



Involvement of auxin in the responses of wheat germination to salt stress

Fateme Masoudi Khorasani, Hilda Besharat and Homa Mahmoodzadeh*

Biology Department, Islamic Azad University, Mashhad Branch, Mashhad, Iran

Abstract

The effects of salt stress and auxin on germination factors of three wheat cultivars viz. Sepahan, c-84-8, and c-83-1 were studied under controlled conditions. Germination was assessed using three replicates of 25 seeds in a factorial lay out in Completely Randomized Design (CRD) testing combinations of three levels of salinity (0, 4, and 8 dSm⁻¹ NaCl) and three levels of auxin (0, 0.5, and 1 ppm IAA) on seeds of three wheat cultivars in 9 cm diameter Petri dishes. Results showed that increasing concentrations of NaCl reduced germination percentage, radicle length, plumule length, seedling fresh and dry weight, and plumule dry weight. Plumule dry weight increased in seeds only at 4 dSm⁻¹. Auxin increased plumule length, seedling fresh and dry weight, and plumule dry weight, but did not influence seed germination percentage and radicle length. C-84-8 cultivar showed high seed germination percentage, radicle length, and plumule length in comparison to other cultivars while c-83-1 cultivar had high radicle dry weights and Sepahan cultivar produced high seedling fresh and dry weights.

Keywords: *Triticum aestivum*; salinity; plant growth regulators; seedling growth; germination indices

MasoudiKhorasani, F., H. Besharat and H.Mahmoodzadeh. 2014. 'Involvement of auxin in the responses of wheat germination to salt stress'. *Iranian Journal of Plant Physiology* 5 (1), 1195-1201.

Introduction

Wheat is the most important staple crop in the world and its productivity in saline soils is considerably reduced due to improper nutrition of plants as well as osmotic and droughtstress (Munn 1993; Shannon, 1998). Inhibition of plant growth by salinity is considered to be due to toxic effects of the NaCl, the ability of the root system to control entry of ions into the shoot, and slowing down water uptake of plants (Lambers, 2003). Jamil et al. (2006) reported that salt stress decreased the germination and also delayed the emergence of seeds in four vegetable species. It is thought that the repressive effect of salinity on

germination could be related to a decline in endogenous levels of plant growth hormones or phytohormones (Debez et al., 2001). It has been reported previously that salt stress reduces the supply of cytokinin from root to shoot (Naqvi and Ansari, 1974) and also the recovery of diffusible auxin from maize coleoptile tips. Indeed, the exogenous application of plant growth regulators (PGRs), e.g., gibberellins (Afzal et al., 2005), auxins (Khan et al., 2004), and cytokinins (Gul et al., 2000) produced some benefits in alleviating the adverse effects of salt stress and they also improved germination, growth, fruit setting, fresh vegetable and seed yields, and yield quality (Saimbhi, 1993). It is also suggested that root colonizing bacteria which produce phytohormones, when bound to the seed coat of

*Corresponding author

E-mail address: homa_mahmoodzadeh@yahoo.com

Received: April, 2014

Accepted: October, 2014

a developing seedling, may act as a mechanism for plant growth stimulation and these organisms can prevent the deleterious effects of stresses from the environment (Frankenberger and Arshad, 1995). The present investigation is designed to determine if the application of plant growth regulators such auxin could alleviate the effects of salinity on the germination and seedling growth of wheat under saline condition.

Materials and Methods

Seeds of three cultivars of wheat, namely, Sepahan, C-84-8, and C-83-1 were used in this study. The trial was conducted at Faculty of Science, Mashhad Branch, Islamic Azad University, Mashhad, Iran in 2013. Seeds were germinated in covered, sterilized, disposable Petri dishes containing Whatman filter paper moistened with either distilled water (control) or different treatment solutions. Germination was assessed using three replicates of 25 seeds in a factorial lay out in Completely Randomized Design (CRD) testing combinations of three levels of salinity (0, 4, and 8 dSm⁻¹ NaCl) (Bracciniet al., 1996) and three levels of auxin (0, 0.5, and 1 ppm IAA) on seeds of three wheat cultivars in 9 cm diameter Petri dishes.

