ISSN 2320-5407 International Journal of Advanced Research (2015), Volume 3, Issue 11, 303 - 311

ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Effect of pre-treatment of chickpea (*Cicer arietinum* L.) seeds on seed germination and seedling growth under salt stress

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Manuscript Info

Manuscript History:

Received: 15 September 2015
Final Accepted: 22 October 2015
Published Online: November 2015

Key words:

Cicer arietinum, Germination, Salicylic acid, Salinity, Seed priming

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Abstract

As germination plays a dominant role in determining the final density of plants so it is one of the critical stages of plant growth cycle. Salinity decreases the seed germination and seedling emergence which is one of the main problems in saline areas. Seed priming technique improves germination and seedling emergence under different environment stresses. On the other side, salicylic acid plays a major role in regulation of many physiological processes like growth, development, germination and ion absorption of plants. The objective of this study was to assess the effectiveness of seed priming in improving growth characteristics, seed germination, seedling vigor index of chickpea (*Cicer arietinum* L.), in response to 4 levels of NaCl (0, 50, 100 and 150 mM) and to 4 levels of priming by Salicylic acid (0, 10^{-4}, 10^{-5} and 10^{-6} M). An experiment was conducted with three replications in research laboratory of Botany department, Kurukshetra University, Kurukshetra. The results showed that plumule length, radicle length, seedling length, plumule fresh weight, radicle fresh weight and seedling fresh weight were significantly ($P<0.01$) reduced by increasing salinity for unprimed seed (control) compared with primed seeds. Also, Salinity conditions reduced seed vigour index and seed germination percent. SA at 10^{-5} M had the maximum beneficial effect followed by 10^{-4} and 10^{-6} M in seed germination in both stressed and non-stressed conditions. SA had shown to exert a possible ameliorative effect under salt stress regarding seed germination testing.

INTRODUCTION

A major problem in almost all the regions of the world, mainly in arid and semi-arid areas is salinity (Munns and James, 2003; Khan and Qaiser, 2006). Amid the stresses, eminent concentrations of salt in the soil can result in harsh detrimental factors, like poor germination, seedling establishment and crop yield (Iqbal and Ashraf, 2006). Plant growth reduction under salt stress is a commonly occurring phenomenon which is chiefly due to low soil water potential and an imbalance in the uptake of mineral nutrients and their accumulation within the plant (Hosseini and Thengane, 2007). Also, salt stress can disrupt growth and photosynthetic processes by causing changes in the accumulation of Na^+, $Cl^−$ and nutrients and interruption in water and osmotic potential (Nafees et al., 2010). Carvalho et al., (2011) expressed that seed priming is an efficient method for increasing seed vigor and integration of germination, as well as the growth of seedlings of many crops under stressful conditions. Hanson (1973) found that priming, generally would cause an effective consolation of the dry seed which is the initiation of metabolic processes that commonly occur during imbibition and which are subsequently fixed by drying the seed. Seed pre-treatment is recommended in farming as a simple, economical technique for counteracting the salinity of soils.

Salicylic acid is a phenolic plant growth regulator having a role in regeneration of physiological processes in plants (Sakhabutinova et al., 2003). As salicylic acid act as signal molecule so it may play a role in improving the growth and development of the crops (Krantev et al., 2008). Senaratna et al., (2000) found that salicylic acid belongs to a group of phenol compounds that are confessed as the mitigating factors of plant responses to environmental stresses. It has been reported that SA has improved some abiotic stresses, e.g. chilling stress (Kang and Saltveit, 2002), heat stress (Dat et al., 1998; He et al., 2002) and heavy elements stress (Metwally et al., 2003). Seeds primed with SA under saline conditions show increase in emergence percentage that may be due to increased oxygen uptake, enhanced α-amylase activity and the efficiency of mobilizing nutrients from the cotyledons to the embryonic axis and enhanced the contents of free amino acids, protein and soluble sugar (Karthiresan et al., 1984; Zhang et al., 1999). According to those researches, SA pre-treatment have improved growth and resulted in higher tolerance of plants to salinity, also increased growth parameters, germination percentage and seed vigor index of the seedlings. Therefore, considering the importance of legumes in the diet of vegetarian people of Indian subcontinent and given the protein value of chickpea (*Cicer arietinum* L.), the objective of the current research was to study the responses of chickpea seedlings to salinity stress and to investigate the possibility of pacifying the effects of salinity stress on this crop by pre-treating its seeds with salicylic acid.

