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Evaluating the Impact of Osmopriming Varying with Polyethylene Glycol Concentrations and Durations on Soybean

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Abstract

The objective of this study was to determine the effects of osmo and hydro-priming on phenology, yield components and biomass yield of soybean (*Glycine max*) cv. William-82. After a laboratory experiment to determine the optimum combination of priming duration and polyethylene glycol 8000 (PEG 8000) concentration, field experiment was conducted in 2003 and 2004 with three priming durations (6, 12 and 18 h) and five different concentrations of PEG 8000 solution (0, -0.2, -0.5, -1.1, -1.8 , -3.0 and -4.2 MPa), together with a dry seed (non primed) control. Primed and non-primed seeds were sown in the field. During both years, plants from primed seed flowered and matured faster than plants from non-primed seed. Primed seed gave taller plants. Averaged over all treatments, priming for 6 h or with -1.1 MPa, were the most beneficial treatments. It is concluded that priming with PEG was much effective but priming with water alone was also better than control.

Keywords: Osmopriming; Hydropriming; Grain legumes; Germination; Phenology; Biomass yield

Introduction

Many developing countries like Pakistan face serious deficiencies in protein and vegetable oil, and must spend a considerable amount of foreign exchange on the import of vegetable oil in particular to meet local demand (MINFAL, 2010-11). Soybean (*Glycine max* (L.) Merrill) has the potential to become the leading oil seed crop in the country. Increased local production would decrease Pakistan's dependence upon costly imports, as well as having other benefits such as fulfilling the increasing demand for this oil by diet-conscious consumers and for soybean meal for the developing poultry industry and livestock sectors. Poor germination and stand establishment are the basic problems of soybean in Pakistan. Soybean seed must absorb 50 percent of its weight in moisture to germinate, compared to only 30 percent for maize. For good water absorption and germination, the seedbed should be fine with enough moisture to provide good contact between seed and soil. Similarly, soybean seed is very perishable and soon loses viability therefore seed older than one year should not be used for planting to ensure good germination (Hatam and Abbasi, 1994).

Seed priming aims to synchronize and improve germination by submitting the seeds to a period of imbibition, and a wide variety of treatments (Bradford, 1986; Ashraf and Foolad, 2006) have been used for this, including soaking in water (Harris *et al.,* 1999) or osmotic solution (Knypl and Khan, 1981). The degree of growth and germination enhancement depends upon the temperature, water potential, duration of soaking and other conditions, although some long duration, low water potential or high temperature priming treatments can have a negative effect on subsequent germination responses (Hardegree, 1998; Carlos and Cantliffe, 1992). Enhancement is particularly valuable under conditions of abiotic stress such as drought (Harris *et al*., 2001) or salinity (Ashraf and Rauf, 2001; Turhan and Ayaz, 2004; Afzal *et al*., 2012; Jafar *et al*., 2012).

The technique of hydropriming consists of soaking seeds in water, which may be aerated or not. Harris *et al.* (2001) advocated a similar technique, "on-farm" seed priming, as a low-cost approach for farmers in dryland areas – the seeds are imbibed without aeration and pre-germination metabolic activities take place, although later stages of germination are inhibited (Pill and Necker, 2001). Osmopriming or osmoconditioning has given promising results for many legumes (Elkoca *et al*., 2007; Ghassemi-

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Golezani *et al*., 2008; Yucel, 2012), and a few studies on soybean are encouraging, although more information is required before its use as a routine practice. It involves soaking seeds in aerated solutions of Polyethylene Glycol (PEG) or sugar followed by drying back to storage weight. The low water potential of the solution partially hydrates the seed, which allows pre-germination metabolism to start, but inhibits germination (Pill and Necker, 2001). The result is usually rapid and uniform germination (Ashraf and Foolad, 2006).

Several physiological and biochemical changes take place in seeds during priming or as a consequence of osmotic conditioning. These include increase vigour and overcoming of dormancy (Jie *et al.*, 2002), and these effects have been noted in soybean (GongPing *et al.*, 2000) and maize (Finch-Savage *et al.*, 2004) using PEG-6000. However, almost all of the work on osmopriming has been conducted under laboratory conditions, and few detailed studies have been reported on the performance of osmotically treated seed in the field. Khalil *et al.* (2010) reported that seed priming with phosphorus solutions enhanced uniformity and speed of germination in wheat.

