

Forage Maize (*Zea mays* L.) Germination, Growth and Yield Gets Triggered by Different Seed Invigoration Techniques

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Abstract: Poor germination is one of the major culprits responsible for lower than potential green forage yield of cereals. A study was therefore conducted to evaluate the effects of different seed priming techniques on emergence, growth and yield of forage maize (*Zea mays* L.). The field trial was carried out at Agronomic Research Area, University of Agriculture Faisalabad, Pakistan, during spring season of 2013. The experiment was laid out in randomized complete block design (RCBD) having three replications. Net plot size was 1.8 m × 7.0 m. Maize seeds were subjected to hydro-priming for 12 hours, on-farm priming and osmopriming with CaCl₂ (1.25%), KCl (1.25%) and KNO₃ (1.25%) solution for 12 hours. Non-primed seeds were also sown in control plots for comparison. Results revealed that priming reduced time to start seed emergence by one day than non-primed seed. Least time taken to 50% emergence (6 days), mean emergence time (6.5 days) and highest final emergence count (30.3 m⁻²) were recorded in plots sown with seed osmoprimed with KCl. The maximum plant height (227.8 cm) was recorded by osmoprimed seed with CaCl₂. Seed osmoprimed with CaCl₂ recorded 40 and 38% higher green forage and dry matter yield, respectively as compared to non-primed seed. The highest crude protein contents were recorded by CaCl₂ priming, while the highest crude fiber and ash contents were given by KNO₃ primed seed that were 77, 40 and 62%, respectively higher than control. It can be concluded from the present study that seed priming with salts especially CaCl₂ can be used to improve emergence, yield and quality of fodder maize.

Key words: Seed priming • CaCl₂ • KNO₃ • Hydro priming • On-farm priming • Osmo-priming

INTRODUCTION

Maize (*Zea mays* L.) is the staple food crop in many countries of the world. It is also an important cereal and fodder crop of Pakistan. It provides bulk of raw material for livestock and other agro-allied industries across the globe [1, 2]. It finds its use in a variety of industrial products like corn oil, corn starch, corn flakes, animal feed, dextrin, sorbitol, lactic acid, sorbic acid, fuel (ethanol) and also as tanning material for leather industry [3]. Maize is called the miracle crop or the Queen of Cereals because it has higher yield potential as compared with other cereals [4]. It is the domestic grass of tropical Mexican origin and belongs to family Gramineae. It ranks third after wheat and rice on hectare basis in Pakistan. It is the crop with high yield and highly nutritious forage produced by less labour and machinery requirement as compared with other forage crops [5-7]. It is grown as

fodder crop alone or in the form of mixture with legumes from mid-February to September, which helps to cope with the forage scarcity problem faced in May-June and October-November. It provides the heavy tonnage of fodder throughout the summer season. It is a nutritious fodder and is the richest feed source for livestock [8]. Although the edaphic and climatic conditions of Pakistan are favorable for maize production, but the quality of forage is one of the major constraint to improve livestock production. There are various factors contributing towards the lower fodder yield like substandard methods of cultivation [9, 10], malnutrition [11, 12], diseases, pests [13], weeds infestation, water scarcity [14], lack of high yielding varieties [15], post-harvest losses and seasonal changes in climate. Among these, poor stand establishment is also one of them. Poor crop establishment in the arid and semi-arid tropics is a major constraint to crop production [16, 17]. Broadly speaking,

