

## **EFFICIENT CLONAL PROPAGATION OF GUAVA CULTIVARS BY MINI SOFTWOOD CUTTINGS**

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### **ABSTRACT**

Guava (*Psidium guajava* L.) is commercially propagated by seeds in Punjab and vegetative propagation systems are lacking. Propagation by stem cuttings is handicapped by the non-availability of plant material in bulk for cuttings and season dependency. Hence, juvenile mini softwood (MSW) shoot cuttings (3"-4") of Gola (Round) cultivar having two to three nodes were treated with plant growth regulators (PGRs) including indole butyric acid 'IBA' or naphthalene acetic acid 'NAA' in combinations with benzyl aminopurine 'BAP' and planted in sand, silt and coco peat (40:40:20 v/v) under CRD layout for PGR optimization. Cuttings treated with BAP and IBA (2 mgL<sup>-1</sup> + 3 mgL<sup>-1</sup>) started early sprouting in 8 days and induced a greater number of branches (5-6), number of leaves (11), number of roots (6-7) and long roots (14 cm). Whereas, sprout length was higher up to 14 cm in cuttings treated with BAP and IBA (3 mgL<sup>-1</sup> + 1 mgL<sup>-1</sup>). In the second part of the study, application of the optimized PGR treatment of BAP and IBA (2 mgL<sup>-1</sup> + 3 mgL<sup>-1</sup>) to MSW cuttings of different commercial strains of cultivars Gola (Round) and Surahi (Pyriform) showed a better number of roots (5, 4), root length (13 cm, 11 cm), bud sprouting (100% and 80%) and number of nodes (5 each) in Gola (Sadabahar 'G<sub>1</sub>') and Surahi (Sindhi Bao 'S<sub>2</sub>'), respectively. The rooted cuttings were maintained under standard growth room conditions and higher humidity till proper plant growth and the established plantlets were transferred to green house. This study established an efficient clonal propagation system using MSW cuttings thus reducing the plant material and PGRs required for mass multiplication of elite guava cultivars.

**Keywords:** Auxin, cytokinin, softwood cuttings, shoot, rooting.

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### **INTRODUCTION**

Guava (*Psidium guajava* L.) is an evergreen tropical fruit tree that exceeds other fruit crops for better production, wider adaptability to diverse environmental conditions and is also known as a "super fruit" (Nimisha *et al.*, 2013). Pakistan is globally ranked 2<sup>nd</sup> for guava production after India. In Pakistan, guava is the third most important fruit crop after citrus and mango. Guava is also a highly nutraceutical fruit crop having diverse uses as fresh and processed food products. Its leaves and other plant parts are also used to treat diseases like diarrhea, diabetes, hypertension, antimicrobial and analgesic properties (Kafle *et al.*, 2018)

Several methods of sexual and asexual propagation (cutting, layering, budding, grafting and tissue culture) have been reported for guava (Singh *et al.*, 2002; Chandra *et al.*, 2004; Usman *et al.*, 2012). In sexual propagation, the genetic purity of the variety cannot be maintained due to segregation and recombination of characters. However, stringent selection amongst sexual progenies may lead to the development of new promising landraces. Vegetative propagation of

guava through stem cuttings is preferred for clonal propagation, to avoid trait segregation (Pereira *et al.*, 2017), and rapid multiplication of economically important trees (Tavares, 1994). Though root induction in the stem cuttings is a well-established and evolved method (Manica *et al.*, 2000), information regarding the efficient rooting ability of the small softwood stem cuttings is scarce in guava.

Auxins like indole butyric acid 'IBA' and naphthalene acetic acid 'NAA' are known to induce rooting, increase the frequency of rooted cuttings, the number and quality of the formed roots and the uniformity in rooting. Auxins stimulate ethylene production thus favoring the emission of roots (Norberto *et al.*, 2001). The application of IBA in guava has been a useful practice for root formation, accelerating the initiation of, the number of rooted cuttings and increasing the quality of the roots (Bacarin *et al.*, 1994). Cytokinin like kinetin and BAP could induce a greater number of shoots, however, there is no report of using cytokinin and auxin for efficient clonal propagation using soft-wood cuttings.

