



Effect of salinity on gas exchange parameters and ionic relations in *bael* (*Aegle marmelos* Correa)

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ABSTRACT

Salt stressed *bael* cultivars showed marginal scorch, necrosis and abscission of leaves under both moderate (6.5 dS m⁻¹) and high (10.7 dS m⁻¹) salinity but control plants (1.3 dS m⁻¹) did not exhibit these injury symptoms. While cvs NB-5 and CB-1 showed delayed onset and gradual progression of the stress symptoms, while NB-9 and CB-2 were worst affected and exhibited severe marginal scorch and necrosis in over 70% of the leaves in saline soils. At high salinity, NB-9 and CB-1 plants did not survive. Salt stress significantly ($p \leq 0.05$) reduced gas exchange and 6.5 dS m⁻¹ salinity caused 28-32% decline in net photosynthesis and 29-39% reduction in transpiration rate in all cultivars relative to control. Although Na⁺ accumulation significantly increased in salt treated plants, cultivar NB-5 exhibited relatively similar distribution of Na⁺ ions in different plant parts and also maintained higher K⁺ concentrations in aerial parts. In spite of significantly high leaf Na⁺ (0.29%) at 6.5 dS m⁻¹ salinity, cultivar NB-5 did not exhibit severe injury symptoms. Although CB-1 cultivar showed the tendency to retain Na⁺ ions in stem and root tissues, it failed to avoid the injury symptoms. Calcium was acquired in high amounts by salinized NB-5 plants as compared to others. Restricted Na⁺ uptake and preferential K⁺ accumulation seemed to contribute to alleviate the salt stress in cultivar NB-5.

Key words: *Aegle marmelos* Correa, gas exchange, salt stress, sodium uptake.

INTRODUCTION

Soil salinity refers to the build-up of soluble salts and/or exchangeable sodium in soil profile in excess amounts resulting in severe limitations for agricultural production. It is estimated that about 6.73 m ha lands in India are affected by salinity and sodicity stresses. In saline soils, initial osmotic stress due to low water potential in root zone followed by nutrient imbalances and toxicities caused by the excessive uptake of Na⁺ and Cl⁻ ions impair the plant growth (Sharma and Singh, 7). Although majority of fruit crops are categorized as sensitive to salinity, wide genetic differences exist and some of the scion and rootstock cultivars may exhibit higher salt tolerance as compared to others. While considerable work has been done to identify salt tolerant types in crops such as citrus (Awasthi *et al.*, 2), some of the underutilized fruits of Indian origin remain under-researched. *Bael* (*Aegle marmelos* Correa; Rutaceae) is such a fruit which is widely distributed throughout the Indian subcontinent, particularly in arid and semi-arid tracts where salinization is a major problem. Different parts of *bael* tree, rich in bioactive compounds such as marmelosin, have long been used as ingredients in traditional Indian medicine. While mature and half-

ripe fruits are used to cure stomach ailments, ripe fruits have restorative and laxative properties (Singh *et al.*, 9).

In recent past, commercial cultivation of *bael* has steadily increased in many arid and semi-arid regions of India, which suffer from constraints such as secondary salinity and increasing scarcity of good quality irrigation water. Previous salinity studies in *bael* have mostly been conducted with seedling plants (Singh *et al.*, 9) and detailed information on commercial cultivars is lacking. Given the growing interest in improved cultivars, their salinity tolerance needs to be worked out to arrive at viable recommendations for commercial cultivation in saline environments. The identification of salt tolerant cultivars may also facilitate their use in future improvement programmes. In this backdrop, observations on growth, gas exchange parameters and mineral nutrition were recorded to evaluate the threshold of salt tolerance and to understand the physiological basis of salt stress alleviation in *bael* cultivars.

MATERIALS AND METHODS

This experiment was carried out during 2012-2013 at ICAR-Central Soil Salinity Research Institute (CSSRI), Karnal, India. One-year-old, grafted plants of four *bael* cultivars, namely, Narendra Bael-5 (NB-5), Narendra Bael-9 (NB-9), CISH Bael-1 (CB-1) and

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CISH Bael-2 (CB-2) procured from the ICAR-Central Institute of Subtropical Horticulture, Lucknow, India were used. The saline soils (6.5 and 10.7 dS m⁻¹) used in this experiment were obtained from the salt-affected CSSRI-Nain Experimental Farm, Panipat, India, while control soil (1.3 dS m⁻¹) was obtained from the crop fields. The soil was filled in large, metallic experimental columns of approximately 74 cm length, 44 cm width and 166 cm circumference (each column containing approx. 76 kg soil). After transplanting, the plants were irrigated with normal water (EC_{iw} 0.5 dS m⁻¹) at weekly intervals till the time of data recording (75 days after planting). Salinity in saturation extract (EC_e) of the experimental soil was evaluated at fortnightly intervals and the values presented here (control- 1.3 dS m⁻¹, moderate salinity- 6.5 dS m⁻¹ and high salinity- 10.7 dS m⁻¹) represent the means of three replicates of the five consecutive measurements over the experimental period (data not presented).

