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Effect of seed priming on germination parameters of Bael (*Aegle marmelos* Corr.) under laboratory conditions

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ABSTRACT

The current experiment was conducted during 2020-21 at the Microbiology Laboratory, CCS Haryana Agricultural University, Regional Research Station Bawal to examine the impact of bio-inoculants and chemicals seed priming on bael seed germination. There were 15 seed priming treatments i.e., control, IBA @ 100 ppm and 50 ppm for 24 hours, NAA @ 50 and 100 ppm for 24 hours, GA_3 @ 50 and 100 ppm for 24 hours, KNO_3 @ 1 per cent for 24 hours, *Azotobacter* (HT 54) for 30 minutes, *Trichoderma viride* for 30 minutes, *Rhizobium* (CK 16) for 30 minutes, PSB (P 36) for 30 minutes, hot water for 30 minutes, nitric acid for 3 minutes, sulphuric acid for 3 minutes. Among different seed priming treatments, shortest germination time (12.7 days) was recorded with sulphuric acid for 3 minutes in agar medium at 28 ºC under laboratory conditions and the highest germination percentage (83.3 %), dry weight per seedling (153.2 mg), seedling length (12.2 cm) and vigour index I (976) and II (12256) were observed when bael seeds primed with GA_3 @ 100 ppm for 24 hours under laboratory conditions.

Introduction

Bael (*Aegle marmelos* Corr.) is an underutilized fruit tree belongs to Rutaceae family. It is a tropical tree native to India and is popularly referred as Indian quince, Bengal quince, Bel, Bilva, Sriphal, Maredo and Stone apple in India. It is sacred tree in Hinduism and Lord Shiva and Parvati offer up its fragrant trifoliate leaves in their prayers. The most important bael growing states in India are Bihar, Orissa, Haryana, Uttar Pradesh, West Bengal and Madhya Pradesh. Bael tree is highly heterozygous in nature (Pati *et al*., 2008). It is a resilient plant that may flourish in a variety of climatic and soil conditions. Although, it flourishes well in subtropical environment having well-drained sandy loam soil, however, tree grow well on lands which are not suitable for other fruit trees. Plants can tolerate extreme pH ranging from 5 to 10, sodicity upto 30 ESP and salinity 9 ds/m (Saroj *et al*., 2006). Fruits are well known for its therapeutic qualities due to the presence of marmelosin. Mature fruits are astringent, digestive and stomachic in nature and often used to treat diarrhoea and dysentery. In ethnomedicine, various plants parts have been utilized as astringent, antidysentric, anticancer, antiulcer, antidiarrhoeal, antipyretic, antidiabetic, antiviral, analgesic, antifungal, radioprotective and anti-helminthic (Patkar *et al*., 2012). Fresh leaves can be utilized as medicine for asthma, dropsy, cataract and beriberi (Maity *et al*., 2009). In its root extract, anti-inflammatory and wound-healing

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properties are found. Bael is typically propagated by seeds and seedlings raised are not true to type and show great degree of variations. Although seedling raised trees produce fruit of varying size and quality, but they usually take long time to start bearing. Nearly 660-hectare area increases from 2020 to 2022 under bael cultivation as well as the usage of vegetative propagation techniques become popular among farmers (Anonymous, 2023). All this leads to create higher demand for healthy and superior rootstocks. To accomplish the increasing demand for superior rootstock, the bael seedlings needs to be grown from seeds, but its seeds cannot germinate easily and took long period because of seed dormancy due to hard seed coat, physiological immaturity of the embryo, impermeability to water and gases and excess of certain endogenous growth inhibitor (Chattopadhyay and Mahanta, 1989). Additionally, bael seeds are recalcitrant in nature, they lose viability, when stored for a long period of time. For farmers, poor germination is a serious obstacle for producing adequate quantity of rootstock with buddable size in short time span. To improve germination, seed priming is employed for breaking dormancy. In seed priming, seeds are soaked in a bioactive chemical to initiate the embryo's pre-germinative metabolism. Primed seed emerges earlier from soil and produces more uniform seedlings than untreated seed. Various seed priming methods employed for breaking dormancy are hot water soaking, scarification, bio-fertilizers treatment and chemical treatments. Growth regulators like NAA and GA_3 also improve seed germination, seedling growth and survival by increasing the supply of reserved mineral elements, water uptake and altering membrane permeability. GA_3 also used to weaken the seed coat, making it easier for the radical of seedling to penetrate. Biofertilizers contains microorganisms which helps in transforming inert nutrients to useable nutrients via biological processes (Athani and Revanappa, 2009). Pre-sowing treatment with chemicals like KNO_3, KH_2PO_4 and plant growth regulators such as NAA and GA_3 and of micronutrient combination increased germination and subsequent seedling vigour in many fruit crops (Ratan and Reddy, 2003). The influence of seed priming treatments is abysmally understood in bael. The primary motive of the researcher to conduct this investigation, was to work out the best seed priming treatment in terms of concentration as well as duration to improve germination percentage and achieve the buddable seedling as early as possible and reduce the germination time for better seedling establishment of bael.

