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Original Article

Studies on Effectiveness of Plant Phytohormones in Reduction of Salinity Effects on Germination of Some Cultivar of Spring Wheat

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Objective: In order to investigate effects of different concentration of gibberlic acid (GA3), kentin and Putrescinepoly amine, on germination of three cultivars of wheat under salinity stress. Methods: The experiment was carried out as factorial based on completely randomized design with four replicates in laboratory of Agronomy and Plant Breeding, department of agriculture in Islamic Azad University, Varamin-Pishva branch in 2014. The first factor was four levels of salinity including S1: 3.78, S2: 5.95, S3: 10.25 and S4: 12.87 ds/m, the second factor was three cultivar of spring wheat including V1: Tajan, V2: Arg and V3: Pishtaz. The third factor was six level of hormone including H0: control, H1: GA3 150 mg/L, H2: Kentin 150 mg/L, H3: GA3 150 mg/L + Kentin 150 mg/L, H4: Putrescine 2.5 mmol/L, H5: GA3 150 mg/L + Kentin 150 mg/L + Putrescine 2.5 mmol/L. Coleoptile and Cleophiza length. Cleophiza number, Coleoptile, Cleophiza and Seedling Dry Weight, Tissue Water Content (TWC), Germination Percent and Number of Damaged Seeds were measured. Results: Analysis of variance showed that Coleoptile and cleorhiza length, cleorhiza number, seedling dry weight and TWC affected by salinity, hormone and Cultivar at 1% of probability level, but not significant in other properties. In general, results of this experiment showed that application of different treatments of hormone and poly amine had no significant effect on reduction of salinity effect in germination process.

1.INTRODUCTION

Salinity of soil and water irrigation is one of the most agents in limitation of agricultural products. Because of low toleration of crop plant to salinity stress and lack of enough data for toleration mechanisms against salinity stress, a large number of areas in the word areout of usage (Merchan *et al.*, 2007). Perivous studies classified wheat plant as semi- susceptible to semi- tolerated to salinity stress (Kirkham *et al.*, 1974). Reduction of wheat yield under environmental stresses is very significant. Responces of plant to these stresses depends on different causes like kind, time and severity of stress, developmental stage and time of occurrence (Argueso *et al.*, 2009). Hence, stress make disorders and changes in plant activity, therefore these stresses maybe were as the means for biochemical and physiological studies in plant (Grattan and Grieve, 2000). Salinity stress affected growth and metabolisms of plant in different ways. There are positive and significant relation between salinity and water limitation. Plants which exposed to salinity, besides osmosis effect of salinity which disturbed water relationships in plant, affected by special effect of salinity, effect of ions on cell metabolism and sometime toxicity of ion accumulation (Barret Lenard *et al.*, 1999). The ways which made plant compatible to stresses like

salinity, drought, cool and heat, affect the growth and improvement of plant. In response to these stress, plants make compatible with different mechanisms like changing morphologically, improvement pattern and physiological and biochemical responses (Sarapatka et al., 2004). Hormone balance under salinity stress, in turn, controls improvement shapes of plant. All of plant hormone affected by osmosis stresses, but the present data supported this hypothesis that absicic acid, cytokinines and ethylene participate in the most opposite reactions and control water balance, while the roll of Indoleasetic and gibberlic acid was low (Mizrahi et al., 1971; Tsonev et al., 2000). It is reported that cytokinines and gibberlic acid content decreased in salinity, while absicic acid increased, which caused changes in membrane permeability and absorption following changing in endogenous hormone content (Hare et al., 1997).

