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Effect of Hormonal Priming (GA_3) and Osmopriming on Behavior of Seed Germination in Wheat (*Triticum aestivum* L.)

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Abstract

In order to evaluate the effect of different seed priming techniques on germination and early growth of two wheat cultivars (Cross Alborz and Sardari) tow experiments carried out at the Razi University Laboratory of Physiology, Faculty of Agriculture and Natural Resources, Kermanshah, Iran. This study was in two separate experiments. The experiments were factorial arranged in a completely randomized design with four replications. The first factor was seed primed for 12, 18, 24 and 30hours, the second was four concentrations of gibberellic acid (50, 100, 150and 200 ppm), PEG-$_{6000}$ (-0.3 MPa) and the third was two wheat cultivars. Seeds were primed for 12, 18, 24 and 30 hours at temperature 25°C in different solutions. Results showed that for hormonal priming, maximum shoot and root length, shoot and root dry weight and germination rate were observed at GA 50 ppm and 24 h treatmeant in Cross-Alborz and Sardari cultivars. For osmmopriming maximum shoot and root length, shoot and root dry weight and germination rate were abserved at 12 h treatment.

Keywords: wheat, osmopriming, germination, hormonal priming

1. Introduction

Winter wheat summer fallow is the dominant rotation on 1.5 million hectares in the low precipitation (<300 mm annual) dry land cropping region of the inland Pacific Northwest (PNW), Stand establishment of winter wheat planted into summer fallow is often hindered by dry seed zone conditions and is a crucial factor affecting grain yield (Bolton, 1983). Gibberellin (GA_3) and cytokinins (CKs) control different developmental processes in plants (Pospíšilová, 2003). CKs act early during shoot initiation and control meristem activity (Pospíšilová, 2003), while GA_3 are responsible for expansion and cell division in shoot elongation, flowering and seed germination. (Pospíšilová, 2003). All phytohormones exert their regulatory role in close relation with each other. Hormone signaling pathways form complex interacting network, which enables perceiving of numerous internal and external stimuli and generating respective plant responses (Pospíšilová, 2003). Additionally, exogenously applied growth regulators can alter the content of endogenous phytohormones (Pospíšilová, 2003). The biosynthesis of GA_3 is regulated by both developmental and environmental (Yamaguchi & Kamiya, 2000). Rapid and uniform field emergence is essential to achieve high yield with respect to both quantity and quality in annual crops (Parera & cantliffe, 1994; Subedi & Ma, 2005). Concentrations to good cost abolishment include improper seedbed preparation (Joshi, 1987), low quality seed, untimely sowing (van Oosterom et al., 1996), poor sowing techniques (Radford, 1983), inadequate soil moisture (Harris, 1996) and adverse soil conditions (Lee et al., 1998). Low seed zone water potential, deep planting depth and soil crusting due to rain before seedling emergence frequently impede winter wheat stands (Giri & Schillinger, 2003). So Farmers place seed as deep as 20 cm below the preplanting soil surface with deep-furrow drills to reach adequate water for germination and emergence (Schillinger et al., 1998). Seed zone water content is the controlling factor for wheat seedling emergence, but soil temperature and depth of soil covering the seed are also important (lindstrom et al., 1976; Kirby, 1993). The three early phases of germination are: (i) imbibition, (ii) lag phase, and (iii) protrusion of the radicle through the testa (Simon, 1984). Seed priming has been found a double technology to enhance rapid and uniform emergence, and to achieve high vigor and better yields in vegetables and floriculture (Farooq et al., 2007). Seed priming is a technique by which seeds are partially hydrated to a point where germination processes begin but radicle emergence does not occur (Bradford, 1986). Priming allows some of the metabolic processes necessary for germination to occur without germination take place. In priming, seeds are soaked in different solutions with high osmotic potential. This prevents the seeds from absorbing in enough water for radicle protrusion, thus suspending the seeds in the lag phase (Taylor et al., 1998). Seed priming has been commonly used to reduce the time between seed sowing and seedling emergence and to synchronize emergence (Parera & Cantliffe, 1994). Seed priming treatments have been used to accelerate the germination and seedling growth in most of the crops under normal and stress conditions. (Basra et al., 2003) reported that primed crops grew more vigorously, flowered earlier and yielded higher (Farooq et al., 2008). It has also been reported that seed priming improves emergence, stand establishment, tillering, allometry, grain and straw yields, and harvest index (Farooq et al., 2008). Typical responses to priming are faster and closer spread of times to germination and emergence over all seedbed environments and wider temperature range of germination, leading to better crop stands and hence improved yield and harvest quality, especially under sub-optimal and stress condition growing conditions in the field (Halmer, 2004). Normally priming is done either in low water potential solution (osmopriming) or in tap water (hydro priming), however, incorporation of plant growth regulators during priming have improved the performance (Afzal et al., 2002). These priming treatments which enhance seed germination include hydro priming (Afzal et al., 2002; Afzal et al., 2004), osmopriming and hormonal priming (Afzal et al., 2006). It was concluded that hydro-primed and/or seed primed in -0.5 MPa osmotic potential solution of PEG were better in phonology and yield than all other treatments (Khan et al., 2008). The seed treatment with hormone and salt solution might have increased the metabolic activity of the plant in such a direction as to result in increased uptake of N, P, K+ and Ca_2+ (Chippa & Lal, 1988). Osmotic solutions are used to impose water stress reproducibly under in vitro conditions (Pandey & Agarwal, 1998). Polyethylene glycol molecules with PEG 6000 are inert, non ionic and virtually impermeable chains that have frequently been used to induce water stress and maintain uniform water potential throughout the experimental period (Hohl & Schopfer, 1991; Lu & Neumann, 1998). Molecules of PEG 6000 are small enough to influence the osmotic potential, but large enough to not be absorbed by plants (Carpita et al., 1979; Saint-Clair, 1976). The present investigation therefore, was designed with the objective to accelerate the germination and seedling emergence of wheat seeds through different seed priming techniques.