In general, softwood cuttings can root more easily and quickly than hardwood cuttings. The success of vegetative propagation through cuttings depends on several factors, such as the genetic potential for rooting, physiological conditions of the mother plant, season, endogenous and exogenous hormonal balance, temperature, light and humidity. The role of PGRs in the successful establishment of plantlets using mini softwood (MSW) cuttings is lacking in guava. So, the present study was conducted to investigate the effect of different PGRs including benzyl amino purine (BAP), indole butyric acid (IBA) and naphthalene acetic acid (NAA) on the successful establishment of MSW cuttings in different commercial varieties (Gola and Surahi) of guava.

## MATERIALS AND METHODS

**Plant Material and PGR Treatments:** The study was conducted in the plant tissue culture cell and fruit plant nursery of the institute during 2020-2021 and was divided into two parts. First, different combinations of plant growth regulators (PGRs) were applied to optimize the dose of PGRs for shoot growth and root induction in MSW cuttings. The 3-4 inches long MSW cuttings of the Gola (Round) cultivar were collected from the Experimental Fruit Garden (EFG) of the Institute. Two half-cut leaves were left at the upper end of the cutting. At the lower end, a slightly slanting cut was given to enhance the rooting area. Before planting, the cuttings were dipped in (2%) copper oxychloride solution for 2-3 minutes to avoid fungal diseases and then kept in different PGR solution treatments including BAP, IBA and NAA combinations for 6 hours to enhance shoot growth and root induction as elaborated in Table 1.

After PGR treatments, the cuttings were planted individually in small plastic pots (10 cm L x 8 cm W) covered with a transparent plastic bag to ensure humid conditions. The pots were filled with sand, silt and coco peat (40:40:20 v/v). After planting PGR-treated cuttings, the pots were immediately irrigated with canal water using a sprinkler and later watered at weekly intervals. Parameters including days to bud sprouting, sprout length (cm), number of leaves, number of branches, root length (cm), rooting percentage (%) were studied. Plant growth regulators (PGRs) were divided into 13 different combinations or treatments including control (Table 1).

In the second part, MSW cuttings having two to three nodes were collected from selected strains of guava cultivars Gola (Round) viz. Sadabahar Gola 'G<sub>1</sub>' and 'G<sub>2</sub>' and Surahi (Pyriform) strains viz. Ramzani 'S<sub>1</sub>', Sindhi Bao 'S<sub>2</sub>' and Sindhi Surahi 'S<sub>3</sub>' available in the germplasm unit (GPU) of the Institute. The best-responding combination of PGRs (BAP 3 mgL<sup>-1</sup> and IBA 2 mgL<sup>-1</sup>) for shoot and root growth from the first experiment was used to screen strains of Gola and Surahi for clonal propagation. Growth parameters described in

experiment 1 were studied and data were collected. After 4 months, the rooted cuttings were planted in the field for further plant growth and development.

**Data Analysis:** Both experiments were laid out in a Completely Randomized Design (CRD) with 20 cuttings per treatment in each variety. Both experiments were replicated three times. In total, there were 520 cuttings per replication in study one (total of 1560 pots) and 100 cuttings per replication were utilized in study two (total of 300 pots). Data were recorded after 60 days of planting, analyzed applying CRD ANOVA using SAS9 software and means were compared using Tukey's HSD (honest significant difference) test.

