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ORIGINAL ARTICLE

The Effects of Different Levels of Salinity and Indole-3-Acetic Acid (IAA) on Early Growth and Germination of Wheat Seedling

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Salt stress as a major adverse factor can lower germination, ion toxicity, reduction in enzymatic and photosynthetic efficiency and other physiological disorders and ultimately lower crop productivity in salinity zones. As growth regulators are involved in altering growth processes in plants, it is possible that they might even reduce the detrimental effects of salinity by stimulating growth. But their physiological roles are not well known. In example, about the roles of IAA in salinity conditions are not similar viewpoints. For this purpose a factorial experiment based on completely randomized design was conducted with influence of foliar application of Indole-3-Acetic Acid (IAA) in farm on germination percent and seedling growth parameters of wheat (cv. Marvdasht) under different levels (0, 40, 80 and 120 mM) of NaCl salinity with 3 replications, during 2012 in the laboratory research of the Department of plant breading and agronomy, Faculty of Agriculture, Maragheh University in Maragheh state in Iran. The results of this study indicate, that salinity decreased the plumule, radicle and seedling length and plumule, radicle and seedling dry weight, seed germination and seedling vigor index, whereas increase in mean germination time and no signification plumule/radicle ratio observed in the cultivar tested. Also, application of IAA at cell division stage of grain growth caused significant increase in seedling growth parameters under different salinity levels. The interaction between application of IAA and salinity levels significantly affected final germination percentage. Highest final germination percentage was recorded with IAA with increasing salinity level from 0 up to 120 mM NaCl (98.3, 98.3, 96.7 and 100% without significant differences between them, respectively).

Key words: Wheat, Grain filling, Indole-3-Acetic Acid (IAA), foliar application, Salinity

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Key words: Wheat, Grain filling, Indole-3-Acetic Acid (IAA), foliar application, Salinity

Abbreviations: Indole-3-Acetic Acid (IAA), Abscisic Acid (ABA), kinetin (KIN), Sodium chloride (NaCl), Germination Percentage (GP), Mean germination time (MGT), Seedling vigor index (SVI).

Salinity is one of the major environmental factors limiting plant growth and productivity. It is estimated that about one-third of world's cultivated land is affected by salinity (Kaya *et al*., 2003). In most arid and semiarid areas (such as Iran) this problem is accentuated by competition for high quality water among agriculture, industry and landscape users has promoted the use of alternative water sources for irrigation.

Germination of seed is an important point in seedling establishment and subsequent plant health and vigor. Salinity can affect germination and seedling growth either by creating an osmotic pressure that prevents water uptake or by toxic effects of sodium and chloride ions on seed germination (Guo *et al*., 2010; Akbarimoghaddam *et al*., 2011). However, screening for seeds with a greater tolerance to salt stress aids in the development of salt tolerant cultivars. Increasing levels of salinity (NaCl) significantly reduced the seedling growth parameters in all studied wheat genotypes (Khan, 2009; Khayatnezhad *et al*., 2010; Ghaloo *et al*., 2011). Bayat et al. (2012) showed that salinity decreased the growth, Chlorophyll reading values, flower number per plant and flower diameter of calendula (*Calendula officinali*s L.).

As growth regulators are involved in altering growth processes in plants, it is possible that they might even reduce the detrimental effects of salinity by stimulating growth. Kaya *et al*. (2010) reported that foliar application of both KIN and IAA significantly reduced Na^+ concentration and increased those of Ca^{2+} and K^+ under salt stress. Hussain *et al*. (2003) found that due to IAA concentrations plant height and dry weight increased in black seeds. Barbieri *et al*. (1991) suggested major role of IAA in promoting the development of root system. In addition, Akbari *et al*. (2007) showed that application of auxin increased hypocotyls length, seedling fresh and dry weigh and hypocotyls dry weight of the three cultivars of wheat plants under salinity. Other researchers also reported that pre-sowing wheat seeds with plant growth regulators like IAA alleviated the growth inhibiting effect of salt stress (Sastry and Shekhawa, 2001; Afzal *et al*., 2005).

