



## Standardization of growing substrate for multiplication of guava (*Psidium guajava*) through cuttings

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### ABSTRACT

Experiments under the polyhouse were conducted for two consecutive years (2021 and 2022) at Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu (SKUAST-J), Jammu and Kashmir to evaluate the effect of different growing substrates on guava (*Psidium guajava* L.) multiplication through cuttings. Twelve substrate combinations were tried in polybags and polytrays under a polytunnel in a polyhouse provided with mist irrigation. The study revealed a positive effect of different growing substrates on growth parameters and successful propagation of guava cuttings. The perusal of the pooled data revealed that T<sub>9</sub> was the most effective growing substrate which recorded maximum rooting percentage, survival percentage, plant height, fresh and dry weight, the diameter of thickest sprout, shoot length, length of longest root, leaf area, and the number of roots, shoots, leaves per longest shoot along with minimum mortality percentage and days to first sprouting. However, some growth parameters such as stem thickness, chlorophyll percentage, number of sprouts, sprouted cuttings, and leaves of planted guava cuttings yielded the best results in T<sub>10</sub>. Cuttings grown in topsoil only without any growing substrate (T<sub>12</sub>) provided the least values of all the growth parameters of guava cuttings.

**Keywords:** Clonal propagation, Coco peat, Growing substrate, Vermiculite

Guava (*Psidium guajava* L.) is one of the major cash crops of India, considered the fifth most important fruit in respect of its area and production after mango, citrus, banana, and apple (Dhaliwal and Rattanpal 2010). In Jammu and Kashmir, the total production of guava is 7.77 thousand tonnes (Anonymous 2019). Both sexual (seed propagation) and asexual (vegetative propagation) methods can be adopted for the propagation of guava under protected and open field conditions. However, seed propagation often results in genetic heterogeneity, late bearing, less tolerance to fungal, bacterial, and viral pathogens, and less adaptability to varying climatic conditions, which makes seed propagation of guava less productive (Pereira *et al.* 2017). In this accordance, vegetative propagation through cuttings is advantageous over other propagation methods in a way that it helps to obtain true-to-type plants and also ensures that economically important plants are produced in a single growth period (Ullah *et al.* 2005). Undoubtedly, the most evolved and expanded method is rooting between methods of vegetative propagation (Manica *et al.* 2002), but

not enough information is available on rooting of cuttings in guava. The addition of amendments in the rooting medium like vermiculite, perlite, sand, and peat enhances the rooting of cuttings independent of auxins like IBA and NAA (Cheng *et al.* 2011). The rooting competency is greatly influenced by the type of growing substrate used and the selection of the most appropriate growing substrate combination should be considered a pre-requisite for successful propagation (Mehmood *et al.* 2013). However, species type, cutting type, planting season, propagation method, and production cost should be considered while selecting any appropriate growing substrate. Keeping these facts in view, the present study was carried out to standardize substrate for the clonal propagation of guava.

### MATERIALS AND METHODS

The present study was carried out at Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu (SKUAST-J), Jammu and Kashmir for two consecutive years (2021 and 2022) under a polytunnel in a polyhouse to standardize substrate for the clonal propagation of guava. The experiment was laid out in a Completely Randomized Design (CRD) (Fig 1). A combination of 12 treatments, viz. T<sub>1</sub>, topsoil+cocopeat (1:1); T<sub>2</sub>, topsoil+vermiculite (1:1); T<sub>3</sub>, topsoil+perlite (1:1); T<sub>4</sub>, cocopeat+vermiculite (1:1);

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Fig 1 Detailed view of propagation of guava cuttings: A, Trimming the guava cuttings; B, Preparation of growing media; C, Filling up of polybags; D, Preparation of guava cuttings; E, Planting of cuttings; F, Planted cuttings under polytunnel; G, Sprouting in cuttings; H, Polytunnel and; I, Data recording.