2. Materials and Methods

The experiment carried out at the Seed Research Laboratory of Faculty of Agriculture of Razi University, Kermanshah, Iran (34º 18´ N and 47º 3´E) in 2011. Seeds of wheat (*Triticum aestivum* L.) cv. Cross alborz and Sardari were obtained from Dry land Agricultural Research Center Sararod, Kermanshah, Iran. To determine seed priming effects on germination, and seedling growth of two winter wheat cultivars (Cross Alborz and Sardari). These cultivars are among widely cultivated bread wheat cultivars under dry land farming conditions in Kermanshah, Iran. Before the start of experiment, seeds were surface sterilized in 1% sodium hypochlorite solution for 3 min, then rinsed with sterilized water and air-dried. Hormone solutions of appropriate concentrations were prepared. 250 g of seeds were soaked in 500 mL of aerated different solutions for 12, 18, 24 and 30 h and retired near to original weight with forced air under shade (Sundstrom et al., 1987). In August of 2011, 250 g of seed of both cultivars was placed in individual nylon net bags and immersed in liquid priming media. Priming media were: (1) Distilled water as control; (2) GA 50ppm; (3) GA 100ppm; (4) GA 150ppm (5) GA 200ppm; and (6) PEG_{6000} (-0.3 MPa). The study consisted of tow experiment. In experiment 1 we tested priming with gibberellin (GA3) in four concentrations (50, 100, 150 and 200 ppm) and in four time (12, 18, 24 and 30 h) on tow wheat cutivar. In experiment 2, we tested osmopriming on tow wheat cultivars using PEG $_{6000}$ (-0.3 Mpa) hn four time (12, 18, 24 and 30 h). All priming media were prepared in distilled water. Seeds were fully immersed in priming media at 20 ºC for 12, 18, 24, and 36 h under dark conditions. After treatment, seeds were given three surface washings with distilled water and retried to original weight with forced air under shade at 23± 2°C (Basra et al., 2002). All seeds were removed from priming media at the same time and then rinsed thoroughly with distilled water and hand dried lightly using blotting paper. Primed and non-primed seeds were placed in 9 cm glass petri dishes on a layer of filter paper (Whatman # 41). Twenty five seeds were placed in each Petri dish. Tow filter paper moistened with 10 ml of distilled water each Petri dish. Experimental units (164 Petri dishes) were arranged factorialy in a completely randomized design with four replications. Seeds were considered germinated when radicle protruded for 2 mm. Seed germination was recorded daily up to day 7 after the start of the experiment. Germination percentage (GP) was calculated based on following equation: (Ashraf et al., 1978)

