Journal of Soil Salinity and Water Quality 7(1), 40-44, 2015

# Salinity Induced Changes in Chlorophyll Pigments and Ionic Relations in Bael (*Aegle marmelos* Correa) Cultivars

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**Abstract**

Bael (*Aegle marmelos*) cultivars NB-5, NB-9, CB-1 and CB-2 were grown in normal ($EC_e$ 1.3 dS $m^{-1}$), moderate (6.5 dS $m^{-1}$) and high (10.7 dS $m^{-1}$) saline soils. The cultivars, evaluated for changes in appearance, leaf chlorophyll pigments and ionic relations, exhibited salt stress symptoms as yellowing, scorching and chlorosis of the leaves. Majority of the affected leaves subsequently abscised from the plants. Salinity significantly increased membrane injury and caused reduction in relative water content in all the cultivars. Accumulation of total soluble sugars in salt treated plants showed genotypic differences with NB-5 plants recording their maximum accumulation. Leaf chlorophyll (a, b and total) values showed a consistent decrease with increase in salinity except for NB-5 plants which exhibited slightly higher chlorophyll contents at moderate salinity. All the cultivars except NB-5 exhibited significantly higher leaf $Na^+$ concentrations with increasing salinity while the plants of cultivar NB-5 maintained a favourable ionic balance in terms of low $Na^+/K^+$ ratio resulting in good plant performance under salinity. The plants of NB-9 and CB-2 varieties did not survive at high salinity. Based on overall performance, NB-5 exhibited tolerance to moderately saline soils ($EC_e$ ~6.5dS $m^{-1}$) and was successfully established when irrigated with normal water.

***Key words*: *Aegle marmelos*, Cultivars, Mineral nutrition, Salinity tolerance, Salt-affected soils**

## Introduction

A sustainable approach for the productive utilization of salt-affected soils (SAS), which cover approximately 6.73 m ha area in India, relates to the use of salt tolerant crop genotypes (Singh *et al.*, 2010). Many plants of economic importance exhibit salt tolerance and are adapted to grow in salt-affected environments. Similarly, many improved crop genotypes have been developed and/or identified for commercial cultivation in SAS (Flowers, 2004). A number of fruit crops perform well under salinity stress and may be commercially grown in saline and sodic soils (Dagar, 2009). Bael (*Aegle marmelos* Correa) is an indigenous underutilized fruit crop valued for its medicinal and processing values and tolerance to different biotic and abiotic stresses. In spite of these strengths, there is no organized cultivation of this fruit in India. Bael cultivation in SAS could be a good option for alternate land use and crop diversification (Dagar, 2009). Salt stressed bael plants suffer from nutrient deficiencies (N, P, K and Ca) which may account for poor plant establishment and growth under salt stress (Shukla and Singh, 1996). The available reports on salinity tolerance in bael provide least information on important physiological and biochemical parameters of the tolerant and susceptible accessions which may explain plant behaviour in relation to salinity and may be correlated with salinity tolerance mechanism in certain cultivar(s). In this backdrop, one-year old plants of four improved bael genotypes were evaluated in saline soils for assessing their salt tolerance so as to appraise their suitability for cultivation in SAS.

## Material and Methods

The present experiment was carried out during 2013-2014 at the experimental facility of 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 CISH Bael-2 (CB-2) procured from the ICAR-Central Institute of Subtropical Horticulture, Lucknow, India were used. The saline soils used in this experiment were obtained from the CSSRI-Nain Experimental Farm, Panipat, India; while control soil was obtained from the crop fields. The soil was filled in large, metallic

**Table 1.** Initial physico-chemical properties of the experimental soil

| Type of soil/soil properties | Control | Moderately saline | Highly saline |
|---|---|---|---|
| $pH_s$ | 7.6 | 8.0 | 8.1 |
| $EC_e$ (dS $m^{-1}$) | 1.3 | 6.5 | 10.7 |
| $HCO_3$* (meq $l^{-1}$) | 0.60 | 0.40 | 0.60 |
| Organic carbon (%) | 0.74 | 0.34 | 0.77 |
| Available N (kg $ha^{-1}$) | 43.12 | 39.2 | 54.88 |
| Available P (kg $ha^{-1}$) | 33.60 | 13.44 | 38.80 |
| Available K (kg $ha^{-1}$) | 100.7 | 92.50 | 117.5 |

