Research Article

Improving the Stand Establishment, Phenology and Yield of Soybean (*Glycine max* L.) by Various Physiological Enhancements

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Abstract | Soybean is one of the most important legumes rich in high quality protein and oil. Its cultivation in Pakistan is restricted to few areas due to poor germination and low seed viability. The optimum yield and quality of this oil seed crop is noticeably affected by its lower germination rate. To address the problem of poor germination and low seed viability, a field study was conducted at Agronomic Research Area, University of Agriculture Faisalabad, during spring 2011. The experiment was composed of two soybean varieties (Faisal Soybean and 95-1) and five priming techniques (control, on-farm priming, hydro priming, hydration inoculation and osmo priming with 0.5% urea solution. Results revealed that hydro priming, on-farm priming and hydration inoculation significantly reduced the time to 50% emergence (E_{50}), mean emergence time (MET) and emergence index (EI) of both soybean varieties. Moreover, osmo priming had no significant effect on E_{50}, MET and EI that was at par with control. On an average, days to maturity (96.42), plant height (25.84 cm), plant population at harvest (25.22), 1000-seed weight (94.97 g), seed yield (1.71 t ha^{-1}) and biological yield (4.37 t ha^{-1}) of both soybean varieties was significantly improved by hydration inoculation and hydro priming techniques. It was concluded that hydration inoculation and hydro priming were best techniques for better stand establishment of soybean and may be recommended for farmers having the problem of less germination and poor stand establishment.

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Introduction

Soybean inherits the character of the most important legume crop which is enriched with 40% protein and 20% oil. Its cultivation remained restricted to limited areas. Poor germination and seed viability are severe problems in soybean which are responsible for low production. High and better crop stand are crucial for obtaining high yield (Junior and Ida, 2015). So, it is very critical to utilize the good quality seed with proper seed rate to achieve the required number of plants. This aim can only be achieved, if farmers are supplied with good quality seed (Mamilla and Mishra, 2017). Mechanical weakening of the seed coat structure such as scarification, seed coat nicking and seed coat lateral splitting has been reported to successfully enhance germination of field crops (Kigel et al., 2015). Similarly, seed invigoration techniques are adopted to enhance germination. The method of seed priming is done before sowing, a single cycle of wetting and drying is performed (Huang et al., 2017). The benefits of priming have also been expressed for numerous field crops like wheat, sugar beet, maize, soybean and sunflower (Sharma et al., 2015). It has been concluded that hydro priming had lowest values for time to 50% emergence (E_{50}) and mean germination time (MGT) (Gu et al., 2017). Whereas the osmo priming is one of the techniques of seed vigor improvement, uniform germination and better initial growth of field crops and priming the most vigorous seed results in the maximum number of normal seedlings and the most rapid growth (Vimala and Pratap, 2014). Moreover, soybean plays an important part in organic food production because it requires less amount of mineral nitrogen and it can fix atmospheric nitrogen by nodule formation in its roots. But in Pakistan, sowing of soybean has been observed with the limitations of germination, stand establishment and other growth related parameters particularly under agro-climatic conditions of Faisalabad. The above mentioned techniques had been proved very beneficial to overcome these problems for cereals and some legumes crops.

Therefore, this study was conducted to evaluate the effect of different seed priming techniques in enhancing germination and vigor of soybean seeds as well as to assess their comparative responses on soybean emergence, phenology and yield behavior of soybean varieties.

