SEED PRIMING IMPROVES THE GERMINATION AND FIELD PERFORMANCE OF SOYBEAN UNDER DROUGHT STRESS

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ABSTRACT

Drought stress is a great challenge to agricultural production worldwide. Seed priming has the potential to improve tolerance in crops against water deficit. This study, consisting of two seprate experiments was conducted to evaluate the role of various seed priming in mitigating the adverse effect of water deficit on germination, biochemical and yield parameters of two soybean cultivars viz. DPX, drought tolerant, and Wiliams, drought sensitive. Seeds either subjected to hormonal priming, osmopriming, halopriming and hydropriming; dry seed being as control. Crops were subjected to 2 and 3 different moisture regimes in growth chamber and field conditions, respectively. Under water deficit, the germination and field performance of tested soybean cultivars was hampered. Seed priming treatments improved the physiological, biochemical, yield and yield parameters under both the optimal and water deficit. Hydropriming for 12 h and hormonal priming with gibberlic acid for 14 h with cultivar DPX was best in this regard. In conclusion, hydropriming, being simple, economical and safe, is recommended to improve germination and seed yield of soybean under both optimum as well as limited water conditions.

Keywords: Biochemical parameters; Priming treatments; Vigour index; Water deficit; Yield.

INTRODUCTION

Soybean (*Glycine max* (L.) Merr) is one of the most important contributors to protein and vegetable oils. Water deficit during growth stages can limit soybean yield (Candogan *et al*., 2013).

Seed priming is one of the farmer's friendly techniques suggested by many researchers for better crop stand establishment and growth under optimal and suboptimal conditions. Seed priming is a pre-sowing treatment in osmotic solutionor with solid-matrix carriers that allows seeds to absorb water and solutes to proceed to the initial stages of germination but prevents radicle protrusion through the seedcoat (Heydecker *et al*. 1973).Seeds can be primed in different media such aswater (hydropriming), aerated low water potential solutions such as polyethylene glycol, or salt solution (KNO_3, KCl,K_3PO_4, KH_2PO_4, $MgSO_4$, $CaCl_2$and NaCl) (osmopriming),solid matrix(matripriming) and priming with plant growth regulators and polyamines (Farooq *et al*. 2008).Seeds are then removed, rinsed 2–3 times and re-driednearer to original weight topermit routine handling and storage if required (Farooq *et al*. 2007). Seed priming has been reported to enhance vigor, rapidanduni form emergence, and improved yields of vegetable, ornamental (Bruggink *et al*. 1999) and field crops (Giri and Schillinger 2003;Murungu *et al*. 2004)underwide ranging field conditions.

Many physiological, biochemical and molecular changes have been found as the possible basisof this performance. These include earlier and increased protein synthesis, aldolase, and isocitrate lyase activity and greaterglucose-6-phosphate dehydrogenase and decreased alcoholdehydrogenase activity in primed sweet corn and sunflower seeds, leading to improved reservemobilization (Wahid *et al*. 2008). In sweetcorn (*Zeamays* L.) seeds, osmopriming and matripriming increased α- and β-amylase activity (Sung andChang1993) and led to increased protein synthesis in wheat (*Triticum aestivum* L.) seeds (Dell'Aquilla and Spada 1992). Koehler *et al*. (1997) reported that osmoprimed corn seeds had higher protein and nucleic acids synthesis. Protein synthesis occurred in the embryo and storage organs of leek (*Allium ampeloprasum*) seeds during osmopriming (Davison and Bray1991). During priming, de novo synthesis of α-amylase is also documented (Lee and Kim 2000), since increased α-amylase activity has been correlated with improved metabolic activities and higher seed vigor (Basra *et al*.2006). Sung and Chang(1993) showed that matripriming of sweet corn seeds with vermiculite enhanced the activities of several lipid peroxide-scavenging enzymes. Higher metabolic activities have been taken as index of higher vigor by many researchers (Basra *et al*. 2006; Wahid *et al*.2008).

For this study, we hypothesized that seed priming might reduce the deleterious effects of drought stress on soybean germination and its field performance. The main objective of this study was to check the influence of seed priming treatments on germination, biochemical traits and seed yield of soybean grown under optimal and suboptimal conditions.

MATERIALS AND METHODS

The study consisted of two independent experiments. Experiment I was laid out in completely randomized design (CRD) in factorial arrangement a controlled environment (growth chamber). Experiment II was conducted in randomized complete block design (RCBD) in factorial arrangement under field condition at the College of Agriculture, Payame Noor University, Gorgan, Golestan province, Iran during 2014-2015. Experiment I consisted of two growth conditions (normal and drought stress),15 seed priming treatments (Table 1), and two soybean cultivars(Williams, drought sensitive and DPX, drought tolerant). After seed priming, the seeds were wiped and air dried to achieved the original moisture content and were stored in at 4°C temperature and 30 ±2% relative humidity until subsequent used. The seeds of both cultivars were obtained from the oil seed Research Institute Gorgan, Golestan province, Iran.