MATERIALS AND METHODS

The current study was carried out in research laboratory of Department of Botany, Kurukshetra University, Kurukshetra, India in 2015 with three replications on chickpea (*Cicer arietinum* L.) var. H-208. The experimental treatments included salinity at four levels (0, 50, 100 and 150 mM) and salicylic acid at four levels (0, 10^{-4}, 10^{-5} and 10^{-6}M). The seeds and petri dishes were sterilized by 10% hypochlorite solution and then, were rinsed with distilled water. The seeds were soaked in 10^{-4}, 10^{-5} and 10^{-6}M SA at 25°C for 4 hours. Distilled water was used as control treatment. Afterwards, the primed seeds were rinsed with distilled water and dried between two layers of paper (23±2°C with relative humidity of 60%). Ten pre-treated seeds were included in each petri dish and received the same required amount of salinity solutions and distilled water (as control). After 24 hours, the seeds were daily checked for 7 days and the number of germinated seeds was recorded. A seed was considered germinated upon emergence of radicle about 2 mm in length. Seed germination percentage (SGP) was determined at the end of the test. To determine the radicle length (RL), plumule length (PL), seedling length (SL), radicle fresh weight (RFW), plumule fresh weight (PFW), seedling fresh weight (SFW) after the 7th day, normal plumules and radicles produced in each petri dish were separated from the seeds, and their lengths and fresh weight were measured. Seedling vigor index (SVI) was calculated by multiplying the germination percentage by the seedling length and then dividing the product by 100 (Abdul-Baki and Anderson, 1970). Analysis of Variance was based on ANOVA procedure by software SAS. Differences among the means of the treatments were estimated using the Duncan's multiple range tests at the 5% probability level.

RESULTS AND DISCUSSION

Germination percentage

Germination percentage was lowered by salinity compared to non-salinity condition. Salinity decreased the germination percentage to about 12, 33 and 46 percent at S_1, S_2 and S_3 levels of salinity respectively compared to controls (fig. 1). Under salinity conditions, germination percentage was significantly increased by SA compared to the seedlings without SA. Germination percentage of seeds increased when primed with 10^{-5} M SA and decreased at SA at 10^{-4} M. SA at 10^{-5} M concentration was the most effective which increased germination percentage by about 10, 13 and 14 percent at different levels of salinity (50, 100 and 150 mM) respectively corresponding to their controls. The same was observed in earlier studies by Shakirova et al., (2003) and El-Tayeb et al., (2005) where they observed that emergence percentage was increased by SA treatment under salinity conditions. Seed germination and early seedling growth are the most sensitive stages to salinity stress in many crop species (Akazawa et al., 1990). Seeds primed with different concentrations of SA proved to be effective in inducing salt tolerance at the germination stage (Afzal et al., 2006), is quite clear from present investigation.

Seed Vigour Index

SVI is the ability of emergence of seed during stress and it decreased with increasing salinity stress in the present study. SA at 10^{-5} M concentration enhanced SVI by about 40, 47 and 54 percent respectively at different levels of salinity (50, 100 and 150 mM) as compared to control. Makawi et al., (1999) reported that survival ability is reduced when seedling cannot make a powerful root system and therefore, root growth can be considered as a screening scale for seed vigour.