Materials and Methods

A field experiment was conducted during 2011 (February to July) at Agronomic Research Area, University of Agriculture, Faisalabad, Pakistan (31°25' lat. N; 73°09'long. E, 185 m above sea level). Before planting, soil samples were collected from experimental area at 0-30 cm depth and physico-chemical analysis report was obtained from soil fertility laboratory, Ayub Agricultural Research Institute, Faisalabad (Table 1). Seed-bed was prepared properly by ploughing the field with cultivator and then with tractor-mounted rotavator followed by planking. Sowing was completed by single-row cotton drill by keeping line to line distance of 30 cm. Priming of seeds was performed by different techniques which were on-farm priming (8 hour water soaking), hydration inoculation (8 hour water soaking followed by inoculation), hydro priming (12 hour water soaking with aeration) and osmo priming (8 hour soaking in 0.5% urea solution). Seed rate was used at the rate 100 kg ha^{-1}. Thinning was done to maintain 5 cm plant to plant distance when crop attained 4-5 leaf stage after germination. Chemical fertilizer was applied at the rate of 25:50:50 kg ha^{-1} of nitrogen, phosphorous and potassium, respectively. All nutrients were applied at the time of planting. Six irrigations were applied till maturity. All other agronomic practices were kept normal and uniform for all the treatments. All necessary plant protection measures were adopted to keep crop free of weeds, insects and diseases.

Table 1: *Results of soil analysis*

Determination	Unit	Value obtained
Sand	%	67
Silt	%	15.6
Clay	%	18.4
EC	dSm^{-1}	1.67
pH	---	8.2
Organic matter	%	0.94
Total N	%	0.22
Available phosphorous	ppm	5.51
Available K	ppm	177

Data were recorded regarding stand establishment, phenology and yield related parameters of soybean crop. E_{50} was calculated by using the following formula of Cool-bear et al. (1984) modified by Farooq et al. (2005):

$$E_{50} = t_i + \left[\frac{N/2 - n_i}{n_j - n_i}\right](t_j - t_i)$$

Where,
N is the final emergence counts, and n_i and n_j are cumulative number of seeds emerged by adjacent count at time t_i and t_j when $n_i < N/2 > n_j$.

MET was calculated by using the following formula of Bewley and Black (1982):

$$MET = \sum(Dn) / \sum n$$

Where,
n is the number of seeds which emerged on day D, and D is the number of days counted from the beginning of emergence.
EI was calculated by using the following formula as described in Association of Official Seed Analysis (1983):

Table 2: *Effect of different priming techniques on stand establishment and phenology of soybean varieties*

	Time to 50% emergence (days)	Mean emergence time (days)	Emergence index	Days to flowering	Days to pod formation	Maturity days
95-1	11.95	8.32	76.08	44.13 b	55.40 b	99.93 a
Faisal Soybean	12.09	8.46	77.41	46.07 a	57.33 a	96.47 b
LSD	NS	NS	NS	0.74	0.58	0.98
Control	12.55 a	8.93 a	61.27 c	46.83 a	57.50 a	100.17 a
On-farm priming	11.58 b	7.93 b	91.41 b	45.33 b	56.67 a	98.83 a
Hydropriming	11.57 b	7.85 b	98.12 a	43.50 c	55.50 b	96.67 b
Hydration Inoculation	11.68 b	8.00 b	90.85 b	43.50 c	54.67 b	96.17 b
Osmo priming	12.72 a	9.23 a	42.07 d	46.33ab	57.50 a	99.17 a
LSD	0.36	0.34	4.58	1.17	0.92	1.55

NS =Non-significant, LSD =Least significant difference

$$\text{Emergence index} = \frac{\text{No. of emerged seeds}}{\text{Days of first count}} + \cdots + \frac{\text{No. of emerged seeds}}{\text{Days of final count}}$$

The measurement of examined characters was done on plants which had been randomly chosen in the second-row from one side of each plot. To calculate yield related attributes, two middle rows of each plot were completely harvested considering the sides. Dried up to 12% final seed moisture, seed dry weight was calculated and considered as final seed yield. To determine biological yield, total plant dry weight was employed as biological yield. The harvest index was calculated from the ratio of seed yield to biological yield of crop. All the data collected were statistically analyzed using computer package Statistical, 8.1. Data was analyzed by Fisher's analysis technique and differences among the treatments were compared by Least Significant Difference (LSD) test at 5% probability level (Steel et al., 1997).

