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ABSTRACT

A series of experiments were conducted in laboratory at The University of Agriculture, Peshawar to determine the optimal duration and maximal concentration of zinc (Zn) and phosphorus (P) for priming wheat seed. The seeds were soaked through a range of durations (0, 12, 18, 24, 36 and 48 h) and tested for germination as surface dry and dried to original moisture (12%). Seed soaked for 18 h resulted in higher mean germination and final germination whereas less time to 50% germination. Primed surface-dry seed performed better than the seed dried to original moisture content. However, primed dried seed were better than the non-primed seed. In other experiments, seeds were primed for 18 h in various concentrations of Zn (0.05%, 0.1%, 1%, 2.0% and 3.0%) and P (0.1%, 0.5%, 1%, 3% and 5%) along with dry and water soaked seeds as control treatments. The seeds were evaluated for germination as surface dry, washed and dried to original moisture, and non-washed dried to original moisture. Seed primed in 0.1% Zn or 0.5% P took less time to 50% germination and resulted in higher mean germination and final germination. The maximal permissible concentrations of Zn and P for priming wheat seed proved to be 0.1% and 0.5%, respectively. The Zn and P primed seeds were also analyzed, as washed and non-washed, for Zn and P concentrations, respectively. Zinc and P concentrations in the seeds were enhanced with increase in Zn and P concentration, respectively, of the priming solution. Non-washed seed contained Zn and P almost in double concentration than that of washed seed.

Key words: Priming duration, wheat, germination, phosphorus, zinc

INTRODUCTION

Achievement of food security is one of the several millennium goals which can be made possible mainly through crop productivity. Crop production is widely limited by nutrient deficiencies and poor stand establishment (Jones and Wahbi, 1992). In drought-prone environment, cereal germination tends to be irregular and can extend over long periods (Bougne et al., 2000). The resulting poor crop stands leave gaps in the canopy, which are rapidly filled by vigorously growing weeds which compete with the crop plants for light, water, and the limiting nutrients (Kropft and Van Laar, 1993). Thus accelerating and homogenizing the germination process is a prerequisite for a good crop establishment and helps to increase yield eventually (Harris, 1996).

Seed qualities (viability and vigor) have profound influence on the establishment and the yield of crops. Healthy plant with well-developed root system can more effectively mobilize limiting nutrients from the soil and can better withstand adverse conditions (e.g. dry spells). Vigorous early seedling growth has been shown to be associated with higher yield (Harris et al., 2000). The vigor of seeds can be improved by techniques generally known as seed priming, which enhances the speed and uniformity of germination (Heydecker et al., 1975). Seed priming induces a range of biochemical changes in the seed, required to start the germination process, such as breaking of dormancy, hydrolysis or metabolization of inhibitors, imbibition and enzyme activation. Some or all of these processes that precede the germination are triggered by priming and persist following the re-desiccation of the seeds (Asgedom and Becker, 2001). Thus upon seeding, primed seed can rapidly imbibe and revive the seed metabolism, resulting in a higher germination rate and a reduction in the inherent physiological heterogeneity in germination (Rowse, 1995).

Unavailability of crop nutrients in appropriate amount and form to crops is one of the major crop productivity constraints in the developing countries (Hussain et al., 2006). Crop production is also affected by low chemical availability of P and Zn (Ryan, 1997). Especially during the early growth stages, a lack of P and Zn retards seedling growth, rendering the young plantlets particularly sensitive to the frequently encountered dry spells (Jones and Wahbi, 1992). A rapid establishment of healthy seedlings and a sufficient supply with P and Zn are prerogative to reduce the risk of crop failure (Brown et al., 1987).

The major soil fertility problems in Pakistani soils are deficiencies of N, P, and Zn (Rashid, 2001). The deficient nutrients may be provided to the crops through soil fertilization but the supply of fertilizers is limited by a range of constraints. Similarly, soil chemical and physical properties also influence nutrients availability. Alternatively, the concept of nutrient seed priming was developed wherein the seeds are primed in solutions containing the limiting nutrients.
Farooq et al. (2006) reported delayed and poor germination and emergence in rice due to over priming. Harris et al. (2001) also stated that damage to seeds of chickpea, in India, due to over-soaking was common. Priming at high nutrient concentration has also been reported to have the potential to damage the seed and inhibit germination (Johnson et al., 2005). Therefore, the present research study was initiated to determine optimal seed priming duration for wheat and to find out maximal P and Zn concentrations in the priming solution.

