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THE EFFECTS OF FOLIAR APPLICATION OF ABA AND CYTOKININE IN DIFFERENT REPRODUCTIVE GROWTH STAGES OF MATERNAL WHEAT PLANTS ON THE SEED TRAITS UNDER SALINE CONDITIONS

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Abstract

In order to study the effects of foliar sprays of Cytokinine (CK) and Abscisic Acid (ABA) in different reproductive growth stages of maternal plant on the seed characteristics under laboratory saline conditions a factorial experiment arranged in RCBD with three replications and two factors was conducted in Agricultural Faculty of Mahabad Azad University. Factor A included three salinity stress levels (0, 0.4 and 0.8 ds/m) and factor B included seven levels; b_1: no spray of hormones as control, b_2: CK foliar spray in pollination stage, b_3: CK and ABA foliar sprays in grain filling and pollination stages, b_4: CK foliar spray in grain filling and pollination stages, b_5: ABA foliar spray in pollination stage, b6: ABA foliar spray in grain filling and pollination stages, b_7: CK and ABA foliar sprays in pollination stages. Results showed that with increasing salinity rate all of the studied traits decreased significantly so that the least germination velocity, rootlet dry weight and rootlet length were obtained from the salinity stress of 0.8 ds/m and their greatest amounts were obtained from the control. The interaction of salinity and plant growth regulators with dry weight and stemlet length was significant. With increasing the salinity percentage at various levels of hormones the stemlet length and dry weight decreased so that the least values were obtained from ABA foliar spray at pollination and grain filling with salinity stress of 0.8 ds/m. The greatest values were obtained from the foliar spray of CK at pollination and grain filling with distilled water (control). The results showed that CK foliar spray at pollination and grain filling had the greatest positive effect on seed vigority under saline conditions.

Key words: *foliar application, cytokinins, abscisic acid, salinity.*

INTRODUCTION

Seed viability and vigor are two important factors affecting seedling establishment, plant growth and yield (Sawan and Copeland, 1997). Many factors such as genotype, seed size, seed weight, environmental stresses including water shortage, high temperatures, salinity, soil acidity, pathogens, nutrients shortage and abundance and anaerobic (no-O_2) conditions which directly affect the growth and nutrition of maternal plant can indirectly affect seed development, food supply and seed quality such as seed viability and vigor (Sawan et al., 1993).
The saline stress causes decrease in yield in field crops in dry and semi dry areas through negatively affecting grain production (Yokoi et al., 2002).
Also, saline stress causes decrease in germination percentage (Epstein et al., 1987) and rootlet length, stemlet length and shoot growth of seedlings (Chi Lin et al., 1995).
The study of effects of salinity on germination (velocity and % germination) and stemlet and rootlet growth in most field crops showed that saline stress at germination is a dependable test for evaluation of tolerance in many species because it decreases % germination and growth of rootlet and stemlet (Ghoulham and Fares, 2001).
Field crops are mostly exposed to restrictive environmental factors such as high salinity of soil or drought which affect plant processes through restricting available water (Grover et al., 2001).
There is a direct and inseparable relationship between salinity stress and water shortage. In saline-exposed plants besides the osmotic effect of saline stress that disrupts water relations in plants, there is also the specific effect of saline stress that shows up through the effect of ions on the cell metabolism and in some cases toxicity from ions accumulation (Hare et al., 1997).

Wide studies in terms of defensive responses to different abiotic and biotic stresses such as thermal stress, salinity, ultraviolet radiation, flooding, chilly stress and pathogen contamination indicate that there are probably several paths of message transmission which have interference with each other and regulate plant's response to the environmental stresses. Such a system of relative interference of response patterns to stress shows that plant's responses to the environmental stresses depend on a total change in the activities of several growth regulators among which abscisic acid and cytokinins are the most dominant ones. Although, other growth regulators such as salicylic acid and gasmonats play an important role in controlling plant's responses to unfavorable environmental conditions (Xiong et al., 2002).

