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Impact of different salinity levels on physiological attributes of *Leucaena leucocephala* Lam. in early growth stage

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Abstract
The present study was carried out in the poly house of College of Forestry, Navsari Agricultural University, Navsari, Gujarat. Two clones of *Leucaena leucocephala* Lam. (clone-C_1 and C_2) were treated with different salinity levels of S_1 (Normal water), S_2 (3 dS m^{-1}), S_3 (5 dS m^{-1}), S_4 (7 dS m^{-1}), S_5 (9 dS m^{-1}), S_6 (11 dS m^{-1}) and S_7 (13 dS m^{-1}). Different physiological parameters such as chlorophyll content, relative water content and total sugar content in plants were found significant for different salinity levels and clones where as non-significant for salinity level and clonal interaction at 180 DAT. The higher chlorophyll content (11.52 mg/g) in S_1 (normal water), relative water content (77.71 %) in S_4 (7 dS m^{-1}) and total sugar content (4.09 %) in S_5 (9 dS m^{-1}) were observed; however, all these values found least in S_7 *i.e.,* 13 dS m^{-1} (9.81 mg/g of chlorophyll content, 63.96 % relative water content and 3.65 % of total sugar content). Clone C_1 exhibited maximum chlorophyll content (11.25 mg/g), relative water content (73.58 %) and total sugar content (4.67 %) as compared to clone C_2. The different physiological parameters of clone C_1 are higher than the clone C_2 which may positively influence on growth and biomass attributes.

Keywords: Chlorophyll content, *Leucaena leucocephala* clone, relative water content, salinity levels, total sugar content

Introduction
The genus *Leucaena* belongs to family Fabaceae and subfamily Mimosoideae and this genus includes about 32 species which are distributed in the most of the tropical areas of the world. *Leucaena leucocephala* Lam. de Wit. is most important species grown in Indian condition and commonly called as Subabul (Pandey and Kumar, 2013) [23]. Subabul is grown in Central and South America, Africa, Australia, Southeast Asia and most of the tropical islands (Brewbaker, 1987) [5]. In India, Subabul is grown mainly in the states of Andhra Pradesh, Tamil Nadu, Karnataka, Himachal Pradesh and Uttar Pradesh (Lohan, 1979) [16]. *L. leucocephala* can used for reduce soil erosion, reclamation of marginal and degraded waste land (due to high nitrogen-fixing potential), as ornamental and to control air pollution along roadside, as live fence along cultivated fields and for shade and support for shade loving plants and vines such as pepper, vanilla and yam *etc*. It has high nitrogen-fixing potential (100-500kg N/ha a year), related to its abundant root nodulation, roughly equivalent to 2500 kg of ammonium sulfate. It also can be used for parquet flooring and small furniture as well as for paper pulp (Despande, 1981) [8].

Abiotic stress such as drought and salinity leads to a series of physiological, biochemical and molecular changes that adversely affect plant growth and productivity (Wang *et al.*, 2003) [29]. Drought and salinity are often interconnected and may induce similar cellular changes, which are manifested largely as osmotic stress, resulted in the disruption of homeostasis and ion distribution in the cell (Chaves *et al.*, 2009; Langridge *et al.*, 2006; Bohnert and Sheveleva, 1998) [6, 14, 4]. High concentrations of NaCl may cause both hyperionic and hyperosmotic stress effects, which lead to a decline of turgor, disordered metabolism and the inhibition of uptake of essential ions, as well as other problems in plant cells (Kim *et al.*, 2007) [13]. Similarly a water deficient condition also disturbs the inherent balance of plant metabolite and renders changes in its metabolic network. Plant growth under saline conditions is usually marginal and is adversely affected either due to high osmotic stress or due to toxicity or deficiency of specific cation. Growth suppression is initiated at some threshold value of salinity which varies with species tolerance.

