Influence of Pre-Sowing Seed Treatments on Vigor and Biochemical Activity in Fresh and Aged Guava (*Psidium guajava* L.) Seeds

ABSTRACT

The study was conducted during 2024–2025 at the Horticulture Laboratory of Shree Guru Gobind Singh Tricentenary University, Gurugram, Haryana, to evaluate the effects of various pre-sowing treatments on germination and seedling growth parameters of fresh and one-year-old guava seeds. Treatments included water soaking (48 hours), hot water soaking (70°C and 80°C for 1 minute), sulphuric acid (10% and 20% quick dip), gibberellic acid (GA_3) at 200 ppm and 400 ppm (24 hours), thiourea at 1000 ppm and 2000 ppm (24 hours), and a control. Parameters assessed included Vigour Index – I and II, speed of germination, accelerated aging test (24, 48, 72, and 96 hours), peroxidase activity, dehydrogenase activity, and electrical conductivity. Results showed that soaking seeds in gibberellic acid (GA_3) at 400 ppm for 24 hours consistently outperformed other treatments, recording the highest Vigour Index – I (205.00 for fresh, 105.67 for aged seeds), Vigour Index – II (11,923.00 for fresh, 7,493.00 for aged), speed of germination (1.88 for fresh, 1.78 for aged), and germination percentages under accelerated aging, alongside the highest peroxidase (710.00 µmol/gm DW for fresh, 640.33 for aged) and dehydrogenase activities (0.23 OD for fresh, 0.18 for aged), and the lowest electrical conductivity (9.43 µS/cm/sample for fresh, 10.08 for aged). The control consistently showed the lowest values across all parameters. Gibberellic acid at 200 ppm and thiourea at 1000 ppm also performed well but were inferior to GA_3 at 400 ppm. These findings demonstrate that pre-sowing treatment with GA_3 at 400 ppm significantly enhances germination, seedling vigour, and seed quality, offering a practical approach for improving guava seedling establishment in nurseries.

Keywords: Guava; Pre-sowing seed treatment; Gibberellic acid (GA_3); Seed germination; Seedling vigour; Accelerated aging; Enzyme activity; Seed quality.

1. INTRODUCTION

Guava (*Psidium guajava* L.), belonging to the family Myrtaceae, is an important small to medium-sized fruit tree widely cultivated in tropical and subtropical regions of the world. It is native to tropical America, particularly the regions of Mexico and Peru, from where it spread to several parts of Asia, Africa, and the Pacific Islands due to its adaptability to diverse agro-climatic conditions. In India, guava occupies a prominent position among fruit crops and ranks fifth after mango, banana, papaya, and citrus. Owing to its wide availability throughout the year, high nutritive value, pleasant flavour, and low market price, guava is popularly known as the “Apple of the Tropics” (Singh *et al*., 2011).

India is the largest producer of guava in the world, contributing nearly 45% of global production, which highlights the crop’s national importance in fruit production systems. Major guava-growing states include Uttar Pradesh, Bihar, Madhya Pradesh, and Haryana, where the crop is cultivated under both commercial orchards and smallholder farming systems (NHB, 2024). In Haryana, guava cultivation has expanded rapidly due to favourable climatic conditions, availability of improved cultivars, and increasing market demand. During the 2023–24 period, the state recorded an estimated production of 183,643 metric tonnes from an area of 16,750 hectares, with commercially important cultivars such as Allahabad Safeda, Hisar Safeda, Hisar Surkha, and Sardar dominating cultivation (NHB, 2023). Guava plays a significant role in the state’s rural economy by generating employment opportunities and supplying raw material to the fruit processing industry, including juice, pulp, and preserve manufacturing units.

From a nutritional perspective, guava is considered a nutrient-dense fruit, rich in vitamin C, dietary fibre, pectin, essential minerals, and natural antioxidants. These attributes make it highly suitable for fresh consumption as well as processing into value-added products such as jams, jellies, nectar, beverages, and dehydrated products, thereby enhancing its commercial value and shelf life (Naseer *et al*., 2018).

Although guava is commercially propagated through vegetative methods such as air layering, grafting, and budding to maintain varietal uniformity, seed propagation remains indispensable for raising healthy, vigorous, and uniform rootstocks used in these methods. However, guava seeds are known to exhibit physical dormancy due to their hard and impermeable seed coat, which restricts water absorption and gas exchange. In addition, guava seeds possess short viability, resulting in delayed, uneven, and poor germination, ultimately affecting seedling vigour and establishment (Nonogaki, 2014).

