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Germination and survival of *Khirni* (*Manilkara hexandra*) seeds influenced by organic seed priming

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Abstract

The study was conducted at the Regional Horticultural Research Station of ASPEE College of Horticulture, Navsari Agricultural University, Gujarat, India to see the impact of organic seed priming on germination and viability of *Khirni* (*Manilkara hexandra*) seeds. The experiment following a Completely Randomized Design was implemented with four replications, featuring five diverse treatments: There were certain soaking treatments: 3% solution of cow dung solution, 3% concentration cow urine solution, 3% *Bijamrut*, 3% *Amritpani* and water (control) where no special treatment is given but only plain water is given for hydration. The seeds treated with 3% *Bijamrut* for 72 hours had the highest germination and survival rates and were quickest to germinate. In addition, the best results were obtained for key growth metrics such as leaf area ratio, leaf area index, crop growth rate, emergence rate index and Bartlett's rate index. The results show that *Bijamrut* is effective for both early growth and general vigour of *Khirni* seedlings and should therefore be considered to improve the establishment of *Khirni* seedlings.

Key words: *Khirni*, *Bijamrut*, cow dung slurry, cow urine, *Amritpani* and seed priming

Introduction

Khirni [*Manilkara hexandra* (Roxb.)] and it belong to the family sapotaceae. It includes about 70 genera and 800 species. It is locally known as '*Khirni*' or '*Rayan*' by tribal people of the different states of India. *Khirni* is believed to be originated in India (Stewart and Brandis, 1992). It is a commercially important tropical fruit tree that provides a considerable source of livelihood and nutritional support for local tribal population. *Khirni* is cultivated in India as an avenue tree in backyards, family gardens, public parks and farmer's fields close to communities because of its economic significance as a fruit tree with health-promoting and therapeutic qualities. *Khirni* is found naturally wild in the South, North and Central India mostly in the states of Rajasthan, Gujarat, Madhya Pradesh and Maharashtra (Malik *et al.*, 2010).

In India, *Manilkara hexandra* (Roxb.) is commonly used as rootstock for sapota. *Khirni* was found to be most vigorous and productive rootstock compared to sapota seedlings (Chadha and Parekh, 2010). Different rootstocks can be used for sapota propagation such as mahua (*Madhuca latifolia*), rayan or *khirni* (*Manilkara hexandra*), mee tree (*Madhuca longifolia*) and sapota (*Manilkara zapota*), *etc.* Plants propagated on mahua rootstock produce vigorous trees, of poor-quality fruits, whereas mee trees shows graft incompatibility. *Manilkara hexandra* is found to be the best rootstock, having better stock-scion compatibility and produces fruits of excellent quality and high yield (Sayed, 1962). Most Indian workers have reported that sapota on *Khirni* rootstocks gave better yield than or equal to those grafted on sapota (Randhawa and Kohli, 1969). *Khirni* induced dwarfing and early bearing in sapota. There is no report of incompatibility. *Khirni* is recommended as an excellent rootstock for shallow soils with a higher pH as well as porous soils with a low or neutral pH.

Seeds are used to propagate *Khirni* commercially. Seeds are taken from freshly harvested fruits and sown during the rainy season. The hard seed coat imposed dormancy and the recalcitrant nature of *Khirni* seeds results in low germination. The hard seed coat prevents water absorption and restricts gaseous exchange. The limited shelf life of seeds makes long-term storage impossible, further reducing the supply of bulk planting supplies. Besides, the slow growth rate of *Khirni* seedlings is also a drawback in its rapid and mass multiplication. The germination of *Khirni* seeds is very poor and the growth of seedlings is also very slow and to attain graftable size, it takes 2 to 3 years. Hence, there is a need to standardize the method of raising the seedlings and improving their growth. Various pre-sowing seed treatments to improve germination and reduce germination time have been widely investigated in tree species (Prasad *et al.*, 2011). With the use of organic seed priming treatments, it is possible to achieve maximum germination and survival of *Khirni* seedlings.