The visible symptoms of salt stress were recorded at fortnightly interval during the course of investigation. The leaves showing stress symptoms were counted at each stage and the extent of damage (%) was expressed as percentage of total leaves per plant. The relative chlorophyll concentration of the fully expanded, attached leaves from middle layer of plants was obtained using a leaf chlorophyll meter (SPAD-502, Minolta, Japan). Chlorophyll readings were taken from the centre of leaves (excluding mid-rib) between 9 and 11.30 AM. To ensure consistency in results, the same leaves were used for gas exchange measurements. Next day, net photosynthesis (P_N), stomatal conductance (g_s) and transpiration rate (E) were measured during the same hours using the portable photosynthetic system (Li-Cor Biosciences, Nebraska, USA). Water use efficiency was obtained as the ratio of P_N and E . After 75 days of salt treatment, the plants were harvested and washed with distil water to remove the dust and salt particles.

After drying by wrapping in paper sheets, the whole plant was divided into different parts (leaves, upper stem, basal stem, primary roots and root hairs) for mineral analyses. These samples were subsequently dried in a forced-draft oven at 60°C for 48 h, weighed and crushed in a hammer mill. Approximately 50 mg of dried and powdered leaf material was extracted with 1 M HNO₃ at 100°C. Na⁺ and K⁺ contents were determined by using the flame photometer (Systronics, India), while Ca²⁺ concentration was determined through atomic absorption spectrometry (Analytik Jena, Germany). The experiment was laid out in a randomized complete block design with three replications. The data were analysed for analysis of variance using the SPSS 11.0.1 for Windows statistical package (SPSS, Chicago, IL, USA). For comparison of the means, the Duncan's multiple range test ($P \leq 0.05$) was used.

RESULTS AND DISCUSSION

Data on salt stress symptoms recorded at fortnightly interval (Table 1) revealed significant differences ($p \leq 0.05$) in the appearance and progression of injury symptoms among the tested cultivars. NB-9 and CB-2 were the most affected cultivars as even moderate salinity caused severe marginal scorch and necrosis in over 70% of the leaves in these cultivars after 75 days of salt treatment. Contrary to NB-5 and CB-1, which showed delayed appearance and gradual development of injury symptoms, NB-9 and CB-2 cultivars exhibited severe injury within a few days of salinity exposure. Between 60th and 75th days, all the leaves became necrotic in NB-9, CB-1 and CB-2 but remained intact. At 10.7 dS m⁻¹ salinity, salt injury appeared as early as 15 days after planting. Initially, the leaves turned chlorotic followed by necrosis and eventual abscission in all the cultivars. However, the pattern and extent of necrosis and abscission varied among the cultivars. The upper leaves were

Table 1. Percentage of leaves with salt stress symptoms after 75 days of salinity exposure.

Cultivar	Salinity (dS m ⁻¹)	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP
NB-5	6.5	1.3e	1.3f	8.4e	16.5g	33f
	10.7	4.2de	11.6e	22d	38.3e	67d
NB-9	6.5	13.7bc	20.3bc	34c	43.2d	70.5cd
	10.7	17b	23b	40b	62b	91.6a
CB-1	6.5	5.4d	15.7d	22d	31.5f	46.3e
	10.7	11.6c	18.3cd	32.3c	52c	80.7b
CB-2	6.5	15.7b	20.8bc	37bc	47.7cd	73.3c
	10.7	26.3a	34.7a	46.7a	70.3a	94.4a

Means with at least one letter common in a column are not statistically significant using Duncan's Test at 5% level of significance. The control plants did not show any injury symptoms and were thus excluded from statistical analysis, DAP = Days after planting.

first to abscise followed by middle and lower leaves. In NB-5 plants, however, some of the lower and middle leaves remained attached to plants till the end of experiment. In citrus, absence of such injury symptoms is frequently used as a criterion for salt tolerance (Lopez-Climent *et al.*, 6). Adverse effects of salinity on plant growth seem to be mainly due to excessive Na⁺ and Cl⁻ accumulation in vegetative tissues as reported in citrus (Lopez-Climent *et al.*, 6) and *bael* (Singh *et al.*, 9). Although all the tested cultivars showed significant increase in toxicity symptoms with increasing salinity, these effects were less pronounced in cv. NB-5 indicating its relatively higher salt tolerance.

