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Clonal Multiplication of Guava (*Psidium guajava*) through Soft Wood Cuttings using IBA under Low-plastic Tunnel

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Abstract

The present investigation on clonal multiplication of guava (*Psidium guajava* L.) soft wood cuttings was done with the aim to determine the most successful media and rooting hormone (IBA) concentration. Guava soft wood cuttings were treated with 0, 200, 400 and 600 mg kg^{-1} IBA solution when planted in three different rooting media (sand, silt and top soil) under low-plastic tunnel. The cuttings were allowed to grow under low-plastic tunnel for 90 day to evaluate the rooting capability and shooting competency. The study showed that the species has a great potential for clonal multiplication through soft wood cuttings. The highest number of roots and root length were observed in the cutting treated with 400 mg kg^{-1} IBA solution in sand, and the lowest in the cuttings without IBA treatment in sand media. In general 400 mg kg^{-1} IBA treatment with silt as rooting media performed better as compared to the other treatments. However, the highest survival percentage (50%) was observed in the cuttings rooted in silt media and treated with 200 mg kg^{-1} IBA solution. Experimental results showed the potential of clonal propagation of guava through soft wood cuttings treated with auxin under simpler and cheaper low-plastic tunnel.

Keywords: *Psidium guajava*; Clonal multiplication; Rooting capability; IBA; Rooting media; Low-plastic tunnel

Introduction

Guava (*Psidium guajava* L.) is a dicotyledonous; evergreen plant belongs to family Myrtaceae. The common guava is usually diploid with chromosomes (2n = 22), but some natural and artificial triploids (2n = 33) also exist in nature. It is one the most important commercially grown fruit crop in the tropics and sub-tropics (Hayes, 1970; Rodriguez *et al*., 2007). It is believed that guava originates Central America and from the southern regions of Mexico. There are approximately 130 genera and 3,000 species present in the family *Myrtaceae*. The genus *Psidium* consists of more than 150 species which includes evergreen shrubs and trees wide spread in different tropical and sub-tropical areas of the world (Jaiswal and Jaiswal, 2005). It is famous as "poor man apple" or "apple of the tropics" because it rich in ascorbic acid, calcium, iron and phosphorous (Prakash *et al*., 2002). It contains 2–5 times more vitamin C as compared to oranges. According to an estimate 100 g of guava fruit contains approximately 260 mg of vitamin C (FAO, 1984; Rahman *et al*., 2003). Pakistan is 2nd largest guava producing country after India and 4th largest horticultural crop grown in Pakistan with respect to production and area following citrus, mango and banana, respectively. Other largest guava producers are Brazil, Mexico and Thailand (Padilla-Ramirez and Gonzalez-Gaona, 2003) and guava fruit production in the last five years has increased up to 10 folds in these countries (Pommer and Murkami, 2009). Guava is now being grown on an area of about 64 thousand hectares with 508 thousand tones production (Anonymous, 2013). But there is still a difference between its potential and actual yield due to poor management, water stress and trees beating to ignore summer crop, improper use of fertilizers, insecticides and poor storage.

Multiplication through stem cutting in fruit crops depend on various factors e.g. mother plant condition, tree age, tree part from where cuttings are taken, planting time, temperature conditions, humidity, rain, media for rooting, care during and after planting (Frey *et al*., 2006). The pattern of traditional propagation is changing with combination of science and technology in nursery

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(Singh and Bajpai, 2003). The planting media must contain pH according to plant growth, a gaseous exchange structure that permit proper aeration for roots and permit water movement (Larson, 1980). When media contains proper environment like good aeration, proper water and sufficient nutrient availability then it helps to develop excessive roots system which results in excellent plant growth (Neelam and Ishtiaq, 2001). The basic initial planting material is one of the essential necessities of a crop for both its quality and quantity (Singh *et al*., 2005).

Guava is propagated by both sexually (Zamir *et al*., 2003) like by seed and asexually methods like budding, grafting, layering and cutting (Chandra *et al*., 2004). Mostly it is propagated through seeds in Pakistan (Maqbool and Khan, 1973) but due to highly cross pollinated (Soubihe and Gurgel, 1962) characteristics goes to segregation. The range of cross pollination varies with amount of 25.7 to 41.3% (Purseglove, 1968). Hence efforts are made to grow guava by cuttings. Cutting is the most economical method of vegetative propagation (Davies and Hartman, 1988). Rooting by vegetative mean of propagation is no doubt the most expanded and efficient method (Awan *et al*., 2012) but the information's related to guava cuttings to produce rooting are very less and rooting through cuttings is a very efficient vegetative propagation method to produce true-to-type plants (Kareem *et al*., 2013).