**MATERIALS AND METHODS**

Optimal soaking duration and maximal concentration of Zn and P for priming wheat seed were determined in a series of laboratory experiments at the University of Agriculture, Peshawar.

**Experiment I: Optimization of Soaking Duration**

Incubation experiments were conducted to determine the optimum priming duration. The effect of hydropriming on time to 50% germination, final germination and mean germination was studied. Seeds, filled into nylon mesh bags, were soaked in distilled water for 0, 12, 18, 24, 36 and 48 h. Half of the soaked seeds were surface dried while the remaining half of the seeds were dried back close to original moisture content (12%). Twenty five seeds from each seed lot were placed on wet blotting paper in Petri dishes and studied for germination in the incubator at 15°C. Each treatment was replicated six times. Germination was determined after every six hours (four times a day) using the standard germination evaluation rules as laid out by ISTA (1983). Time to 50% germination was determined by plotting graph of the germination data. Germination recorded at the last interval was considered to be the final or total germination as there was no further germination. Number of seeds germinated at all the intervals, at which the data were recorded, were summed and then divided by the number of intervals at which the germination was recorded. This gave the mean germination of all the intervals. The data were analyzed using CR design through MS Excel and Statistix software.

**Experiment II: Optimization of Priming Concentration**

The maximum permissible concentrations of P and Zn in the priming solution were determined in these experiments. Seeds were soaked in solutions, having various concentrations of Zn and P, for the predetermined optimum soaking duration (Experiment 1). Five concentrations of Zn (0.05%, 0.1%, 1%, 2.0%, and 3.0%) and five concentrations of P (0.1%, 0.5%, 1%, 3% and 5%); using ZnSO$_4$ and KH$_2$PO$_4$ as Zn and P source, respectively; were included in the experiments. Half of the primed seeds were sown, as surface dry, in Petri dishes and the remaining half of the seeds were dried back close to original moisture content (12%). Half of the remaining seeds were dried without washing while half of them were washed with distilled water before drying. Two control treatments (dry and water soaked seeds) were also included in the experiment. Zinc and phosphorus were determined in the washed and non-washed seeds to know if the nutrients are removed from the seed by washing. Data on time to 50% germination, final germination, mean germination, and P and Zn concentrations of the primed washed and non-washed seeds were recorded. The dried and powdered seeds were subjected to digestion with nitric acid and perchloric acid, and then the aliquots were used for the determination of P and Zn by spectrophotometer and atomic absorption spectrophotometer, respectively (A.O.A.C., 1990). The data were analyzed using completely randomized design through MS Excel and Statistix software.

**RESULTS AND DISCUSSION**

**Experiment I: Optimization of Soaking Duration**

The results of wheat seed primed for different time intervals and dried by two methods (surface drying and drying to original moisture content) before germination are presented in Table 1. The results were significantly (P≤0.05) affected by soaking intervals and higher mean germination (87%) occurred where the seeds were primed for 18 h and surface dried. Lower mean germination (28%) was recorded for seed soaked for 48 h followed by non-soaked dry seed (35%). Significantly higher mean germination (76%) also occurred after 18 h soaking when the seeds were dried to original moisture content (12%). Lower mean germination was noted for non-soaked dry seed followed by seed soaked for 48 h.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean germination (%)</th>
<th>Surface dry</th>
<th>Dried to original moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry seed</td>
<td>35 d</td>
<td>55 e</td>
<td></td>
</tr>
<tr>
<td>12 h soaking</td>
<td>76 c</td>
<td>73 b</td>
<td></td>
</tr>
<tr>
<td>18 h soaking</td>
<td>87 a</td>
<td>76 a</td>
<td></td>
</tr>
<tr>
<td>24 h soaking</td>
<td>82 b</td>
<td>65 c</td>
<td></td>
</tr>
<tr>
<td>36 h soaking</td>
<td>76 c</td>
<td>70 b</td>
<td></td>
</tr>
<tr>
<td>48 h soaking</td>
<td>28 e</td>
<td>62 d</td>
<td></td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>1.77</td>
<td>2.44</td>
<td></td>
</tr>
</tbody>
</table>

