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The effect of GA3 on seed germination and early growth of lentil seedlings under salinity stress

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Abstract

Seed germination is the most important sensitive stage to stress, so that early seedling growth and final crop yield can be inhibited by effects of salinity. The present experiment was developed in order to evaluate salinity effects and plant growth regulator (GA3) on seed germination in two lentil genotypes (Kimia and Ilam). Statistical analysis on germination characters and seedlings growth indicated that, salinity caused significant reduction on seeds germination rate and seedlings growth in both genotypes. TWC and RWC had been increased in seedlings following increase in salt concentration. However, results showed that inhibitory effects of salt on early growth of Kimia seedlings, that is significantly more than Ilam genotype. Regarding to the results, GA3 had stimulant effects on germination rate and root to shoot ratio in Kimia genotypes. Also, it is well known that using of GA3 had positive response for germination percentage, TWC and RWC at salinity stress.

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Introduction

Beans are dry edible seeds which belong to *Fabaceae* Family. Meanwhile, lentil (*Lens Culinaris*) is a plant with lots of branches, soft fur, light green, herbaceous perennial, with short branched stems with height between 15-75 cm (Parsa and Bagheri, 2008).

Crops often exposed to environmental limiting factors like high soil salinity or drought, which affect all its processes with restricting the amount of available water for plant (Grover *et al*., 2001).

Germination of seeds and Seedling establishment may be inhibited under salt stress (Almansouri *et al*., 2001, Ghorbanli *et al*., 2006, Omidi *et al*., 2009). Furthermore, Salinity stress can decrease water uptake by seeds (Murillo-Amador *et al*., 2002, Demir Kayaa *et al*., 2006).

Between salt stress and shortage of water there is a direct and impartible relationship. Seed germination is one of the critical and determining steps in growth cycle of plant species; because plays a role in successful establishment and final performance of the plantlets. Three distinguishable steps in seed germination are: 1) The seed turgescence step, in which seed absorbs water (Hare *et al*., 1997), 2) Delay phase in which activation of the enzyme and beginning of meristem activities happen and 3) Beginning of growth with elongation of root and stem growth, and their departure from the seed coat, the amount and rate of germination reduce with reduction of external water potential, and there is special water potential for each of species, which in less than that germination would not happen (Mehrabi *et al*., 2006).

The reduction of growth under stress conditions is the result of the prevention of cell division, cell growth, or both of them; which these inhibitory effects can be the result of change in balance of plant growth regulators (phytohormone) in response to the stress (Morgan, 1984). It has been specified that under unfavorable environmental conditions, the endogenous levels of phytohormons show major changes (lopez-Carbonell *et al*., 1996, Hare *et al*., 1997).

Reduction in amounts of cytokinins and gibberellic acid, and increase in the abscisic acid content, in several species of herbs, under salinity and drought stresses, has been reported (Mizrahi *et al*., 1971, Bozcuk, 1981). In spite of the fact that hormonal balance mechanisms in herbs is weak, but it has been specified that absolute concentrations of cytokinins and other growth regulators, which mutually affect their synthesis and metabolism (Hare *et al*., 1997). The beneficial effects of growth regulators on seed germination under salinity, which can also maintain optimum yield levels have been demonstrated for many species and genuses such as tomato, barley, cotton (Bozcuk, 1981) and Suaeda genus (Bucaud and Unger, 1976).

The purpose of this research was studying regulator growth, giberellic acid, on seed germination and early growth of lentil seedlings under salinity stress.

Materials and methods

Plant material

The healthy seeds of two lentil genotypes, Ilam and Kimia, were provided and were sterilized by benomyl and ampicillin.

Establishment of cultures

After washing 3 times with sterile distilled water, under sterile conditions, 35 numbers of seeds were cultured in glass containers (petri dishes, with a diameter of 9cm) on a layer of filter paper. Experiment was arranged in a factorial based on a CRD (Completely Randomized Design).

In the experiment, two factors consist of four concentration of salinity caused by NaCl (0, 60, 120 and 180 $mMLit^{-1}$) and GA3 in four concentration (0, 2, 4 and 6 $\mu MLit^{-1}$) were used. Finally, all cultures were protected in a 16:8 h (L/D) photoperiod at 20±1°C.

One week following to cultures, characteristics on plantlets were evaluated as follow: number of germinated seeds, length of root, length of shoot, seedling fresh and dry weight, TWC (Tissue Water Content, by equation 1) and RWC (Relative Water Content, by equation 2).

1) TWC= [(FW-DW) / FW] ×100

To determine the relative water content of seedlings, both control herbs after measuring 1g their wet weight, they immersed in distilled water and were incubated in temperature of 4 °C, after four hours the turgid weight of herbs were measured, turgid weight per each germ of the wet weight of control herbs for both types was calculated and in this way the corresponding turgid weight to each wet weight unit was calculated. With existing information, the relative content water for each testing unit can be calculate by the relation number 2; which in fact is the ratio between the actual amount of absorbed water by herbs and the actual amount of impossible absorbed water by herbs.