$$\mathrm{GP} = \frac{\text{Total germinated seed after 8days}}{\text{Total number of seed}}$$

Then the mean germination rate was calculated according to the following equation: (Ellis et al., 1987)

$$\mathrm{MGR} = n / Dn$$

Where MGR is the mean germination rate, n is the number of seeds germinated on day and Dn is the number of days from the start of test. Final seedling length, radicle length, shoot length, seedling dry weights were recorded 8 days after cessation of the experiment. Data were in per experiment separated analyzed using SAS (Statistical software, SAS institute, 2002) and treatment means were compared using Duncan's multiple range test at 1% level of probability.

3. Results and Discussion

Seed priming had significant positive effect on different aspects of germination seed. Comparison of means c.v Cross Alorz (Table 3) indicated that almost of the adjectives under study including shoot length, radicle length, shoot dry weight, radicle dry weight, speed of germination were significant ($P<0.05$). Maximum shoot length, radicle length, shoot dry weight, radicle dry weight, Speed of germination and germination percentage in cv.Cross Alborz was observed in hormonal priming when the seeds primed by GA 50 ppm for 24h . In general, in different concentrations at 18 and 24 hours were better than other times (Table 1 and 3). The means comparison among various times in osmopriming treatment (Table 1) indicated that all of the adjectives except germination percentage under study including shoot length, radical length, shoot dry weight, radical dry weight and speed of germination were significant ($P<0.05$). Maximum shoot length, radicle length, shoot dry weight, and germination rate in cv.Cross Alborz was observed in osmopriming when the seeds primed by PEG (-0.3Mpa) for 12h. Comparison of means indicated that in cv. Sardari some priming treatments were significant compared to control (Table 3). In adjectives shoot length, radical length, shoot dry weight, radicle dry weight and Speed of germination in concentration 50ppm for 24hwas the best treatment ($P<0.05$) . Also means comparison between various times in osmopriming treatment (Table 1) indicated that osmmopriming maximum shoot and root length , shoot and root dry weight and germination rate were abserved at 12 h treatment. Results also showed that shoot length and radicle length in osmspriming improved compared to hormonal priming. Both GA_3 and PEG6000 seed priming improved germination rate compared to the control treatment (1 and 3). The two cultivars responded same with respect to germination percentage, speed of germination, radicle dry weight and radicle length in osmopriming (Table 2). Cross Alborz cultivar showed the highest radicle length, radicle dry weight, germination percentage and speed of germination compared to cultivar Sardari (Table 4). Germination rate and radicle length in both cultivars decreased with increasing hormone concentration (Figure 1 & 2). The results of the present study are in agreement with observations of yari et al. (2010) who reported that maximum radicle length of cultivar Sardari was obtained at 20% PEG6000 solution primed for 24h. Zareh et al. (2006) indicated that priming of wheat seed with GA_3 germination decreased but has a positive effect on shoot growth. WenGuang et al. (2009) reported that when pelleted seeds tobacco were germinated under 25°C, the priming treatment of 100 mg/L GA_3 under 25°C and 20°C for 36 hours were better than other treatments. Ghana et al. (2003) reported that seed priming has limited practical worth for enhancing emergence and yield of winter wheat planted deep into summer fallow. Pre-treatment of seeds with different type of hormones and plant growth regulators is much effective in alleviating stress effects of salinity on the plants at different stages especially at early stage and it has been shown to improve crop germination as reported earlier under salt stress (Ashraf & Foolad, 2005; Ashraf et al., 2008). The results of the present study are in agreement with observations of Chauhan et al. (2009) who reported that seeds treated with GA_3 showed significant difference with control indicated that the germination percentage decreases when the concentration increased, which shows that higher concentration inhibit germination and the longest radicle length was observed under GA_3 50 ppm. Also The results of the present study are in agreement with observations of Xingru (2009) who showed that shoot growth was promoted by GA_3 and root growth were promoted by GA_3 (in cv. Cross alborz). Afzal et al. (2004) also found that the osmopriming (jutemat) proved to be the best in reducing the time to 50% germination and mean germination time among all priming treatments. During emergence test, priming treatments i.e; osmopriming (jute mat) for 24 hours reduced the time to 50% emergence and mean emergence time. In this study, PEG6000 caused the maximum shoot and radical length, shoot and radical dry weight, speed of germination (Table 1 and 2). This result is not agreement with Moradi Dezfuli et al. (2008) who indicated that PEG6000 soaked seeds did not act well from germination point of view, possibly due to low osmotic potential of the solution or long priming duration. Sharifzadeh et al. (2006) also found that osmopriming of wheat had no positive significant effect on germination characteristics. Enzymes such as amylase, protease and lipase have a great role in initial growth and development of embryo. Every increase in activity of these enzymes results in faster initial growth of seedling therefore its establishment improvement result in higher yield. As Singh et a1. (1999) reported that osmotic priming of muskmelon with PEG result in higher amylase and dehydrogenises activity and germination rate in saline condition increased. However, Jie et al. (2002) reported that osmopriming of the wild rye seeds with PEG6000 in resulted in higher Super Oxide Dismotase (SOD) and Peroxidase (POD) activity that ultimately resulted in higher germination rate. By result of this experiment and other experiments, we can conclude that suitable priming period, osmotic and hormonal priming.

