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Improving Seed Germination and Seedling Growth of Guava under Protected Conditions by Pre-Sowing Chemical and Hormonal Seed Treatments

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ABSTRACT: Germination of the guava seeds is very poor, uneven and takes a long time because of the hard seed coats. Therefore, the present studies were carried out with the objective to reduce the difficulties in seed germination. Different pre-sowing seed dip treatments *viz.*, GA_3 @ 400 and 600 ppm for 24 hours, HCl @ 2.5 and 5.0 % for 2 minutes, H_2SO_4 @ 2.5 and 5.0 % for 2 minutes, KNO_3 @ 0.5% and 1.0% for 24 hours, Cow urine @ 50 and 100 % purity for 24 hours and Control (direct sowing) in Completely Randomized Design with three replications were tried. It has been observed that among different pre-sowing treatments, when guava seeds were dipped in 1.0 % solution of KNO_3 for 24 hours, resulted in maximum (83.00%) guava seed germination. However, minimum time for seed germination (18.67 days), maximum mean daily germination (3.09), survival percent (91.67 %) and growth characteristics *viz.*, seedling height, seedling diameter and number of leaves at 30, 60, 90 and 120 days after transplanting were observed, when guava seeds were dipped in 600 ppm solution of GA_3 for 24 hours.

Keywords: Guava, seed, KNO_3, GA_3, germination.

INTRODUCTION

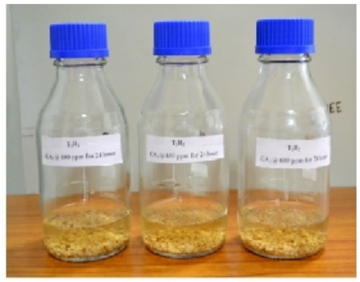
Guava (*Psidium guajava* L.) is one of the most delicious fruit grown both in tropical and sub-tropical regions of the world and native to tropical America. It is also recognized as "poor man's apple" owing to its cheaper cost and nutritional value (Dhaliwal and Singla, 2002). Guava has gained popularity all over world due to its nutritional importance and health benefits. It is a rich source of vitamins C, A, B_2 (riboflavin) and minerals like calcium, potassium, phosphorous and iron. It can be cultivated efficiently up to an elevation of 1500 m above mean sea level. However, temperature ranges between 20-30°C, well distributed rainfall ranges from 100 to 200 cm throughout the year, soils with good drainage, high levels of organic matter and pH ranges between 5.0 to 7.0 are ideal for successful guava cultivation (Yadava, 1996). In recent times, guava has witnessed a commendable growth in area expansion and its cultivation across the country. Guava occupies an area of 2,65,000 hectare with a production of 40,54,000 metric tonne in India (Anonymous, 2018a). In Himachal Pradesh, guava occupies an area of 2,292 hectares with annual production of 2,607 metric tonnes (Anonymous, 2018b). As the area under guava is increasing rapidly, the demand for vegetatively propagated plants has increased manifold and nurserymen has to produce a greater number of graft-able seedling rootstock in shorter period. However, due to the dormant cytological enzymes, presence of inhibitors and hard seed coat, there are more difficulties in the seed germination (Hejazi *et al.*, 2018). In recent years, attention has been shifted to the use of different chemicals and plant growth regulators to achieve higher, earliest seed germination and better seedling growth (Manthri and Bharad, 2017).

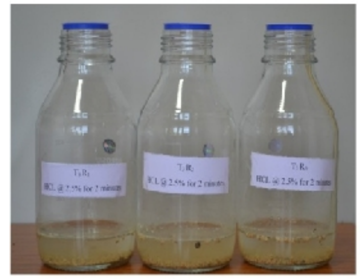
MATERIALS AND METHODS

The present experiment was carried out in the nursery area of Department of Fruit Science, College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh during the academic year 2019-20. The experimental block of fruit nursery is located at an elevation of 618 m above mean sea level, between 31°41′49.98″ North latitude and 72°28′02.21″ East longitude. The experiment was laid out in Completely Randomized Design (CRD) with three replications using five grams (500 seeds approximately) of seeds. Guava seeds were treated with different pre-sowing treatments (Plate 1) *viz.*, T_1: (GA_3 @ 400 ppm for 24 hours), T_2 : (GA_3@ 600 ppm for 24 hours), T_3: (HCl @ 2.5% for 2 minutes), T_4: (HCl @ 5.0% for 2 minutes), T_5: (H_2SO_4 @ 2.5% for 2 minutes), T_6: (H_2SO_4 @ 5.0% for 2 minutes), T_7: (KNO_3 @ 0.5% for 24 hours), T_8 : (KNO_3 @ 1.0% for 24 hours), T_9: (Cow urine @ 50% for 24 hours), T_{10}: (Cow urine @ 100% for 24 hours) and T_{11}: (Control). The treated seeds were shade dried for 15 minutes and then sown in raised beds up to 0.5