Growth reduction in stress conditions is caused by prevention from cell division and growth or both of them which these preventive effects may come from change in plant growth regulators balance caused by stress (Stavir et al., 1998). It's been revealed that under unfavorable environmental conditions the inner levels of phytohormones make fundamental changes (Hare et al., 1997). Reduction in the amount of cytokinines and gyberelic acids and increase in abscisic acid content in variant species under drought and saline stress have been reported (Stavir et al., 1998). Although the mechanisms of hormone balance is weak in plants, but it's been revealed that absolute concentrations of cytokinins and other growth regulators reciprocally affect their synthesis and metabolism (Xiong et al., 2002). Thus, external treatment of growth regulators as reciprocal factor on stress-affected plants can be a possible method to improve the effects of abiotic environmental stresses (Stavir et al., 1998).

The present article is a report of the effects of two growth regulators, abscisic acid and cytokinines, on the seed germination and initial growth of the wheat seedlings under saline stress conditions. The aim of the present research was to study the effects of these two growth regulators foliar spray at pollination and grain filling on the seed vigor obtained from saline stress conditions.

MATERIALS AND METHODS

In order to study the effects of plant growth hormones, ABA and CK, at different plant growth stages of wheat on the seed vigor a factorial experiment arranged in CRBD with three replications was done on the farm of Mahabad Azad University. This experiment contained two factors: factor a, comprised of three levels of saline stress: 0 (distilled water), 0.4 and 0.8 ds/m. Factor b, hormone foliar sprays containing seven levels; b1: no-spray (check), b2: CK foliar spray at pollination, b3: ABA and CK foliar spray at pollination and grain filling, b4: CK foliar spray at pollination and grain filling, b5: ABA foliar spray at pollination, b6: ABA foliar spray at pollination and grain filling, b7: ABA and CK foliar spray at pollination.

Each experimental unit (plot) contained one sterile Petri dish with a filter paper medium. The seeds were sterilized with 50% ethanol for 30 seconds and then with 10% sodium hypochlorite (bleach) for 10 minutes.

After each sterilization the seeds were washed at least three times with distilled water. After sterilization ten seeds were put in each Petri dish. To generate the saline stress, NaCl was used in three tested concentrations 0, 0.4 and 0.8 ds/m and as much as 5 ml per each Petri dish. Then, their covers were tightly covered with Parafilm and transferred to germinator at 25°C.

The germinated seeds were counted daily. Seeds with a 2mm-long rootlet were considered as germinated. After 10 days the following traits were calculated:

germination velocity, average germination, % germination, seed vigor, rootlet dry weight, stemlet dry weight, rootlet length, stemlet length and the ratio of rootlet length/stemlet length under saline conditions in the laboratory of the Agricultural College.

Some traits were calculated as follows:

Function 1: Germination velocity, GR (germinated seeds per day) (Bajji et al., 2002)

$GR = \Sigma N_i / T_i$

in which,

N_i: number of germinated seeds in i^{th} day,

T_i: number of das to the i^{th} count.

Function 2: Mean Daily Germination (MDG) based on Magoyer's formula (Bajji *et al.*, 2002).
MDG= FGP/d
in which,
FDG: final % germination,
d: number of days to max final germination.

Function 3: Final % germination (FGP), (Abdul Baki and Anderson, 1973).
FGP=Σ (Ni × 100)/total seeds

Function 4: Seed vigor index (VI), (Abdul Baki and Anderson, 1973).
VI= [mean stemlet length (mm) × %germination]/100

Analysis of variance and statistical calculations

The data were analyzed using the program MSTAT-C and mean comparisons were done by means of LSD test at p≤5 and the tables and figures were drawn by means of the program Excel.

RESULTS AND DISCUSSIONS

The effect of different salinity treatments on the germination velocity of wheat seeds was significant at p≤1 (Table 1) so that with increasing salinity from 0 to 0.8 ds/m the germination velocity of the seeds decreased (Figure 1).

According to mean comparisons the greatest germination velocity belonged to the check and the least GV belonged to the saline stress of 0.8 ds/m. Of the mechanisms of salinity effect on germination velocity are the toxicity of ions like Na and Cl and disrupting the ion balance including K/Na ratio (Lianes et al., 2005). Decrease in germination velocity caused by salt concentration has been reported in different plants.

