

INFLUENCE OF SEED INVIGORATION TECHNIQUES ON GERMINATION AND SEEDLING VIGOR OF MAIZE (*ZEA MAYS* L.)

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ABSTRACT. The objective of this study was to assess the comparative efficiency of different priming techniques on germination and early seedling growth of maize cultivars. Laboratory experiments were conducted to examine the efficacy of different seed invigoration treatments on seed germination and vigor traits of three maize hybrids, Pioneer 3025, Pioneer 70 and Baber at Agronomy research laboratory, University of Agriculture, Peshawar, Pakistan. The experiment was laid in completely randomized design (CRD) and was replicated thrice. The treatments included control treatment (T₁) unsoaked/dry seed, (T₂) hydropriming with distilled water for 24 hrs, (T₃) halo priming treatments with NaCl (3% solution) for 24 hrs, (T₄) osmopriming with PEG-6000 for 24 hrs and (T₅), hormonal priming with GA₃ for 24 hrs. The results showed that seed invigoration treatment with gibberellic acid (GA₃) PEG-6000 and hydropriming with distilled water for 24 hrs gave higher germination, decreased days to 50% germination, increased shoot length, root length, seedling fresh and dry weight, as

compared to halo priming with NaCl and control treatment. It is concluded that seed invigoration with GA₃, osmopriming with PEG-6000 and hydropriming with distilled water for 24 hrs serve as an appropriate treatment for accelerating the emergence and growth parameters of maize hybrid. Pioneer 3025 showed its superiority over other cultivars in all the studied parameters.

Keywords: priming; seed germination; seedling growth; vigor; maize.

INTRODUCTION

Seed enhancement through priming has led to great improvements in farmer's ability to achieve this goal in the field and under controlled environment/greenhouse (Amin *et al.*, 2016). Numerous cereals, vegetable and ornamental crop species have been primed successfully for improving seed quality and longevity. Rapid and

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uniform field emergence are two vital rudiments to increase yield, quality and ultimately profits in crops. Uniformity and percentage of seedling emergence of direct-planted crops have a major impact on final yield and quality (Khan *et al.*, 2016). Slow emergence results in smaller plants and weak seedlings, which are more vulnerable to soil-borne diseases. Extended emergence periods expose the planting bed to deterioration and increased soil compaction, particularly under unfavorable environmental conditions. Poor germination is a common phenomenon at suboptimal temperatures, which is a great concern of farmer's community that grows wheat in late winter and maize in early spring. Optimum seed germination and seedling emergence occur at relatively high temperatures (20-30°C) for several species of wheat, barley, tomato, watermelon, cucumber and melon nowadays. Different seed priming techniques have been developed, including hydropriming (soaking in water), halopriming (soaking in inorganic salt solutions), osmopriming (soaking in solutions of different organic osmotica), thermo priming (treatment of seed with low or high temperatures), solid matrix priming (treatment of seed with solid matrices), and biopriming (hydration using biological compounds) (Ashraf & Foolad, 2005).

Each treatment has advantages and disadvantages and may have varying effects depending upon plant species, concentration/dose of priming

agent, incubation period and stage of plant development. The use of a salt, as an osmoticum, can lead to an increase in fresh weight of a seed. In this case, germination is delayed through increased solute potential of the embryo. Osmoconditioning is also used to describe the same treatment when materials, such as polyethylene glycol (PEG), are used as the osmoticum. Solid matrix priming (SMP) is a term used for a pre-sowing seed treatment in which a solid matrix (press mud) is used, instead of an osmotic solution to enhance germination. Matrix conditioning was proposed as an alternative term to SMP, to distinguish seed conditioning by matrix and osmotic forces. Biopriming is a treatment where sweet corn seeds are coated with a bacterium and soaked in warm water until the seed moisture content increases to 35-40%. Seed priming treatments (pre-sowing seed treatment) using moist chilling, growth regulators (gibberellic acid, GA₃) and plant-derived smoke compounds (El-Dengawy, 2005; van Staden *et al.*, 2006), magnetic fields (Eşitken, 2003) and salts, such as KNO₃, have been effective in improving seed germination at low and high temperatures (Demir & Mavi, 2004). Seed maturation stage can also be an influential factor in germination performance at low temperatures and response to priming treatment (Demir & Oztokat, 2003).

