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Effect of salinity stress on proline content in *Prosopis cineraria* and *Prosopis juliflora*

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ABSTRACT
The objective of the present study is to find out the effect of salinity stress on proline content in two species of genus Prosopis i.e., Prosopis cineraria and Prosopis juliflora.Prosopis is a plant that is well adapted to grow in the challenging conditions of arid and semi-arid region. Proline plays an important role in mitigating the abiotic stress conditions in plants. Therefore, experiments were designed to find out the role of proline and its content in Prosopis cineraria and Prosopis juliflora.Proline content was determined by method given by Bates et al. (1973). Proline content of the seedling under different salinity treatments (ranging from 0.0mM to 300.00mM concentrations) were observed after two weeks and then a regular interval. The observations showed that with increase in salinity, the level of proline increased in both the species up to a certain limit. After that when the salinity was increased further the proline content starts decreasing. In case of Prosopis cineraria as well as Prosopisjuliflora proline content was highest at 150mM salt concentration that is 10.46±0.41[a] and 12.97±0.13[a] respectively. The proline content in Prosopis juliflora was higher than Prosopiscineraria, in controlled as well as in different salt treatment.
Keywords: salinity stress, proline content, *Prosopis cineraria*, *Prosopis juliflora*, arid region

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INTRODUCTION

Prosopis is a very important plant of arid and semi-arid regions. Survival of this genus in the adverse environmental conditions of desert make it an important plant of this region. Timbers of these plants are used as fuel and for many other purposes. Pods of these trees are highly nutritious, used to feed the cattle. Pods of Prosopis cineraria are also consumed by local people, it becomes the only food during the famine in these regions. The two species of Prosopis, Prosopis cineraria and Prosopisjuliflora are the lifeline trees of the arid region. Prosopis cineraria is worshiped by many communities of these regions for its usefulness and hence is called 'Kalpvriksha' [1], [2]. It withstands lots of abiotic stress conditions which is offered to this plant by nature. This makes these plants interesting for physiological studies [3]. Not many reports are available on the study of physiological parameters of this genus.Both of these plants can tolerate a high level of soil salinity. Sodium chloride contributes largely to soil salinity [4]. Salinity leads to the accumulation of $Na+$ and $Cl-$ ions and causes the water deficiency in plants. This osmotic stress ultimately leads to the death of plants [5]. To avoid the osmotic stress plant requires the osmo-protectors. Osmo-protectors are the nontoxic molecules which accumulate in the plant cell up to the required level to protect it from dehydration without affecting the metabolism of plants [6]. Synthesis of some macromolecules such as proline is affected in response to salt stress [7]. Proline is an osmolyte which actively participate to maintain the osmotic balance in plants. Proline is known to be the only osmolyte which can scavenge singlet oxygen as well as free radicals such as hydroxyl ions (OH-) [5]. It increases the turgor to help the plant in cell expansion [8], [9]. Proline helps in biochemical reactions [10], [11] and does not have any adverse effect on enzymatic activity [12]. Plants are having the ability to accumulate as well as degrade proline quickly when required [13], [14]. According to the study of many researchers, accumulation of proline is a physiological process which increases under abiotic stress [15], [16], [17], [18], [19]. This indicates that proline is a sign of stress resistance. During salt stress the osmotic adjustment is primarily the function of proline [20], [21]. Synthesis of proline takes place under Glu pathway which dominates under stress [22].It protects proteins, DNA and other macromolecules and also helps to regulate redox potential. Enzyme denaturation due to stress can be reduced by proline [8]. Proline biosynthesis, accumulation and transportation under salinity stress studied by KaviKishor and Sreenivasulu, 2014 [23].There are many researchers who believes that proline accumulation often related with the salt tolerance. According to them salt tolerant genotype shows higher accumulation of proline as compare to the salt sensitive ones. According to [24] salinity does not affect the proline accumulation significantly. It is reported by [5] that over-expression of an enzyme, P5CS (D1-pyrroline-5-carboxylate synthetase) in transgenics enhances oxidative stress tolerance caused by drought and salt stresses. This is the enzyme used in the rate limiting step of proline biosynthesis reaction. Over expression of this enzyme during stress which leads to proline accumulation and enhanced stress tolerance reveals that proline has some role in stress tolerance. This is supported by different scientists in different transgenics plants such as in rice [25], [26], chickpea [27], tobacco [28], wheat [29], potato [30]. The purpose of this study is to investigate the effect of salinity on proline content in Prosopis cineraria and Prosopisjuliflora and to find out that if proline plays some role in the salt tolerance of these plants.