Laboratory Experiment: Polyethyleneglycol (PEG) 6000 was used to induce drought stress. Osmotic potential of the PEG solution was -0.75MPa. Distilled water was used for normal condition.

A total of 200 seeds in four replicates (50 seeds per replicate) were placed between two germination papers and incubated in a seed germinator in dark at constant 25±1 ◦ C temperature. Seeds were considered germinated when at least 2 mm long radicle raised through the seed coat. The terminal germination percentage was computed on the 8th day of planting the seeds (ISTA, 2011). Germination counts were taken every 12 h and mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981).

For calculating the seedling vigour and vigour index, a total of 60 seeds in four replicates (15 seeds per replicate) were sown between papers and were maintained in a germinator, for seven days at a constant 25°C temperature. After every 3 days the germination papers of the petri dishes were replaced to prevent the accumulation of PEG in petri dishes. The root and shoot vigour were evaluated as the sum of total shoot length (cm) and root length (cm) of all the seedlings of a replicate divided by 15. The seedling vigour was displayed as the sum of mean of the shoot and root vigour. Vigour index was obtained by multiplying the seedling vigour (shoot and root length) and germination percentage. According to highest vigour index out of 15, four promising seed priming treatments were selected from each priming treatment utilized along with non-primed seeds for further biochemical and field performance studies. The promising treatments selected were P1 (hydropriming for 12 h), P3 (osmopriming with 13.5% solution of PEG 6000 for 24 h), P8 (halopriming with 1% solution of Potassium nitrate for 18 h), and P11 (hormonal priming with gibberlic acid50ppm for 14 h) along with (P0) (Table 1).

Membrane stability of primed seeds was assessed by electrical conductivity of the seed leachates. Loss of membrane integrity (a sign of cellular damage) was evaluated in terms of ion (electrolyte) leakage from the seed measuring conductivity of the bathing medium, as per the method of Duke and Kenyon (1993).

This includes measuring the content of a product of lipid peroxidation i.e. malonealdehyde (MDA) by the colorimetric method (Heathand Packer, 1968).

The three antioxidant enzymes peroxidase, catalase and superoxide dismutase wereassayed by the methods described by Shannon *et al*. (1966), Chance and Maehly (1955) and Beauchamp and Fridovich (1971), respectively.

The α-amylase and β- were estimated by the method described by Chrispeels and Varner (1967) and Bernfeld (1955) using starch as the substrate, respectively. Theacid and alkaline phosphatases were determined using p-nitrophenol phosphate as the substrate (Leigh and Walker, 1980).

Assay of dehydrogenase was in triplicate, using a standard procedure (Kumar *et al*., 2000) in which the conversion of NAD to NADH was determined spectrophotometrically.

Total chlorophyll and free proline was computed according to the methods given by Nagata and Yamashita (1992) and Bates *et al*. (1973), respectively, also the relative water contents (RWC) were measured following the method of Turner (1981).

Field Experiment: On the basis of higher vigour index, seeds with selected priming treatments (P0- P1- P3- P8- P11) were used to evaluate their performance. Research Farm, at the College of Agriculture, (36 50´N; 54 22´E, altitude: 14 m) characterized by a typicalsemi-arid climate with hot dry summer and cool winter, for two growing seasons i.e. 2014 and 2015.

Irrigations were done when a quantity of evaporated water from class "A pan" evaporation reached 50 (I1; optimum conditions of irrigation), 100 (I2; mild water deficit) and 150 (I3; high water deficit) mm, respectively. Total irrigation water applied in optimum irrigation (I1), mild and high drought stress levels (I2, I3) were 465, 234.5 and 146.56 m^3, respectively. After sowing time, the plots were irrigated based on their prescribed treatment.

Data for field emergence, 1000-seed weight, number of pods per plants and seed yield are presented here. Before thinning after 15 days of sowing, field emergence of seeds [(No. of seeds emerged/100) × 100] were recorded.

All data were statistically analyzed using SAS software. Since the trend was alike in both growing seasons, the data recorded on field of 2years, were pooled to get a average value and was statistically analysed. Difference among treatment means ($p \leq 0.05$) were evaluated by using least significant difference test (LSD).

RESULTS

Laboratory Experiment: There was significant effect of drought stress and seed priming on the germination attributes (Table 2). Both cultivars also differed for germination attributes. Drought stress significantly reduced germination and early seedling growth of both soybean cultivars while seed priming improved germination and related attributes i.e. terminal germination percentage and mean germination time and time taken to 50% germination in comparison to non-primed both under normal and drought condition (Table 2).P1, P2, P3and P11were equally effective to increase the germination in comparison to non-primed seeds i.e. control, under both normal and drought condition (Table 2).Under drought condition, halopriming decreased final germination for both soybean cultivars in comparison to non-primed seed while reduced germination time for both soybean cultivars than non-primed seed as indicated by lower MGT values(Table 2).This finding can be useful for farmers to have better seedling establishment in DPX under undesirable conditions. Mean germination time also reduced considerably due to above seed priming treatments from 4.1 days to 1.1 days (in combined over normal and drought conditions and two cultivars). Although, all the hydropriming treatments were beneficial to increase the seed germination under both drought and normal conditions, but only P2decreased the MGT remarkably, which was atpar with P3and P11 (Table 2). This trait helps soybean to emerge quickly and complete canopy at early growth.