In this regard, Keshavarzi et al. (2008) studied the effects of salt on different varieties of sesame and achieved similar results. Demir-Kaya et al. (2006) studied the effects of saline stress on sunflower and reported that with increase in saline stress the germination velocity decreased.

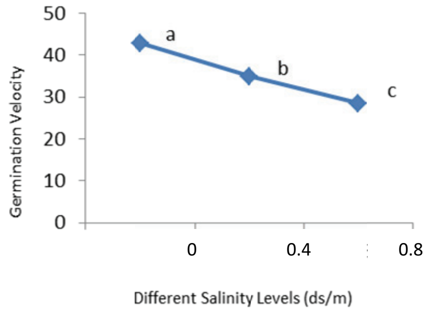

Figure 1. Effects of different salinity levels on germination velocity

The analysis of variance showed that the effect of foliar application of plant growth hormones at different growth stages of the maternal plant on germination velocity of the obtained seeds was significant at p≤1 (Table 1).

According to mean comparison of data (Figure 2) the foliar spray of CK at pollination and grain filling had the greatest increase in germination velocity by 12% relative to the check. Foliar application of CK to seeds during the growth stage leads to increase of CK in these organs (Yang et al., 2003).

Acceleration of germination velocity is probably due to high concentration of CK in seeds during cell division period. The lowest germination velocity belonged to ABA spray at pollination and grain filling which decreased by 30.7% relative to the Check.

Mehrabi et al. (2008) reported that ABA had a severe preventive effect on germination and growth of wheat seedlings under saline stress so that all the studied traits from ABA spray decreased significantly relative to the check. The results of the ANOVA showed that the interaction of saline levels and growth regulator hormones aren't significantly different (Table 1).

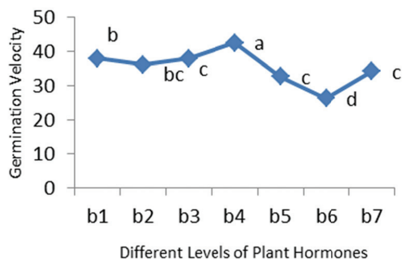

Figure 2. Effects of plant growth hormones on germination velocity

Table 1. Analysis of variance of studied salinity and plant hormones traits on seeds of wheat

S.O.V	df	Germination Velocity	Mean Germination Velocity	Seed Vigor	Root Length	Shoot Length	Shoot Dry Weight	Root Dry Weight
Salinity	2	1087.450**	582.159**	27.213**	26.894**	26.213**	0.001^{**}	0.0001^{**}
Plant Hormones	6	234.611**	315.692**	13.677**	16.810**	16.374**	0.001^{**}	0.001^{**}
Salinity* Plant Hormones	12	31.385^{ns}	166.434**	0.917*	0.426^{ns}	0.920*	0.0001^{**}	0.0001^{ns}
Error	42	44.892	33.426	0.384	0.588	0.415	0.0001	0.0001
C.V.	62	8.92	5.99	4.48	5.61	4.54	7.12	5.99

ns, * and **: non-significant and significant at p≤5% and 1%, respectively.

Average Germination Velocity

The analysis of variance showed that the interaction effect of foliar application of plant growth hormones at different growth stages of the maternal plant on Average Germination Velocity of the obtained seeds was significant at p≤1 (Table 1). The results of means comparison showed that the no-stress treatments had more mean germination velocity than saline stress treatments (Figure 3). The foliar application of plant hormones at different growth stages and at different salinity levels in treatment compounds of a_1b_1, a_1b_2, a_1b_3 and a_1b_5 had the highest mean germination velocity without any significant difference. The CK foliar application had a positive significant effect on the mean germination velocity relative to ABA in most treatment compounds. The ABA spray at pollination and grain filling with 0.8 ds/m stress had the lowest mean germination velocity. Increase in mean germination velocity by kinetin under stress conditions can be due to increase in water uptake because of increasing membrane permeability or the inner concentration of active osmotic salts (Stavir et al., 1998).