Radicle Length

In the present study, radicle length decreased progressively with increasing salinity. It may be due to toxic effects of the unbalanced nutrient uptake by the seedlings as well as NaCl used. SA at 10^{-5} M concentration increased radicle length by about 60, 56 and 48 percent respectively at S_1, S_2 and S_3 levels of salinity corresponding to their controls. SA at 10^{-6} M too increased the radicle length but the magnitude was less compared to 10^{-5} M concentration. Root elongation may be inhibited by high salinity due to slowing down the uptake of water by the plants (Werner et al., 1995). The seeds primed with 10^{-5} M SA showed maximum radicle length (6.93 cm). The seeds treated with SA produced higher radicle length in all salinity levels than untreated seeds (Fig.3). Similarly Jaleel et al., (2007) on *Catharanthus roseus* that root length was affected by different salinity levels.

Plumule Length

Plumule length decreased significantly with increase in salinity levels. The shortest plumule length goes to salinity level of 150 mM. Salicylic acid at 10^{-5} M caused significant adjustment in decrement of length of plumule caused by salinity (Fig.4). SA at 10^{-5} M mitigated the effects of salinity by increasing plumule length by about 76, 62 and 55 percent respectively at S_1, S_2 and S_3 levels corresponding to their controls. Decrease in length can be due to decrease in cell elongation and decrease in turgor. Similar results have been observed in salinity treatment of two sensitive and resistant types of rice (Kang et al., 2002) and cowpea (Dantas et al., 2005).

Radicle Fresh Weight

Radicle fresh weight decreased under salinity treatment by 12.56 %, 34.15 % and 51.5 % at S_1, S_2 and S_3 levels compared with control (Fig.5). Earlier studies have shown that NaCl treatment decreased the some growth parameters such as fresh weight of root of plants (Yildirim et al., 2008). In this study, we found that the presence of NaCl reduced the radicle fresh weight of chickpea compared to the control as a consequence of salt osmotic effects, which reduced water availability (Fig.5). During imbibition, water entry occurs through aquaporins, which have reduced expression in the presence of salt (Boursiac et al., 2005). Seed priming with different concentrations of salicylic acid alleviated the radicle fresh weight of salt stressed chickpea seeds but 10^{-5} M concentration was the most effective which increased radicle fresh weight by about 28, 29 and 34 percent respectively at S_1, S_2 and S_3 levels corresponding to their controls.

Plumule Fresh Weight

Salinity reduced the fresh weight of plumule of chickpea compared to the control by 26.13, 31.35 and 61.69 % at S_1, S_2 and S_3 levels respectively. Priming of seeds by different concentrations of salicylic acid mitigated the effect of salt stress. 10^{-5} M SA was the most effective and alleviated the salt stressed plumule fresh weight of chickpea by about 44, 28 and 13 percent respectively at S_1, S_2 and S_3 levels corresponding to their controls. Khodary (2004) also reported that SA increased fresh weight of shoot of salt stressed maize plants. Similarly, Mori et al., (2011) reported that salinity reduced the plumule fresh weight of plants.

Seedling Length

Data showed the effect of seed pretreatment by different concentrations of salicylic acid on seedling length during stress (Fig.7). Seed pre treatment with salicylic acid alleviates the seedling length in both stress and non-stress conditions. 10^{-5} M concentration of SA increased the salt stressed seedling length at S_1, S_2 and S_3 levels by 128.2, 88.77 and 76.5 percent corresponding to their controls.

Seedling Fresh Weight

Fig. 8 indicated the effect of SA on seedling fresh weight on chickpea seedling during salinity stress. Three different salinity levels (50, 100 and 150 mM) decreased seedling fresh weight by 17.5, 33.12 and 55.2 percent respectively of control. Priming with different concentration of salicylic acid mitigated the effect of salt stress on seedling fresh weight. 10^{-5} M SA concentration was the most effective and increased the seedling fresh weight by 33.6, 29.12 and 20.71 % at S_1, S_2 and S_3 salinity levels respectively of their controls.