Seedling vigour and vigour index of both cultivars was also decreased with drought stress (Table 2). In general, considering all the seed priming treatments together and on the basis of seed germination and seedling vigour, remarkably positive effects of P1, P3, P8 and P11 were recorded for both normal and drought grown seedlings cultivars (Table 2). The highest seedling vigour (28.2 cm) was recorded in P5followed byP11 and P1 as 27.2 and 27.1 cm, for cultivar DPX under normal conditions, respectively (Table 2). Significantly, higher vigour index was showed in P1 andP11 in comparison to other seed priming treatments as well as non-primed seeds in combined over normal and drought conditions and two cultivars (Table 2).

Drought stress significantly disturbed the membrane stability and comparatively higher electrical conductivity was found for drought sensitive than drought tolerant cultivar (Table 3).Although there was no difference for electrical conductivity between seed priming treatments under normal condition in both soybean cultivars (Table 3); under drought, P1 substantially increased the membrane stability (the lowest EC between seed priming treatments) in both soybean cultivars (Table 3).

Data on lipid peroxidation as affected by various seed priming treatments, drought stress and cultivars are shown in Table 3. Malondialdehyde contents (MDA), an index of lipid peroxidation, were substantially increased under drought in both soybean cultivars, but the lowest MDA accumulation was observed in cultivar DPX than cultivar Wiliams (Table 3). The data revealed that the significantly low value of lipid peroxidation was recorded in P3 in comparison to other treatments (Table 3). The seeds treated inP8 had increased lipid peroxidation value which was significantly higher than P0 in both conditions.

Seed priming, stress and cultivars had significant effect on all three antioxidants enzyme tested (Table 3). The most antioxidant enzymes activity was achieved under drought condition compared to the normal condition (Table 3).Among the cultivars, cultivar DPX had more antioxidant activity than cultivar Wiliams, indicating that this cultivar can be more tolerance to drought stress (Table 3). Seed priming treatments increased the antioxidant enzymes activity in this experiment; although, the enhancement in the activity of the three enzymes was observed due to different treatments such as hydropriming for (P1) for catalase, osmopriming (P3) for Ascorbate peroxidase and hormonal priming (P11) for superoxide dismutase, the negative influence exhibited by halopriming (P8) was common for all the enzymes (Table 3).

Drought stress significantly decreased the α-amylase and β-amylase and acid and alkaline phosphatase activityin soybean compared with normal conditions (Tables 3 and4).Seed priming treatments were effective in improving the physiological attributes under water stress. A close perusal of the data revealed that the maximum activity of α- and β-amylases was recorded in P1 which was statistically higher than that of other treatments under both conditions in both cultivars (Table 3). The α-amylase activity was similar to that in seeds conditioned in P11.All the seed priming treatments increased the acid and alkaline phosphatase activities in the seeds (Tables 3 and 4). It is evident from the data presented in table 3 that the activity of acid phosphatase was maximum in the seeds conditioned in P11 whereas maximum activity of alkaline phosphatase was recorded in hydroprimed seeds (P1).Although, there was no considerable difference in physiological attributes in both cultivars in normal condition, but, α- and β-amylase and acid and alkaline phosphatase activity was higher in cultivar DPX in drought condition (Tables 3 and4).

The activity of this enzyme was increased under drought in both cultivars (Table 4). All the seed priming treatments tested in present study, except P8, increased the activity of this enzyme. The hydroprimed seeds (P1) exhibited the highest activity of dehydrogenase which was significantly higher than other treatments (Table 4). There was difference between both cultivars for dehydrogenase activity; however, under normal condition, different activities of this enzyme were not considerable in both cultivars, but under drought stress condition, activities were higher in cultivar DPX (Table 4).The present study suggests that drought tolerance ability in soybean is associated with increased dehydrogenase activities under drought condition.

Seed priming, stress and cultivar had significant effect on proline, chlorophyll and relative water content, the most proline content was achieved under drought condition compared to the normal condition (Table 4). Seed priming treatments decreased proline content in this experiment; this means without stress treatments had more proline level than seed priming agents. Halopriming with Potassium nitrate (P8) resulted in higher proline content compared to the other seed priming treatments; this might have been due to the role of nitrogen in proline. Under normal condition, variations among cultivars were low, among the cultivars; cultivar DPX had more proline content than cultivar Wiliams, indicating that this cultivar might be more resistant to drought stress (Table4).

