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Effect of Water and Salt Stress on Germination Indices and Proline Accumulation in *Cyamopsis tetragonoloba* (L.)

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Abstract
Abiotic stresses like water stress and salt stress are the crucial limiting factors affecting the growth and yield of field crops. The aim of the study was to examine the impact of water stress and salt stress on germination and proline accumulation in *Cyamopsis tetragonoloba* (L.), an important food legume. Seeds of *C. tetragonoloba* were exposed to distilled water for the control or with aqueous solutions of Polyethylene glycol (PEG) 6000 (three levels of osmotic potentials i.e. -0.3MPa, -0.6MPa and -0.9MPa) and Sodium chloride (NaCl) (50mM and 100mM) for different water stress and salt treatments. The seed germination percentage and growth reduced in severe stressed seedlings. On the other hand, the proline content in the leaf tissue of stressed seedlings increased significantly in response to stress. Seed germination of Cyamopsis is less sensitive to mild and moderate stress. This suggests the possibility of growing Cyamopsis in moderately salt affected and water stressed areas.

Keywords: Water stress, Salt stress, Proline, *Cyamopsis tetragonoloba*

INTRODUCTION

Food and nutritional security is vital for the development of any nation. Though there was an impressive growth in agriculture, ensuring food and nutritional security was still a challenge due to imbalance growth in agriculture biased towards wheat and rice (Shalendra et al., 2013). Food legumes are important both nutritionally and economically for being a major source of protein in Indian diet and eco-friendly. Cluster bean or guar, one of the food legumes, was native of India where it was used as a vegetable, fodder and green manure. Guar gum is an extract of the guar bean, where it acts as a food and water store.

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Abiotic stresses and their effect on plants in both natural and agricultural settings is an area that is receiving increasing attention because of the potential impacts of climate change on rainfall patterns and temperature extremes, salinization of agricultural lands by irrigation and the overall need to increase agricultural productivity on marginal lands. In the field, crop plants may be exposed to several distinct abiotic stresses either concurrently or at different times through the growing season which affect their growth factor and crop productivity. In the face of global scarcity of water resources and the increased salinization of soil and water, salinity and water stress are considered the most severe stresses.

Salinity not only decreases the agricultural production of most crops, but also, as a result of its effect on soil physicochemical properties, adversely affects the establishment, growth and development of plants leading to huge losses in productivity (Mathur et al., 2007). The adverse effects of salinity are attributed to the osmotic effect, ion effect, alterations in ionic composition and diversion of photosynthates and nitrogenous metabolites from growth to energy supply (Greenway and Munns, 1980; Flowers, 2004).

Water stress is characterized by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata and decrease in cell enlargement and growth. Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally the death of plant (Jaleel et al., 2008c).

Biochemical and physiological changes in plants growing under salt or water-deficit conditions have been broadly investigated in many crop species (Lutts et al., 2004; Wahid, 2004; Luo et al., 2005; Castillo et al., 2007; cha-um, et al., 2010). Those parameters in crop species cultivated in water deficit or salt stresses have been developed as effective indices for tolerant selection in breeding programs (Parida & Das, 2005; Ashraf & Foolad, 2007; Zhao et al., 2008).

Many plants accumulate high levels of solutes such as mannitol, free proline (Pro) in response to water stress, salinity and other abiotic plant stresses (Ashraf and Harris, 2004; Munns and Tester, 2008, Lu et al., 2009), indicating an essential role for these solutes in tolerance to these stresses. Proline is widely believed to function as a protector or stabilizer of enzymes or membrane structures that are sensitive to dehydration or ionically induced damage (Watanabe et al., 2000, Chen et al., 2007).

Hence, an attempt has been made to determine the impact of water and salt stress on germination and proline accumulation in *C. tetragonoloba (L.)*

MATERIALS AND METHODS

Plant material

Certified seeds of *Cyamopsis tetragonoloba (L.)* were purchased from the commercial seed vendors in the market at Kavali.