The objective of this work was to assess the effects of seed priming on the yield components, biological yield and harvest index of field-grown soybean, compared with non-primed controls, as a preliminary to the development of protocols for the use of the technique in Pakistan.

Materials and Methods

Germination Assay

An initial laboratory experiment was conducted to determine optimum polyethylene glycol 8000 (PEG) concentration and seed priming duration for maximum germination. Seeds of soybean cv. Williams-82 were primed for 6, 12 and 18 h using PEG 8000 solutions with concentrations of 0, 100, 200, 300, 400, 500 and 600 g PEG L^{-1} water. These were equivalent to molarities of 0, 0.013, 0.025, 0.038, 0.050, 0.0625 and 0.075 moles L^{-1} respectively (osmotic potential 0, -0.2, -0.5, -1.1, -1.8, -3.0 and -4.2 MPa) at 25°C, oxygenated with an aquarium pump in order to prevent damage to the seed. The control treatment was dry seeds (non primed). Osmotic potentials were determined according to Michel (1983). After priming, seeds were rinsed with tap water for two min to facilitate handling. Twenty seeds of each treatment were placed in 14 cm diameter Petri dishes, covered with 2 cm of canal silt (particle size 0.002–0.02 mm) as the growing medium, replicated six times. The dishes were uniformly moistened and were placed in an incubator (Model No. 2020-2E, SHELAB, USA) at a constant temperature of 25°C to simulate temperatures in the area in April and May, the usual sowing time for soybean. Germination counts were made daily for 7 days, after which no further germination occurred.

Field Trials

Field experiments were conducted at the Agricultural Research Farm of the University of Agriculture, Peshawar, Pakistan during 2003 and 2004. The experimental site was located at 34°0’ N, 71° 35’ E and an altitude of 450 meters above sea level. The soil was a silty clay loam with a clay type montmorillonite, low in nitrogen (0.03-0.04%) and organic matter (0.7-0.9%), and alkaline in reaction (pH 8.0-8.2).

Seeds of the same variety were primed in the laboratory for 6, 12 and 18 h using water, or 100, 200, 300 and 400 g PEG L^{-1} water (osmotic potentials 0, -0.2, -0.5, -1.1 and 1.8 MPa, respectively). The seeds were air dried under a fan for half an h to facilitate clump free sowing after aerated priming and were sown in the field on 3rd May 2003 and 5th May 2004. The control treatment was dry seed (non-primed). The experiment was a randomized complete block (RCB) design with four replications, plus an additional unprimed control plot in each replicate: there were a total of 4 x ((5 x 3) + 1) = 64 plots each year. The field was ploughed with cultivator twice followed by rotavator to convert soil clods into small pieces. Plot size was 2 x 3 m, with row to row distance of 50 cm and plant to plant distance 10 cm. One hundred and twenty seeds were sown in each plot, and were not thinned. Plots were hoed twice by hand to control weeds, and were watered when needed. A basic fertilizer dose of 30 kg nitrogen (N) and 90 kg phosphorus (P) per hectare was applied as urea and triple superphosphate, broadcast before sowing. The experiments were harvested in the 2nd week of September in each year, when the majority of the leaves had dropped, the lowest pods had turned yellow, and seed moisture content was estimated to be 14–15%. Data were recorded on germination percentage, plant height, number of branches per plant, number of grains pod, thousand grain weights, days to flowering and maturity, biological yield and harvest index. For days to emergence, emergence m^{-2} and grain yield data please see Arif *et al.* (2008).

Statistical Analysis

The data for the laboratory and field experiments were analyzed as a general analysis of variance, taking years, duration of soaking and PEG concentration as factors, and again with an additional level in duration of soaking to take account of the unprimed control (Table 1). Standard errors of differences were used to assess the significance of differences between treatment and interaction means. Germination percentages were transformed using the angular transformation in order to account for non-normality of the data.

