VOL. 10, NO. 9, SEPTEMBER 2015 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

www.arpnjournals.com

KINETIN AND ABSCISIC ACID EFFECTS ON SEED GERMINATION AND SEEDLINGS GROWTH OF MAIZE (*Zea mays* L.) UNDER SALT STRESS CONDITION

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ABSTRACT

Soil salinity severely affected establishment of seedlings at early growth stages of crop plants. Therefore, high germination rate and vigorous early growth under salty soils is preferred. In this study germination and seedling growth of a maize (*Zea mays* L.) cultivar was assessed using three replicates of 50 seeds in a factorial laid out in two separate experiments as Completely Randomized Design (CRD) testing combinations of three levels of salinity (0, 80, 160 and 240 mMol NaCl Lit) and four levels of kinetin (0, 10, 20 and 30 m mMol Lit) in the first experiment and the same salinity levels with three levels of abscisic acid (0, 2, 4 and 6 m Mol Lit) in the second experiment. Germination percentage was reduced by salinity compared to non-salinity condition. Salinity decreased germination percentage to about 15, 43 and 89% of controls. Kinetin increased germination percentage to 10 m gr Lit, but applying more decrease it. ABA decreased germination percentage to about 44% in 6 m Mol than control. On average at 80, 160 and 240 mMol NaCl salinity germination rate was about 80, 400 and 700 %, respectively of control. Kinetin and ABA decreased germination rate. When seeds were in 160 and 240 m M NaCl kinetin significantly improved germination. Priming with kinetin and ABA decreased radicle length and hypocotyl length relatively. Priming with kinetin could not improve radicle and hypocotyl length in all salinity levels. Dry weight of radicle and hypocotyls decreased significantly under salinity condition compared with non salinity condition. Priming with 10 m Mol Lit kinetin showed maximum radicle and hypocotyl length and higher amount decreased both traits. ABA decreased seedling fresh and dry weight to about 10 and 12% in 4 m Mol Lit than control. In general, Increasing of kinetin and ABA levels more than 10 and 2 m Mol Lit caused decrease in all traits under salinity condition.

Keywords: maize (*Zea mays* L.), kinetin, abscisic acid, germination rate, seedling weight, radicle, hypocotyl.

INTRODUCTION

More than 800 million hectares of land throughout the world are salt-affected (including both saline and sodic soils), equating to more than 6% of the world's total land area (FAO, 2013). Furthermore there is also a dangerous trend of a 10% per year increase in the saline area throughout the world. Iran is one of the countries that suffer from sever salinity problems. For example 18 million ha or 10% of total land area in Iran is salinity or sodicity soil (Rogers *et al*., 1995).

The germination of seeds is one of the most crucial and decisive phases in the growth cycle of plant species since it determines plant establishment and final yield of the crops. Poor germination and seedling establishment are the results of soil salinity. It is an enormous problem adversely affecting growth and development of crop plants and results in to low agricultural production (Garg and Gupta, 1997). Therefore, any treatment which could be used to improve seed germination and subsequent seedling establishment under saline conditions would be highly desirable. Pre-sowing seed treatments have been shown to enhance stand establishment in non-saline areas (Khan, 1992) and have potential in saline areas as well (Ashraf and Rauf, 2001; Basra *et al*., 2005). Prior to selecting these alternatives, it seems necessary to examine seed vigor enhancement techniques leading to better and synchronized stand establishment under stress conditions. Physiological treatments to improve seed germination and seedling emergence under various stress conditions have been intensively investigated in the past two decades (Bradford, 1986). It is thought that the depressive effect of salinity on germination could be related to a decline in endogenous levels of hormones (Debez *et al*., 2001). However, presoaking seeds with optimal concentration of phytohormones has been shown to be beneficial to growth and yield of some crop species growth under saline conditions by increasing nutrient reserves through increased physiological activities and root proliferation (Singh and Dara, 1971). Concerted attempts have been made to mitigate the harmful effects of salinity by application of plant growth regulators (Datta *et al.*, 1998). Thus the detrimental effects of high salts on the early growth of wheat seedlings may be reduced to some extent by treating seeds with the proper concentration of a suitable hormone (Darra *et al.*, 1973).

