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European Journal of Experimental Biology, 2012, 2 (5):1680-1684

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Effect of salicylic acid and seed weight on germination of Wheat (CV. BC ROSHAN) under different levels of osmotic stress

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ABSTRACT

To study the effect of salicylic acid and seed weight on Wheat germination (CV. BC ROSHAN) under different levels of osmotic stress, an experiment was conducted in the research laboratory of Shahid Chamran University of Ahvaz, Iran in 2012. The experimental design was factorial based on completely randomized design (CRD) with three replications. The treatments was included of salicylic acid (SA) (1mM without and with using) 1000 weight kernel and four levels of osmotic stress by PEG (Polyethylene glycol). In this experiment, in standard germination test traits of seed germination percentage, germination rate and in seedling growth test traits of seedling length and dry weight of seedlings have been measured. The results showed that osmotic stress decreased seed germination of wheat cultivars in general concentration of PEG (12 bar) and 1000 Weight kernel (22 gr) decreased germination over % as compared with control. Also, the SA increased the seedling length and dry weight of seedlings. SA increased length and weight of radicle and plumule in treatments of low seed weight (1000 grain weight =22 g).The lowest germination index were also observed in the treatment of severe stress and without pre-treated with SA and minimum seed weight.

Keywords: Germination, Osmotic stress, Salicylic acid, Seed weight, Wheat

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INTRODUCTION

Wheat is the major crop in Iran [13]. In Iran, 6.2 million hectares are under wheat cultivation, of which 33% is irrigated and 67% is rain fed, the irrigated wheat growing areas (2 million hectares) are located mostly in southern, central and eastern of Iran [13]. (PEG) widely used to induce water stress, is a non-ionic water polymer, which is not expected to penetrate into plant tissue rapidly [11].Polyethylene glycol (PEG) compounds have been used to simulate osmotic stress effects in Petri dish (Iin vitro) for plants to maintain uniform water potential throughout the experimental period [15]. One of the most important abiotic factors limiting plant germination and early seedling stages is water stress brought about by drought and salinity [1],which are widespread problems around the world [25]. Salinity and drought affect the plants in a similar way [10]. Reduced water potential is a common consequence of both salinity and drought [16]. Water stress acts by decreasing the percentage and rate of germination and seedling growth [4]. Seed germination and seedling establishment of winter wheat is often hindered by dry soil conditions and is a crucial factor affecting grain yield. The methods to increase plant's tolerance to stress include breeding and genetic engineering and the use of plant growth regulators (PGR). In contrast to the breeding approach which is difficult and costly, seed treatment with plant PGRs is an easy, low cost and low risk technique and an alternative approach to overcome agricultural problems. Many molecules such as salicylic acid (SA) and polyamines have been suggested as signal transducers and messengers which may have profound effects on plant growth and development [14]. SA, as a natural signal molecule has been shown to play important roles in regulating a number of physiological processes in plants. It's exogenous application has promoted plant performance under biotic and abiotic stresses. SA is a common plant-produced phenolic compound known as an important signal molecule for modulating plant responses to environmental stresses [22]. It is now clear that SA provides protection against a number of abiotic stresses such as heat stress in mustard seedlings [3], chilling damage in different plants [26], heavy metal stress in barley seedlings [18] and drought stress in wheat plants [9]. Reducing the uptake and accumulation of toxic ions and maintaining the cellular membrane integrity [7]. Studies of the relationship between seed size and early growth have been reported since early this century [27]. Seedling establishment and speed of emergence influence the time required for seedling to reach the autotrophic phase. Most investigators have reported a positive relationship between seedling vigor, improved stand establishment and higher productivity of cereal crops with plants originating from large seed compared to those grown from smaller seed. Baalbaki and [2] reported that in wheat, seed size not only influence emergence and establishment but also affected yield components and ultimately grain yield. Results also are in conformity with reference [23]in wheat. Also, these results indicated that seed size had a greater effect on percent than index of germination and emergence. With increased seed size, higher germination and emergence were determined in triticale [13].

The aim of this experiment was the effect of salicylic acid and seed weight on germination of Wheat (CV. BC ROSHAN) under different levels of osmotic stress.

MATERIALS AND METHODS

In order to study the effect of salicylic acid and seed weight on germination of Wheat (CV. BC ROSHAN) under different levels of osmotic stress, an experiment was conducted in the Research Laboratory of Shahid Chamran University of Ahvaz, Iran in 2012. The experimental design was factorial based on completely randomized design (CRD) with three replications. The treatments have been used including of Treatments of salicylic acid (SA) (1mM without and with using) 1000 weight kernel (22 and 42gr) and four levels of osmotic stress was inducted by PEG (Polyethylene glycol) (0, 4, 9 and 12 bar) (Tab 1) [19].

