2000). Certain genotypes preferentially accumulate K^+ in leaf and stem

tissues to lessen the salt injury (Zou and Wu 2011). Higher photosynthetic assimilation in mango rootstock *M. zeylanica* than *M. indica* 13-1 at 60 mM NaCl was due to a higher root K^+/Na^+ ratio and lower leaf/root Na^+ in *M. zeylanica* (Schmutz 2000). Several rootstock and scion cultivars in fruit crops also exhibit partial salt exclusion at the root level and/or tend to retain salt ions in the roots and stem tissues to prevent salt transport to the photosynthesizing leaves. Nonetheless, salt exclusion capacity often breaks down at excess salinity levels. For example, grape rootstock Dog Ridge believed to tolerate up to 6.5 dS/m NaCl-induced salinity shows excessive uptake of salts when continuously irrigated with saline water (Sharma *et al.* 2011). Reduced uptake of Ca^{2+} in salinized plants results due to Na^+ induced reduction in the binding of Ca^{2+} to the plasma membrane, inhibition of influx and a higher efflux of Ca^{2+} , and loss of the Ca^{2+} from endomembranes (Rengel 1992). Genotypes maintaining relatively higher Ca^{2+} levels can partly reduce the injurious effects of Na^+ enhancing the interest in supplemental use of Ca additives to mitigate the salt stress in fruit crops. In strawberry, application of 5 mM Ca (as $CaCl_2$) ameliorated the negative effects of NaCl (35 mM) on growth and fruit yield by decreasing the membrane permeability, correcting leaf Ca^{2+} deficiency and improving the plant water use (Kaya *et al.* 2002).

Correlation studies

Among all the parameters studied, the significant highest positive correlation was noted between SFW and RFW ($r = 0.950^{**}$) while the highest negative correlation was observed between MII and RWC ($r = -0.973^{**}$) for all the varieties studied. RWC had a strong positive correlation with different growth parameters implying that maintenance of adequate leaf hydration was critical to sustaining biomass production under saline conditions. Conversely, a highly negative correlation between RWC and MII indicated that leaf hydration, and consequently plant growth, gradually diminished with the increase in salt induced MII in bael

Table 4 Pearson's correlation coefficients for association among plant height (PH), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), membrane injury index (MII), relative water content (RWC), total chlorophyll (TC), leaf Na/K ratio (Na/KL), shoot Na/K ratio (Na/KS), root Na/K ratio (Na/KR) and leaf proline (PRL)

Trait	PH	SFW	RFW	SDW	RDW	MI	RWC	TC	Na/KL	Na/KS	Na/KR	PRL
PH	1	0.676 ^{**}	0.764 ^{**}	0.688 ^{**}	0.773 ^{**}	-0.516 ^{**}	0.559 ^{**}	0.483 ^{**}	-0.442 ^{**}	-0.581 ^{**}	0.107	-0.341 [*]
SFW		1	0.950 ^{**}	0.944 ^{**}	0.894 ^{**}	-0.876 ^{**}	0.856 ^{**}	0.628 ^{**}	-0.802 ^{**}	-0.585 ^{**}	-0.056	-0.683 ^{**}
RFW			1	0.881 ^{**}	0.910 ^{**}	-0.789 ^{**}	0.777 ^{**}	0.580 ^{**}	-0.722 ^{**}	-0.604 ^{**}	0.009	-0.562 ^{**}
SDW				1	0.889 ^{**}	-0.834 ^{**}	0.835 ^{**}	0.684 ^{**}	-0.717 ^{**}	-0.591 ^{**}	-0.172	-0.761 ^{**}
RDW					1	-0.859 ^{**}	0.883 ^{**}	0.740 ^{**}	-0.717 ^{**}	-0.736 ^{**}	-0.242	-0.725 ^{**}
MI						1	-0.973 ^{**}	-0.851 ^{**}	0.897 ^{**}	0.568 ^{**}	0.313	0.724 ^{**}
RWC							1	0.864 ^{**}	-0.846 ^{**}	-0.564 ^{**}	-0.396 [*]	-0.705 ^{**}
TC								1	-0.732 ^{**}	-0.531 ^{**}	-0.449 ^{**}	-0.587 ^{**}
Na/KL									1	0.428 ^{**}	0.157	0.540 ^{**}
Na/KS										1	0.176	0.674 ^{**}
Na/KR											1	0.415 [*]
PRL												1

cultivars. A negative correlation between leaf Na^+/K^+ ratio and plant growth parameters indicated that higher leaf Na^+ was inimical to plant growth. A significantly higher negative correlation between leaf Na^+/K^+ ratio and RWC ($r = -0.846^{**}$), and between Na^+/K^+ ratio and TC ($r = -0.732^{**}$) suggested that accumulation of Na^+ ions in leaves adversely affected leaf water relations and chlorophyll biosynthesis (Table 4).

Conclusions

Salinity hampers plant growth by disrupting the metabolic processes in plants. A better understanding of salt-induced changes in plant metabolism can help design efficient agronomic interventions for lessening the salt damage. Screening of commercial bael cultivars indicated salt tolerance in NB-5. Investigations revealed that NB-5 endures prolonged exposure to salinity by maintaining higher leaf chlorophyll and proline levels coupled with retention of Na^+ ions in stem and root tissues and preferential accumulation of K^+ and Ca^{2+} ions to overcome the deleterious effects of Na^+ . Failure of other tested cultivars to adapt to salinity was due to accumulation of Na^+ to the toxic levels in leaves and a consistent drop in leaf hydration, leaf chlorophyll and tissue K^+ levels adversely affecting the growth.

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