Total chlorophyll percentage (TCP) also decreased with drought and comparatively higher TCP was found for cultivar DPX than cultivar Wiliams cultivar (Table 4). Chlorophyll contents were substantially improved by seed priming under both drought and normal conditions. Among the seed priming treatments, maximum total chlorophyll contents was obtained for P3 and was followed by P8 in both cultivars in comparison to other seed priming treatments including untreated control (Table4).

Drought significantly decreased the relative leaf water (RWC) contents in both cultivars (Table 4).Seed priming significantly improved the RWC in both cultivars under both well-watered and drought conditions (Table 4); but the effect of P1 and P3 was very pronounced particularly in improving RWC. Although, there was no considerable difference in RWC under normal condition in both cultivars, but under drought condition, RWC was higher in cultivar DPX (Table4).

Field Experiment: Analysis of variance on field performance indicated significant differences among irrigation levels, soybean cultivars and seed priming treatments (Table 5). Field emergence and other agronomical traits decreased from optimum condition of irrigation to mild and high water deficit stress levels in both cultivars, significantly (Table 5). Seed priming treatments significantly improved the final emergence count in all irrigation levels. Field emergence in all the treatments ranged from 49% to 89%. Maximum final emergence counts were recorded from P11 followed by P1 (Table 5). Likewise seed priming treatments significantly improved the number of pods per plants, thousand seed weight and seed yield. At the optimum irrigation (I1), the difference in field performance among cultivars were not considerable, whereas, at the mild and high water deficit stress conditions (I2 and I3 respectively), the highest and lowest reduction in field performance were observed in cultivars of Wiliams and DPX, respectively (Table 5).

Table 1. Seed priming treatments applied to seeds of soybean cultivars.

Method	Treatment code	Duration in (h)	Treatment substrate	Concentration	Reference for method use
Non-primed (Control)	P0	-	-		
Hydropriming	P1	12	Distilled water		Harris *et al*. (2001)
	P2	18	Distilled water		
Osmopriming	P3	24	Polyethylene glycol (PEG-6000)	13.5% (-0.25MPa)	Michael and Kaufman (1973)
	P4	48	Polyethylene glycol (PEG-6000)	13.5% (-0.25 MPa)	
	P5	24	Polyethylene glycol (PEG-6000)	20.2% (-0.50 MPa)	
	P6	48	Polyethylene glycol (PEG-6000)	20.2% (-0.50 MPa)	
Halopriming	P7	12	Potassium nitrate	1.0%	Kulkarni and Eshanna (1988)
	P8	18	Potassium nitrate	1.0%	
	P9	12	Calcium chloride	2.0%	
	P10	18	Calcium chloride	2.0%	
Hormonal priming	P11	14	Gibberlic acid	50 ppm	Eisvand *et al*. (2010)
	P12	21	Gibberlic acid	50 ppm	
	P13	14	Gibberlic acid	100 ppm	
	P14	21	Gibberlic acid	100 ppm	

Table 2. Influence of seed priming treatments on germination, mean germination time, seedling vigour and vigour index in soybean cultivars under normal and drought conditions.

