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RESEARCH ARTICLE

EFFECT OF DIFFERENT PRIMING METHODS ON GERMINATION BEHAVIOUR OF BROADLEAF MUSTARD CV. MARPHA CHAUDA PAATE

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ABSTRACT

An experiment was conducted in IAAS to study the effectiveness of different priming methods on germination & seedling characters of Marpha Chauda Pate variety of broadleaf mustard. The experiment was laid in CRD with 6 treatments each replicated 5 times. Treatments used were GA_3 (100 ppm), PEG (10%), NaCl (2%), Mannitol (4%), controlled, and hydro-priming. The highest germination percentage was 91.2% with GA_3 & PEG priming while the lowest germination was achieved at 77.6% from NaCl. Lower germination in NaCl might be due to the toxic effect of salinity. Hydro-priming gave the highest fresh weight. Unprimed seeds gave the highest fresh weight (0.338 gm). The highest vigor index (1032.74) was achieved with PEG. Highest R: P. ratio was recorded with hydro priming and lowest with GA3. Overall, priming with PEG (10%) gave better performance. Priming with PEG would be beneficial for germination and seedling establishment of mustard.

KEYWORDS

Priming, PEG, vigor index, fresh weight.

1. INTRODUCTION

Broadleaf mustard (*Brassica juncea)* is a cool-season crop that is grown for its leafy green and in its maturity, it produces a seed that is used for oil production. This crop of the Brassicaceae family is a fast-growing, green-colored oval-shaped broadleaf plant, and reaches its maturity at 35-65 days after transplanting. It has many uses such as seed oil, the crushed seed used in the production of mustard, and a variety of vegetable uses. It is also used for forages and medicinal purposes. Mustard greens are good sources of dietary fiber, provitamin A, vitamin K, thiamine, riboflavin, Vitamin B6, folate, and mineral nutrient (Van wky, 2005). Morphologically, the leaves are 40-50 cm long and 20-25 cm wide with flat petioles, light green color, non-hairy, fleshy smooth to crumple with wavy margins.

The broadleaf Mustard commonly known as 'Rayo,' is found to be the most common vegetable crop in Nepal; about 70% of vegetable cultivating farmers cultivate this crop (CBS, 2010). It occupies the first position in terms of production and coverage area among the leafy vegetables; however, it holds 4.2% of the total area under vegetables and 4.1% of total vegetable production (CBS, 2010). The productivity of mustard is highly affected by prevailing weather conditions throughout its lifecycle. In addition, maintaining optimum seed germination is also important to improve crop productivity. Brassica juncea is commonly grown as a transplanting crop, optimum seedling density also helps to obtain vigorous seedlings for uniform standard and better seedling establishment and adds more to the productivity of the crop.

Seed priming involves soaking the seed in water, surface drying, and sowing on the same day. The rationale is that sowing soaked seed decreases the time needed for germination and may allow the seedlings to escape from deteriorating soil physical conditions. In addition to the better establishment, farmers reported that primed crops grew more vigorously, flowered earlier, and yielded higher. (Farooq et al., 2008). It has also been reported that seed priming improves emergence, tillering, allometry, grain, straw yields, and harvest index (Farooq et al., 2008). Seed priming has been found a double technology to enhance rapid and uniform emergence and to achieve high vigor and better yields in vegetables and floriculture (Dear man et al., 1987). Mustard seed is grown in the dry season of the year. One of the major problems in mustard seed germination and seedling establishment is water shortage. The viable seeds do not get germinated due to restrictions on water uptake and that losses metabolism. Seed priming could be the best reason to overcome drought in the seeds. Seed priming is a technique for controlling seeds for slow absorption and post-dehydration (Heydecker and Coolbear, 1977). Seed priming is defined as a pre-sowing treatment that allows the seed to imbibe water and proceed to the first stages of germination but prevents radicle protrusion through the seed coat (Bewely and Black, 1982; Chen and Sung, 2001; Farooq et al 2009; Silva et al., 2015). Improved seed priming techniques are applied to reduce emergence time and obtain uniform emergence and optimum crop geometry in many horticultural and field crops (Ashraf and Foolan; 2005). Seed priming is widely adopted as a tool to improve seed germination under both optimal and adverse conditions, but benefits have also been found for subsequent seedling growth (Caliskan et al., 2012). Priming is one of the most important physiological methods which improves the performance of seed and provides faster and synchronized germination. The primed seed gives earlier, more uniform, and sometimes greater germination and seedling establishment and growth as compared to unprimed ones (Bradford, 1986). After treatment with initiators, plant seeds not only exhibit enhanced emergence rate and even emergence of seedlings but also improved resistance or tolerance to cold, drought and salt (Ruan et al., 2003; Harris et al., 1999; Bradford, 1986; Li and Fu, 1990; Wang et al., 2004). Initiators have an obvious effect on seed germination and osmotic stress resistance (Wang and Shen, 1991; Liao and sun, 1994).