Material and Methods

The study was carried out during 2020-21 at Microbiology laboratory of Regional Research Station, Bawal, CCS Haryana Agricultural University. For seed extraction, completely matured, healthy, and disease-free bael fruits were collected from orchard of Regional Research Station, Bawal. Dead, immature and non-viable seeds floated on saline in water were eliminated. The extracted viable seeds were rinsed with tap water. After washing, the seeds were kept in shade for drying. The healthy bael seeds (225 per treatment) were exposed to various chemicals and bio - inoculants of varying concentrations and duration based on various researcher's findings on different crops. The treatments consist of GA_3 @ 50 and 100 ppm for duration of 24 hours, NAA @ 50 and 100 ppm for 24 hours, IBA @ 50 and 100 ppm for 24 hours, KNO_3 @ 1.0% for 24 hours, *Azotobacter* (HT 54) for 30 minutes, *Trichoderma viride* for 30 minutes, *Rhizobium* (CK 16) for 30 minutes, PSB (P 36) for 30 minutes, Hot water treatment for 30 minutes, Conc. Nitric acid (98%) for 3 minutes, Conc. Sulphuric acid (98%) for 3 minutes and control. Observations were recorded for germination percentage, mean germination time, dry weight of seedling, seedling length and vigour index. For calculating germination percentage, treated bael seeds were planted in one per cent agar solution at a temperature of 28ºC in a seed germinator (Caltan-NSW-192) (Fig 1). After 20 days of sowing the final count of normal seedling was done and per cent germination was calculated on basis of total seeds sown as per the guidelines of International Seed Testing Association (ISTA, 2010).

$$\text{Germination per cent (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

The duration of germination (in days) was recorded from the date of sowing of seeds to the date of germination of last seed in a treatment. For measuring seedling length (cm), ten normal seedlings were chosen randomly per treatment and length was measured with the help of meter rod from tip of root to the tip of shoot. Five seedlings selected from every treatment for measuring dry weight. Dry weight of individual seedlings was measured with digital electronic balance (A&D weighing- Galaxy HR-150AZ). The seedlings were kept at 60 ± 2ºC in hot air oven for drying, until a constant weight achieved. Formula proposed by Abdul-Baki and Anderson (1973) was used for calculating vigour index I and II.

Vigour Index I = Standard germination (%) × Average seedling length (cm)

Vigour Index II = Standard germination (%) × Average seedling dry weight (mg)

The data collected throughout the investigation was statistically evaluated using Fishers (1958) analysis of variance (ANOVA) technique. The mean value of different parameters was compared using critical difference. The statistical software OPSTAT was used to carry out the entire statistical analysis.