Seeds were incubated in a germinator at 25°C and were considered germinated with the emergence of the radicle. Germination was scored when a 1 mm radicle had emerged from the seed coat. After one week, radicle and plumule lengths and seedling fresh weights were immediately determined. To determine the impact of the treatments on seed germination, all seedlings were separated from the remaining seeds. Seedling dry weights were evaluated after 72 h in an oven at 80°C.

Germination percentage (GP, %), germination rate (GR), relative germination percentage (RGP), mean germination time (MGT), germination index (GI), and weighted germination index (WGI) were the parameters under investigation in the study. Final percentage of germination (GP) for each treatment was calculated after seven days. The germination index (GI) was based on the number of seeds germinated and the germination rate. These

parameters were also calculated based on the following formulas proposed by Bu et al. (2008):

$$GP = 100 \times GN / SN$$

where GN is the total number of germinated seed and SN is the total number of seeds tested;

$$GR = \sum_{D_i} N_i$$

where N_i is the number of seeds germinated on day i;

$$RGP = GP \text{ treatment} / GP \text{ control} \times 100$$

Germination index (GI) is a synthetic measure designed to reflect the synthetic germination ability including germination rate and germination numbers. Where I is the number of days since the day of sowing and G_i is the number of seeds germinated on day i.

The weighted germination index (WGI) as described by Bu et al. (2008) was calculated with maximum weight given to the seeds germinating early and the minimum weight given to those germinating late.

$$WGI = [N \times n_1 + (N - 1) \times n_2 + (N - 2) \times n_3 + \dots] / N \times N'$$

where n₁, n₂, ..., n₆₀ are the number of seeds that germinated on the first, second, and subsequent days until the 60th day, respectively; N is the total days of experiment and N' is the total number of seeds placed in incubation.

Data were analyzed with SPSS software using analysis of variance (ANOVA) and Duncan's multiple range test for comparison of the treatment means.

Results

Analysis of variance revealed seed germination percentage was increased in high salinity conditions with the presence of 0.5 ppm auxin for Sepahan and C-84-8 cultivars. Also the presence of 0.5 ppm auxin for C-83-1 cultivar decreased seed germination percentage at higher levels (8 dSm⁻¹) of salinity (Table 1). Increasing concentrations of NaCl reduced germination rate, relative germination percentage, germination

Table 1
Means values of seed germination indices for three wheat cultivars, auxin, and salinity stress