The most common seed priming treatments used to increase seed germination are osmo priming (immersing the seeds in the solution with osmotic potential), halo priming (placing the seeds in a salt solution), hydropriming (placing the seeds in water) (Basra et al., 2003; Khan, 1992; Mcdonald, 2000). Seed priming has been found a double technology to

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enhance rapid and uniform emergence and to achieve high vigor and better yields in vegetables and floriculture. Seed priming with different solutions plays multiple roles in seed germination, plant growth, functioning in cell division, cell wall expansion, and other developmental process (Asada,1990). It is the best medium that can be taken to counteract the adverse effects of abiotic stress (Patade et al., 2009).

2. Literature Review

2.1 Seed Priming and its Effect on Germination

Germination and seedling growth are critical stages in a plant's life cycle. Insufficient seedling emergence and failure in crop establishment are two major constraints in the production of crops, especially in rainfed conditions. For many years, strategies to improve the growth and development of crop species have been investigated. Among many techniques developed, seed priming is one of the most effective ways for farmers to reduce the period between seed sowing and germination and improve seedling growth. The theory of seed priming was proposed by (Heydecker, 1973). Seed priming is a technique in which seeds are partially hydrated to a point where germination-related metabolic processes are initiated but radicle emergence does not occur (Farooq et al., 2006). Primed seeds exhibit increased germination rate, greater uniformity in germination, and sometimes greater total germination percentage (Kaya et al., 2006). Seed vigor enhancement through priming treatments has proven to be very effective to achieve rapid and uniform seed germination of several vegetable species (Taylor et al., 1998). Primed seeds complete their germination process earlier as compared to non-primed seeds (Dezfuli et al., 2008). Studies on different crops showed that seed priming techniques had effectively enhanced germination and early seedling growth mainly in abnormal conditions and also developed against stress conditions (Pouramir and Hosseini, 2014). Seed priming is a simple and low-cost hydration technique to improve seed germination and early seedling growth leading to faster and more uniform crop stand with yield-associated benefits in many field crops (Rehman et al., 2011).

2.2 Hydro Priming

In hydro priming, seeds are soaked in water and then re-dried before completing germination which minimizes the use of chemicals and avoids discarding materials (Soon et al., 2000). This a defined hydro priming as a phenomenon in which seeds are hydrated in a column of aerated water to a moisture content close to that required for radicle protrusion (Thornton and Powel, 1992). Hydro-priming plays an important role in seed germination, and radical and plumule emergence in different crop species and also has a beneficial effect on enzyme activity required for rapid germination.

Hydro priming affects DNA and RNA synthesis, α-amylase activities, and better embryo growth. Improving germination rate, growth consistency, seedling vigor and establishment by hydro-priming improves field performances of crops (Ruan et al., 2002). In many studies, hydro priming has been found to improve crop performance under different stress and non-stress conditions. There found that hydro-priming was the most effective method for improving the germination of onions while comparing three priming techniques (Caseiro et al., 2004). An experiment conducted by to find out the effects of seed hydro priming on chickpea were significantly affected by seed priming with significantly greater plant height, dry matter accumulation (22%), and grain yield (23%) as compared to unprimed seeds (Gupta and Singh, 2012).

2.3 Halo Priming

Halo priming refers to the soaking of seeds in a solution of inorganic salts: NaCl, KNO_3, etc. several studies have shown that halo priming has significant improvement in seed germination, seedling emergence, and final crop yield. Priming with KCl and NaCl helped remove the deleterious effects of salts (Iqbal et al., 2006). Halopriming have a very important role in germination, seedling emergence, and plant growth at all developmental stage of the plants. Halopriming not only promotes seed germination but also stimulates subsequent growth, thereby enhancing final crop yield (Eleiwa, 1989; Sallam, 1999). Priming with inorganic salts may insignificantly affect the activity of enzymes in a germinating seed. For example, the seed of muskmelon soaked with KNO_3 solution showed enhanced activity of dehydrogenase and α-amylase under low temperature (Singh et al., 1999). Similarly, seed priming with NaCl, have sown to improve the germination and growth of many crops under stressed conditions (Sivritepe et al., 1999). Improvement of germination in pepper plants by priming with water and NaCl has also been reported (Smith and Cobb, 1991). Moreover, halopriming enables seedlings to cope with the stress imposed by NaCl or PEG. Halopriming with NaCl was found to be an effective pre-germination practice for overcoming salinity and drought-induced negative effects in sugarcane (Patade et al., 2009).