1.1.Tajan Wheat

Tajan cultivar (pedigree: Bow S / Nkt S) obtained from International Center of Wheat and Maize Breeding (CIMMYT). Potential of yield, resistance against yellow and brown rust, spike fusarium and toleration to germination on the spike made it a suitable cultivar for cultivation in valley area of Khazarbanks. Tajan with recorded yield of 7.3 Ton/ha and average yield of 6.3 ton/ha was superior to Falat cultivar up to 18%. The seeds of Tajan were solid and brown. Tahjan with Protein of 12% had well quality for baking different kinds of bread. 90-95 cm height made it resistance to lodging. Early maturity and toleration to spike germination were reported for Tajan cultivar, also. Wheat Arg Cultivar: Arg cultivar is new, tolerated to salinity cultivar which is appropriated in salty soil and moderated climate. Arg cultivar with 1-66-22/Iniapedigree is hybrid between Inia wheat, symit originated with well quality, as male parent and salinity tolerated line 1-66-22 as female parent. The average yield of Arg during compatibility experiments was 5.470 tone/ha in salinity stress (Ec_{water}=8-12ds/m ,Ecs_{oil}=9-14ds/m). The yield of this cultivar increased 820, 971 and 508 kg to Kavir, Roshan and Bam, respectively and 766 kg (16.3%) to control. In research experiment- adapting and researchingextension, the Arg cultivar was superior against seed yield of control cultivar of salty moderated area including Bam, Kavir, Roshan and Neishaboori with yield average of 3.95 and 4.232 Ton/ha and 16 and 13%, respectively. High yield, well compatibility in moderated climate with salty water and soil, resistance to lodging, seed abscission and high quality are Arg cultivar properties. This cultivar had significant revolution in wheat production in salty water and soil parts.

1.2. Pishtaz Wheat

Pishtaz cultivar obtained by crossing an improved line (1-27-6275/Cf1770), which later named Alvand, with resistance to yellow rust line, A1dan/Ias58, which originated from Brazil, in 1998 Selection steps of Pishatz were implemented in two generation in year, between Iranshahr and Clardasht station. Resistance to disease and favorable baking quality, made Pishtaz named and introduced in 2004. Pishtaz had the highest yield, so that the average yield of 7396 kg and record of 9646 kg/ha reported for this cultivar.

2. MATERIALS AND METHODS

The experiment was conducted in laboratory of Agronomy and Plant Breeding, department of agriculture in Islamic Azad University, Varamin-Pishva branch in 2014. The experiment was carried out as factorial based on completely randomized design with four replicates. The first factor was four levels of salinity including S1: 3.78, S2: 5.95, S3: 10.25 and S4: 12.87 ds/m, the second factor was three cultivar of spring wheat including V1: Tajan, V2: Arg and V3: Pishtaz. The third factor was six level of hormone including H0: control, H1: GA3 150 mg/L, H2: Kentin 150 mg/L, H3: GA3 150 mg/L + Kentin 150 mg/L, H4: Putrescine 2.5 mmol/L, H5: GA3 150 mg/L + Kentin 150 mg/L + Putrescine 2.5 mmol/L.

2.1. Germination Experiment

After weighting the above hormone, they poured in container volume: 50 ml. GA3 and Kin solved in NaOH (1N). An aliquot of control water (obtained from well) poured to each hormone treatments to reach the interested volume. Different levels of salinity were obtained from agricultural wells of Gomrod, in Gome Province. 10 seeds of Tajan cultivar were placed in Petri dishes which covered with filter paper, regularly. Then 10 ml of the treatments added to each Petri dishes. To avoid respiration and transpiration, the door of Petri dishes covered with parafilm. The samples were transferred to germinator without any light in 20 °C and 50% humidity. 7 days later, the length of coleoptile and cleorhiza, number of radicle and length of longest radicle and plumule measured. Then the coleoptile and cleorhiza of each petri placed in separated paper and placed in the pocket and all information of Petri written of it. On each pocket, the fresh weight (FW) of coleoptile and cleorhiza added, also. Then two papers pressed to each other and all samples transferred to oven at 70 °C for 48 h, then the dry weight (DW) of them measured. The cultured seeds were maintained at 25±1 and absolutely no light. After 7 days, number of germinated seeds, radicle and plumule length, coleoptile length, fresh and dry weight of seedlings measured. Tissue Water Content calculated using the following formula: TWC=((FW-DW)/FW)×100. The collected data analyzed using SPSS, SAS and MSTAT-C softwares. The means were separated using Duncan Multiple Range Tests at 5% of probability level.

