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Impact of salt stress on plant establishment, chlorophyll and total free amino acid content of ber (*Zizyphus mauritiana* Lamk.) cultivars

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Abstract
Salinity is considered as the most important abiotic stress limiting crop production and plants are known to be able continuing survive under this stress by involving many mechanisms. In this content, the present study was carried out to evaluate the impact of salt stress on some physiological and biochemical parameters in five cultivar of ber cultivars viz. Banarsi Karaka, Narendra Ber Sel-1, Narendra Ber Sel-2, Gola and Ponda The present experiment was carried out under pot at Department of Horticulture, Narendra Deva University of Agriculture & Technology, Kumarganj, Faizabad. The stress was induced by different salts i.e. $CaCl_2$, $MgCl_2$, NaCl and Na_2SO_4 and different ratio (1:1:1:2) of these salts were used. The chloride dominated salinity was maintained at four concentrations of ECe (0, 4, 8, 12, 16 dSm^{-1}). Results indicated that increasing salinity stress in all cultivars had significantly negative impact on plant establishment, survival and chlorophyll content. However, protein hydrolysis is activated resultant accumulation of free amino acids is increased in leaves. Banarsi Karaka, Narendra Ber Sel-2 and Ponda cultivars successfully tolerated up to salinity level $12 dSm^{-1}$ by accumulating more proline in leaves and maintaining usually higher values in all parameters in opposition to Gola. Based on the findings of experiment, it was concluded that Banarsi Karaka, Narendra Ber Sel.-2 and Ponda can be placed in tolerant and cvs. Narendra Ber Sel.-1 and Gola as semi-tolerant group or susceptible.

Keywords: Amino acids, Chlorophyll, Plant establishment, Salt stress, *Zizyphus mauritiana*

Introduction
Indian jujube or ber (*Zizyphus mauritiana* Lamk.) is one of the most ancient and common fruit Indigenous to India. It belongs to family Rhamnacae and it is popularly called the king of arid zone fruits. Ber fruit is quite popular in arid and semi arid region due to its high economic returns, low cost of cultivation, wider adaptability and ability to withstand drought [1]. The fruits of ber are not only eaten fresh but also used in other forms such as dried, candied, pickled, squash, juice, syrup and butter. The fruits are rich in vitamin C, A and B complex. It is underutilized fruits of India and cultivated in an area about 48000 ha with an annual production of about 6.62 lakh tones in India [2]. This crop mainly grown in Rajasthan, Gujarat, Madhya Pradesh, Haryana, Punjab, Uttar Pradesh, Bihar, Maharashtra, Andhra Pradesh, Tamil Nadu etc.

Salinity is a major environmental factor determining plant productivity and plant distribution. It affects more than 10 percent of arable land and salinization is rapidly increasing on a global scale, declining average yield for major crop plants by more than 50 percent [3]. Salt stress occurs in areas where soils are naturally high in salt and precipitation is low where irrigation, hydraulic lifting of salty underground water or invasion of sea water in coastal areas brings salt to the surface soil that inhabit plants. Globally 20% of irrigated land and 2.1% of dry land agriculture suffers from the salt problem and NaCl is the predominant salt causing salinization [4]. Salinity adversely affects germination, growth, physiology and productivity by reducing the ability of plants to take up water causing imbalance in osmotic potential, ionic equilibrium and nutrient uptake. Further, it facilitates severe ion toxicity by depositing high concentration of Na^+ which causes membrane disorganization, inhibition of cell division and expansion. In addition, it impairs a wide range of cellular metabolism including photosynthesis, protein synthesis and lipid metabolism.). The salt stress was responsible for decreased biosynthesis of chlorophyll and inefficiency of photosynthesis, all of which ultimately leading to lowered economic productivity [5, 6]. The decline in photosynthesis due to salinity stress could be due to lower stomata conductance, depression in carbon uptake and metabolism, inhibition of photochemical capacity or a combination of all these factors [7]. To perceive the incoming stresses and rapidly regulate their physiology and metabolism, plants evolved mechanisms that allow them to cope with them by synthesis and accumulation of a number of compatible solutes called "osmolytes" [8]. These osmolytes include proteins, carbohydrates, amino acids and quaternary ammonium compounds which are accumulated in plants at high concentrations to alleviate enzyme inactivity or loss of membrane integrity due to water deficiency [9]. Proline is a key osmolytes which helps plants to maintain cell turgor [10, 11]. A large number of plant species accumulate proline in response to salinity stress and this accumulation may play a role in defense against salinity stress that is why in some cases higher proline content could be correlated with abiotic stress tolerance. The role of proline in cell osmotic adjustment, membrane stabilization and detoxification of injurious ions in plants exposed to salt stress is widely reported [12, 13].