Cultivar	Salinity (dSm ⁻¹)	Auxin (ppm)	Germination percentage(%)	Germination rate(NDay ⁻¹)	Relative Germination percentage	Mean Germination Time	Germination Index	Weighted Germination Index
Sepahan	0	0	97 ^{ab}	23.2 ^{bcd}	94 ^{ab}	3.5 ^{de}	41 ^d	0.55 ^{abc}
Sepahan	0	0.5	95.5 ^{bcd}	24.1 ^{ab}	92.5 ^{def}	3.8 ^{bcd}	40.5 ^{ef}	0.52 ^{bc}
Sepahan	0	1	94.2 ^{defg}	21.4 ^{defg}	93.2 ^{bcd}	3.2 ^e	40 ^{fg}	0.50 ^{bcd}
Sepahan	4	0	93.33 ^{defgh}	18.5 ^{hi}	91.5 ^{fgh}	3.9 ^{bc}	38.5 ^{gh}	0.46 ^d
Sepahan	4	0.5	93.33 ^{defgh}	18.8 ^h	91.8 ^{fg}	3.8 ^{bcd}	39 ^{fgh}	0.43 ^{def}
Sepahan	4	1	93.33 ^{defgh}	18.4 ^{hi}	91.5 ^{fgh}	3.5 ^{de}	40.4 ^{ef}	0.45 ^{de}
Sepahan	8	0	87 ^j	16.6	88.5 ^{ij}	4.5 ^a	36 ^{hi}	0.41 ^{ef}
Sepahan	8	0.5	92 ^{gh}	18.2 ^{hij}	90.4 ^h	3.2 ^e	37.7 ^h	0.43 ^{def}
Sepahan	8	1	89.33 ^j	17.3 ^{kl}	91.2 ^{gh}	3.75 ^{bcd}	38.2 ^{gh}	0.40 ^f
C-84-8	0	0	98.33 ^{abc}	24.5 ^a	95 ^a	2.5 ^{hi}	43 ^{ab}	0.6 ^a
C-84-8	0	0.5	96.13 ^{bcd}	23.2 ^{bcd}	93.5 ^{bcd}	3.2 ^e	44.1 ^a	0.58 ^{ab}
C-84-8	0	1	95.4 ^{bcd}	22.8 ^{cde}	94 ^{ab}	3.5 ^{de}	43.8 ^{ab}	0.55 ^{abc}
C-84-8	4	0	95.4 ^{bcd}	21.5 ^{def}	93.2 ^{bcd}	3.2 ^e	41.7 ^{cd}	0.52 ^{bc}
C-84-8	4	0.5	95.4 ^{bcd}	21.9 ^{def}	92.6 ^{def}	3.0 ^{fg}	42 ^{cd}	0.50 ^{bcd}
C-84-8	4	1	95.4 ^{bcd}	22.6 ^{cde}	92 ^{ef}	2.8 ^{gh}	42.9 ^{bc}	0.53 ^{bc}
C-84-8	8	0	91.3 ^{efgh}	18 ^{hijk}	90.5 ^h	3.6 ^{cde}	39 ^{fgh}	0.42 ^{ef}
C-84-8	8	0.5	95 ^{bcde}	23.4 ^{bc}	92.8 ^{cde}	3.7 ^{cd}	40.2 ^{efg}	0.54 ^{abc}
C-84-8	8	1	93 ^{efgh}	18.25 ^{hij}	92.5 ^{def}	3.6 ^{cde}	40.8 ^{de}	0.44 ^{de}
C-83-1	0	0	97 ^{ab}	23.6 ^{bc}	93.8 ^{bc}	3.4 ^{def}	41.7 ^{cd}	0.53 ^{bc}
C-83-1	0	0.5	95.3 ^{bcd}	22.4 ^{cde}	92.8 ^{cde}	3.75 ^{bcd}	42.3 ^{bcd}	0.52 ^{bc}
C-83-1	0	1	95 ^{bcde}	24.5 ^a	93 ^{bcd}	3.6 ^{cde}	42.9 ^{bc}	0.54 ^{abc}
C-83-1	4	0	94 ^{cdef}	21.8 ^{def}	92 ^{ef}	4.2 ^{ab}	39.5 ^{fg}	0.48 ^{cd}
C-83-1	4	0.5	93.3 ^{defgh}	18.3 ^{hij}	89 ⁱ	4.1 ^{ab}	40.4 ^{ef}	0.46 ^d
C-83-1	4	1	93 ^{efgh}	18.25 ^{hij}	90.5 ^h	3.9 ^{bc}	41.3 ^d	0.46 ^d
C-83-1	8	0	92.2 ^{fgh}	17.5 ^{kl}	88 ^{jk}	3.7 ^{cd}	40.2 ^{efg}	0.35 ^{gh}
C-83-1	8	0.5	91.3 ^{hi}	17.5 ^{kl}	89.5 ⁱ	3.2 ^e	41.5 ^{cd}	0.37 ^{fgh}
C-83-1	8	1	86.2 ^j	16.4 ^l	90.2 ^{hi}	3.5 ^{de}	41 ^d	0.39 ^{fg}

Means by the same letter in each column are not significantly different based on the Duncan test (P = 0.05)

index, and weighted germination index but increased mean germination time in all cultivars (Table 1). These factors were increased with the presence of auxin.