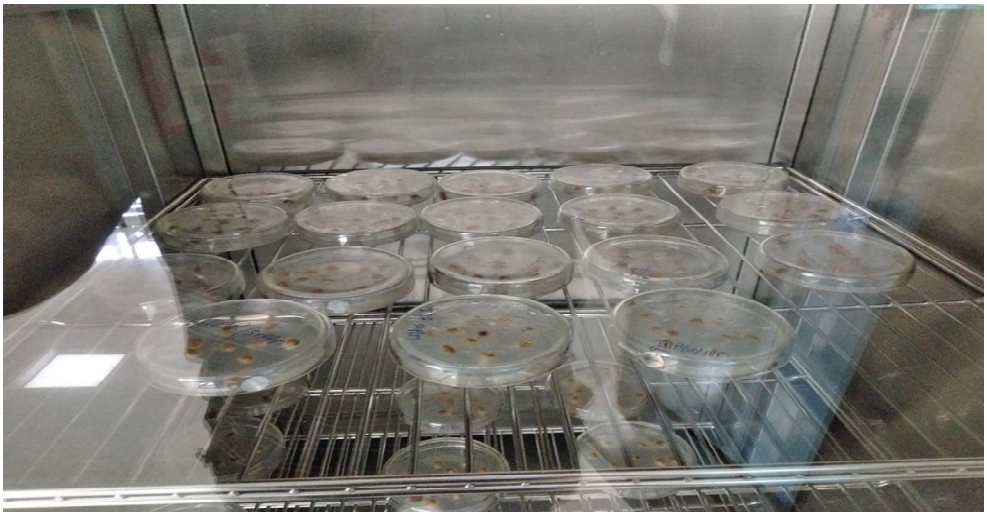

Fig. 1: Photo showing seeds in seed germinator at 28°C

Results and Discussion

Germination percentage

The seed priming treatments significantly influenced the germination percentage of bael (Table 1). Germination per cent ranged from 63.3 to 83.3 per cent. Seeds treated with GA_3 @ 100 ppm for a period of 24 hours resulted in highest germination per cent (83.3 %) while the lowest germination per cent (63.3%) was observed in control. The findings showed that bael seeds are physically dormant. The enhanced germination observed with GA_3 application may be attributed to the exogenous stimulation of aleurone layers within the seeds. GA_3 aids in the production of α-amylase enzyme, which facilitates the conversion of insoluble sugars into soluble forms. Moreover, GA_3 also acts as a catalyst for initiating radical growth by alleviating certain metabolic impediments. (Kolumbina *et al*., 2006, Babu *et al*., 2010). GA_3 also enhanced the seed germination by alleviating the inhibitory effects exerted by endogenous seed inhibitors (Wareing *et al*., 1968). The results mentioned align with the conclusions of Boricha *et al.* (2020). According to their findings, guava seeds treated with 150 mg/l GA_3 for period of 24 hours resulted in maximum germination percentage (80.77%). Similarly, Joshi *et al*. (2015) revealed that GA_3 has a substantial impact on the germination of acid lime seeds.

Duration of Germination

The results mentioned in Table 1 strongly indicates the significant impact of priming treatments on duration of germination. The duration of germination varied from 12.7 to 18.7 days. Among different priming treatments seeds dipped in sulphuric acid for 3 minutes took lowest duration of germination (12.7 days), that was statistically akin

with GA_3 @ 100 ppm for duration of 24 hours (13 days) and KNO_3 @ 1.0 % for 24 hours (13.3 days). However, maximum duration of germination (18.7 days) was observed in control. Early germination with sulphuric acid might be due to the fact that acid softens the rigid coating of seed by dissolving the accumulated lipids and pectic compounds. These compounds are sole responsible for seed hardiness. This softening enhances the permeability of seed coat to gases and water, thereby encouraging early germination (Chattopadhyay and Dey, 1992). Brijwal and Kumar (2014) and Kumar *et al.* (2022) in guava and Sharma (2016) in chironji also reported similar results.

Table 1: Effect of seed priming treatments on germination (%) and duration of germination in bael (*Aegle marmelos* Corr.)

Sr. No.	Treatments	Germination (%)	Duration of germination (days)
1	Control	63.3	18.7
2	IBA @ 100 ppm for 24 hours	76.0	14.3
3	IBA @ 50 ppm for 24 hours	76.0	14.7
4	GA_3 @ 100 ppm for 24 hours	83.3	13.0
5	GA_3 @ 50 ppm for 24 hours	76.0	14.7
6	NAA @ 100 ppm for 24 hours	73.3	14.7
7	NAA @ 50 ppm for 24 hours	73.3	14.7
8	KNO_3 @ 1.0% for 24 hours	80.0	13.3
9	Hot water for 10 minutes	66.0	16.0
10	*Azotobacter* (HT 54) for 30 minutes	70.0	15.7
11	*Trichoderma viride* for 30 minutes	73.3	15.7
12	*Rhizobium* (CK 16) for 30 minutes	80.0	15.3
13	PSB (P 36) for 30 minutes	76.0	15.3
14	Nitric acid (HNO_3) for 3 minutes	70.0	14.0
15	Sulphuric acid (H_2SO_4) for 3 minutes	66.0	12.7
	Range	63.3-83.3	12.7-18.7
CD (p = 0.05)		1.7	1.02

Seedling length (cm)

Data on seedling length varied from 7.5 to 12.2 cm (Table 2). The maximum seedling length (12.2 cm) was observed when seed primmed with 100 ppm GA_3 for 24 hours duration, followed by 50 ppm GA_3 concentration for 24 hours (11.6 cm). Results showed that the minimum seedling length (7.5 cm) was reported in control that was statistically at par with the hot water treatment for 10 minutes. The observed increase in seedling with GA_3 treatment can be attributed to hormones ability to enhance osmotic uptake of nutrients that leads to cell multiplication in the internodal cambium tissue (Krishnamoorthy and Sandooja, 1981). Gibberellic acid plays pivotal role in regulating stem elongation by influencing various cellular processes. It enhances cell wall extensibility by promoting the synthesis of cell wall components and reducing cell wall rigidity. This increased extensibility allows for greater cell expansion and elongation, contributing to overall stem growth. Gibberellic acid also stimulates cell division, leading to an increase number of cells available for elongation. Apart from this, it increases the synthesis of IAA, which has an immediate effect on stem elongation (Leopold and Kriedeman, 1983). Similar observations were reported by Santos *et al.* (2022) in pitahaya.

