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EFFECT OF SEED PRIMING AGENTS (GA_3, PEG, HYDROPRIMING) IN THE EARLY DEVELOPMENT OF MAIZE

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ABSTRACT
Maize (*Zea mays*), the second most staple crop in Nepal is predominantly cultivated in the hills during the spring and summer, thus, facing moisture stress during the early seedling stages. Seed priming has proved to be an effective technique to resolve the issue. Early development response of maize due to eight different seed priming levels viz. hydropriming, three PEG concentrations (10%, 15%, 20%), three GA_3 concentrations (50 ppm, 75 ppm, 100 ppm) and control exposed to non-stress and stress environment using 10% PEG was studied in a two-factor factorial CRD replicated thrice. The study was conducted in the Agronomy Laboratory of Lamjung Campus. GA_3 was effective in improving the germination percentage with 91.6% germination at 100 ppm. However, under the stressful environment, there was a significant 16% reduction in germination. GA_3 @ 50 ppm had the lowest mean germination time (MGT) under the non-stress environment, while, increased GA_3 concentration resulted in quicker germination under the stress environment. A similar trend of increased concentration favoring quicker germination was observed for PEG as well. Non-stress environment showed a balanced root: shoot ratio (1.00) while the stress environment increased the R:S ratio (1.51) considering the progressive increase in root development under stress. The lowest SVI was observed in the seeds treated with 20% PEG in both environments. GA_3 priming has a better impact on germination under stress environment for its effect in countering the germination inhibitors and accelerating the metabolic plasticity over the osmotic adjustments provided by PEG-priming.

KEY WORDS
GA_3, PEG, metabolic plasticity, osmotic adjustment, seed priming, water-stress.

Maize is the second most important principle food crop in Nepalese agriculture both in terms of area and production (Karki et al., 2015). During spring and summer planting of maize, germination, and plant foundation is compromised due to moisture deficit, especially in arid and semi-arid regions, which leads to the use of increased seed requirement as insurance for optimal plant population. Germination plays an important role in the successful growth and seedling development of a new plant (Wolny et al., 2018). Faster and uniform germination of healthy seedlings signifies successful plant establishment (El-Sanatawy et al., 2021). The delayed or very early sowing of maize outturns to the retarded or no germination due to differences in optimum temperatures required for the maize seeds (Shrestha et al., 2018). Although all the conditions like imbibition, respiration, synthesis of nucleic acids/ proteins, and other metabolic activities are completed; there is no protrusion of the radicle; for which still the reasons are not scientifically clarified. Such dormant seeds can be changed into germinable seeds by priming technique (Bewley and Black, 1994). Seed priming is an economic pre-sowing treatment that allows partial hydration to carry out all the metabolic activities before germination and then redried to the initial dry weight (Ur Rehman et al., 2011). Seed priming not only improves germination and better seedling establishment but also helps to reduce fertilizer use and increases seed vigor (Carvalho et al., 2011; Ghassemi-Golezani et al., 2012; Laware et al., 2018). Priming with Polyethylene Glycol (PEG) was proved to be effective for ameliorating seed germination and plant establishment (Zhang et al., 2015). Priming with PEG decreased the mean germination time while significantly increasing the leaf surface area along with the other plant growth parameters (Salah et al., 2015). PEG priming has improved the rate, speed, and energy of germination compared to non-primed seeds but 5% PEG priming showed better performance than 10% PEG priming (Subedi et al., 2015). In maize seeds, the rate of imbibition was greater in PEG priming than in hydropriming while 15% PEG priming showed increased root and shoot biomass with a significant increase in hundred-grain weight (Tian et al., 2014). Osmo-priming with PEG significantly enhanced the germination percentage, germination rate, shoot length and shoot fresh and dry weights compared to control when experimented in fields (Mirmazloum et al., 2020). PEG priming enhances root length and can maintain uniform water potential; also it speeds up the rate of imbibition, enhances seed metabolism and germination rate, and maintains germination homogeneity (Laware et al., 2018). As a plant growth regulator hormone, gibberellic acid (GA_3) has many advantageous results on seed germination, stem prolongation, foliar growth, and flowering initiation (Cornea-Cipcigan et al., 2020). GA_3 priming significantly increased germination percentage, root and shoot lengths, seedling vigor index, and relative water content while decreasing the mean germination time as compared to control (Gnawali & Subedi, 2021). Gibberellic acid treatment increased the root and shoot length along with leaf length and width compared to untreated seeds. Gibberellic acid both priming and foliar application was proved to be the most effective method for the overall growth and development of maize under salt-stressed conditions (Shahzad et al., 2021). Gibberellic acid treatment significantly increased the germination rates, plant height, and biomass; subsequently enhancing the production (Ma et al., 2018). The objective of the experiment is to assess the impacts of hydropriming, various concentrations of polyethylene glycol and gibberellic acid priming in maize under water abundant and PEG 10% stressed environment in controlled laboratory conditions.