In general, mature seeds tend to show a better germination

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performance at stress temperatures than those of earlier and later harvests, while advancement obtained by priming was greater in earlier harvests (Khan *et al.*, 2016). Several studies on seed germination and emergence revealed the beneficial effects of seed priming by several ways (heat, smoke, soaking, leaching, temperature, scarification and NaCl salinity) (Ahmed *et al.*, 2006). Solid matrix priming improved germination of hot pepper seed by 10-16% depending on temperature, and this effect enhanced when SMP was followed by halopriming and osmopriming (Pandita *et al.*, 2007). Moreover, hydropriming (48 hrs) for tomato and sand matrix priming (80% water holding capacity, 3 days) for eggplant and chilli were established as best methods of priming treatment capable of improving seed vigour (Venkatasubramanian & Umarani, 2007). Furthermore, a low frequency magnetic field (16 Hz) can be used as a method of post harvest seed improvement for different plant species, especially for seeds of temperature-sensitive species germinating at low temperatures (Rochalska & Orzeszko-Rywka, 2005).

The effect of magnetic field and or KNO_3 on asparagus seeds resulted in increased seed germination and seedling growth, as well as it changed the direction of radicle growth (Soltani *et al.*, 2006). Increasing NaCl concentration (200 mM) reduced the germination percentage, the growth parameters and the relative water

content of four lentil genotypes (Sidari *et al.*, 2007) and also affected seed germination for several species (carrot, chile pepper, tomato and guayule) (Miyamoto *et al.*, 2004). Moreover, several factors, such as dehydration temperature and/or relative humidity, may affect pepper seed emergence and/or germination (Demir *et al.*, 2005). High temperatures during sowing may delay or inhibit seed germination in most crops. Consequently, seed invigoration techniques may be used as an important tool to improve seed performance and stand establishment in the field (e.g. maize, wheat), especially during the summer and winter (Shah *et al.*, 2007). Therefore, the aim of this study was to evaluate the potential of seed invigoration techniques in improving germination and early seedling growth of maize hybrids under the agro-environmental conditions of Peshawar.

MATERIALS AND METHODS

The experiment was conducted to study the influence of different seed invigoration techniques on germination and vigor attributes of the maize hybrids in the seed technology laboratory, Department of Agronomy, University of Agriculture, Peshawar, Pakistan, during the year 2016. Seeds of maize hybrids, Pioneer 3025 and Pioneer 70 were obtained from their respective seed companies, whereas Baber was obtained from Cereal Crop Research Institute (CCRI), Nowshera-Pakistan. Before the start of experiment, seeds of all cultivars were surface sterilized in 10% sodium hypochlorite solution for 10 min, then

rinsed with distilled water and dried closer to original seed moisture content of 9.24% with forced air at room temperature. Seeds of uniform size were used in the experiment. The experiment was laid out in completely randomized design with three replications. Seed were treated with the following seed-soaking media: (T₁) unsoaked seed (control), (T₂) hydropriming with distilled water for 24 hrs, (T₃) halopriming with NaCl (3% solution) for 24 hrs, (T₄) osmopriming with PEG-6000 for 24 hrs, (T₅) hormonal priming with GA₃ for 24 hrs. After each treatment, seeds were given three surface washings with distilled water and dried closer to original moisture with forced air at room temperature.

For germination of the maize seeds, three replicates of 100 seeds each were placed on moistened filter paper in Petri

dishes (11.0 x 1.5 cm), then placed in an incubation chamber at 25°C. A seed was considered to have germinated when coleoptile and radical lengths have reached 2 mm. Counts of germinating seeds were made daily, starting on the first day of imbibition and terminated when maximum germination was achieved. The time to reach 50% germination (T₅₀) of final germination was calculated according to the following formula of Coolbear et al. (1984):

$$T_{50} = t_i + \frac{[N/2 - n_i] \times (t_j - t_i)}{n_j - n_i},$$

where, N is the final number of germination and n_i, n_j cumulative number of seeds germinated by adjacent counts at time t_i and t_j when n_i < N/2 < n_j.