Table 1: Effect of duration of soaking and PEG concentration on final germination (%) of soybean (data show the angular transformation of means of the percentage germination for all days of measurement, non-soaked control = 47.3

Duration of soaking (h)	Osmotic potential (MPa)							
	0	-0.2	-0.5	-1.1	-1.8	-3.0	-4.2	Mean
6	56.9	60.8	63.4	65.6	56.6	43.9	46.8	56.1
12	50.7	52.1	54.3	58.5	59.4	44.1	42.8	51.7
18	48.1	43.5	50.4	54.2	51.8	42.8	43.5	47.7
Mean	51.6	52.1	56.0	59.4	55.9	43.6	44.4	

Standard errors of a difference of a mean:PEG Concentrations (n = 72) = 1.68 (for difference with non-primed control = 2.38); Duration of soaking (n = 168) = 1.10 (for difference with non-primed control = 2.21); Interaction (n = 435) = 2.92

Table 2: Main effect of duration of priming on traits of soybean (mean of 2 years data 2003 and 2004, and of all concentrations)

Parameters	Duration of priming (h)					
	0	6	12	18	s.e.[a]	s.e.[b]
Plant height (cm)	83.0	90.1	90.8	90.4	2.13ns	1.23ns
No. of branches per plant	3.92	4.27	4.07	3.89	0.192**	0.111**
No of grain per pod	2.88	3.03	2.98	2.98	0.117ns	0.053ns
Days to flowering	53.3	50.2	51.0	51.4	1.27*	0.73ns
Days to maturity	126	120	121	122	1.17***	0.67*
Thousand grain weight (g)	148	154	154	150	4.0ns	2.3ns
Total biomass (t ha^{-1})	10.1	11.4	10.9	10.7	0.53ns	0.31*
Harvest index (%)	30.2	31.8	31.6	29.5	2.15ns	1.24ns

s.e.[a] = standard error for a difference between primed and non-primed treatments, *n* = 30 versus *n* = 6
s.e.[b] = standard error for a difference between priming treatments, *n* = 30

Table 3: Values of recorded traits in soybean, mean of all treatments and concentrations, for each of years 2003 and 2004

Parameters	Year		
	2003	2004	s.e.
Plant height (cm)	89.0	91.0	0.97*
No. of branches per plant	4.30	3.82	0.088***
No of grain per pod	3.06	2.93	0.042**
Days to flowering	52.0	50.0	0.58***
Days to maturity	119	124	0.5***
Thousand grain weight (g)	153	152	1.8ns
Total biomass (t ha^{-1})	10.0	11.9	0.24***
Harvest index (%)	31.8	30.1	0.98ns

s.e. = standard error for a difference between year means, *n* = 48

Results

Laboratory Experiments

Seed priming with either water or PEG significantly increased final germination (Table 1) compared with control. Priming with solutions of -1.1 or -0.5 MPa were most effective, averaged over the germination period, soaking for 6 h was optimum. Soaking for 18 h had no effect compared with not priming, and was detrimental at high osmotic potentials. As a result, we selected PEG concentrations of 0, -0.2, -0.5, -1.1 and -1.8 MPa for the subsequent experiments.

Field Experiments

On average (Table 2), priming had significant effects on days to maturity, days to flowering (P = 0.042), and plant height (P <0.001). Plant height was increased by 9%, from 83.0 to 90.5 cm. Priming reduced the time to flowering from 53.3 to 50.9 days, and days to maturity from 126 to 121 days.

The duration of priming had highly significant effects on number of branches per plant (P=0.004), days to maturity (P=0.042), and total biomass (P=0.047). Priming for 6 h was more effective for improving establishment than priming for 18 h, led to greater branching than other durations of priming (Table 2).

Differences between years were significant for most of phenological and yield traits (Table 3). Time to flowering was two days less in 2004 than 2003, but time to maturity was almost 5 days longer. Both the number of branches, and number of grains per pod, was less in 2004, by 12.6% for branches, and by 4.2% for grains per pod. Similarly, total biomass was higher in year 2004 (11.9 t ha^{-1}) as compared to 2003 (10.0 t ha^{-1}).

