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Effect of salinity on leaf health and total leaf chlorophyll contents of Bael (*Aegle marmelos* Correa.) Cultivars

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Abstract
Salinity is considered as the most important abiotic stress limiting fruit production and fruit plants such as bael is known to survive under this stress by involving many mechanisms. In this context, the present study was carried out to evaluate the impact of salinity on leaf health and chlorophyll content in five bael cultivars i.e. NB-5, NB-9, CISHB-1, CISHB-2 and Pant Aparna. Thus, an experiment was carried out at horticultural farm and laboratory of Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The salinity levels induced stress on bael seedlings were 5.40 dSm^{-1}, 9.20 dSm^{-1}, 13.30 dSm^{-1} and 16.80 dSm^{-1}. Results showed that increasing salinity stress, for all cultivars, had a negative impact on leaf and chlorophyll contents. Cultivar NB-5 satisfactorily tolerated highest salinity level (16.80 dSm^{-1}) comparing to other cultivars by less accumulation of salts in their leaves and showed higher level (1.85 mg/g fresh weight basis) of chlorophyll content in bael leaves. NB-9 and CISHB-^{1}also maintaining usually higher values of both the parameters and consider as medium salinity tolerant.

Keywords: Bael, chlorophyll, leaves, salinity, soil.

Introduction
The bael (*Aegle marmelos* Correa) is a deciduous, aromatic and an important indigenous fruit of India belongs to the family Rutaceae and different species of this genus exists naturally all over India (Singh *et al*., 2014) [10]. It is one of the most sacred trees of India. Hindus hold the tree in great adorations and especially leaf of bael tree is consumed in latria of Lord Shiva, whose worship can't be accomplished without offering its leaves (Ariharan and Prasad, 2013) [1].

Ramachandra and Kumar (2003) [9] suggested planting of *Aegle marmelos* is advantageous for sustainable management of degraded or waste land. Considering this bael cultivars *viz*., NB-5, NB-9, CISHB-1, CISHB-2 and Pant Aparna were transplanted at different salinity levels (i.e. 5.40 dSm^{-1}, 9.20 dSm^{-1}, 13.30 dSm^{-1} and 16.80 dSm^{-1}) to assess the effect of salinity on leaves character and chlorophyll content.

However, the osmotic effects of salinity contribute to reduced growth rate, changes in leaf color, and developmental characteristics. Ionic effects are manifested more generally in leaf and meristem damage or as symptoms typical of nutritional disorders. Thus, high concentrations of Na or Cl may accumulate in leaves or portions thereof and result in 'scorching' or 'firing' of leaves. Leaf injury and death is probably due to the high salt load in the leaf that exceeds the capacity of salt compartmentation in the vacuoles, causing salt to build up in the cytoplasm to toxic levels (Lauchli and Grattan, 2007) [5]. Salinity levels also decide the extent of damage caused by stress which principally has effect on the chlorophyll content of plants and it also affects photosynthesis mainly through a reduction in leaf area and impairment in chlorophyll.

However, the information on the effect of salinity levels which influence the leaf and chlorophyll contents particularly of bael cultivars is meager. The present investigation is, therefore, an attempt to see the salt injury symptoms on the leaf and the effect of salinity stress on total leaf chlorophyll content of different bael cultivars at different salinity levels.

Materials and Methods

The present investigation was carried out at Horticulture Research Farm and Laboratory of the Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, U.P. (India) during the year 2013-2014 and 2014-2015 with a view to assess the tolerance limit of five commercially grown cultivars of bael to saline soils of the northern India. The materials used and methodologies adopted in this experiment are as follows:

Soil collection, sampling and analysis

Top soil upto 15 cm depth was scraped from the site which was normal in nature. Soil thus obtained was mixed well and all the waste materials such as weed, roots, sand and gravels were removed. Five hundred gram soil was drawn from it using quartering method. The sample was dried in oven and passed through 2mm sieve. Finally, sample was analyzed for various physico-chemical characteristics of soil.

Preparation of artificial saline soil

Artificially salinization of soil was done by using the mixture of salts, i.e. $CaCl_2$, $MgCl_2$, NaCl and Na_2SO_4 in the ratio of 1: 1: 1: 2 and then chloride dominated salinity was maintained. The ratio between Cl^- and SO_4^- was observed 60: 40. Salt solution containing above salts was prepared on the basis of saturation percentage of the soil. For easy and uniform distribution of salts, half of the soil was spread on a polythene sheet in about 3 cm thick layer.

The salt solution was thoroughly sprayed with a fine nozzle sprayer. Remaining soil was spread over the treated layer of the soil. The treated soil was covered with polythene sheet for two days to facilitate the upward and downward movement of the salt in the soil and thereafter it was raked thrice and mixed thoroughly. Thus, four levels of salinity (ECe) i.e., 5.40, 9.20, 13.30 and 16.80 dSm^{-1} were maintained. For control, the soil was treated only with ordinary water in which no salt was added.

Eight month to one year old bael seedlings of average height about 15 cm in each cultivar of NB-5, NB-9, CISHB-1, CISHB-2 and Pant Aparna were transplanted in pots (40 ×40 cm size) in which all four levels of salinity along with control was separately maintained. After adjustment of different salinity levels, soil samples were collected from each salinity level and ECe as well as pH was initially checked (Table-1) before the plantation, which were estimated again at the time of termination of experiment with no marked variations in their values.

Total Chlorophyll content estimation

Mature leaves were plucked in July and the fresh leaves were used for the estimation of total chlorophyll content. The chemical analysis was conducted at the termination of the experiments and total leaf chlorophyll was determined by the method as suggested by Arnon (1949) [2].