T<sub>5</sub>, cocopeat+perlite (1:1); T<sub>6</sub>, cocopeat+vermiculite+perlite (2:1:1); T<sub>7</sub>, cocopeat+vermiculite (2:1); T<sub>8</sub>, cocopeat+perlite (2:1); T<sub>9</sub>, cocopeat+vermiculite+perlite (3:1:1); T<sub>10</sub>, cocopeat+vermiculite (3:1); T<sub>11</sub>, cocopeat+perlite (3:1); and T<sub>12</sub>, control (top soil only) were considered into study with each treatment replicated thrice and three plants were tagged and analyzed in individual repetitions. A five-year-old guava orchard located in the organic farming field, SKUAST-J, grown in soil pH 8.5–10.0 was considered as source material for the present study. The apical shoot cuttings of guava (var. L-49), approximately 10 cm long, 0.4–0.6 cm thick, and each with 4 buds and 2 cut leaves were taken into the study. The cuttings were trimmed with an 8-inch heavy-duty plant cutter in the month of August and the leaves were removed from that part which had to be buried in planting substrate. A basal slanting cut was given 0.4 cm below a bud and an upper cut about 1 cm above the bud. The cuttings were washed thoroughly before planting. The cuttings were planted in black polyethylene bags (11.80 inches in diameter) at a depth of about 1.5–1.9 inches. The polybags and polytrays were afore prepared and filled with different treatment combinations. The prepared polybags and polytrays were placed under a polytunnel in polyhouse in a proper manner equipped with mist irrigation. The cuttings were immediately irrigated after planting. The subsequent irrigations were carried out at interval of 1–2 days. The data on different growth parameters, viz. rooting, survival and mortality percentage (%), fresh and dry weight of cuttings

(g), shoot length (cm), leaf area (cm<sup>2</sup>), chlorophyll percentage (%), number of primary, secondary and total roots, shoots and sprouted cuttings, length of longest root (cm), days to first sprouting, leaves per longest shoot, stem thickness (cm), fresh weight of leaves (g), diameter of thickest sprout (cm) was recorded after 120 days of planting. However, data on height of plant (cm), number of sprouts and leaves were recorded from 30–120 days after planting of cuttings.

*Statistical analysis:* Data obtained under various categories were subjected to statistical analysis using one-way ANOVA in a Completely Randomized Design. SPSS version 20.0 software was used to calculate the critical difference at 5% level of significance. Tukey HSD test was used to determine whether the difference between two means between treatments exceeded the critical difference (CD) value (Panse and Sukhatme 1978).

## RESULTS AND DISCUSSION

*Pooled effect of growing substrate on rooting, survival, and mortality percentage of cuttings:* The pooled data revealed that T<sub>9</sub> recorded highest rooting (84.43%) and survival percentage (75.85%) with minimum mortality percentage (24.15%) which was statistically different from all other growing substrates. The lowest rooting (37.57%) and survival percentage (29.80%) with maximum mortality percentage (70.20%) were recorded in control (topsoil) (Table 1). Our results are supported by Rani *et al.* (2018) who recorded highest rooting and survival percentage in coco peat as a medium. The better performance of coco peat as a medium can be attributed to its better aeration as a result of the presence of coarser materials (Awang *et al.* 2009). The phenomenon of aeration allows the removal of carbon dioxide from the soil to the outer atmosphere and the addition of atmospheric oxygen into the soil, leading to better rooting and survival of the cuttings (Jeyaseeli and Paul 2010). The observations that Ansari (2013) recorded on the highest efficiency of sand + vermiculite on the rooting percentage of pomegranate cuttings also corroborate our present findings. Shiri *et al.* (2019) documented a significant effect on survival percentage by the addition of growing substrate.

*Pooled effect of growing substrate on the fresh and dry weight of cuttings, fresh weight of leaves, shoot length, length of longest root and stem thickness:* T<sub>9</sub> recorded maximum fresh weight (5.03 g/cutting), dry weight (2.53 g/cutting), shoot length (15.00 cm), length of longest root

Table 1 Pooled effect of growing substrate on rooting, survival, mortality percentage, fresh, dry weight of cuttings, fresh weight of leaves, length of shoot and longest root and stem thickness after 120 days