Seed priming with organic substances acts as an anti-bacterial, anti-fungal and anti-viral. Seed priming plays an important role in promoting germination and roots protection giving high strength to the seedlings. During seedling stages, it can add strength to the nursery plants during transplanting. Beneficial microorganisms present in cow dung slurry, cow urine, *Amritpani* and *Bijamrut* protect the seed from seed-borne and soil-borne pathogens. *Bijamrut* is a liquid formation used for seed treatment from locally available resources like agricultural farm resources. *Bijamrut* is effective in protecting young roots from soil-borne and seed-borne disease that commonly affect plants after the monsoon period and initial stages of germination and establishment. Different

beneficial microorganism like nitrogen fixers, phosphorus solubilizers, actinomycetes and fungi are present in the *Bijamrut* solution (Devakumar *et al.*, 2014). *Bijamrut* is a very effective organic and natural product that helps in quick and large number of seed germination and plant roots and protects the germinated seed and shoots and seedling roots from soil-borne pathogens, thereby increasing the growth of seedlings (Chaudhary, 2023). Therefore, there is a need to use standard organic seed priming treatment to get early seed germination with maximum germination percentage, and survival percentage and attain graftable size of seedling with minimum time.

Materials and methods

The present experiment was carried out at the Regional Horticultural Research Station, ASPEE College of Horticulture, Navsari Agricultural University, Navsari, Gujarat, during the year 2022. An experiment was laid out in a Completely Randomized Design, which included four repetitions and five treatments, namely, T_1 - Cow dung slurry 3 %, T_2 - Cow urine 3 %, T_3 - *Bijamrut* 3 %, T_4 - *Amritpani* 3 % and T_5 - Water soaking. According to agro-climatic conditions of Gujarat state, Navsari falls under 'South Gujarat Heavy Rainfall Zone, Agro Ecological Situation III'. The climate of this zone is typically tropical. The average rainfall of the tract is about 1500 millimeters (mm), which is normally received by June and ceases by the end of September. The experiment was carried out by sowing seeds in 6 " × 3 " size polyethylene bags. The polyethylene bags were punched for drainage and filled with potting mixture, which was prepared by mixing soil, FYM and vermicompost in 1:1:1 (v/v) proportion. The polyethylene bags were placed in the polyhouse at proper space. Seeds were soaked for 72 hours as per treatment then dried for 2 hours under shade conditions. The significance levels of generated data were decided using method of Panse and Sukhatme (1985). For the preparation of figures, the data were analyzed using GRAPES software, and the mean was compared using Duncan's Multiple Range Test (DMRT).

Cow dung slurry was prepared by adding water to cow dung and the proportion was 1:2 of cow dung and water. Freshly collected cow urine was used for cow urine treatment.

Bijamrut was prepared by cow dung, cow urine, lime and a handful soil from the bund of the farm. For the preparation of *Bijamrut*, first 5 kg fresh cow dung was put in a cloth bag and suspended it in a container filled with water to extract the soluble ingredients of the cow dung. Fifty grams of lime was dissolved separately in 1 liter of water. After 12 to 16 hours, the bag was squeezed to extract the liquid, which was then mixed with 5 liters of cow urine, 50 grams of soil, the prepared lime water, and an additional 20 liters of water. This mixture was left to stand for 8 to 12 hours before being filtered for use in seed priming and foliar application.

Amritpani was prepared by mixing cow dung, ghee, honey and water. For the preparation of *Amritpani* first 10 kg of cow dung was mixed with 500 g honey and mixed thoroughly to form a creamy paste. Then added 250 g of desi cow ghee and mixed well to dilute with 200 litres of water.

