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Effect of different physical and chemical methods of seed treatment on germination and seedling growth attributes of guava (*Psidium guajava* L.)

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Abstract
The present study was carried out to investigate the effect of different physical and chemical methods of seed treatment on germination and growth of guava seedling (*Psidium guajava* L.). Among the different physical and chemical methods of seed treatment, seeds treated with GA_3 at 1000 ppm for 24 hours resulted in maximum germination percentage (88.56%), highest seedling length (65.73 cm), highest girth of the seedling (2.42 mm) and highest survivability (70.58%). Sowing of seed without seed treatment showed poor performance in terms of germination and seedling growth.

Keywords: GA_3, survival, seed, germination, SEM

Introduction
Guava (*Psidium guajava* L.) is considered as fifth most important fruit crop in India after mango, banana, papaya and citrus. Guava is commercially cultivated in tropical and subtropical regions of India. Major guava producing states are Madhya Pradesh, Uttar Pradesh, Bihar, Maharashtra, West Bengal, Tamil Nadu, Karnataka, Gujarat and Haryana. Guava has gained considerable prominence on account of its high nutritive value, pleasant aroma, good flavour, high amount of vitamin C, pectin and more importantly remunerative price even without much care. Considered as "poor man's apple", the guava truly happens to be the fruit for masses in terms of its availability in the market and accessibility to the poor (Jayachandran *et al*., 2005) [4]. In the recent past, the techniques like high density planting and meadow orcharding are becoming popular among the farmers. High density planting requires huge quantity of planting material. Nowadays demand for quality planting material and non-availability of budded and grafted plants has adversely affected the production and productivity levels in guava. Non-availability of superior rootstock due to poor seed germination and seedling growth is attributed to inconsistent production of quality planting material.

The germination of guava seed takes long time due to hard seed coat (Singh, 1967) [13], impermeability of the hard seed coat, resulting in poor seedling emergence establishment Kavitha *et al*., (2015) [5]. Sadeghi and Khaef (2012) [10] reported that hard seeds are more suitable for storage and offers resistance to adverse which results poor germination percentage. The dormancy in seed might be due to hard seed coat and impermeability to water and gases. Hence, an attempt was made to break the seed dormancy and improve germination capability of guava and also to produce highly vigorous rootstocks with wider adaptability through different seed treatments like mechanical scarification, acid treatment and chemical treatment using GA_3 raising seedling rootstock of guava.

Material and methods
The present study was carried out at Department of Fruit Crops, Horticultural College and Research Institute, TNAU, Coimbatore, Tamil Nadu during 2018 – 2019. The treatment details are given below;

Fully ripened fruits of guava were collected and seeds were extracted. The seeds were washed with tap water and only sinking seed were taken for seed treatment. Polythene bags of 200-gauge thickness (20 X 10 cm) were used for raising guava seedlings. The pot mixture comprised of sand, red soil and farm yard manure in (4:2:1) proportion. Healthy and uniform sized seeds were selected for sowing in polybags and kept in shade net with necessary care. Light irrigation was provided regularly till the start germination. Seed germination was recorded on 45thday after sowing on complete cessation of germination and expressed in percentage.

Five seedlings were randomly selected from each replication and observation on total height of the seedling (cm) were recorded. Stem girth (mm) was recorded with digital Vernier calliper by measuringthe girth of the seedling at 60, 90,120 and 150 days after sowing. Survival percentage was recorded after 150 days after sowing. A micrograph of seed was taken before and after seed treatment and germination to compare the morphological changes that occur during the breaking of dormancy by using dissecting microscope equipped with a digital camera. The seed coat structure was analysed by using scanning electron microscopy image by following the method suggested by Serrato-Valenti *et al*., (2000) [11].