Treatment	Germination (%)				MGT (days)				Seedlig vigour (cm)				Vigour index			
	C1		C2		C1		C2		C1		C2		C1		C2	
	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D
P0	86.3bc	71^{b}	85.2^{bc}	61b^{c}	3.5^{a}	3.9^{a}	3.7^{a}	4.1^{a}	15^{g}	12.6^{f}	13.5^{g}	8.9^{hi}	1298.4gh	853gh	1149^{gh}	549^{f}
P1	96a	80^{a}	96^{a}	70.3^{a}	1.5^{ef}	1.8^{de}	1.6^{ef}	2.1^{bc}	27.1^{b}	24.5^{a}	25.1^{bc}	21.5^{b}	2606.1a	2001a	2411^{a}	1480^{a}
P2	95.3a	80^{a}	94.3^{a}	70.1^{a}	1.3^{gh}	1.6^{fg}	1.5^{fg}	1.9^{de}	22^{d}	19.8^{c}	20^{d}	16^{d}	2090.6c	1598c	1888^{d}	1122^{c}
P3	95a	81^{a}	95^{a}	71^{a}	1.2^{hi}	1.5^{gh}	1.3^{h}	1.7^{fg}	24.5^{c}	21.5^{b}	23.5^{c}	19.3^{c}	2325.7^{b}	1700^{b}	2235^{b}	1373^{b}
P4	78d	63^{c}	76^{d}	53^{d}	1.1^{i}	1.3^{i}	1.3^{h}	1.8^{ef}	22^{d}	19.7^{c}	20^{d}	16.5^{d}	1713^{ef}	1260de	1522^{f}	875^{d}
P5	81c	65^{cb}	80^{c}	59^{bc}	1.4^{fg}	1.7^{ef}	1.6^{ef}	2^{cd}	28.2^{a}	24.9^{a}	26.1^{a}	22.2^{a}	2270^{b}	1610^{bc}	2077^{c}	1295^{b}
P6	62e	47^{d}	62^{e}	37^{e}	1.7^{cd}	1.9^{cd}	1.8^{cd}	2.1^{bc}	17.3^{f}	14.2^{e}	15.7^{ef}	11.1^{g}	1054^{i}	658^{i}	958.4^{i}	445^{g}
P7	82c	67^{bc}	83^{c}	57^{c}	2^{b}	2.2^{b}	2^{b}	2.4^{a}	17^{f}	14^{e}	15.3^{f}	11^{g}	1393^{g}	939^{fi}	1243^{g}	630^{f}
P8	84c	69^{b}	84^{c}	59^{bc}	1.3^{gh}	1.4^{hi}	1.4^{gh}	1.6^{g}	25.4^{c}	22.5^{b}	24^{c}	20^{c}	2100^{c}	1587^{c}	2018^{c}	1181^{c}
P9	84c	69^{b}	84^{c}	59^{bc}	1.7^{cd}	1.8^{de}	1.8cd	2^{cd}	15^{g}	12.1^{f}	13.5^{g}	10^{gh}	1262^{h}	827^{h}	1132^{h}	591^{f}
P10	60e	45^{d}	61^{e}	35^{e}	1.4^{fg}	1.6^{fg}	1.4^{gh}	1.7^{f}	14^{g}	11.4^{f}	12.6^{g}	8.5^{i}	838^{j}	496^{j}	793^{j}	295^{h}
P11	96a	81^{a}	96.3^{a}	71^{a}	1.3^{gh}	1.5^{gh}	1.5^{fg}	1.8^{ef}	27.2^{b}	24.7^{a}	25.8^{ab}	21.5^{b}	2591^{a}	2025^{a}	2504^{a}	1530^{a}
P12	93a	75^{a}	92.7^{a}	65^{ab}	1.6^{de}	1.7^{ef}	1.8^{cd}	1.9^{de}	21de	17.2^{d}	19.9^{d}	14.9^{e}	1955^{d}	1257^{d}	1852de	945^{d}
P13	91ab	69^{b}	91^{ab}	59^{bc}	1.6^{de}	1.7^{ef}	1.7^{de}	1.9^{de}	18.2^{f}	15.2^{e}	16.8^{e}	12.8^{f}	1640^{f}	1035^{f}	1545^{f}	768^{e}
P14	91ab	69^{b}	91^{ab}	58^{cd}	1.7^{cd}	1.8^{d}	1.8^{cd}	2^{cd}	20.1^{e}	17.1^{d}	19.9^{d}	14.5^{e}	1821^{d}	1175^{e}	1775^{e}	850de

Means superscripted with different letter in same column are significantly different at $P \leq 0.05$ (LSD).

P0: untreated seed (control), P1: hydropriming for 12 h, P2: hydropriming for 18 h P3: osmopriming with PEG (13.5%) for 24 h, P4: osmopriming with PEG (13.5%) for 48 h, P5: osmopriming with PEG (20.2%) for 24 h, P6: osmopriming with PEG (20.2%) for 48 h, P7: halopriming with potassium nitrate (1%) for 12 h, P8: halopriming with potassium nitrate (1%) for 18 h, P9: halopriming with calcium chloride (2%) for 12 h, P10: halopriming with calcium chloride (2%) for 18 h, P11: hormonal priming with gibberlic acid50 ppm for 14 h, P12: hormonal priming with gibberlic acid 50 ppm for 21 h, P13: hormonal priming with gibberlic acid 100 ppm for 14 h, P14: hormonal priming with gibberlic acid 100 ppm for 21 h,MGT: Mean germination time, C1: cultivar DPX, C2: cultivar Wiliams, N: Normal condition, D: Drought condition.

Table 3. Effect of seed priming treatments on membrane stability, lipid peroxidation, antioxidant enzymes and reserve mobilising enzymes in soybean cultivars under normal and drought conditions.

Treatment	Electrolyte leakage (%)				Malondialdehyde (nmol g^{-1}fr.wt)				Catalase (unit g^{-1}fr.wt)				Ascorbate peroxidase (unit g^{-1}fr.wt)			
	C1		C2		C1		C2		C1		C2		C1		C2	
	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D
P0	53^{a}	71^{a}	54^{a}	96^{a}	15.47^{b}	20.56^{b}	17.97^{b}	24.03^{bc}	101^{c}	125^{d}	100^{c}	102^{c}	6.2^{b}	6.7^{b}	5.6^{b}	5.7^{b}
P1	33^{b}	49^{c}	36^{b}	74^{b}	14.1^{c}	18.29^{c}	15.89^{c}	22.75^{d}	160^{a}	195^{a}	150^{a}	160^{a}	7.1^{ab}	8.1^{ab}	6.3^{ab}	6.4^{ab}
P3	35^{b}	57^{b}	37^{b}	76^{b}	13.9^{c}	17.56^{c}	15.7^{c}	22.2^{d}	154^{a}	175^{b}	140^{a}	154^{a}	7.7^{a}	8.9^{a}	6.8^{a}	7^{a}
P8	39^{b}	68^{a}	42^{b}	91^{a}	17.57^{a}	22.03^{a}	20.1^{a}	26.81^{a}	97^{c}	110^{e}	89^{c}	93^{c}	4.1^{c}	4.5^{c}	3.5^{c}	3.7^{c}
P11	37^{b}	62^{b}	39^{b}	78^{b}	14.2^{c}	18.35^{c}	16.5^{c}	23^{cd}	133^{b}	167^{c}	125^{b}	131^{b}	6.5^{b}	7.2^{b}	5.8^{b}	6^{ab}