IAA has been reported that it responds to salinity in crop plants. However, little information seems to be available on the relationship between salinity stress and auxin levels in plants. The variations in IAA content under stress conditions appeared to be similar to those of ABA (Ribaut and Pilet, 1991), and increased levels of IAA have also been correlated with reduced growth (Ribaut and Pilet, 1994). Keeping this in mind, the present project was undertaken to find out whether the foliar application of indole-3-acetic acid (IAA) on wheat can alleviate the harmful effect of salinity on plant growth.

MATERIALS AND METHODS

Experimental procedure and design

In the experiment, influence of foliar application of Indole-3-Acetic Acid (IAA) was used at the beginning of cell division (from anthesis until 14 days after anthesis) in farm on germination percent and seedling growth parameters of wheat (cv. Marvdasht) under different levels (0, 40, 80 and 120 mM) of NaCl salinity, during 2012 in the laboratory research of the Department of plant breading and agronomy, Faculty of Agriculture, Maragheh University in Maragheh state in Iran. In order to this, in the form of experiment was factorial, using a completely randomized design (CRD) with three replications. Wheat grains were disinfected with 96% ethanol for 30 seconds and 10% sodium hypochlorite solution for 50 seconds. 25 grains were germinated on one layers of filter paper in 9 cm petri dishes. The Petri dishes were covered to prevent the loss of moisture by evaporation under laboratory condition (25±1 °C) for 7 days.

Seedling growth parameters measurements

Grains were considered germinated when they exhibited radicle extension of > 2 mm. Every 24 hours after soaking, germinated grains were made daily during the course of the experiment to determine following germination parameters. Where the number of germinated seeds was recorded 7 days after planting as Germination Percentage (GP) according to ISIA (1993) and ISIA (1999): $GP = (N_g / N_t) \times 100$, N_g=Total number of germinated seeds, N_t=Total number of seeds evaluated.

Mean Germination Time (MGT) or Mean Emergence Time (MET) was calculated according to Ellis and Roberts (1981): $MGT = \sum Dn / \sum n$, Where n is the number of seeds, which were emerged on day D, and D is the number of days counted from the beginning of emergence.

The seedling vigor index was calculated according to following formula (Abdul-Baki and Anderson, 1970): SVI = (seedling length (cm) × germination percent)/100.

The experiment was terminated by harvesting seedlings 7 days after grains soaking and traits including plumule length, radicle length, plumule dry weight, radicle dry weight were measured. Dry weight was determined after drying samples in oven at 70 °C for 48 hours.

Statistical analyses

Statistical analyses were performed using EXCEL and SAS statistical software (version 9.0). The significant differences between treatments were compared with the critical difference at 5% probability level by the Duncan's test.

RESULTS AND DISCUSSION

The results of this study showed significant main effects of different salinity levels and exogenous application of IAA was used at the beginning of cell division on germination and seedling growth parameters (Table 1). The highest plumule dry weight, radicle dry weight and seedling dry weight were observed in control, while salinity at 120 mM decreased significantly germination and seedling growth parameters. Highest values of plumule dry weight were recorded with the control followed by those treated with control and 40 mM NaCl without significant differences between them (5.70 and 5.58 mg, respectively). A correlation was found between seedling dry weight and the seedling length (Table 3).

Increasing salinity concentrations from 0 to 120 mM NaCl gradually decreased averages of plumule length, radicle length and seedling length. Maximum decrease was observed at 120 mM of NaCl salinity (Table 1). Highest seedling length (13.6 cm) was recorded with the control treatment compared with other salinity levels, Treated seed with 40 and 80 mM NaCl came in the second and third ranks (10.6 and 6.52 cm, respectively). Highest salinity levels i.e. 120 mM NaCl recorded the lowest seedling length (3.36 cm). High level of salinity may have also inhibit the root and shoot elongation due to slowing down the water uptake for overall osmotic adjustments of the plant body under high salt stress condition. Decrease and delay in germination in saline medium has also been reported by (Bishnoi and Pancholy, 1979; Guo *et al*., 2010; Fethi *et al*., 2011). Therefore, the reduction in plant growth under stress conditions could be an outcome of altered hormonal balance. Hence, their exogenous application provides an attractive approach to counter the stress conditions. However, Prakash and Prathapasenan (1990) reported that NaCl caused a significant reduction in indole-3-acetic acid concentrations in rice leaves. Sakhabutdinova *et al*. (2003) reported that salinity causes a progressive decline in the level of IAA in the root system of plants. Other researchers also reported that pre-sowing wheat seeds with plant growth regulators like IAA alleviated the growth inhibiting effect of salt stress (Sastry and Shekhawa, 2001; Afzal *et al*., 2005).