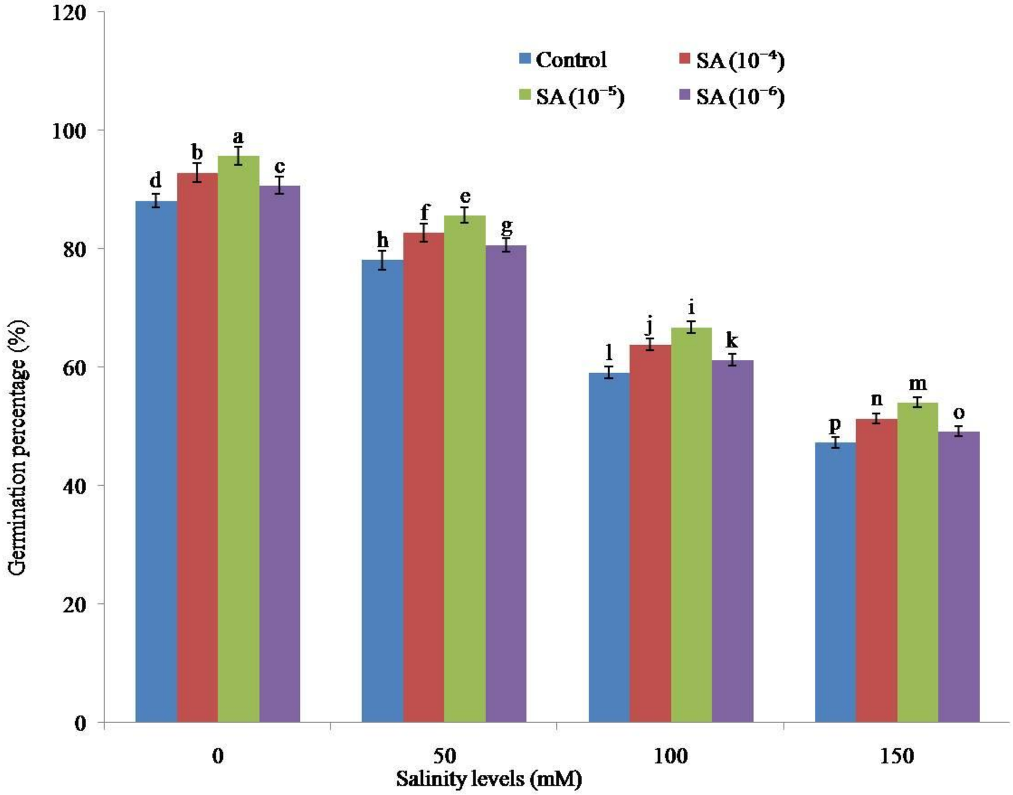

Fig.1 Effect of priming by salicylic acid on germination percentage in chickpea (*Cicer arietinum*) during salt stress.

