Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not. Bot. Hort. Agrobot. Cluj 38 (1) 2010, 109-113

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Effects of Hydro-Priming Duration on Seedling Vigour and Grain Yield of Pinto Bean (*Phaseolus vulgaris* L.) Cultivars

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Abstract

Seeds of pinto bean (*Phaseolus vulgaris* L.) cultivars ('Talash', 'COS_{16}' and 'Khomain') were divided into four sub-samples, one of which was kept as control (non-primed, P_1) and three other samples were soaked in distilled water at 20°C for 7 (P_2), 14 (P_3) and 21 (P_4) hours and then dried back to initial moisture content. In the laboratory, the lowest mean germination time and the highest germination percentage and seedling dry weight were achieved with P_2, which was not significantly different from P_3. The mean time of seed germination for 'Khomain' was significantly higher than that for 'Talash' and 'COS_{16}'. In the field, 1000 grain weight of 'Khomain' was significantly higher than that of other cultivars, but mean grains/plant, grains/m^2, grain yield/plant and grain yield/m^2 of 'COS_{16}' and 'Talash' were significantly higher than those of 'Khomain'. Therefore, grains/plant was the most important yield component affecting grain yield of pinto bean cultivars. Hydro-priming for 7 and 14 hours resulted in lower mean emergence time and higher seedling emergence percentage, grains/m^2 and grain yield/m^2, compared with P_1 and P_4. Grains/plant, 1000 grain weight and grain yield per plant were not significantly affected by hydro-priming. However, hydro-priming for 7 and 14 hours improved grain yield per unit area indirectly through enhancing seedling establishment and grains/m^2. The extended priming duration negatively affected laboratory and field performance of pinto bean cultivars.

Keywords: germination, grain yield, priming, seedling emergence, vigour

Introduction

Germination and seedling establishment are critical stages in the plant life cycle. In crop production, stand establishment determines plant density, uniformity and management options (Cheng and Bradford, 1999). A method to improve the rate and uniformity of germination is the priming or physiological advancement of the seed lot (Finch-Savage, 2004; Halmer, 2004). Seed priming is the soaking of seeds in a solution of any priming agent followed by drying of seeds that initiates germination related processes without radicle emergence (McDonald, 1999). Various seed priming techniques have been developed, including hydro-priming (soaking in water), halo-priming (soaking in inorganic salt solutions), osmo-priming (soaking in solutions of different organic osmotica), thermo-priming (treatment of seeds with low or high temperatures), solid matrix priming (treatment of seed with solid matrices) and bio-priming (hydration using biological compounds) (Ashraf and Foolad, 2005).

Seed priming has been used to improve germination, reduce seedling emergence time and improve stand establishment and yield (Khan, 1992). The beneficial effects of priming have been demonstrated for many field crops such as barley (Abdulrahmani *et al.*, 2007), maize (Parera and Cantliffe, 1994), lentil (Ghassemi-Golezani *et al.*, 2008a), chickpea (Ghassemi-Golezani *et al.*, 2008b), sugar beet (Sadeghian and Yavari, 2004) and sunflower (Singh, 1995). These effects of priming are associated with the repairing and building up of nucleic acids, increased synthesis of proteins as well as the repairing of membranes (McDonald, 2000). Priming also enhances the activities of anti-oxidative enzymes in treated seeds (McDonald, 1999; Wang *et al.*, 2003; Hsu *et al.*, 2003). Moreover, priming increases the activities of glyoxysome enzymes in primed bitter gourd seeds (Lin and Sung, 2001).

Earlier works showed that the success of seed priming is influenced by the complex interaction of factors including plant species, water potentiality of priming agent, duration of priming, temperature, seed vigour and storage conditions of the primed seeds (Parera and Cantliffe, 1994). Since hydro-priming is a very simple, economical and environmental friendly type of seed priming (Thornton and Powell, 1992; Ghassemi-Golezani *et al.*, 2008a), the objective of this research is to investigate the effects of hydro-priming duration on seed invigoration and field performance of pinto bean cultivars.