Treatment details

Treatment	Treatment details
T_1	Control
T_2	Mechanical scarification using sand paper
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

Result and discussion

Days taken for initiation of germination: The data on initial germination of each physical and chemical methods of seed treatmentshowed marked influence on seed germination. The days taken for emergence of guava seedlings ranged from 16.66 to 30.33 days under different physical and chemical methods of seed treatment. The minimum number of days (16.66 days) required for seedling emergence was recorded in 1000ppm of GA_3 soaking for 24 hours followed by in 500ppm of GA_3 soaking for 24 hours(18.33 days). GA_3 enhanced seed germination, which might have antagonized the effect of inhibitors present in anloa seeds (Kumari *et al*., 2007) [6]. Similar results were also reported by Suryakanth *et al.,* (2005) [15] and Singh and Soni, (1974) [14] and Singh, (1967) [13] that guava seeds have hard seed coat resulting in delayed germination. Hayes, (1953) [3] reported that acid scarification of guava seeds shortened the germination period without adverse effect of germination percentage.

Days taken for 50 per cent of germination: Among the all pre sowing treatments 50 per cent of seedling emergence obtained at 22.34 and 23.33 days in 1000ppm of GA_3 soaking for 24 hours and 500 ppm of GA_3 soaking for 24 hours respectively. The maximum 35.00days taken for 50 per cent of germination was recorded in control.

Germination percentage: The results of the experiment indicated significant response among various physical and chemical methods of seed treatmentin terms of germination percentage. Seeds treated with 1000ppm of GA_3 soaked for 24 hours (T_5) give maximum germination percentage (88.56%) at 45 days after sowing which was found to be on par with T_3 (80.94%), followed by T_7 (74.28%), T_6 (72.37%) and T_2 (61.9%). Whereas minimum germination percentage was recorded in T_3 (18.09%) (Table.1). The increase in germination percentage with GA_3 might be due to involvement of GA_3 in the activation of cytological enzymes along with increase in cell wall plasticity and better absorption of solvents (Padma lay *et al*., 2013) [7]. GA_3 treatment would have stimulated germination, due to increase in relative growth rate. (Powell, 1990) [8] Reported that decreased germination percentage at higher water temperature could be attributed to embryo damage. Teketay, (1998)[16] reported lower availability of O_2 with higher water temperature which resulted in destruction of certain enzymatic component leading to poor germination percentage.

Table 1: Effect of different physical and chemical methods of seed treatment on germination attributes of guava

Treatment details	Days taken for initiation of germination	Days taken for 50 % of germination	Germination percentage (%)
T_1(Control)	30.33	35.00	50.47
T_2 (Mech. Sand paper)	26.33	30.00	61.9
T_3(Hot water100º10sec)	28.00	33.33	18.09
T_4(GA_3 500ppm-24 hr)	18.33	23.33	80.94
T_5(GA_3 1000ppm-24 hr)	16.66	22.34	88.56
T_6(HCl 5%-2 min)	21.00	26.00	72.37
T_7(HCl 10%-2 min)	19.33	25.33	74.28
T_8($H_2SO_4$5%- 3 min)	22.67	28.67	59.99
T_9($H_2SO_4$10%- 3 min)	23.33	28.66	61.89
SEd	5.33	5.33	5.33
CD(p=0.05)	11.20	11.20	11.20

Total length of the seedling: The effect of different physical and chemical methods of seed treatment on total seedling height at 60, 90,120 and 150 days after sowing was recorded and discussed (Table.1).The maximum length (65.73 cm) of guava seedling at 150 days after sowing was observed in 1000ppm of GA_3 soaking in 24 hours (T_5) followed by T_7 (64.03), T_6 (63.20) and T_4 (62.90). Which was on par with each other while minimum height of seedling (49.63) observed in seeds quick dipped in hot water at 100º for 10 sec (T_3). Increased seedling length in GA_3treated seedlings might be due to the stimulating effect on cell wall to release and transmit its calcium into cytoplasm that provides a condition for absorption of water by increase in cell size and growth Aatla and Srihari, (2013)[1]reported that treatment of mango cv. Alphonso kernel with GA_3 500 ppm resulted in greater seedling height and internodal length. Increased shoot length with GA_3 treatment might be because it activated amylase, which digested the available carbohydrate into simple sugars so that energy and nutrition were easily available to faster growth of seedling (Vishwakarma, 2013) [18].