MATERIALS AND METHODS OF RESEARCH

The research was conducted in the Agronomy laboratory of the Institute of Agriculture and Animal Science (IAAS), Lamjung Campus located at 28.1448° N, 84.4120° E. 960 maize seeds of variety Arun-4 were collected from the Maize Research Station, Rampur, Chitwan. Surface sterilization was conducted using 95% ethanol for all types of equipment used before the initiation of the experiment. 96 Petri dishes of 12 cm diameter and the germination chamber (PRC 1200 WL) were used for the lab trials. The experiment was conducted in a two-factorial design with two factors (priming agents and environments) in a completely randomized design (CRD) replicated thrice. The priming reagents were control (unprimed), hydropriming, 10% PEG solution, 15% PEG solution, 20% PEG solution, 50 ppm GA_3 solution, 75 ppm GA_3 solution, and 100 ppm GA_3 solution. The environments were hydration (water abundant) and 10% PEG solution (induced water stress environment). Twenty maize seeds were used in every petri dish in three concentric circles (13 in outer circles, 6 in inner, and one in the center). The Petri dishes and pots were moisturized or irrigated during the one-day interval. Each Petri dish was rinsed with its respective environment solution to wet the filter paper at the base to be neither dry nor get excess water on the Petri dish. Seed germination was recorded on the daily basis. A seed was considered germinated if the radicle emergence was 5mm. Germination was carried out in the germination chamber at 24 ± 1^0C. A one-foot scale was used to measure the root and shoot lengths after one simple washing with the tap water. Plant weights were evaluated by using an electric laboratory weighing balance. Tap water is poured and left for 24 hours in the Petri plates to measure the turgor weight while plants are subjected to an oven at 72^0C for 24 hours to measure the dry weight. Data analysis was conducted using MS Excel 2019 and R (version 4.1.0). The analysis of variance was carried out and the means were compared using Duncan's multiple range test.

The 'environments' term here refers to the moisturizing/irrigating reagents used in the experiment. There are two moisturizing reagents (hydration (water abundant) and 10% PEG solution (water stress environment)) used in the Petri- dishes which are the two environments. 10% solution of PEG was prepared by pouring 100gm of PEG 6000 in 1 liter of water for the preparation and normal tap water is used as the water abundant environment.

Normal tap water was used for hydropriming. The three solutions of PEG were prepared by pouring 100gm, 150gm, and 200 gm of PEG 6000 in 1 liter of water for the preparation of 10%, 15%, and 20% of PEG solutions respectively. Firstly, a stock solution of 5000 ppm gibberellic acid was prepared; by pouring 5 gm of gibberellic acid powder in ethanol to dissolve completely then making thus prepared solution 1 liter by the additional pouring of water. From the stock solution of 5000 ppm; 10 ml, 15 ml, and 20 ml of GA_3 solution were extracted differently by the pipette and made 1/1-liter solution by pouring additional water into three beakers to prepare 50 ppm, 75 ppm, and 100 ppm GA_3 solutions respectively.

Research parameters:

1. Germination percentage: Germination percentage was calculated according to Scott et al. (1984) and Ahammad et al. (2014):

$$\text{Germination percentage} = \frac{\text{The total number of seeds germinated}}{\text{Total number of planted seeds}} * 100\% \quad (1)$$

2. Mean Germination Time: Mean germination time was calculated as per the formula given by Orchard (1977):

$$\text{MGT} = \frac{\sum fx}{\sum f} \quad (2)$$

Where: MGT - Mean Germination Time; f - Number of seeds germinated in ‘x’ days.

3. Root- shoot ratio: Root and shoot lengths were measured by using a simple one-foot scale and measured in centimeters (cm) on the eighth day of the experiment and the ratio was obtained.