the term invigoration is implied to any seed treatment i.e. chemical, physical or physiological which is done to facilitate and enhance germination, vigor of seedling and to improve stand establishment in field, vegetable and horticultural crops [18]. Recently, infusion of pesticides, fungicides, bio-ingredients, bio-products, growth regulators, agro-chemicals, halogens and herbicidal antidotes have made this more attractive and cheap technology to get profitable results [19]. Seed priming is considered as one of the most practical approach in crop production [20]. Early germination and uniform crop stand establishment can be obtained by using different seed priming techniques [21]. Seed priming is a pre-sowing technique by which seeds are partially hydrated up to a limit where the germination processes get started but radical protrusion does not occur [22, 23, 24]. Most commonly used priming treatments are on-farm priming, hydropriming and osmopriming. For on-farm seed priming, firstly seeds are soaked in water overnight following surface-drying under shade and then sowing is done in imbibed state [25]. In hydropriming seeds are soaked in distilled water for specific time duration with aeration, then seeds are dried to its original weight and sown in the field. The most widely used priming type is osmopriming in which seed soaking is done in aerated solution of low water potential [26]. Priming allows rapid DNA replication, enhances protein and RNA synthesis, repairs damaged parts of seed, increases embryo growth and reduces metabolites leakage [27]. Seed primed with nitrate solution has been reported to increase the germination rate and germination index of many cereals. The radicle and plumule appeared very fast due to high water uptake efficiency and rapid metabolic activity in primed seeds [28]. Osmopriming with CaCl_2 and KCl shows osmotic benefits as both K^+ and Ca^{2+} play important role in water saturation improvement of cell and both act as co-factor of many enzymes [29]. So osmopriming can be used as a suitable technique to improve crop salt tolerance.

The present study was therefore, planned to evaluate the influence of seed priming techniques on growth and yield of forage maize (*Zea mays* L.) under the agro-climatic conditions of Faisalabad.

MATERIALS AND METHODS

In order to investigate the effects of different seed priming techniques on growth and yield of forage maize (*Zea mays* L.), the seeds were primed in the laboratory and then a field trial was conducted at Agronomic Research Area, University of Agriculture Faisalabad (30.35-31.47°N

latitude and 72.08-73.0°S longitude, 184 m above sea level). The experiment was laid out in a randomized complete block design (RCBD) having three replications. The gross plot size was 1.8 m × 7.0 m and net plot size was 1.8 m × 6.0 m. The seeds of forage maize (var. Pak. Afgoyee) were obtained from Ayub Agricultural Research Institute, Faisalabad. While, the chemicals for priming i.e. Calcium Chloride (CaCl_2), Potassium Chloride (KCl) and Potassium Nitrate (KNO_3), were purchased from the local market of Faisalabad.

Experimental Protocol: Maize seed were subjected to Hydro-priming for 12 hours, On-farm priming, osmopriming with CaCl_2 (1.25%) solution for 12 h, osmopriming with KCl 1.25% solution for 12 h and osmopriming with KNO_3 (1.25%) solution for 12 h. For seed priming, seed was soaked in respective solutions or water, keeping seed to solution ratio 1:5 (w/v) at $25 \pm 2^\circ\text{C}$ for 12 h. The untreated seeds were sown for control treatment. After respective soaking period, seeds were removed from solution and washed with the distilled water three times and dried near to original seed moisture content with forced air. Then seeds were sealed in air tight polythene bag. Maize fodder var. Pak. Afgoyee was sown on 28th of May, 2013. Sowing was done by manual method using a seed rate of 100 kg ha⁻¹. The crop was sown at 30 cm spaced rows.

Half dose of nitrogen along with full dose of phosphorous was applied by broadcast method at the time of sowing in the form of Urea and Diammonium Phosphate (DAP), respectively, while remaining half dose of nitrogen was applied with 2nd irrigation. All the agronomic operations were kept uniform for all treatments. Total five irrigations were applied. First irrigation was applied 15 days after sowing the crop while the remaining irrigations were applied according to the crop requirement. Weeds were controlled manually by hand hoeing twice at 20 and 35 days after sowing. Hand weeding was done at proper soil moisture condition. Furadan (3-G) at 20 kg ha⁻¹ was applied to protect the crop from maize borer and shoot fly. Crop was harvested manually after three months of sowing and tied into bundles.

Procedure for Recording Data

Stand Establishment

Time to Start Emergence (days): In each plot at randomly selected places, time to start emergence was recorded by visiting the field daily. The time when the first seed started germination was recorded as time to start germination.