3. RESULTS

3.1. Coleoptile Length

Analysis of variance showed that coleoptile length affected by main effects of salinity, cultivar and hormone at 1% of probability level (Table 1). Also, the effect of salinity× cultivar, hormone× cultivar, salinity× hormone and hormone× cultivar× salinity was significant at 1% of probability level (Table 1). The longest coleoptile (6.7 cm) was in salinity of 3.87 ds/m, while the shortest was in 10.25 ds/m with 4.04 cm (Table 2). Pishtaz had the longest coleoptile with 5.12 cm, and the shortest was seen in Tajan with 4.49 cm (Table 3). The longest coleoptile was in GA3 150 mg/L with 5.51 cm, while the shortest was in Kin 150 mg/L with 4.27 cm (Table 4). The shortest coleoptilewas in Tajan treated in 12.87 ds/m of salinity with 3.48 cm, while the longest was

obsereved in Arg and salinity of 3.87 ds/m with 7.38 cm. The shortest coleoptile was in application of Kin 150 mg/L and salinity of 10.25 ds/m with 3.32 cm, and the longest was in salinity of 3.87 and GA3 150 mg/L with 8.35 cm. The shortest coleoptile was in Tajan treated with GA3+Kin 150 mg/L with 3.55 cm, while the longest was in Pishtaz treated with GA3 150 mg/L with 6.28 cm. The longest coleoptile was in Pishtaz treated with GA3 150 mg/L with 6.28 cm. The longest coleoptile was in Pishtaz treated with GA3 150 mg/L with 6.28 cm. The longest coleoptile was in Pishtaz treated with GA3 150 mg/L and salinity of 3.87 ds/m with 9.04 cm. The shortest coleoptile was in salinity of 12.87 ds/m and Kin 150 mg/L with 2.84 cm. These results were in agreement with (Abdullah and Ahmad, 1990; Afzal *et al.*, 2006; Akman, 2009).

Analysis of variance for studied traits of wheat variety under salt stress										
S.O.V.	DF	Length	Length	Number	Dried weight	Dried weight	Dried weight	Interstitia l water	Percentage of germination	Percentage of Damage seeds
		Coleoptil	Coleorhiz a	Coleorhiz a	of Coleorhiz a	of Coleopti l	of seedling s	seedlings	germinition	
Salinity	3	124.503**	12.428**	0.294ns	0.000ns	0.011ns	0.014*	610.851*	159.944ns	1.569ns
variety	2	9.550**	16.525**	5.731**	0.000ns	0.019*	0.025**	410.724	62.847ns	0.628ns
Hormone	5	12.944**	270.367**	34.757**	0.008**	0.001ns	0.008ns	899.556**	1677.222**	161.772**
Salinity. variety	6	4.916**	3.125**	0.076ns	0.001ns	0.007ns	0.007ns	200.130ns	243.403ns	2.433ns
Salinity . Hormone	15	6.141**	12.850**	0.731**	0.001ns	0.005ns	0.008*	349.143**	231.667*	2.317*
Variety. Hormone	10	2.057**	4.521**	1.394**	0.001ns	0.003ns	0.004ns	72.251ns	95.347ns	0.953ns
Salinity. Variety. Hormone	30	1.692**	1.609**	0.701**	0.001ns	0.003ns	0.005ns	208.335ns	188.125ns	1.881ns
Error	14 8	0.345	0.463	0.223	0.000	0.004	0.004	162.492	136.343	1.363
%CV	-	10	12	15	9	8	7	10	13	14

Table 1.

^{ns}: Non significant, ** and * significant at 0.01 and 0.05 probability levels, respectively.

Table 2.

Means comparison for studied traits

S.O.V.	Length of Coleoptil (cm2)	Length of Coleorhiza (cm2)	Dried weight of seedlings (g)	Interstitial water content of seedlings
Salinity3.87(ds.m)	6.70a	2.41c	0.11a	86.24a
Salinity5.95(ds.m)	4.64b	3.36a	0.08b	83.88a
Salinity10.25(ds.m)	4.04c	2.78b	0.09b	79.23b
Salinity12.87(ds.m)	3.80d	2.56ab	0.10ab	83.22ab