Highest values of plumule/radicle ratio were recorded with the control followed by those treated with 40, 80 and 120 mM NaCl without significant differences between them (0.91, 0.88 and 0.87, respectively).

Increasing salinity concentrations from 0 to 120 mM NaCl gradually decreased averages of germination and seedling vigor index (Table 1). Highest final germination percentage (93.3%) was recorded with the control treatment compared with other salinity levels, Treated seed with 40 and 80 mM NaCl came in the second and third ranks (89.2% and 80.8%, respectively). Bijeh Keshavarzi (2012) showed that by increasing salinity, percentage and race of germination decreased, So that, in the 150 mM of salinity level, germination reached to minimized (8.33%). Hegarty (1977) indicated that stress at germination stage can result in delayed and reduced germination or may prevent germination completely. Soltani *et al*. (2006) expressed that probably reduced germination percentage and seed germination index under low osmotic potential due to endosperm material decompose or slower transfer of this material to the seedling. Sadat noori *et al*. (2007) reported that canola seeds that were under stress, more time needed for germination. Salinity can affect seedling growth either by creating an osmotic pressure that prevents water uptake or by toxic effects of sodium and chloride ions on seed germination (Guo *et al*., 2010; Akbarimoghaddam *et al*., 2011).

MGT was increased in the wheat plants in the salt treatment compared to the non-stressed plants (Table 2). In application of IAA a negative correlation was found between MGT and the plumule length, radicle length, seedling length, plumule dry weight, radicle dry weight and seedling dry weight. But, a correlation was found between SVI and the all seedling growth parameters (Table 3). Also, application of IAA a negative correlation was found between MGT and the SVI (r=-0.82**).

Exogenous application of IAA at cell division stage of grain growth caused significant increase in radicle length and plumule, radicle and seedling dry weight, seed germination and seedling vigor index (Table 1). It caused rapid plant stand during germination and ultimately production of more dry matter. In addition, Akbari *et al*. (2007) showed that application of auxin increased hypocotyls length, seedling fresh and dry weigh and hypocotyls dry weight of the three cultivars of wheat plants under salinity. Babu *et al*. (2012) reported that endogenous bioactive IAA content significantly increased with application of elevated NaCl treatment. It suggests that the growth and development of tomato plants under salt stress is supported by increased production of IAA.

The interaction between application of IAA and salinity levels significantly affected final germination percentage as illustrated in Fig 3 D.

Table 1. Analysis of variance of the effects of salt condition, application of IAA and their interactions on germination and seedling growth parameters

S.O.V	df	Mean Square									
		Plumule length (cm)	Radicle length (cm)	Seedling length (cm)	Plumule dry weight (mg)	Radicle dry weight (mg)	Seedling dry weight (mg)	Plumule/Radicle ratio	Mean germination time (day)	Germination (%)	Seedling vigor index
Salt condition (SC)	3	27.6 **	33.6 **	122 **	13.9 **	11.9 **	48.9 **	0.015 ns	0.40 **	376 **	124 **
Treatment IAA (T)	1	2.94 ns	9.59 *	23.1 ns	14.1 *	9.25 *	46.1 *	0.091 ns	0.47 **	4401 **	76.3 **
SC×T	3	2.99 ns	5.34 *	15.8 ns	3.83 ns	3.72 *	14.6 *	0.021 ns	0.04 ns	420 **	12.2
Error	16	2.61	1.99	8.76	1.89	1.46	5.68	0.038	0.04	52.1	7.51
CV (%)		**39.5**	**31.9**	**34.7**	**30.9**	**28.6**	**27.5**	**21.6**	**10.4**	**8.51**	**36.3**

ns, * and **: Non significant, significant at 5 % and 1 % levels of probability, respectively.