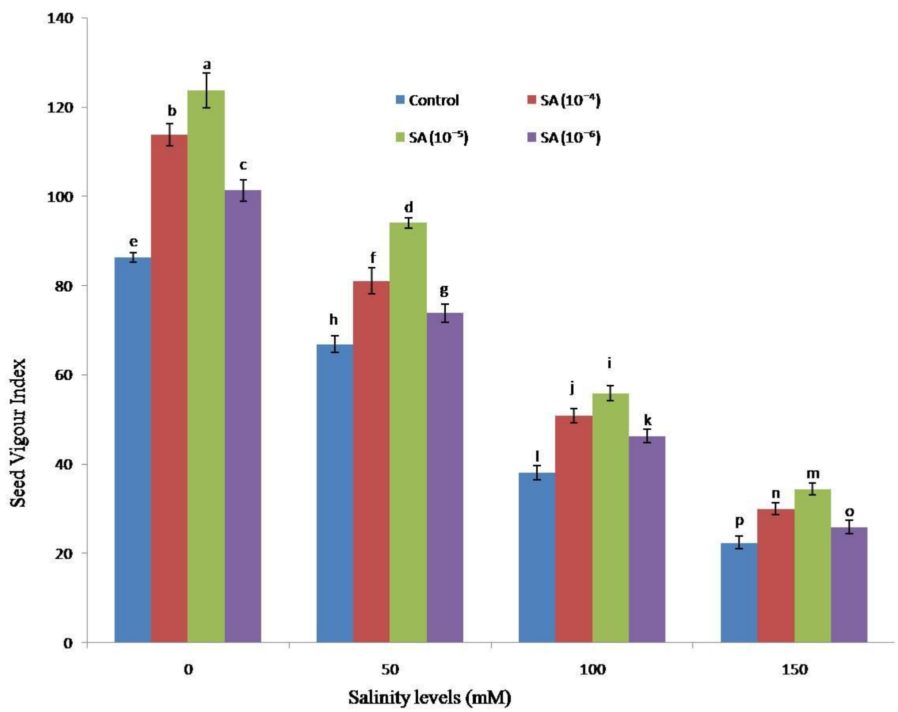

Fig.2 Effect of priming by salicylic acid on seed vigour index in chickpea (*Cicer arietinum*) during salt stress.

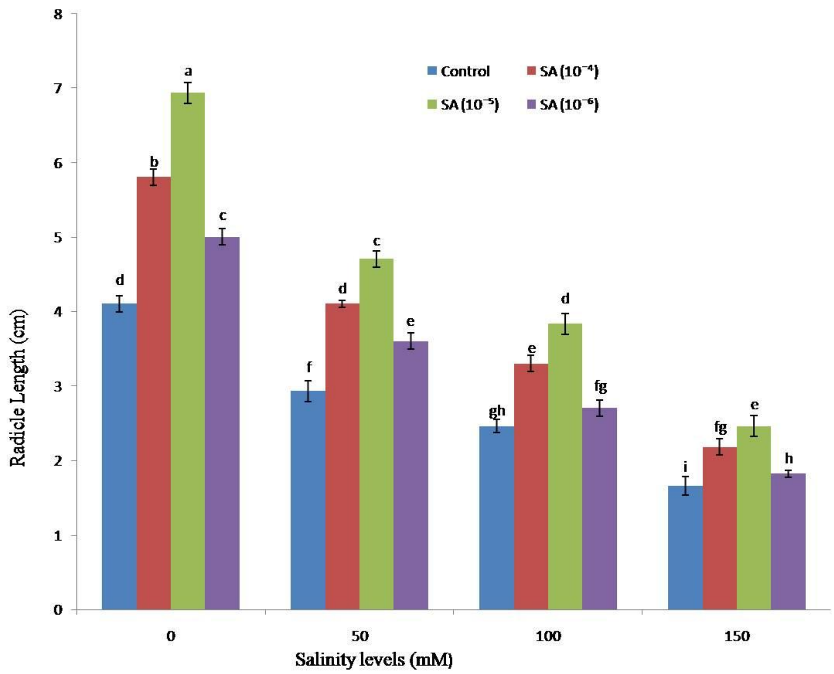

Fig.3 Effect of priming by salicylic acid on radicle length in chickpea (*Cicer arietinum*) during salt stress.