cm depth under polyhouse conditions in first week of August. Guava seedlings were transplanted at 4-6 leaves stage after germination in polythene bags of 20 × 30 cm size and kept in polyhouse. Observations on seed germination (%), time taken for seed germination (days), mean daily germination (Samir *et al.*, 2015), survival and growth characteristics *viz.*, seedling height, seeding diameter and number of leaves at 30, 60, 90 and 120 days after transplanting were recorded.


T_1 (GA_3@ 400 ppm for 24 hours)

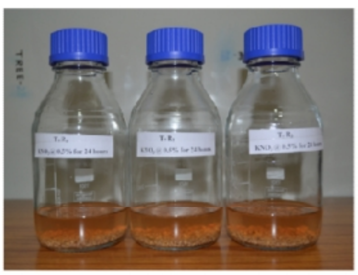

T_2 (GA_3@ 600 ppm for 24 hours)

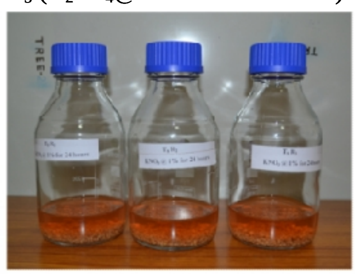

T_3 (HCL@ 2.5% for 2 minutes)


T_4 (HCL@ 5.0% for 2 minutes)

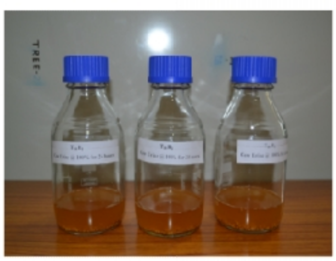

T_5 (H_2SO_4@ 2.5% for 2 minutes)


T_6 (H_2SO_4@ 5.0% for 2 minutes)


T_7 (KNO_3@ 0.5% for 24 hours)


T_8 (KNO_3@ 1.0% for 24 hours)


T_9 (Cow urine@ 50% for 24 hours)


T_{10} (Cow urine@ 100% for 24 hours)


T11 (Control)

Plate 1. Pre-sowing treatments in Guava seeds.

RESULT AND DISCUSSION

A. Seed germination (%)

The data pertaining to the effect of pre-sowing treatments are depicted in Table 1 showed a significant influence on percent seed germination. The maximum seed germination (83.00%) was observed in seeds which were dipped in 1.0 % solution of KNO_3 for 24 hours (T_8) and minimum seed germination (17.33%) was recorded with untreated control (T_{11}). The increase in germination might be due to the presence of nitrogenous compounds in KNO_3 which triggers the germination in soil by stimulating the pentose phosphate pathway in seeds as it triggers the oxidation of NADPH to NADH. Nitrate present in KNO_3 can alter hormonal levels by inducing the expression of enzymes that catalyze the inactivation of abscisic acid and biosynthesis of gibberellins (Finch *et al.*, 2007). KNO_3 also removes the outer thin leathery seed coat, increase water absorption and led to enzyme activation and hydrolysis of food material (Aatla and Srihari 2013). These results are in conformity with those of Owino and Ouma (2011); Parab *et al.* (2017); Dwivedi *et al.* (2015) in papaya.

B. Time taken for seed germination (days)

The different pre-sowing treatments exerted a significant effect on the time taken for seed germination (Table 1). The minimum time for seed germination (18.67 days) was recorded in the seeds, which were treated with 600 ppm of GA_3 for 24 hours (T_2), while, maximum time taken for seed germination (29.67 days) was observed with control (T_{11}). The earliest seed germinations were attained due to gibberellins, which have a stimulatory effect in the formation of enzymes that induces faster radicle protrusion. Gibberellins acts on the embryo and causes denova synthesis of hydrolyzing enzymes particularly amylase and protease. The hydrolyzed food is utilized for growth of embryo and thereby enhanced the seed germination in guava (Kalyani *et al.,* 2014). These results are in harmony with those of Tandon *et al.* (2019) in Tamarind, Patel *et al.* (2016) in mango, Desai *et al.* (2017); Pratibha *et al.* (2015) in papaya.