Girth of the seedling: Different physical and chemical methods of seed treatment had significant effect on stem girth of guava seedlings (Table.2). Girth of the seedling at 150 days after sowing was maximum (2.24 cm) in 1000ppm of GA_3 soaking for 24 hours (T_5) and minimum(1.39 cm) in seeds quick dipping in hot water at 100º for 10 sec(T_3) (Table.2). Girth at 60, 90, and 120 days also followed the same trend.

The maximum stem girth of seedling obtained from GA_3 treated seeds might be due to the fact that GA_3 treatment enhancedthe rate of cell division and cell elongation of stem portion. Reshmi *et al.*, (2007) [9] reported similar results of GA_3 pre-soaking treatment to increase the stem girth in aonla.

Survival percentage: Different physical and chemical methods of seed treatment had significant effect on survival percentage of guava seedling. It was maximum (70.58%) at 150 days after sowing in 1000ppm of GA_3 soaking in 24 hours (T_5) and minimum (24.29%) in seeds quick dipped in hot water at 100° for 10 sec (T_3). (Fig.1) The probable cause of high survival percentage of seedlings might be due to early germination of seeds which helped in successful acclimatization of seedlings in field conditions and vigour of seedlings ultimately leading to better growth, thus, higher survival percentage. Similar results has been reported by (Shrivastava *et al.*, 2002) [12] in aonla.

Table 2: Effect of different physical and chemical methods of seed treatment on growth attributes of seedling

Treatment	Girth of the seedling (mm)			Total seedling Length (cm)				
	60 DAS	90 DAS	120 DAS	150 DAS	60 DAS	90 DAS	120 DAS	150 DAS
T_1(Control)	0.46	1.10	1.28	1.60	4.7	18.80	29.47	52.27
T_2(Mech. Sand paper)	0.46	1.16	1.49	1.93	5.93	19.47	32.60	53.13
T_3(Hot water-100°10sec)	0.47	1.02	1.22	1.39	5.33	18.40	28.33	49.63
T_4(GA_3 500ppm-24 hr)	0.77	1.87	2.17	2.28	8.27	27.93	44.03	62.90
T_5(GA_3 1000ppm-24 hr)	0.79	2.06	2.36	2.42	8.90	29.00	46.89	65.73
T_6(HCl 5%-2 min)	0.66	1.46	2.23	2.31	8.50	22.87	44.03	63.20
T_7(HCl 10%-2 min)	0.64	1.48	2.26	2.33	8.40	27.33	46.37	64.03
T_8($H_2SO_4$5%- 3 min)	0.58	1.26	1.54	2.09	6.40	23.93	36.87	57.00
T_9($H_2SO_4$10%- 3 min)	0.56	1.37	1.47	2.14	6.78	23.10	38.37	58.67
	T	D	T X D	T		D	T X D	
S.Ed	0.022	0.015	0.045	0.317		0.211	0.635	
CD (p=0.05)	0.045	0.030	0.091	0.633		0.422	1.266	