The wheat seed disinfected with sodium hypochloride for 5 min and 96% ethanol for 30 seconds, rinsing well with distilled water. Then he was transferred to sterile Petri dishes containing 30 seeds on filter paper. It also was used for osmotic stress PEG solution of 10 ml per Petri dish. Then placed in petri dishes and the door were closed with Para film and were in germinator at 25° C temperature. Germinated seeds were counted every 12 hours and 5 days. The germination percentage was obtained from the ratio of the number of seeds germinated after 5 days to the total number of seeds [5]was also calculated from the difference between a humdrum germination time up to 10 percent to 90 percent germination and germination. Uniformity of germination in the (absolute value) is smaller than the numbers indicate that more uniform germination [24]. Root and shoot lengths were measured after five days by the ruler and the estimation of root and shoot dry weight, were placed in the oven with a temperature of 70 ° C for 48 hours after it was weighed using digital scales with Resolution 0.01 gr. And speed of germination index was also used to calculate the equations 1 and 2, respectively [20].

Equation (1):

$$RS = \sum_{i=1}^{n} \frac{Si}{Di}$$

RS= germination rate, Si
RS= germination rate, Si = number of germinated seeds in each count, n= times counting, Di= the number of days to (n) counts
Equation (2):
Germination index = number of germinated seeds / day one of count) . . . + (Number of germinated seeds / last day of counting)

Analysis of variance was performed using PROC ANOVA of SAS (21). Each treatment was analyzed in three replications. The comparison of the means was done by Duncan test at a probability level of 5 percent.

RESULTS AND DISCUSSION

The maximum percentage of germination: The results of analysis of variance showed (Table 2) that drought stress and seed weight had significantly (at 1% probability level) effect on the maximum percentage of germination and so SA in the various treatments not caused to significant differences (at the level 5%). The germination percent decreased with increasing in PGA concentration (Table 3). This reduced was over 20% compared to control and severe stress. Pretreatment of seeds with SA under drought stress caused an increase in germination percentage (Table 3). The highest percentage of germination was obtained in treatments without stress and also treatments with 0.1 mM SA and without stress and the lowest percentage of germination were also seen in severe stress (Table 4). Seed pretreatment with SA in severe osmotic stress increased germination between 6 (seed weight 22 grams) to 9 (42 mg seed weight) percent (Table 4). The germination percentage increasing of wheat in the drought and treatment with SA was observed in the research of Soltanii et al [25].

Table 1.Values of PEG for stress levels

NO.	PEG (g/lit)	Stress level (bar)
1	0	0
2	154	-4
3	213	-9
4	251	-12

Table 2. Variance analysis the effect of salicylic acid and seed weight on wheat germination under different osmotic stress levels

Sov	Df	GP	GR	MGT	GI	LS	LR	GU	RDW	SDW	RDW/ SDW
PEG	3	798.91**	0.10**	1.11**	149.64**	1920.35**	1988.4**	1237.02**	0.001**	0.0003**	0.11**
SA	1	108.00*	0.013**	0.14**	32.02**	204.18**	295.02**	275.52**	0.0001**	0.00004**	0.02**
Ws	1	675.00**	0.013**	0.14**	24.95**	667.52**	1092.52**	212.52**	0.0005**	0.0001**	0.001*
PEG× SA	3	93.61*	0.001^{ns}	0.074*	4.20*	19.576^{ns}	14.96^{ns}	14.57*	0.00001*	0.000002*	0.023**
PEG× Ws	3	21.50^{ns}	0.002^{ns}	0.002^{ns}	0.27^{ns}	28.24*	29.79*	15.57*	0.00002**	0.00007**	0.001*
SA×Ws	1	28.08^{ns}	0.0005^{ns}	0.0004^{ns}	0.013^{ns}	1.68^{ns}	3.52^{ns}	1.68^{ns}	0.0000007^{ns}	0.000001*	0.00003^{ns}
PEG× SA× Ws	3	89.91*	0.003^{ns}	0.032*	3.50*	21.96*	1.46^{ns}	11.85*	0.00001*	0.00005**	0.003*
CV	-	5.18	7.46	7.92	9.15	9.54	7.8	4.57	9.47	7.74	8.7

***, * and ns: significant at the 1%, 5% probability levels and non significant respectively.*
PEG: poly Ethylene Glycol 6000, SA: salicylic acid, Ws: Weight Seed, CV: Coefficient Variation
GP: Germination Percent GR: Germination Rate MGT: The mean of Germination time GI: Germination Index LR: length Root LS: length Soot GU: Germination uniformity RDW: Root Dry Weight SDW: Shoot Dry Weight.