Materials and methods

Seeds of pinto bean (*Phaseolus vulgaris* L.) cultivars ('Talash', 'COS_{16}' and 'Khomain') were divided into four sub-samples, one of which was kept as control (non-primed, P_1) and three other samples were soaked in distilled water at 20°C for 7 (P_2), 14 (P_3) and 21 (P_4) hours and then dried back to initial moisture content at a room temperature of 20-22°C. Laboratory tests were carried out as factorial, based on RCB design at the Seed Technology Laboratory of the University of Tabriz, Iran. Four replicates of 25 seeds were placed between moist filter papers and germinated in an incubator adjusted at 20°C for 9 days. Germination (protrusion of radicle by 2 mm) was recorded in daily intervals. At the end, percentage of normal seedlings and seedling dry weight were determined. Mean germination time (MGT) was calculated based on the following equation of Ellis and Roberts (1980):

$$MGT = \frac{\sum (D * n)}{\sum n}$$

where *n* is the number of seeds germinated on day *D* and *D* is the number of days counted from the beginning of the test.

Four replications of 50 pre-weighed seeds of each seed lot were soaked in 250 ml deionized water in plastic containers covered with caps to prevent evaporation loss and entry of foreign matter. A container of deionized water without seeds was prepared as the control. All the containers were then incubated at 20°C for 24 hours. Conductivity was measured with an electrical conductivity meter (WTW LF90). The results were expressed on a seed dry-weight basis (µS/cm/g).

The field experiment was conducted at the Research Farm of the University of Tabriz (Latitude 38˚05' N, Longitude 46˚17' E, Altitude 1360 m above sea level) in 2008. All the seeds were treated with benomyl at a rate of 2 g kg^{-1} before sowing. Seeds were hand sown in about 5 cm depth with a density of 50 seeds m^{-2} on 19th April 2008.

Tab. 1. Means of pinto bean seed quality parameters affected by hydro-priming duration and cultivar

Treatments	Electrical conductivity (µS/cm/g)	Mean germination time (day)	Germination percentage	Seedling dry weight (mg)
Hydro-priming				
P_1	10.51^a	3.03^a	95.33^b	84.92^b
P_2	9.65^b	2.51^b	99.00^a	94.42^a
P_3	9.60^b	2.65^b	97.00^{ab}	91.75^a
P_4	9.50^b	3.07^a	94.67^b	83.67^b
Cultivar				
'Talash'	9.84^a	2.77^b	96.25^a	88.62^a
'COS_{16}'	9.75^a	2.73^b	96.75^a	88.87^a
'Khomain'	9.83^a	2.95^a	96.50^a	88.56^a

different letters at each column for each treatment indicating significant difference at p≤0.05. P_1, P_2, P_3 and P_4: non-primed and hydro-primed seeds for 7, 14 and 21 h, respectively.

Each plot consisted of 6 rows with 4 m length, spaced 25 cm apart. The experiment was arranged as factorial, based on RCB design with three replications. All plots were irrigated immediately after sowing and then fertilized with 30 kg ha^{-1} urea (46% N). Subsequent irrigations were carried out after 70 mm evaporation from class A pan. Weeds were controlled by hand during crop growth and development. Plants were protected from heliothis caterpillar attack by spraying Diazinon at a rate of 2 ml l^{-1} before flowering.

Seedling emergence was recorded in daily intervals up to final establishment in each plot. Subsequently, mean emergence time and percentage of seedling emergence were calculated. At maturity, 10 plants were harvested from each plot and grains per plant, grains/m^2 and 1000 grain weight were determined. Finally, plants of 1 m^2 in the middle part of each plot were harvested and grain yield per plant and grain yield/m^2 were recorded.

Analysis of variance of the data in the experimental design and comparison of means at p≤0.05 were carried out, using MSTATC software.

Results and discussion

The analysis of variance of the laboratory data showed significant effects of hydro-priming duration on electrical conductivity of seed leachates, germination percentage, mean germination time and seedling dry weight (P≤ 0.01). The electrical conductivity of non-primed seeds (P_1) was significantly higher than that of hydro-primed seeds (P_2, P_3 and P_4). However, hydro-priming duration had no significant effect on the electrical conductivity of seed leachates (Tab. 1). The lowest germination time and the highest germination percentage and seedling dry weight were achieved with P_2 (hydro-priming for 7 h), which was not significantly different from P_3 (hydro-priming for 14 h). These traits were statistically similar for P_1 (non- primed seeds) and P_4 (hydro-priming for 21 h) (Tab. 1).