The osmotic potential of the priming solution had highly significant effects on days to maturity, number of branches (P

Table 4: Effect of priming medium (OP of water or PEG 8000 solution[1]) on traits of soybean (mean of 2 years data 2003 and 2004, and of all durations)

Parameters	Osmotic potential (MPa)							
	Not-primed	0	-0.2	-0.5	-1.1	-1.8	s.e.[a]	s.e.[b]
Plant height (cm)	83.0	89.1	88.4	91.2	91.3	92.3	2.01***	1.59ns
No. of branches per plant	3.92	3.87	4.10	4.14	4.29	3.94	0.184ns	0.143*
No of grain per pod	2.88	2.90	2.97	3.02	3.13	2.98	0.086ns	0.068*
Days to flowering	53.3	50.4	51.6	51.7	48.8	51.7	1.19*	0.95*
Days to maturity	126	125	121	120	119	122	1.10***	0.87***
Thousand grain weight (g)	147	151	153	154	155	151	3.7ns	3.0ns
Total biomass (t ha^{-1})	10.1	10.4	11.1	11.2	11.6	10.7	0.504ns	0.399*
Harvest index (%)	30.2	29.2	31.6	31.7	31.7	30.7	2.03ns	1.60ns

s.e.[a] = s.e.d for the difference between non-primed and primed, *n* = 6 versus *n* = 90
s.e.[b] = s.e.d. for the difference between priming treatments, *n* = 18

Table 5: Effect of priming duration and osmotic potential on days to flowering (DF). Mean of 2003 and 2004, DF = 53.3 days

Priming duration (h)	Osmotic potential (MPa)				
	0	-0.2	-0.5	-1.1	-1.8
	DF	DF	DF	DF	DF
6	50.0	49.7	48.7	50.0	52.5
12	51.8	53.2	51.0	47.2	52.0
18	49.5	52.0	55.3	49.3	50.7

s.e.d for differences between concentration x duration means. DF = 1.64***

Table 6: Interaction of years with duration of priming on days to flowering and to maturity

Duration	Year	Duration of priming (h)						
		0	6	12	18	s.e.[a]	s.e.[b]	s.e.[c]
Days to flowering	2003	53.0	51.5	50.3	54.1	2.31***	1.79***	1.04***
	2004	53.7	48.8	51.8	48.6			
Days to maturity	2003	125	118	118	121	2.1*	1.7*	1.0*
	2004	127	123	124	124			

s.e.[a] = standard error of a difference between non-primed treatments
s.e.[b] = standard error of a difference between non-primed and primed treatments
s.e.[c] = standard error of a difference between primed treatments

= 0.039), days to flowering (P=0.012), total biomass (P=0.045) and number of grains per pod (P=0.023). The greatest branching occurred where most of the seeds established, and the greatest number of grains per pod were recorded at -1.1 MPa (Table 4). Days to flowering were also shortest when primed with a solution of -1.1 MPa: although there was no effect of different osmotic potentials on days to maturity, PEG in the priming solution accelerated maturity compared with water alone, from 125 to 120.5 days. Total biomass was also highest when primed at -1.1 MPa: this trait increased with osmotic potential to this level, but reduced when a solution of -1.8 MPa was used.

Interactions of years with priming (data not shown) were significant for total biomass (P=0.042) and harvest index (P=< 0.001). In 2003, the primed biomass (10.14 t ha^{-1}) was 24.5% higher than non-primed, but in 2004 there was no significant difference (1.19 and 1.20 t ha^{-1} respectively). These differences were reflected in harvest index – this was 37.9% for control and 31.4% for primed plots in 2003, but 22.6 and 30.6% respectively in 2004.

The interactive effect between priming duration and osmotic potential was significant for days to flowering (P=0.003). There was little or no effect of priming duration when primed with water, or with solutions of -0.2, -1.1 or -1.8 MPa, but when primed a solution of -0.5 MPa priming for 18 h was less effective than priming for 6 or 12 h (Table 5).

The years x priming duration interaction was significant for days to flowering and days to maturity (Table 6). Although priming for 6 or 12 h accelerated flowering in 2003, when priming was extended to 18 h flowering was similar to the control situation. However, in 2004, all durations of priming (6, 12 or 18 h) accelerated flowering compared to the controls. In the case of days to maturity, this was accelerated by all durations in both years, but to a greater extent in 2003 (almost 6 days) than in 2004 (3 days).