Table 1. Means comparison of germination characteristics in Cross Alborz and Sradari cultivars in osmopriming treatments compared to the control

cultivar	Times	shoot length(cm)	Radicle length(cm)	shoot dry weight(mg)	Radicle dry weight(mg)	Speed of germination	Germination percentage (%)
Cross Alborz	control	12.68^{b}	10.40^{bc}	203^{b}	117.4^{bc}	17.73^{b}	96^{a}
	12	16.62^{a}	14.10^{a}	231^{a}	141^{a}	22.43^{a}	100^{a}
	18	15.98^{a}	12.20^{b}	210^{b}	122^{b}	16.20^{c}	86^{b}
	24	15.63^{a}	11.20^{bc}	190^{c}	112^{c}	16.20^{c}	87^{b}
	30	15.3^{a}	8.70^{c}	180^{c}	87^{e}	15.03^{c}	84^{b}
Sardari	control	12.10^{b}	11.70^{bc}	165.7^{d}	111.75^{c}	18^{b}	96^{a}
	12	15.54^{a}	13.40^{a}	180.21^{c}	127^{b}	22^{a}	100^{a}
	18	16.01^{a}	9.08b^{c}	182^{c}	113.5^{c}	16^{c}	84^{b}
	24	16.60^{a}	8.58^{d}	185^{c}	100^{d}	15^{c}	80^{b}
	30	15.62^{a}	8.40^{c}	181^{c}	83^{e}	13^{d}	76^{bc}

*Values with at least one similar letter in a column, do not have significant difference (P<0.05).