2.4 Osmo-priming

Osmo-priming is also known as osmotic conditioning. In this method of priming, seeds are soaked for a certain period in solutions of sugar, polyethylene glycol (PEG), glycerol, sorbitol, or mannitol followed by air drying before sowing. Osmo-priming not only improves seed germination but also enhances crop performance under non-saline or saline conditions. Several reports are under different environmental stresses such as salinity, drought, and high/low temperatures. Osmo priming leads to cellular, sub-cellular and molecular changes in seeds and finally promotes seed vigor during germination and emergence in different plant species (Numjun et al., 1997). It is reported that cucumber seeds osmoprimed in -0.2 MPa PEG solution promoted a vigorous initial seedling growth with a higher speed of germination through the fruit yield was not affected (Lima and Filho, 2009). The reported that the capsicum seedlings obtained from PEG primed seeds tolerated both cold and salt stresses whereas seedlings from control seeds did not survive either of the stresses (Yadav et al., 2011). The studied the Osmo priming effect of PEG in tomato seeds and observed the best results with a priming duration of 7 days (Araby and Hegazi., 2004). Has are treated sugar beet seeds with polyethylene glycol (PEG 8000) and found seeds primed with PEG gave higher germination and root yield than unprimed seeds (Jalali and Saheli, 2011).

2.5 Hormonal Priming

Soaking seeds in hormone solution before sowing is generally known as hormonal priming. Gibberellic acid, salicylic acid, ascorbic acid, abscisic acid, and cytokinins are the most commonly used hormones for priming. Priming seeds with plant growth regulators has the potential to further enhance the uniformity of germination, stand establishment, growth, and harvestable yield (Farooq et al., 2009). Several experiments have been carried out on seeds of different crops to evaluate the effect of these hormones in germination and seedling establishment and observed that those hormones play important role in germination as well as stress response and adaptation. These hormones play an important role in stress adaptation. According to, it controls the development of vascular tissue, cell elongation, and apical dominance (Wang et al., 2001). Gibberellins (GAs) are involved in growth and development. They control seed germination, leaf expansion, stem elongation, and flowering. Gibberellic acid is well known for breaking down starch stored in seeds to be utilized by growing embryos during germination (Taiz L and Zeiger E, 2006).

This reported that wheat seeds primed with GA_3 (20 ppm) resulted in minimum mean germination and emergence time as compared to control (Khan et al., 2011). Maize seeds subjected to hormonal priming with 100 mg/L GA_3 for 24 hours resulted in a higher germination percentage and germination index, lower germination time, and mean emergence time (Afzal et al., 2008).

3. Materials and Methods

3.1 Location of Experiment

The experiment had been conducted in the Horticulture lab of IAAS Lamjung Campus, Sundarbazar 7, Lamjung district.

3.2 Design of Experiment

The experiment had been carried out in Complete Randomized Design having 5 replications and 6 treatments. A seed of Marfa cauda pate variety of broadleaf mustard was used which was collected from the nearby agro vet. Seeds were subjected to priming with 6 different solutions; GA3 (100ppm), PEG 10%, NaCl 2%, Mannitol 4%, Controlled (no priming), and hydro priming. Seeds were left to prime for 24 hours and then dried in the air for the next 12 hours. After air-drying 25 seeds per treatment were set in Petri plates and kept in a germinator. Seed germination was observed daily and the final germination percentage was calculated after 8 days. After 8 days 15 seedlings from each treatment were used for calculating average shoot length, average root length, and fresh weight.

Table 1: Treatment Details

Treatments	Chemicals Used
T1	GA3(100ppm)
T2	PEG (10%)
T3	NaCl (2%)
T4	Mannitol (4%)
T5	Controlled
T6	Distilled water

3.3 Methodology

3.3.1 Layout of Experiment

The layout was done in 2 steel trays by keeping 15 plastic Petri plates in each tray.

1 tray = 15 Petri plates

2 tray = 30 Petri plates

1 Petri plate = 25 mustard seeds

30 Petri plates= 750 seeds

3.4 Observation taken

3.4.1 Germination Parameters

3.4.1.1 Germination Percentage

Each petri dish was observed every 24 hours and the number of germinated seeds was recorded. A seed was considered to be germinated when the seed coat ruptured, plumule and radicle emerged out and measured >2 mm. germination count was expressed in percentage using the formula:

Germination percentage = (Number of germinated seeds × 100)/ Total number of seeds used.

3.4.1.2 Germination Energy

Germination energy was calculated using the formula:

Germination energy = Percentage of seeds germinated at 72 hrs

3.4.1.3 Mean germination time (MGT)

MGT is calculated to enable the knowledge of the velocity of germination. The lower the value of MGT, the faster a population of seeds germinated (Orchard, 1977). MGT was calculated using the formula:

MGT = $\sum fx \,/\, \sum f$ where f is the number of seeds germinated on day x.

3.4.2 Seedling Parameters

3.4.2.1 Radicle plumule ratio

On the 8th day of the experiment, fifteen seedlings were randomly selected from each Petri dish and their radicle and plumule length were measured using a scale and the radicle plumule ratio was calculated.