*Carbonates were not present in experimental soil.

experimental columns of approximately 74 cm length, 44 cm width and 166 cm circumference (each column containing approximately 76 kg soil). There were three salinity treatments: control (soil $EC_e$ 1.3 dS $m^{-1}$), moderate ($EC_e$ 6.5 dS $m^{-1}$) and high ($EC_e$ 10.7 dS $m^{-1}$). After transplanting, the plants were irrigated with normal water ($EC_{IW}$ 0.5 dS $m^{-1}$) till the time of data recording.

Soil $pH_s$ and $EC_e$ were determined by a glass electrode pH meter and electrical conductivity meter, respectively in the supernatant. The organic carbon content in soil was determined by wet oxidation method of Nelson and Sommer (1982). Carbonate and bicarbonate in soil water extract were determined using 0.01N $H_2SO_4$ as described by Richards (1954). The available N in soil was determined by alkaline permanganate method (Subbiah and Asija, 1956), available P by colorimetric method (Olsen *et al.*, 1954) and available K by flame photometry (Jackson, 1973).

One-year after salt treatment, the leaves were collected and analysed for estimating salinity induced physio-biochemical changes. The 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. Total soluble sugars were measured by the colorimetric method with antrone reagent (Yemm and Wills, 1954). Total soluble protein content was determined according to Bradford (1976). The leaf chlorophyll (chlorophyll a, b, and total) values were estimated using the method of Hiscox and Israelstam (1979). For ionic relations, leaves were dried in a forced-draft oven at 60 ºC 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 ºC. $Na^+$ and $K^+$ contents were determined by using the flame photometer (Systronics, India).

## Results and Discussion

### Selected soil properties

The initial physico-chemical properties of the experimental soil (Table 1) indicated its low organic carbon (0.34-0.77 %) status, extreme deficiency of available nitrogen (39.2-54.88 kg $ha^{-1}$), moderate quantities of available P (13.44-38.8 kg $ha^{-1}$) and low to moderate available K (92.5-117.5 kg $ha^{-1}$). The $pH_s$ in saturation extract was in range of 7.6-8.1. The electrical conductivities of experimental soils in soil saturation paste extract were 1.3, 6.5 and 10.7 and the soils were accordingly categorized as normal, moderately saline and highly saline. The saturation extract of soils used had measurable amount of $HCO_3^-$ but $CO_3^{2-}$ was not detectable. Plants growing in such saline soils face osmotic stress and nutrient toxicities (primarily due to excess accumulations of $Na^+$ and $Cl^-$ ions) which result in poor growth (Flowers, 2004). The traditional practice of leaching the excess soluble salts below root zone to facilitate crop production in saline soils requires huge amounts of good quality water. Given the high environmental footprint of this technology and limited availability of fresh water, identification of salt tolerant genotypes can greatly help in their productive utilization (Dagar, 2009; Sharma *et al.*, 2014).

### Morphological symptoms of salt stress

Morphological symptoms (data not shown) revealed that salt stressed plants initially showed yellowish appearance and marginal scorching in leaves as compared to their non-salinized counterparts. With increase in duration of salt treatment, these symptoms spread to entire leaf which eventually became chlorotic and abscised from the plants. At 6.5 dS $m^{-1}$ salinity, NB-9 and CB-2 plants were severely affected while those of CB-1 recorded relatively lesser injury but salt treated NB-5 plants maintained growth comparable to their non-salinized counterparts. At high salinity (10.7 dS $m^{-1}$), all the cultivars exhibited severe reduction in growth and those of NB-9 and CB-2 did not survive. These results are consistent with previous salinity studies in bael (Pandey *et al.*, 1985). Salt stressed bael plants suffer from nutritional deficiencies and ion toxicities which seem to cause injury symptoms such as scorching, chlorosis and necrosis of leaves and in extreme cases eventual abscission from the plants (Shukla and Singh, 1996) resulting in poor plant growth and establishment.