Table 2. Means comparison of germination characteristics in two wheat cultivars

Cultivar wheat	shoot length(cm)	Radicle length(cm)	shoot dry weight(mg)	Radicle dry weight(mg)	Speed of germination	Germination percentage (%)
Cross Alborz	15.24^{a}	11.32^{a}	202.80^{a}	115.88^{a}	17.51^{a}	90.60^{a}
Sardari	15.17^{a}	10.23^{a}	178.78^{b}	107.05^{b}	16.80^{a}	87.20^{a}

*Values with at least one similar letter in a column, do not have significant difference (P<0.05).

Table 3. Means comparison of germination characteristics in GA_3 priming treatments in two wheat cultivars

GA Concentrations (ppm)	Times	shoot length(cm)	Radicle length(cm)	shoot dry weight(mg)	Radicle dry weight(mg)	Speed of germination	Germination percentage (%)
Cross Alborz	control	13.38^{b-h}	9.50^{b}	205.50^{c}	119^{b}	18.81^{b}	98^{ab}
50	12	13.86^{b-f}	6.80^{c-f}	156.50^{def}	73.25^{c}	10.05^{cd}	68^{d-j}
50	18	13.84^{b-f}	4.70^{def}	156.25^{def}	66.25^{cd}	12.94^{c}	81^{a-h}
50	24	16.85^{a}	12.20^{a}	232.19^{a}	160^{a}	21.05^{a}	100^{a}
50	30	12.10^{e-i}	4.77^{d-g}	143.25^{d-h}	63.50^{cde}	8.58^{cd}	60^{g-n}
100	12	14.40^{a-e}	4.27^{ed}	150.25^{d-g}	50.50^{d-g}	9.25^{cd}	66^{d-k}
100	18	15.35^{abc}	5.17^{d-g}	150.50^{d-g}	52.25^{c-f}	10.24^{cd}	62^{e-m}
100	24	16.21^{ab}	4.22^{d-g}	142^{d-h}	52^{c-f}	20.63^{a}	94^{abc}
100	30	13.66^{b-g}	4.07^{e-i}	153.25^{def}	55.75^{c-f}	9.60^{cd}	63^{e-l}
150	12	13.36^{b-h}	3.11^{f-j}	116^{df-i}	38.50^{f-j}	10.43^{cd}	57^{g-o}
150	18	15.20^{abc}	3.55^{f-j}	149.25^{d-g}	41^{f-i}	8.67^{cd}	76^{a-h}
150	24	15.54^{abc}	4.49^{edf}	164de	27^{h-m}	21.02^{a}	97^{ab}
150	30	14.76a^{-d}	2.96^{edf}	117^{e-i}	42.75^{e-i}	8.33^{cd}	54^{h-o}
200	12	13.42^{c-g}	4.40^{edf}	120.75^{e-i}	42^{e-h}	8.67^{cd}	59^{g-o}
200	18	13.84^{b-f}	3.11^{f-j}	137.25^{d-h}	36.50^{f-k}	9.07^{cd}	70^{b-i}
200	24	14.36^{a-e}	2.25^{g-k}	106.25^{g-j}	22.50^{i-m}	9.07^{cd}	57^{g-o}
200	30	13.59^{c-g}	3.55^{e-i}	82^{i-l}	33.50^{f-k}	9.67^{cd}	58^{g-o}
Sardari	control	11.32^{f-k}	9.43^{b}	168.25^{cd}	110.75^{b}	18.18^{b}	96^{ab}
50	12	10.81^{h-k}	2.52^{g-k}	106.25^{ghi}	45.50^{d-g}	5.68^{d}	33no
50	18	12.34^{d-i}	5.59^{cd}	125.50^{d-i}	66.75^{cd}	7.18^{cd}	43^{i-o}
50	24	15.58^{abc}	10.72^{ab}	221.25^{b}	150^{a}	19.69^{a}	91^{a-d}
50	30	11.73^{e-i}	2.42^{h-k}	92^{ijk}	37^{f-k}	6.13^{d}	40^{k-o}
100	12	9.80^{jk}	2.03^{ijk}	54.50^{k-n}	20.25^{i-m}	6.30^{d}	37^{l-o}
100	18	10.65^{ijk}	1.59^{jk}	65^{j-n}	24.25^{h-m}	6.35^{d}	36^{l-o}
100	24	11.82^{e-i}	1.75^{ijk}	68.75^{j-m}	24.75^{h-m}	12.94^{c}	81^{a-h}
100	30	10.78^{h-k}	0.69^{k}	41.25^{lmn}	10.50^{m}	5.82^{d}	31^{o}
150	12	9.1^{k}	0.98^{k}	44^{lmn}	15^{klm}	6.65^{cd}	42^{j-o}
150	18	10.79^{h-k}	1.07^{k}	81.75^{ijk}	24.75^{h-m}	7.61^{cd}	38^{l-o}
150	24	12.14^{e-i}	2.41^{h-k}	89^{ijk}	31.75^{h-m}	9.07^{cd}	70^{b-i}
150	30	11.97^{e-i}	1.98^{ijk}	41^{lmn}	10.07^{m}	5.86^{d}	34^{mn}
200	12	8.96^{k}	0.88^{k}	27^{nm}	13^{lm}	9.40^{cd}	43^{i-o}
200	18	10.78^{h-k}	1.40^{jk}	39.75^{lmn}	11.25^{lm}	6.80^{cd}	54^{h-o}
200	24	11.18^{g-k}	1.44^{jk}	40.50^{lmn}	16.25^{j-m}	8.33^{cd}	55^{h-o}
200	30	6.14^{l}	0.73^{k}	23^{n}	10.25^{m}	7.27^{cd}	41^{j-o}