Ber grows on a wide range of soils from gravelly, shallow soils to deep aridisols and to some extent on entisols. Even in soils underlain with *murram* (calcic sub horizon) within one meter depth, roots were found to penetrate up to 4.5 m. *Zizyphus* rooting depth is critical for the maintenance of high rates of assimilation and conductance throughout the day making it tolerant to drought conditions. Cultivated species of *Zizyphus* are tolerant to a degree of salinity up to 6 dSm^{-1}. Ber is of great interest for production and reclamation of the saline soils of India, which have high pH, low organic carbon, low fertility, excessive exchangeable sodium and indurated $CaCO_3$ [14]. Ber is known to survive even in soils having pH as high as 9.2. The productivity of plants is greatly affected by various environmental stresses. Soil salinity affects plant growth and development by way of osmotic stress, injurious effects of toxic ions and nutrient imbalance caused by excess of Na^+ and Cl^- ions. There are several cultivar/genotypes which are commercially grown in eastern part of Uttar Pradesh. Indications are available that ber can survive well in sodic soils, but there is little information for its tolerance limit to salinity. No systematic work has been done on the salinity tolerance limit of these genotype / cultivars. Beside this, information pertaining to adaptability, survival, nutrient uptake and growth behavior etc. are also lacking. Hence the present study was initiated to evaluate the effect of NaCl treatment in five accessions of ber on plant establishment, chlorophyll content and total free amino acids in order to better understand their differences on salt stress tolerance and select tolerant accession.

Method and materials

The study was carried out during February, 2012 to September, 2013 at Main Experiment Station, Department of Horticulture, Narendra Deva University of Agriculture & Technology (NDUA&T), Faizabad, Uttar Pradesh with a view to assess the tolerance performance of five ber cultivars i.e., Banarsi Karaka, Narendra Ber Selection-1, Narendra Ber Selection -2, Ponda and Gola to variable salinity levels. The collection of soil for filling in the pots was done from the area having normal soil at Main Experiment Station, Department of Horticulture. Top soil up to 15 cm depth was scrapped from the selected site and after well polarization it was passed through 2 mm sieve and the waste material was discarded. The soils as mixed thoroughly, five random samples were collected from the heap of the soil. The samples were analyzed for various physical and chemical constituents are given below.

Initial characteristics of soil used in the experiment

Sl. No.	Components	Values
1	Sand %	58.60
2	Silt	24.50
3	Clay	16.90
4	Soil Texture	Sandy loam
5	pH (1:2.5 soil water suspension)	7.60
6	Saturation Per cent	36.00
7	Exchangeable Sodium Percentage	10.24
8	Cation Exchange Capacity	10.12
9	Electrical conductivity (dSm^{-1})	0.58
10	Organic carbon (%)	0.56
11	Bulk Density (g/cc)	1.23
12	Available chlorine(me/litre)	4.21
13	Available nitrogen (Kg/ha)	214.86
14	Available phosphorus (Kg/ha)	18.30
15	Available potassium (Kg/ha)	248.65
16	Available calcium (me/litre)	4.53

For the preparation of artificially salinized soil, a mixture of salts i.e. $CaCl_2$, $MgCl_2$, NaCl and Na_2SO_4 were used in the ratio of 1:1:1:2 and thus chloride dominated salinity was maintained. The ratio between Cl^- and SO_4^{--} was observed to be 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. This layer was again saturated with rest of the salt solution. The treated soil was covered with polythene sheet for two days to facilitate the up and downward movement of salt in the soil and thereafter, it was raked thrice and mixed thoroughly. Thus, four levels of salinity (ECe) i.e, 4.0, 8.0, 12.0 and 16.0 dSm^{-1} were maintained. In addition, a control was also maintained. For control, the soil was treated only with normal water having (pH 7.4 and 700 µmhos/cm ECe. After adjustment of different salinity levels, soil samples were taken out for each salinity level and the ECe and pH were initially checked before the plantation which were estimated again at the time of termination of the experiment. Data on initial establishment and survival plants were recorded after 30 days of planting. The chlorophyll contents (total chlorophyll) of leaves were analyzed following the DMSO method of Barnes *et al*. [15]. The content of free amino acids were determined by Yemm and Cocking [16].