Germination tests

The seeds were surface sterilized using 4% hypo solution for 3 minutes and later washed repeatedly with sterile distilled water. A completely randomized design was adopted for the experiment with three replicas of 15 seeds for each water stress and salt treatments were placed on filter paper in petri-dishes. The filter paper was moistened with distilled water for the controls or with aqueous solutions of PEG 6000 (three levels of osmotic potentials i.e. -0.3MPa, -0.6MPa and -0.9 MPa) and NaCl (50mM and 100mM) for different water stress and salt treatments. PEG 6000 solutions were prepared according to Michel and Kaufmann (1973). Water or treatment solutions were added twice in the day to maintain the filter paper wet during the experiment. The number of seeds germinated was counted every day from the start of the experiment.

Germination energy, Speed of germination index, Average time of germination and Emergence energy value were then calculated using the recorded data on seed germination.

Germination percentage was calculated according to the formula G% =100 x A/N where A is the number of seeds germinated and N is the total number of seeds used in the germination test (Fanti and Perez, 1998). Germination energy was calculated after Maguire (1962) using the formula GE =

$$\frac{X_1}{Y_1}+\frac{X_2-X_1}{Y_2}+\ldots\ldots\ldots\ldots\ldots\ldots\ldots\frac{(X_n-X_{n-1})}{Y_n}$$

where X_n = number of seeds germinated on the n^{th} counting date and Y_n = the number of days from sowing to the n^{th} count. Speed of germination index was calculated by the formula S =

$$\frac{E_1}{N_1}+\frac{E_2}{N_2}+\ldots\ldots\ldots\frac{E_n}{N_n}$$

where E_n = number of emerged seedlings observed in the n^{th} daily counting and N_n = number of days after the seeds were put to germinate in the n^{th} counting (Jose et al.1999). Average time of germination was calculated by the formula

$$\frac{G_1T_1+G_2T_2+\ldots\ldots+G_nT_n}{G_1+G_2+\ldots\ldots+G_n}$$

where G is the germination count on any counting period and T = time (Santana and Ranal, 2006). Emergence energy value is the highest value obtained when the germination percentage on a day is divided by the number of days since test when that germination percentage was reached.

Proline accumulation during seedling development

The seeds were placed in germination boxes filled with dried soil. Seeds were subjected to water stress by using different concentrations of PEG 6000 and salt stress by using NaCl as described above. After 15 days the seedlings were harvested and the accumulation proline in the leaves was measured by Bates et al., (1973) method.

RESULTS & DISCUSSION

Germination Percentage

The germination percentage of seeds at different treatments is shown in Fig1.

Figure-1. Effect of PEG-induced water stress on proline content of *Cyamopsis tetragonoloba*

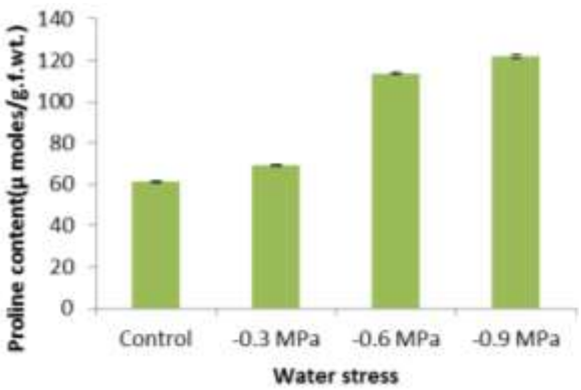


Table1: Effect of Water stress on germination indices of *Cyamopsis tetragonoloba*

Treatments	G%	GE	S	ATG	EEV
Control	100.00 ± 0.00	25.0 ± 0.0	61.24 ± 0.00	3.83 ± 0.00	100 ± 0.00
-0.3MPa	92.88 ± 0.44	11.24 ± 0.22	32.42 ± 0.66	4.05 ± 0.00	23.22 ± 0.11
-0.6MPa	86.66 ± 1.3	10.27 ± 0.22	29.63 ± 0.69	4.06 ± 0.01	21.66 ± 0.33
-0.9MPa	78.66 ± 1.3	6.47 ± 0.07	18.34 ± 0.17	4.51 ± 0.00	19.66 ± 0.33