Considering the increasing demand for quality planting material and the importance of strong rootstocks for successful vegetative propagation in Haryana’s expanding guava orchards, the adoption of pre-sowing seed treatments becomes essential. Treatments such as water soaking, scarification, and the application of plant growth regulators have been reported to effectively overcome seed dormancy, enhance germination percentage, and improve seedling growth and seed quality. Therefore, the present investigation was undertaken to evaluate the effect of different pre-sowing seed treatments on germination behaviour, seedling growth, and seed quality parameters of guava seeds of varying ages, with the aim of improving nursery performance and planting material production.

2. METHODOLOGY

The laboratory experiment was conducted during 2024–2025 at the Horticulture Laboratory, Faculty of Agricultural Sciences, Shree Guru Gobind Singh Tricentenary University, Gurugram, Haryana, India (28º27′ N, 77º00′ E; 217 m above mean sea level). Seeds of wild guava (*Psidium guajava* L.) belonging to two age groups—fresh and one-year-old—were used for the study. Healthy seeds were extracted from fully ripe fruits, separated from the mesocarp, thoroughly washed, and shade-dried to achieve a moisture content of approximately 12–15%. The dried seeds were stored in cloth bags under ambient laboratory conditions until further use. Prior to the application of treatments, seeds were surface-sterilized with 1% sodium hypochlorite (NaOCl) solution for 10 minutes, rinsed under running tap water for 3 minutes, and air-dried.

The experiment was laid out in a Completely Randomized Design (CRD) with ten treatments replicated three times, each replication consisting of 30 seeds. The treatments included: untreated control (T_1), water soaking for 48 hours (T_2), hot water soaking at 70°C for 1 minute (T_3), hot water soaking at 80°C for 1 minute (T_4), sulphuric acid quick dip at 10% (T_5) and 20% (T_6), gibberellic acid (GA_3) soaking at 200 ppm (T_7) and 400 ppm (T_8) for 24 hours, and thiourea soaking at 1000 ppm (T_9) and 2000 ppm (T_{10}) for 24 hours. After each treatment, seeds were thoroughly rinsed with tap water to remove any chemical residues and then surface-dried before sowing.

For standard germination test, 30 seeds of each treatment in three replicates were placed in moist sand medium and kept at 27 ± 1°C with 80–85% RH in a seed germinator. Germination count was recorded at regular intervals up to 30 days, and normal seedlings were considered as per ISTA (2001) rules. Mean germination time (MGT) was calculated using the formula of Moradi Dezfuli *et al.* (2008). Seedling length was measured from 10 randomly selected normal seedlings, and dry weight was recorded after oven drying at 65 ± 2°C for 48 h.

3. RESULTS AND DISCUSSION

Vigour Index – I- The data presented in Table no.1 revealed that pre-sowing seed treatments had a significant effect on Vigour Index – I of guava seeds. The highest values were recorded in seeds soaked in GA_3 at 400 ppm for 24 hours, achieving 205.00 in fresh seeds and 105.67 in one-year-old seeds. These values were statistically comparable with those obtained from seeds soaked in GA_3 at 200 ppm for 24 hours and thiourea at 1000 ppm for 24 hours. The minimum vigour index values (136.00 and 87.00, respectively) were observed in the untreated control. The results clearly demonstrate that GA_3 pre-soaking markedly enhanced seedling vigour by promoting higher germination and better seedling growth, even in aged seed lots.

Vigour Index – II- Similarly, pre-sowing treatments significantly influenced Vigour Index – II (Table no. 1). The highest values were observed in seeds soaked in GA_3 at 400 ppm for 24 hours (11,923.00 in fresh seeds and 7,493.00 in one-year-old seeds), which were statistically on par with GA_3 at 200 ppm for 24 hours. The lowest indices (7,153.00 and 5,857.33, respectively) were recorded in the control. The superior performance of GA_3-treated seeds may be attributed to enhanced germination, increased shoot elongation, and improved metabolic activity, which together facilitated efficient mobilization and utilization of seed reserves (Bewley,1994 ; Copeland, 2001). These findings corroborate earlier reports in aonla (Singh, 2014), papaya (Hassan, 2014), Indian gooseberry (Farooq *et al*., 2012), tamarind (Manoharan, 2018), and khirni (Ratna *et al*., 2018).

