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Effect of different pre-sowing treatments on seed germination, seed coat morphology and survival of guava (*Psidium guajava* L.)

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Abstract
Effect of different pre-sowing treatments on germination of *P. guajava* seeds revealed that all the treatments had play a significant role on seed germination, earlier germination, seedling girth, number of leaves, height of the seedling, root length and vigour index of the seedling. The highest percentage of germination was recorded in GA_3 @ 1000 ppm for 24 hours. Germination start in 16.15 days after sowing and completed in 30.33 days. The minimum duration 16.15 days for germination when the sowing of seeds treated with GA_3 @ 1000 ppm for 24 hours. Height 32.50 cm and girth of seedling 2.42 mm varied significantly with different sowing treatments. Number of leaves per seedling also influenced by different sowing treatments and maximum (20.00) number of leaves per seedling was recorded in seed soaked with GA_3 @ 1000 ppm for 24 hours. Different sowing treatment greatly influenced the seedling survival in nursery. Maximum (85.57%) seedling survival was obtained, when seeds soaked with GA_3 @ 1000 ppm for 24 hours. The transplanting success ranged between 28.42 and 97.32 per cent and highest transplanting success was noted in seed soaked with GA_3 @ 1000 ppm for 24 hours.

Keywords: Seed, germination, vigour, enzyme, SEM

Introduction
Guava (*Psidium guajava* L.) is an important fruit crop of India. The genus *Psidium* belongs to the family Myrtaceae, order Myrtales and its basic chromosome number is X=11. Fifth most important fruit in area and production after banana, mango, papaya and citrus. It possess high amount of nutrient value, heavy bearing capability in every year and also results in good economic returns without much care (Singh *et al.*, 2000) [17]. This promotes involve to more farmer for commercial guava cultivation. Currently, cultivated guava plants are propagated through budding (Gupta and Mehrotra, 1985; Kaundal *et al*., 1987) [6], air layering (Manna *et al*., 2004) [8] and layering methods (Pathak and Saroj, 1988) [12]. These plants are still not, economically viable due to varying rate of success, life span of the tree, without tap root system and cumbersome process. As another methods, rapid multiplication technique through wedge grafting has been developed by CISH, Luck now for fulfilling the requirement of quality planting material.
Currently, increases the area of guava cultivation day-by-day, eventually the demand for *wild spp* rootstock used grafted plants is also increasing, but this demand not fulfilled because of unavailability of quality seedling rootstocks which might be due to poor seed germination and seedling growth. The production of healthy, wider adaptable and vigours rootstock is most important factor for successful plant propagation. Guava seeds are in common used for raising the rootstock for propagation. Seeds are hard seed coat play role in poor germinated, unevenly germination and require more time to seedling emerge (Doijode, 2001) [5]. To follow various seed treatment used to raising the guava rootstock. Hence present investigation was conducted to evaluate various seed treatment techniques on the performance of guava seedlings.

Material and Methods
Seed were pre-treatment as given below for germination studies were research trail was conducted in Department of Fruit Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India during 2018 – 2019. The seeds of guava variety ayakudi local (red flesh) were extracted from fully ripened fruits and the pulp was removed from seed. The extracted seed were sieves and wash with tap. Seeds without pulp were allowed for shade drying at room temperature (22ºC) for few minutes. Only sunken seeds in water selected after overnight soaking to impose the treatments. Seeds were pre-treatment as per the following treatment *viz.*

Treatment details

Number of Treatment	Treatment details
T_1	Control
T_2	Tap water soaking @ 48 hours
T_3	Seed soaking in hot water (100 ^{0}C) for 10 sec
T_4	Seed treated with GA_3 at 500ppm for 24 hours
T_5	Seed treated with GA_3 at 1000ppm for 24 hours
T_6	Seed treated with HCl at 5% for 2 minutes
T_7	Seed treated with HCl at 10% for 2 minutes
T_8	Seed treated with conc.H_2SO_4 at 5%for 3 minutes
T_9	Seed treated with conc.H_2SO_4 at 10% for 3 minutes

Proper care was taken to raise seedling with regular watering for morning and evening hours. The pot mixture comprising of sand, red soil and farm yard manure as (3:2:1) were used to fill polythene bag of 200 gauge thickness (20 cm X 10 cm) used for transplanting of guava seedlings.