Time to 50% Emergence: The time to reach 50% germination (T_{50}) of final germination was calculated according to the formula suggested by Coolbear *et al.*, [30]:

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

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

Mean Emergence Time (Days): Mean emergence time (MET) was calculated according to the equation of Ellis and Roberts [31] as under:

$$EI = \frac{\text{No. of seedlings at first count} + \dots + \text{No. of seedlings at last count}}{\text{Days to first count} + \dots + \text{Days to last count}}$$

Agronomic and Yield Attributes

Plant Height (cm): At the time of fodder harvesting data on plant height was recorded. Ten plants were randomly selected from each plot at harvest and plant height was measured from the soil level to the tip of the highest leaf with the help of measuring tape and then their averages were calculated. Plant height was taken in centimeter.

Leaf Area per Plant (cm²): Randomly ten plants were harvested from each plot. After that their leaves were removed and weighed. Then a sub sample of 10 g was kept over the screen of leaf area meter (Licor model 3100) to record leaf area. Then this leaf area was used for calculating leaf area per plant.

Green Forage Yield at Harvest (t ha⁻¹): All the plots of each replication were harvested with the help of sickle and were weighed to calculate the green forage yield of each plot in kg. After that the yield was converted into t ha⁻¹.

Dry Matter Yield (t ha⁻¹): Dry matter yield per plot was obtained by multiplying the average dry weight of a plant from each plot with number of plants present in that plot and then yield was converted into t ha⁻¹.

$$\text{Dry matter (\%)} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

Quality Attributes

Crude Protein (%): The crude protein percentage was determined by using standard procedures as given below,

$$N\% = \frac{\text{Volume of N/10 sulphuric acid used} \times 0.0014 \times \text{volume of sample dilution}}{\text{Wt. of sample} \times \text{volume of sample solution used (10 ml)}} \times 100$$

$$\text{Crude protein (\%)} = N\% \times 6.25$$

Crude Fiber (%): The fiber percentage was calculated by following formula.

$$\text{Crude fiber \%age} = \frac{W1 - W2}{\text{Sample weight (g)}} \times 100$$

Ash (%): Total ash (%) calculated by given formula.

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

where

MET = Mean emergence time (days)

n = Number of seedlings, which emerged on day D

D = Number of days counted from start of emergence

Final Emergence Count (m²): When the seedling emergence became constant in each plot, then total number of emerged seedlings was counted in square meter from each respective plot.

Emergence Index: Emergence index was calculated by using the following formula:

$$\text{Ash (\%)} = \frac{\text{Total weight of crucible and ash} - \text{Weight of empty crucible}}{\text{Weight of the sample}} \times 100$$

Statistical Analysis: Data collected on various crop attributes were statistically analyzed using Fisher's analysis of variance technique using "DASAAT" statistical program [32]. Difference among treatments' means was compared using Fisher's least significant test (LSD) at 5% probability level [33].

RESULTS AND DISCUSSION

Stand Establishment: All the treatments showed significant differences regarding time to start emergence. Primed seed emerged earlier than non-primed seed as maximum TSE (5.33 days) was calculated when non-primed seeds were sown, while all other treatments of hydro-priming, on-farm priming and osmopriming with CaCl_2 , KCl and KNO_3 showed least TSE (4.33 days). So, it is concluded that the priming techniques reduced the time to start emergence (Table 1).

Time to 50% emergence is a good index of seed vigor. Vigorous seeds germinate earlier and provide uniform stand establishment and photosynthesis faster just after the emergence in seedlings emerged from primed seed. Data regarding E_{50} the effect of seed priming is depicted in Table 1. It is clear from the table that seed priming with different agents significantly ($p=0.05$) improved seed emergence by decreasing days to 50% emergence. All primed treatments showed less time taken to 50% emergence than non-primed seed. It is clear from the table that osmopriming with KCl 1.25% solution for 12 hours performed the best and took least time (6.10 days) for the completion of 50% emergence closely followed by CaCl_2 recording 7.32 days for 50% seeds to be emerged. Both of these are also statistically at par ($p=0.05$) with each other. After that osmopriming with KNO_3 (9.44 days) took least time for E_{50} , which was also at par with on-farm priming (11.45 days). The control (14.30 days) and hydro-primed (12.67 days) seeds took maximum time for E_{50} , both of

these also shared the same letter which represented that these were not statistically different from each other but varied significantly from other treatments.