Germination (%): The total number of germinated seeds was counted 30 days after sowing. It was calculated as per below

$$\text{Germination (\%)} = \frac{\text{Total number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

Survival Percentage: The survival percentage was calculated at 45 days after sowing. formula.

$$\text{Survival Percentage} = \frac{\text{Total number of seedlings survived}}{\text{Total number of seeds germinated}} \times 100$$

Leaf area ratio (cm^2/g): The leaf area ratio was calculated by the leaf area of the plant to total plant biomass. It was calculated based on the below-mentioned formula.

$$\text{LAR} = \frac{\text{Leaf area of plant}}{\text{Total dry weight of plant}}$$

Leaf area index (cm^2/plant): Leaf area index was calculated by dividing the leaf area per plant by land area occupied by the plant.

$$\text{LAI} = \frac{\text{Leaf area}}{\text{Ground area}}$$

Crop growth rate (g/m^2/day): It represents dry weight gained by a unit area of crop in a given time. It was calculated based on below mentioned formula.

$$\text{CGR} = \frac{W_2 - W_1}{(t_2 - t_1)\, S}$$

Where,

W_1 and W_2 are plant dry weight (g) at time t_1 and t_2, respectively

S is land area (m^2) over which dry matter was recorded

Emergence rate index (ERI): The emergence rate index (ERI) was calculated by the formula of Evetts and Burnside (1972).

$$\text{ERI} = \frac{G_1}{T_1} + \frac{G_2}{T_2} + \cdots\cdots + \frac{G_n}{T_n}$$

Where,

G_1 - Percent of seed germinated at first count T_1

G_2 - Additional percent of seeds germinated at second count T_2

G_n - Additional percent of seeds germinated at final count T_n

T_1 - Weeks from sowing to first count

T_2 - Weeks from sowing to second count

T_n - Weeks from sowing to last count

Bartlett rate index (BRI): The Bartlett rate index (BRI) refers to the earliness of germination and was worked out by the following

$$\text{BRI} = \frac{P_1 + (P_1 + P_2) + \cdots\cdots + (P_1 + P_2 + \cdots P_n)}{N\,(P_1 + P_2 + \cdots P_n)}$$

Where,

P_1, P_2...P_n = Germination percent at 1, 2….n weeks, respectively

N = Total number of weeks in the test

Results and discussions

Days required for germination: The data presented in Table 1 clearly indicated that the days (9.00) taken for germination was significantly reduced by 3 % *Bijamrut* (T_3). This might be due to *Bijamrut* contains macro and essential micronutrients, many vitamins, essential amino acids, growth-promoting substances like indole acetic acid (IAA), gibberellic acid (GA) and beneficial microorganisms (Palekar, 2006 and Sreenivasa *et al.*, 2009). *Bijamrut* also helped in increasing general metabolic activities and initiated the occurrence of seedlings from seeds (Subramaniyan and Malliga, 2016). Similar results were also observed by Sujin *et al.* (2021) in mango and Boricha *et al.* (2020) in guava.

Germination (%): A perusal of data presented in Fig. 1 clearly revealed that there was a significant effect on germination

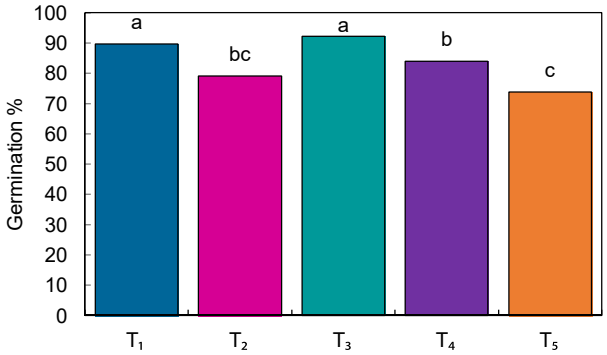

Fig. 1. Effect of organic seed priming on germination percentage of *Khirni* seedlings. T_1 = Cow dung slurry 3 %, T_2 = Cow urine 3 %, T_3 = *Bijamrut* 3 %, T_4 = *Amritpani* 3 %, T_5 = Water soaking

percentage by different seed priming treatments. After 30 days of sowing, the maximum germination (92.16%) was recorded in T_3 treatment which was statistically at par with T_1 treatment (89.66%). The remarkable effect of *Bijamrut* on maximum seed germination might be due to the presence of beneficial bacteria in *Bijamrut*, which may produce indole acetic acid (IAA) and gibberellic acid (GA) as reported by Sreenivasa *et al.* (2009). Shakuntala *et al.* (2012) observed that the *Bijamrut*-treated seeds recorded more amylase enzyme activity, and due to that, they found a high germination percentage. A similar finding was also reported by Boricha *et al.* (2020) in guava and Sujin *et al.* (2021) and in mango.