Determination of germination percentage (GP) and Mean germination time (MGT)

Emergence of radical and plumule were considered as seed germination. Observation of seed germination and MGT percentage was done on 45th day after sowing, when germination completely ceased and was expressed in percentage using the formula:

$$\text{Germination percentage} = \frac{\text{Total no of seed germinated}}{\text{Total number of seeds sown}} \text{X100}$$

Mean germination time was calculated by using formula:

$$\text{Mean germination time} = \sum(\text{nt})/\sum \text{n}$$

Where, n= time taken for germination, t = days from sowing (Nichols and he decker, 1968) [10].

Post germination studies

Five seedlings from each replication were select randomly for taking observation was recorded. Root and shoot length was measured by using ruler and expressed in centimetre (cm). Total length of the seedling calculated by addition of shoot length and root length of respective seedling and expressed in centimetre (cm). Vigour index reflect the health of the seedling of the seedling and calculated by using formula

$$\text{Vigour index} = \text{Germination percentage (\%) X Total seedling length (cm)}$$

(Abdulal-baki and Anderson, 1973)

The stem girth (mm) was recorded by using digital vernier callipers. The number of leaves per seedling was recorded by counting the total number of leaves at 60, 90,120 and 150 days after sowing. Seed coat images taken from Scanning electron microscope (SEM). Seed coat morphology images on control.

Experimental design and statistical analysis

An experiment was conducted in Completely Randomized Design and nine treatment with three replication.

Results and Discussion

Mean germination time: The data on initiation of germination of each different pre-sowing treatment methods significantly reduced the day's emergence of guava seedling as compared to control. The mean germination time of guava seedlings ranged from 16.15 to 30.33 days under different pre-sowing treatment. The minimum number of days (16.15 days) required for seedling emergence was recorded in 1000ppm of GA_3 soaking in 24 hours (T_5) followed by 18.33 days in 500ppm of GA_3 soaking in 24 hours (T_5) significantly superior compared with other treatments. GA_3 might have acted on embryo causing *de nova* synthesis of hydrolysing enzyme particularly amylase and proteases and this hydrolysed food energy is utilized growth embryo for enhancing early germination (Paleg, 1965) [11]. GA_3 treatment increase the germination by changing the internal biochemical reaction known to be enhanced by endogenous GA level by synthesis of hydrolases (α −amylase) in the endosperm of seed. Its breakdown is generally believed to be an essential process for germination. Similar results were also reported by (Suryakanth *et al.,* 2005) (Singh and Soni, 1974) [18, 19] in guava. And (Kolumbina *et al.,* 2006) [7] in wheat grains among all treatment compare delayed germination was observed in (30.33 days) control (T_1) reported that guava seeds have hard seed coat and thickness of seed coat also higher (Fig.2). (Hayes, 1953) reported that seed acid scarification of guava seeds shorten the germination period without adverse effect of germination percentage. Similar research finding was given by Singh, (1967) [20].

Germination percentage: The data represented in table.1 Significant response among the different pre-sowing treatment was observed for germination per cent. Seed treated with 1000ppm of GA_3 soaking in 24 hours (T_5) give maximum germination (88.56%) at 45 days after sowing which was found to be on par with T_4 (80.94%), followed by T_7 (74.28%), T_6 (72.37%) and T_2 (61.90%). Whereas minimum germination percentage (18.09%) recorded in T_3 seeds quick dipping in hot water at 100^o C for 10 sec. Improve the germination percentage with GA_3 treatment increase the cell-wall plasticity and better abortion of water to increase the moisture content of seed and endogenous, exogenous application of GA_3 which helped to increase endogenous synthesis of GA_3 and trigger synthesis of hydrolysing enzymes (α −amylase) for conversion of starch to sugar energy making them available to utilized growth embryo for enhancing early germination. Same findings reported by (Ramchandran and Sheogovind, 1990) [15] in guava. Decreased germination percentage by seed treated with different pre-sowing treatment included higher water temperature could be attributed to embryo damage and availability of O_2 level was low at higher water temperature which resulted in destruction of certain enzymatic component, hence poor germination percentage. Same findings reported by Powell, (1990) [14] and Teketay, (1998) [22] in *acacia*.