Discussion

During germination trial, while priming with either water or PEG was effective, the most effective method to improve germination was soaking for 6 h with either 300 or 400 g L^{-1}

PEG (-1.1 or -1.8 MPa). Higher osmotic potentials were detrimental, particularly when soaking period was extended. Several authors have found that soaking with high PEG concentrations is detrimental to germination, e.g. Murungu *et al.* (2005) in cotton (*Gossypium hirsutum*) and maize. The lower germination with high osmotic potentials could be due to solute leakage during priming (Hegarty, 1978) or water may have come out of the primed seed into the PEG solution, possibly by osmosis, thereby arresting the germination process (Murungu *et al*., 2005).

The results from the field confirm the generally beneficial result of both hydro- and osmopriming on a number of crop parameters, and on average soaking for 6 h (with any concentration), or with a solution with an osmotic potential between -0.2 and -1.1 MPa were the most effective treatments.

Basra *et al.* (2003) and Arif *et al.* (2008) have reported improved germination, emergence and establishment in field trials of PEG primed seed. Improvements in later growth have been noted in chickpea (*Cicer arienatum*) using mannitol (Kaur *et al.,* 2002) or water, without aeration, (Musa *et al.,* 2001; Harris *et al.,* 2005b) and in sorghum (*Sorghum bicolor*) and Italian ryegrass (*Lolium multiflorum*) using PEG and aeration (Hur, 1991).

Many other reports have also shown the beneficial effect of priming on improved and earlier seedling emergence, and subsequent benefits (yield, earliness. etc) of many beans and cereals (Harris *et al.,* 2001; Musa *et al.,* 2001; Murungu *et al.,* 2004a, 2004b; Arif *et al.,* 2005; Rashid *et al.,* 2006; Shah *et al*., 2012).

The improved establishment in primed seed might be due to the completion of pre-germinative metabolic activities which makes the seed ready for radicle protrusion so that the seed germinates soon after planting compared with untreated dry seed. It may also be due to metabolic repair processes, a build up of metabolites or osmotic adjustments during priming (Bray *et al*. 1989), or improved membrane integrity and enhanced physiological activities at germination (Sung and Chang, 1993).

Priming generally accelerated both flowering and maturity, and the results suggest that the greatest acceleration was by priming for 6 h with a solution of -1.1 MPa rather than with water alone. Earlier flowering and maturity have considerable importance for farmers, particularly if, as was the case here, there is no yield penalty. They may enable the crop to escape from a situation of terminal drought (Musa *et al.,* 2001) or maximum pest or disease activity (Harris *et al.,* 2005a). In addition, they may enable an extra crop to be grown over the course of a season, as has occurred with the widespread adoption of chickpea instead of fallow after rice in the High Barind tract of Bangladesh (Musa *et al.,* 2001). There may also be a socioeconomic advantage to farmers in some parts of Pakistan, as elsewhere, as an early harvest may enable them to migrate earlier to cities or elsewhere in the country, and so be in a better position in the labour market (Harris *et al.,* 2001).

It is possible that differences between years were related to differences in rainfall. There was no rain at all in May or June 2004, compared with 23 and 10 mm in 2003, and in July, August and September rainfall was much higher in 2003 than in 2004 (156 v 7 mm, 114 v 57 mm, and 111 v 35 mm). The biomass of the primed plots was greater than of the non-primed in 2003, although there was no difference between the two in 2004. This suggests that in the wetter year more resources were devoted to leaf production than to grain, and that this was assisted by a priming effect. This is consistent with the work of Desclaux and Roumet (1996), who noted that the switch from vegetative to reproductive growth in soybean was earlier under water-stress than under wetter conditions. The lack of significant interactions involving thousand grain weight, grains per pod and branches per plant also support this conclusion.

Taking the results as a whole, it is clear that priming with any of the tested concentrations for 6 h, or with an osmotic potential of -1.1 MPa, were the most beneficial treatments. By lowering the osmotic potential gradient between the seed and the priming solution, PEG improved number of branches plant^{-1}, and grains pod^{-1} and reduced the days to flowering and maturity. Seed priming in water alone also performed better than control and improved most of the yield components and biomass yield with no cost which represents an attractive option for resource-poor farmers in Pakistan and elsewhere.

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