Results and Discussions

Nature and extent of salt injury

The visual symptoms of salt injury were recorded regularly from planting to termination of the experiment. The injury symptoms started with change in colour of leaves from dark green to either light or whitish or yellowish green. Burning and necrosis of apical portion of leaves and drying of shoot tip were also observed in some cases. Leaf injury symptoms were first observed in younger leaves. Cell elongation and to a lesser extent cell division, are reduced leading to lower rates of leaf growth (Lauchli and Grattan, 2007) [5]. Some of the leaves were completely burnt and lastly abscised which account for the ultimate death of the plants.

The main visual symptoms of salt injury at higher salinity levels were the inhibition of plant growth. The process of salinization attributed to increase in level of soluble salts (i.e. mostly Ca and Mg are cations and Cl^- and SO_4^+ are anions) and their accumulation on the soil surface, resulted in devastating effect on plant metabolism through disrupting major physiological and biochemical process (Mane *et al*., 2011) [7]. Generally the salinity effect appears firstly on leaves of the fruit plants. Exclusively salinity reduces the growth rate resulting in smaller leaves, shorter stature, and sometimes fewer leaves. The initial and primary effect of salinity, especially at low to moderate concentrations, is due to its osmotic effects (Jacoby, 1999) [4]. The comparative performance of bael cultivars in terms of their injury at different salinity level are given in Table-2. None of bael cultivars showed leaf injury in control and 5.40 dSm^{-1} salinity levels. However, there was no symptom of toxicity in NB-5, NB-9 and CISHB-1, while CISHB-2 and Pant Aparna showed change in leaf colour from dark green to yellowish green at 9.20 dSm^{-1} but at 13.30 dSm^{-1} all the cultivars (except NB-5) showed change the leaves colour from dark green to yellowish or whitish green along with burning and necrosis of apical portion of leaves was noted only in Pant Aparna. At 16.80 dSm^{-1} level, all cultivars showed change in leaves colour from dark green to yellowish or whitish green along with burning and necrosis of apical portion of leaves excluding NB-5 in which change in leaves colour from dark green to yellowish or whitish green appeared. NB-5 showed to have better tolerance to salinity than other cultivars.

Chlorophyll content

It is evident from the data presented in Table-3 that all salinity level and cultivars of bael significantly differed with respect to total chlorophyll content in leaves. The Highest chlorophyll content (1.825 mg/g) was noted in cv. NB-5. It was followed by NB-9 (1.718 mg/g). Salinity levels showed deteriorative effect on chlorophyll content as compared to control. The maximum 1.85 mg/g chlorophyll was recorded on normal soil. The chlorophyll content consistently declined with increasing salinity levels. The minimum chlorophyll (1.370 mg/g) was noted at 16.80 dSm^{-1} levels. Chartzoulakis *et al*. (2002) [3] proposed that by increasing level of salinity, decreased the chlorophyll content and has detrimental effect on olives leaf.

Interaction effect revealed notable differences between cultivars and salinity levels and the value ranged between 1.957 and 1.570 mg/g under the normal soil condition. Cultivar CISHB-2 was found to have the highest (1.957 mg/g) chlorophyll content under normal soil condition and the minimum (1.307 mg/g) with cultivar Pant Aparna at 16.80 dSm^{-1} level of salinity. Data clearly revealed that chlorophyll content decreased significantly in all cultivars with increase in salinity level as compared to plant grown in normal soil condition. Lichtenthaler *et al*. (2005) [6] found that salt stress was responsible for decreased biosynthesis of chlorophyll and inefficiency of photosynthesis (Munns, 2002) [8].

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Table 1: Showing the desired and obtained salinity levels.

Desired level of ECe dSm^{-1}	Obtained level of ECe	
	dSm^{-1}	pH (1:2.5soil-water suspension)
Control	0.76	7.3
5.40	5.60	7.5
9.20	9.30	7.8
13.30	13.00	8.3
16.80	16.50	8.5

Table 2: Leaf injury in relation to salinity of bael cultivars

Salinity Level (T) / Variety (V)	(Control)	5.40 dSm^{-1}	9.20 dSm^{-1}	13.30 dSm^{-1}	16.80 dSm^{-1}
(V_1) NB-5	A	A	A	A	B
(V_2) NB-9	A	A	A	B	B,C
(V_3) CISHB-1	A	A	A	B	B,C
(V_4) CISHB-2	A	A	B	B	B,C
(V_5)Pant Aparna	A	A	B	B,C	B,C

***A**-No symptoms appeared,
B-Change in leaves colour from dark green to yellowish or whitish green,
C-Burning and necrosis of apical portion of the leaves

Table 3: Effect of salinity on chlorophyll content (mg/g fresh weight basis) in bael leaves

Salinity Level (T) / Variety (V)	T_1 (Control)	T_2 5.40 dSm^{-1}	T_3 9.20 dSm^{-1}	T_4 13.30 dSm^{-1}	T_5 16.80 dSm^{-1}	Mean
(V_1) NB-5	1.930	**1.900**	1.887	1.730	1.680	1.825
(V_2) NB-9	1.910	1.840	1.697	1.610	1.533	1.718
(V_3) CISHB-1	1.760	1.713	1.530	1.403	1.167	1.515
(V_4) CISHB-2	1.957	1.617	1.273	1.313	1.167	1.465
(V_5)Pant Aparna	1.570	1.567	1.540	1.413	**1.307**	1.479
Mean	1.825	1.727	1.585	1.493	1.370	
	V		**T**		**V×T**	
SEm±	0.018		0.018		0.039	
CD(P=0.05)	0.050		0.050		0.112	

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