## RESULTS

**Selection of PGRs for enhanced bud sprouting and plant growth in MSW cuttings:** In this study for PGR selection and dose optimization, significant differences were found amongst most of the treatments used and different parameters studied. PGR treatment T<sub>4</sub> (BAP 2 mgL<sup>-1</sup> + 3 IBA mgL<sup>-1</sup>) induced the earliest bud sprouting and reduced time to 8.33 days followed by T<sub>13</sub> (3 mgL<sup>-1</sup> NAA) compared with non-treated control (T<sub>0</sub>) required a maximum of 17 days (Table 1) to sprout. A higher sprout length of 13.87 cm was induced by treatment T<sub>3</sub> (BAP 3 mgL<sup>-1</sup> + IBA 1 mgL<sup>-1</sup>) followed by T<sub>12</sub> (NAA 2 mgL<sup>-1</sup>) compared with minimum sprout length at control T<sub>0</sub> (3.21 cm). The number of branches induced was a maximum of 5.67 at T<sub>4</sub> followed by T<sub>7</sub> (BAP 3 mgL<sup>-1</sup> + NAA 2 mgL<sup>-1</sup>). Regarding number of leaves, a maximum of 10-11 leaves were developed in T<sub>3</sub> and T<sub>4</sub> compared with 2 leaves at control (T<sub>0</sub>). The maximum number of roots induced was 6.33 and the longest roots up to 13.60 cm were developed at T<sub>4</sub> followed by T<sub>13</sub> (NAA 1 mgL<sup>-1</sup>). Overall, early sprouting, a higher number of leaves and root development, the longest roots and greater sprout length were found in T<sub>4</sub> (BAP 2 mgL<sup>-1</sup> + IBA 3 mgL<sup>-1</sup>). Hence, this treatment T<sub>4</sub> was utilized to screen MSW cuttings of different strains of guava cultivar Round and Pyriform for enhanced root and shoot growth.

**Genotypic responses for bud sprouting and plant growth in MSW cuttings:** MSW cuttings of different strains of cv. Surahi viz. S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> when treated with better performing selected PGR treatment T<sub>4</sub> (BAP 2 mgL<sup>-1</sup> + 3 IBA mgL<sup>-1</sup>) showed early sprouting (13-14 days) and greater sprout length (10-14 cm) compared with strains of cv. Gola viz. G<sub>1</sub> and G<sub>2</sub> (Fig. 2a, c). Bud sprouting (%), number of nodes, number of leaves, number of roots and root length were greater in cv. Gola strain G<sub>1</sub> and cv. Surahi strains S<sub>2</sub> compared with other strains (Fig. 2b, d-g). Whereas rooting (%) was greater in MSW cuttings of strains of cv. Surahi than cv. Gola and rooting was a maximum of up to 60% in S<sub>3</sub>.

Table 1. Effect of PGRs on bud sprouting, sprout growth and rooting in MSW cuttings of guava cultivar Gola (Round).