Result and Discussion

Stand establishment

Table 2 indicates E_{50}, MET and EI values when treatments are compared individually, both of the soybean varieties responded well to priming treatments and showed significant results with hydro priming, on-farm priming and hydration inoculation except osmo priming (urea) and the control. Results show that E_{50} was minimum for hydro priming which was followed by on-farm priming and hydration inoculation. Maximum E_{50} was noted for osmo priming which was at par with the control. Almost same trend was observed for MET and EI.

The overall results regarding E_{50} proved that priming techniques improved germination and emergence of seeds. Minimum E_{50} values for hydro priming, on-farm priming and hydration inoculation might be due to the increased metabolic activity of germination enzymes within the seeds. These enzymes not only worked to emerge the plumule earlier than the untreated seeds but also speed up the germination process. Priming enhances the production of germination metabolites (Huang et al., 2014) which helps in the restoration of better genetic make-up (Sibande et al., 2015) of seeds. Hydro priming (Toklu et al., 2015) aided to maintain lowest values for E_{50} and mean germination time (MGT). Similarly, Kanto et al. (2015) observed the role of priming the carrot seeds which grow faster due to an increase in their embryo length. Moreover, emergence time was less for the plots exhibiting earlier plumule and radicle protrusion. This might be the result of active growth of cells involved in germination which are activated by the hydration of seeds with or without some chemicals. This practice also helped to improve the seed vigor and showed better results of successive crop growth stages. Similarly, faster emergence was observed in maize under field conditions when seeds were tested against priming treatments (Mazzilli et al., 2014) and findings of Wang et al. (2015) were also in line which enhanced the germination speed in soybean and plant growth in the field when followed by hydro priming. This study supports to our work. It was concluded that priming treatments always perked up the germination and emergence of soybean by shortening the MET (days) and all the successive growth parameters when it was compared with the control and osmo priming technique.

Seed priming helped to emerge the plumule and radicle earlier than the non-primed seeds and similar results were observed by Zulueta-Rodriguez et al. (2015). This early emergence of seedlings has provided with even crop stand and higher plant population which proved the more vigor of primed seeds. As root growth is enhanced due to priming so it enabled the plants to uptake nutrients from the soil layers more effectively and significantly affected the growth of the plants. This technique ultimately provides plants with faster growth and development. Higher values for E_{50} and MET gave rise to lower emergence index of seedlings which accounted for lower vigor of seeds and a retarding effect on the plant's growth appeared. This can be concluded that more vigorous seeds produced a dense crop stand and better yield. The effect of osmo priming was not proved much beneficial. Emergence of seeds was disturbed due to negative effect of salt solution during priming. As a result, emergence and growth of the young seedlings hindered and produced fewer plants. This effect was might be due to salt toxicity of the urea which negatively affected the vigor of the soybean seeds. Similar observations were recorded (Faqenabi et al., 2014) in rice seeds which support this study.

Phenology

Seed priming techniques proved best and primed seeds have shown a promotive effect on all these stages of both soybean varieties. The response of Faisal soybean was significant than 95-1 variety and similar results were computed for all the above described parameters. Data indicate that the least time to start flowering was recorded for hydration inoculation which was at par with the hydro priming but the response of other priming treatments and control was non-significant (Table 2). Similarly, for varieties, Faisal soybean has less value for flower initiation and more for 95-1. The same trend was recorded for the both other parameters.

The earlier emergence of the plants and healthy vigor of the seeds helped them to complete their vegetative phase faster than the non-primed seeds. Significant results were obtained in producing better flowering which refer to hydration inoculation and hydro priming of seeds. Similar findings were witnessed by Rodrigues et al. (2014) using hydro priming technique. Earlier flowering produced the pods more rapidly and the results of earlier pod formation relative to hydro priming and hydration inoculation were again attributed to earlier germination and healthy vigor of the seeds than the non-primed seeds. Similar results were obtained for hydro priming (Brunner et al., 2015) technique in wheat crop. While more time to pod formation was due to their late emergence, late flowering and poor growth which proved the less significant response of on-farm priming, control and osmo priming treatments when compared with other techniques. Same trend was observed for hydro priming and hydration inoculation treatments in attaining earlier maturity of plants as compared to other three treatments. The rate of earlier flower initiation and pod formation was less significant in Faisal soybean than 95-1 while days to maturity were significant. Faisal soybean gained maturity earlier than the 95-1 which might be attributed to its genetic variability.