The transplanting success ranged between 28.42 and 97.32 per cent and highest transplanting success was noted in seed soaked with GA_3 @ 1000 ppm for 24 hours. Different pre-

sowing treatments had significant effect on seedling girth of guava. Girth of the seedling at 150 DAS (days after sowing) was maximum (2.24 mm) in 1000 ppm of GA_3 soaking in 24 hours (T_5) and minimum (1.39 mm) in seeds quick dipping in hot water at 100° C for 10 sec (T_3). Girth at 60, 90, and 120 days also followed in same trend. (Table 2.) The maximum stem girth of seedling obtained from GA_3 seeds treatment might be due to the enhancement of the rate of cell division and cell elongation of stem portion. (Reshmi *et al.*, 2007) [16] reported similar results increase the stem girth by GA_3 pre-soaking treatment in Anola.

Table 1: Mean germination time and Germination% of different pre-germination treatment

Treatment details	Mean time germination (MGT)	Germination Percent	Vigour index	Survival percentage	Transplanting success
T_1	30.33	50.47	2634.33	24.76	34.16
T_2	26.33	61.90	3289.67	36.19	52.12
T_3	28.00	18.09	892.67	17.14	28.42
T_4	18.33	80.95	5097.67	76.18	90.44
T_5	16.15	88.57	5825.00	85.57	97.32
T_6	21.00	72.38	4579.67	78.09	84.34
T_7	19.33	74.28	4757.67	76.19	86.22
T_8	22.67	59.99	3418.00	39.04	53.23
T_9	23.33	61.90	3630.00	54.28	66.36
Mean	22.88	63.17	3791.63	54.49	65.84
S.Ed	5.33	5.22	331.82	6.41	7.13
CD (0.05%)	11.20	11.19	696.82	13.46	14.21

Number of leaves per seedling: Pre-sowing treatment gave significant effect on number of the leaves per seedling. The maximum number of leaves (20.00) was registered at 150 days after sowing in 1000 ppm of GA_3 soaking in 24 hours (T_5) followed by 18.33 T_4 and T_6 which were on par whereas minimum number of leaves (9.66) was observed in seeds quick dipping in hot water at 100° for 10 sec (T_3). GA_3 increased cell division and cell growth apparently leading to increased development of young leaves. Therefore, from the present study it is evident that the maximum number of leaves per seedling with GA_3 may be attributed to increase the physiological process and stimulatory action of GA_3 to form new leaves at faster rate. Archana *et al.*, (2015) [3] reported similar results in *Annona squasmosa*, Ramchandar and Govind, (1990) in guava.

Table 2: Girth of the seedling (mm) and Number of leaves of pre-sowing treatment on

Days / Treatments	Girth of seedling (mm)				Number of leaves			
	30 days	60 days	90 days	120 days	30 days	60 days	90 days	120 days
T_1	0.46	1.10	1.28	1.60	2.00	9.33	10.67	12.67
T_2	0.47	1.16	1.49	1.94	2.00	9.33	14.67	15.33
T_3	0.47	1.12	1.22	1.39	2.00	7.33	9.33	9.67
T_4	0.77	1.88	2.17	2.29	2.67	13.33	17.00	18.33
T_5	0.79	2.06	2.36	2.42	3.33	14.67	19.33	20.00
T_6	0.66	1.46	2.24	2.31	2.00	10.67	17.33	18.33
T_7	0.64	1.48	2.27	2.33	2.67	12.00	16.00	17.67
T_8	0.58	1.27	1.55	2.10	2.00	10.00	12.67	15.33
T_9	0.56	1.38	1.48	2.14	2.00	8.67	12.33	17.33
Mean	0.60	1.43	1.78	2.05	2.29	10.59	14.37	16.07
S.Ed	0.02	0.06	0.03	0.04	0.54	1.13	1.34	1.07
CD (0.05%)	0.05	0.14	0.07	0.08	1.14	2.37	2.81	2.26

Fig 1: Effect of pre-sowing treatment on height of the seedling

Height of the seedling (cm): The maximum height of guava seedling (32.50 cm) at 150 day after sowing was noticed in 1000 ppm of GA_3 soaking in 24 hours (T_5) followed by T_7 (31.30 cm), T_4 and T_6 (30.93 cm) which were on pair and minimum height of seedling (24.76) observed in seeds quick dipped in hot water at 100° C for 10 sec (T_3). Increase shoot length with GA_3 treatment to the activated amylase enzymes, which digested the available carbohydrate into simple sugar energy and nutrition were easily available to embryo for faster growth of seedling. Same findings reported by (Vishwakarma, 2013) [24] in acid lime. Aatla and Srihari, (2013) reported that treatment of mango cv. Alphonso kernel with GA_3 @ 500 ppm resulted in greater seedling height and intermodal length.