3.4.2.2 Vigor index

The vigor index was calculated by using the following formula given by (Abdul-Baki and Anderson, 1973):

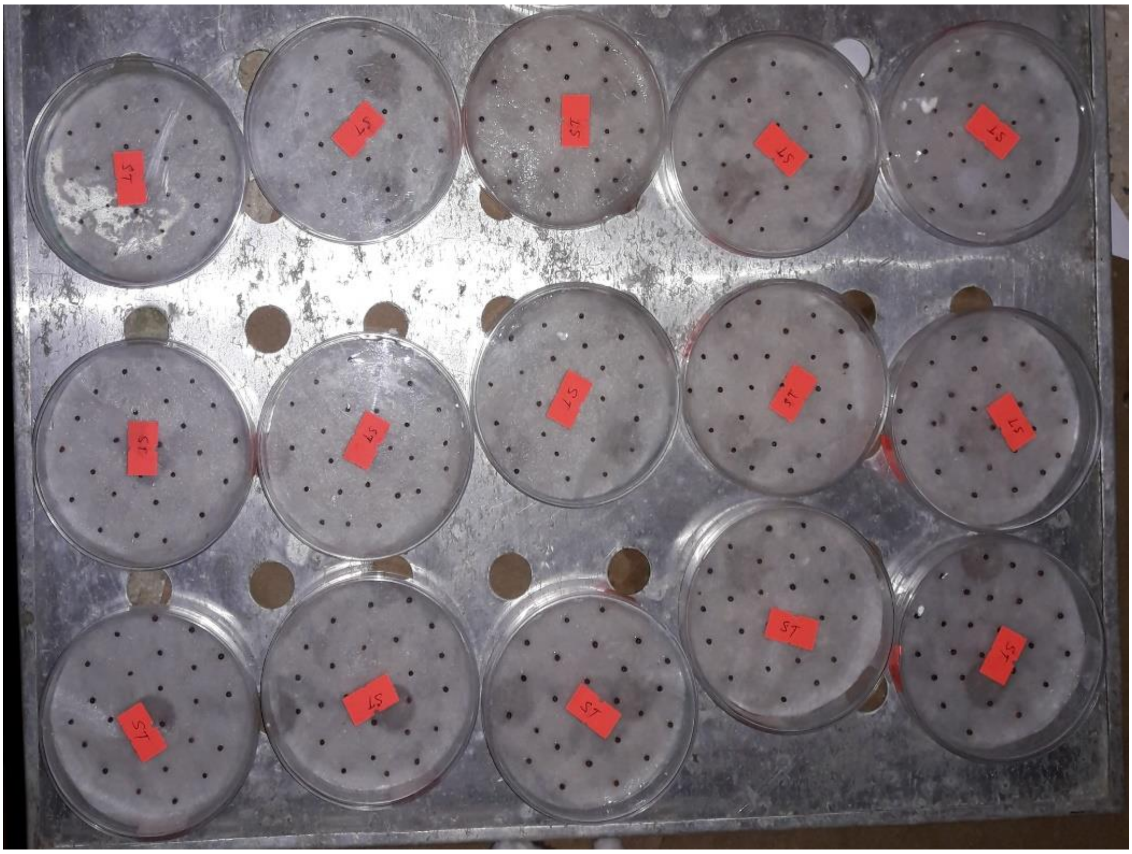
Vigor Index (VI) = Germination percentage × mean seedling length (cm)

3.4.2.3 Fresh weight of the seedling

After the 8th day of the experiment, the random fifteen seedlings from each petri dish were taken and their weight was measured by weighing balance and expressed in gram (gm).

3.5 Statistical Analysis

For the statistical analysis of the observed data, we used R-Studio version 1.3.959



4. Results and Discussion

4.1 Germination Parameters

The effect of priming treatments on the germination percentage of mustard was found highly significant. But the treatment with GA_3 (100 ppm) and PEG (10%) gave the highest germination while the treatment with NaCl (2%) gave the lowest germination. Priming induces a range of biochemical changes in the seed required for initiating the germination process i.e., hydrolysis or metabolism of inhibitors, imbibition of enzyme activation which increases germination percentage (Aljouri et al., 2004). Some processes required for germination are triggered by priming and persist during re-drying the seeds (Asgedom and Becker, 2001). Thus sowing those seeds can imbibe faster and restart the seed metabolism and resulting in a higher germination percentage and a reduction in physiological heterogeneity of seeds during germination (Rowse, 1995). Improvement of germination by priming with PEG and GA_3 might be directly related to the modification of seed water relation as reported by (Nagarajan et al., 2005). It is suggested that vacuums created inside the seed as a result of osmopriming make water flow easier which contributes to tissue hydration allowing a maximum number of seeds to germinate. Results of in cucumber showed NaCl led to increased germination and seedling emergence (Esmaielpour et al., 2006). But in the present study, there was a reduction in overall germination due to NaCl priming. This may be attributed to the salinity stress created by NaCl. This can also be due to the toxic effects of Na^+ and Cl^- on the germination process (Hosseini et al., 2003). Thus, a lower concentration would have given better results via priming in germination behavior.

GE and MGT were found comparatively lower on GA_3 (100 ppm), PEG (10%), and NaCl (2%) primed seeds as compared to mannitol (4%), hydro-primed, and controlled (unprimed) seeds. Lower germination energy might be due to the slowing of initial physiological activities inside the cell due to osmotic stress; later after 72 hours, the same treatments increased the physiological activity to help for the faster germination of the seedling. The reason behind the faster germination of seeds may be due to earlier metabolic activities, faster imbibition, and a lesser mechanical restriction of seed coats as a result of softening of the seed coat (Mcdonald, 2000).