Abscisic acid (ABA) has been proposed to act as a mediator in plant responses to a range of stresses, including drought and salt stress. ABA is also the major internal signal enabling plants to survive adverse environmental conditions such as salt stress (Cabot *et al*., 2009). Keksin *et al*., (2010) reported that the MAPK4-like, TIP1 and GLP1 genes were induced much faster in response to ABA treatment in wheat. This result could be evidence for the possible role of these genes in the ABA-

induced pathways. Thus, in many ways ABA plays vital roles in whole plant responses to salt stress.

Kinetin regulate several plant growth aspects and developmental processes, including cell division, apical dominance, chloroplast biogenesis, nutrient mobilization, leaf senescence, vascular differentiation, photomorphogenic development, shoot differentiation and anthocyanin production (Mok and Mok, 2001; Davies, 2004). Seed enhancement (seed priming) with kinetin is reported to increase plant salt tolerance (Iqbal *et al*., 2006). Kinetin receptor genes of other species are regulated by changes in the osmotic conditions as well, indicating that their function in the osmotic stress response might be common although mechanistically not well understood (Merchan *et al*., 2007).

Seed germination and stand establishment in wheat farms is very often poor due to high level of salinity of irrigation water in Iran. Therefore, the aim of this experiment was to study the effect of seed pre-sowing treatment with kinetin and ABA on germination and seedling growth of a wheat cultivar under salinity stress conditions.

MATERIAL AND METHODS

Plant material: The experiment carried out in Islamic Azad University of Ramhormoz, Khuzestan, Iran in March 2012. Similar seed size and weight of a hybrid maize (*Zea mays* L.) cultivar (Single Cross 704) was selected to exclude effect of that on the seedling establishment. Seeds were surface sterilized in 1.5% (v/v) sodium hypochloride for 10 min and thoroughly washed with sterile tap water. Seeds were germinated in covered, sterilized, disposal pertri dishes containing Whatman No. 1 filter paper moistened with either distilled water (control), or different treatment solutions. Germination was assessed using three replicates of 20 seeds in a factorial laid out in two separate experiments as Completely Randomized Design (CRD) testing combinations of three levels of salinity (0, 80, 160 and 240 mMol NaCl Lit) and four levels of kinetin (0, 10, 20 and 30 m mMol Lit) in the first experiment and the same salinity levels with three levels of abscisic acid (0, 2, 4 and 6 m Mol Lit) in the second experiment. The seeds were kept for 6 hours in the kinetin and ABA solutions, after which the solution was eliminated and the seeds were dried lightly by depositing them on filter paper that absorbed most of the solution left on the seeds, and then finally they were deposited in separate petri dishes between two filter papers.

Growth conditions: Seeds were incubated in a growth chamber (Type 8194, VINDON) and were considered germinated with the emergence of the radical. Temperature was maintained during the 10-d duration of the germination tests at 25ºC (±0.5). In order to maintain adequate moisture, 5 mL of the original salt solutions were added to each petri dish every three days. Germination was scored when a 2 mm radical emerged from the seed coat. Every three days, the germinated seeds were removed from the petri dishes. The seeds to germinate in each replicate were retained for measurements of radical and hypocotyl lengths at the end of the experiment. After 240 h, final germination percentages were recorded and seedling fresh weight immediately determined. To determine the impact of the treatments on seed germination, all seedlings were separated from the remaining seeds. Seedlings were harvested after ten days and washed with deionized water after harvest. Five washed seedlings from each replication were separated into root and shoot for the determination of their fresh and dry weight. Dry weight was determined after oven drying the samples at 65ºC. Stem diameter was measured above the first real leaf by using caliper ruler with 0.001 mm.

Growth parameters: Germination percentage, germination rate, radicle and hypocotyl length, seedling fresh and dry weight, radicle and hypocotyls dry weight and total dry weight were measured.

Germination rate: A germination index was calculated for each subpopulation as GR:

$$GR = X1/Y1+(X2-X1)/Y2+\ldots+ (Xn-Xn-1)/Yn$$

Where Xn is the germination percentage on n^{th} day and Yn in the number of day from first day experiment (Mguire, 1962.).

Data analysis: Data were analyzed using the GLM procedure of SAS program (SAS Institute, 2001). Significant differences between treatments were determined using Duncans multiple range test at 0.05 level.