C. Mean daily germination

The different pre-sowing treatments exhibited a marked effect on mean daily germination (Table 1). The highest mean daily germination (3.09) was recorded in the seeds treated with 600 ppm of GA_3 for 24 hours (T_2). Whereas, lowest mean daily germination (0.39) was observed in the seeds of control (T_{11}). These findings are in conformity with those of Samir *et al.* (2015), where they found maximum mean daily germination when khirni seeds were presoaked in GA_3, because gibberellins resulted in higher germination percent and lower time for final germination.

D. Survival percent

The pre-sowing treatments showed a significant impact on the final survival of guava seedlings (Table 1). The maximum survival (91.67%) was obtained when guava seeds are treated with 600 ppm of GA_3 for 24 hours (T_2). However, minimum survival (25.67%) was recorded in control (T_{11}) treatment. The probable cause of higher survival percent of guava seedlings might be due to the easy root and shoot development and making the seedling stouter, withstanding the transplanting shock, resisting root diseases and leading to better growth (Palepad *et al.,* 2017). Our results are also in conformity with those of Barche *et al.* (2010) in papaya and Dinesh *et al.* (2019) in guava.

Table 1: Effect of pre-sowing treatments on percent seed germination, time taken for seed germination, mean daily germination and survival percent of guava seedlings.

Treatment	Percent seed germination (%)	Time taken for seed germination (days)	Mean daily germination	Survival percent (%)
T_1 (GA_3@ 400 ppm for 24 hours)	50.67	25.00	1.52	75.00
T_2 (GA_3@ 600 ppm for 24 hours)	75.33	18.67	3.09	91.67
T_3 (HCL@ 2.5% for 2 minutes)	25.33	26.33	0.63	50.00
T_4 (HCL@ 5.0% for 2 minutes)	30.33	25.67	0.77	70.00
T_5 (H_2SO_4@ 2.5% for 2 minutes)	28.33	26.00	0.70	66.67
T_6 (H_2SO_4@ 5.0% for 2 minutes)	35.66	25.33	0.94	72.67
T_7 (KNO_3@ 0.5% for 24 hours)	68.33	23.00	2.38	83.33
T_8 (KNO_3@ 1.0 % for 24 hours)	83.00	19.67	3.08	85.00
T_9 (Cow urine@ 50% for 24 hours)	24.67	28.00	0.60	45.00
T_{10} (Cow urine@ 100% for 24 hours)	58.67	24.67	1.89	83.00
T_{11} (Control)	17.33	29.67	0.39	25.67
$CD_{0.05}$	**3.22**	**2.54**	**0.14**	**10.90**

E. Seedling height

The different pre-sowing treatments exerted a significant influence on seedlings height at 30, 60, 90 and 120 days after transplanting (Table 2 and Plate 2). The maximum seedling height (7.92 cm) was recorded with T_2 (GA_3@ 600 ppm for 24 hours) treatment at 30 days after transplanting. Whereas, minimum seedling height (1.94 cm) was registered in control (T_{11}).

At 60 days after transplanting, maximum seedling height (10.34 cm) was recorded with T_2 (GA_3@ 600 ppm for 24 hours) treatment and minimum seedling height (2.90 cm) was recorded with control (T_{11}).

At 90 days after transplanting, maximum seedling height (13.89 cm) was recorded when guava seeds were dipped in 600 ppm of GA_3 solution 24 hours (T_2), while, minimum seedling height (4.48 cm) was recorded with control (T_{11}), which was statistically at par with T_3 (5.56 cm) and T_5 (6.07 cm) treatment.

At 120 days after transplanting, maximum seedling height (18.89 cm) was recorded when guava seeds were dipped in 600 ppm of GA_3 solution 24 hours (T_2) and minimum seedling height (6.27 cm) was recorded in control (T_{11}).

The increase in seedling height might be due to the cell multiplication and elongation in the cambium tissue of the internodal region, as gibberellins apparently activates the metabolic processes or nullifies the effect of inhibitors on growth (Singh *et al.,* 1989). The gibberellin hormone increased the osmotic uptake of nutrients, causing cell multiplication and cell elongation in the cambium tissue of the internodal region; thus increased height of the plant (Patil *et al.,* 2018). These results are also in agreement with the findings of Biradar *et al.* (2005) in guava, Sheoran *et al.* (2018) in ber, Vasantha *et al.* (2014) in tamarind and Gurung *et al.* (2014) in passion fruit.