3.2. Cleorhiza Length

Analysis of variance showed that cleorhiza length affected by main effect of salinity, cultivar and exogenous application of hormone and their two-fold and three-fold opposite effects at 1% of probability level (Table 1). The longest cleorhiza was in salinity of 5.95 ds/m with 3.36 cm, while the shortest was in 3.87 ds/m with 2.41 cm (Table 2). The longest cleorhiza was in Tajan with 3.87 ds/m, while the shortest was in Arg with 2.43 cm (Table 3). The longest cleorhiza was in not application of hormone with 6.69 cm. and the shortest was in Kin 150 mg/L with 0.62 cm (Table 4). The longest cleorhizawas in salinity of 5.95 ds/m in Tajan with 3.78 cm, while the shortest was in Pishtaz and salinity of 3.87 ds/m with 1.78 cm. The longest cleorhiza was in not application of hormone and salinity of 5.95 ds/m with 8.62 cm, while the shortest was in Kin 150 mg/L and salinity of 5.95 ds/m with 0.5 cm. The longest cleorhiza was in Tajan and not application of hormone with 7.9 cm, and the shortest was in Pishtaz treated with Kin 150 mg/L with 0.49 cm. The longest cleorhiza was in Tajan, salinity of 5.95 ds/m and not application of hormone with 9.7 cm, while the shortest was in Pishtaz, salinity of 5.95 ds/m and Kin 150 mg/L with 0.37 cm. These results were in agreement with (Argueso et al., 2009; Ashraf, 2004, Afzal et al., 2005).

3.3. Cleorhiza Number

Analysis of varianceindicated thatcleorhiza number affected by cultivar, hormone and their two-fold and three-fold opposite effects at 1% of probability level (Table 1). Cleorhiza number not affected by salinity and salinity× cultivar treatments (Table 1). The most number was in Arg with 4.26 and the lowest was in Tajan with 3.77 Table 3). The most and lowest number of cleorhiza was in not application of hormone and GA3+Kin 150 mg/L with 4.79 and 3.17 (Table 4). The most number of cleorhiza was in not application of hormone and salinity of 3.87 ds/m with 5.1, while the lowest was in GA3+ Kin 150 mg/L and salinity of 3.87 ds/m with 2.9. The most number of cleorhiza was in Pishtaz treated with Putrescine 2.5 mmol/L with 5.1, while the lowest was in Tajantreated with Putrescine 2.5 mmol/L with 2.52. The lowest cleorhiza number was in salinity of 3.87 ds/m, in Tajan and GA3+ Kin 150 mg/L with 1.1. The most number of cleorhiza was in salinity of 12.87 ds/m, in Pishtaz and Putrescine 2.5 mmol/L with 5.4. These results were in agreement with (Chakrabarti and Mukherji, 2003; Blumwald, 2000; Alonso-Ramirez et al., 2009).

Means comparison for studied traits.									
S.O.V.	Length	Length	Number	Dried weight	Dried weight				
	of Coleoptil	of Coleorhiza	of Coleorhiza	of Coleoptil	of seedlings(g)				
	(cm2)	(cm2)		(g)					
Variety(Taian)	4.49c	3.24a	3.77c	0.05b	0.08b				
Variety(Arg)	4.77b	2.43c	4.26a	0.06ab	0.10ab				
Variety(pishaz)	5.12a	2.67b	4.08b	0.07a	0.11a				

	Tab	le 3.
	Means comparison	n for studied trait
Length	Length	Number

S.O.V.	Length	Length	Number	Dried weight	Interstitial water	Percentage of	Percentage of Damage
	of Coleoptil	of Coleorhiza	of Coleorhiza	of	content of seedlings	germination	seeds
	(cm2)	(cm2)		Coleorhiza (g)	<u>-</u>		
Hormone(0)	5.36a	6.69a	4.79a	0.05a	89.26a	91.67a	0.83c
Hormone(Ga150mg.l)	5.51a	3.67b	4.77a	0.04a	84.25ab	90.42ab	0.96bc
Hormone(Kin150mg.l)	4.27d	0.62d	3.21c	0.02b	75.90c	85.62b	1.44b
Hormone(Ga150&Kin150mg.l)	4.37cd	0.78d	3.17c	0.02b	83.88ab	80.42c	1.96a
Hormone(Putrescine2.5mmol)	4.68b	3.71b	4.86a	0.04a	83.74ab	90.00ab	1.00bc
Hormone(Ga150mg.l&Kin150mg.l&Putrescine2.5mmol)	4.58bc	1.19c	3.41b	0.02b	81.85b	77.29c	2.27a

Table 4.

3.4. Cleorhiza Dry Weight

cleorhiza was affected by hormone treatment at 1% of probability level, while the effect of salinity, cultivar and their opposite effects werenot significant (Table 1). The highest cleorhiza dry weight was in not application of hormone with 0.05 g, and the lowest was in Kin 150 mg/L, GA3+Kin 150 mg/L and GA3+Kin 150 mg/L+ Putrescine2.5 mmol/L with 0.02 g (Table 4). These results were in agreement with (Davies, 2004; Chartzoulakis and Klapaki. 2000).