RESULTS AND DISCUSSIONS

Germination percentage. Variance analysis results of germination percentage are shown in Tables 1 and 2. According to Tables 1 and 2, salinity ($P<0.01$), kinetin ($P<0.05$) and abscisic acid ($P<0.01$) affected germination percentage significantly. However, there was no significant interaction between kinetin and abscisic acid to salinity (Tables 1 and 2). Germination percentage was reduced by salinity compared to non-salinity condition. Salinity decreased germination percentage to about 15, 43 and 89% of controls (Table-3). Kinetin increased germination percentage to 10 m gr Lit, but applying more decrease it. ABA decreased germination percentage to about 44% in 6 m Mol than control (Table-3). In rice, cellular division in endosperm is closely related with endogenous concentration of cytokinins and exogenous application of kinetin can increase the number of endosperm cells (Warner *et al*., 2010).

Table-1. Analysis of variance for the traits investigated in a maize cultivar in response to salinity stress and kinetin.

Sources of variation	df	Germination percentage	Germination rate	Radicle length	Plumule length	Radicle dry weight	Plumule dry weight	Seedling fresh weight	Seedling dry weight
Salt stress	3	**	**	*	*	**	**	**	**
Kinetin	3	*	*	**	**	*	*	*	*
S. stress ×kinetin	9	ns	*	**	**	ns	ns	ns	ns

ns Non- significant, * Significantly at $p < 0.05$, ** Significantly at $p < 0.01$.

Table-2. Analysis of variance for the traits investigated in a maize cultivar in response to salinity stress and abscisic acid.

Sources of variation	df	Germination percentage	Germination rate	Radicle length	Plumule length	Radicle dry weight	Plumule dry weight	Seedling fresh weight	Seedling dry weight
Salt stress	3	**	**	*	*	**	**	**	**
ABA	3	**	**	**	**	**	**	**	**
S. stress ×ABA	9	ns	**	**	**	**	**	**	**

ns Non- significant, * Significantly at $p < 0.05$, ** Significantly at $p < 0.01$.

Table-3. Mean values of characters measured at germination and seedling growth stages of seed treated with kenitin and ABA under NaCl stress in a maize cultivar.

Treatment	Germination percentage	Germination rate	Radicle length (mm)	Plumule length (mm)	Radicle dry weight (mg)	Plumule dry weight (mg)	Seedling fresh weight (mg)	Seedling dry weight (mg)
Salinity level mMol NaCl								
0	90.17 a	8.32 a	112.12 a	69.39 a	25.32 a	28.25 a	280.26 a	53.52 a
80	79.83 b	5.14 b	88.65 b	51.17 b	21.41 b	20.39 a	246.67 b	41.64 a
160	63.18 c	1.78 c	31.24 c	29.18 c	15.12 c	12.87 b	184.57 c	27.58 b
240	48.24 d	0.12 d	16.63 d	13.54 d	6.45 d	6.62 c	83.87 d	12.19 c
Kenitin mMol lit								
0	85.24 ab	7.54 a	116.12 a	70.89 a	25.25 b	29.54 b	284.27 b	53.38 b
10	89.16 a	5.12 b	90.45 b	67.11 b	27.17 a	31.62 a	290.51 a	56.47 a
20	80.04 b	4.32 c	61.23 c	65.11 b	24.52 c	27.89 c	279.46 c	49.23 c
30	80.28 b	4.16 c	59.24 c	60.05 c	23.86 c	27.21 c	278.52 c	48.29 c
abscisic acid mMol lit								
0	91.52 a	7.40 a	108.21 a	106.62 c	21.61 a	22.54 a	261.43 a	52.32 a
2	82.24 b	4.29 b	88.18 b	78.44 b	16.49 b	14.42 a	258.19 a	51.62 a
4	69.61 c	2.12 c	74.26 c	65.61 a	13.18 c	11.58 b	239.57 b	47.83 b
6	65.44 c	1.97 c	74.19 c	58.26 c	12.26 c	9.61 b	239.41 b	46.84 b

Means with similar letters in each column are not significantly different at 5 % level of probability (Duncan).

Table-4. Mean values of characters measured at germination and seedling growth stages of seed treated with interaction of ABA and NaCl stress in a maize cultivar.