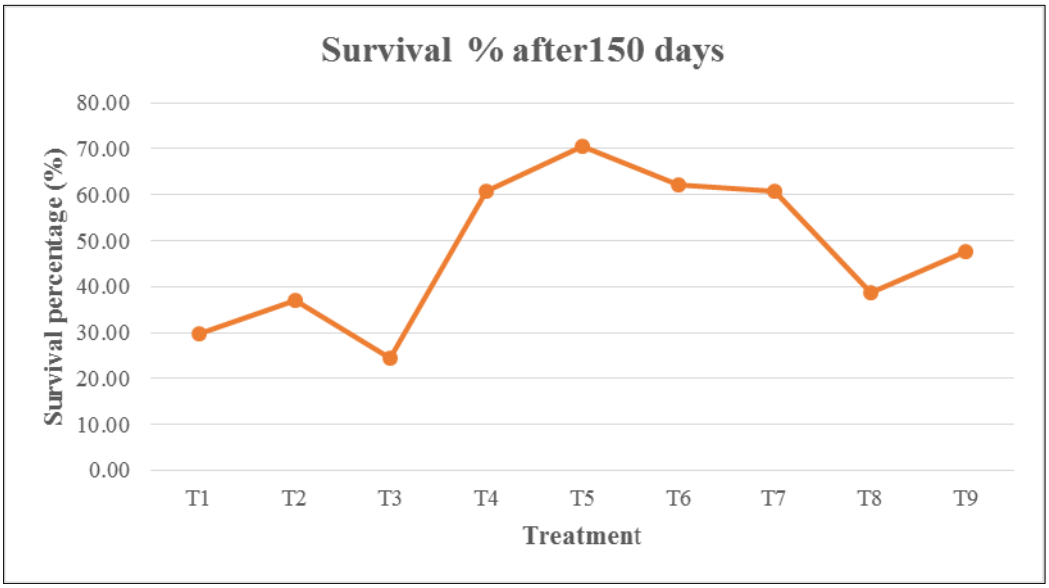
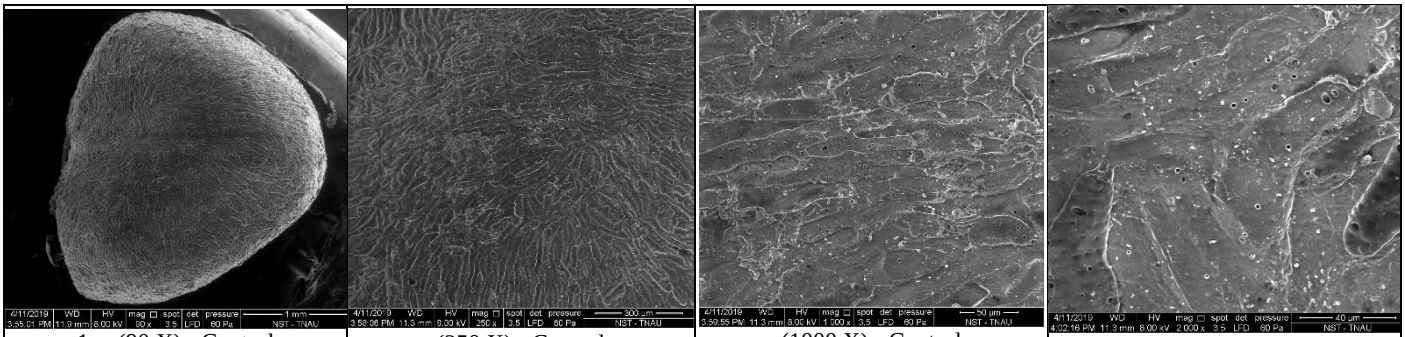


Fig. 1: Effect of different physical and chemical methods of seed treatment on Survival (%)



1 a. (90 X) - Control | (250 X) - Control | (1000 X) - Control | (2000 X) - Control