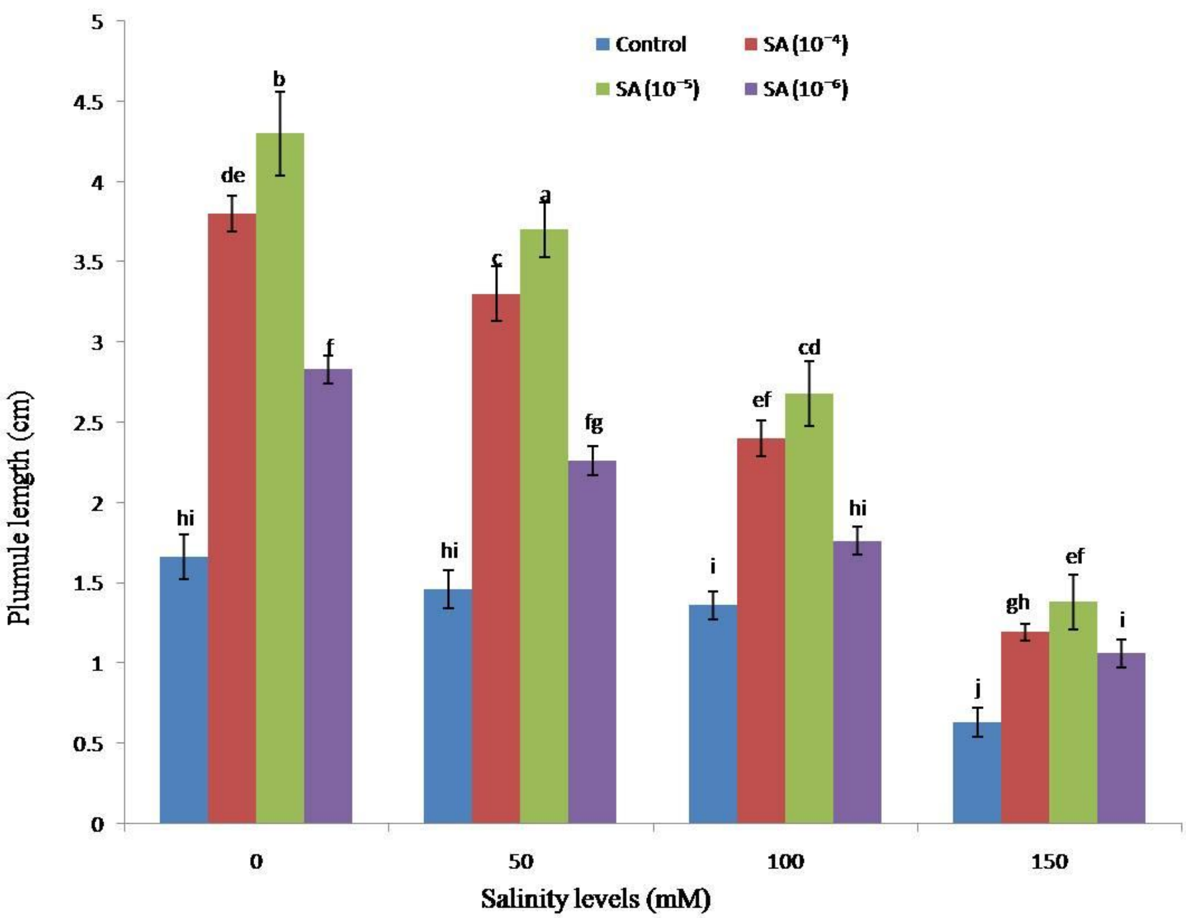

Fig.4 Effect of priming by salicylic acid on plumule length in chickpea (*Cicer arietinum*) during salt stress.