Treatment	Superoxide dismutase (unit g^{-1}fr.wt)				α-Amylase (decrease in OD/min/g/fwt)				β-Amylase (mg maltose/h/g/fwt)				Acid phosphatase (µg p-nitrophenol/h/g/fwt)			
	C1		C2		C1		C2		C1		C2		C1		C2	
	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D
P0	120^{a}	136^{b}	105^{b}	118^{b}	6.29^{c}	5.18^{c}	6.07^{c}	4.88^{c}	3206.8^{e}	3006.4^{e}	3096.2^{d}	2748.3^{d}	439.5^{e}	383.4^{d}	415.5^{d}	315.1^{e}
P1	133^{a}	140^{ab}	120^{a}	130^{a}	9.09^{a}	7.89a	9.04^{a}	6.71^{a}	9193.6^{a}	8970.3^{a}	9000.8^{a}	8643.8^{a}	663.4^{b}	594.1^{a}	639.6^{a}	525.9^{b}
P3	138^{a}	143^{ab}	123^{a}	131^{a}	8.01^{b}	6.9^{b}	7.72^{b}	5.58^{b}	8579.4^{b}	8247.9^{b}	8397.8^{a}	8036.9^{b}	600.4^{c}	561.4^{b}	574.6^{b}	456.5^{c}
P8	95^{c}	115^{c}	84^{c}	100^{c}	5.81^{c}	4.38^{d}	5.8^{d}	3.85^{d}	3678.4^{d}	3104.8^{d}	3348.7^{c}	2844.3^{d}	470.7^{d}	408.3^{c}	449.9^{c}	346.8^{d}
P11	141^{a}	149^{a}	130^{a}	140^{a}	8.83^{a}	7.54^{a}	8.3^{ab}	6.07^{ab}	8160.2^{c}	7650.2^{c}	7679.5^{b}	6929.1^{c}	674.4^{a}	610.1^{a}	651.3^{a}	557.1^{a}

Means superscripted with different letter in same column are significantly different at $P \leq 0.05$ (LSD).610.1a

P0: untreated seed (control), P1: hydropriming for 12 h, P3: osmopriming with PEG (13.5%) for 24 h, P8: halopriming with potassium nitrate (1%) for 18 h, P11:hormonal priming with gibberlic acid50 ppm for 14 h,C1: cultivar DPX, C2: cultivar Wiliams, N: Normal condition, D: Drought condition.

Table 4. Effect of seed priming treatments on Alkaline phosphatase, dehydrogense, relative water contents, chlorophyll and free proline contents in soybean cultivars under normal and drought conditions.

Treatment	Relative water contents (%)				Total chlorophyll (%)				free proline contents (µmol g-1 FW)			
	C1		C2		C1		C2		C1		C2	
	N	D	N	D	N	D	N	D	N	D	N	D
P0	85.1^{d}	81.5^{d}	81.1^{c}	69^{d}	25.3^{d}	10.6^{e}	23.5^{d}	7.8^{d}	7.87^{c}	13.37^{c}	6.5^{b}	9.1^{d}
P1	96.2^{a}	93.1^{a}	90.9^{a}	81.1^{a}	41.2^{c}	19.5^{d}	39.4^{c}	13.1^{b}	8.5^{b}	15.3^{b}	6.9^{b}	11^{c}
P3	95.5^{a}	92.9^{a}	89.9^{a}	80.5^{a}	57.5^{a}	28^{a}	53.5^{a}	17.3^{a}	8.9^{b}	15.9^{b}	7.1^{b}	12.3^{b}
P8	87.1^{d}	83.1^{b}	82.2^{c}	75.5^{c}	48.3^{b}	25^{b}	45.4^{b}	15.9^{a}	9.5^{a}	17.5^{a}	8.4^{a}	13.5^{a}
P11	90^{b}	87.5^{c}	84.3^{b}	79.1^{b}	39.8^{c}	22^{c}	37.9^{c}	9.9^{c}	8.3^{b}	15.5^{b}	7^{b}	12^{bc}