Table 2: Effect of Different Priming Treatments on Germination Parameters of Mustard Seed

Treatment	Germination Percentage	Germination Energy	Mean Germination Time
T1 ($GA_3$100ppm)	91.2^{a}	22.4^{c}	2.554^{cd}
T2 (PEG 10%)	91.2^{a}	28.0^{c}	2.531^{d}
T3 (NaCl 2%)	77.6^{c}	28.8^{bc}	2.955^{a}
T4 (Mannitol 4%)	86.4^{ab}	40.8^{ab}	2.845^{ab}
T5 (Control)	82.4^{bc}	44^{a}	2.811^{abc}
T6 (Hydro Priming)	88.8^{ab}	$41.6^{41.6}$	2.627^{bcd}
Grand Mean	86.226	34.267	2.720
LSD	7.831	12.789	7.770
CV	6.955	28.593	0.276

Different letters indicate a significant difference at $p < 0.05$.

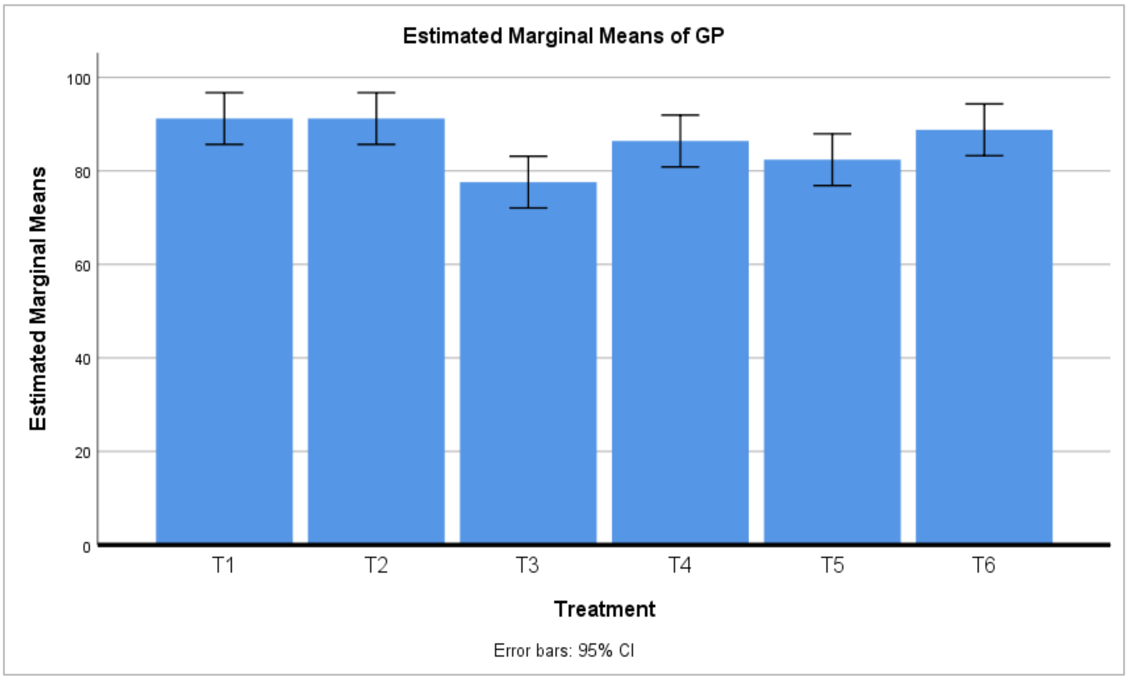


Figure 1: Effect of Different Priming Methods on Germination Percentage

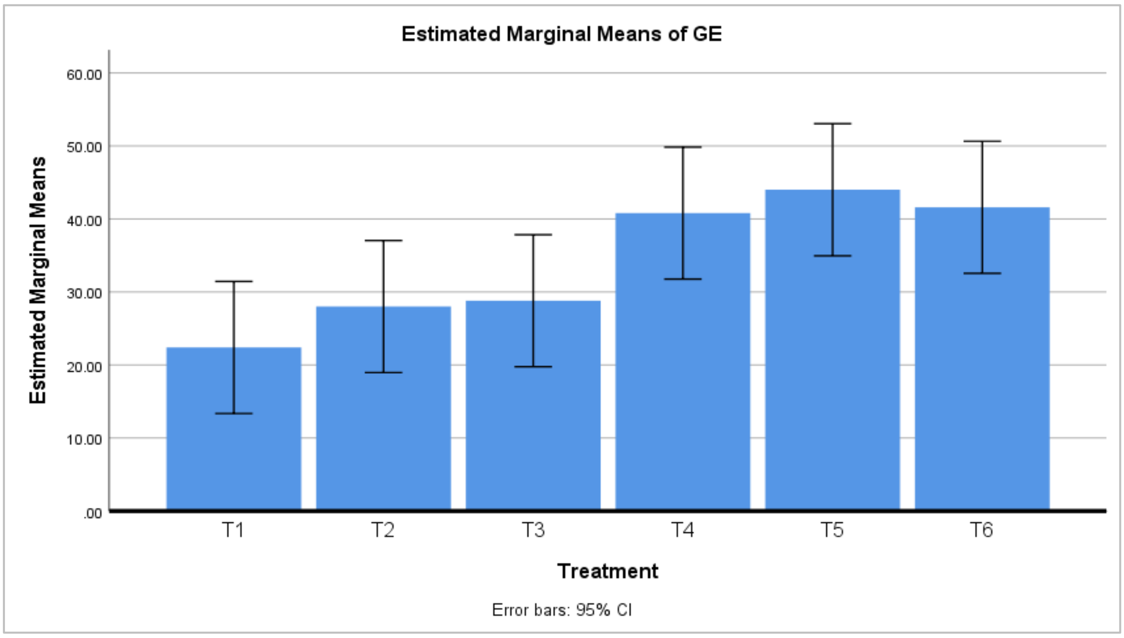


Figure 2: Effect of Different Priming Methods on Germination Energy

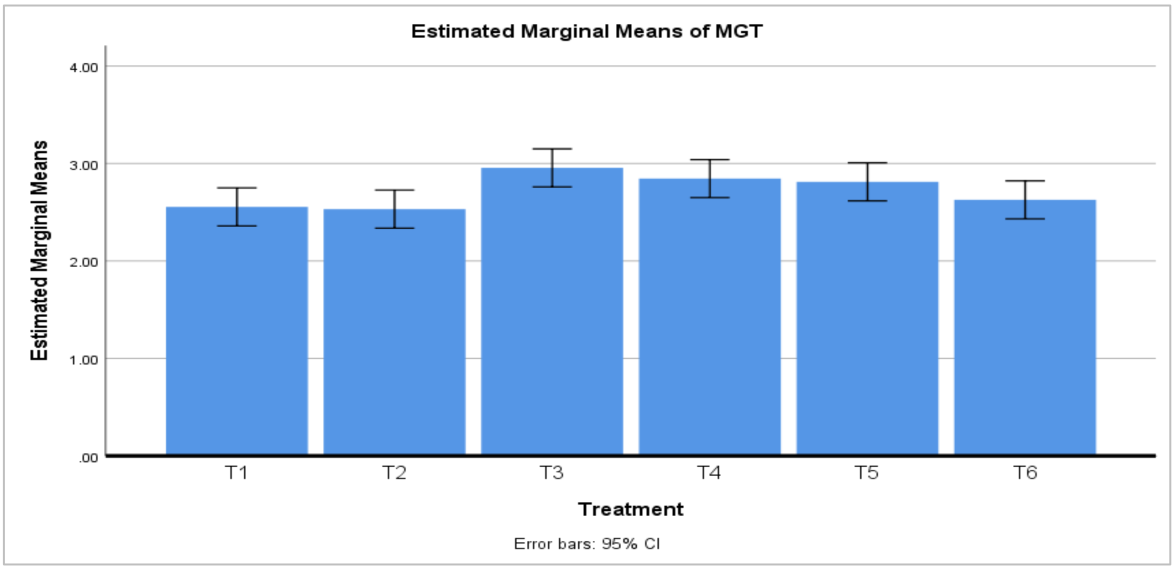


Figure 3: Effect of Different Priming Methods on Mean Germination Time

4.2 Seedling Parameters

From the study of the ANOVA table, highly significant differences were found in R:P ratios among various treatments. The results exhibited higher radicle length than plumule. Priming showed enhanced radicle development and thus a better chance for field seedling establishment. Enhanced germination and increased cell division of the root region as a result of priming might be the reason behind longer roots than plumules. Priming the seeds may increase the capacity of root growth by extending the wall of root cells. Many studies have shown reduced radicle to plumule ratio in unprimed than primed. Thus, a higher radicle: plumule ratio induced by priming can be helpful for the better establishment of seedlings under water deficit soils (Price et al., 1997). In contrast to the previous studies, the R:P ratio in our study was similar to other treatments. This might be due to the reason that the hybrid seed was used for research purposes.

Vigor index is obtained as a product of germination percentage and seedling length. VI was found highest at PEG (10%). Primed seeds showed a comparatively higher vigor index than unprimed seeds except for GA3 (100 ppm) which showed the lowest vigor index. That while working on rice seeds, also found that the primed seeds showed a higher seedling vigor index than non-primed ones (Ruan et al., 2002). The significant improvement in seedling length might be a result of earlier induced germination by primed seeds over unprimed seeds as reported by, which results in vigorous seedlings (Farooq et al., 2005). Seedling fresh weight was significantly influenced by priming. The unprimed seed has the highest fresh weight as compared to primed seeds. A fair trend of increasing fresh weight with priming was not seen which is contrary to the finding of while studying germination behavior of wheat where fresh weight was found higher in primed than unprimed seeds. This may be due to the reason that the seed we used is vigorous and has higher GE and VI (Baque et al., 2016).