By comparing the time taken to 50% germination, it was observed that 43 and 46 h were taken by seeds when soaked for 18 and 24 h, respectively, and surface dried (Fig. 1). Seed soaked for 48 h took higher time (87 h).
followed by dry seed to reach 50% germination. Similarly, wheat seed soaked for 18 h and dried to original moisture took less time (67 h) whereas non-soaked dry seed took more time (83 h) to 50% germination.

Higher final germination of 100% and 99% was obtained for seed soaked for 18 and 24 h, respectively and surface dried whereas lower final germination (60%) was achieved by soaking seed for 48 h (Fig. 1). Highest and lowest final germination was 100% and 85% for seed soaked for 18 h and 48 h, respectively, and dried to original moisture.

The results showed that all the priming treatments, surface dry or dried to original moisture, had faster germination and took less time to 50% germination than non-soaked dry seed. The least time was taken by 18 h soaking treatment to achieve 50% germination, both as surface dry and dried to original moisture content. It was revealed that drying seed to original moisture, after soaking in water, increased time to 50% germination as compared to surface dry seeds. Soaking seed for 18 h achieved higher final germination and higher mean germination, both as surface dry and dried to original moisture. It was also noted that increasing priming duration beyond 18 h caused an increase in time to 50% germination and a decrease in final germination and mean germination probably due to anoxic conditions that may have occurred due to prolonged soaking durations.

Harris et al. (2004) reported that priming seed for 8 h resulted in significant benefits to yield in many crops including wheat. Rehman et al. (2007) suggested 12 h soaking as optimum priming duration for wheat. Yari et al. (2010) primed wheat seed for 12, 24 and 36 hours and concluded that maximum speed of germination, germination percent and VI were observed on 12 h. Kahlon et al. (1992) stated that hydropriming for 24 h in wheat resulted in increased grain yield compared with sowing untreated seeds. Basra et al. (2002) concluded that hydropriming for 48 h showed maximum invigoration followed by hydropriming for 24 h. This limited review of literature summed that there is variation in timing compared with present work that may be due to variation in seed variety or temperature.

![Germination of water soaked surface-dry wheat seed. Vertical bars are standard errors of mean.](image)

Fig 1. Germination of water soaked surface-dry wheat seed. Vertical bars are standard errors of mean.

Priming up to a limit can have positive effect, while extended priming duration will negatively affect germination (Bradford, 1986). Hydrolysis and leaching of certain chemicals from the seed to the aqueous solution, due to priming beyond certain limit, may act as germination inhibitors (Hopkins, 1995). Farooq et al. (2006) reported delayed and poor germination and emergence due to over priming in rice. Similarly, Harris et al. (2001) also observed damage to seeds of chickpea due to over-soaking. Farooq et al. (2010) evaluated different seed priming treatments for rice and investigated whether re-drying is essential or not. Results showed that surface drying was more effective than re-drying close to original weight. The benefits obtained with
priming were partially lost with seed drying and the dried primed seeds had lower performance and higher conductivity than fresh primed seeds (Brancalion et al., 2008).

Experiment 2: Optimization of Priming Concentration: Zn

A. Zinc concentration for priming

1. Germination tests

After determining the safe limit in experiment 1 (i.e. 18 h), wheat seed were primed with different concentrations of Zn solution and sown in Petri dishes for germination test after ‘surface dried’, ‘washed and dried to original moisture’ and ‘dried to original moisture without washing’. Mean germination percentage was significantly (P≤0.05) affected by different Zn concentrations and higher mean germination (81%) was recorded for water soaked seed (Table 2). Among the Zn primed surface dry seed, mean germination was higher (73%) for 0.05% Zn that was at par with 0.1% Zn and lower (28%) for 2.0 and 3.0% Zn primed seed. Zinc primed washed dried seed showed higher mean germination (69%) for water soaked seed followed by 0.05% and 0.1% Zn primed seed (63%) whereas lower mean germination (45%) was noted for 3.0% Zn. Likewise, mean germination of non-washed dried seed was higher (69%) for water soaked followed by 0.1% Zn and 0.05% Zn whereas lower mean germination (25%) was recorded for 3.0% Zn.