MATERIAL AND METHODS

To study the proline content under control and stress condition, 500 seeds of each of the selected plants (Prosopis cineraria &Prosopisjuliflora) were soaked in separate beakers. The imbibed seeds were transferred to the petri dishes of 5cm diameter containing three layers of moist filter papers. After seven days the germinated seeds with a length more than 3.5cm were transferred to the pots containing Hoagland solution. Ten seedlings were placed in each pot. After two weeks (when each of the plants got at least one leaf) the Hoagland solution was supplemented with salt solution (i.e., Hoagland with different salt concentrations). Proline content of the seedling under different salinity treatments (control, 25mM, 50mM, 75 mM, 100 mM, 125 mM, 150 mM, 175 mM, 200 mM, 250 mM, 300 mM) were observed after two and four weeks. For this the leaves of the seedling under control and different salt concentrations were harvested after two and four weeks.

Proline determination

Proline content was estimated by total proline content determination assay by Bates et al. (1973) [31]. Standard curve was prepared using proline. Plant extract was prepared by blending 250mg of fresh leaves of the treated plants with 5ml of 3% sulpho-salicylic acid. The mixture was centrifuged at 10,000 rpm for 20 minutes. 2ml of supernatant was mixed with equal amount of glacial acetic acid and ninhydrin. The mixture was boiled for 1hour. After cooling 4ml of toluene reagent was added using cyclo-mixture. Optical density of the upper layer was read using spectrophotometer at 520nm. The experiment was done in triplets and was repeated five times.

RESULT

Proline content:

The experiments were conducted to determine the proline content of the plants (*Prosopis cineraria* and *Prosopis juliflora*) after two and four weeks. Initially theproline content in the plants under study increased with the increase in salinity and time, it started decreasing when the salinity increased beyond 150 mM (fig. a, b). The proline content was always higher in *Prosopis juliflora* than *Prosopiscineraria* (fig. c) The increase in the particular treatments was also more prominent in case of *Prosopisjuliflora*. Under controlled condition the proline content in *Prosopisjuliflora* were $5.47±0.31^{g}$, $6.16±0.13^{h}$ while in*Prosopiscineraria* $3.43±0.28^{h}$, $4.69±0.22^{f}$ after two and four weeks respectively. After two weeks proline content was recorded $4.29±0.26^{g}$ and $6.33±0.11^{f}$ in *Prosopiscineraria* and *Prosopisjuliflora* respectively under 25 mM salt concentration. At 50mM salt concentration proline content was $5.74±0.17^{f}$ and $6.86±0.05^{e}$ in *Prosopiscineraria* and *Prosopisjuliflora* respectively. At 75 mM salt concentration proline content was $7.24±0.20^{d}$ and $7.70±0.16^{d}$ in *Prosopiscineraria* and *Prosopisjuliflora* respectively. At 100 mM salt concentration also proline content was higher in *Prosopisjuliflora*$8.52±0.19^{c}$ as compared to *Prosopiscineraria*$7.80±0.12^{c}$. Proline content recorded at 125 mM was higher in *Prosopisjuliflora*$10.6±40.31^{b}$ as compared to *Prosopiscineraria*$8.39±0.25^{b}$. At 150 mM salt concentration proline content was $10.46±0.41^{a}$ and $12.47±0.34^{a}$ in *Prosopis cineraria* and *Prosopis juliflora* respectively. At 175 mM salt concentration proline content was $6.33±0.28^{e}$ and $7.41±0.33^{d}$ in *Prosopis cineraria* and *Prosopi sjuliflora* respectively. At 200mM salt concentration also proline content was higher in *Prosopisjuliflora*$4.39±0.32^{h}$ as compared to *Prosopiscineraria*$5.37±0.38^{f}$. After four weeks proline content was recorded $4.69±0.22^{f}$ and $7.26±0.16^{f}$ in *Prosopiscineraria* and *Prosopisjuliflora* respectively under 25 mM salt concentration. At 50mM salt concentration proline content was $6.69±0.22^{d}$ and $7.81±0.17^{e}$ in *Prosopis cineraria* and *Prosopis juliflora* respectively. At 75 mM salt concentration proline content was $8.62±0.31^{c}$ and $8.56±0.23^{d}$ in *Prosopis cineraria* and *Prosopis juliflora* respectively. At 100 mM salt concentration also proline content was higher in *Prosopisjuliflora*$9.77±0.15^{c}$ as compared to *Prosopiscineraria*$9.32±0.29^{b}$. Proline content recorded at 125 mM was higher in *Prosopisjuliflora*$11.50±0.28^{b}$ as compared to *Prosopiscineraria*$10.28±0.23^{a}$. At 150 mM salt concentration proline content was $9.60±0.25^{b}$ and $12.97±0.13^{a}$ in *Prosopis cineraria* and *Prosopis juliflora* respectively. At

175 mM salt concentration proline content was 5.54±0.32[e] and 6.76±0.47[g] in *Prosopis cineraria* and *Prosopis juliflora* respectively. At 200mM salt concentration proline content was 3.50±0.33[i] in *Prosopis juliflora*, *Prosopiscineraria* seedling did not survive at 250mM salt concentration. In *Prosopis juliflora* proline content was highest under 150mM (12.97±0.13[a]) (Table. 1), lowest under 250mM (3.21±0.14[i]) after two weeks. In case of *Prosopis cineraria* proline content was highest (10.46±0.41[a]) under 150mM salt concentration and lowest (5.37±0.38[f]) under 200mM after two weeks (Table. 2).