All the cultivars exhibited significant decrease ($P \leq 0.05$) in relative chlorophyll (SPAD) values (Table 2) with increasing salinity. The maximum (50.5%) and the minimum (36.7%) reductions in SPAD at 6.5 dS m⁻¹ salinity occurred in CB-2 and NB-5, respectively, as compared to control. High salinity (10.7 dS m⁻¹) caused drastic reductions in SPAD with cultivar CB-2 exhibiting as high as 73% decrease relative to control. The observation that SPAD only marginally decreased in NB-5, while other cultivars exhibited moderate to high reductions is supported by previous findings on *bael* (Singh *et al.*, 9) and citrus (Anjum, 1). Leaf chlorophyll relations in salt stressed plants vary with the magnitude of salinity, genotype and growth stage and often depend on the integrity of cell membranes and the activities of key enzymes (Singh *et al.*, 18; Anjum, 2). As salt tolerant types often exhibit significantly lower membrane damage, they succeed in maintaining favourable chlorophyll

levels under salt stress (Singh *et al.*, 8; Singh *et al.*, 9). Similar to SPAD, P_N of attached leaves significantly decreased ($P \leq 0.05$) in salt treated plants (Table 2). At moderate salinity, P_N decreased by about 28 to 32% in different cultivars. At high salinity, the maximum (67.11%) reduction in P_N was noted in CB-2 and the minimum (59.29%) in NB-5 plants. Salinity induced photosynthetic decline in studied cultivars coincided with a significant decrease ($p \leq 0.05$) in stomatal conductance (g_s) and transpiration rate (E , Table 2). At moderate salinity, E decreased by 39% in NB-5, 33% in CB-1 and 29% each in NB-9 and CB-2 plants as compared to control. Such genotypic variations in photosynthetic relations have also been reported in fruit crops such as citrus (López-Climent *et al.*, 6) and olive (Chartzoulakis, 3). Salt tolerant types in citrus either maintain equilibrium or alternatively exhibit substantial declines in gas exchange and CO₂ assimilation under salinity (López-Climent *et al.*, 6). A decrease in stomatal conductance and the concurrent lowering of transpiration rate was noted in all the cultivars but this effect was more pronounced in NB-5. It probably implied a strategy by NB-5 plants to improve water use efficiency under salt stress. The high water use efficiency of NB-5 plants at moderate salinity (about 13% high as compared to control) explains their relative salt tolerance as decline in stomatal conductance may allow the plants to economize water use to partially alleviate the effects of salinity (Chaves *et al.*, 4).

Salt treated plants recorded significantly ($p \leq 0.05$) higher Na⁺ ions in all plant parts as compared to control (Table 3). Nevertheless, data on Na⁺

Table 2. Salinity induced changes in gas exchange parameters in *bael* cultivars.

Cultivar	Soil salinity (dS m ⁻¹)	SPAD	P_N	g_s	E	WUE
NB-5	1.3	44.1b	10.12ab	215.33a	2.34a	4.32b
	6.5	27.91c	7.07c	78.33d	1.42d	4.97a
	10.7	19.61f	4.12d	55.33e	0.86e	4.81a
NB-9	1.3	45.4a	9.89b	218.33a	2.39a	4.15bc
	6.5	25.65d	6.73c	186b	1.7c	3.96c
	10.7	14.62h	3.28e	106cd	1.33d	2.47d
CB-1	1.3	46.21a	10.44a	212.33a	2.18b	4.8a
	6.5	25.67d	7.06c	94bc	1.46d	4.86a
	10.7	16.26g	3.81d	82.33cd	0.9e	4.23bc
CB-2	1.3	45.95a	9.82b	209a	2.4a	4.09bc
	6.5	22.74e	7.05c	105.67b	1.71c	4.13bc
	10.7	12.44i	3.23e	91cd	1.38d	2.34d

Means with at least one letter common in a column are not statistically significant using Duncan's test at 5% level of significance. Note: SPAD= relative chlorophyll, P_N =net photosynthesis ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$), g_s = stomatal conductance ($\text{mol H}_2\text{O}/\text{m}^2/\text{s}$), E = transpiration ($\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$), WUE= water use efficiency (P_N/g_s).

Table 3. Effect of salinity on Na⁺ (% DW) partitioning in different plant parts in *bael* cultivars after 75 days of salt treatment.