Mean emergence is an important index of synchronized emergence, uniformity and seedling emergence. Earlier and synchronized emergence gives uniform seedling establishment, which ensures better crop stand. Seedlings with lower value of mean emergence time are considered more vigorous because they can complete their emergence in less time period. There was significant effect of seed priming on (MET) of forage maize. The minimum mean emergence time was recorded in seeds osmoprimed with KCl 1.25% solution for 12 h (6.47 days) which was statistically at par ($p=0.05$) with seeds hydro-primed for 12 h. (7.12 days), on-farm primed (7.27 days) and osmoprimed with CaCl_2 1.25% solution for 12 h. (6.90). The maximum mean emergence time was observed in control (8.37 days) which was also at par with seeds osmoprimed with KNO_3 1.25% solution for 12 h.

Optimum plant population and good crop stand can be achieved by higher seed emergence. In the present study, seed priming had significant ($p=0.05$) effect on final emergence count (Table 1). The maximum number of seeds (30.33 m^{-2}) was emerged in plots sown with seed treated with KCl which was statistically similar to osmoprimed with KNO_3 , on-farm and hydro primed seed which recorded 52% higher number of emerged seeds than control. Higher number of seeds was emerged in plots where seeds, osmoprimed with CaCl_2 were sown that were 32% more than control.

Emergence index explicates the speed of emergence of seeds. It is also an important index of determining seed vigor. Higher value of emergence index indicates the higher seed vigor. The maximum emergence index was recorded in seeds osmo-primed with KCl 1.25% solution for 12. (25.07) which was at par with seeds osmo-primed with KNO_3 1.25% solution for 12 h. (24.32).

Table 1: Influence of different seed priming techniques on emergence attributes of forage maize.

| Treatments | TSE | E_{50} | MET | FEC | EI |
|--------------------------------------------------------------|---------|----------|---------|----------|----------|
| Control (unprimed) | 5.333 a | 14.30 a | 8.37 a | 19.00 c | 15.60 c |
| Hydro-priming for 12 hours | 4.333 b | 12.67 ab | 7.12 bc | 28.67 ab | 23.87 ab |
| On-farm priming | 4.333 b | 11.45 bc | 7.27 bc | 29.00 a | 22.78 ab |
| Osmopriming with CaCl_2 1.25% solution for 12 hours | 4.333 b | 7.32 de | 6.90 bc | 25.00 b | 19.79 bc |
| Osmopriming with KCl 1.25% solution for 12 hours | 4.333 b | 6.105 e | 6.47 c | 30.33 a | 25.07 a |
| Osmopriming with KNO_3 1.25% solution for 12 hours | 4.333 b | 9.437 cd | 7.46 ab | 29.00 a | 24.32 a |
| LSD ($p=0.05$) | 1.10 | 2.33 | 0.94 | 3.94 | 4.37 |

Means not sharing the same letter differ significantly at 5% level of probability (LSD).

Table 2: Influence of different seed priming techniques on agronomic and yield attributes of forage maize

| Treatments | Plant height (cm) | Leaf area (cm ²) | Green forage yield (t ha ⁻¹) | Dry matter yield (t ha ⁻¹) |
|----------------------------------------------------------------|-------------------|------------------------------|------------------------------------------|----------------------------------------|
| Control (unprimed) | 192.87 c | 4033 e | 57.78 d | 12.29 d |
| Hydro-priming for 12 hours | 203.67 bc | 5136 d | 62.05 cd | 13.34 cd |
| On-farm priming | 201.67 bc | 7007 b | 66.33 bc | 14.54 bc |
| Osmopriming with CaCl ₂ 1.25% solution for 12 hours | 227.87 a | 8465 a | 80.96 a | 17.03 a |
| Osmopriming with KCl 1.25% solution for 12 hours | 217.73 ab | 8795 a | 73.61 ab | 16.73 a |
| Osmopriming with KNO ₃ 1.25% solution for 12 hours | 218.47 ab | 6101 c | 72.65 b | 15.44 ab |
| LSD ($p=0.05$) | 19.51 | 794.03 | 7.43 | 1.81 |

Means not sharing the same letter differ significantly at 5% level of probability (LSD).