Table 2. Mean germination and Zn concentration of seeds of the Zn-primed wheat seed

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean germination (%)</th>
<th>Zn (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S Dried</td>
<td>W Dried</td>
</tr>
<tr>
<td>Dry seed</td>
<td>37 d</td>
<td>56 c</td>
</tr>
<tr>
<td>Water Soaked</td>
<td>81 a</td>
<td>69 a</td>
</tr>
<tr>
<td>0.05% Zn</td>
<td>73 b</td>
<td>63 b</td>
</tr>
<tr>
<td>0.1% Zn</td>
<td>70 b</td>
<td>61 b</td>
</tr>
<tr>
<td>1.0% Zn</td>
<td>48 c</td>
<td>48 de</td>
</tr>
<tr>
<td>2.0% Zn</td>
<td>28 e</td>
<td>52 cd</td>
</tr>
<tr>
<td>3.0% Zn</td>
<td>28 e</td>
<td>45 e</td>
</tr>
</tbody>
</table>

LSD (0.05) 3.78 4.24 3.75 14.29 14.43

S Dried= Surface dried; W Dried= Washed dried; NW Dried= Non-washed dried

Seed soaked in water and sown as surface dried took less time (52 h) to achieve 50% germination followed by 0.05% Zn (54 h) and 0.1% Zn (56 h) solution (Fig. 2). Seed primed in 2.0% Zn solution took more time (114 h) to 50% germination. In case of seed primed in Zn solutions, washed and dried to original moisture; water soaked seed spent less time (82 h) to 50% germination followed by 88 h for 0.05% and 83 h for 0.1% Zn. Results of the non-washed dried seed showed that water soaked seed took minimum time (80 h) to 50% germination. Among the Zn primed seed, less time of 87 h was recorded for 0.05% Zn followed by 0.1% Zn and dry seed. Seed primed in 3.0% Zn solution showed the slowest germination (more time to 50% germination). Graphs of washed and non-washed dried seed have been excluded to shorten the paper.

![Fig 2. Germination of Zn-primed surface-dry wheat seed. Vertical bars are standard errors of mean.](image-url)

Higher final germination of 92% was noted for water soaked seed followed by 87% for 0.1% Zn primed and 85% for 0.05% Zn primed and dry seed whereas lower final germination percentage (55%) was recorded for seed primed in 2.0% Zn solution and surface dried (Fig. 2). Data pertaining to washed dry seeds revealed that water soaked seed had higher final germination (99%) followed by dry seed (97%). Among the Zn priming...
treatments. 0.1% Zn primed washed dried seed resulted in higher final germination followed by seed primed in 0.05% Zn. Lower final germination (65%) was recorded for seed primed in 3.0% Zn. Similarly, data on the non-washed seed showed that water soaked seed resulted in higher total germination (92%) followed by dry seed (89%) and 0.1% Zn primed seed (85%) while seed primed in 3.0% Zn solution resulted in lower total germination (45%).

Wheat seed primed in 0.05% and 0.1% Zn solutions took less time to 50% germination and achieved higher final germination and higher mean germination as compared to other Zn priming treatments. Priming seed in solution having more than 0.1% Zn adversely affected speed of germination and final germination. Time to 50% germination was increased and final germination percentage was decreased when the concentration of Zn in the priming solution was increased beyond 0.1%. Delayed germination was noted for seed dried to original moisture as compared to surface dry seed. The germination was faster, compared to dry seed, for seed primed in lower concentrations of Zn and dried to original moisture, whether washed or non-washed. Furthermore, germination at higher concentrations of Zn was slower and final germination percentage and mean germination percentage was lower for non-washed dried seed as compared to washed dried seed. Since our objective was to find out maximal Zn concentration in the priming solution, therefore, it was concluded that wheat seed may be safely primed in solution having Zn from 0.05% to 0.1%.