Table no. 1 Vigour Index –I and vigour index - II

Treatment details	**Vigour Index – I**		**Vigour Index – II**	
	Fresh	**1 yr old**	**Fresh**	**1 yr old**
Control	136.00	87.00	7,153.00	5,857.33
Water soaking for 48 hrs.	192.33	97.67	10,555.33	6,889.00
Hot water soaking at 70°C for 1 min.	179.33	94.33	10,036.67	6,421.33
Hot water soaking at 80°C for 1 min.	187.67	96.33	10,218.67	6,573.00
10% Sulphuric acid treatment (Quick Dip)	149.33	88.00	8,274.33	5,937.67
20% Sulphuric acid treatment (Quick Dip)	165.00	91.33	9,838.33	6,226.33
Soaking seeds in GA_3 @ 200 ppm for 24 hrs.	203.00	103.67	11,615.33	7,376.33
Soaking seeds in GA_3 @ 400 ppm for 24 hrs.	205.00	105.67	11,923.00	7,493.00
Soaking seeds in thiourea @1000ppm for 24 hrs.	200.33	101.33	11,229.67	7,280.33
Soaking seeds in thiourea @2000ppm for 24 hrs.	198.00	100.00	10,990.00	7,154.67
C.D. @ 5%	6.53			

Peroxidase Activity Test - The data presented in Table no.2 indicated that pre-sowing treatments significantly affected germination percentage under accelerated aging conditions in guava. In the fresh seed lot, the highest germination percentages were recorded in seeds soaked in GA_3 at 400 ppm for 24 hours (T_8), with 14.63%, 10.00%, 4.67%, and 1.95% at 24, 48, 72, and 96 hours, respectively, followed by GA_3 at 200 ppm (T_7) with 14.37%, 9.78%, 4.50%, and 1.88%. The lowest germination values were observed in the control (T_1) with 6.00%, 4.00%, 1.35%, and 0.68%, respectively. A similar trend was recorded in the one-year-old seed lot, where T_8 exhibited the highest germination percentages of 9.98%, 5.00%, 2.69%, and 0.67% at 24, 48, 72, and 96 hours, respectively, while the control (T_1) consistently recorded the lowest values of 4.63%, 3.00%, 0.69%, and 0.00%. These findings confirm the superiority of GA_3 at 400 ppm in maintaining seed vigour and viability by enhancing metabolic efficiency and delaying aging-induced deterioration.

Table no. 2 Peroxidase Activity Test.

Treatment details	**@24 hrs**		**@48 hrs**		**@72 hrs**		**@96 hrs**	
	Fresh	**1yr old**	**Fresh**	**1yr old**	**Fresh**	**1yr old**	**Fresh**	**1yr old**
Control	6.00	4.63	4.00	3.00	1.35	0.69	0.68	0.00
Water soaking for 48 hrs.	11.54	7.78	7.98	4.51	3.42	1.95	1.43	0.57
Hot water soaking at 70°C for 1 min.	11.93	6.51	6.78	4.31	2.46	1.59	1.25	0.45

Hot water soaking at 80°C for 1 min.	10.34	6.99	7.27	4.45	3.02	1.83	1.35	0.53
10% H_2SO_4 treatment (Quick Dip)	7.19	4.95	4.72	3.48	1.98	0.87	0.80	0.21
20% H_2SO_4treatment (Quick Dip)	8.25	5.69	5.64	3.94	1.51	1.06	1.04	0.33
Soaking seeds in GA_3@200ppm for 24 hrs.	14.37	9.61	9.78	4.94	4.50	2.55	1.88	0.64
Soaking seeds in GA_3@400ppm for 24 hrs.	14.63	9.98	10.00	5.00	4.67	2.69	1.95	0.67
Soaking seeds in thiourea @1000ppm for 24hrs.	13.50	8.82	9.21	4.78	4.12	2.42	1.67	0.62
Soaking seeds in thiourea @2000ppm for 24hrs.	12.92	8.15	8.47	4.64	3.87	2.26	1.58	0.60
C.D. @ 5%	**0.44**	**0.33**	**0.38**	**0.13**	**0.15**	**0.10**	**0.08**	**0.05**

Dehydrogenase Activity Test - The results (Table no.3) revealed that pre-sowing treatments significantly influenced dehydrogenase activity in guava seeds. The highest activity was recorded in seeds soaked in GA_3 @ 400 ppm for 24 hours (0.23 in fresh and 0.18 in one-year-old seeds), which was statistically at par with GA_3 @ 200 ppm (0.22 and 0.17) and thiourea @ 1000 ppm (0.22 and 0.18), while the lowest was observed in the control (0.09 and 0.06). The enhanced dehydrogenase activity under GA_3 and thiourea treatments indicates improved enzymatic metabolism and respiratory efficiency, which are vital for better seed vigour and germination (Khan, 1971; Hassan, 2014; Farooq *et al.,* 2012).