Survival percentage: The data presented in Table 1 clearly indicated that different seed priming treatments had a significant effect on survival percentage. The maximum survival (80.33%) was recorded when the seeds of *Khirni* were soaked in *Bijamrut* for 72 hours (T_3) treatment. A possible reason for the higher survival percentage of Khirni seedlings could be the higher germination rate observed in the same treatments, which ultimately contributed to improved seedling survival. The soaking of seed in *Bijamrut* helps to protect the seed from seed-born and soil-borne pathogens during monsoon periods and microorganisms associated with it thus, high survival (Kruppaswamy and Peruma, 2013). It may also be due to the overall performance concerning growth parameters being good in the same treatment, which ultimately increased the survival percentage. Similarly, the present findings are supported by the results of Boricha *et al.* (2020) in guava.

Table 1. Effect of organic seed priming on germination, seedling growth and survival of *Khirni* seedling

Treatments	Days required for germination	Leaf area ratio (cm^2/g)	Leaf area index (cm^2/ plant)	Survival (%) at 45 DAS
T_1	9.50	63.43	0.080	74.83
T_2	11.25	61.99	0.077	51.33
T_3	9.00	66.61	0.084	80.33
T_4	11.00	59.30	0.079	62.50
T_5	13.00	58.81	0.075	42.50
S.Em. ±	0.25	1.39	0.001	1.17
CD at 5 %	0.75	4.21	0.003	3.53

Leaf area ratio (cm^2/g): The highest leaf area ratio (66.61 cm^2/g) was recorded in treatment T_3, which was statistically at par with T_1 treatment (63.43 cm^2/g). This might be due to the *Bijamrut* treatment stimulating increased photosynthate mobility, which increased the leaf area of the seedling. *Bijamrut* was also found to include advantageous biochemical groups, such as bacteria that produce substances that promote plant growth, free-living N_2-fixers, P-solubilizers and increased amylase enzyme activity, which results in increased vigour of the seedlings. This, in turn, raises the dry weight of the seedlings, which, in turn enhances the leaf area ratio (Devakumar *et al.*, 2014).

Leaf area index (cm^2/plant): Data presented in Table 1, showed significant difference in leaf area index by different seed priming treatments. It is clear from the result that treatment T_3 (*Bijamrut* 3 %) recorded the maximum leaf area index (0.084 cm^2/plant). This might be due to the microorganisms present in the organic formulation that convert raw nutrients into an easy-to-digest form that plants can absorb efficiently for better crop growth, more number of branches, and more leaves, which facilitates the production of increased leaf area, which ultimately increases the leaf area index (Sreenivasa *et al.*, 2009).

Crop growth rate (g/m^2/day): The data in Fig. 2 clearly indicate a significant effect of different seed priming treatments on crop growth rate. It is clear from the result that treatment T_3 (*Bijamrut* 3 %) recorded the maximum crop growth rate (0.304 g/m^2/day). It might be due to the production of IAA and GA by bacteria present in *Bijamrut* that could have stimulated seedling growth and macro and micronutrients also help to increase vigour of seedlings by increased height and girth of seedlings, which leads to increased dry weight of seedlings, it turns to increases in crop growth rate (Devakumar *et al.*, 2014).