Miraj (2005) noted early germination in maize seed primed with water followed by seed primed with 1% Zn solution as compared to non-primed seed. Similarly, final germination was also higher in non-primed seed followed by seed primed in water and 2% Zn solution whereas lower germination was recorded for seed priming in 1% Zn solution (Miraj, 2005). Jalwat (2005) noted earlier germination of mungbean seed primed in 0.02% Zn solution followed by water priming. Total germination was 100% for dry and water soaked seed whereas seed primed in 0.01 and 0.02% Zn resulted in 94 and 91% germination, respectively. Roberts (1948) reported the potential to damage the seed and inhibit germination by priming at high nutrient concentrations. Germination of barely was negatively affected by priming in solutions having more than 50 mM Zn and the germination rate was particularly reduced to below the level of unprimed seed by 100 mM Zn solution (Ajouri et al., 2004).

2. Zinc concentration in Zn-primed seed

Chemical analysis of the Zn-primed wheat seed revealed that the Zn concentration was significantly (P≤0.05) affected by the different concentrations of Zn in the priming solution (table 2). Increasing Zn concentration in the priming solution caused an increase in the Zn concentration of the primed seed. Higher concentration of Zn was noted in the seed primed in the highest concentration of Zn, both as washed and non-washed. However, Zn adhered to the seed surface was removed from the seed as a result of washing with water. Johnson et al. (2005) indicated 350 mg kg⁻¹ Zn in wheat seed primed for 12 h in 0.004 M Zn solution. Zinc content was increased in the seeds and on the seed coat of barley due to priming in Zn solution (Ajouri et al., 2004). Miraj (2005) revealed that Zn concentration in the seed was increased with increasing Zn concentration in the priming solution and the non-washed seed had Zn concentration more than double of the washed seed. Shah et al. (2012) reported similar trend for mungbean seed. Harris et al. (2007) revealed that simply rinsing the primed seeds reduced the Zn content suggesting that about half the extra Zn was adhering to the seed surface or just below the seed coat and was relatively easy to remove.

B. Phosphorus Concentration for Priming

1. Germination tests

Germination results of wheat seed primed, for the predetermined safe limit (18 h), in different concentrations of P and sown in Petri dishes as ‘surface dried’, ‘washed and dried to original moisture’ and ‘dried to original moisture without washing’ are shown in table 3. Germination was significantly (P≤0.05) affected by different concentrations of P in the priming solution and higher mean germination (85%) was achieved by 0.1% P primed surface dried seed that was at par with water primed seed whereas lower mean germination (28%) was achieved by 5% P primed seed (Table 3). Seed primed in 0.5% P solution, dried without and after washing, achieved higher mean germination that was at par with 0.1% P whereas lower mean germination was recorded for 5% P primed seed.

Table 3. Mean germination and P concentration of seeds of the P-primed wheat seed

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean germination (%)</th>
<th>P (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S Dried</td>
<td>W Dried</td>
</tr>
<tr>
<td>Dry Seed</td>
<td>38 d</td>
<td>52 d</td>
</tr>
<tr>
<td>Water Soaked</td>
<td>81 a</td>
<td>63 b</td>
</tr>
<tr>
<td>0.1% P</td>
<td>85 a</td>
<td>69 a</td>
</tr>
<tr>
<td>0.5% P</td>
<td>71 b</td>
<td>71 a</td>
</tr>
<tr>
<td>1% P</td>
<td>59 c</td>
<td>59 c</td>
</tr>
<tr>
<td>3% P</td>
<td>34 e</td>
<td>51 d</td>
</tr>
<tr>
<td>5% P</td>
<td>28 f</td>
<td>42 e</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>3.97</td>
<td>3.96</td>
</tr>
</tbody>
</table>