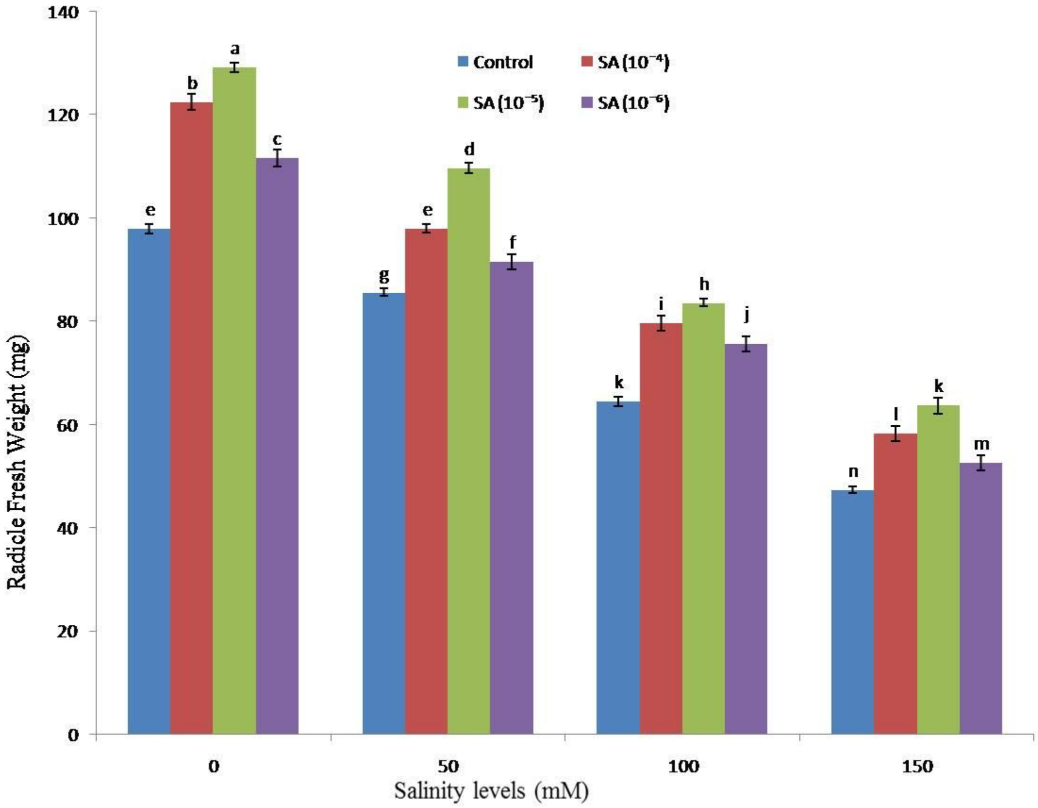

Fig.5 Effect of priming by salicylic acid on radicle fresh weight in chickpea (*Cicer arietinum*) during salt stress.

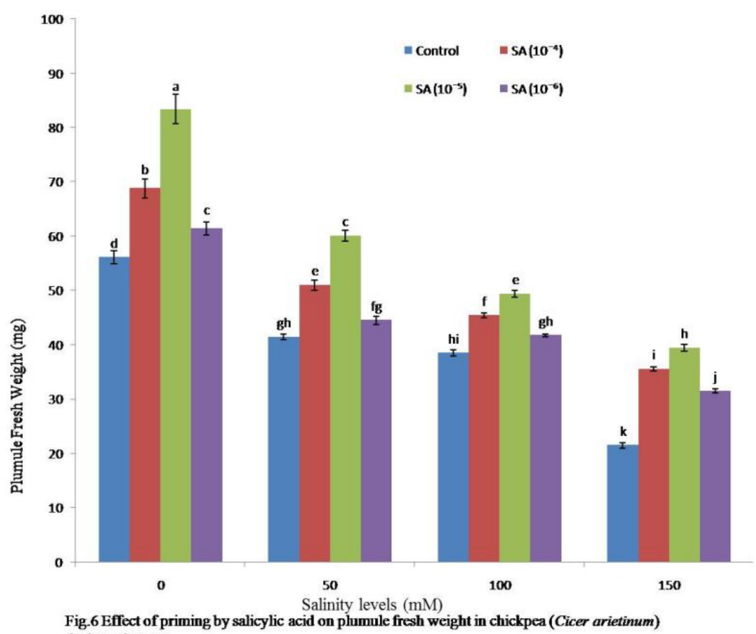

Fig.6 Effect of priming by salicylic acid on plumule fresh weight in chickpea (*Cicer arietinum*) during salt stress.

