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ORIGINAL ARTICLE

Effect of Some Stresses on Free Proline Content During Pigeonpea (*Cajanas cajan*) Seed Germination

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Plants accumulate proline under a variety of stress conditions thereby preventing stress-caused damages. The proline accumulation in germinating seeds of pigeonpea in response to NaCl, Boron and Aluminium treatments were studied. Results showed increased accumulation of proline in salinity stressed germinating seeds. As compare to 100 ppm boron, 10 and 50 ppm of boron treatments promoted proline levels at different stages of seed germination. The content of proline was found to be induced by all aluminium concentrations particularly at 48, 72 and 120 hrs. of seed germination. Whereas reverse pattern of proline accumulation was observed at 96 hrs stage under all studied stresses.

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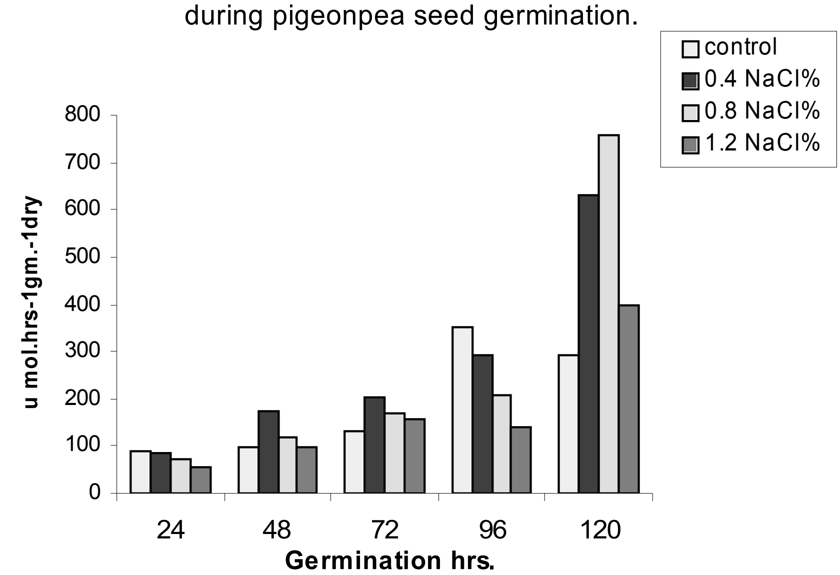
Abiotic stresses influences survival, biomass production and crop yield in negative manner. Proline is an electro cyclic low molecular weight protein amino acid of glutamate family has attracted considerable attraction of many ecophysiologists in last two decades. It is an organic osmoprotectant accumulates in a large number of plant species exposed to environmental stresses such as salinity, drought, extreme temperature, UV radiations and heavy metals (Hare and Cress 1997; Zhu *et al.,* 1998; Rhodes *et al.,* 1999; Taylor 1996; Yamada *et al.,* 2005; Eraslan *et al*., 2007). Proline is involved in the regulation of osmotic homeostasis, scavenging of reactive oxygen species and protection of cell structures (Srinivas and Balasubramanian 1995; lyer and Caplan 1998). Proline is a typical adaptive response in plants and it may be a part of stress signal (Maggio *et al*., 2002; Yang *et al*., 2009). Accumulation of proline has been reported from increased proline biosynthesis, lower proline utilization, decreased degradation of proline and proteins (Delauney and Verma 1993). The level of proline accumulation varies from species to species and also among the plant organs, its highest levels found in flowers and seeds and lowest levels in roots. (Verbruggen *et al*., 1993; Nathalie and Christian 2008). Proline ameliorates the stress tolerance by preventing membrane and protein damage (Santoro *et al*., 1992; Santarius 1992). Catabolism of proline in stress free conditions generates reducing equivalents especially ATP to repair stress-induced damage (Hare and Cress 1997; Hare *et al*., 1998). Ashraf and Foolad (2007) suggested that the application of proline successfully improved stress tolerance in plants. Hence it is worthwhile to study the fate of free proline under the influence of NaCl, Boron and aluminium treatment at the time of seed germination.

Thus the present work was carried out to investigate the effect of salinity and Boron and Aluminium toxicities on proline accumulation so as to assess the significance of proline in stress tolerance in germinating seeds of pigeonpea.

MATERIAL AND METHOD

Healthy seeds of Pigeonpea (var. ICPL-87) were sorted out and surface sterilized with 1% sodium hypochloride for two mins. Petri-plates were sterilized with absolute alcohol and lined with filter paper at bottom. Twenty seeds of Pigeonpea were allowed to germinate on Whatman filter paper No- 1 in sterilized petridishes at 30^0 C in BOD under the influence of various concentrations of NaCl (0.4, 0.8 and 1.2%), Boron (10, 50 and 100 ppm) and Aluminium (5, 10 and 50 ppm) and investigations were covered at different stages of germination from 24 to 120 hrs. The free proline content was determined by employing the method of Bates *et al*. (1973).