Table 3. Mean comparisons the effect of salicylic acid and seed weight on wheat germination under different osmotic stress levels.

Treatment	GP(%)	GR(in 24 hours)	MGT	GI	LS (millimeter)	LR (millimeter)	GU	RDW (gram)	SDW (gram)	RDW/ SDW %
PEG										
PEG 1	96.0a	0.55a	1.82a	13.61a	44.66a	56.75a	-33.25a	0.039a	0.023a	0.56b
PEG 2	91.0b	0.51b	1.68b	11.56b	38.91b	47.66b	-37.08b	0.034b	0.019b	0.59b
PEG 3	87.41b	0.43c	1.42c	9.06c	25.25c	40.58c	-45.83c	0.022c	0.016c	0.73a
PEG 4	76.75c	0.34d	1.13d	5.40d	16.91d	26.25d	-56.08d	0.015d	0.010d	0.74a
SA										
SA1	86.29b	0.44b	1.46b	9.09b	29.37b	40.33b	-45.45b	0.026b	0.016b	0.68a
SA2	89.29a	0.47a	1.57a	10.72a	33.50a	45.29a	-40.66a	0.029a	0.018a	0.63b
WS										
WS1	84.04b	0.44b	1.46b	9.19b	27.70b	38.04b	-45.16b	0.024b	0.015b	0.66a
WS2	91.54a	0.47a	1.57a	10.63a	35.16a	47.58a	-40.95a	0.031a	0.019a	0.65b

Means followed by similar letters in each column are not significantly different at the 5% level of probability according to Duncan test. PEG 1= 0 bar PEG 2= -4 bar PEG 3= -9 bar PEG 4= -12 bar SA1=without salicylic acid SA2= 0.1 mM salicylic acid WS1= seed weight 22g WS2= seed weight 42g.

Germination rate and Germination uniformity: The results of analysis of variance showed (Table 2) that with increasing drought stress and reduce of seed weight decreased (at 1% probability level) germination uniformity and germination rate. The SA treatment also influenced the germination rate and uniformity at 1% probability level (Table 2). According to Table 3 with increased osmotic concentration decreased germination rate and uniformity, so in compared to severe stress and control treatments that was seen 40% reduction in germination rate and 60% reduction in the uniformity of germination. Pretreatment of seeds with salicylic acid could partly compensate for damages caused by drought and low seed weight and enhances the germination rate and uniformity (Table 3). The highest germination rate and germination uniformity was in treatments without stress, maximum seed weight and seed priming with SA and also the lowest germination rate and germination uniformity was observed in the treatment of severe stress, the minimum seed weight and without SA. Seeds need to have enough water for critical activities and the start of germination. If water absorption is impaired or proceeds slowly activities inside the seed will be too slow and increases duration exiting the radicle of seeds and the expression decreases germination rate and uniformity. So with a more negative osmotic potential solution been troubled water absorption in dry conditions and reduced of germination rate and uniformity than the control treatment. Gholamin and Khayatnezhad [6], Maghsodi and Arvin [17]and Homayoun and colleagues [8]reached similar conclusions about the wheat plant.

Table 4. Mean comparisons the triple interaction of the effect of salicylic acid and seed weight on wheat germination characteristics under different osmotic stress levels