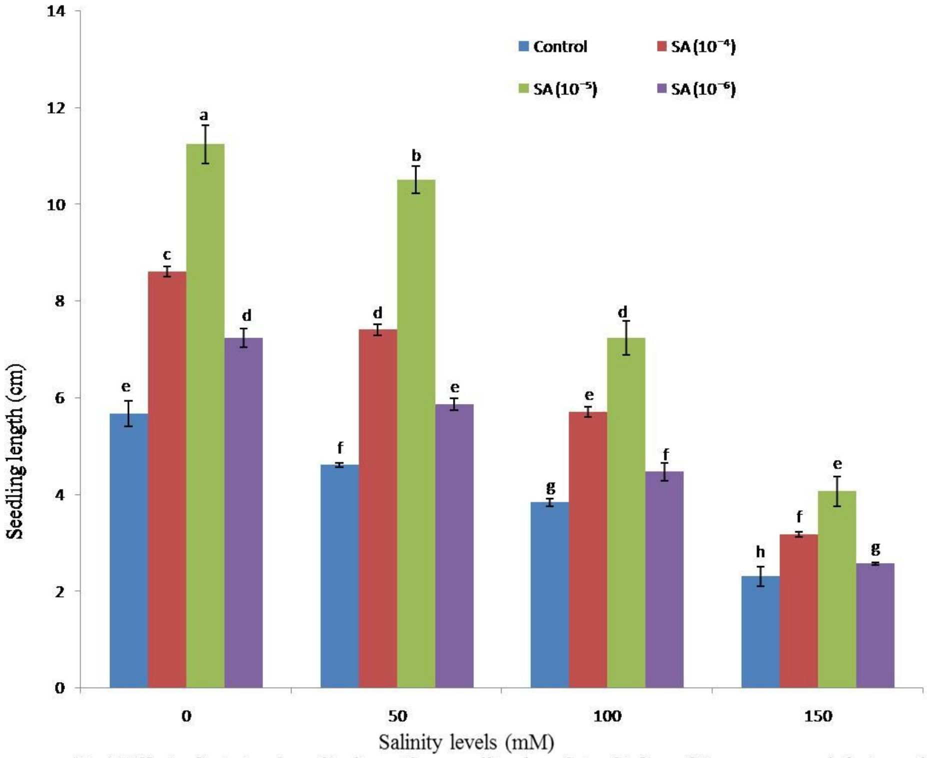

Fig.7 Effect of priming by salicylic acid on seedling length in chickpea (*Cicer arietinum*) during salt stress.

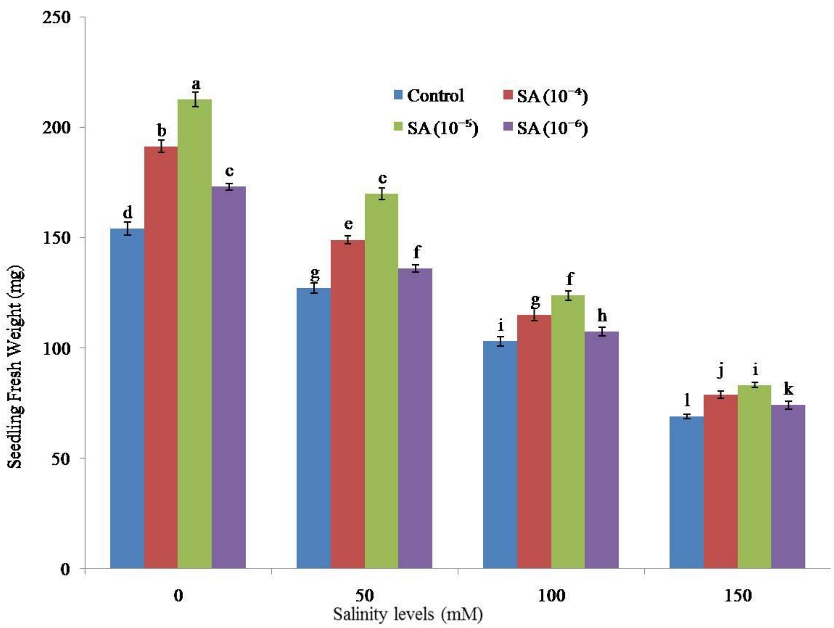

Fig.8 Effect of priming by salicylic acid on seedling fresh weight in chickpea (*Cicer arietinum*) during salt stress.

Conclusions

Priming increases seed and seedling quality so this technique can be beneficial for chickpea seedling. Primed seeds germinate faster and this will enhance their competition power against the salinity stress. The priming by 10^{-4} and 10^{-6} M salicylic acid was less effective than 10^{-5} M salicylic acid. Priming also increases growth parameters like radicle length, plumule length, seedling length, radicle fresh weight, plumule fresh weight, seedling fresh weight and seed vigour index of chickpea under salt stress conditions.

Acknowledgements

Authors are highly acknowledged to CCS Haryana Agriculture University, Hisar, India for providing certified seeds of chickpea.

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