Table 2. Mean comparison of salt stress and application of IAA on germination and seedling growth parameters

Treatment	Plumule length (cm)	Radicle length (cm)	Seedling length (cm)	Plumule dry weight (mg)	Radicle dry weight (mg)	Seedling dry weight (mg)	Plumule/Radicle ratio	Mean germination time (day)	Germination (%)	Seedling vigor index
Salt condition (mM)										
0	6.39 a	7.22 a	13.6 a	5.70 a	6.05 a	11.8 a	0.98 a	1.53 c	93.3 a	12.8 a
40	5.26 a	5.35 b	10.6 a	5.58 a	4.57 b	10.2 ab	0.91 a	1.81 b	89.2 ab	9.65 a
80	3.10 b	3.41 c	6.52 b	3.98 ab	3.51 bc	7.49 bc	0.88 a	1.84 b	80.8 bc	5.28 b
120	1.61 b	1.76 c	3.36 b	2.48 b	2.81 c	5.29 c	0.87 a	2.16 a	75.8 c	2.53 b
Treatment										
Control	3.74 a	3.80 b	7.54 a	3.67 b	3.61 b	7.28 b	0.97 a	1.97 a	71.3 b	5.78 b
IAA	4.44 a	5.07 a	9.50 a	5.20 a	4.85 a	10.1 a	0.85 a	1.69 b	98.3 a	9.34 a

Means followed by the same letter within columns are not significantly different ($P < 0.05$) according to Duncan's test.

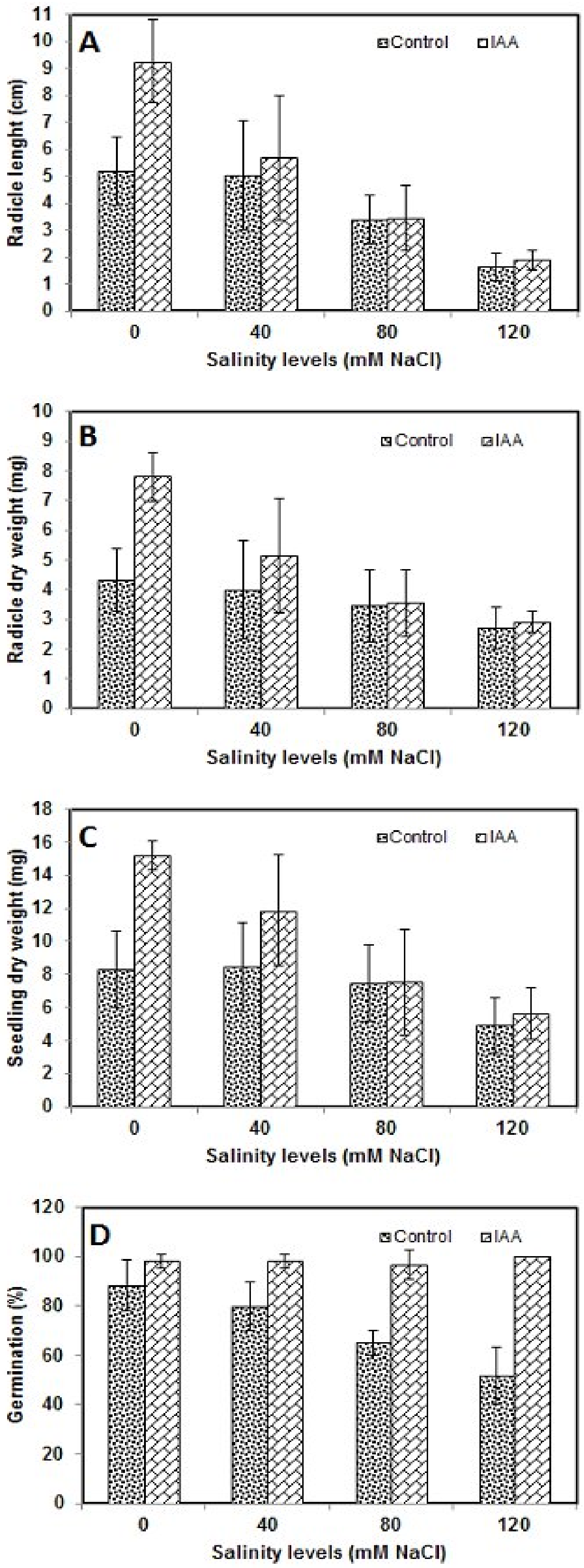

Figure 1. Radicle length (A), radicle dry weight (B), seedling dry weight (C) and germination (D) of wheat in response to foliar Indole-3-Acetic Acid (IAA) applications under different salinity levels. Vertical bars represent ± SD.