4. Seedling Vigor Index II: Seedling Vigor Index II was calculated by using the following formulae Anderson (1973) as stated in Anupama et al. (2014):

$$\text{SVI II} = \text{DW} * \text{GP} \quad (3)$$

Where: SVI II - Seedling Vigor Index; DW - Dry Weight of seedling; GP - Germination Percentage.

RESULTS AND DISCUSSION

The highest germination percentage was recorded in the 75 ppm GA_3 treated seeds in water abundant environment while this highest GP abated significantly by 16% in the case of PEG stressed environment which coordinates with the conclusion exhibited by Afzal et al. (2008) and Bhatt et al. (2022). Hydropriming has also been efficacious in escalating the germination percentage giving the second highest GP. Similar trend was also reported by Lara-viveros et al. (2018). In stressed environments, the GP of all eight treatments was significantly diminished which complements with the outcomes obtained by Gnawali & Subedi (2021). The least germination was documented in the 15% PEG solution treated seeds in the stressed environment. GA_3 treatments have revealed positive response for germination percentage in both water-abundant and stressed environments. GA3 being a natural regulator stimulates the production of A- amylase: a hydrolytic enzyme that helps in germination (Gupta & Chakrabarty, 2013). In addition to this, catabolism activities of GA_3 also play the vital role in the metabolic pathways of germination inhibitors like ABA. In addition to this, GA_3 also acts as an antioxidant and reduces lipid peroxidation thus helping in enhancing germination (Marthandan et al., 2020). PEG has been observed to ameliorate the germination performance in the case of stressed conditions. This is due to the osmotic adjustment by accumulating the osmolytes; decreasing the osmotic potential in response to stress (Saha et al., 2022).

Mean Germination Time in the stressed condition is found to be longer than the water abundant conditions irrespective of the treatments used. Similar results were also reported by Khodarahmpour (2011). GA_3 100 ppm treated maize seeds required the least time for germination.

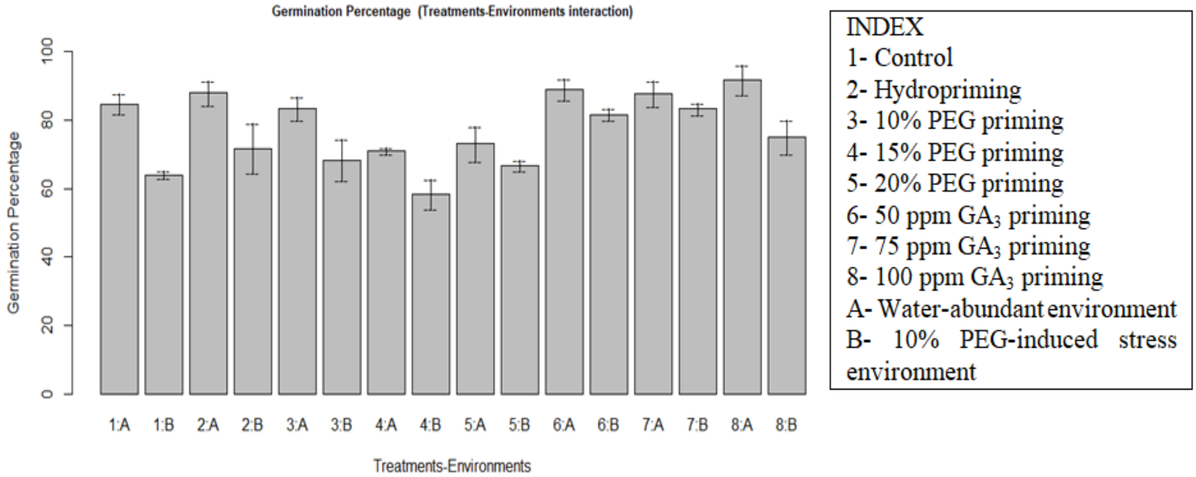

Figure 1 – Germination Percentage (GP)