Cultivar	Soil salinity (dS m ⁻¹)	Leaf	Upper stem	Basal stem	Primary roots	Root hairs
NB-5	1.3	0.17de	0.08h	0.08e	0.08g	0.18ef
	6.5	0.29b	0.49c	0.23c	0.15f	0.17fg
	10.7	0.16de	0.21g	0.2cd	0.15f	0.15g
NB-9	1.3	0.06g	0.07hi	0.05ef	0.08g	0.11h
	6.5	0.21c	0.29f	0.17d	0.22d	0.44b
	10.7	0.21c	0.39d	0.28b	0.29c	0.33c
CB-1	1.3	0.05g	0.05ij	0.05ef	0.09g	0.2e
	6.5	0.14ef	0.34e	0.2cd	0.2de	0.24d
	10.7	0.12f	0.48c	0.36a	0.34b	0.49a
CB-2	1.3	0.12f	0.04j	0.03f	0.03h	0.08h
	6.5	0.36a	0.6b	0.2cd	0.19e	0.44b
	10.7	0.18d	0.88a	0.32b	0.57a	0.49a

Means with at least one letter common in a column are not statistically significant using Duncan's test at 5% level of significance.

partitioning indicated a strong tendency for its restricted uptake with increasing salinity in NB-5 (Table 3). While, leaf Na⁺ concentrations were statistically similar at both moderate and high salinities in NB-9 and CB-1, NB-5 and CB-2 exhibited significantly lower ($p \leq 0.05$) leaf Na⁺ concentrations (about 50% in each case) at high salinity as compared to moderate salinity. In upper stem (primary and secondary branches; excluding the leaves) tissues, cultivar NB-5 exhibited significantly lower Na⁺ (0.21% DW) at high salinity as compared to moderate salinity (0.49% DW). In contrast, other cultivars exhibited significantly higher ($p \leq 0.05$) Na⁺ concentrations in upper stems at high salinity. In basal stems (5-6 cm above the graft union; below the branching point), primary roots and root hairs, cultivar NB-5 exhibited non-significant differences for Na⁺ accumulation at both moderate and high salinity treatments. Although leaf Na⁺ concentration in NB-5 (0.29%) was almost two-fold high relative to CB-1 (0.14%) at 6.5 dS m⁻¹ salinity, it did not exhibit severe injury symptoms while the latter showed severe chlorosis, marginal scorch and downward leaf curling. It appears, therefore, that besides restricted uptake and subsequent translocation to foliage, NB-5 cultivar had a higher threshold for Na⁺ induced toxicity. Na⁺ partitioning under salinity indicated distinct behaviour of the tested cultivars. Cultivar NB-5, in spite of higher leaf Na⁺ concentration at moderate salinity, did not exhibit pronounced injury symptoms till the end of experiment as compared to other cultivars. It points to relative cellular tolerance for Na⁺ as well as probable osmotic adjustment in NB-5 plants. At high salinity, NB-5 plants showed almost similar distribution of

Na⁺ ions in leaf, stem and root tissues and this could have prevented excessive Na⁺ accumulation in aerial parts. Cultivar CB-1 showed partial retention of Na⁺ ions in stem and root tissues, but failed to avoid the injury symptoms. Both NB-9 and CB-2 cultivars showed excessive Na⁺ build up in leaves and stems. The different patterns of sodium uptake and accumulation observed in these cultivars highlight the existence of separate mechanisms, which operate to limit the transport of Na⁺ from the roots to aerial parts. In a nutshell, cultivar NB-5 exhibited higher tolerance threshold for Na⁺ at moderate salinity and restricted Na⁺ uptake at high salinity.

The leaf, stem and root K⁺ concentrations significantly decreased with increasing salinity in all the cultivars (Table 4). At both moderate and high salinities, cultivar NB-5 maintained significantly higher leaf K⁺ as compared to others. Moreover, it also exhibited a tendency for higher K⁺ accumulation at high salinity as compared to moderate salinity with significant differences for upper and basal stems and primary roots and non-significant differences for leaves and root hairs. At moderate salinity, 1.25 to two-fold more K⁺ ions were retained in the root hairs of NB-9, CB-1 and CB-2 plants as compared to those of NB-5. At moderate salinity, the highest K⁺ concentration in upper stems was noted in CB-1 (0.61% DW) and the lowest (0.49% DW) in NB-9. At high salinity, the highest K⁺ concentration in upper stems was noted in NB-5 (0.68% DW) and the lowest (0.45% DW) in NB-9. The higher K⁺ concentrations in leaf and stem tissues of NB-5 indicated an easier translocation of K⁺ from root to aerial parts where it seems to have partially substituted for toxic Na⁺ ions

Table 4. Effect of salinity on K⁺ (% DW) partitioning in different plant parts in *bael* cultivars after 75 days of salt treatment.