2) RWC = [(FW-DW) /(FW-DW)]×100

Statistical analysis

The results were achieved using analysis of variance and mean comparison of experimental treatments by SAS software (Ver.9.1)

Results

Interactions of genotypes and salinity on germination characters

According to analysis of variance for different parameters (Table 1), Ilam and Kimia genotypes had different responses for length of root, length of seedling, root to shoot ratio and fresh weight in different concentrations of salt. So that, Kimia genotype in control level of salinity was found positively rather than Ilam for all the investigated characters, while the least of them, was achieved by different concentrations of salt in Ilam genotype (Fig.

Response of lentil genotypes to different GA3 concentrations

In current research the results showed that the interaction of genotype and GA3 was significant for germination characters such as root to shoot ratio, germination rate, TWC (Tissue water content) and RWC (Relative water content) (Table 1).

Table 1. Mean of squares for germination characteristics of lentil genotypes treated with GA3, at salinity stress induced by NaCl.

SOV	df	Mean of Square										
		Length of root	Length of Shoot	Length of seedling	Root / Shoot	Fresh weight (g)	Dry weight (g)	Number of root	Germination percentage	Germination rate	TWC	RWC
Genotype	1	15.27**	1.37*	8.83**	2.74**	0.01^{ns}	0.00**	9.39*	465.47**	142.22**	297.47**	15404.69**
NaCl	3	34.52**	29.96**	129.63**	0.07^{ns}	0.40**	0.01**	77.34**	1363.46**	117. 14**	201.83**	18.62**
GA3	3	0.30^{ns}	0.25^{ns}	0.09^{ns}	0.20**	0.03*	0.00^{ns}	3.12^{ns}	17.10**	21.18^{ns}	12.49^{ns}	0.79^{ns}
Genotype ×NaCl	3	4.05**	0.23^{ns}	2.90*	0.39**	0.01^{ns}	0.00*	1.96^{ns}	21.57^{ns}	20.89^{ns}	11.05^{ns}	0.42^{ns}
Genotype×GA3	3	0.64^{ns}	0.50^{ns}	0.88^{ns}	0.11*	0.01^{ns}	0.00^{ns}	0.29^{ns}	28.04^{ns}	41.64**	28.01*	4.13*
NaCl×GA3	9	0.59 ^{ns}	0.26^{ns}	1.50^{ns}	0.03^{ns}	0.01^{ns}	0.00^{ns}	0.83^{ns}	46.10**	12.02^{ns}	21.80*	2.44*
Genotype×NaCl× GA3	9	0.58^{ns}	0.54**	1.85^{ns}	0.06^{ns}	0.00^{ns}	0.00^{ns}	2.29^{ns}	37.15*	13.92^{ns}	16.52^{ns}	1.73^{ns}
Error	64	0.47	0.19	1.00	0.04	0.00	0.00	1.84	14.15	7.88	10.04	1.06

ns, * and ** are not significant, significant at the 5 and 1 % levels of probability, respectively.

According to Fig. 2, control level of GA3 was more affective for rate of germination in Ilam genotype. The same genotype with 2μMLit^{-1} of GA3, gave the best result for TWC, whereas control level of GA3, did not prove beneficial to both parameters in Kimia genotype.

Furthermore, different concentrations of GA3 had less difference for RWC in each of genotypes, and Ilam genotype was significantly higher than Kimia to this trait and TWC. But, it was observed that the most of root to shoot ratio was related to Kimia genotype and 6μMLit^{-1} of GA3, so that the same genotype had been successfully to improve it in all investigated concentration.

Relation between salinity and GA3 on lentil seed germination

Interaction between GA3 and salt was developed to find relation between them on seedling growth and germination characters. The results indicated that percentage of germination, TWC and RWC were influenced by salinity and GA3 (Table 1). Comparing means of salinity interaction and GA3 (Fig. 3), showed that applying of 0, 2, or 6mMLit^{-1} of hormone (GA3) without salinity was more positively for germination percentage, and use of 2 µMLit^{-1} of it, resulted in the increase in of RWC (in 180mMLit^{-1} of salt) and TWC (in 120mMLit^{-1} of salt).

Interactions of salinity and GA3 on germination of lentil genotypes

Accordingly to results (Table 1, Fig. 4), Ilam and Kimia genotypes had change significantly in different levels of salinity and GA3 for germination percentage and shoot length. Where, the control level of salt related to Kimia genotype, had 100% germination in all GA3 concentration, also, it was followed by the use of 60mMLit^{-1} of salt without using of GA3 in Kimia genotype.

Besides, the higher length of shoot produced without applying salt in both genotypes, and GA3 at 2 & 4µMLit^{-1}, induced taller shoots in Ilam and Kimia genotypes, respectively.