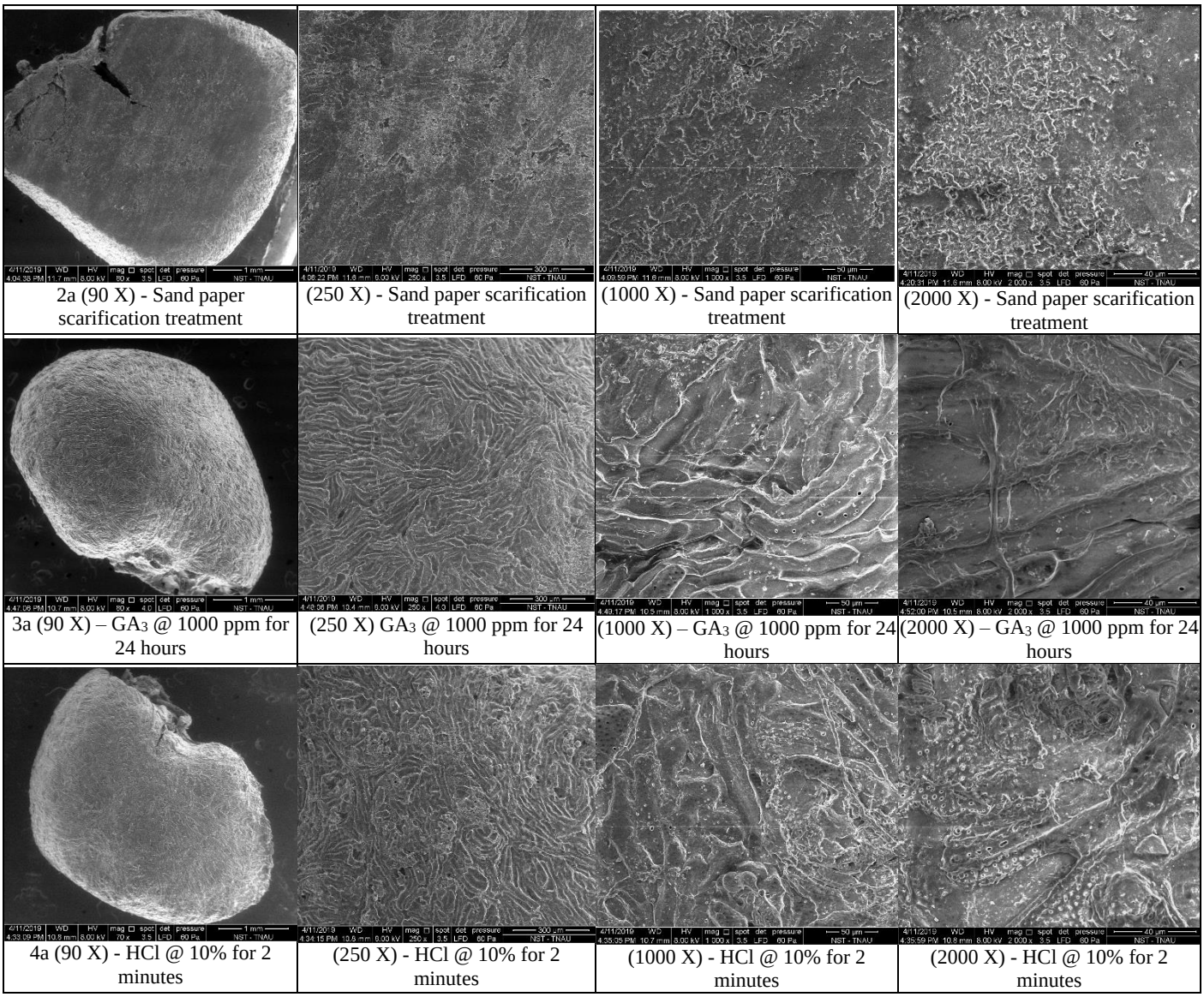


Fig 2: SEM image of physically and chemically treated seed coat morphology

Seed coat morphology

According to our present investigation, the highest response to physical and chemical treatments was observed in germination percentage and growth of seedlings. The hormonal treatment of GA_3 resulted in flattening of the ridges and softening of seed coat to permeablewaterand facilitate gaseous exchange (Fig.3a). Hartman and Kester, (1979) [2] reported that GA_3, helps in the synthesis of enzymeslike α-amylase which converts the starch into simple sugars during the process of germination. The sugar provide energy that is required for various physiological process of germination. Other enzymes activated by GA_3 include those which weaken the seed coat allowing the axis to burst through.GA_3 also enhanced cell elongation, so that the radicle pushed through the endosperm through hard seed coat and improve germination. The sand paper scarification treatment caused cracking on surface water gap area of seed coat to increase the germination percentage compared with control (Fig.2a). Sand paper scarification caused ultra structural damage which is formed by deep cracking through lens rupture. Usually, the weakest part of testa and micropyle together form structure called as water gap. Turner *et al*., (2009) [17] reported that the water gap at the seed served as a signal detector of environment, and on provision of water this gap will open and this environment provides a high probability of the seeds to germinate and grow better. The HCl scarified treatment helped the seeds to attain superior germination percentage. HCl treated seed coat dissolve the ridges and increase number of holes and size in seed coat facilitating the to permeability to water and enhanced germination (Fig.4a).Non-treated seed did not after the seed coat permeability because there is no ultra structural changes causing damage of seed coat (Fig.1a).

Conclusion

Seeds treated with GA_3 at 1000 ppm for 24 hours (T_5) germinated earlier and seedling growth parameters were found to be superior compared with other treatments. Studies on seed coat morphology indicated the GA_3 accelerated the synthesis of digestive enzymes like α-amylase which improved the germination percentage and seedling growth of guava.

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