Cultivar	Soil salinity (dS m ⁻¹)	Leaf	Upper stem	Basal stem	Primary roots	Root hairs
NB-5	1.3	0.95b	1.05b	0.73a	0.51b	1.01a
	6.5	0.34d	0.54g	0.34e	0.37ef	0.27g
	10.7	0.39d	0.68de	0.4d	0.51b	0.28g
NB-9	1.3	0.92b	0.73d	0.57b	0.45c	0.98a
	6.5	0.17f	0.49gh	0.34e	0.44cd	0.53d
	10.7	0.24e	0.45h	0.4d	0.4de	0.25g
CB-1	1.3	0.83c	0.88c	0.6b	0.73a	0.68c
	6.5	0.22ef	0.61f	0.32ef	0.34fg	0.34f
	10.7	0.11g	0.63ef	0.29f	0.36ef	0.47de
CB-2	1.3	1.31a	1.13a	0.47c	0.5b	0.75b
	6.5	0.26e	0.5gh	0.31ef	0.3h	0.42e
	10.7	0.18f	0.61f	0.43cd	0.31gh	0.34f

Means with at least one letter common in a column are not statistically significant using Duncan's test at 5% level of significance.

and thus created a favourable K⁺/Na⁺ ratio for plant growth under elevated salinity.

Data on Ca²⁺ partitioning in different plant parts (Table 5) indicated that moderate salinity induced slight decrease in NB-5 and CB-2 while marginal increase in leaf Ca²⁺ in NB-9 and CB-1 cultivars. In contrast, at high salinity, only NB-5 plants showed significantly higher Ca²⁺ in leaves while other cultivars exhibited significant decrease relative to control. In upper stems, Ca²⁺ concentration either showed significant increase (NB-5 and CB-2) or decrease (NB-9) or no change (CB-1). In basal stems, Ca²⁺ concentration significantly decreased in all the cultivars with increasing salinity except in NB-5 which showed higher Ca²⁺ as compared to control.

Ca²⁺ concentration in primary roots increased in salinized plants of all cultivars except NB-9. Cultivar NB-5 almost invariably showed increase in Ca²⁺ concentrations with increasing salinity except the slight reduction in leaf as compared to control. Cultivar NB-5 either maintained Ca²⁺ concentrations in different plant parts at par with control or even showed significantly higher Ca²⁺ levels under elevated salinity. It pointed to preferential accumulation and easy translocation of Ca²⁺ to foliage tissues under saline conditions. On the contrary, salinity induced reduction in Ca²⁺ levels hampered the growth in other cultivars. Similar findings have earlier been reported in citrus (García-Sánchez *et al.*, 5) where salt tolerant lines did not show impaired Ca²⁺ nutrition under salinity.

Table 5. Effect of salinity on Ca²⁺ (% DW) partitioning in different plant parts in *bael* cultivars after 75 days of salt treatment.

Cultivar	Soil salinity (dS m ⁻¹)	Leaf	Upper stem	Basal stem	Primary roots	Root hairs
NB-5	1.3	2.8efg	1.8f	3.88d	1.8fg	2.04h
	6.5	2.33h	2.36d	3.95d	3.55b	9.64a
	10.7	3.03cde	3.43b	4.5c	4.5a	9.61a
NB-9	1.3	3.89b	4.12a	5.45a	2.92c	2.54fg
	6.5	4.51a	3.33b	3.24e	1.67gh	3.29cd
	10.7	3.26c	1.88ef	3.23e	2.37d	6.29b
CB-1	1.3	2.94def	2.4d	4.97b	1.08i	2.23gh
	6.5	3.11cd	2.33d	2.32g	2.03ef	3.65c
	10.7	2.65g	2.4d	2.62f	1.56gh	2.87ef
CB-2	1.3	2.95def	1.98e	3.87d	1.08i	1.12i
	6.5	2.93def	2.73c	2.23g	1.45h	3.22de
	10.7	2.76fg	2e	2.12g	2.25de	2.26gh

Means with at least one letter common in a column are not statistically significant using Duncan's test at 5% level of significance.

Based on the above results, it can be said that salt stressed *bael* cultivars tend to arrest gas exchange in leaves so as to economize water use as well as to restrict the uptake of toxic sodium ions through transpiration stream. Ionic partitioning in salt treated plants indicated restricted Na⁺ uptake and preferential K⁺ accumulation in NB-5 cultivar. Based on the extent of salt injury symptoms in leaves, gas exchange characteristics and ionic distribution in leaf, stem and root tissues, cultivar NB-5 outperformed other tested cultivars in moderately saline soils (EC_e ~6.5 dS m⁻¹).

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