Treatment	Dehydrogenase nmol NADH oxidized s-1 mg protein-1				Alkaline phosphatase (µg p-nitrophenol/h/g/fwt)			
	C1		C2		C1		C2	
	N	D	N	D	N	D	N	D
P0	15.1^{b}	59.1^{c}	12.8^{b}	30.9^{b}	383.3^{e}	343.8^{d}	365.4^{e}	290.1^{e}
P1	22.2^{a}	77.9^{a}	18.8^{a}	38.1^{a}	728.2^{a}	697.8^{a}	700.9^{a}	650.1^{a}
P3	18.3^{a}	68.9^{b}	16.9^{a}	35.8^{a}	592.9^{c}	562.8^{c}	576.1^{c}	516.1^{c}
P8	14.2^{b}	58.4^{c}	12.7^{b}	31^{b}	401.2^{d}	350.8^{d}	384.1^{d}	325.8^{d}
P11	21.2^{a}	74.1^{b}	16.1^{b}	37.2^{a}	625.2^{b}	595.6^{b}	603.5^{b}	558.2^{b}

Means superscripted with different letter in same column are significantly different at $P \leq 0.05$ (LSD).

P0: untreated seed (control), P1: hydropriming for 12 h, P3: osmopriming with PEG (13.5%) for 24 h, P8: halopriming with potassium nitrate (1%) for 18 h, P11: hormonal priming with gibberlic acid50 ppm for 14 h, C1: cultivar DPX, C2: cultivar Wiliams, N: Normal condition, D: Drought condition.

Table 5. Effects of seed priming treatments on field performance in soybean cultivars under different irrigation regimes(data averaged over 2 years, 2014–2015).

Treatment	Field emergence (%)						Number of pods per plant					
	C1			C2			C1			C2		
	I1	I2	I3	I1	I2	I3	I1	I2	I3	I1	I2	I3
P0	80^{c}	72.1e	60^{c}	79.1^{d}	54.3e	48.6^{c}	30.07^{a}	21.39^{c}	15.87^{c}	19.92e	8.5^{d}	2.9^{d}
P1	88.2^{a}	78.9^{b}	69.5^{a}	87.1^{b}	60.1^{b}	51.3^{b}	33.8^{a}	23.11^{b}	17.02^{b}	23.61^{b}	10.8^{b}	4.6^{b}
P3	85.9^{b}	77.5^{c}	66.7^{b}	84.8^{c}	59.2^{c}	51.2^{b}	32.7^{b}	22.53^{b}	16.5^{b}	22.4^{c}	9.5^{c}	6.3^{a}
P8	84.3^{b}	73.1^{d}	63.4^{b}	84.2^{c}	56.9^{d}	48.7^{c}	31.1^{c}	22.01^{c}	16.3^{bc}	20.49^{d}	8.8^{d}	3.7^{c}
P11	88.7^{a}	79.8^{a}	70.1^{a}	89.9^{a}	62.1^{a}	53.2^{a}	34.1^{a}	24.82^{a}	18.72^{a}	26.51^{a}	12.24^{a}	6.7^{a}

Treatment	Thousand seed weight (g)						Seed yield (kg.ha-1)					
	C1			C2			C1			C2		
	I1	I2	I3	I1	I2	I3	I1	I2	I3	I1	I2	I3
P0	135.3e	106^{d}	82^{b}	135^{c}	72^{b}	61^{b}	2458^{c}	1210^{d}	453^{d}	2320^{c}	765^{c}	140^{c}
P1	154.1^{b}	125^{b}	102^{a}	148^{ab}	81^{a}	79^{a}	3011^{b}	2100^{a}	1025^{b}	2800^{a}	1450^{a}	315^{b}
P3	144.2^{c}	111^{cd}	87^{b}	142^{bc}	79^{a}	77^{a}	2986^{b}	1903^{b}	853^{c}	2750^{a}	1321^{b}	295^{b}
P8	142.5^{d}	109^{d}	85^{b}	139^{c}	77^{ab}	63^{b}	2502^{c}	1640^{c}	506^{d}	2410^{b}	820^{c}	182^{c}
P11	158.7^{a}	133^{a}	107^{a}	155^{a}	80^{a}	79^{a}	3100^{a}	2161^{a}	1197^{a}	2810^{a}	800^{c}	498^{a}

Means superscripted with different letter in same column are significantly different at $P \leq 0.05$ (LSD).

P0: untreated seed (control), P1: hydropriming for 12 h, P3: osmopriming with PEG (13.5%) for 24 h, P8: halopriming with potassium nitrate (1%) for 18 h, P11: hormonal priming with gibberlic acid50 ppm for 14 h, C1: cultivar DPX, C2: cultivar Wiliams, I1: optimum conditions of irrigation, I2; mild water deficit and I3; high water deficit.

DISCUSSION

Drought stress during the imbibition phase of germination is the primary reason for both inhibition or delayed seed germination and seedling establishment (Pirasteh *et al*., 2011).Better germination rate due to seed priming helps soybean to fast emergence and canopy closure at early growth. P1, P3 and P11 substantially improved the seed germination and seedling vigour under drought and well-watered conditions owing to early completion of pre-germination metabolic activities during seed priming. Modulation of hydrolases during lag phase of germination by PEG 6000 helped to build germination metabolites (Sharma *et al*. 2014), resulting in earlier and uniform stand establishment (Table 2).