Table 2: Effect of pre-sowing treatments on seedling height at 30, 60, 90 and 120 days after transplanting of guava seedlings.

Treatment	Seedling height (cm)			
	30 days after transplanting	60 days after transplanting	90 days after transplanting	120 days after transplanting
T_1 (GA_3@ 400 ppm for 24 hours)	6.55	8.71	10.24	13.90
T_2 (GA_3@ 600 ppm for 24 hours)	7.92	10.34	13.89	18.89
T_3 (HCL@ 2.5% for 2 minutes)	2.06	3.82	5.56	7.32
T_4 (HCL@ 5.0% for 2 minutes)	4.03	5.61	9.45	12.52
T_5 (H_2SO_4@ 2.5% for 2 minutes)	2.74	4.40	6.07	7.93
T_6 (H_2SO_4@ 5.0% for 2 minutes)	2.98	5.63	9.82	12.69
T_7 (KNO_3@ 0.5% for 24 hours)	4.90	7.30	10.82	14.73
T_8 (KNO_3@ 1.0 % for 24 hours)	5.70	7.50	11.71	15.52
T_9 (Cow urine@ 50% for 24 hours)	4.98	5.83	7.48	10.47
T_{10} (Cow urine@ 100% for 24 hours)	6.34	8.30	11.14	14.33
T_{11} (Control)	1.94	2.90	4.48	6.27
$CD_{0.05}$	**2.00**	**2.36**	**2.64**	**2.94**

F. Seedling diameter

The different pre-sowing treatments had a significant effect on seedling diameter at 30, 60, 90 and 120 days after transplanting (Table 3 and Plate 2). The maximum gain in seedling diameter (1.63 mm) was recorded in the plants raised from the seeds treated with 600 ppm of GA_3 for 24 hours (T_2), while, minimum growth in seedling diameter (0.92 mm) was recorded in control (T_{11}), at 30 days after transplanting.

At 60 days after transplanting, the maximum seedling diameter (2.11 mm) was obtained in the plants raised from the seeds treated with 600 ppm of GA_3 for 24 hours (T_2), however, minimum seedling diameter (1.23 mm) was observed in control (T_{11}).

At 90 days after transplanting, maximum growth in seedling diameter (2.49 mm) was obtained in the plants raised from the seeds treated with 600 ppm GA_3 for 24 hours (T_2), While, minimum seedling diameter (1.51 mm) was recorded in plants raised under control (T_{11}).

Maximum growth in seedling diameter (3.17 mm) was attained when seeds were treated GA_3@ 600 ppm for 24 hours (T_2), however, minimum seedling diameter (1.74 mm) was recorded under control (T_{11}) at 120 days after transplanting.

The increase in seedling diameter might be due to fact that indigenous levels of gibberellins were increased with application of GA_3 that results in faster cell division, cell elongation and cell multiplication in the cambium tissue and reflects as increase in seedling diameter (Manthri and Bharad, 2017). Gibberellins might have increased the somatic uptake of nutrients, causing cell elongation and thus increasing height and diameter of the seedlings. These results are also in conformity with those of Rai *et al.* (2018) in khirni, Deb *et al.* (2010) in papaya and Al-Hawezy (2013) in loquat.

Table 3: Effect of pre-sowing treatments on seedling diameter at 30, 60, 90 and 120 days after transplanting of guava seedlings.

Treatment	Seedling diameter (mm)			
	30 days after transplanting	60 days after transplanting	90 days after transplanting	120 days after transplanting
T_1 (GA_3@ 400 ppm for 24 hours)	1.49	1.97	2.27	2.53
T_2 (GA_3@ 600 ppm for 24 hours)	1.63	2.11	2.49	3.17
T_3 (HCL@ 2.5% for 2 minutes)	1.05	1.33	1.64	1.83
T_4 (HCL@ 5.0% for 2 minutes)	1.20	1.38	1.66	2.14
T_5 (H_2SO_4@ 2.5% for 2 minutes)	1.11	1.33	1.54	1.86
T_6 (H_2SO_4@ 5.0% for 2 minutes)	1.46	1.86	2.22	2.52
T_7 (KNO_3@ 0.5% for 24 hours)	1.52	1.89	2.30	2.86
T_8 (KNO_3@ 1.0 % for 24 hours)	1.61	2.05	2.47	2.95
T_9 (Cow urine@ 50% for 24 hours)	1.13	1.37	1.59	1.80
T_{10} (Cow urine@ 100% for 24 hours)	1.29	1.72	2.24	2.61
T_{11} (Control)	0.92	1.23	1.51	1.74
$CD_{0.05}$	**0.30**	**0.35**	**0.38**	**0.33**