Auxins are very helpful to overcome difficulties in cuttings for root induction. Indole butyric acid (IBA) is a plant growth hormone among the auxin family and is used in many horticultural plants for root induction. Mukhtar *et al*. (1998) and Hafeez *et al.* (1988) succeeded in growing guava softwood cutting by using root growth regulators. Debnath and Maiti (1990) obtained best rooting in Baruipur cultivar when dipped in 2500 mg kg^{-1} IBA. However, there is convincing evidences that auxins are essential for root development (Hartmann and Kester, 1982).

Thus keeping in view all above considerations, study was planned with the objective, to develop asexual multiplication method to avoid segregation and evaluate best rooting media and IBA concentration for guava rooting under low-plastic tunnel.

Materials and Methods

The present study was carried out over a period of three months from mid-September to mid-December 2013–2014 for two consecutive years at Nursery Sanitation Project, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Highly productive and phenotypically superior healthy mother plants of Gola cultivar (6-7 year-old) having excellent bearing and fruit quality were selected from Post-graduate Agricultural Research Station (PARS), Faisalabad as plant material for the study. Apical shoot cutting at juvenile stage of length 12 cm, each with 2–4 nodes carrying 2–4 pair of leaves, were taken during the study. After collection, cuttings were thoroughly washed and their bark was injured on lower portion with budding knife to facilitate callusing process. After preparation, basal portion of cuttings was quickly dipped in copper oxychloride (David Gray and Co. Ltd., Australia; 1 g per liter of water) solution to avoid any fungal infection for 30 sec and kept in open air for 5 min. The cuttings were then treated with 0, 200, 400 and 600 mg kg^{-1} IBA (Indole 3-butyric acid) to investigate the rooting ability. For IBA treatment basal portion of the cuttings was dipped into solution for 5 min. Three nursery grade rooting media such as sand, silt and top soil were used separately by making 6 inches layer on ground for root induction under non-misting low-plastic plastic tunnel (Fig. 1). Before using, all rooting media were sun sterilized under the polyethylene for 2 weeks in order to dry and kill harmful pathogens (Elmore *et al*., 1997). The plastic tunnel had a covering of 0.10 mm thick poly sheet of Jilani Poly Industries (Pvt) Ltd, Pakistan.

A total of 432 cuttings were placed under twelve treatments with three replications in a non-misting low-plastic tunnel. Cuttings were planted in 36 plots, 12 plots for each replication (0, 200, 400 and 600 mg kg^{-1} IBA solution in sand, silt and top soil) and each plot containing 12 cuttings. Cuttings were watered with manual sprinkler once in a week after setting into blocks under non-misting low-plastic tunnel. Every morning light misting was done with a hand sprayer to maintain the humidity till the cuttings were rooted and transferred from the low-plastic tunnel.

Finally the number of roots, root length (cm), root fresh and dry weight (g), number of leaves, number of sprouts, shoot length, sprout length and survival percentage (%) of each cutting were recorded 90 days after planting. The experiment was laid out according to Completely Randomized Block Design (CRD) with two factors layout. The data collected was analyzed statistically by applying the Fisher analysis of variance (ANOVA) and difference among the treatments means were compared by applying Tukey HSD test.

Results

Rooting Capability of Cuttings

Primary roots per cutting: Number of roots developed per cutting was significantly affected by the rooting media, IBA concentrations and their interactions in guava cuttings (Table 1). The highest roots per cutting were observed in silt and lowest roots in the top soil. Among IBA treatments, the highest roots per cutting were observed in the cuttings treated with 400 mg kg^{-1} IBA solution and the lowest without IBA treatment (Table 1). However, interaction between rooting media and IBA concentrations revealed the highest roots per cutting planted in sand and silt, respectively with 400 mg kg^{-1} IBA solution. No roots were observed in the cuttings without IBA treatment (control) and 600 mg kg^{-1} IBA solution in sand media.