Germination index (GI) was calculated according to ISTA (2009) formula:

$$GI = \frac{\text{No. of germinated seeds at first count}}{\text{Days of first count}} + \dots + \frac{\text{No. of germinated seeds at final count}}{\text{Days of final count}}$$

Seedling emergence

Control and treated seeds were sown in plastic trays (25 in each) having moist sand, replicated three times and were placed in growth chamber at 25°C temperature in completely randomized design. Emergence was recorded daily according to ISTA rules (2009). Mean emergence time (MET) was calculated according to the equation of Ellis and Roberts (1981), as under:

$$MET = \frac{\sum Dn}{\sum n}$$

where, n is the number of seeds, which were germinated on day D and D is the number of days counted from the beginning of emergence. The plants were harvested 12 days after planting and seedling vigor data were recorded according to ISTA (2009).

Measurement of root and shoot length

Root and shoot length of randomly selected 25 seedlings were measured after 12 days of experiment. It was measured with a measuring scale and expressed in cm. These seedlings were kept in brown paper and weighed the fresh weight first and were dried in oven at 70°C for 48 hrs to record the dry weight. These were measured with electronic balance and expressed in mg.

Statistical analysis

Data on different parameters were subjected to analysis of variance (ANOVA), according to the methods described for completely randomized design (CRD) and means between treatments were compared using LSD test at 5% probability level (Steel & Torrie, 1997).

RESULTS AND DISCUSSION

Germination (%)

The data revealed that all seed invigoration treatments significantly ($p < 0.05$) affected the germination (%) and early seedling growth in all maize cultivars. The interaction between C x P was found non-significant. Hormonal priming with GA₃ and osmopriming with PEG-6000 gave faster and earlier germination, followed by hydropriming (Fig. 1). A significant decline in germination (%) was observed by halopriming, when seed were treated with NaCl for 24 hrs (Fig. 1). The decrease in germination (%) by NaCl might be due to accumulation of salts in tissue, which cause toxicity (Smith & Cobb, 1991). Germination index also showed similar trend of improvement (40.2) in hormonal and osmopriming, followed by hydropriming, when seed were soaked in distilled water for 24 hrs. A gradual decrease in germination % and germination index took place, when seed were soaked in NaCl for 24 hrs. Non-primed seeds/control treatment gave minimum germination % and germination index. The improvement in germination % and germination index by hormonal and osmopriming of GA₃ and PEG-6000 may be attributed to stimulation of hydrolytic enzyme activity, known to be induced by these pre-sowing chemical agents. The reason for higher germination % and germination index may be due to

greater hydration of colloids, higher viscosity and elasticity of protoplasm, offer an increase in bound water content, lower water deficit and increased metabolic activity (Tariq *et al.*, 2017). These findings support the earlier work on wheat (*Triticum aestivum*) (Amin *et al.*, 2016) and rice (*Oryza sativa*) (Lee & Kim, 2000), who reported improved germination rate and percentage in seeds subjected to seed hardening and hydropriming for 24 hrs. Seed priming with GA₃, osmopriming with PEG-6000 and hydropriming with distilled water for 24 hrs significantly reduced the germination time (T₅₀) and improved the emergence rate and seedling vigor despite that the seed lot provided by the respective companies was of high vigor (Table. 1). It shows that priming with GA₃ and PEG-6000 for 24 hrs not only improve the performance of low vigor seeds, but also invigorate and induce early, synchronized and healthier crop stand. Among maize cultivars, Pioneer 3025 and Pioneer 70 had the highest germination percentage, as compared to Pioneer 70 and Baber cultivars (Fig. 1). Baber gave minimum germination percentage in all pre-sowing treatments. Statically, similar trend of increase in germination index was observed in cultivar Pioneer 3025 and Pioneer 70, as compared to Baber cultivar. The variation among cultivars with respect to germination and its related attributes may be due to genetic potentiality of these newly released maize cultivars and their positive response to different priming

techniques. The findings of this study support the earlier work on wheat (*Triticum aestivum*) (Khalil *et al.*, 2015), who stated that osmopriming

with PEG (20%) for 24 hrs may be used as tool to improve germination and seedling growth of wheat under drought condition.