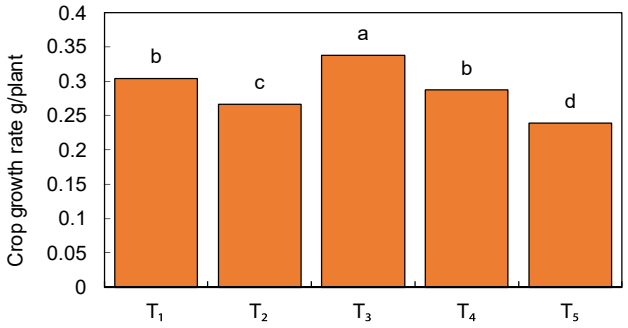

Fig. 2. Effect of organic seed priming on crop growth rate (g/plant) of *Khirni* seedlings. T_1 = Cow dung slurry 3 %, T_2 = Cow urine 3 %, T_3 = *Bijamrut* 3 %, T_4 = *Amritpani* 3 %, T_5 = Water soaking

Emergence rate index (ERI): The data in Fig. 3 show that seed priming treatments had a significant effect on the emergence rate index. The T_3 treatment showed the highest emergence rate index (44.74). This might be due to *Bijamrut* contains macro and essential micronutrients, many vitamins, essential amino acids, growth-promoting substances like IAA, GA and beneficial microorganisms (Palekar, 2006 and Sreenivasa *et al.*, 2009). *Bijamrut* also helps increase general metabolic activities and initiates the occurrence of seedlings from embryos, resulting in minimum days required for the germination and maximum germination percentage, which leads to an increase in the emergence rate index (Subramaniyan and Malliga, 2016).

Bartlett rate index (BRI): A perusal of data presented in Fig. 4 clearly revealed that the data there was a significant effect on the Bartlett rate index by different seed priming treatments. The maximum Bartlett rate index (0.82) was recorded in T_3 treatment. This might be due to the presence of beneficial bacteria in *Bijamrut*, which may produce Indole Acetic Acid (IAA) and gibberellic acid as reported by Sreenivasa *et al.* (2009).

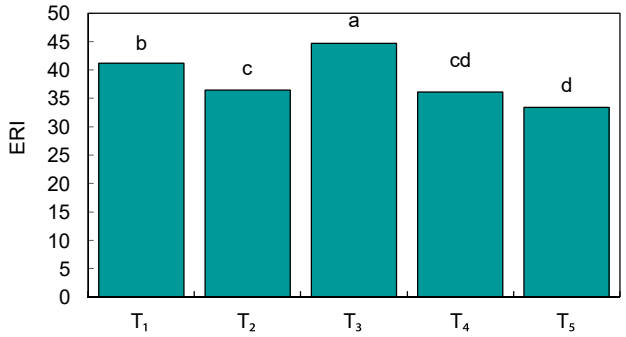

Fig. 3. Effect of organic seed priming on emergence rate index (ERI) of *Khirni* seedlings. T_1 = Cow dung slurry 3 %, T_2 = Cow urine 3 %, T_3 = *Bijamrut* 3 %, T_4 = *Amritpani* 3 %, T_5 = Water soaking.

Shakuntala *et al.* (2012) observed that the *Bijamrut*-treated seeds recorded more amylase enzyme activity due to that they found a high germination percentage, which ultimately increased the Bartlett rate index.

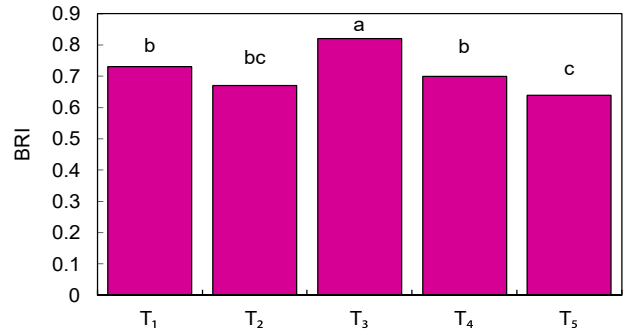

Fig. 4. Effect of organic seed priming on Bartlett rate index (BRI) of *Khirni* seedlings. T_1 = Cow dung slurry 3 %, T_2 = Cow urine 3 %, T_3 = *Bijamrut* 3 %, T_4 = *Amritpani* 3 %, T_5 = Water soaking

On the basis of results obtained from the experiments, it can be concluded that the *Khirni* seeds soaked in 3% of *Bijamrut* for 72 hours gave maximum germination, seedling growth and survival of *Khirni* seedlings.

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