S Dried= Surface dried; W Dried= Washed dried; NW Dried= Non-washed dried
Seed primed in 0.1% P and water, and then surface dried, took less time to 50% germination followed by 0.5% P primed seed (Fig. 3). Dry non-primed seed took more time (90 h) to 50% germination whereas seed primed in 3% and 5% P solutions did not achieve 50% germination. Germination of seed dried to original moisture, after washing with distilled water, showed that less time (76 h) to 50% germination was spent by 0.5% and 0.1% P primed seed whereas more time to 50% germination was 100 h for 5% P primed followed by 88 h for dry seed. Germination of non-washed seed, dried to original moisture, revealed that less time (76 h) was taken by 0.5% P primed seed followed by 1.0% P (77 h). Seed primed in 5% P solution did not achieve 50% germination and 3% P took more time (90 h) to 50% germination.

Final germination was higher (95%) for 0.1% P primed surface dried seed followed by water and 0.5% P primed seed whereas lower germination (44%) was noted for 5% P primed seed (Fig. 3). Seed primed in 0.1% P, and dried to original moisture after washing with water, resulted in higher final germination (92%) followed by 0.5% P primed seed. Higher final germination (53%) was recorded for 5% followed by 3% P. Seed primed in 0.1% P, and dried without washing, had higher final germination (96%) followed by water soaked (93%) and 0.5% P primed seed (91%) whereas seed primed in 5% P had lower (44%) final germination.

It was noted in the results that wheat seed primed in 0.1% and 0.5% P solutions achieved 50% germination in less time and resulted in higher final germination and higher mean germination as compared to other P priming treatments. Increasing concentration beyond 0.5% P in the priming solution increased time to 50% germination and decreased final germination and mean germination. Seed primed in solutions having concentrations higher than 3% P, surface dry or dried to original moisture without or after washing, took more time to 50% germination as compared to non-soaked dry seed or even did not reach to 50% germination. Germination was delayed for seed dried to original moisture as compared to surface dry seed. However, as compared to dry seed, the germination was faster for seed primed in P solution having concentrations lower than 3% and dried to original moisture, whether washed or non-washed. The objective was to find out maximal P concentration in the priming solution, therefore, it was concluded that wheat seed could be primed in 0.1% to 0.5% P solutions.

Miraj (2005) tested germination of maize seed primed in water, 1% and 2% P solutions. It was reported that seed primed in water has the earliest germination followed by lower level of P (1%). Total germination was higher for non-primed seed followed by 1% P and water priming. Jalwat (2005) studied germination of mungbean seed primed in water, 0.01% and 0.02% P. Early germination was noted for 0.01% P followed by water soaked and 0.02% P, respectively. Total germination was 100% for dry and water soaked seed whereas 0.01% and 0.02% P achieved 94% and 95% total germination, respectively. Iqbal et al. (2012) reported a decrease in time to 50% germination and mean germination time but no effect on final germination when seeds of two wheat cultivars were primed in 0.01% B solutions. Both wheat cultivars showed an adverse effect on the germination and seedling growth beyond this concentration.
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2. Phosphorus concentration in P-primed seed

Analysis of the P-primed wheat seed revealed that P concentration was significantly (P≤0.05) affected by the different concentrations of P in the priming solution (Table 3). Phosphorus concentration in the primed seed was increased with increasing P concentration in the priming solution. Seed primed in solution of the highest concentration of P, both as washed and non-washed, resulted in higher concentration of P. However, about half of the P was removed as a result of washing the seed with water.

Miraj (2005) recorded higher P concentration in maize seed, both as washed and non-washed, primed in the highest concentration of P and the non-washed seed had more P concentration than the washed seed. Similar trend was found for mungbean seed (Jalwat, 2005 and Shah et al., 2012). Nutrient adhering to the seed surface or just below the seed coat could be easily removed by simply rinsing the primed seed in water (Harris et al., 2007).

CONCLUSION AND RECOMMENDATIONS

It was concluded that 18 h was the optimum priming duration for wheat and the seed be safely primed in nutrient solutions containing up to 0.1% Zn or 0.5% P

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