Table 1: Effect of different rooting media and IBA concentrations on number of roots of guava cuttings

IBA (mg kg^{-1})	Rooting media: Sand	Silt	Top soil	Mean
0	0.00±0.00 h	3.83±0.28 g	5.43± g	3.09±0.13 D
200	17.94±0.92 d	17.22±2.22 de	9.33± f	14.83±1.23 B
400	31.17±1.26 a	28.00±1.05 b	15.00± e	24.72±0.92 A
600	0.00±0.00 h	24.00±1 c	8.67± f	10.88±0.84 C
Mean	12.27±0.54 B	18.26±1.12 A	9.61±0.68 C	

*Means with same letters do not differ significantly at p 0.05 by Tukey HSD test; ± values indicate the standard deviation

Table 2: Effect of different rooting media and IBA concentrations on root length (cm) of guava cuttings

IBA (mg kg^{-1})	Rooting media: Sand	Silt	Top soil	Mean
0	0.00±0.00 g	5.19±0.31 f	7.36±0.05 cd	4.18±0.12 C
200	9.35±0.23 b	6.92±0.15 de	6.29±0.08 e	7.52±0.15 B
400	12.62±0.60 a	8.01±0.21 c	7.88±0.17 c	9.51±0.32 A
600	0.00±0.00 g	6.72±0.03 de	6.65±0.05 e	4.46±0.03 C
Mean	5.49±0.20 C	6.72±0.18 B	7.04±0.09 A	

*Means with same letters do not differ significantly at p 0.05 by Tukey HSD test; ± values indicate the standard deviation

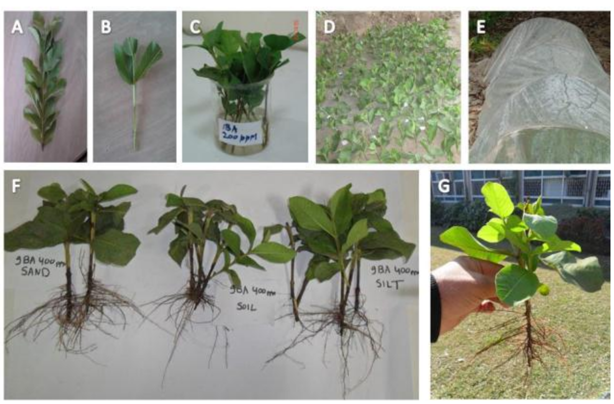

Fig. 1: Multiplication of guava through soft wood cutting under low-plastic tunnel. (A) Soft wood cutting collected from the mother plant (B) Standard cutting (C) Cutting treatment with IBA solution (D) Cuttings planted (E) Low-plastic tunnel to conserve moisture (F) Rooting ability of stem cutting under various treatments (G) Developed plant

Root length (cm): Rooting media has significant effect on the root length and the highest root length was obtained in top soil whereas lowest root length in sand (Table 2). For IBA treatments, the maximum root length was observed in the cuttings treated with 400 mg kg^{-1} IBA solution and lowest in the cuttings without IBA treatment (control). Interaction of two factors showed that the highest root length was obtained in the sand with 400 mg kg^{-1} IBA treatment followed by with 200 mg kg^{-1} IBA solution in the sand media (Table 2). No root formation was observed in the sand media with 600 mg kg^{-1} IBA and control treatment.