Table no. 3 Dehydrogenase Activity Test and Electrical Conductivity Test

Treatment details	**Dehydrogenase Activity**		**Electrical Conductivity (µS/cm)**	
	Fresh	1 yr old	Fresh	1 yr old
Control	0.09	0.06	16.20	24.15
Water soaking for 48 hrs.	0.12	0.08	11.51	13.73
Hot water soaking at 70°C for 1 min.	0.15	0.14	13.74	16.29
Hot water soaking at 80°C for 1 min.	0.17	0.17	12.85	14.67
10% H_2SO_4treatment (Quick Dip)	0.10	0.07	15.72	20.44
20% H_2SO_4treatment (Quick Dip)	0.12	0.07	14.88	18.33
Soaking seeds in GA_3@200ppm for 24 hrs.	0.22	0.17	9.68	10.66
Soaking seeds in GA_3@400ppm for 24 hrs.	0.23	0.18	9.43	10.08
Soaking seeds in thiourea@1000ppm for 24 hrs.	0.22	0.18	10.11	11.08
Soaking seeds in thiourea@2000ppm for 24 hrs.	0.18	0.11	10.73	12.65
C.D. @ 5%	0.01	0.01	0.29	0.48

Electrical conductivity - Electrical conductivity (Table no.3) was significantly affected by pre-sowing treatments. The lowest values were obtained in GA_3 @ 400 ppm for 24 hours (9.43 µS/cm in fresh and 10.08 µS/cm in one-year-old seeds), which was statistically comparable with GA_3 @ 200 ppm (9.68 and 10.66), whereas the highest values were recorded in the control (16.20 and 24.15). Lower electrical conductivity values in GA_3-treated seeds indicate reduced membrane leakage and higher seed vigour, signifying better membrane integrity and metabolic efficiency (Copeland, 2001; Manoharan, 2018; Singh, 2014).

4. CONCLUSION

The present investigation clearly demonstrated that pre-sowing seed treatments play a crucial role in enhancing seed vigour, germination performance, and physiological quality of guava **(***Psidium guajava* L.) seeds, irrespective of seed age. Significant improvements were observed in both fresh and one-year-old seed lots, indicating that appropriate seed treatments can effectively mitigate the adverse effects of seed aging and dormancy associated with hard seed coats and declining metabolic activity.

Among the various treatments evaluated, soaking seeds in gibberellic acid (GA_3) at 400 ppm for 24 hours emerged as the most effective and consistent treatment. This treatment recorded the highest Vigour Index–I and Vigour Index–II, reflecting enhanced germination percentage, increased seedling length, and greater seedling dry matter accumulation. The superior performance of GA_3-treated seeds can be attributed to its role in stimulating cell elongation, promoting enzyme synthesis, and facilitating efficient mobilization of stored food reserves, which collectively contributed to improved seedling growth and vigour.

Biochemical assessments further substantiated the beneficial effects of GA_3 treatment. Higher dehydrogenase activity observed in GA_3-treated seeds indicated enhanced respiratory metabolism and cellular activity, which are essential indicators of seed viability and vigour. In addition, lower electrical conductivity values recorded under GA_3 treatments suggested improved membrane integrity and reduced solute leakage, signifying better physiological stability of seeds. These results confirm that GA_3 not only enhances germination but also strengthens the internal biochemical and cellular mechanisms associated with seed quality.

The peroxidase activity test under accelerated aging conditions revealed that GA_3-treated seeds maintained significantly higher germination percentages even after prolonged exposure to stress, compared to untreated control seeds. This highlights the ability of GA_3 to delay aging-induced deterioration, possibly through improved antioxidant activity and metabolic efficiency, thereby extending seed longevity and maintaining viability under adverse conditions.

Although treatments such as GA_3 at 200 ppm and thiourea at 1000 ppm also showed statistically comparable improvements in several parameters, their effects were consistently inferior to GA_3 at 400 ppm. In contrast, untreated control seeds and acid scarification treatments exhibited comparatively lower vigour indices, reduced enzymatic activity, and higher electrical conductivity values, indicating inferior seed quality and reduced physiological performance.

In conclusion, soaking guava seeds in GA_3 at 400 ppm for 24 hours can be recommended as a simple, economical, and effective pre-sowing treatment for improving germination, seedling vigour, and physiological stability in both fresh and moderately aged guava seed lots. Adoption of this treatment can significantly enhance nursery establishment, rootstock production, and overall planting material quality, thereby supporting the sustainable expansion of guava cultivation, particularly in regions such as Haryana where quality rootstocks are essential for successful vegetative propagation.

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