Treatments (BAP + IBA + NAA) mgL <sup>-1</sup>	Days to bud sprouting	Sprout length (cm)	Number of branches	Number of leaves	Number of roots	Root length (cm)
T <sub>0</sub> (0+0+0)	17.00 <sup>±0.94a</sup>	3.21 <sup>±0.27c</sup>	1.67 <sup>±0.38b</sup>	2.00 <sup>±0.47c</sup>	0.00 <sup>±0.00c</sup>	0.00 <sup>±0.00c</sup>
T <sub>1</sub> (3+0+0)	11.67 <sup>±1.19ab</sup>	8.83 <sup>±0.47b</sup>	3.00 <sup>±1.23ab</sup>	6.33 <sup>±0.72b</sup>	1.67 <sup>±0.42b</sup>	7.80 <sup>±0.82bc</sup>
T <sub>2</sub> (2+1+0)	10.67 <sup>±1.19ab</sup>	11.83 <sup>±0.72a</sup>	3.67 <sup>±1.05ab</sup>	9.33 <sup>±0.72a</sup>	4.00 <sup>±0.94ab</sup>	8.83 <sup>±0.81ab</sup>
T <sub>3</sub> (3+1+0)	11.00 <sup>±0.94ab</sup>	13.87 <sup>±0.47a</sup>	3.33 <sup>±1.26ab</sup>	10.67 <sup>±0.98a</sup>	3.67 <sup>±1.19b</sup>	9.27 <sup>±1.13ab</sup>
T <sub>4</sub> (2+3+0)	8.33 <sup>±1.19b</sup>	8.07 <sup>±0.72b</sup>	5.67 <sup>±0.58a</sup>	11.00 <sup>±0.94a</sup>	6.33 <sup>±0.88a</sup>	13.60 <sup>±1.00a</sup>
T <sub>5</sub> (2+0+1)	10.33 <sup>±1.19ab</sup>	11.10 <sup>±0.72a</sup>	3.33 <sup>±1.12ab</sup>	9.00 <sup>±1.25a</sup>	4.67 <sup>±0.88ab</sup>	8.47 <sup>±0.95b</sup>
T <sub>6</sub> (3+0+1)	11.00 <sup>±0.94ab</sup>	11.90 <sup>±0.47a</sup>	3.00 <sup>±1.11ab</sup>	8.67 <sup>±1.19a</sup>	3.33 <sup>±1.44b</sup>	10.50 <sup>±0.90ab</sup>
T <sub>7</sub> (3+0+2)	10.33 <sup>±1.19b</sup>	11.73 <sup>±0.72a</sup>	5.33 <sup>±0.76a</sup>	8.67 <sup>±0.98a</sup>	5.00 <sup>±0.42a</sup>	10.93 <sup>±1.30ab</sup>
T <sub>8</sub> (0+1+0)	11.00 <sup>±1.42ab</sup>	12.30 <sup>±0.47a</sup>	3.00 <sup>±1.12ab</sup>	7.00 <sup>±0.94ab</sup>	3.67 <sup>±1.19b</sup>	9.43 <sup>±1.06ab</sup>
T <sub>9</sub> (0+2+0)	11.33 <sup>±0.72ab</sup>	9.37 <sup>±0.72a</sup>	3.33 <sup>±0.88ab</sup>	6.67 <sup>±0.94ab</sup>	5.13 <sup>±1.20a</sup>	10.50 <sup>±0.88ab</sup>
T <sub>10</sub> (0+3+0)	9.33 <sup>±1.19b</sup>	10.10 <sup>±0.47a</sup>	3.00 <sup>±0.60ab</sup>	7.00 <sup>±0.72ab</sup>	3.67 <sup>±0.88b</sup>	11.17 <sup>±1.25a</sup>
T <sub>11</sub> (0+0+1)	10.67 <sup>±0.98ab</sup>	10.32 <sup>±0.72a</sup>	3.33 <sup>±1.11ab</sup>	7.67 <sup>±0.94ab</sup>	5.33 <sup>±1.20a</sup>	10.64 <sup>±1.09ab</sup>
T <sub>12</sub> (0+0+2)	9.67 <sup>±0.94b</sup>	12.77 <sup>±0.47a</sup>	3.00 <sup>±1.26ab</sup>	6.00 <sup>±0.94b</sup>	4.67 <sup>±1.19ab</sup>	9.80 <sup>±1.11ab</sup>
T <sub>13</sub> (0+0+3)	9.00 <sup>±1.19b</sup>	10.31 <sup>±0.27a</sup>	3.33 <sup>±1.09ab</sup>	7.00 <sup>±0.94ab</sup>	4.43 <sup>±0.91ab</sup>	11.33 <sup>±1.13a</sup>

Means sharing similar letters in a column are statistically non-significant at P&gt;0.05 (Tukey HSD test) and ± values indicate SE.



Fig. 1. Enhanced rooting and shoot growth induction in mini softwood (MSW) cuttings of Guava cultivars by PGR treatments i.e., a) T4; b) T3; c) T11, d) T10, e) T4 and f) G<sub>1</sub> plantlet established after treatment with T4.