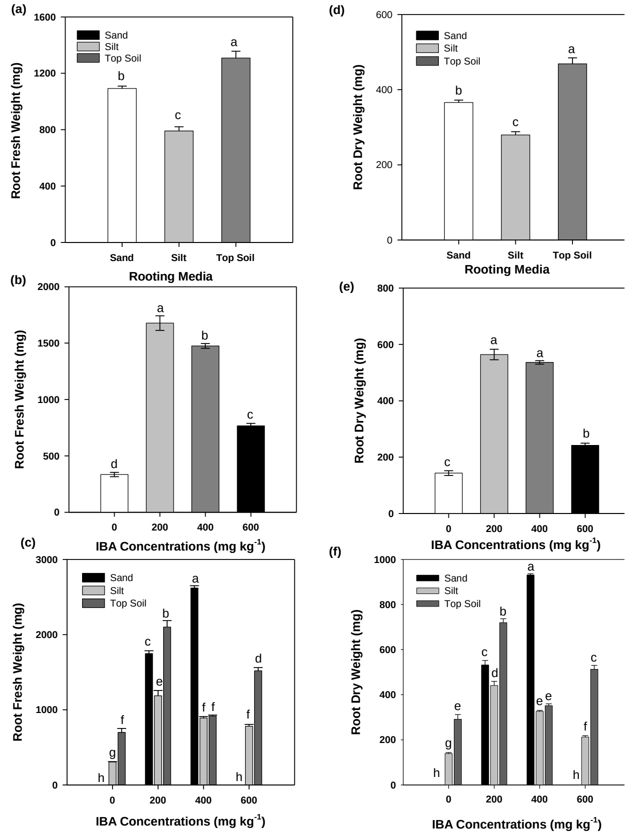

Fig. 2: Effect of different rooting media and IBA concentrations and their interaction on (a, b, c) fresh weight and (d, e, f) dry weight of guava cuttings

Root fresh and dry weights (g): According to (Fig. 2) media and different concentrations of IBA had significant effect on root fresh and dry weights. The high and low fresh weight was observed in top-soil and silt, respectively. These observations revealed that there is a positive relationship between fresh and dry weight of root so that by increase in fresh weight, dry weight also increased. Among different concentrations of IBA, the highest root fresh and dry weights was recorded in cuttings treated with 200 mg kg^{-1} IBA solution and lowest root fresh and dry weights observed in the control treatment. However, interaction of media and IBA concentrations indicates that highest root fresh and dry weights of guava cutting was achieved in sand media with 400 mg kg^{-1} IBA solution, followed by top-soil with 200 mg kg^{-1} IBA solution and lowest with sand control treatment (Fig. 1).

Shooting Competency of Cutting

Number of leaves per cutting: Rooting media and IBA treatments individually and in combination had significant effect on number of leaves per cutting (Table 3). For rooting media, the highest number of leaves per cutting was observed in silt followed by topsoil and lowest in sand.

For auxin treatments, the highest number of leaves per cutting was obtained treated with 400 mg kg^{-1} IBA solution followed by 200 mg kg^{-1} and lowest number of leaves per cutting without IBA treatments (control). Overall comparison of rooting media and growth regulator treatments indicated that highest number of leaves per cutting was obtained from cuttings treated with 400 mg kg^{-1} IBA and grown in silt and sand, respectively. However, the cutting treated with 0 and 600 mg kg^{-1} IBA solution and grown in sand media was unable to produce any leave (Table 3).

Number of sprouts per cutting: Rooting media and growth regulator treatments had significant effect on number of sprouts per cutting. The highest number of sprouts per cutting was obtained in top soil followed by silt and lowest was obtained in sand media (Table 4). The number of sprouts per cutting increased with increasing concentration of IBA up to 400 mg kg^{-1} and then decreased on higher concentrations. The highest number of sprouts per cutting was obtained on the cuttings treated with 400 mg kg^{-1} IBA solution followed by 200 mg kg^{-1} and lowest number of sprouts per cutting was observed in control treatment. In interaction, the highest number of sprouts per cutting was observed where cuttings treated with 400 mg kg^{-1} IBA solution and grown on silt and top soil, respectively (Table 4). However, no sprouts were observed on cuttings grown in sand media and treated with 0 and 600 mg kg^{-1} IBA solution.

Shoot length: The highest shoot length was observed in silt whereas lowest in sand (Table 5). For IBA treatments, the maximum shoot length was observed in the cuttings treated with 200 mg kg^{-1} IBA solution and lowest in the cuttings treated with 600 mg kg^{-1} IBA solution. Interaction of two factors showed that highest shoot length was obtained in the top-soil with 200 mg kg^{-1} IBA solution and no shoot formation was observed in the control treatment (Table 5).

Sprout length: The highest sprouting length per cutting was obtained in the top-soil followed by silt and lowest in sand media. Different IBA treatments indicated that, the highest sprouting length per cutting was obtained with 200 mg kg^{-1} IBA solution and lowest sprouting length in the cuttings treated with 600 IBA solutions. In the interaction, the highest sprouting length per cutting was achieved in sand media treated with 200 mg kg^{-1} IBA solution (Table 6). However, no sprouts were observed in the cuttings grown in control treatment.