Table. 1 Proline content of *P. cineraria*

	N	*P. cineraria* after two weeks	Std. Error	*P. cineraria* after four weeks	Std. Error
Control	8	3.43±0.28[h]	0.10	4.69±0.22[f]	0.08
25	8	4.29±0.26[g]	0.09	5.34±0.30[e]	0.11
50	8	5.74±0.17[f]	0.06	6.69±0.22[d]	0.08
75	8	7.24±0.20[d]	0.07	8.62±0.31[c]	0.11
100	8	7.80±0.12[c]	0.04	9.32±0.29[b]	0.10
125	8	8.39±0.25[b]	0.09	10.28±0.23[a]	0.08
150	8	10.46±0.41[a]	0.15	9.60±0.25[b]	0.09
175	8	6.33±0.28[e]	0.10	5.54±0.32[e]	0.11
200	8	5.37±0.38[f]	0.14	0.00±0.00[g]	0.00
250	8	0.00±0.00[i]	0.00	0.00±0.00[g]	0.00

Table. 2 Proline content of *P. juliflora*

	N	*P. juliflora* after two weeks	Std. Error	*P. juliflora* after four weeks	Std. Error
Control	8	5.47±0.31[g]	0.11	6.16±0.13[h]	0.05
25	8	6.33±0.11[f]	0.04	7.26±0.16[f]	0.06
50	8	6.86±0.05[e]	0.02	7.81±0.17[e]	0.06
75	8	7.70±0.16[d]	0.06	8.56±0.23[d]	0.08
100	8	8.52±0.19[c]	0.07	9.77±0.15[c]	0.05
125	8	10.6±40.31[b]	0.11	11.50±0.28[b]	0.10
150	8	12.47±0.34[a]	0.12	12.97±0.13[a]	0.04
175	8	7.41±0.33[d]	0.12	6.76±0.47[g]	0.17
200	8	4.39±0.32[h]	0.11	3.50±0.33[i]	0.12
250	8	3.97±0.11[i]	0.04	3.21±0.14[i]	0.05