It is necessary to determine the threshold limit of salinity exposure within which a species can grow suitably at a given site, before significant loss of planting stock occurs. Salt stress is one of the major abiotic stress factors that affect almost every aspect of plant growth, physiology and finally its yield (Fooland, 2004) [10]. Salinity tolerance is species specific and differs in ability how well they tolerate salt-affected soils. Furthermore the systematic studies on physiological parameters under saline conditions are scanty for this species. Subabul is a potential salt tolerant plant and salinity levels generally affect the physiological parameters. Therefore this trial was conducted with an objective to know the effect of different salinity levels on physiological attributes in clones of *L. leucocephala*.

Material and Methods

The present investigation was conducted in the poly house of College of Forestry, Navsari Agricultural University, Navsari, Gujarat. Planting material of Subabul was acquired from private paper mill of Gujarat and the experiment was conducted in Completely Randomized Design (CRD) comprising of different salinity levels (S_1-Normal water, S_2-3 dS m^{-1}, S_3-5 dS m^{-1}, S_4- 7 dS m^{-1}, S_5- 9 dS m^{-1}, S_6-11 dS m^{-1} and S_7- 13 dS m^{-1}) on two clones of Subabul (clone-C_1 and C_2). Physiological observations such as chlorophyll content, relative water content and total sugar content were measured at the 180 days after imposing of treatment (DAT). The irrigation water of different salinity levels were applied periodically (1 litre/ pot at each alternate day) up to the field capacity level. The physiological parameters were determined by following standard procedures. The chlorophyll content and total sugar content were calculated as per Thimmaiah (2012) [28] whereas relative water content was determined as given by Morgan (1984) [18]. Data were subjected to statistical analysis following factorial completely randomized design (FCRD) as described by Panse and Sukhatme (1967) [24].

Results and Discussion

There was a significant impact of different salinity level on chlorophyll content, relative water content (RWC) and total sugar content in Subabul clones at nursery condition. Chlorophyll content of leaves (Fig. 1) procured from S_1 (11.52 mg/g), followed by S_2 (11.47 mg/g), S_3 (11.24 mg/g), S_4 (11.08 mg/g) and S_5 (10.80 mg/g) showed higher values as compared to S_6 and S_7 (10.52 and 9.81 mg/g, respectively). Subabul plants irrigated with saline water showed a decrease in chlorophyll content associated with increase in relative water content and sugar contents in leaves.

Maximum leaves chlorophyll content was observed in control whereas lowest chlorophyll content was found in leaves of plants treated with higher salinity (S_7). In line with our study, several researchers reported that salinity decreases the chlorophyll and increases the sugar levels in *Azadirachta indica* (Ahmad *et al.*, 1985) [2], *Simarouba glauca* (Rajmane and Gaikwad, 2014) [25], *Dalbergia sissoo* (Nisha, 2015) [22] and in leaves of Pistachio tree (Mehdi *et al.*, 2008) [17]. The content of total chlorophyll in *Salvinia auriculata* decreased when the plants were subjected to saline treatments (100 and 200 mM, respectively of NaCl and Na_2SO_4); however, they reported significant difference were noticed in *S. auriculata* subjected to control and 200 mM Na_2SO_4 treatments (Gomes *et al.*, 2011) [11]. This decrease can be attributed to an increase in water content (Strogonov, 1970), as we observed in our non-stressed plants. Furthermore, reduction may probably due to the inhibitory effect of the accumulated ions (Ali *et al.*, 2004) [3] and iron containing enzymes which activates the bio-synthesis of chlorophyll (Rubin and Artikhovakaya, 1964) [26]. Reduction in leaf chlorophyll content under salt stress has also been attributed to the destruction of chlorophyll pigments and instability of the pigment protein complex (Levit, 1980) [15].