Survival percentage (%): Rooting media differ significantly for the survival percentage of guava soft wood cuttings (Fig. 3). The highest survival percentage of guava cutting was achieved in silt, top soil and sand, respectively. The exogenous application of IBA significantly improved the survival percentage of guava cutting. The highest survival percentage was achieved for the cutting treated with 200 mg kg^{-1} IBA solution followed by 400 mg kg^{-1} and lowest without IBA treatment (control).

Table 3: Effect of different rooting media and IBA concentrations on number of leaves of guava cuttings

IBA	Rooting media			Mean
(mg kg^{-1})	Sand	Silt	Top soil	
0	0.00±0.00 g	4.00±0.5 f	7.00±1.00 e	3.67±0.50 D
200	11.83±0.76 ab	8.22±0.51 de	7.33±0.58 e	9.13±0.61 B
400	12.17±1.04 a	12.67±0.58 a	9.67±0.28 cd	11.50±0.63 A
600	0.00±0.00 g	10.33±0.58 bc	9.50±0.50 cd	6.61±0.36 C
Mean	6.00±0.45 B	8.80±0.54 A	8.38±0.59 A	

*Means with same letters do not differ significantly at p 0.05 by Tukey HSD test; ± values indicate the standard deviation

Table 4: Effect of different rooting media and IBA concentrations on number of sprouts of guava cuttings

IBA	Rooting media			Mean
(mg kg^{-1})	Sand	Silt	Top soil	
0	0.00±0.00 d	0.83±0.29 cd	2.17±0.29 ab	1.00±0.13 B
200	2.28±0.25 ab	1.99±0.33 ab	2.00±0.19 ab	2.09±1.23 A
400	2.33±0.57 ab	2.77±0.25 a	2.50±0.5 a	2.53±0.91 A
600	0.00±0.00 d	2.33±0.57 ab	1.33±0.58 c	1.22±0.84 B
Mean	1.15±0.21 B	1.980±0.36 A	2.00±0.34 A	

*Means with same letters do not differ significantly at p 0.05 by Tukey HSD test; ± values indicate the standard deviation

Table 5: Effect of different rooting media and IBA concentrations on shoot length (cm) of guava cuttings

IBA	Rooting media			Mean
(mg kg^{-1})	Sand	Silt	Top soil	
0	0.00±0.00 f	17.02±0.22 c	18.58±0.63 b	11.87±0.28 B
200	18.38±0.19 b	19.12±0.51 b	22.07±0.90 a	19.86±0.54 A
400	18.07±0.40 bc	18.58±0.38 b	15.50±0.25 d	17.38±0.34 A
600	0.00±0.00 f	20.967±0.47 a	13.51±0.42 e	11.49±0.30 B
Mean	9.11±0.15 C	18.92±0.40 A	17.42±0.55 B	

*Means with same letters do not differ significantly at p 0.05 by Tukey HSD test; ± values indicate the standard deviation

The interactive effect of both factors also had pronounced effect on the survival percentage of guava cuttings. The highest survival percentage was recorded for the cuttings treated with 200 mg kg^{-1} of IBA solution and grown on silt media. Moreover, no cutting survived when grown on sand treated with 0 and 600 mg kg^{-1} of IBA solution.

Discussion

In vegetative propagation, adventitious root development is a key step which contains root induction, root initiation and projection, related to the emergence of root primordial (Berthon *et al*., 1990; Heloir *et al*., 1996). In present study, rooting was significantly affected by auxin concentration, highest number of root per cutting, rooting length; root fresh and dry weight was achieved at 400 mg kg^{-1} IBA solution. Indole butyric acid (IBA) is a root promoting hormone that stimulates the activity of cambium to initiate roots (Rahman *et al*., 1991). Application of rooting hormone to enhance the rooting capability of cutting has been suggested by many authors (Wahab *et al*., 2001; Rahman *et al*., 2004; Ullah *et al*., 2005; Manan *et al*., 2008; Abbas *et al*., 2013; Kareem *et al*., 2013).