G. Number of leaves

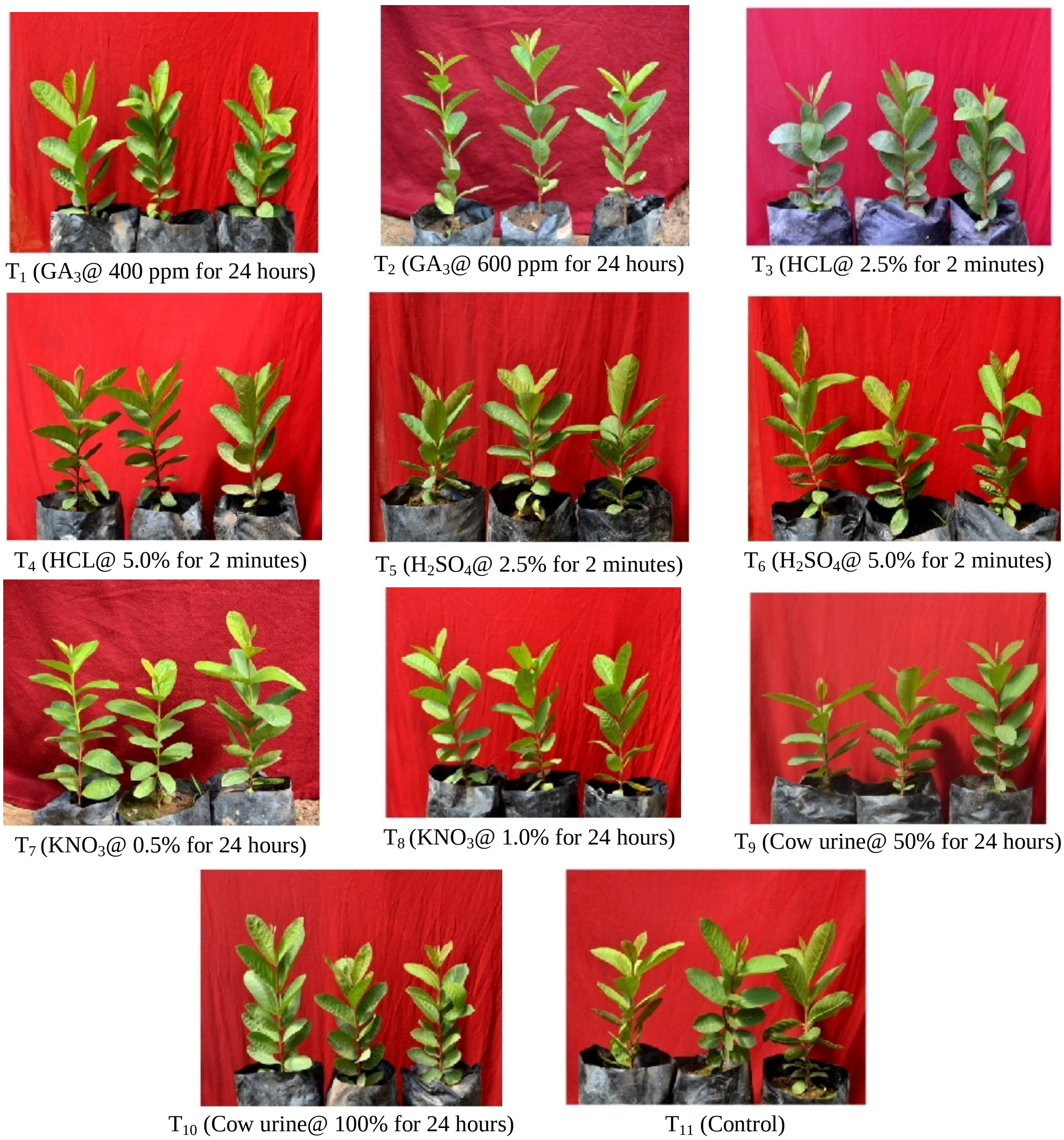
The pre-sowing treatments showed a significant effect on production of number of leaves at 30, 60, 90 and 120 days after transplanting (Table 4 and Plate 2). At 30 days after transplanting, maximum number of leaves (9.80) were formed in the plants raised from seed treated with GA_3@ 600 ppm for 24 hours (T_2), while, minimum number of leaves (5.89) were formed in control (T_{11}).