Significant differences were found in radicle lengths depending on cultivar, salinity, and auxin levels. Increasing salt concentrations severely affected radicle elongation (Table 2). The 4 and 8 dSm⁻¹ NaCl treatments reduced radicle length in all cultivars in comparison with control. The length of radicle was not decreased under high salinity conditions (8 dSm⁻¹) with the presence of 0.5 and 1 dSm⁻¹ auxin for C-84-8 cultivar (Table 2) in comparison with 0 ppm auxin. It seems that C-84-8 cultivar response to auxin that can decrease high salinity effects on some

growth traits such as radicle length. There were significant differences in plumule lengths depending on cultivars, salinity, and auxin levels. Increasing salt concentrations severely affected the length of plumule (Table 2). The 4 dSm⁻¹ NaCl treatment reduced plumule length in C-84-8 cultivar while the 8 dSm⁻¹ NaCl reduced plumule length in Sepahan cultivar. The plumule length increased in salt stress conditions with the presence of 0.5 dSm⁻¹ auxin for all cultivars (Table 2). Thus, all cultivars are appropriate for auxin application and auxin presence can reduce salinity effects on their growth traits.

Increasing salt concentrations severely affected seedling fresh weight. Sepahan cultivar under 1 ppm auxin without salinity and C-84-8

Table 2
Means values of seedling growth for three wheat cultivars, auxin and salinity stress

Cultivar	Salinity (dSm ⁻¹)	Auxin (ppm)	Radicle length (mm)	Plumule length (mm)	Seedling freshWeight (g)	Seedling dryWeight (g)
Sepahan	0	0	8.3 ^b	8.32 ^d	1.1 ^{cdefg}	0.045 ^{bc}
Sepahan	0	0.5	5.6 ^e	11.2 ^a	1.2 ^{bcd}	0.051 ^a
Sepahan	0	1	2.67 ^{gh}	7.02 ^e	1.35 ^a	0.045 ^{bc}
Sepahan	4	0	5.47 ^e	7.05 ^e	1.14 ^{cde}	0.04 ^d
Sepahan	4	0.5	2.03 ^{ijk}	8.6 ^d	1.12 ^{cdef}	0.048 ^{abc}
Sepahan	4	1	2.01 ^{ijk}	7.5 ^e	1.26 ^{ab}	0.048 ^{abc}
Sepahan	8	0	3.2 ^g	5.2 ^f	0.86 ^j	0.033 ^{gh}
Sepahan	8	0.5	2.02 ^{jk}	7.6 ^e	1 ^{hi}	0.039 ^{de}
Sepahan	8	1	2.36 ^{ijk}	4.5 ^g	1.06 ^{efgh}	0.038 ^e
C-84-8	0	0	9.35 ^a	11.09 ^a	1.2 ^{bcd}	0.046 ^{bc}
C-84-8	0	0.5	7.74 ^{bc}	9.7 ^b	1.18 ^{bcd}	0.048 ^{abc}
C-84-8	0	1	2.75 ^{gh}	8.6 ^d	1.13 ^{dce}	0.051 ^a
C-84-8	4	0	5.6 ^e	8.2 ^d	1.02 ^{fgh}	0.036 ^{efg}
C-84-8	4	0.5	5.4 ^e	9.5 ^{bc}	1.01 ^{hig}	0.038 ^e
C-84-8	4	1	2.54 ^{igh}	8.2 ^d	1.09 ^{defg}	0.037 ^{ef}
C-84-8	8	0	2.09 ^{ijk}	3.6 ^h	0.63 ^l	0.034 ^{fg}
C-84-8	8	0.5	2.38 ^{ijk}	5.7 ^f	0.86 ^j	0.036 ^{efg}
C-84-8	8	1	1.7 ^k	5.72 ^f	0.92 ^{ij}	0.035 ^{fg}
C-83-1	0	0	7.33 ^{dc}	8.5 ^d	1 ^{hig}	0.049 ^{ab}
C-83-1	0	0.5	7.1 ^d	9.5 ^{bc}	1.21 ^{bc}	0.045 ^{bc}
C-83-1	0	1	6.75 ^d	6.8 ^e	1.16 ^{bcde}	0.04 ^d
C-83-1	4	0	5.05 ^e	8.2 ^d	1 ^{hi}	0.034 ^{fg}
C-83-1	4	0.5	4.21 ^f	9.5 ^{dc}	1 ^{hig}	0.037 ^{ef}
C-83-1	4	1	2.51 ^{igh}	7.3 ^e	1.06 ^{ehfg}	0.037 ^{ef}
C-83-1	8	0	2.42 ^{igh}	4.2 ^g	0.75 ^k	0.032 ^{ghi}
C-83-1	8	0.5	0.95 ^l	5.3 ^f	0.81 ^{jk}	0.034 ^{fg}
C-83-1	8	1	1.32 ^l	5.45 ^f	0.81 ^{jk}	0.037 ^{ef}