The present study revealed that seed priming significantly ($p=0.05$) affected emergence attributes. Priming resulted in early seed emergence, decreased time take to 50% emergence (E_{50}) and mean emergence time (MET), improved final emergence count (FEC) and emergence index (Table 1). More water absorption and faster production of metabolites in primed seed might have resulted in its early emergence [34]. Priming increased seed emergence because higher rate of amylose and protein formation and metabolic processes was observed in primed seeds [35]. Our findings are supported by Rehman *et al.* [36]. Various priming techniques used in the present study varied regarding TSE, E_{50} , MET and FEC. Early emergence of maize seed primed with different priming agents was also reported by Harris *et al.* [37]. They reported that seed priming improved rate of germination significantly as compared with untreated seeds.

Least E_{50} and MET and highest FEC were recorded by CaCl₂ and KCl treated seed. In primed seeds, lower value of E_{50} might be due to the reason of production of germination metabolites earlier. Zheng *et al.* [38] also recorded early and uniform emergence of rice when seeds were primed with CaCl₂ and KCl. Moreover, seed priming techniques resulted in seedling vigor enhancement as well, as indicated by high energy of emergence, emergence index and final emergence count in primed seed. Ruan *et al.* [39] also reported improved emergence count and emergence index in primed seed as compared to non-primed seed.

Agronomic and Yield Attributes: Plant height was significantly ($p=0.05$) affected by various seed priming techniques (Table 2). The maximum plant height (227.87 cm) was recorded in plots sown with seeds osmoprimed with CaCl₂ 1.25% solution for 12 h. It was followed by osmopriming with KNO₃ (218.47 cm) which was at par ($p=0.05$) with the treatment in which seeds were osmoprimed with KCl 1.25% solution for 12 h.

Leaf area is the measure of the size of assimilatory system of the plant and is the product of leaf length and width. Although, it is considered to be mainly concerned with accumulation and partitioning of photosynthesis to the economic parts of the plant but it has also an important role in the final biomass of the crop. Table (2) showed that there was a significant effect of seed priming on leaf area per plant of forage maize. The maximum leaf area (8795 cm²) was observed in seeds osmoprimed with KCl 1.25% solution for 12 h. It was also at par with the treatment in which seeds were osmoprimed with 1.25% solution of CaCl₂ (8465 cm²). Thereafter, on-farm priming showed the best results regarding leaf area per plant. It was followed by osmopriming with KNO₃ 1.25% solution (6101 cm²) and hydropriming for 12 h (5136 cm²). The minimum leaf area per plant was observed in the control treatment Green forage yield was significantly ($p=0.05$) improved by seed priming as compared to control plots where non-primed seed was sown. Highest green forage yield was observed in plots where CaCl₂ treated seeds (80.96 t ha⁻¹) were sown which was statistically at par ($p=0.05$) with KCl treated seed (73.61 t ha⁻¹). These were followed by seed, osmoprimed with KNO₃ and on-farm primed seed recording 26 and 12% more green forage yield as compared to control. Moreover, hydro-primed seed recorded higher green forage yield than control to the tune of 7%.

Seed priming techniques varied significantly ($p=0.05$) regarding dry matter yield. The maximum and statistically similar ($p=0.05$) dry matter yield was recorded in plots sown with CaCl₂ (17.02 t ha⁻¹), KCl (16.73 t ha⁻¹) and KNO₃ (15.44 t ha⁻¹) treated seeds that was 38, 36 and 26%, respectively higher than control. These were followed by on-farm and hydro primed seeds which recorded dry matter yield of 14.54, 13.34 t ha⁻¹, respectively.