At 60 days after transplanting, the maximum number of leaves (12.17) were obtained in the plants raised from seed treated with GA_3@ 600 ppm for 24 hours (T_2), which was statistically at similar with T_8 (11.70), T_7 (11.08) and T_{10} (10.65) treatment. However, minimum number of leaves (6.73) were emerged in control (T_{11})

and was statistically at par with T_3 (7.34), T_9 (7.58) and T_5 (8.13) treatment. While, maximum number of leaves (14.50) were obtained in the plants raised from seed treated with GA_3@ 600 ppm for 24 hours in (T_2) treatment and minimum number of leaves (8.10) were emerged in control (T_{11}), at 90 days after the transplanting.

Whereas, the maximum number of leaves (18.76) were formed the plants raised from seed treated with GA_3@ 600 ppm for 24 hours (T_2), while, minimum number of leaves (9.18) was obtained in control (T_{11}) at 120 days after transplanting.

Gibberellins moves into the shoot apex, increases cell division and cell growth apparently leading to increased development of young leaves (Salisbury and Ross, 1988). Therefore, the maximum number of leaves per seedlings in the present study with gibberellins may be due to acceleration of physiological processes and stimulatory action of gibberellins to form new leaves at a faster rate (Brijwal and Kumar 2013). These results are also in agreement with the findings of Jaiswal *et al.*, (2018) in kagzi lime and Singh and Maheswari (2017) in soursop.



T_1 (GA_3@ 400 ppm for 24 hours)
T_2 (GA_3@ 600 ppm for 24 hours)
T_3 (HCL@ 2.5% for 2 minutes)
T_4 (HCL@ 5.0% for 2 minutes)
T_5 (H_2SO_4@ 2.5% for 2 minutes)
T_6 (H_2SO_4@ 5.0% for 2 minutes)
T_7 (KNO_3@ 0.5% for 24 hours)
T_8 (KNO_3@ 1.0% for 24 hours)
T_9 (Cow urine@ 50% for 24 hours)
T_{10} (Cow urine@ 100% for 24 hours)
T_{11} (Control)

Plate 2. Vegetative growth of Guava seedlings after 120 days.

Table 4: Effect of pre-sowing treatments on number of leaves at 30, 60, 90 and 120 days after transplanting of guava seedlings.

Treatment	Number of leaves			
	30 days after transplanting	60 days after transplanting	90 days after transplanting	120 days after transplanting
T_1 (GA_3@ 400 ppm for 24 hours)	7.78	9.57	11.77	14.76
T_2 (GA_3@ 600 ppm for 24 hours)	9.80	12.17	14.50	18.76
T_3 (HCL@ 2.5% for 2 minutes)	6.12	7.34	8.56	9.85
T_4 (HCL@ 5.0% for 2 minutes)	6.43	8.95	10.86	12.70
T_5 (H_2SO_4@ 2.5% for 2 minutes)	6.33	8.13	10.30	12.28
T_6 (H_2SO_4@ 5.0% for 2 minutes)	6.45	9.60	11.37	14.58
T_7 (KNO_3@ 0.5% for 24 hours)	8.83	11.08	13.17	16.42
T_8 (KNO_3@ 1.0 % for 24 hours)	9.32	11.69	13.26	17.09
T_9 (Cow urine@ 50% for 24 hours)	6.32	7.58	8.73	9.74
T_{10} (Cow urine@ 100% for 24 hours)	8.57	10.65	12.78	15.92
T_{11} (Control)	5.89	6.73	8.10	9.18
$CD_{0.05}$	**1.70**	**1.87**	**2.46**	**2.76**

CONCLUSION

The demand for the quality planting material of guava is increasing at faster pace across the whole country and it becomes hard to fulfill the requirement especially, seedling rootstock. Use of plant growth regulators/chemicals, that enhances the seed germination and their survival in fruit nurseries will boost the income of the growers. In the present study, it has been observed that guava seeds soaked in 600 ppm solution of GA_3 for 24 hours found most effective for earliest seed germination (18.67 days), improving mean daily germination (3.09), survival percentage (91.67%), seedling height, seedling diameter and number of leaves. However, guava seeds soaked in 1.0 percent solution of KNO_3 for 24 hours resulted in highest per cent seed germination (83.00%).

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Conflict of Interest. None.

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