Treatment	Rooting %*	Survival %*	Mortality %*	Fresh weight of cutting (g)*	Dry weight of cutting (g)*	Fresh weight of leaves (g)*	Shoot length (cm)*	Length of longest root (cm)*	Stem thickness (cm)*
T <sub>1</sub>	53.68±0.57 (7.30)	55.70±0.58 (7.53)	44.30±1.13 (6.73)	4.68±0.02	1.63±0.20	0.42±0.02	9.13±0.23	6.37±0.47	1.03±0.02
T <sub>2</sub>	52.23±0.53 (7.20)	52.66±0.35 (7.32)	47.33±0.95 (6.95)	4.62±0.04	1.49±0.05	0.41±0.02	10.90±0.75	5.67±0.48	1.02±0.01
T <sub>3</sub>	49.23±0.57 (6.99)	51.33±0.64 (7.23)	48.66±0.56 (7.04)	4.58±0.02	1.16±0.05	0.39±0.01	10.66±0.11	5.26±0.46	1.00±0.04
T <sub>4</sub>	62.57±1.05 (7.87)	59.33±1.15 (7.76)	40.66±0.41 (6.45)	4.83±0.05	1.84±0.10	0.48±0.01	11.79±0.26	9.65±0.60	1.04±0.02
T <sub>5</sub>	57.53±0.55 (7.55)	57.73±0.65 (7.66)	42.26±0.50 (6.57)	4.70±0.00	1.67±0.02	0.44±0.01	11.33±0.28	7.21±0.39	1.05±0.01
T <sub>6</sub>	79.23±0.59 (8.86)	67.26±0.32 (8.26)	32.73±0.79 (5.80)	4.99±0.01	2.39±0.10	0.53±0.02	14.50±0.30	11.55±0.45	1.08±0.01
T <sub>7</sub>	65.86±0.95 (8.08)	65.36±1.11 (8.14)	34.63±0.76 (5.96)	4.83±0.05	2.00±0.10	0.49±0.01	13.46±0.35	10.99±0.01	1.07±0.01
T <sub>8</sub>	59.93±0.57 (7.71)	62.33±0.57 (7.95)	37.67±0.57 (6.21)	4.76±0.05	1.77±0.02	0.47±0.02	12.96±0.35	9.70±0.56	1.06±0.02
T <sub>9</sub>	84.43±0.75 (9.14)	69.83±1.15 (8.41)	30.16±0.92 (5.58)	5.03±0.06	2.53±0.01	0.54±0.01	15.00±1.00	13.76±0.68	1.08±0.01
T <sub>10</sub>	81.57±0.57 (8.99)	75.85±0.37 (8.76)	24.15±0.34 (5.01)	5.00±0.00	2.50±0.10	0.54±0.01	13.96±0.05	12.84±0.73	1.09±0.01
T <sub>11</sub>	73.40±0.50 (8.53)	72.33±0.64 (8.56)	27.66±0.63 (5.35)	4.90±0.00	2.30±0.05	0.50±0.01	13.80±0.17	12.39±0.45	1.08±0.02
T <sub>12</sub>	37.57±1.15 (6.11)	29.80±1.10 (5.55)	70.20±1.11 (8.43)	2.20±0.20	0.90±0.00	0.19±0.01	5.36±0.55	2.50±0.43	0.46±0.11
SEm±	0.02	0.03	0.04	0.04	0.05	0.01	0.26	0.29	0.02
CD (P≤0.05)	0.08	0.08	0.11	0.11	0.15	0.02	0.76	0.86	0.06

\*Mean of 3 replications ± Standard deviation; CD, Critical difference; SEm±, Standard error mean. The figures in parenthesis are square root transformed values. Treatments details are given under Materials and Methods.