The positive effect of seed priming with GA may be due to improvement of germination rate, cellelongation, and cell division in seedling (Dasilva*et al*., 2005).

The reduction of lipid peroxidation in the seedlings of primed soybean seeds might be associated with better membrane repair during osmotic priming process and inductive responses of antioxidant enzymes observed in the present study which can provide protection against oxidative damage (Sharma *et al*., 2014).

Proline is one of the most current osmolytes, which helps in instigating water retention and mitigating the unsuitable effect of drought on plants (Serraj and Sinclair,2002). There was strong positive correlation of proline with RWC, seedling vigour, chlorophyll and membrane stability under drought (data not shown), indicating the proline accumulation modified the RWC, thus avoiding the oxidative damages. Increased content of intracellular proline thus increases the plant's ability to survive under water deficit (Taylor 1996).

Many researchers have reported the presence of several antioxidative and hydrolytic enzymes in dry cereal grains, and activities raised sorely after the start of seed imbibition (Morohashi, 2002). The present study involving priming of soybean seeds by various methods lead to improvement in overall faster and uniform germination and increased seedling vigour particularly by hydropriming (P1), osmopriming (P3) and hormonal priming (P11) in both in normal and drought conditions, which may be probably due to enhanced enzymatic activities leading to reduced oxidative stress.

The control unprimed plants grown in PEG experienced severe oxidative stress (high electrical conductivity of the seed leachates and high MDA levels) and their anti-oxidant defense mechanisms possibly were overwhelmed as shown by lower antioxidant enzymes activity (Table 3). Poor growth in unprimed control plants under drought could also be connected to the impairment of antioxidant enzyme levels. These results bear similarity to the findings of Goswami *et al*. (2013) in rice responding to drought conditions.

It is assumed that seed priming enhances the mobilization of seed reserves as a result of activation or synthesis of key enzymes (Fu *et al*., 1988). In present research, maximum activities of α- and β- amylase and alkaline phosphatase were recorded in hydroprimed seeds of soybean. The results are in agreement with the findings of Wattanakulpakin *et al*. (2012) in maize and Sharma *et al*. (2014) in okra. Nasri *et al*. (2011) reported that seed priming with KNO_3 resulted in an increment of acid phosphatase at a level close to that of the control in roots, shoots and cotyledons under saline conditions in lettuce seedlings. Instead of, in soybean, seed priming with KNO_3 did not produce any beneficial effect. This could be due to the crop-specific effects of diverse treatments/environments.

Dehydrogenases are considered important in generating reducing powers which are utilized in various metabolic activities (Kumar *et al*., 2000).Results suggest varying behavior of dehydrogenase in two sets of soybean cultivars differing in drought tolerance and that lower in the activities of dehydrogenases in drought sensitive soybean cultivar due to drought condition may be one of the possible reasons for decreased growth of soybean plants under drought conditions. These findings are in agreement with the increased dehydrogenase activity observed by other workers under stressful conditions and seed priming (Kumar *et al*., 2000; Farooq *et al*., 2006).

Our results showed that by decreasing irrigation hampered yield and yield attributes of soybean in both cultivars. The reductions in seed yield are associated with linear decreases in yield components, especially the pods per plant. Various seed priming treatments were beneficial in mitigating the adverse effect of water deficit.The highest yield was recorded in seeds primed with hormone followed by hydroprimed seeds in all irrigation levels. Similar trend was observed for field emergence of seeds, number of pod/plant and 1000-grain weight. This may be attributed to early and synchronized field emergence, which resulted in more leaf area and early canopy development. Better ground cover provided by these treatments reduced the evaporation from the soil, saving sufficient water for transpiration. Moreover, early emergence resulted in vigorous plants that may have deeper and more extensive root systems capable of extracting water efficiently, even under lower irrigation regimes (Ali *et al*., 2013). Hydroprimed seeds reported to be effective in early germination, better establishment and increased yield in okra (Sharma *et al*., 2014).

The present study was an attempt to investigate whether seed priming techniques could help in improving the field performance when water availability is low. The results of the present study show that adverse effects of water stress on germination, plant growth, and

establishment can be minimized by using different seed priming treatments. The seed priming technique promoted early emergence and improved the total germination count and growth of soybean cultivars. Moreover, increase in the number of pods, grain weight, and biological yield by seed priming increased the seed yield. On the basis of above discussion, hydropriming and hormonal priming had significant effects on uniform field emergence, yield and related traits of soybean cultivars. Hydropriming is reported to be a simple, economical and a safe technique, whereas hormonal priming and osmopriming are relatively expensive. From farmer's point of view, we recommend the hydropriming treatment for soybean seeds which can be effective to increase the grain yield up to 55% under varying moisture conditions ranging from moderate to no drought stress in northern Iran and similar environments in other parts of the world.

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