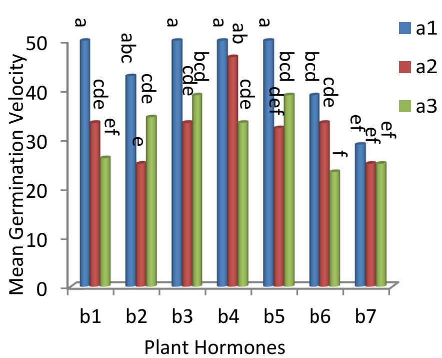

Figure 3. The interaction of salinity and plant hormones with average germination velocity

Seed Vigor

Regarding seed vigor there wasn't a significant difference among different saline stress levels and different levels of plant hormones at p≤1% and interaction between them at p≤5%. The results of means comparison showed that saline stress causes seed vigor to decrease so that the greatest seed vigor belonged to no-stress treatment (distilled water) and the least seed vigor belonged to 0.8 ds/m saline stress (Figure 4). The effects of ABA and CK sprays on the seed vigor at different plant growth stages and different salinity levels was significant. CK foliar application at pollination and grain filling together with the no-salinity stress (*a_1b_4*) had the greatest seed vigor among treatments. ABA foliar application at different growth stages caused decrease in seed vigor so that the seeds from ABA foliar application at pollination and grain filling with 0.8 ds/m saline stress had the least vigor (Figure 4).

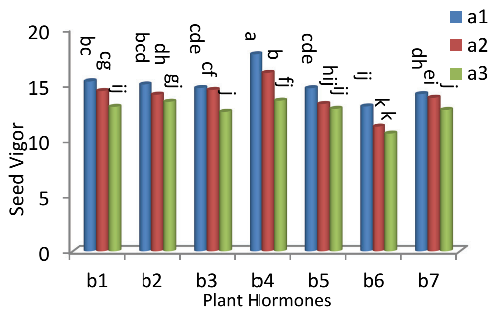

Figure 4. Effects of plant growth hormones and salinity on seed vigor

Rootlet Dry Weight

According to the analysis of variance (Table 1) the saline stress had a significant effect on the mean rootlet dry weight at p≤1% so that with increasing salinity from 0 to 0.8 ds/m the mean rootlet dry weight reduced.

The least mean rootlet dry weight was of the 0.8 ds/m saline stress. The greatest mean rootlet dry weight was obtained from no-salinity treatment (Check). The results of this study showed that the 0.4 ds/m saline stress and the check were in the same statistical group and in terms of rootlet dry weight they were not significantly different (Figure 5).

Keshavarzi et al. (2009) studied the effects of saline stress on seven sesame cultivars and achieved the same results as the present study so that with increasing the salinity level the root and shoot weights of all cultivars reduced significantly. In another study, Jamil et al. (2006) showed that with increasing salinity the root and shoot weights of the canola plant reduced. Hatami and Galeshi (1998) studied the impacts of different levels of NaCl on bareley and wheat and observed a significant reduction in all germination traits including root and shoot weights relative to the check with increasing salinity level.

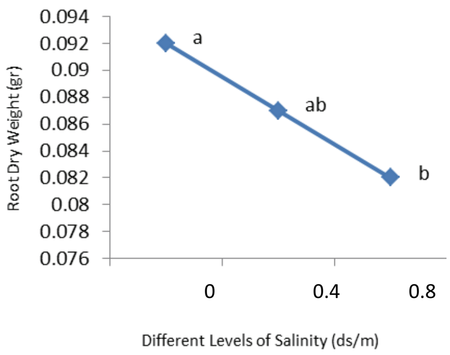

Figure 5. Effects of salinity on root dry weight

Results revealed that the impact of plant growth regulators spray at pollination and grain filling of maternal plant was significant on its seed's root dry weight at p≤1% (Table 1). According to means comparison the treatments of CK and ABA foliar application at pollination and grain filling (b3) and foliar application of CK at pollination and grain filling without any significant difference with 0.094 and 0.103 gr had the greatest root dry weight.