(13.76 cm) whereas T<sub>9</sub> and T<sub>10</sub> witnessed maximum fresh weight of leaves (0.54 g/leaf) and stem thickness (1.09 cm), respectively which was statistically different from other treatments. Control (topsoil) recorded minimum values of all the growth parameters (Table 1). Our results are in line with Rani *et al.* (2018) who obtained highest dry weight of planted cuttings in cocopeat. The highest fresh weight of leaves in perlite+FYM+garden soil (2:1:1) was reported by Monika *et al.* (2021) whereas lowest leaf fresh weight was recorded in topsoil. Our results are also in line with Nazari *et al.* (2011) and Gohil *et al.* (2018) who recorded maximum leaf fresh weight in hyacinth and chrysanthemum, respectively when coco peat was used as a medium. Monika *et al.* (2021) documented the importance of growing substrate in achieving maximum stem thickness in planted guava cuttings. Manila *et al.* (2017) reflected significant effect of growing substrate on length of longest root wherein they recorded maximum length of the longest root in vermiculite. Khalil and Aly (2013) also recorded the highest length of the longest root in vermiculite as a

growing medium.

*Pooled effect of growing substrate on cutting height, number of sprouts and number of leaves:* The maximum height of guava cuttings was recorded in T<sub>9</sub> from 30–120 days of planting. This was followed by T<sub>6</sub> and T<sub>10</sub> respectively. However, maximum number of sprouts and leaves were recorded in T<sub>10</sub> which was statistically at par with T<sub>10</sub> and T<sub>9</sub>. All the treatments were significantly different from control (topsoil) which recorded least cutting height, number of sprouts and leaves after 120 days of planting (Table 2). Our findings are in corroboration with Rani *et al.* (2015) who recorded maximum plant height in vermiculite+sand+FYM (1:1:1) after 120 days of planting. Manila *et al.* (2017) recorded higher number of sprouts and leaves in vermiculite as a growing medium. The maximum number of leaves were recorded in coco peat as a growing medium (Dhatrikarani 2019). Rani *et al.* (2018) recorded highest number of leaves in cocopeat as a growing medium after 135 days of planting.

*Pooled effect of growing substrate on leaf area and*

Table 2 Pooled effect of growing substrate on the number of leaves, sprouts and height of guava cuttings

Treatment	Height of cuttings (cm)*			Number of sprouts*			Number of leaves*			
	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP	120 DAP
T <sub>1</sub>	6.4±0.43	10.40±0.51	15.40±0.60	2.76±0.55	4.26±0.73	6.43±0.83	4.00±0.00	14.63±2.28	21.13±2.45	26.13±0.95
T <sub>2</sub>	6.23±0.25	10.23±0.35	15.23±0.45	2.73±0.58	4.23±0.77	6.40±0.87	4.00±0.00	14.63±2.28	21.33±2.46	26.13±1.00
T <sub>3</sub>	6.13±0.15	10.13±0.25	15.13±0.35	2.66±0.55	4.16±0.73	6.33±0.83	3.67±0.57	14.30±2.85	21.00±2.68	25.80±2.68
T <sub>4</sub>	6.90±0.40	10.90±0.45	15.90±0.52	2.96±0.64	4.46±0.83	6.63±0.92	4.66±0.57	15.30±2.04	22.00±1.85	26.80±0.80
T <sub>5</sub>	6.60±0.43	10.60±0.51	15.60±0.60	2.86±0.55	4.36±0.73	6.53±0.83	4.33±0.58	14.96±2.60	21.66±2.42	26.46±2.43
T <sub>6</sub>	7.60±0.69	11.60±0.78	16.60±0.87	3.16±0.55	4.66±0.56	6.83±0.45	5.33±0.57	15.96±2.62	22.66±2.48	27.26±2.45
T <sub>7</sub>	7.16±0.76	11.17±0.66	16.27±0.56	3.10±0.51	4.60±0.55	6.76±0.45	5.33±0.58	15.97±2.60	22.66±2.42	26.46±2.43
T <sub>8</sub>	7.13±0.32	11.13±0.40	16.13±0.49	3.00±0.60	4.50±0.65	6.66±0.55	5.00±0.00	15.63±2.28	22.33±2.11	27.13±2.10
T <sub>9</sub>	7.83±1.04	11.83±1.06	16.83±1.10	3.23±0.49	4.73±0.51	6.90±0.40	5.66±0.57	16.30±2.85	23.00±2.68	27.80±2.68
T <sub>10</sub>	7.27±0.92	11.26±1.02	16.26±1.11	3.33±0.57	4.83±0.61	7.00±0.50	6.33±0.58	16.96±2.62	23.66±2.48	28.46±2.45
T <sub>11</sub>	7.20±1.31	11.20±1.25	16.20±1.20	3.26±0.55	4.76±0.56	6.93±0.45	6.00±0.00	16.63±2.28	23.33±2.11	28.13±2.10
T <sub>12</sub>	5.50±0.40	9.50±0.50	14.50±0.60	1.06±0.11	1.36±0.05	2.50±0.10	1.00±0.00	2.33±0.57	3.33±0.57	4.66±0.57
SEm±	0.39	0.41	0.43	0.31	0.37	0.37	0.25	0.76	0.69	0.54
CD (P≤0.05)	1.16	1.21	1.28	0.91	1.08	1.10	0.74	2.24	2.04	1.60