Table 2: Effect of Salt stress on germination indices of *Cyamopsis tetragonoloba*

Treatments	G%	GE	S	ATG	EEV
Control	100.00 ± 0.00	25.0 ± 0.0	61.24 ± 0.00	3.83 ± 0.00	100 ± 0.00
50mM	77.33 ± 1.33	6.38 ± 0.09	18.13 ± 0.27	4.51 ± 0.00	19.33 ± 0.33
100mM	74.66 ± 2.66	4.66 ± 0.16	11.51 ± 0.41	5.0 ± 0.00	18.66 ± 0.66

The germination percentages decreased with rise of osmotic potentials and salinity. Germination in the control commenced on day1 whereas the seeds under mild (-0.3MPa) and moderate (-0.6MPa) water stress germinated on day 2, seeds under severe water stress (-0.9MPa) germinated on day3. At NaCl treatments, the germination was delayed till day 4 with 100mM concentration. Seed germination was significantly lower under severe salt stress. Elevated water stress decreases water uptake by seeds there by inhibiting their imbibition and germination. Similar decrease in seed germination of wheat under moisture stress has been reported (Khayatnezhad and Gholamin, 2011) in the recent past.

The inhibitory effects of NaCl on seed germination could be due to its direct effect on the growth of the embryo. Similar results has been reported by El-Tayeb, 2005; Azhdari et al., 2010.

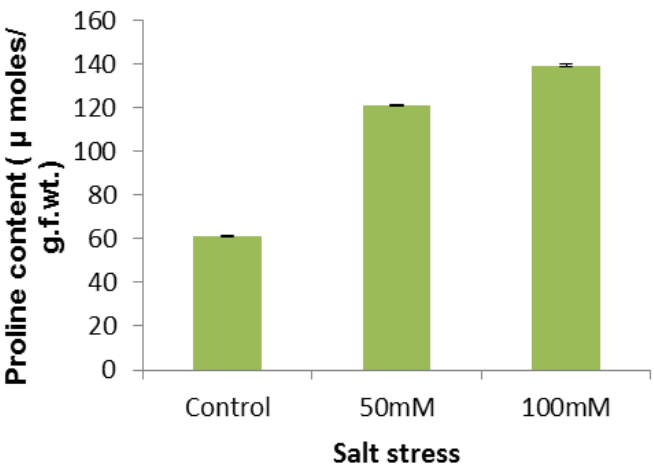
Germination indices

Average time of germination increased as osmotic potential decreased. Speed of germination index, emergence energy value and germination energy reduced with the elevation of stress (Table 1, 2). Similar observation about germination indices was made by Homa Mahmoodzadeh et al, 2013. Germination indices under severe water stress and 50Mm NaCl stress were almost similar. Investigations of this type are essential for selection of crops with seed characteristics acceptable for cultivation in stress-affected areas.

Proline content

Proline content increased with the increase in stress levels under both the stresses (Fig 1, 2). These results revealed that *Cyamopsis* presented variation within the control and stress treatments, having the capacity of adjusting to the unfavourable conditions. Similar results were found by Tarun et al, 2007 demonstrating that this metabolite works as an osmotic adjuster in plants under abnormal abiotic conditions. The increase in proline levels may be due to an increase inP-5CR enzyme activity or concentration and a decrease of these amino acids in the mitochondria (Taiz and Zeiger, 1998; Kerbauy, 2004)

Figure-2. Effect of salt stress on proline content of *Cyamopsis tetragonoloba*



Conclusion

Results indicated that germination is sensitive to both the severe stress levels. However, germination was more sensitive to salt stress than to water stress induced by PEG. It appears that final germination percentage and emergence energy value were the most resistant and sensitive components respectively to stress.

Competing interests

The authors have declared that no competing interests exist.

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