Such inference also recorded for RWC of leaves (Fig. 2) procured from Subabul clones exposed to different salinity levels. It was found the S_4 (77.71 %), followed by S_3 (75.67 %) and S_2 (74.19 %) recorded significantly higher RWC than treatments with higher salinity levels (S_7 with RWC of 63.96 %). However, significant reductions were not reflected in relative water contents at lower salt concentration *i.e.* upto 7 dS m^{-1}. Similar decline in the relative water content at 4 dS m^{-1} to 12 dS m^{-1} were also observed by Nisha (2015) [22]. Relative water content directly reflects the water status of plants and its decrease in present study suggests that salinity caused water deficit in Subabul plants. The negative effect on plant water relations was induced probably by an increase in soluble salts in Subabul plants, which slowed down the uptake of water and nutrients, causing osmotic effects and toxicity. Similar observations on decreased water potential and osmotic pressure with increase in salinity stress were also reported by Nabil and Coudret (1995) [21] and Kumari and Toky (2008) [12] in *Acacia nilotica* and concluded that lower water potential enabled the plant to maintain the turgor.

Similarly, total sugar content of leaves (Fig. 3) was found to be significant among different salinity level treatments. S_5 (4.09 %) and S_4 (4.07 %) recorded significantly higher total sugar in the leaves of Subabul clones as compared to S_7 (3.65 %) and other treatments. Total sugar content represents the sum total of organic, inorganic and secondary metabolites that accumulate in the plant tissue and in the leaf, and contributes towards specific leaf weight. In the present study, total sugar content increased significantly in *L. leucocephala* upto certain level of increase in salinity (upto 9 dS m^{-1}) after that decrease gradually with increase in salinity levels. Similar result of soluble sugar accumulation in response to salt stress has also been reported earlier in *Dalbergia sissoo* by Nisha (2015) [22] where total sugar content increased significantly *i.e.* 11.06% and 22.74%, respectively at 4 and 8 dS m^{-1} as compared to control. The energy yielding products thus produced could control ion fluxes during mineral uptake (Chimiklis and Karlander, 1973; Ahmad and Abdullah 1982) [7, 1]. The cell membrane is a phospholipids bi layer, which acts as a semi permeable barrier to solutes. Any stress, particularly ionic stress, causes membrane injury and ion leakage from the cell, which is proportional to the magnitude of stress (Dionisio-Sese and Tobita, 1998; Nisha, 2015) [9, 22]. This leakiness quantified as relative stress injury in *Melia composita* shows that it has an injury index of 67.7 and 104.0 per cent, respectively at 4 and 8 dS m^{-1} respectively when compared to control. In some other studies, Munns (2002) [19] and Munns and James (2003) [20] also reported that when plants were subjected to salinity stress, conditions of physiological drought were created which resulted in the decreased water status of plant. This trend has also been reflected in our study, where a significant decline (12.08 %) in the relative water content was observed at 13 dS m^{-1} compared to control.

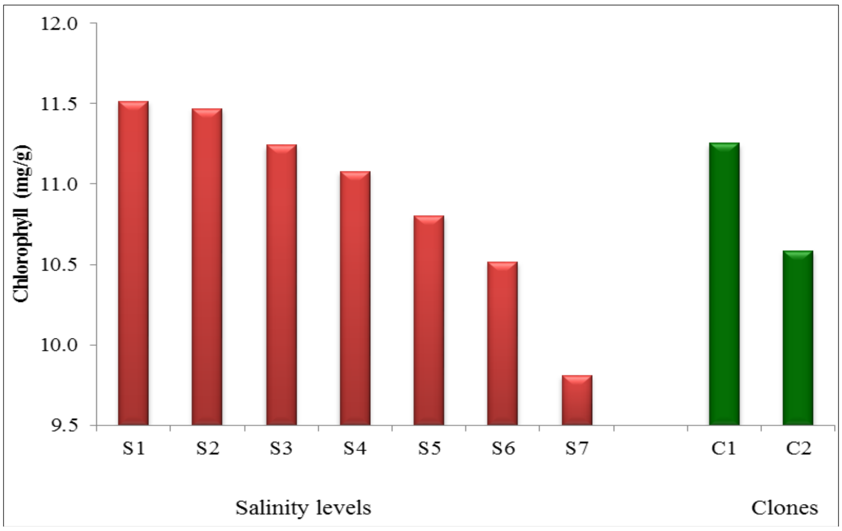

Fig 1: Impact of different salinity levels and clones of Subabul on chlorophyll content.