Means by the same letter in each column are not significantly different based on the Duncan test (P = 0.05)

cultivar under 8 dSm⁻¹ salinity without auxin produced the most (1.35 g) and the least (0.92 g) seedling fresh weights, respectively (Table 2). Also increasing salt concentration influenced seedling dry weight. Sepahan cultivar under 0.5 ppm auxin without salinity and the same cultivar under 8 dSm⁻¹ salinity with no auxin produced the most and the least seedling dry weights, respectively. It seems that reduction in seedling fresh and dry weights is due to a decrease in water uptake by seedlings under salt stress condition.

The correlations between each pair of studied factors (germination percentage, germination rate, relative germination percentage, germination index, weighted germination index, mean germination time, seedling dry weight, radicle length, plumule length, and seedling fresh weight) were calculated (Table 3) and the findings suggested

that germination percentage, germination rate, relative germination percentage, germination index, and mean germination time positively correlated with all factors but did not correlate with seedling dry weight and radicle and plumule length. Weighted germination index did not correlate with radicle and plumule lengths while it correlated with the other measured traits (Table 3). The seedling fresh weight positively and significantly correlated with seedling dry weight ($r = 0.69^{**}$), plumule length ($r = 0.45^*$), and radicle length ($r = 0.49^*$).

There were highly significant ($p < 0.01$) positive correlations between seedling dry weight and seedling fresh weight and also between plumule and radicle length. Radicle length did not correlate ($r = 0.2$) with plumule length and the other traits except seedling fresh and dry weights (Table 3).

Table 3
Correlation coefficient among seed germination factors for three wheat cultivars, auxin and salt stress

Factors	Germination %	Germination rate	Relative Germination percentage	Mean Germination Time	Germination Index	Weighted Germination Index	Seedling Fresh Weight	Seedling Dry Weight	Radicle Length
Germination rate	0.56*								
Relative Germination percentage	0.46*	0.45*							
Mean Germination Time	0.32 ^{ns}	0.54**	0.44*						
Germination Index	0.58*	0.66**	0.55*	0.68**					
Weighted Germination Index	0.67**	0.45*	0.47*	0.55*	0.45*				
Seedling Fresh Weight	0.73**	0.48*	0.54*	0.45*	0.65**	0.45*			
Seedling Dry Weight	0.04 ^{ns}	0.15 ^{ns}	0.23 ^{ns}	0.18 ^{ns}	0.35 ^{ns}	0.42*	0.69**		
Radicle Length	0.13 ^{ns}	0.22 ^{ns}	0.18 ^{ns}	0.25 ^{ns}	0.10 ^{ns}	0.28 ^{ns}	0.49*	0.76**	
Plumule Length	0.14 ^{ns}	0.18 ^{ns}	0.12 ^{ns}	0.35 ^{ns}	0.32 ^{ns}	0.30 ^{ns}	0.45*	0.86**	0.2 ^{ns}