Result and discussion

Data pertaining to the plant establishment and plant survival presented in Table 1. The establishments of ber cultivars were recorded after 30 days after planting, result showed that the plant establishments were non-significantly and varied from 96.67 to 90.00 per cent. Cultivars Banarsi Karaka recorded 96.67 per cent establishment followed by Narendra Ber Sel.-2 (96.67 per cent) and Gola and Ponda (93.33 per cent) while cultivar Narendra Ber Sel.-1 showed minimum establishment (90.00 per cent). However, per cent establishment decreased as the salinity increased from 12 ECe to 16 ECe of salinity level. Minimum plant establishment (66.67 per cent) was observed with cultivar Narendra Ber Selection-1 with 16 ECe level. In respect to percentage survival were recorded at monthly interval till the termination of experiment and have been presented in Table 1. It is evident from result that the plant survival was statistically non-significant for all cultivars. The salinity effect showed considerable variation in plant survival. The 100 cent per cent plant survival was obtained in normal soil and 4 ECe level, whereas it reduced to 94.44, 77.83 and 66.66 per cent at 8, 12 and 16 ECe levels of salinity, respectively. Soil having higher salinity levels may reveal poor plant establishment and survival due to high osmotic pressure of soil substrate or due to accumulation of toxic ions with the plant cells. Higher osmotic pressure of soil solution inhibits the intake of water by the root which causes exo-osmosis, which results in the movement of water from cell sap towards the root zone and subsequently to the soil solution. In the mean time, endo-osmosis also occurs at a slower rate and as a result of gap between protoplasm and cell wall is occupied by the external salt solution of higher concentration. If this process is allowed to continue for a long time, all the metabolic processes are inhibited and it results in the death of plant cell and eventually causes mortality of the plants. The experiment conducted to evaluate the effect of different sodicity and salinity level on wild jujube (*Ziziphus mauritiana* var. *rotundifolia*), rootstock and budded plants of Indian jujube (*Z. mauritiana*) and found the decreased plants establishment and survival of seedling and budded plants with increasing levels of sodicity and salinity levels [17]. The present findings had conformity with other workers [18, 19].

Data presented in Table 2 showed marked difference in ber cultivars with respect to total chlorophyll contents in leaves. Higher chlorophyll contents (3.20 mg/g) were noted in cv. Banarsi karaka followed by Narendra Ber Sel.-2 (3.15 mg/g). Salinity level had also considerable deterioration effect on chlorophyll contents as compared to control. The maximum chlorophyll content of 3.29 mg/g was recorded at normal soil. It was suggested that decrease in chlorophyll content in response to salt stress is a general phenomenon which led to disordering synthesizing chlorophyll and appearing chlorosis in plant. NaCl stress decreased total chlorophyll content of the plant by increasing the activity of the chlorophyll degrading enzyme: cholorophyllase, inducing the destruction of the chloroplast structure and the instability of pigment protein complexes [20]. It was reported that the reduction in chlorophyll concentration by NaCl to the inhibitory effect of the accumulated ions of various salts on the biosynthesis of the different chlorophyll fractions [21]. In saline soil, decrease in chlorophyll contents may be attributed to reduced uptake of nitrogen and increased accumulation of chloride and sodium in the plant leaves. In addition to this in saline soil too, few of the micronutrient viz., Fe, Mn, Cu, Zn are present in unavailable form which are known to influence the enzymatic activity in the plants. The decreasing trend in chlorophyll contents has also been viewed in the leaves of aonla [22]. Inspite of these crops, decrease in total chlorophyll content has also been estimated in bael [23].