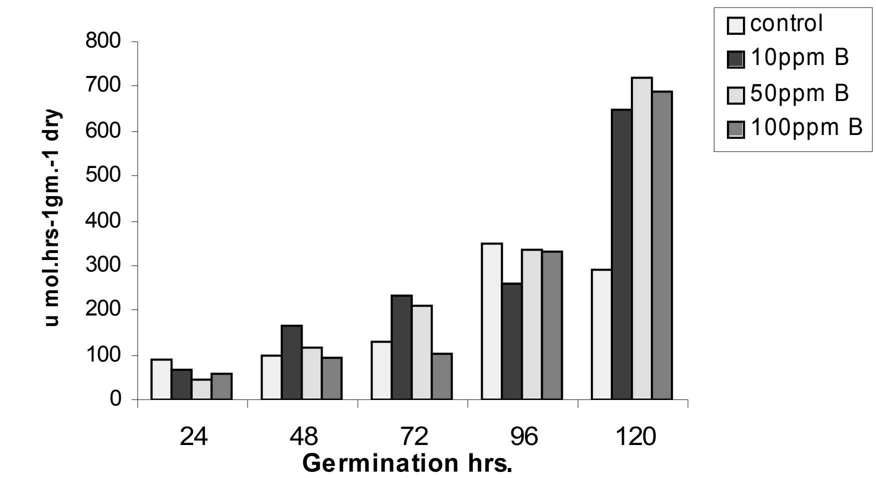
Fig.1 Effect of salt stress on the free proline content during pigeonpea seed germination.



RESULTS AND DISCUSSION

The results revealed highest proline content at 120 hrs of seed germination by lower doses of NaCl as compared to 24 whereas decrease in proline content was found at 24 and 96 hrs. (Fig. 1). The role of proline as osmolyte in salt stressed tissues is supported by many physiologists. The increase in proline content due to NaCl treatment has been reported by some workers (Durgaprasad *et al.*, 1996; Kuzhetsov and Shevyakova 1997). Increased proline contents and reduction in proline dehydrogenase enzyme activity has been reported in *Brassica juncea* (Madan *et al*., 1995) and in *Solanum tuberosum* (Rahnama and Ebrahimzadeh 2004). Similarly Wang *et al*. (2011) found higher proline contents, increased activity of orn-δ-aminotransferase and reduced proline dehydrogenase in the leaves of *Saussurea amara* under salinity stress. In contrast to it decrease in proline content under salinity noticed by Singh and Jain (1982). Murumkar (1986) speculated that decrease in proline content under salt stress reflects salt sensitive nature of chickpea seeds at germination stage. It is evident from our observations that accumulation of proline at 120 hrs may be playing a significant role against the salt stress.

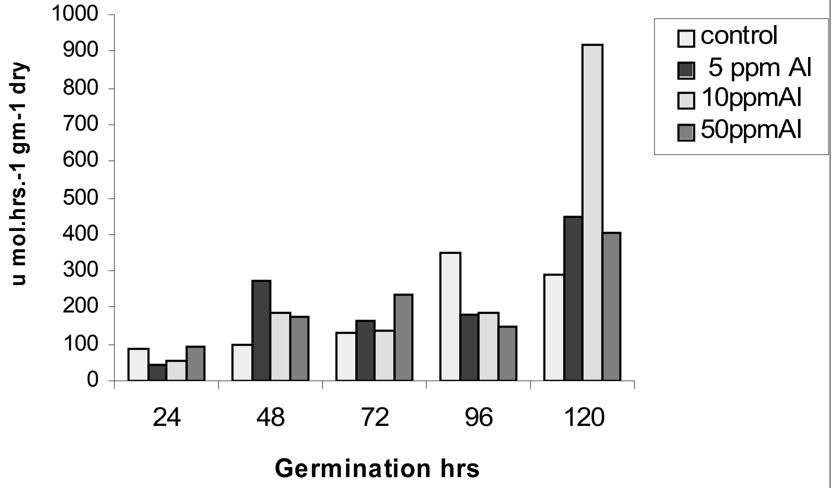
Fig.2 Effect of various concentrations on Boron on free proline content during pigeonpea seed germination



Excess boron can limit plant growth on soils of arid and semiarid environments throughout the world. There was no attention has been paid so far to the influence of boron in the medium on proline level. Induction in proline accumulation was found at different stages of pigeonpea seed germination more particularly by 10 and 50 ppm of boron. However higher concentrations of boron (100 ppm) in the medium adversely affect the proline concentration (Fig. 2). Boron toxicity results into osmotic imbalances, photooxidative damage, memberane leakiness, lipid peroxidation and proline accumulation (Reid 2007; Eraslan 2007; Herrera-Rodriguez *et al*., 2007, 2010). Papadakis *et al*. (2003) has been emphasized increased proline contents in the leaves of Cleementine mandarin plant grafted on sour orange and *Swingle citrumelo* under boron toxic condition. If a level of proline is related to stress tolerance potential it can be concluded that boron concentrations above 50 ppm are not desirable for the pigeonpea seed germination.

The problem of soil acidity is becoming acute in the world. In acidic soil major problem is aluminium toxicity involved in limiting growth in plants (Tang et al., 2001; Kochiana et al., 2005; Valle et al., 2009). Soil acidity is reported to cause accumulation of proline in pea, soybean, wheat, barley, maize, sorghum and Polygonum (Klimashevskii 1984; Guo et al., 2004). The proline accumulation was found to be increased by aluminium treatments at 48, 72 and 120 hrs, while opposite trend was noticed at 24 and 96 hrs of pigeonpea seed germination (Fig. 3). The initial decrease in proline content is in agreement with the earlier report of Satokopan et al. (1990). They observed that increasing concentrations of $Al_2(SO_4)_3$ from 1µM to 100 µM reduced proline content during chickpea seed germination. Guo et al. (2004) stated that Al and Cd toxicity caused increase in proline levels in barley seedlings. Aluminium decreased proline accumulation in leaves of Sorghum bicolor plant (Rodrigues da Cruz et al., 2011). It is difficult to pinpoint the exact role of proline under the aluminum toxicity.

Fig. 3 Effect of various concentrations of Aluminium on free Proline content during pigeonpea seed germination.



From present work it has been concluded that the stimulation in proline accumulation may reflect a common strategy to overcome the stresses due to NaCl, boron and aluminium in pigeonpea .

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