Table 3: Effect of Different Priming Treatments on Seedling Parameters of Mustard Seed

Treatment	Radicle: Plumule Ratio	Vigor Index	Fresh Weight (gram)
T1 (GA3100ppm)	1.492 [b]	772.901[c]	0.096[d]
T2 (PEG 10%)	2.109[a]	1032.74[a]	0.134[cd]
T3 (NaCl 2%)	1.577[b]	828.506[bc]	0.174[bc]
T4 (Mannitol 4%)	2.210[a]	871.029[bc]	0.304[a]
T5 (Control)	1.618[b]	800.469[bc]	0.338[a]
T6 (Hydro priming)	2.14[a]	902.074[b]	0.216[b]
Grand Mean	1.858	867.953	0.210
LSD	0.4529	127.407	0.059
CV	18.669	11.245	21.578

Different letters indicate a significant difference at $p < 0.05$.

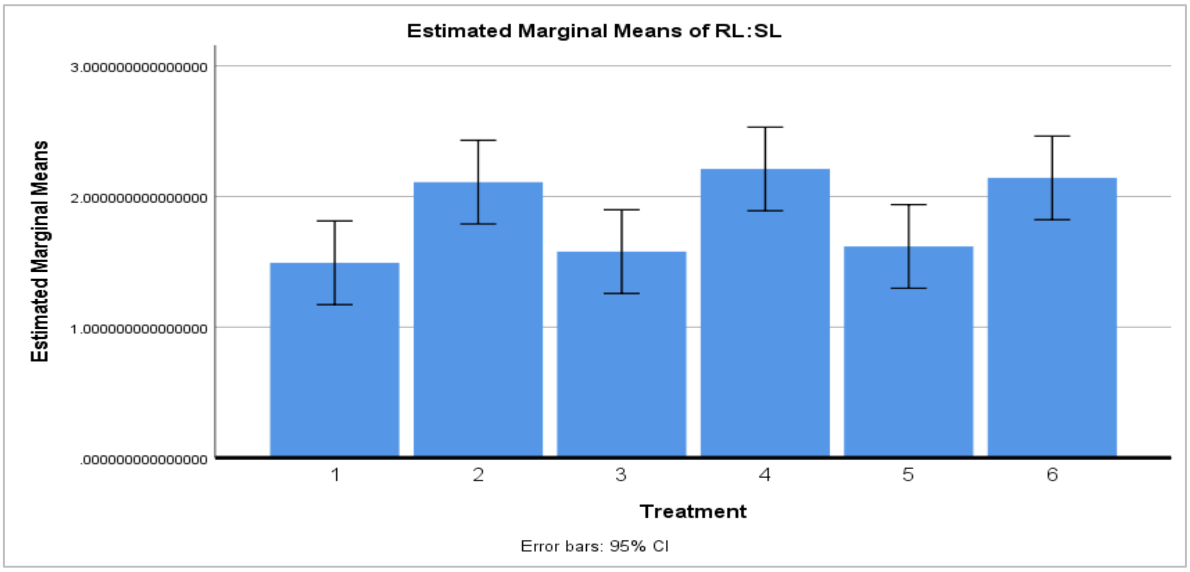


Figure 4: Effect of Different Priming Methods on Radicle: Plumule Ratio.