Although all priming treatments required a lower mean germination time compared to control, maize seeds treated with all three gibberellic acid concentrations and 10% PEG treated seeds have significantly lower mean germination time. These results have similarities with the results obtained from Tian et al. (2014). There is also a significant difference in mean germination time when only environments are considered. With the increasing concentration of PEG treatments, germination time required also soared while there is an exactly opposite trend in the case of gibberellic acid. These results complements with the consequences obtained by Tian et al. (2014) and Gnawali & Subedi (2021). Slanting graphs are observed in 15% PEG and 75 ppm GA_3 as observed in the facet wrap graph shown below. With the increasing GA_3 concentration, cytological enzymes have been activated which resulted in higher cell plasticity with rapid water absorption that caused quicker germination (Chauhan et al., 2019), and with the decreasing PEG concentration, the time required for seeds to germinate increased as a higher concentration of PEG have negative effects in germination (Yuan et al., 2010). So, for prompt germination, a higher concentration of GA_3, lower concentration of PEG, or hydropriming can be recommended while the best results can be obtained from priming by GA_3 100 ppm solution.

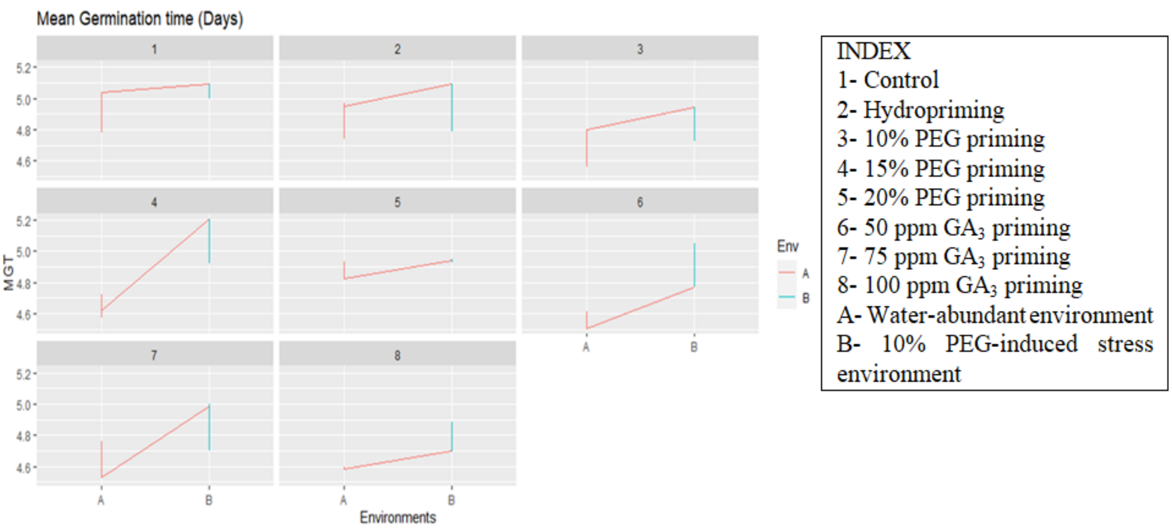

Figure 2 – Mean Germination Time (MGT)

The root-shoot ratio is remarkably impacted by both the seed priming treatments and the environments used in the experiment. A balanced root-shoot ratio was found in water abundant environment while a 1.51 R-S ratio was observed in the 10% PEG-induced stress environment. This result is obtained because, in the induced environment, root length increased significantly in search of water which directly influenced the R-S ratio. Similar results were also demonstrated by Magar et al. (2019) and Queiroz et al. (2019). The highest R-S ratio was noted in the maize seeds pre-treated with 50 ppm GA_3 which significantly decreased in the other two higher concentrations viz. 75 ppm and 100 ppm. As previously discovered, gibberellic acid enhances leaf expansion but impedes root growth (Stowe & Yamaki, 1957) although low GA_3 concentration is essential for root elongation as per Tanimoto (2012) and higher GA concentrations are inhibitory for root growth (Hedden & Sponsel, 2015). These studies have exactly matched with our experiment's results. There was no significant difference in the results of the R-S ratio observed in the different concentrations of PEG as treatments. Similarly, hydropriming and control seeds also showed a similar R-S ratio.