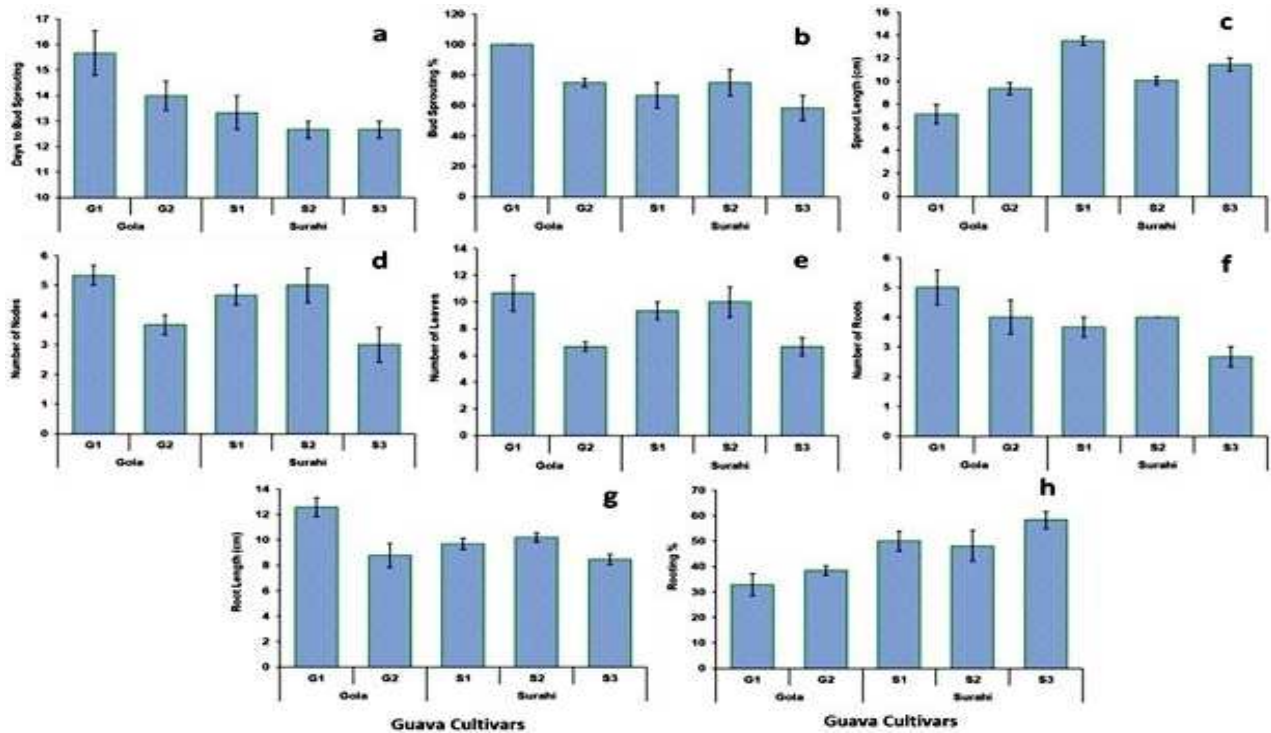


Fig. 2. Genotypic responses in mini softwood (MSW) cuttings of Guava cultivars Gola (Round) and Surahi (Pyriform) for root and shoot growth attributes. Figures show a) days to bud sprouting, b) bud sprouting (%), c) sprout length, d) number of nodes, e) number of leaves, f) number of roots and g) root length and h) rooting (%) in response to treatment with PGRs (BAP 2 mgL<sup>-1</sup> + IBA 3 mgL<sup>-1</sup>).

## DISCUSSION

**PGRs enhancing bud sprouting, shoot growth and rooting in MSW cuttings:** Stem and root cuttings have been used to propagate woody fruit crops including guava for varietal maintenance and nursery development using auxins including IBA, NAA and IAA (Pereira, 1995; Pereira *et al.*, 2017). Auxins like IBA and NAA are known to induce adventitious rooting in stem cuttings under humid conditions. However, excessive humidity may lead to the incidence of fungal diseases like damping off. Different studies have been reported in guava for clonal propagation using standard size (5–8-inch stem cuttings) treated with higher doses of auxins like IBA and NAA (0.2-1 g/100 g of talcum powder) as reported by Kareem *et al.* (2016) and Arafat *et al.* (2020). In the current study, the size of the softwood cuttings has been further reduced to 5-8 cm and the auxins (IBA and NAA) and cytokinin (BAP) have been used in different combinations at the lowest concentrations (1-3 mgL<sup>-1</sup>) to make the propagation system cost-effective and commercially more viable. In this study, data revealed better root induction and shoot growth at BAP 2 mgL<sup>-1</sup> and NAA 3 mgL<sup>-1</sup> indicating that IBA individually and in combination with BAP performed better for early bud sprouting. The early sprouts observed in MSW cuttings by the application of BAP and IBA could be attributed to enhanced physiological functions in cuttings that favored early sprouting in guava (Singh *et al.* 2014) and pomegranate (Kaur and Kaur, 2016). The application of bioactive compounds BAP and IBA may robustly activate meristem and enhance nitric acid signaling which provides essential energy for the initiation of root and shoot growth in stem cuttings (Karim and Muhammad, 2020). The sprout length and maximum number of leaves were observed in response to treatments T<sub>4</sub> (BAP 2 mgL<sup>-1</sup> + IBA 3 mgL<sup>-1</sup>) and T<sub>3</sub> (BAP + IBA: 3 mgL<sup>-1</sup> + 1 mgL<sup>-1</sup>) Table 1. The reason may be that the treatment of BAP and IBA in MSW cuttings of guava can increase the utilization of stored carbohydrates; nitrogen and increase the cell division and callus formation in cuttings as explained by Prakash *et al.*, (2018). In other similar reports cuttings of conocarpus when treated with BAP+IBA developed more roots and four times more leaves than the untreated cuttings (Fattorini *et al.*, 2017; Abdel-Rahman *et al.*, 2020). In this study, the maximum number of roots and root length were observed in response to the T<sub>4</sub> treatment, while no root induction was noted in the control (Table 1). It has been reported that IBA has an impact on the rooting of cuttings. IBA enhanced the percentage of root cuttings, root length, and roots per cutting when treated with 1000 to 1500 ppm (Shahzad *et al.*, 2019). As reported in the pomegranate for higher number of roots per cuttings and root length may also follow the same pattern in the guava (Kaur and Kaur, 2016). The root formation in a better way can also