Electerical conductivity, germination percentage and seedling dry weight were not significantly affected by cultivar (P≤ 0.05), but the effect of cultivar on mean germination time was significant (P≤ 0.05). Mean germination time of 'Khomain' seeds was significantly higher than that of 'COS_{16}' and 'Talash' seeds. However, the difference in mean germination time between 'COS_{16}' and 'Talash' seeds was not significant (Tab. 1).

The effects of hydro-priming on seedling emergence percentage, mean emergence time, grains/m^2 and grain yield/m^2 in the field were significant, but the effect of hydro-priming on grains per plant, 1000 grain weight and grain yield per plant was not significant (Tab. 2). Seedling emergence percentage for P_2 (hydro-priming for 7 h) and P_3 (hydro-priming for 14 h) was significantly higher than that for P_1 (non-primed seeds) and P_4 (hydro-priming for 21 h). Seedlings of P_2 emerged about 4 and 5 days earlier than those of P_1 and P_4, respectively. However, mean emergence time for P_2 and P_3 was statistically similar (Tab. 3).

Although, grains per plant, 1000 grain weight and grain yield per plant were not significantly influenced by hydro-priming, grains/m^2 and grain yield/m^2 for P_2 and P_3 treatments were considerably higher than those for P_1 and P_4 treatments (Tab. 3).

Seedling emergence percentage and mean emergence time were not significantly affected by cultivar, but the effect of cultivar on grains per plant, grains/m^2, 1000 grain weight, grain yield per plant and grain yield/m^2 were significant (Tab. 2). Mean grains per plant, grains/m^2, grain yield per plant and grain yield/m2 of 'COS$_{16}$' and 'Talash' were significantly higher than those of 'Khomain'. In contrast 'Khomain' produced the largest grains among the cultivars (Tab. 3).

Hydro-priming for 7 and 14 hours improved seed quality of pinto bean cultivars in the laboratory. This improvement was reflected in low electrical conductivity (EC) of seed leachates and mean germination time and high germination percentage and seedling dry weight (Tab. 1). Similar results were reported for wheat (Basra *et al*., 2003), barley (Abdulrahmani *et al*., 2007), lentil (Ghassemi-Golezani *et al*., 2008a) and chickpea (Ghassemi-Golezani *et al*., 2008b). It was shown that seed priming causes metabolic changes in germinating seed, such as cell cycle related events (De Castro *et al*., 2000), endosperm weakening by hydrolase activities (Groot *et al*., 1988; Bradford *et al*., 2000) and mobilization of storage proteins (Job *et al*., 2000). However, positive effects of seed priming on seed invigoration depends on priming duration (Ashraf and Foolad, 2005; Ghassemi-Golezani *et al*., 2008b). When seeds imbibe, the water content reaches a plateau and changes little until radicle emergence (Bradford, 1986). Priming up to this point can have a positive effect, while extended priming duration as shown in this research (Tab. 1) will negatively affect germination.

Better emergence of seedlings from hydro-primed seeds for 7 and 14 hours (Tab. 3) suggests that proper priming duration can ensure optimum plant establishment of pinto bean cultivars in the field. Under field conditions, the rate of seedling emergence and establishment is critical (Kaufmann and Eckard, 1977). Rapid and uniform field emergence of seedlings are two essential pre-requisites to increase yield, quality and ultimately profit in annual crops (Finch-Savage, 1993). Rapid emergence of seedlings could lead to the production of vigorous plants (Ghassemi-Golezani *et al*., 2008b). The efficiency of seed hydro-priming for better seedling emergence is also reported in barley (Ab-

Tab. 2. Analysis of variance of the effects of hydro-priming duration on field performance of pinto bean cultivars