The least root dry weight with 0.076 gr was obtained from ABA foliar application at pollination and grain filling which was in the same statistical group as the treatments b2, b5 and b7 (Figure 6). Mehrabi et al. (2008) reported that significant interactions of salinity with ABA in relation with most growth traits show that growth responses of seedlings under simultaneous effects of salinity and ABA are different but with increasing ABA the growth of the seedlings and their wet weight reduced obviously.

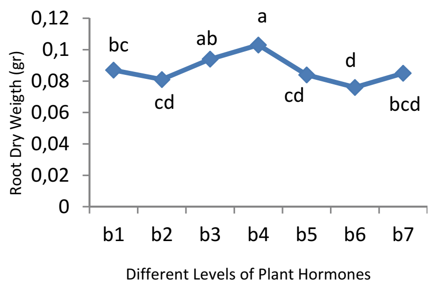

Figure 6. Effects of plant growth hormones on root dry weight

Shoot Dry Weight

Results of analysis of variance showed that among different levels of salinity, different levels of plant hormones and their interactions with shoot dry weight there wasn't significant difference at p≤1% (Table 1). The results of mean comparison of interaction of salinity and plant hormones showed that with increasing %salinity at different levels of plant hormones the dry weight of shoot decreased so that the least shoot dry weights were obtained from ABA foliar applications at pollination and grain filling with 0.4 ds/m (a2b6) and 0.8 ds/m (a3b6) salinity as much as 0.076 gr and 0.064 gr, respectively which reduced by 24% and 36%, respectively relative to the check (no-salinity and no-spray).

Ekiz and Yilmaz (2003) studied the dry weight and height of barley seedlings under saline conditions and concluded that plant dry weight is a better index than plant height to show sensitivity to salinity. The studies of Keshavarzi et al. (2009) on the impacts of salinity on sesame cultivars showed that with increasing salinity the shoot weight decreased significantly. The greatest shoot dry weight was obtained from the treatment of no-salinity together with CK foliar application at pollination and grain filling that with 0.112 gr increased the shoot dry weight by 12% relative to the check (Figure 7).

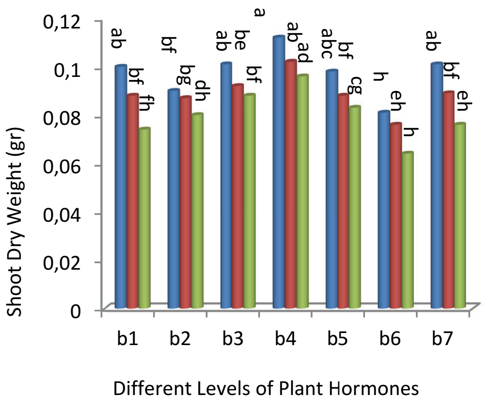

Figure 7. Interaction of salinity and plant hormones with shoot dry weight

Root Length

According to the ANOVA table different levels of salinity and plant hormones influenced the root length significantly at p≤1% (Table 1). With increasing salinity this trait decreased significantly so that the greatest root length and the least root length were obtained from the Check (distilled water) with 14.80 mm and 0.8 ds/m saline stress with 12.54 mm, respectively (Figure 8). Niu et al. (1995) believe that after or simultaneous with water uptake a series of hormones and certain important enzymes inside the seed including lipaz, proteaz, amylaz, etc. are secreted that decompose the stored food stuffs including starch and dissolve them in water. Thus, the energy necessary for the emergence and growth of root and shoot is supplied which in high levels of salinity the activity mechanisms inside the seed are changed and these processes get disrupted and with growth cessation and decrease due to lack of transfer of nutrients from cotyledon to root and shoot of seedling their dry weight reduces. The growth retardation by saline stress has been reported by other researchers. For instance, Soltani et al. (2001) reported that saline stress reduced the growth of root and shoot of pea and with increasing salinity level the growth reduced more.