**Membrane injury index and relative water content**

Salt stressed bael plants exhibited significant cultivar differences for membrane injury index and relative water content (Table 2). At moderate (6.5 dS $m^{-1}$) salinity the membrane injury ranged from 38% (CB-2) to 74% (NB-9) as compared to control plants. Similarly differences were also observed among the cultivars for relative water content (RWC). Among cultivars, moderate salinity caused the maximum decrease (23.8%) in RWC in CB-2 while the minimum (7.8%) decrease occurred in CB-1. In citrus, cultivar differences have earlier been reported for salinity induced membrane damage and reduction in RWC in leaves. Salt stressed plants exhibit damage of lipid membranes which often results in increased cell permeability and electrolyte leakage from cells (Singh *et al.*, 2014). High salt concentration in root zone, which causes osmotic stress, restricts water absorption by the plants and causes cellular dehydration, seems to be primarily responsible for decrease in RWC (Greenway and Munns, 1980). These results are in agreement with findings of Singh *et al.* (2014) in citrus.

**Table 2.** Effect of salinity on membrane injury index (MII), relative water content (RWC) and total soluble sugars (TSS) in bael cultivars

| Cultivar | Soil salinity (dS $m^{-1}$) | MII | RWC (%) | TSS (mg $g^{-1}$ DW) |
|---|---|---|---|---|
| NB-5 | 1.3 | 14.46g | 77.78a | 13.36f |
| | 6.5 | 21.61e | 71.08b | 22.4c |
| | 10.7 | 37.03b | 59.89c | 31.43a |
| NB-9 | 1.3 | 16.3fg | 78.37a | 13.1f |
| | 6.5 | 28.35c | 64.04c | 21.69cd |
| | 10.7 | PNS | PNS | PNS |
| CB-1 | 1.3 | 14.52g | 79.53a | 14.08f |
| | 6.5 | 24.46d | 73.34b | 18.4e |
| | 10.7 | 42.71a | 55.25d | 28b |
| CB-2 | 1.3 | 17.44f | 79.96a | 13.39f |
| | 6.5 | 24.08de | 60.94c | 20.26d |
| | 10.7 | PNS | PNS | PNS |

Means with at least one letter common in each column are not statistically significant using Duncan's Test at 5% level of significance. PNS= Plants not survived (NB-9 and CB-1 plants did not survive at 10.7 dS $m^{-1}$ salinity).

**Total soluble sugars**

Salt treated bael cultivars exhibited different accumulation patterns of total soluble sugars (TSS) in leaves (Table 2). Although TSS concentration in leaves increased with salinity in all the cultivars, NB-5 plants had significantly higher TSS at both moderate and high salinity as compared to other cultivars. It suggested the salt tolerant nature of NB-5 plants as soluble sugars accumulate in higher concentrations in salt stressed plants and contribute to osmotic adjustment (Bolarín *et al.*, 1995).

**Chlorophyll pigments**

All the cultivars showed significant reductions in chlorophyll (a, b and total) values with increasing salt stress except NB-5 which showed slightly higher chlorophyll contents at moderate salinity (Table 3). At 6.5 dS $m^{-1}$ salinity, decrease in chlorophyll 'a' was 30% in NB-9, 56% in CB-1 and 47% in CB-2 as compared to respective control plants. Similarly, chlorophyll 'b' content decreased by 13% in NB-9, 35% CB-1 and 22.45% in CB-2 as compared to control. In general, high salinity causes a decrease in chlorophyll in both tolerant and susceptible genotypes in different crop (Misra *et al.*, 1997; Singh *et al.*, 2014) but in certain cases low-to-moderate salt concentrations may favour preferential accumulation of these pigments in tolerant types (Misra *et al.*, 1997). The effects of salt stress on chlorophyll degradation, presumably due to increased activity of the enzyme chlorophyllase (Misra *et al.*, 1997), were characterized as the yellowing of leaves which failed to produce the optimum amounts of photosynthates leading to reduced plant growth and vigour.