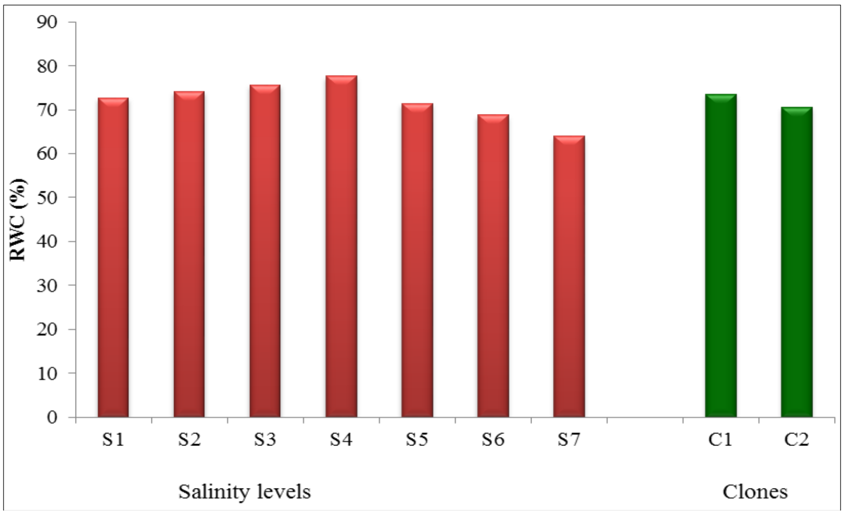

Fig 2: Impact of different salinity levels and clones of Subabul on relative water content

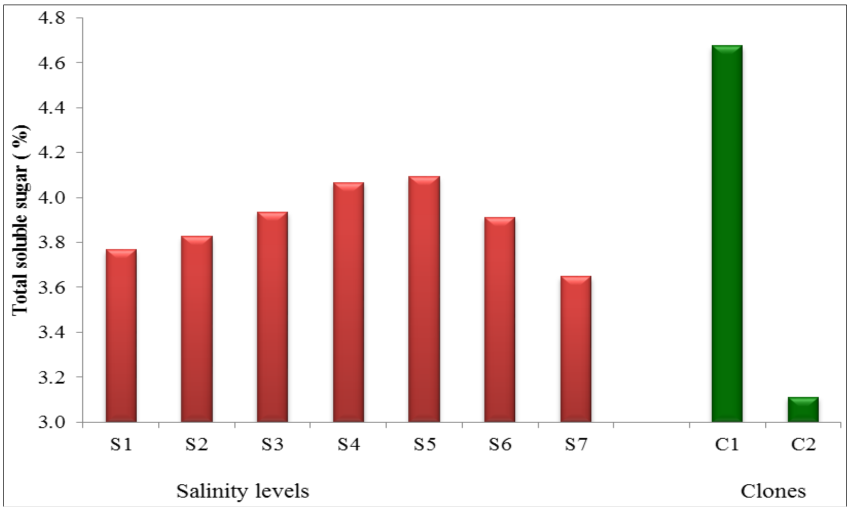

Fig 3: Impact of different salinity levels and clones of Subabul on total sugar content

Conclusion

Different saline irrigation water affected the physiological attributes of Subabul clones at 180 DAT. It is concluded that Subabul clones performed better in terms of physiological attributes upto up to 5 dS m^{-1} saline irrigation water which was comparable with plants treated with normal water at the age of 180 DAT. Among two clones, clone C_1 performed better as compared to clone C_2 for physiology attributes.

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