The increase in plant height due to hydro priming and inoculation may be ascribed to earlier emergence and pronounced vegetative growth (Table 3). Similar findings for the soaking of seeds prior to sowing were reported by Masciarelli et al. (2014). For hydration inoculation, the increase in plant height might refer to start the nitrogen fixation during early growth stages of crop. Furthermore, the use of inoculation singly or with varying levels of NPK fertilizers, aided to improve plant growth (Lee and Chang, 2017) which was significantly different from the control. The reduction in plant height due to osmo priming was observed due to reduction in germination and plant growth which was attributed to use of chemicals (Soon et al., 2014). These findings strengthen this study. It was concluded that hydration inoculation, on-farm priming and hydro priming treatments ensured higher plant height when compared with control and osmo priming in soybean. Similarly, maximum plant population was observed in hydration inoculation and hydro primed seed plots. As early emergence and higher cop growth rate was recorded (Meriem et al., 2014) with higher plant population. This interpretation can be concluded that hydration inoculation and hydro priming treatments resulted in higher plant population as compared to on-farm priming, control and osmo priming in soybean (Table 3). Likewise, the varietal response of Faisal soybean was superior to 95-1.

Yield and yield components

It is evinced from the mean effects of that hydration inoculation and hydro priming exhibited a positive trend towards the pod bearing trait and significantly differed in behavior than the remaining priming techniques including the control (Table 3). Hydration inoculation plots gave maximum number of pods

Table 3: *Effect of different priming techniques on agronomic traits and yield components of soybean varieties*

	Plant poula-tion (m^{-2})	Plant height (cm)	Number of pods per plant	1000-seed weight (g)	Biolog-ical yield (t ha^{-1})	Seed yield (t ha^{-1})
95-1	21.40 b	23.77	31.33 b	93.06	3.39	1.21 b
Faisal Soybean	22.59 a	24.36	33.06 a	93.62	3.42	1.28 a
LSD	0.83	NS	1.64	NS	NS	0.06
Control	21.18 c	21.79 b	25.17 d	92.28 b	2.95 c	0.99 d
On-farm priming	22.83 b	25.72 a	29.00 c	94.35 a	3.37 b	1.24 c
Hydro priming	25.10 a	27.37 a	40.67 b	94.87 a	4.33 a	1.66 b
Hydration Inoclation	25.35 a	24.32 ab	45.83 a	95.07 a	4.42 a	1.77 a
Osmo priming	15.50 d	21.17 b	20.33 e	90.13 c	1.96 d	0.58 e
LSD	1.32	3.67	2.59	1.03	0.33	0.09

NS = Non-significant, LSD = Least significant difference

per plant which were significantly differed from hydro priming plots. The number of pods per plant for on-farm priming treatment was found significantly different from the control. While very less number of pods per plant was observed for the osmo priming. Faisal soybean performed best and number of pods per plant was more than 95-1. The maximum seed weight was recorded for hydration inoculation plots which were in line with the results of hydro priming and on-farm priming plots. While for 1000-seed weight, non-significant differences were observed in hydro priming, on-farm priming and hydration inoculation and performed better than the control and osmo priming technique. But control plots showed significant difference than the osmo primed seed weight. Both varieties also remained insignificant in this regard. Seed priming techniques also significantly affect the biological yield in both hydration inoculation and hydro priming plots while on-farm priming plots had less biological yield that was also significantly different from the control plots and the least value of biological yield was recorded for the osmo priming plots. But in case of varieties both performed similar in producing total biological mass of soybean crop. Maximum seed yield was recorded where the seeds were treated with hydration inoculation and significantly differed from hydro priming (Table 3). While on-farm priming also produced less seed yield that was also significant from the seed yield of the control. The minimum seed yield was recorded for the osmo priming technique.