Treatment	GP (%)	GR(in 24 hours)	MGT	GI	LS (millimeter)	LR (millimeter)	GU	RDW (gram)	SDW (gram)	RDW/ SDW %
PEG× SA× Ws										
PEG1× SA1×Ws1	90.0b-f	0.53abc	1.75abc	11.21de	37.0cd	47.66cd	-38.0d	0.033de	0.019cd	0.60ef
PEG1× SA1×Ws2	99.33a	0.56ab	1.86ab	13.34bc	51.66a	59.66b	-31.66ab	0.046a	0.025b	0.54f
PEG1× SA2×Ws1	95.0abc	0.53abc	1.75abc	14.22ab	41.0c	52.33c	-33.33ab	0.036cd	0.021c	0.58f
PEG1× SA2×Ws2	99.66a	0.59a	1.94a	15.66a	49.0ab	67.33a	-30.0a	0.043ab	0.027a	0.63def
PEG2× SA1×Ws1	86.66c-f	0.48c-f	1.6c-f	10.42ef	33.66de	42.33de	-40.33de	0.03ef	0.017e	0.57f
PEG2× SA1×Ws2	94.66abc	0.51bcd	1.7bcd	12.06cd	40.33c	51.33c	-37.0cd	0.036d	0.021c	0.58f
PEG2× SA2×Ws1	87.0c-f	0.50b-e	1.67b-e	11.15de	35.66cde	43.66de	-37.0cd	0.032ed	0.018de	0.55f
PEG2× SA2×Ws2	95.66ab	0.53abc	1.76abc	12.61cd	46.0b	53.33c	-34.0bc	0.041bc	0.021c	0.53f
PEG3× SA1×Ws1	81.66fg	0.39gh	1.28gh	7.83gh	20.0fg	33.33f	-49.66g	0.018gh	0.013f	0.74abc
PEG3× SA1×Ws2	90.33b-e	0.42fg	1.4fg	8.96fg	25.0f	41.33e	-47.00fg	0.022g	0.016e	0.74abc
PEG3× SA2×Ws1	85.33def	0.44efg	1.47efg	9.21fg	25.33f	40.0e	-45.0f	0.022g	0.016e	0.72bcd
PEG3× SA2×Ws2	92.33a-d	0.46def	1.51def	10.23ef	30.66e	47.66cd	-41.66e	0.027f	0.019cd	0.72bcd
PEG4× SA1×Ws1	71.00h	0.29i	0.97i	4.09i	12.66h	20.0h	-63.0i	0.011i	0.009g	0.84a
PEG4× SA1×Ws2	76.66gh	0.33hi	1.11hi	4.82i	14.66h	27.0g	-57.0h	0.013i	0.011g	0.82ab
PEG4× SA2×Ws1	75.66gh	0.36h	1.18hi	5.37i	16.33gh	25.0gh	-55.0h	0.014hi	0.010g	0.69cde
PEG4× SA2×Ws2	83.66efg	0.38gh	1.27gh	7.34h	24.0f	33.0f	-49.33g	0.021g	0.013f	0.63def

Germination index: The results of analysis of variance (Table 2) showed that drought stress reduced the germination index at 1% probability level. The SA treatment and seed weight were also caused a significant difference (at 1% probability level) in germination index. According to table (3) increased osmotic concentration (PGA) decreased germination index (this lower is more than 60% for comparison the control and severe stress). Pretreatment with SA increased germination index in treatments (Table 3). The best germination index was obtained in the treatment of without stress, highest seed weight and treated with a solution of 0.1 mM SA and the lowest germination index were also observed in the treatment of severe stress and without pre-treated with SA and minimum seed weight (Table 4). Pretreatment of seeds with SA (14%) and the seeds with more endosperm increased the germination index (16%).

Length and dry weight of radicle and plumule: The analysis of variance results (Table 2) showed deficit stress, SA and seed weight reduced Length and dry weight of Root and shoot (in the 1% probability level). The triple interaction of stress, seed weight and SA was significant for length and weight of shoot and radicle weight. But triple interaction was not significant for root length. According to table (3) with increasing of osmotic potential decreased root and shoot length and weight, which were harder to attribute this reduction in length and weight of shoot. Hence we learn shoot growth is more sensitive to drought. Pretreatment of seeds with SA will increase root and shoot length in drought condition. Increase the length and weight of root and shoot of wheat in drought and treatment with SA was observed in the research of Maghsodi and Arvin [17]. Also SA increased length and weight of radicle and plumule in treatments of low seed weight (1000 grain weight =22 g) (Table 4).

Root/ shoot dry weight ratio: According to results of analysis of variance (Table 2) drought stress and SA were significantly for Root/ shoot dry weight ratio (1% probability level), But seed weight was affected this trait in the 5% level. The triple interaction was also significant for the trait at the 5% level (Table 2). Pretreatment seeds with SA decrease the ratio of root to shoot dry weight. In Severe drought treatments (12 - bar) the decrease value of dry weight ratio of root to shoot in the effect of SA for minimum seed weight (22 g) was nearly 18% and for maximum seed weight (42 g) was close to 24% (Table 4). Uttermost Dry weight ratio of root to shoot was obtained in treatments of severe drought.

CONCLUSION

Overall test results showed significant effect of SA (0.1 mM) concentrations for reducing the unfavorable drought stress effect and low seed endosperm. As it was observed for all traits, (in all levels of drought stress) the seed treatment with SA and minimum seed weight (22 g) were in a one of statistical category with maximum seed weight (42 g) and without pre-treated with SA (the same level of drought stress).

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