*Values with at least one similar letter in column, do not have significant difference (P<0.05).

Table 4. Means comparison of cv. Cross Alborz compared with cv. Sradari in hormonal priming treatments

Cultivar wheat	shoot length(cm)	Radicle length(cm)	shoot dry weight(mg)	Radicle dry weight(mg)	Speed of germination	Germination percentage (%)
Cross Alborz	15.29^{a}	11.79^{a}	160.3^{a}	67.42^{a}	16.79^{a}	73.35^{a}
Sardari	11.94^{b}	8.50^{b}	124.2^{b}	52.10^{b}	13.30^{b}	53.82^{b}

*Values with at least one similar letter in a column, do not have significant difference (P<0.05).

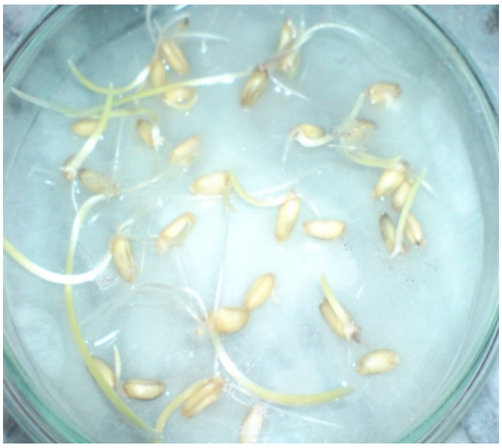

Figure 1. Inhibitory effect of GA3 (200ppm) in high concentrations on seed germination wheat

Figure 2. Positive effect of GA3 (50ppm) in low concentrations on seed germination wheat

4. Conclusion

Our results showed significant improvement in germination and early growth of wheat (cv. Cross Alborz and cv. Sardari) due to hormonal priming treatment and osmopriming Compared with control. The results of priming among species, varieties, and seed lots have been variable (Heydecker, 1977). Because of this variability in response, Bradford (1986) has suggested that treatment conditions must be optimized for each seed lot. However, maximum priming can be achieved in a particular seed lot through various combinations of temperature, water potential and treatment duration.

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