Table 3: Effect of pre-sowing treatment on height of the seedling and root length (cm) on

Days / Treatments	Root length (cm)				Height of the seedling (cm)			
	30 days	60 days	90 days	120 days	30 days	60 days	90 days	120 days
T_1	2.47	10.67	15.64	26.23	2.23	8.13	13.83	26.03
T_2	2.90	11.03	16.90	26.50	3.07	8.43	15.70	26.73
T_3	2.73	10.67	15.50	24.87	2.60	7.73	12.83	24.77
T_4	4.23	15.50	22.27	32.03	4.03	12.43	21.77	30.92
T_5	4.67	15.80	23.89	33.23	4.50	13.20	23.00	32.50
T_6	4.20	11.77	22.40	32.27	4.30	11.10	21.83	30.93
T_7	4.20	15.10	23.70	32.73	4.20	12.23	22.67	31.30
T_8	3.33	13.27	19.10	29.27	3.07	10.67	17.77	27.73
T_9	3.67	14.00	20.03	30.50	3.13	9.37	18.33	28.17
Mean	3.60	13.09	19.93	29.73	3.45	10.56	18.63	28.78
S.Ed	0.19	0.46	0.51	0.39	0.14	0.48	0.34	0.49
CD (0.05%)	0.41	0.98	1.07	0.82	0.31	1.01	0.73	1.02

Root length: The effect of pre sowing treatment on root length was found to be significantly. Maximum root length of guava seedling (33.23 cm) at 150th day after sowing was observed in 1000 ppm of GA_3 soaking in 24 hours (T_5) followed by T_7 (32.73 cm), T_6 (32.26 cm) and T_4 (30.03 cm) which were on pair and minimum length T_3 (24.86 cm) was observed in seeds quick dipped in hot water @ 100° C for 10 sec. Similar results of increased root growth with GA_3 pre sowing treatment was reported by Pampanna and Sulikeri (2001) [13] in sapota cv. Kalipatti. The maximum root length in case of seedling obtained from GA_3 pre-soaked seeds might due to increase the elongation of the cells in the sub apical region of the roots as reported by Salisbury and Ross, (1988) [21].

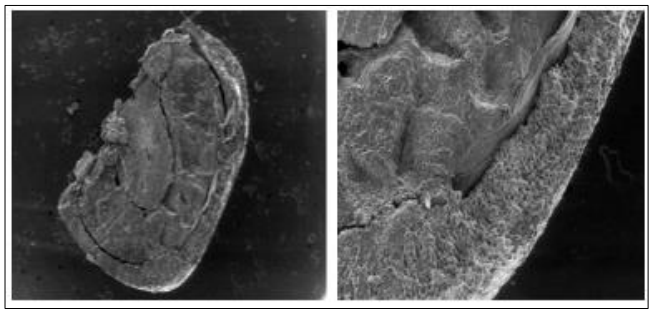

Fig 2: Effect of pre-sowing treatment on seed coat on guava

Vigour index: The maximum vigour of seedling(5825) at 150 day after sowing was observed in 1000ppm of GA_3 soaking in 24 hours (T_5) followed by T_4 (5097), T_7 (4757) and T_6(4579) and minimum root length T_3(97.00) was observed in seeds quick dipped in hot water at 100° for 10 sec. Similar results reported by Avinash Norman and Manivannan, (2012) reported that treating noni seeds in GA_3 100 ppm for 24 hours improved germination, early germination and seedling vigour. GA_3 seed treatment showed highest seedling vigour, increase in germination and seedling height which have contributed to higher vigour index.

Conclusion

On the basis of experimental findings, it can be concluded that among the different seed scarification treatment soaking in GA_3 1000 ppm soaked for 24 hours (T_5) was the most effective treatment for early germination, germination percentage, height of the seedling, girth of the seedling, number of leaves per plant, shoot length, and vigour index.

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