The number of pods per plant in case of hydration inoculation and hydro priming were more that can be attributed towards more number of flowers due to higher growth and nodulation. Seed inoculation provided more availability of nitrogen and initiation of early nitrogen fixation for both soybean varieties. Nitrogen fixation resulted in stimulating the vegetative growth and to supply better phosphorous which enhanced the photosynthetic efficiency in terms of more number of pods per plant. Growth and yield increased significantly with more N_2 availability which ensures improved phosphorous utilization for setting of pods (Mouradi et al., 2016). These results were also supported by the findings of Malik et al. (2016), they reported the significant effect of inoculation on growth and yield components of soybean. The higher seed weight refers to improved growth and development of crop plants due to the regular supply of N through *Rhizobium* inoculation. As rhizobia strains enable to fix N_2 which help to secure the required N supply of soybean (Kang et al., 2014). The hydration technique not only facilitated root extension and nodule formation but also might have increased the accumulation of assimilates in the seeds and thus develops a strong source-sink relationship which yielded seeds with more weight and showed a linear relationship for inoculated seeds when compared with the control.

Moreover, hydro priming aided to enhance the root proliferation and enhanced the nutrient translocation towards the developing pods and increases the 1000-seed weight. Similarly, Tamagno et al. (2017) proved that priming had significantly increased the 1000-seed weight and yield of soybean crop. Contrarily in this study, osmo priming decreased the seedling emergence and affected all successive growth parameters including 1000-seed yield. But Kubala et al. (2015) also concluded that osmo priming was more effective than the on-farm priming treatments. They reported that seed priming always acts to enhance the 1000-seed weight of brassica either it is done alone or with inoculation. The higher seed yield of priming treatments including hydration inoculation and hydro priming might be attributed to early and improved emergence. This further gave earlier flowering, number of pods plant^{-1} and seeds pod^{-1} and ultimately higher seed weight. The similar effects were in orthodoxy with (Huang et al., 2017) who verified that seed priming enhanced the grain yield. Not only priming but also the nitrogen affected the pod formation, branches per plant, 1000-seed weight which increased the seed yield of soybean. As Gu et al. (2017) concluded that when soybean seeds were treated with an inoculum of *Rhizobium japonicum* before sowing, higher seed yield was obtained which is in line to this research. While Chen and Chang (2015) found positive trend between the inoculation and the growth parameters and yield in *Glycine max* when they used different nutrient combinations with microbial inoculants. In our study, low yield in osmo primed plots depicted the negative effect of low emergence on the growth and yield of soybean. Similarly, Cabrera-Orozco et al. (2013) narrated that there were no beneficial effects of the seed-priming treatments on corn grain yield but some constructive effects of seed priming on seedling vigor and stand establishment were observed.

Increase in biological yield of soybean was due to early germination, uniform emergence (Table 2, 3), and higher plant height when subjected to different priming techniques which ultimately gave higher biological yield. While response of osmo priming and control treatment showed uneven emergence of plants that finally reduced the biological yield. Moreover, nitrogen fixation through *Rhizobium* inoculation hastened the vegetative growth and post germination parameters and finally improved the biological yield of two soybean varieties.

Conclusion

Seed priming techniques prove best to ensure rapid seedling emergence, better plant stands and have substantial effects on performance of soybean cultivars under field conditions. Both vegetative and reproductive development of soybean crop depends on better seed germination and emergence. However, keeping in view the least understood use of NPK fertilizers in combination with the priming techniques and different varieties to optimize their interaction suggests that further studies are required in this area.

Authors Contribution

MFS and MAC designed the experiments. MTS performed the experiments, collected data and wrote the manuscirpt. MAW, MFS and MAC provided experiment inputs. A Sattar assisted MTS in writing results and discussion. MS helped in writing introduction and abstract. A Shakoor provided technical inpot in data entry and analysis.

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