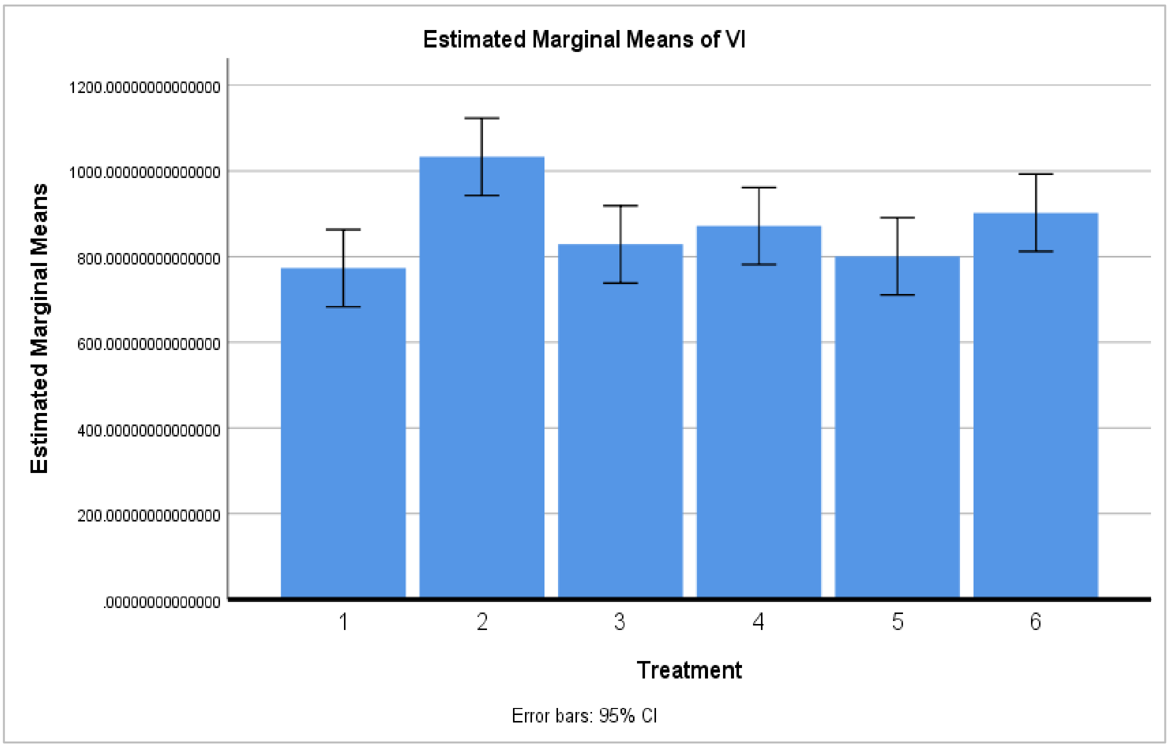


Figure 5: Effect of Different Priming Methods on Vigor Index.

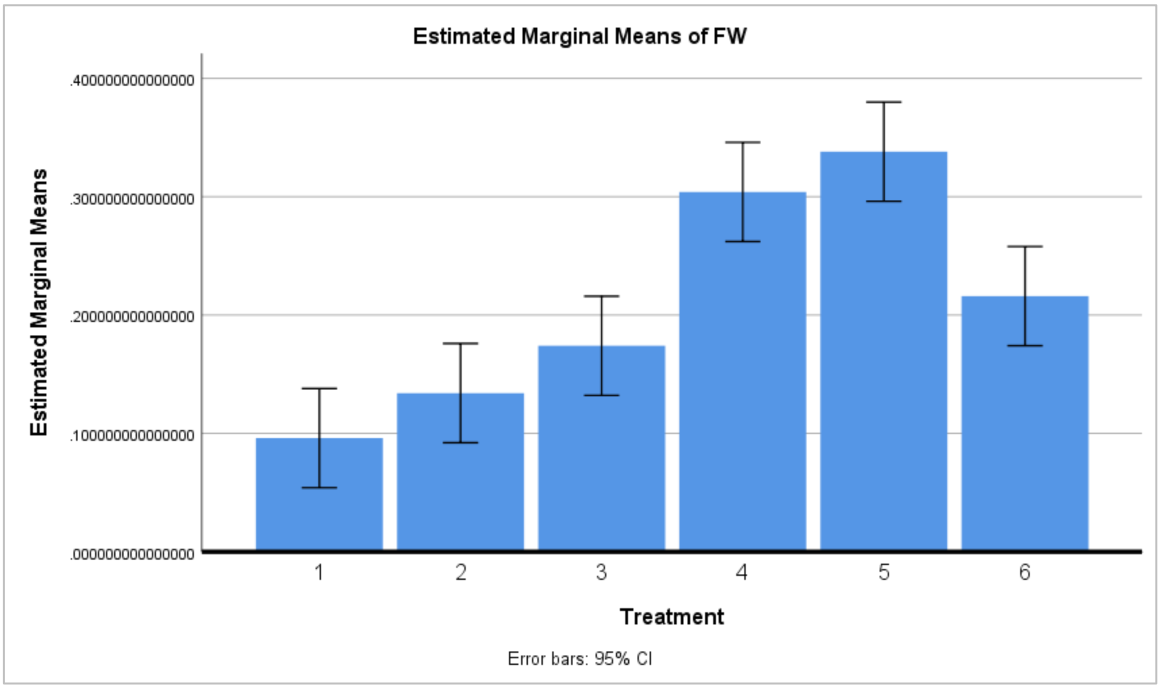


Figure 6: Effect of Different Priming Methods on Fresh Weight

Conclusion

The observed results help to conclude that priming can be used as a simple and cost-effective technique to enhance germination and seedling growth. GA_3 (100 ppm) and PEG (10%) were helpful for faster and higher germination but later the growth was retarded which caused a lower R:P ratio and fresh weight. NaCl (2%) showed inhibitory action upon seed germination. Hydro-priming showed comparatively higher germination energy and vigor index which may be due to the reduced osmotic stress. Hence, overall PEG (10%) priming gave a better result. So, priming the broadleaf mustard seed with PEG (10%) would be beneficial for improving germination and seedling behavior.

Limitations of Research

The research was carried out only once at only one location. Therefore, this experiment should be further verified by conducting similar research at different locations at different periods. The increase in the number of replications could also be done in this research but wasn't carried out due to time constraints.

Suggestions for Further Research

The present investigations should be repeated to confirm the findings. Similar research can be done with more replications and more treatment dosages. The performance in combination with different priming sources could be carried out in the future.

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