Discussion

Salinity is one of the major stresses, especially in arid and semi arid regions it can severely limit crop production (Shannon, 1998). Successful seedling establishment and vigor seedlings at stress conditions depends on ability of the seed species to germinate (Roundy, 1987, Alizadeh *et al.*, 2005)

These results are in close conformity with such reported: Zakeri, *et al* (1990), mentioned that salinity caused dwarfism and stopping growth of roots. Asadian and Miyamoto (1987) following investigate the effects of salinity on alfalfa germination, reported that increase in salt concentration caused a decrease in different characters (i.e. root and shoot length). The inhibitor effect of salinity on growth rate and development of seedling as found earlier by Larcher, 1995 and Mehrabi *et al.*, 2006.

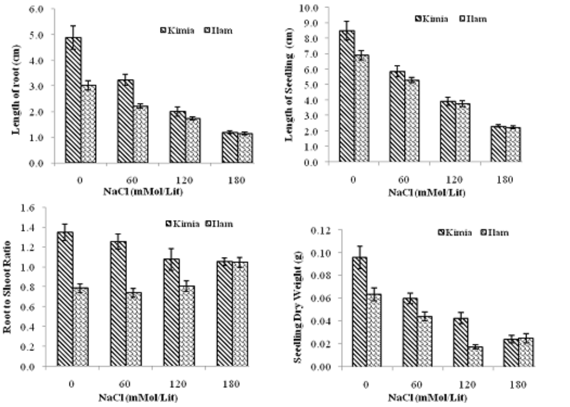

Fig. 1. Effect of salinity stress (NaCl) on lentil germination in Ilam & Kimia genotypes.

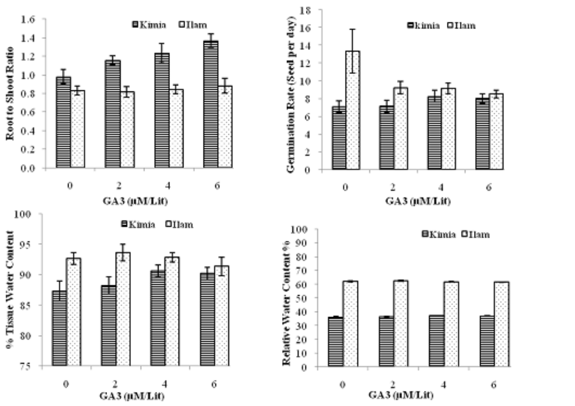

Fig. 2. Effect of different GA3 concentrations and genotypes on germination characters.

Besides, genotype interactions and GA3 recognized that higher root to shoot ratio obtained follow increase in GA3 concentration in Kimia genotype, also obtained similar results by 6µMLit^{-1} of GA3 in Ilam genotype. According to previous studies, seedling growth regulators can protect herb against salt damage, and salinity stress (Raou, 1961). Lessani and Mojtahedi (2011) found that GA3 can help to elongation of root. In this research also obtained similar results and interaction of treatment showed that the maximum of germination was obtained by using 6µMLit⁻¹ of GA3 in both genotypes at high salinity stress (120 & 180 mMLit⁻¹)

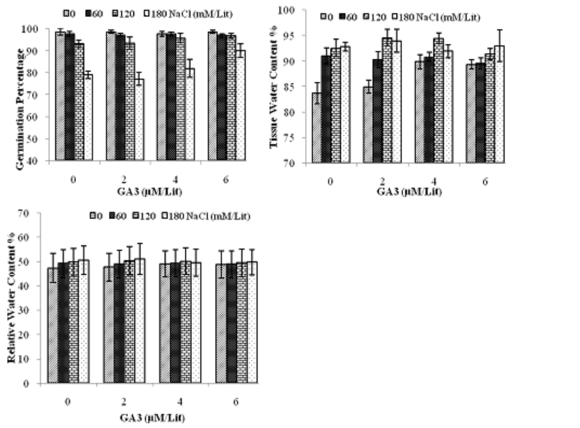

Fig. 3. Hormonal Effect (GA3) on percentage of germination, TWC and RWC at stress salinity (NaCl).

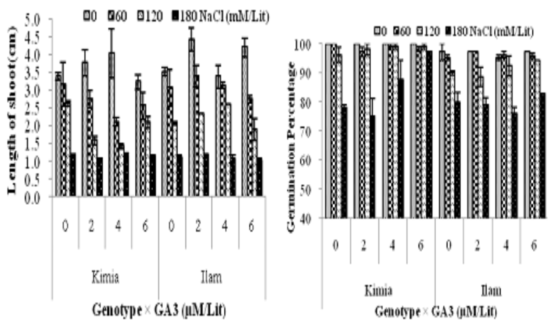

Fig. 4. Comparisons of GA3 concentrations on shoot length and germination percentage of lentil genotypes at salinity stress (NaCl).

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