be influenced by biochemical changes in plant metabolic pathways with source-sink relationship manipulation, translocation of metabolites, total phenolic contents, polyphenol oxidase activity, IAA-oxidase activity, and peroxidase enzymatic activity accumulation (Dash *et al.*, 2011). The accumulation of proteins and total soluble sugars from the upper parts of plants also increases root growth (Shao *et al.*, 2018). These results were also found consistent with the previous study in which IBA treatment increased 2-3 times root growth in conocarpus (Abdel-Rahman *et al.*, 2020). This might be due to the synergetic effect of BAP and IBA as explained earlier BAP swells the root primordial part and enhances the level of IBA in the root tips which may increase the root length (Tognoni *et al.*, 1967). The activity of IBA in the root tissue translocates and hydrolysis the carbohydrate, and nitrogenous substances at the cell level which results in cell division and cell elongation in favorable environmental conditions (Singh *et al.*, 2002; Kareem *et al.*, 2016).

Secondly, different strains of guava cv. Surahi and Gola were treated with the selected treatment of PGRs T<sub>4</sub> (BAP 2 mgL<sup>-1</sup> + 3 IBA mgL<sup>-1</sup>) and found an early sprouting, greater sprout length and maximum rooting percentage in Surahi as compared with strains of Gola. Whereas bud sprouting (%), number of nodes, number of leaves, number of roots and root length were greater in cv. Gola. Genotypes of honeybush (*Cyclopia subternata*) have a significant impact on the rooting percentage of cuttings in which SGD1, SGD7, and SGD9 were exposed to different PGRs of the same concentrations SGD1 and SGD7 has the highest rooting percentage (Mabizela *et al.*, 2017). The interaction among genotypes of *Pongamia* had a significant effect on root induction and differentiation (Kesari *et al.*, 2010). Similarly in another study, five hybrid aspens (*Populus tremula* L. × *Populus tremuloides* Michx.) were used for propagation through cuttings and explained that age of the plant does not significantly affect the rooting and sprout, but the selection of suitable genotype has a significant impact on the rooting and sprouting of cuttings (Stenvall *et al.*, 2004).

**Conclusions:** This study describes that the treatment of PGRs has a great impact on inducing rooting in mini softwood cuttings of guava. Amongst, different combinations of BAP, IBA, and NAA, T<sub>4</sub> (BAP and IBA) performed well and had more synergetic effects on the successful establishment of MSW cuttings. Genotypic variability was also noticed for successful rooting and shoot growth responses in cuttings when treated with selected PGRs. Clonal propagation of guava through MSW cuttings is recommended for enhanced propagation of guava saplings with better genetic purity and minimum space consumption. The established approach is easy, fast

and economical for commercial propagation of the selected genotypes.

**Conflict of Interest:** The authors declare no conflict of interest.

**Author's Contribution Statements:** BAA and OM executed the work in the field, FUR analyzed the data and wrote the manuscript, whereas MU and BF conceived the idea and supervised the whole research work, MU provided funds for research and edited the article.

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**Novelty statement:** The manuscript highlights efficient clonal propagation of elite guava cultivars using mini softwood cuttings and optimal combinations of cytokinin (BAP) and auxin (IBA).

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