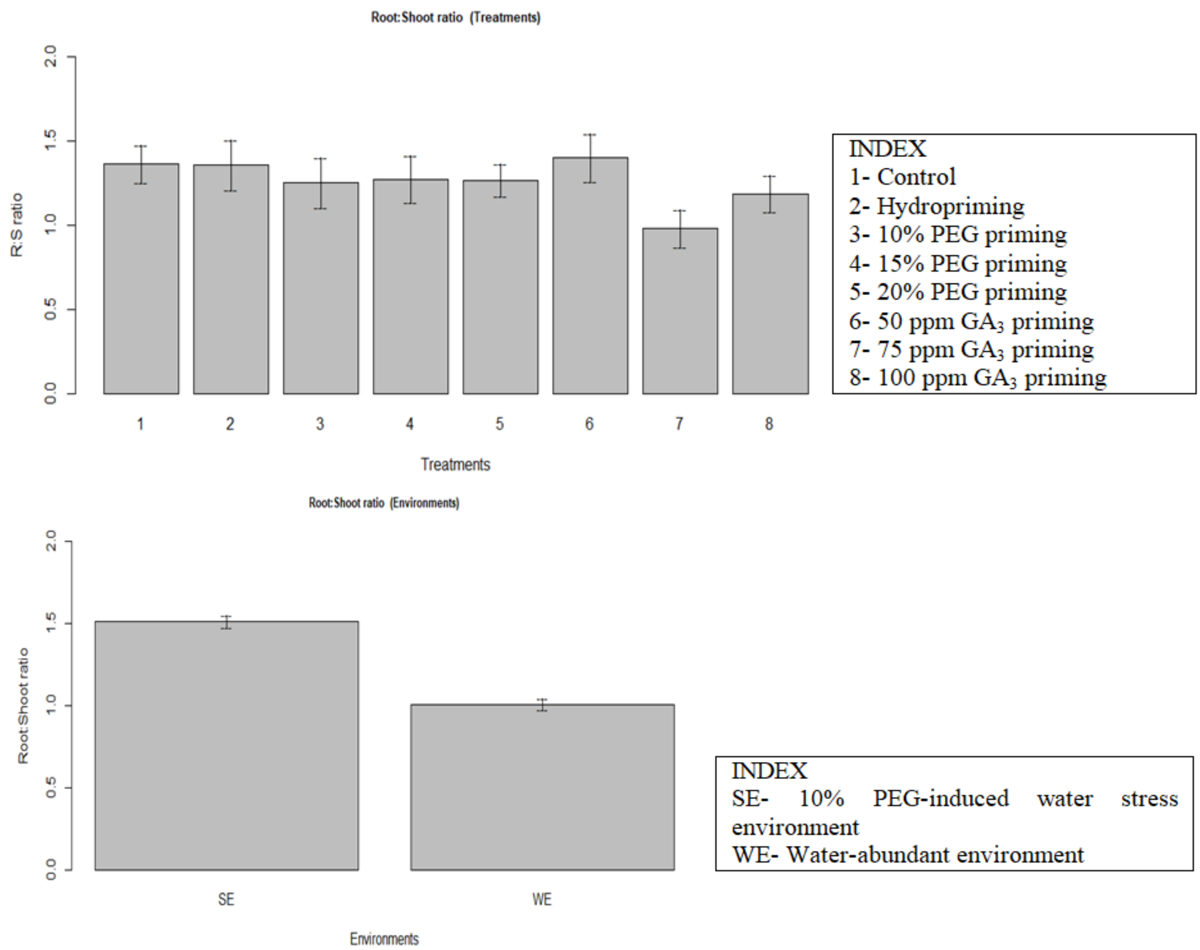

Figure 3 – Root-shoot ratio (R-S ratio)

Seedling Vigor Index II is not significantly affected by the priming agents and environments. The highest SVI II was found to be in the maize seeds pre-treated with 75 ppm GA_3. Similar results were also obtained in the other two concentrations (50 ppm and 100 ppm) of gibberellic acid which matches with the results exhibited by Chauhan et al. (2019). Increasing the PEG concentration of priming treatments decreased the SVI II (Khodarahmpour, 2011) while hydropriming caused to increase in SVI II. Similar trends were also followed by the experiments conducted by Khodarahmpour (2011), ur Rehman et al. (2015), and Marthandan et al. (2020).