Treatments		Germination rate	Radicle length (mm)	Plumule length (mm)	Radicle dry weight (mg)	Plumule dry weight (mg)	Seedling fresh weight (mg)	Seedling dry weight (mg)
Salinity level mMol NaCl	abscisic acid mMol lit							
	0	8.32 a	112.12 a	69.39 a	25.32 a	28.25 a	280.26 a	53.52 a
0	2	5.14 b	88.65 b	51.17 b	21.41 b	20.39 a	246.67 b	41.64 a
	4	1.78 c	31.24 c	29.18 c	15.12 c	12.87 b	184.57 c	27.58 b
	6	0.12 d	16.63 d	13.54 d	6.45 d	6.62 c	83.87 d	12.19 c
	0	7.54 a	116.12 a	70.89 a	25.25 b	29.54 b	284.27 b	53.38 b
80	2	5.12 b	90.45 b	67.11 b	27.17 a	31.62 a	290.51 a	56.47 a
	4	4.32 c	61.23 c	65.11 b	24.52 c	27.89 c	279.46 c	49.23 c
	6	4.16 c	59.24 c	60.05 c	23.86 c	27.21 c	278.52 c	48.29 c
	0	7.40 a	108.21 a	106.62 c	21.61 a	22.54 a	261.43 a	52.32 a
160	2	4.29 b	88.18 b	78.44 b	16.49 b	14.42 a	258.19 a	51.62 a
	4	2.12 c	74.26 c	65.61 a	13.18 c	11.58 b	239.57 b	47.83 b
	6	1.97 c	74.19 c	58.26 a	12.26 c	9.61 b	239.41 b	46.84 b
	0	3.76 a	62.42 a	60.08 c	14.02 a	14.58 a	172.6 a	32.25 a
240	2	2.20 b	52.40 b	4599 b	11.47 b	10.52 a	170.01 a	31.90 a
	4	112 c	45.44 c	39.50 a	9.81 c	9.1 b	161.60 b	29.99 b
	6	1.45 c	45.41 c	35.90 a	9.35 c	8.11 b	161.59 b	29.51 b

Germination rate: The data Tables 1 and 2 indicated that there is significant difference in salinity levels, kinetin, ABA and interaction effect of these two hormones and salinity. On average at 80, 160 and 240 mMol NaCl salinity germination rate was about 80, 400 and 700 %, respectively of control Table-3. Kinetin and ABA decreased germination rate Table-3. When seeds were in 160 and 240 mMNaCl kinetin significantly improved germination Figure-1. The lowest germination rate was achieved in seeds primed without kinetin Figure-1. Regarding the effect of ABA results from Table-4 indicated that the applications of ABA (2 and 4 m Mol) were mitigated the negative effect of salinity levels on germination rate. The abscisic acid (ABA) has been proposed to act as a mediator in plant responses to a range of stresses, including drought and salt stress. ABA is the major internal signal enabling plants to survive adverse environmental conditions such as salt stress (Keskin *et al*., 2010).

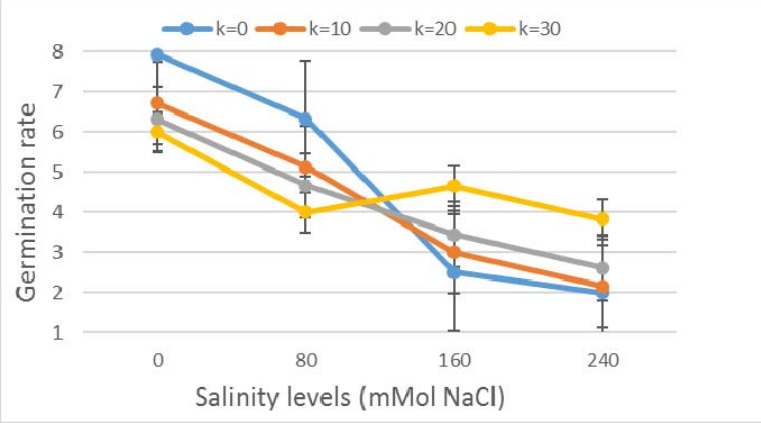

Figure-1. Interactive effects of salinity and kinetin on germination rate in wheat.

Radicle and hypocotyl length: These two traits were highly sensitive to salt with about 26% reduction even at the lowest concentration of 50 mMol NaCl (Table-3). With increasing salinity, radicle and hypocotyl length decreased progressively. The reason for reduced shoot and root development may be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings. High salinity may inhibit root and shoot elongation due to slowing down the water uptake by the plant (Pessarakli and Szabolcs, 1999). Priming with kinetin and ABA decreased radicle length and hypocotyl length relatively (Table-3). Priming with kinetin could not improve radicle and hypocotyl length in all salinity levels (Figures 2 and 3). It was hypothesized that cytokinins could increase salt tolerance in wheat plants by interacting with other plant hormones, especially auxins and ABA (Iqbal *et al*., 2006b). Application of ABA enhanced radicle and hypocotyl length in all salinity levels compared to untreated seeds with treatment (Table-4). The increase of ABA concentration in roots, when root growth continues, suggests that these tissues may have different sensitivities to the localized concentration of ABA either in endogenous form, or when exogenously applied (Jia *et al*., 2002).