Dry weight per seedling (mg)

Data in Table 2 illustrates that seeds treated with GA_3 at concentration of 100 ppm for 24 hours resulted in highest dry weight per seedling (153.2 mg), which was statistically at par with KNO_3 at concentration of 1.0 % for 24 hours (151.8 mg) and 100 ppm IBA treatment (151 mg). However, in control, lowest dry weight per seedling (142.0 mg) was noticed. The significant increase in dry weight with GA_3 treatment might be attributed to improved water and nutrient transportation, leading to enhanced photosynthesis and the efficient movement of photosynthates within the seedlings, resulting in greater overall growth and therefore greater dry weight per seedling. Sasikala and Srimathi (2006) observed similar pattern in papaya, whereas, Patel *et al*. (2016) also observed such variations in custard apple.

Table 2: Effect of seed priming treatments on seedling length (cm) and dry weight (mg) per seedling in bael (*Aegle marmelos* Corr.)

Sr. No.	Treatments	Seedling length (cm)	Dry weight/seedling (mg)
1	Control	7.5	142.0
2	IBA @ 100 ppm for 24 hours	10.5	151.0
3	IBA @ 50 ppm for 24 hours	10.1	149.0
4	GA_3 @ 100 ppm for 24 hours	12.2	153.2
5	GA_3 @ 50 ppm for 24 hours	11.6	149.2
6	NAA @ 100 ppm for 24 hours	9.9	149.6
7	NAA @ 50 ppm for 24 hours	9.1	148.0
8	KNO_3 @ 1.0% for 24 hours	11.8	151.8
9	Hot water for 10 minutes	8.2	146.1
10	*Azotobacter* (HT 54) for 30 minutes	9.5	147.6
11	*Trichoderma viride* for 30 minutes	9.7	148.6
12	*Rhizobium* (CK 16) for 30 minutes	9.6	148.0
13	PSB (P 36) for 30 minutes	9.6	149.0
14	Nitric acid (HNO_3) for 3 minutes	9.9	146.6
15	Sulphuric acid (H_2SO_4) for 3 minutes	10.2	148.4
	Range	7.5-12.2	142.0-153.2
CD (p = 0.05)		1.1	2.3

Vigour Index

The highest vigour index I (976) was observed in GA_3 @ 100 ppm seed treatment, closely followed by KNO_3 @ 1.0 per cent. In contrast, control had the lowest vigour index I (448). The value of vigour index I varied from 448 to 976 (Table 3). The value of Vigour index II fluctuates between 8520 and 12256. Maximum vigour index II was noticed in GA_3 @ 100 ppm for duration of 24 hours, followed by KNO_3 @ 1.0% for 24 hours and IBA @ 100 ppm for 24 hours, while, minimum (8520) was noticed in control. In the current study, seeds which are treated with GA_3 @ 100 ppm for 24 hours has higher vigour index I and II by 118.34 and 438.49 per cent, in comparison to control. The increased length and dry weight of seedlings in GA_3 seed treatment may be the potential cause of the rise in vigour indexes. Sheoran *et al.* (2018) noticed that seed soaking with GA_3 resulted in maximum vigour index in ber.

Table 3: Effect of seed priming treatments on vigour index in bael (*Aegle marmelos* Corr.)

Sr. No.	Treatments	Vigour index - I	Vigour index - II
1	Control	448	8520
2	IBA @ 100 ppm for 24 hours	773	11068
3	IBA @ 50 ppm for 24 hours	742	10922
4	GA_3 @ 100 ppm for 24 hours	976	12256
5	GA_3 @ 50 ppm for 24 hours	850	10936
6	NAA @ 100 ppm for 24 hours	694	10472
7	NAA @ 50 ppm for 24 hours	637	10360
8	KNO_3 @ 1.0% for 24 hours	907	11537
9	Hot water for 10 minutes	520	9206
10	*Azotobacter* (HT 54) for 30 minutes	634	9845
11	*Trichoderma viride* for 30 minutes	680	10402
12	*Rhizobium* (CK 16) for 30 minutes	676	10360
13	PSB (P 36) for 30 minutes	706	10922
14	Nitric acid (HNO_3) for 3 minutes	660	9778
15	Sulphuric acid (H_2SO_4) for 3 minutes	646	9394
	Range	448-976	8520-12256
CD (p = 0.05)		52	696

Conclusion

Results of the experiment indicated that minimum time required for germination (12.7 days) was noticed in seed priming treatment with sulphuric acid for 3 minutes, while other parameters such as germination percentage, dry weight per seedling, seedling length and vigour index were recorded maximum in GA_3 treatment at 100 ppm for 24 hours under laboratory conditions. These values highlight the potential of gibberellic acid as an effective treatment to promote seedling growth and overall plant performance.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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