**Table 3.** Effect of salinity on chlorophyll pigments in bael cultivars

| Cultivar | Soil salinity ($dS\ m^{-1}$) | Chlorophyll 'a' ($mg\ g^{-1}$ FW) | Chlorophyll 'b' ($mg\ g^{-1}$ FW) | Total chlorophyll ($mg\ g^{-1}$ FW) |
|---|---|---|---|---|
| NB-5 | 1.3 | 2.02d | 1.38b | 3.41b |
| | 6.5 | 2.4bc | 1.59a | 4a |
| | 10.7 | 0.85g | 1.12d | 1.97d |
| NB-9 | 1.3 | 2.32c | 1.56ab | 3.87a |
| | 6.5 | 1.63e | 1.36bc | 2.99c |
| | 10.7 | PNS | PNS | PNS |
| CB-1 | 1.3 | 2.69a | 1.17cd | 3.86a |
| | 6.5 | 1.17f | 0.76e | 1.93d |
| | 10.7 | 0.68h | 0.49f | 1.17e |
| CB-2 | 1.3 | 2.46b | 0.98d | 3.44b |
| | 6.5 | 1.3f | 0.76e | 2.06d |
| | 10.7 | PNS | PNS | PNS |

Means with at least one letter common in each column are not statistically significant using Duncan's Test at 5% level of significance. PNS= Plants not survived (NB-9 and CB-1 plants did not survive at 10.7 $dS\ m^{-1}$ salinity).

**Sodium, potassium and sodium: potassium ratio**

At both salinity levels, there was a significant increase in leaf $Na^+$ accumulation irrespective of the cultivar (Table 4). At moderate salinity, there was a three-fold increase in $Na^+$ concentration in leaves of NB-5 relative to control while the corresponding increase was five-times in NB-9 and four-times in both CB-1 and CB-2 cultivars. Cultivar NB-5 not only prevented the accumulation of $Na^+$ to toxic levels but also exhibited higher $K^+$ concentrations and thus maintained a favourable ionic balance in terms of low $Na^+/K^+$ ratio resulting in good plant performance.

**Table 4.** Ionic relations in bael cultivars under salinity stress

| Cultivar | Soil salinity ($dS\ m^{-1}$) | $Na^+$ (% DW) | $K^+$ (% DW) | $Na^+/K^+$ ratio |
|---|---|---|---|---|
| NB-5 | 1.3 | 0.06e | 0.27g | 0.22a |
| | 6.5 | 0.18d | 1e | 0.18cd |
| | 10.7 | 0.29b | 1.46b | 0.2bc |
| NB-9 | 1.3 | 0.06e | 0.45f | 0.13e |
| | 6.5 | 0.3b | 1.35c | 0.22a |
| | 10.7 | PNS | PNS | PNS |
| CB-1 | 1.3 | 0.06e | 0.42f | 0.14e |
| | 6.5 | 0.24c | 1.07d | 0.23a |
| | 10.7 | 0.35a | 1.65a | 0.21ab |
| CB-2 | 1.3 | 0.06e | 0.46f | 0.12e |
| | 6.5 | 0.24c | 1.41bc | 0.17d |
| | 10.7 | PNS | PNS | PNS |

Means with at least one letter common in each column are not statistically significant using Duncan's Test at 5% level of significance. PNS= Plants not survived (NB-9 and CB-1 plants did not survive at 10.7 $dS\ m^{-1}$ salinity).

The plants of NB-9 and CB-2 varieties, due to $Na^+$ toxicity, did not survive at high salinity. Most of the fruit crops are sensitive to excessive concentrations of $Na^+$ ions in the growing medium. Excessive $Na^+$ concentration reduces the uptake of $K^+$ and $Ca^{2+}$ by plants. Available reports in citrus point to genotypic differences which may explain better performance by some scions and/or rootstocks as compared to others (Levy and Syvertsen, 2004; Murkute *et al.*, 2005).

## Conclusions

The bael cultivars tested in this experiment exhibited tolerance to low and moderate salinity. Cultivar NB-5 exhibited relatively higher salinity tolerance and showed better performance as compared to other cultivars. Although salinity adversely affected some of the physiological traits in NB-5 plants, they showed better salt tolerance owing to favourable chlorophyll concentration, restricted uptake of $Na^+$ ions and higher $K^+$ accumulation in leaves. In concluding remarks, the commercial cultivation of NB-5 is feasible in moderately saline soils ($EC_e$ ~6.5).

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Received: January, 2015; Accepted March, 2015