The result presented in Table 2 clearly showed a variation in total free amino acid contents of different ber genotypes. The maximum free amino acid contents were recorded with cultivar Banarsi Karaka (179.43 µg/100 mg), which was found to be significantly higher than all genotypes. The minimum total free amino acids were recorded with Gola (163.26 µg/100 mg). Total amino acids increased significantly with increase salinity levels of soil. The maximum content of total free amino acids (187.99 µg/100mg) was recorded at 16 ECe level, whereas minimum value (147.97 µg/100mg) was in normal soil condition. The result clearly revealed that total free amino acid contents increased significantly in the leaves with increasing salinity level as compared to plant grown under normal soil condition [24]. Probably, the above findings revealed in saline soil might be due to that protein hydrolysis resulted in accumulation of free amino acids in the leaves. During hydrolysis, protein used to break down amino acids and as a result, status of protein bound amino acids decreased and that of total free amino acids increased [25].

Table 1: Effect of salt stress on plant establishment and survival of ber cultivars

Cultivar	Plant establishment (%)						Plant survival (%)					
	Soil Salinity level					Mean	Soil Salinity level					Mean
	Normal Soil	4	8	12	16		Normal Soil	4	8	12	16	
Banarsi Karaka	100.00	100.00	100.00	100.00	83.33	96.67	100.00	100.00	100.00	83.33	83.33	93.33
Narendra Ber Sel.-1	100.00	100.00	100.00	83.33	66.67	90.00	100.00	100.00	83.33	66.67	50.00	80.00
Narendra Ber Sel.-2	100.00	100.00	100.00	100.00	83.33	96.67	100.00	100.00	100.00	83.67	66.67	90.00
Ponda	100.00	100.00	100.00	83.33	83.33	93.33	100.00	100.00	83.33	66.67	66.67	83.33
Gola	100.00	100.00	100.00	83.33	83.33	93.33	100.00	100.00	100.00	83.33	66.67	90.00
Mean	100.00	100.00	100.00	88.88	80.55		100.00	100.00	94.44	77.83	66.66	
	Cultivar (C)		ECe Level (S)		Interaction (C X S)		Cultivar (C)		ECe Level (S)		Interaction (C X S)	
C.D. (P=0.05)	11.78 (N.S.)		9.96		26.35 (N.S.)		13.03 (N.S.)		11.01		29.15 (N.S.)	

Table 2: Effect of salt stress on chlorophyll and total free amino acids contents in ber leaves

Cultivar	Chlorophyll content (mg/g fresh weight basis)						Total free amino acids (µg/ 100 mg dry weight basis)					
	Soil Salinity level					Mean	Soil Salinity level					Mean
	Normal Soil	4	8	12	16		Normal Soil	4	8	12	16	
Banarsi Karaka	3.41	3.37	3.24	3.10	2.89	3.20	154.83	174.27	184.53	192.63	195.58	179.43
Narendra Ber Sel.-1	3.22	3.23	3.17	2.83	2.60	3.01	146.37	163.43	171.43	185.10	184.13	170.63
Narendra Ber Sel.-2	3.39	3.35	3.22	3.05	2.75	3.15	151.23	171.87	181.47	191.60	191.90	171.77
Ponda	3.20	3.18	3.10	2.80	2.63	2.98	145.13	164.40	172.63	182.60	185.10	172.70
Gola	3.24	3.23	3.18	2.86	2.71	3.04	150.27	165.47	174.87	185.70	187.20	178.23
Mean	3.29	3.26	3.17	2.90	2.70		147.97	167.14	176.43	186.84	187.99	
	Cultivar (C)		ECe Level (S)		Interaction (C X S)		Cultivar (C)		ECe Level (S)		Interaction (C X S)	
C.D. (P=0.05)	0.029		0.027		0.065		1.34		1.22		3.01	

Conclusion

In conclusion, our study showed that salt stress at higher concentration, especially 16 ECe levels of salinity NaCl is harmful to plants of the five cultivars of ber since the plant establishment and survival decreased significantly. In addition to this morphological feature, photosynthetic parameters (chlorophyll content) were adversely affected while total free amino acids amount in leaves was activated and increased by increasing salinity level. Banarsi Karaka, Narendra Ber Sel.-2 and Ponda can be placed in tolerant and cvs. Narendra Ber Sel.-1 and Gola as semi-tolerant group or susceptible based on various parameters studied. It's apparent from the present investigation that salinity stress tolerance in ber is attributed to the biosynthesis of proline which makes plants able to continue growth even under higher salinity level. However, there was no single parameter could be suggested as sole factor responsible for salinity stress tolerance; it is the combination of many characters.

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