\*Mean of 3 replications ± Standard deviation; CD, Critical difference; SEm±, Standard error mean; DAP, Days after planting. Treatment details are given under Materials and Methods.

*chlorophyll per cent*: The highest average leaf area (22.67 cm<sup>2</sup>) was recorded in cocopeat T<sub>9</sub> whereas maximum chlorophyll percentage (2.31%) was recorded in cuttings grown in cocopeat T<sub>10</sub>. These results were statistically different from all other treatments and control wherein minimum leaf area (9.33 cm<sup>2</sup>) and chlorophyll percentage (1.10%) were recorded in cuttings grown in topsoil only (control-T<sub>12</sub>) (Table 3). Our findings are supported by Dhatrikarani (2019) who recorded highest average leaf area and chlorophyll content in cocopeat after 135 days of planting. The findings of Rani *et al.* (2018) who recorded the highest chlorophyll content in cocopeat as a medium, also support our present findings. Improved water retention capacity, aeration, and proper drainage conditions of cocopeat can be attributed to its highest efficiency (Khayyat *et al.* 2007). Ratnakumari (2014) documented that higher leaf number in cuttings improvised nutrient uptake by plant and thus, enhanced photosynthate assimilation improvising plant metabolic activities by enhancing chlorophyll amount, an important light harvesting plant pigment.

*Pooled effect of growing substrate on primary, secondary, and total number of roots*: The maximum number of primary (12.67), secondary (26.50), and total roots (39.13) were recorded in T<sub>9</sub> and T<sub>6</sub> after 120 days of planting which was at par with T<sub>10</sub>. All the treatments were significantly superior over control (topsoil) which recorded minimum number of 3.67, 12.13, 15.80 primary, secondary and total roots per cutting after 120 days of planting, respectively (Table 3). Our results are in corroboration with Manila *et al.* (2017) who recorded vermiculite as a highly efficient medium for providing the maximum number of primary and secondary roots. Khalil and Aly (2013) also recorded the highest number of primary and secondary roots when pomegranate cuttings were planted in vermiculite. The highest number of primary and secondary roots were obtained in sphagnum moss+coco peat+vermicompost when used as a growing medium (Parmar *et al.* 2018).

*Pooled effect of growing substrate on diameter of thickest sprout, leaves per longest shoot, number of sprouted cuttings, number of shoots and days to first sprouting*: The cuttings grown in T<sub>9</sub> recorded maximum diameter of thickest sprout (2.40 cm), leaves per longest shoot (2.16 leaves/shoot), and number of shoots (4.10 shoots/cutting) with minimum days to first sprouting (14.06 days). However, cuttings grown in T<sub>10</sub> recorded the maximum number of sprouted cuttings (52.00 cuttings) after 120 days of planting. All the treatments were significantly superior over control which recorded minimum value of all the growth parameters (Table 3). Our results are in conformation with Manila *et al.* (2017) who recorded vermiculite as an effective substrate for providing the maximum number of sprouted cuttings and also the maximum diameter of sprouts per cutting. Ratnakumari (2014) recorded the best sprouting of pomegranate cuttings in vermiculite as a medium. Rani *et al.* (2018) reported cocopeat as the most efficient medium in providing maximum number of shoots after 135 days of planting. Rani *et al.* (2015) recorded minimum days to

Table 3 Pooled effect of growing substrate on number of roots, diameter of thickest sprout, leaves per longest shoot, sprouted cuttings, shoot number, days to first sprout, leaf area and chlorophyll percentage of guava cutting after 120 days