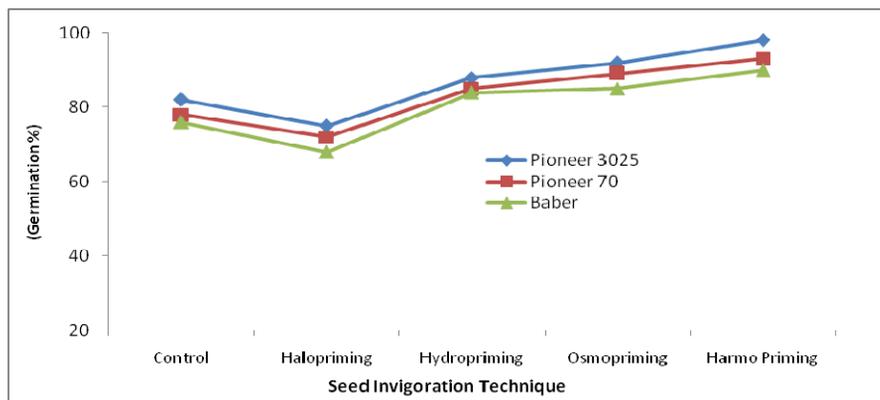


Figure 1 – Influence of different seed invigoration techniques on germination of maize cultivars

Table 1 - Effect of different invigoration treatments on germination and vigor attributes of maize cultivars

Pre-sowing treatment	T50 days	E50 days	Germ. index	S.f. wt (mg)	S.d. wt (mg)
Control	4d	5c	20.1d	6.7c	4.9c
Hydropriming	2b	3b	24.3c	10.5ab	8.7b
Halopriming	3c	4bc	22.3c	9.1b	7.9b
Osmopriming	1a	3a	34.9b	11.34a	9.2a
Hormonal priming	1a	2a	40.2a	12.89a	10.21a
Maize cultivars					
Pioneer 3025	1.5a	2.8a	31.7a	11.7a	9.7a
Pioneer 70	1.4a	3.0b	31.1ab	10.4b	8.23b
Baber	2.0b	3.4b	28.5b	9.3b	7.3b
Interaction between (CxP)					
	Ns	ns	Ns	Ns	Ns

*Means of the same category, followed by different letters, are significantly different at 0.05 level of probability using LSD test.

Seedling vigor

Data regarding seedling growth and vigor are reported in tables and figures. Significantly, maximum time (5 days) to E₅₀ was recorded in

halopriming with NaCl and control treatment/non-primed seed, whereas minimum day to E₅₀ was observed in hormonal and osmopriming (2 days), followed by hydropriming (Table. 1).

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Maximum emergence time was observed in NaCl and control treatment/non-primed seed, which resulted in delayed and scattered germination. Decrease in T_{50} by hormonal treatment of gibberellic acid (GA_3) and osmopriming of PEG-6000 may be due to faster production of germinative metabolites and better genetic repair, i.e. earlier and faster synthesis of DNA, RNA and protein

(Bray *et al.* 1989). Significant increase in lengths of root and shoot were observed in 24 hrs hormonal priming with GA_3 and osmopriming with PEG-6000, which was statistically at par to water soaked seeds (*Fig. 2*). A half-way increase in shoot/root length was observed in NaCl (2%), haloprimed seed, as compared to non-primed/dry seed.