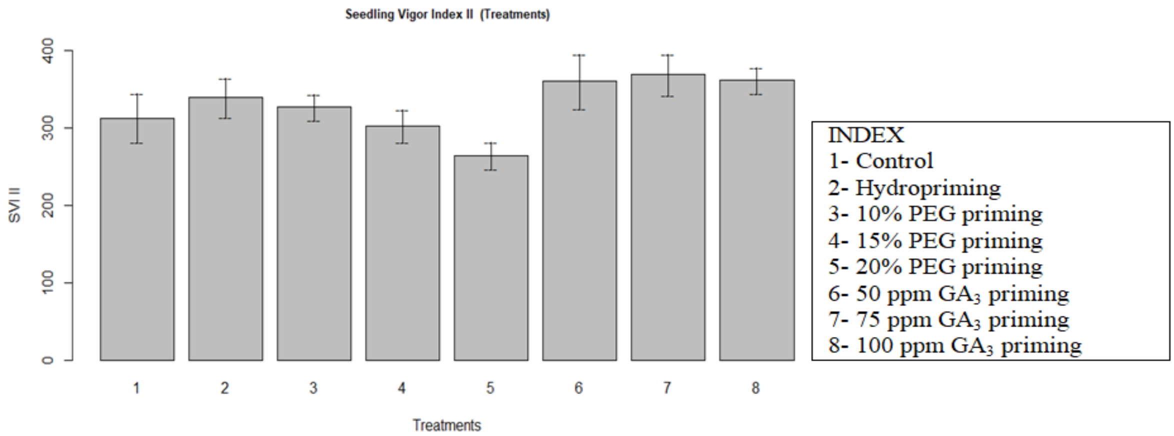

Figure 4 – Seedling Vigor Index II (SVI II)

Table 1 – Interaction effect of priming agents and environment on GP, MGT, RS ratio and SVI II of Arun-4 maize seeds

Treatment	Gemination Percentage (GP)(%)	Mean Germination time (MGT) (Days)	Root shoot ratio (RS ratio)	Seedling vigor index II (SVI II)
Priming agent				
Control	73.20 ab	4.97 a	1.37 a	312.80 ab
Hydro priming	76.45 a	4.89 ab	1.36 a	339.13 a
PEG (10%)	75.83 ab	4.77 bcd	1.25 ab	326.71 ab
PEG(15%)	61.67 bc	4.86 abc	1.27 ab	302.68 ab
PEG(20%)	58.38 c	4.91 a	1.27 ab	263.76 b
GA_3 (50 ppm)	75.83 ab	4.75 cd	1.40 a	360.23 a
GA_3 (75 ppm)	83.33 a	4.77 bcd	0.98 c	368.78 a
GA_3 (100 ppm)	82.50 a	4.67 d	1.19 b	361.48 a
$LSD_{0.05}$	14.46	0.13	0.17	71.96
p- value	* 0.05	**0.05	***0.05	. 0.05
Environment				
Water	76.71	4.72 b	1.00 b	323.08 a
PEG (10%)	70.08	4.92 a	1.51 a	335.81 a
$LSD_{0.05}$	7.23	0.06	0.08	35.98
p-value	. 0.05	*** 0.05	***0.05	- 0.05
CV%	16.75	2.33	11.64	18.57
Grand mean	73.39	4.82	1.26	329.44

Gibberellic acid increases metabolic plasticity which is the principal cellular mechanism responsible for inducing growth. Polyethylene glycol induces electrolyte leaching due to increased membrane plasticity that decreases the seed vigor. Thus, GA_3 is recommended for increasing seedling vigor. In addition to this, hydropriming can also be an effective economic method for increasing seedling vigor.

CONCLUSION

Gibberellic acid priming with 75 ppm provided the best results in the early development of maize among all the treatments. Increasing GA_3 concentration while priming also gave better results compared to control. In contrast to this, rising the concentration of polyethylene glycol reduced the germination parameters. Thus, 5% PEG is the best pre-treating agent when different levels of PEG are to be considered. For the consideration of the environments, a PEG-induced stress environment resulted in a worsening of the germination parameters. As indicated by the results of the experiment, the PEG-induced stress environment negatively affected the germination percentage, mean germination time, and seedling vigor index II. Application of GA_3 priming in stressed environments exhibited better germination performance than non-primed seeds. Thus, water abundant environment is the best condition for enhancing germination; however in water stressed environment GA3 priming is the best priming reagent that provides better germination performance even in water scarce conditions. Hydropriming also gave better results almost similar to that of GA_3 priming. Thus, hydropriming practiced by farmers for better germination should be continued and if available and affordable, GA_3 priming should be practiced.

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