In the present study all seed priming techniques significantly increased plant height, plant weight, number of leaves, fresh and dry weight of maize (Table 2). Our findings are supported by Nagar *et al.* [40]. It might

Table 3: Influence of different seed priming techniques on quality attributes of forage maize

| Treatments | Crude protein (%) | Crude fiber (%) | Total ash (%) |
|----------------------------------------------------------------|-------------------|-----------------|---------------|
| Control (unprimed) | 7.85 e | 25.45 e | 9.0 d |
| Hydro-priming for 12 hours | 9.4 d | 27.46 d | 11.1 c |
| On-farm priming | 10.4 c | 29.80 c | 12.7 bc |
| Osmopriming with CaCl ₂ 1.25% solution for 12 hours | 13.9 a | 31.63 b | 13.8 ab |
| Osmopriming with KCl 1.25% solution for 12 hours | 13.2 ab | 31.40 b | 13.1 b |
| Osmopriming with KNO ₃ 1.25% solution for 12 hours | 12.9 b | 33.25 a | 14.6 a |
| LSD ($p=0.05$) | 0.80 | 1.3 | 1.9 |

Means not sharing the same letter differ significantly at 5% level of probability (LSD).

be due to early emergence of seedling and better stand establishment due to priming. Plant height was increased due to priming because it produced vigorous seedlings and provided an energetic start to seedling growth. More respiration and carbohydrate metabolism in seedlings emerged from primed seed resulted in improved plant growth and weight. Srivastava [41] also reported increased plant height of Indian mustard in plots sown with primed seed as compared to non-primed seed. Sedgi *et al.* [42] reported increased fresh and dry weight of sorghum by primed seed to the tune of 28% as compared to non-prime seed. In the present study, osmo-priming salts with CaCl₂ proved to be more effective in increasing plant growth, fresh and dry weight as compared to on-farm and hydro priming. Green forage and dry matter yield was also significantly improved by seed priming (Table 3). These findings are in line with those obtained by Capron *et al.* [43], who found that 14% higher yield was recorded by primed seed than non-primed seed. Due to priming, enzymes involved in seed germination became more active that caused breakdown of metabolites in endosperm of seed resulting in higher plant growth and increasing final yield.

Quality Attributes: Protein contents are one of the most important parameter affecting the nutritional value of forage crops. Seed priming had significant ($p=0.05$) effect on crude protein contents of fodder Maize seeds (Table 3). The highest protein contents were recorded in seed treated with CaCl₂ (13.90%) which was statistically at par ($p=0.05$) with KCl (13.20%) treated seeds which were 77 and 68% higher than control. These were followed by osmopriming with KNO₃ and on-farm priming recording 12.9 and 10.40%, respectively protein contents.

Crude fiber contents were significantly ($p=0.05$) affected by various seed priming techniques. The maximum crude fiber contents were observed in seed treated with KNO₃ (33.25%) which was 31% higher as compared to control. It was followed by KCl (31.40) and CaCl₂ (31.60%) treated seeds. Moreover, plots sown with

hydro- and on-farm primed seed recorded higher crude contents as compared to control to the tune of 8 and 17%, respectively.

Significant ($p=0.05$) effect of seed priming on total ash contents suggested that least percentage of total ash contents was observed in plots sown with non-primed seed (9.00%) (Table 3). Nevertheless, the highest ash contents were recorded by seed osmoprimed with KNO₃ (14.60%) which was statistically at par ($p=0.05$) with CaCl₂ treated seeds (13.80%). Furthermore, on-farm primed and hydro primed seeds recorded higher ash contents to an extent of 41 and 22%, respectively as compared to control.

In the present study, all seed priming techniques significantly improved quality of fodder maize (Table 3). It might be due to better net assimilation rate and better assimilate partitioning in primed seed. These results are supported by Sathish and Sundareswaran [44]. Crude protein, fiber and ash contents were increased in plots sown with primed seed as compared to non-primed seed. This might be due to increased seed emergence in these plots. Long sprouting resulted in loss of carbohydrates and increase in crude protein contents. Moreover, long radicle allowed high uptake of minerals which resulted in increased fiber and ash contents. Natural and artificial seed priming mobilize and solubilize the globulin and also enhances the production of different late embryogenesis abundant proteins

CONCLUSIONS

It can be concluded from the present study that seed priming can improve emergence, yield and quality of fodder maize. However, osmo-priming with salts (CaCl₂, KCl and KNO₃) proved to be the most effective technique of priming which realized highest FEC, yield and quality of fodder maize. There is a dire need to explore the potential of priming techniques in increasing the crops germination as well as yield by studying priming effect on seed germination in relation to varying ranges of soil temperature and moisture.

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