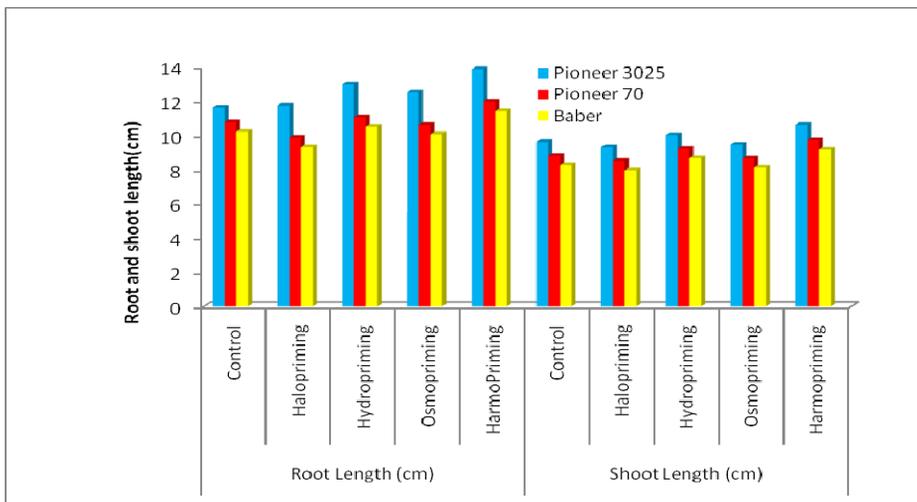


Figure 2 – Comparative performance of different seed invigoration techniques on root and shoot length of maize cultivars

This is in line with the findings of Gulnaz *et al.* (1999), who stated that both quantitative and qualitative responses of plants to different hormones/chemicals may differ considerably at different plant growth stage. Pre-sowing invigoration treatment gave maximum fresh and dry seedling weight, except halopriming with NaCl (*Table 1*). Control treatment gave minimum fresh dry weight. This shows that during invigoration procedure, seeds

were simultaneously subjected to the processes of repair and deterioration and force between the two determinants of success or failure of the treatment. Improved seedling dry weight by hormonal and osmopriming treatment may be attributed to more synchronize germination, which resulted in early stand establishment. Higher shoot fresh weight and length in seedlings from 24 hrs invigoration in GA_3 and PEG 6000 seed is an indication of early emergence and

more rapid rate of emergence of wheat seed (*Table 1; Fig. 2*). Earlier work of Shah (2007) support the findings of current research in which hydropriming and hardening resulted in higher fresh and dry weight of seedlings. Longest shoots were noted in 24 hrs seed hardening. The findings of present study are in agreement with the results of Kaya *et al.* (2006) and Basra *et al.* (2006), who observed that hardening and hydroprimed seeds of sunflower and wheat could germinate faster and produced longer seedling under drought/salinity stress, as compared with untreated seeds. Hardening of wheat seeds in NaCl and Na₂SO₄ could not strengthen the wheat seeds rather these reduced the vitality and increase emergence time. Maize cultivars Pioneer 3025 and Pioneer 70 gave maximum root and shoot length (13.85 and 10.6 cm) than Baber under different seed invigoration treatments (*Fig. 2*). Both cultivars performed better under hormonal and hydropriming. The response of osmo and halopriming was statistically at par in the said parameters. Minimum root and shoot length was observed in control treatment. Maximum seedling fresh and dry weight were obtained from seed treated with GA₃ (hormonal priming) and PEG-6000 (osmopriming), followed by hydropriming, as compared to control treatment/ non-primed seed (*Table 1*). Improved seedling FW might be due to increased cell division within the apical meristem of seedling roots, which cause an increase in plant

growth, which resulted in early stand establishment (Farooq *et al.*, 2007).

CONCLUSION

Generally, the overall performance of seed invigoration with GA₃ (hormonal priming), PEG-6000 (osmopriming) and traditional soaking of seeds in water (hydropriming) for 24 hrs was good and helpful in reducing the risk of poor stand establishment.

Our findings revealed that among seed invigoration techniques, the above are useful technique for enhancing seedling emergence rate and percentage of maize. These can improve seedling establishment and field performance of this important cereal.

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