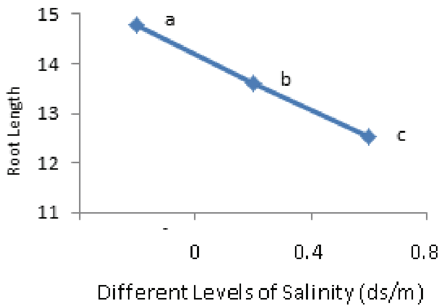

Figure 8. Effects of salinity on root length

According to means comparison of plant hormones the foliar application of CK at pollination and grain filling with an average of 16.14 mm had the greatest root length that increased by 13.18% relative to the check.
The ABA foliar application at pollination and grain filling decreased root length relative to the check through its preventive effect so that it had the least root length with 11.62 mm (Figure 9).

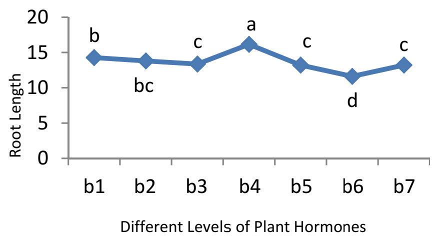

Figure 9. Effects of plant hormones on root length

Shoot Length

Results of analysis of variance showed that among different levels of salinity, different levels of plant hormones (p≤1%) and their interactions with shoot length (p≤5%) there wasn't significant difference (Table 1). Mean comparison of treatments interaction showed that with increasing salinity level the shoot length at different levels of ABA and CK sprays decreased during maternal plant growth stages. In the treatment of 0 ds/m salinity (distilled water) the foliar application of CK at pollination and grain filling with 17.47 cm had the greatest shoot length that increased by 13.44% relative to the check. The least shoot length belonged to the ABA foliar application at pollination and grain filling together with 0.8

ds/m salinity and a shoot length of 10.43 cm that reduced by 32.33% relative to the check (Figure 10).

Of factors causing shoot length to reduce under saline conditions, decrease in or lack of transfer of nutrients from cotyledons to the nucleus have been reported. Besides, decrease in water uptake by the seed in saline conditions leads to decrease in enzymes activity and secretion of plant hormones and eventually disruption in growth of root and shoot (Kafi et al., 2006).

Apparently, the shoot length is associated with seed vigor and sufficient nutrients supply. Jamil et al. (2006) reported that increase in salinity level results in decrease in % germination and % germination velocity, root and shoot length and root and shoot dry weights of canola.

According to the above-mentioned results it seems that increase in salinity decreased the wheat seedlings height significantly through disrupting the shoot function.

According to Stavir et al. (1998) findings it is reasoned that harmful effects of saline stress on plant growth and amylase activities can be reversed through adding synthetic kinetin and ABA to the growth medium of pea seeds. These compounds neutralize the saline effects and improve seedlings growth via strengthening or enhancing starch metabolism and amylase activity.

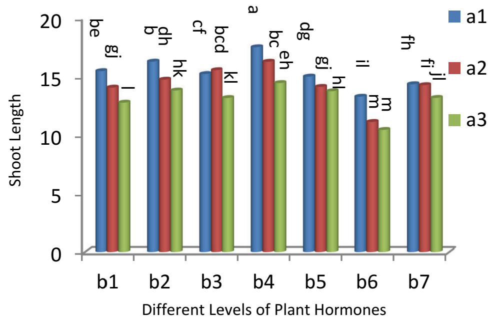

Figure 10. Interaction of plant hormones and salinity with shoot length

CONCLUSIONS

Utilization of with high vigor and establishment potential on the field is of great importance. In the present experiment maternal treatments foliarly sprayed with CK had greater seed quality which can be a new method of seed production on fields of maternal plants. In the present study the Zarrin cultivars of wheat were used which is susceptible to draught and saline stresses. The application of this technique promoted the germination quality of the CK-treated treatments in the above mentioned cultivar relative to different levels of salinity being promising in the future. Of course, fidelity of this method requires continuous studies which the author is pursuing it. Another strong point of this experiment is the significance of the interaction of CK and ABA which indicates that increase in CK concentration under water stress conditions regulates the effects of ABA which, in turn, increases plant establishment of the field.

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