Table 3. Correlation coefficients among germination and seedling growth parameters in improved wheat cultivar under different salinity levels

		PL	RL	SL	PW	RW	SW	S\R	MGT	PG	SVI
PL	Control	1									
	IAA	1									
RL	Control	0.95**	1								
	IAA	0.97**	1								
SL	Control	0.99**	0.99**	1							
	IAA	0.99**	0.99**	1							
PW	Control	0.91**	0.84**	0.88**	1						
	IAA	0.93**	0.85**	0.90**	1						
RW	Control	0.89**	0.82**	0.87**	0.74**	1					
	IAA	0.96**	0.99**	0.99**	0.87**	1					
SW	Control	0.96**	0.89**	0.94**	0.94**	0.92**	1				
	IAA	0.98**	0.95**	0.97**	0.97**	0.96**	1				
S\R	Control	0.47	0.21	0.34	0.59*	0.43	0.55	1			
	IAA	0.49	0.29	0.39	0.63*	0.32	0.49	1			
MGT	Control	-0.43	-0.52	-0.48	-0.30	-0.47	-0.41	0.20	1		
	IAA	-0.79**	-0.82**	-0.82**	-0.73**	-0.79**	-0.78**	-0.19	1		
PG	Control	0.60*	0.74**	0.68*	0.34	0.59*	0.49	-0.29	-0.67*	1	
	IAA	-0.01	-0.06	-0.03	-0.18	-0.03	-0.11	0.21	-0.01	1	
SVI	Control	0.94**	0.99**	0.98**	0.77**	0.84**	0.86**	0.18	-0.54	0.80**	1
	IAA	0.99**	0.99**	1.00**	0.89**	0.99**	0.97**	0.39	-0.82**	0.01	1

* and ** Significant at the 5 and 1 percent levels, respectively and another no significant.
PL: Plumule Length (cm), RL: Radicle Length (cm), SL: Seedling Length (cm), PW: Plumule Weight (mg), RW: Radicle Weight (mg), SW: Seedling Weight (mg), P/R: Plumule/Radicle, MGT: Mean Germinatiom Time (day), GP: Germination percent (%), SVI: Seedling Vigor Index.

Highest final germination percentage was recorded with IAA with increasing salinity level from 0 up to 120 mM NaCl (98.3, 98.3, 96.7 and 100% without significant differences between them, respectively).

Maximum radicle length and radicle dry weight was obtained from control (Non application of IAA) and application of IAA at without salt treatment as presented in Fig 1 A, B and C.

Increasing salinity concentrations from 0 to 120 mM NaCl gradually decreased averages of seedling dry weight in the both control and application of IAA treatment (Fig 1 C). Our current findings are in agreement with the previous reports of, who demonstrated that IAA generally increases in plants in response to elevated salt stress. Other researchers also reported that pre-sowing wheat seeds with plant growth regulators like IAA alleviated the growth inhibiting effect of salt stress (Sastry and Shekhawa, 2001; Afzal *et al*., 2005). In wheat seed germination decreased with increasing salinity level, while the adverse effect of salinity was alleviated by treatment of seeds with IAA or NAA (Balki and Padole, 1982; Gulnaz *et al*., 1999).

CONCLUSION

It is concluded that salinity stress can considerably decreased germination and seedling growth parameters of wheat. The highest plumule, radicle and seedling length and dry weight, germination and seedling vigor index were observed in control, while salinity at 120 mM decreased significantly germination and seedling growth parameters. Exogenous application of IAA at cell division stage of grain growth caused significant increase in radicle and seedling length and also plumule, radicle and seedling dry weight, seed germination and seedling vigor index. Also, in wheat seed germination decreased with increasing salinity level, while the adverse effect of salinity was alleviated by treatment of seeds with IAA.

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