* and ** significant at 5 and 1%, respectively and ns not significant

Discussion

There are several reports (e.g., Gupta 1971; Gregorio et al., 1995; Naidu, 2001) showing the positive effect of plant growth regulators gibberellic acid, auxin, and kinetin on seed germination. In fact, seed priming with auxin had positive effects on some growth traits of wheat seeds in the present study. There is also evidence of growth under saline conditions by using plant growth hormones as priming agents, such as GA and ascorbic acid in wheat (Al-Hakimi and Hamada, 2001).

The present results are in agreement with those reported by Xue et al. (2004) who found that high levels of salinity can significantly inhibit seed germination. Furthermore, Waisel (1972) found that increasing salinity concentrations during germination often caused osmotic and/or specific toxicity which could reduce or retard germination percentage.

Salt induced inhibition of seed germination could be attributed to osmotic stress or to specific ion toxicity (Huang and Redmann, 1995). Also, it is thought that the repressive effect of salinity on germination could be related

to a decline in endogenous levels of hormones (Debez et al., 2001). Wheat plants grown under saline conditions from seed soaked in IAA, NAA, and GA showed increased seed germination and growth compared to control plants (Balki and Padole, 1982). Numerous research studies indicated that application of hormones such as auxins may increase the germination ability of seeds and seedling vigour in different plants (Balestri and Bertini, 2003). Thus it seems that soaking wheat seeds in different concentrations of auxin before germination counteracted adverse effects of NaCl on adult plant growth.

Some researchers reported that salinity results in a decline in metabolic activity of plant cells, which should be inevitably reflected in inhibition of their growth (Kurth et al., 1986; Cicek and Cakirlar, 2002). High salinity may inhibit root and shoot elongation due to slowing down the water uptake by the plant and this may be another reason for some other studies which also reported that pre-soaking wheat seeds in plant growth regulators like IAA and gibberellins alleviated the growth inhibiting effects of salt stress (Afzal et al., 2005). Khan and Weber (1986) and Gul et al. (2000) also observed that the plant

growth stimulating compounds such gibberellic acid, zeatin, and ethephon can alleviate the effects of salinity on germination and growth of *Ceratoides lanata*, *Salicornia apacifica*, and *Allenrolfea accidentalis* (Khan et al., 2004). It is also possible that under high salt concentrations, naturally present hormones may be suppressed (Afzal et al., 2005). Sakhabutdinova et al. (2003) also reported that salinity resulted in a progressive decline in the level of IAA in the root system of plants. In this condition, seed soaking with plant growth regulators and application of additional natural phytohormones supplied sufficient hormones for normal plant development and growth in saline conditions (Afzal et al., 2005). Although salinity can induce a rapid reduction in root and shoot growth (Neumann, 1997), radicle length decreases proportionally more than plumule length, causing a reduction in the root/shoot ratio. Salinity significantly reduces the total dry matter and the degree of reduction in total dry matter depending on genotypes and salt concentrations (Pessaraki, 1991).

Conclusion

Seed germination is an elaborate developmental process that is regulated through intricate signalling networks integrating diverse environmental cues into endogenous hormonal signalling pathways. The bulk of studies in recent years support the role of auxin in seed germination. In fact, priming the seeds with optimal concentrations of plant growth hormones is shown to effectively improve germination as well as growth and yield performance of various crop species under both normal and stress conditions. Growth hormones normally used for seed priming include auxins (IAA, TBA, NAA), gibberellins (GA), kinetin, and abscisic acid. This study demonstrated the positive effects of IAA hormone on some germination traits. It seems that moderate levels of auxin (0.5 ppm) could reduce negative salinity effects on seed germination.

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