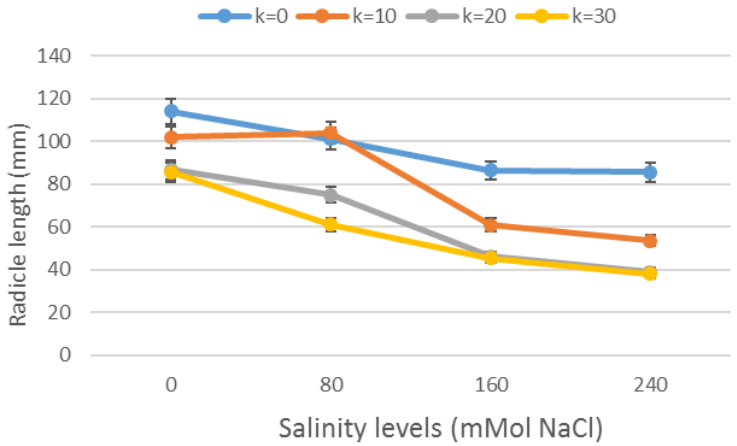

Figure-2. Interactive effects of salinity and kinetin on radicle length in wheat.

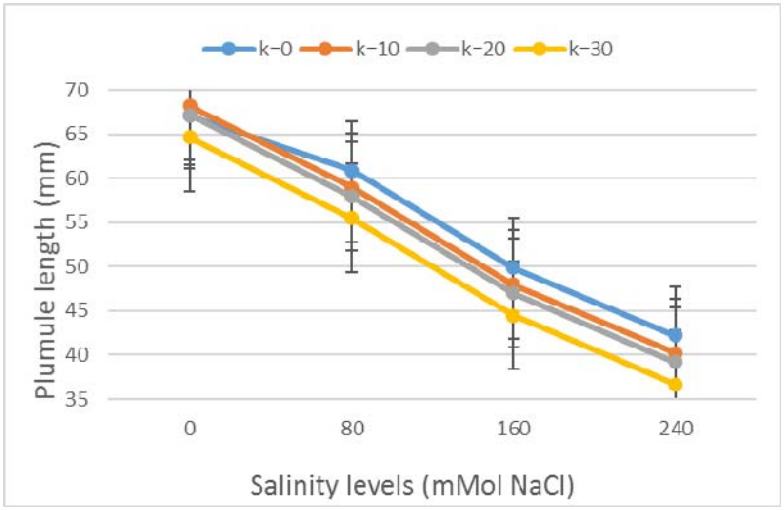

Figure-3. Interactive effects of salinity and kinetin on plumule length in wheat.

Radicle and hypocotyls dry weight: Variance analysis results of radicle and plumule dry weight are shown in Tables 1 and 2. Dry weight of radicle and hypocotyls decreased significantly under salinity condition compared with non salinity condition (Table-3). According to Table-3 kinetin and ABA influenced on radicle and hypocotyls dry weight significantly. These results are similar to those reported by and Afzal *et al*., (2005) who found that dry weight was reduced by salt stress in wheat.

Seedling fresh and dry weight: Significant difference in seedling fresh and dry weight between salinity levels, salicylic acid and gibberellic acid was observed in this study (Tables 1 and 2). Seedling fresh weight at 80, 160 and 240 mMol NaC was reduced to about 12, 55 and 120 %, respectively of the control (Table-3). On average at 80, 160 and 240 mMol NaCl seedling dry weight were about 30, 95 and 160 %, respectively of the control (Table-3). Priming with 10 m Mol Lit kinetin showed maximum radicle and hypocotyl length and higher amount decreased both traits (Table-3). ABA decreased seedling fresh and dry weight to about 10 and 12% in 4 m Mol Lit than control (Table-3).

CONCLUSIONS

Increasing of kinetin and ABA levels more than 10 and 2 m Mol Lit caused decrease in all traits under salinity condition. Germination percentage was significantly increased by kinetin under salinity conditions compared to non treatment. When seeds were in the highest NaCl level kinetin significantly improved germination. Priming with ABA could not improve radicle length in all salinity levels.

ACKNOWLEDGMENTS

Funding support for this research was provided by the Islamic Azad University of Ramhormoz, Khuzestan, Iran.

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