Treatment	Number of roots per cutting*	Primary number of roots*	Secondary number of roots*	Diameter of thickest sprout (cm)*	Number of leaves per longest shoot*	Number of sprouted cuttings*	Number of shoots per cutting*	Days taken to first sprouting (no.)*	Leaf area (cm <sup>2</sup> )*	Chlorophyll (%)*
T <sub>1</sub>	29.80±0.36	8.00±1.00	21.60±0.70	1.83±0.11	1.76±0.20	32.33±0.57	3.53±0.11	18.73±1.02	13.16±0.72	1.92±0.05
T <sub>2</sub>	28.46±1.28	7.33±1.15	20.93±0.72	1.80±0.10	1.73±0.15	32.00±0.00	3.46±0.15	19.70±0.66	15.72±0.70	1.91±0.03
T <sub>3</sub>	27.80±1.98	7.00±1.00	20.80±1.38	1.73±0.20	1.60±0.17	22.66±1.52	3.13±0.12	20.60±0.87	14.30±0.57	1.89±0.05
T <sub>4</sub>	33.33±1.24	9.77±1.07	23.56±0.98	2.12±0.04	1.90±0.10	32.66±0.57	3.66±0.30	16.63±0.55	17.66±0.20	2.06±0.05
T <sub>5</sub>	31.13±1.12	8.67±1.15	22.46±0.81	1.96±0.05	1.83±0.20	32.33±0.58	3.63±0.20	17.33±0.57	16.40±0.91	1.96±0.05
T <sub>6</sub>	39.13±1.96	12.67±0.57	26.46±1.72	2.27±0.06	2.03±0.05	42.33±0.57	4.06±0.11	14.80±0.26	15.83±1.04	2.15±0.02
T <sub>7</sub>	35.06±1.04	10.63±1.09	24.43±0.83	2.14±0.05	1.96±0.05	40.00±1.00	3.73±0.15	16.30±0.51	19.83±0.25	2.13±0.02
T <sub>8</sub>	34.20±0.95	10.20±0.95	24.00±0.95	2.03±0.05	1.86±0.15	42.00±0.57	3.73±0.15	16.67±0.48	18.53±0.41	2.11±0.01
T <sub>9</sub>	39.13±1.96	12.67±0.57	26.50±1.72	2.40±0.01	2.16±0.15	42.33±0.58	4.10±0.10	14.06±0.15	22.67±0.51	2.17±0.02
T <sub>10</sub>	37.53±0.32	11.87±0.96	25.66±1.70	2.39±0.01	2.06±0.05	52.00±1.00	3.90±0.10	15.90±0.11	21.86±0.20	2.31±0.01
T <sub>11</sub>	36.73±0.72	11.47±0.89	25.26±0.90	2.22±0.08	2.03±0.05	42.66±0.76	3.83±0.20	16.00±0.10	18.76±0.82	2.21±0.01
T <sub>12</sub>	15.80±3.36	3.67±1.52	12.13±1.85	0.38±0.07	0.70±0.36	15.70±0.430	2.03±0.05	29.30±2.85	9.33±0.57	1.10±0.10
SEm±	0.91	0.49	0.35	0.05	0.09	0.46	0.09	0.57	0.36	0.02
CD (P≤0.05)	2.69	1.44	1.02	0.15	0.28	1.36	0.27	1.67	1.08	0.07

\*Mean of 3 replications ± Standard deviation; CD, Critical difference; SEm±, Standard error mean. Treatment details are given under Materials and Methods.

first sprout in substrate composition of perlite+sand+FYM (1:1:1) and the maximum number of shoots in guava cuttings propagated in vermiculite+sand+FYM (1:1:1).

The perusal of the present study revealed that the prerequisite for successful guava propagation is planting the cuttings in appropriate growing substrate wherein T<sub>9</sub> and T<sub>10</sub> provided the highest value of growth parameters under the present study. Hence, guava cuttings should be planted in T<sub>9</sub> as a preliminary growing substrate for more successful propagation under protected conditions.

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