3.5. Coleoptile Dry Weight

Coleoptile dry weight was not affected by salinity, hormone and their opposite effects, while affected by cultivar treatments at 5% of probability level (Table 1). Pishtaz had the highest dry weight with 0.07 g, and Tajan had the lowest with 0.05 g (Table 3). These results were in agreement with (Iqbal and Ashraf, 2005; Dewey, 1962; Gul and Khan, 2008).

3.6. Seedling Dry Weight

Analysis of variance showed that Seedling dry weight was affected by salinity (P<0.05), cultivar (P<0.01), salinity× hormone (P<0.05) (Table 1). The highest of seedling dry weightwas in salinity of 3.87 ds/m with 0.11 g, and the lowest was in salinity of 5.95 ds/m with 0.08 g (Table 2). The highest dry weight was in salinity of 3.87 ds/m and Putrescine 2.5 mmol/L with 0.16 g, while the lowest was in salinity of 10.25 ds/m and GA3+Kin 150 mg/L + Putrescine2.5 mmol/L with 0.06 g. The above results were in agreement with (Iqbal *et al.*, 2006).

3.7. Tissue Water Content (TWC)

Analysis of variance showed that TWC was affected by salinity (P<0.05), hormone (P<0.01) and salinity × hormone (P<0.01) (Table 1). The highest content of TWC was in salinity of 3.87 ds/m with 86.240 while the lowest was in salinity of 10.25 ds/m with 79.23 (Table 2). The highest of TWC was in not application of hormone with 89.26 and the lowest was in Kin 150 mg/L with 75.9 (Table 4). The highest TWC was in not application of hormone and salinity of 10.25 ds/m with 90.35, and the lowest was in Kin 150 mg/L and salinity of 10.25 ds/m with 58.59. These results were in agreement with (Kirkham *et al.*, 1974; Ismail, 2003).

3.8. Germination Percent

Analysis of variance showed that germination percent was affected by hormone (P<0.01) and salinity× hormone (P<0.05) (Table 1). It was no significant difference among salinity, cultivar, salinity× cultivar, cultivar× hormone and salinity× cultivar× hormone (Table 1). The highest germination was in not application of hormone with 91.67%, while the lowest was in GA3+Kin 150 mg/L+ Putrescine2.5 mmol/L with 77.29% (Table 4). The highest germination was in not application of hormone and salinity of 10.25 ds/m with 93.33%, while the lowest was in salinity of 10.25 ds/m and GA3+Kin 150 mg/L+ Putrescine2.5 mmol/L with 68.32%. These results were in agreement with [27]. .Damaged seeds: Analysis of variance showed that damaged seeds affected by hormone (P<0.01) and salinity × hormone (P<0.05). It was no significant difference among salinity, cultivar, salinity× cultivar, cultivar× hormone and salinity× cultivar× hormone (Table 1).The highest damaged seeds were in GA3+Kin 150 mg/L+ Putrescine 2.5 mmol/L with 2.27, while the lowest was in not application of hormone with 0.83 (Table 1). The highest

damaged seeds were in salinity of 10.25 ds/m and GA3+Kin 150 mg/L+ Putrescine 2.5 mmol/L with 3.1 and the lowest was in salinity of 10.25 ds/m and not application of hormone with 0.66. These results were in agreement with (Boucaud and Ungar, 1976).

4. DISCUSSION

In this paper salinity stress from chemical stress was investigated. Hence, salinity stress caused drought stress in plant naturally, so they investigated together. Salinity caused physiological drought in plant. The literatures showed that plants have precise mechanisms which defense themselves. When an agent threat plant survival, plants defense themselves with different mechanisms including decreasing plant life cycle, changing in aleometry ratio, phylotaxy, Albedo coefficient, retransformation, changing in concentration of endogenous hormone, osmotic add justmen and etc. The results of this experiment indicated that even application of exogenous treatment with hormone and poly amine, no significant reduction in salinity effect in germination process of wheat seeds was not observed and salinity had similar effect on three cultivars, even in the presence of above treatments.

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