Source of variation	Degrees of freedom	MS							
		Seedling emergence percentage	Mean emergence time	Grains per plant	Grains/m^2	1000 Grain weight	Grain yield per plant	Grain yield/m^2
Replication	2	148.78*	3.22	11.63	20226.08	30.44	0.19	1504.35
Hydro-priming(Hp)	3	434.19**	47.36**	4.19	106344.03**	14.85	0.01	4439.26*
Cultivar(C)	2	1.78	3.31	928.07**	705693.58**	8103.02**	22.95**	21619.84**
C×Hp	6	2.52	1.58	0.07	2605.36	3.24	0.03	51.77
Error	22	36. 90	8.55	28.88	14821.33	897.16	0.40	1145.81
%CV	-	10.06	10.37	15.81	12.40	9.19	8.31	14.99

*, **: statistically significant at p≤0.05 and p≤0.01, respectively

Tab. 3. Means of pinto bean field traits influenced by hydro-priming duration and cultivar

Treatments	Seedling emergence percentage	Mean emergence time (day)	Grains per plant	Grains/m^2	1000 Grain weight(g)	Grain yield per plant(g)	Grain yield/m^2 (g m^{-2})
Hydro-priming							
P_1	55.11^b	29.68^a	33.52^a	896.2^b	326.8^a	7.553^a	209.1^b
P_2	68.22^a	25.75^b	34.94^a	1106.0^a	324.7^a	7.630^a	251.7^a
P_3	64.22^a	26.80^b	34.06^a	1040.0^a	324.7^a	7.612^a	236.8^{ab}
P_4	54.00^b	30.57^a	33.48^a	884.7^b	327.0^a	7.581^a	205.7^b
Cultivar							
'Talash'	60.16^a	27.92^a	38.63^a	1115.0^a	317.0^b	8.385^a	248.1^a
'COS$_{16}$'	60.83^a	27.87^a	39.52^a	1128.0^a	305.4^b	8.400^a	252.5^a
'Khomain'	60.16^a	28.80^a	23.86^b	701.7^b	355.1^a	5.997^b	176.9^b

different letters at each column for each treatment indicating a significant difference at p≤0.05; P_1, P_2, P_3 and P_4: non-primed and hydro-primed seeds for 7, 14 and 21 h, respectively dulrahmani *et al*., 2007), lentil (Ghassemi-Golezani *et al*., 2008a) and chickpea (Ghassemi-Golezani *et al*., 2008b).

Ghassemi-Golezani *et al.* (2008b) showed that hydro-priming of chickpea seeds for 8, 16 and 24 hours enhanced seedling emergence, biological yield, grains/m^2 and grain yield per unit area, but the best improvement was achieved with 16 hours priming duration. Beneficial effects of hydro-priming on grain yield were also reported in wheat (Kahlon *et al*., 1992), safflower (Bastia *et al*., 1999), sunflower (Hussain *et al*., 2006) and rice (Farooq *et al*., 2006). The results of our research indicated that early emergence of seedlings from P_2 (hydro-priming for 7 h) and P_3 (hydro-priming for 14 h) seeds was compensated by efficient use of individual plants of P_1 (non-primed seeds) and P_4 (hydro-priming for 21 h) treatments from light and soil resources during growth and development, due to poor stand establishment. Consequently, grains per plant, 1000 grain weight and grain yield per plant were statistically similar for plants from non-primed (P_1) and hydro-primed (P_2, P_3 and P_4) seeds. Therefore, the superiority of hydro-priming for 7 and 14 hours (P_2 and P_3, respectively) in improving grain yield per unit area indirectly resulted from higher seedling establishment and production of more grains per unit area, compared with P_1 and P_4 treatments (Tab. 3).

Although mean grain weight of 'Talash' and 'COS_{16}' cultivars was lower than that of 'Khomain', they produced more grains per plant and per m^2, leading to higher grain yield per plant and per unit area (Tab. 3). Therefore, grains per plant are more important than 1000 grain weight in determining the yield potential of pinto bean cultivars in the field. Similar results were reported in black bean (Nielsen and Nelson, 1998), common bean (Ghassemi-Golezani and Mardfar, 2008) and sesame (Eskandari *et al*., 2009).

Conclusions

Hydro-priming for 7 and 14 hours can be successfully applied to enhanced seed and seedling vigour, stand establishment and grain yield of pinto bean cultivars in the field. Yield improvement is mainly achieved indirectly through increasing seedling establishment and grains/m^2. The number of grains per plant is the most important yield component in determining grain yield potential of pinto bean cultivars in the field.

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