Figure 1. Proline content of P.juliflora

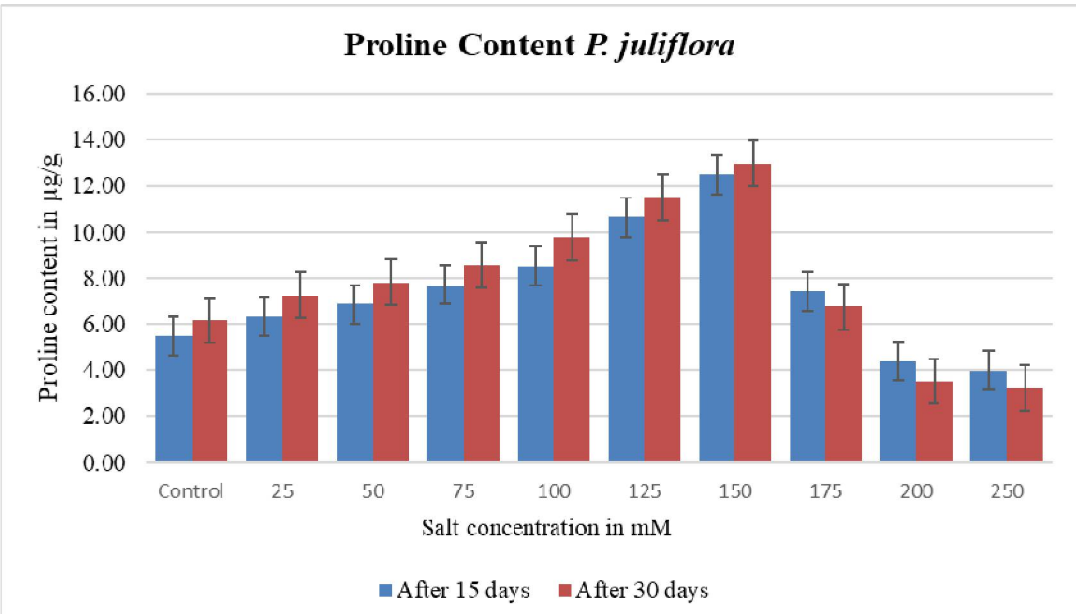


Figure 1. Proline content of P.cineraria

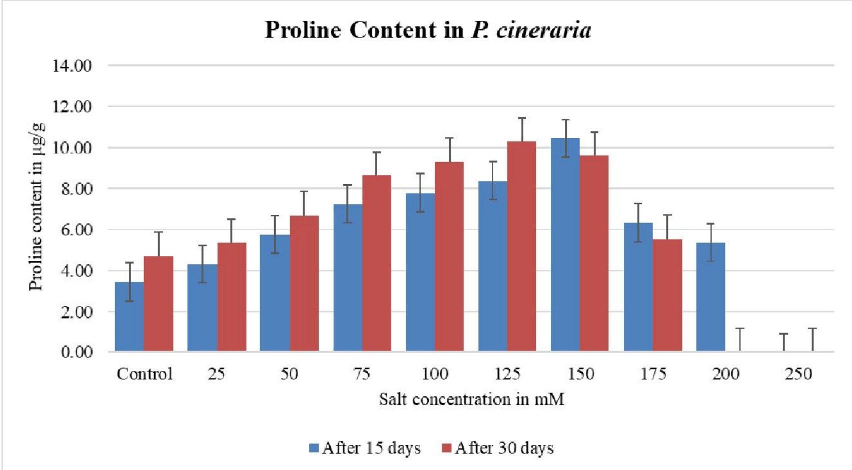
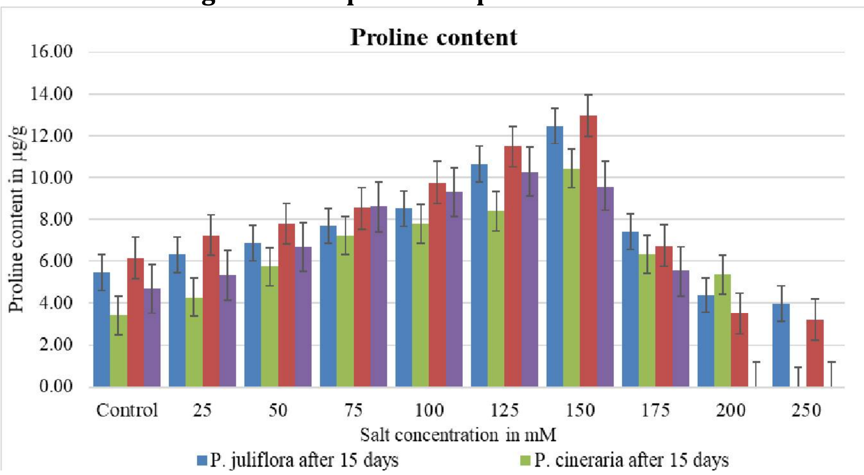


Figure 3. Comparison of proline contents



DISCUSSION

Accumulation of proline as osmolyte is the most common phenomenon used by the plants and other organisms to maintain their osmotic stability [32]. The scientists believe that proline accumulation makes the plants more tolerant and somehow helps the plant to overcome stress, here are some supporting views by different scientists. Proline is a compatible solute [12], must have some important role in osmotic adjustment during stress condition [33], [34] that is why metabolic stress leads to proline accumulation [35]. According to [36] proline can improve salt tolerance. Even the exogenous application of proline can improve the plant growth [37]. It was reported by [38] that foliar application of proline showed positive effect on plant growth and yield characteristics under salt stress. The level of proline accumulation was found higher in the tolerant plant species as compared to the sensitive ones. [23], [39], [40]. Increased proline content was recorded with increasing salinity in *Phaseolus vulgaris,* L. [41]. Proline content was found to increase in maize when treated with NaCl concentrations higher than 100mM [42]. Proline content of rice seedlings increased with the increase in salinity [43]. Proline content increased with the salt concentration in *Acacia auriculiformis* when this plant species was treated with different salt concentrations (0.3, 3.9, 6.0, 7.9, 10.0, 12.1, and 13.9 dS m−1) [44]. The similar results were reported in tobacco [45], [28]), sorghum [46], green gram [47], wheat [48] and mulberry [49].On the other hand, some scientists found the contrasting results. They recorded that excess of salinity inhibits the proline accumulation in Citrus [34]. Excessive use of proline could be the reason behind the decline in proline content under higher salinity stress. This view is supported by many scientists in Sorghum [50], rice [51] cotton [52], tomato [53]. The above studies explain the increase in proline content with the increase of salinity and then decrease in proline content at higher salinity in the plant under the present study.

CONCLUSION

The present studies reveal that proline has an important role in mitigating stress in many plant species. Change in level of proline content is directly related to stress condition. The *Prosopis juliflora* is more tolerant as compare to *Prosopis cineraria*. As the proline content of *P. juliflora* were reported higher as compare to *P. cineraria* under all the treatment as well as controlled conditions.

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