Table 6: Effect of different rooting media and IBA concentrations on sprouting length (cm) of guava cuttings

IBA	Rooting media			Mean
(mg kg^{-1})	Sand	Silt	Top soil	
0	0.00±0.00 g	1.92±0.38 b-e	1.49±0.26 ef	1.14±0.21 C
200	3.98±0.47 a	1.50±0.16 ef	2.50±0.32 b	2.66±0.32 A
400	2.21±0.16 b-d	2.3±0.10 bc	1.63±0.02 d-f	2.05±0.08 B
600	0.00±0.00 g	1.05±0.12 f	1.68±0.03 c-f	0.91±0.05 C
Mean	1.55±0.16 B	1.69±0.19 AB	1.82±0.15 A	

*Means with same letters do not differ significantly at p 0.05 by Tukey HSD test; ± values indicate the standard deviation

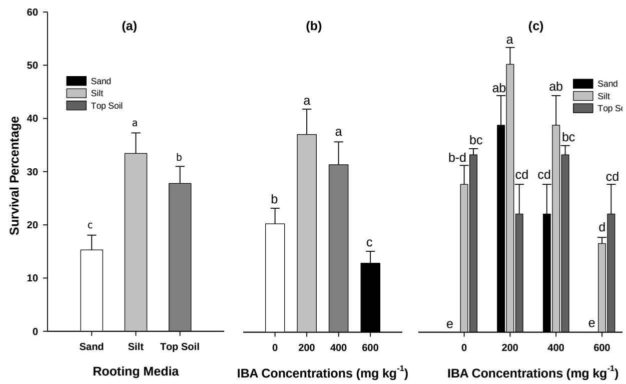

Fig. 3: Effect of different (a) rooting media (b) IBA concentrations and (c) their interaction on survival percentage of guava cuttings

Sen (2006) reported that the average number of root of *Flacourtia jangomas* cuttings was significantly escalated due to the application of IBA. The maximum number of root was achieved in 0.4% IBA treated cuttings and the minimum with no treatment. Hossain *et al.* (2002) reported that exogenous auxin (0.4% IBA) application appreciably ($p<0.05$) increased the number of roots per cuttings of jackfruit. In another experiment Hossain *et al.* (2004) reported the highest number of roots per cutting treated with 0.4% IBA solution compared with less in with no IBA treatment in *Chukrasia velutina.*

Similarly, in shooting competency parameters, highest number of leaves and number of sprouts per cutting was also achieved with 400 mg kg^{-1} IBA solution in silt and topsoil, respectively. However, maximum shoot length, sprout length and survival percentage was observed with 200 mg kg^{-1} IBA solution in silt, topsoil and silt media, respectively. It was found that 1.5% of IBA produced similar maximum leaves of guava (Abbas *et al*., 2013), semi hardwood cutting at 3000 mg kg^{-1} concentration of IAA (Wahab *et al.,* 2001) while at 1000 mg kg^{-1} IBA treatment similar results were reported by Rahman *et al*. (2004). Wahab *et al.* (2001) also reported the best survival percentage with IBA and NAA at 6000 mg kg^{-1}. Abbas *et al*. (2013) mentioned the survival percentage by using 1.5 percent of IBA.

Sand is a porous media which helps in the formation and expansion of roots. Sardeoi (2014) noted maximum number of roots in soil loam media among eight different rooting media. The reason may be more porosity and aeration that enhances roots penetration more deeply in sand media due to geotropism and attains more length. Ramtin *et al*. (2010) reported that longer roots are produced in beds with lower capacity of water retention.

The maximum root length and root fresh weight in lavender cuttings was achieved in perlite bed but non-significant results was observed between perlite and sand perlite (Sardoei, 2014). Badrzad *et al*. (2012) observed no significant difference between sand and sand-perlite (1:1 v/v) regarding rooting traits of cuttings of apple MM106; on the other hand sand was more effective than sand-perlite [1:1 v/v] concerning root dry weight. The highest root number, average root length, dry and fresh weight was achieved in soil loam (Salehi *et al*., 2012).

In the present study juvenile apical shoot cuttings of guava were selected for rooting induction. Goel and Behl (2004) reported that physiologically mature tissues have lower rooting potential and take more time to initiate roots and develop fewer roots than does physiologically juvenile material. Selection of optimal sizes of cutting for rooting induction is very essential in case of valuable super materials. Puri and Swamy (2000) mentioned that most of earlier workers used 20–22 cm size as the optimum size for root induction in woody plants. We have accomplished a propagation procedure through which small cuttings (12 cm long) could be multiplied easily under low-plastic tunnel. The phenomenon behind this may be that there are carbohydrates stored in the cuttings, which supply food for bud sprouting. After inducing roots these growth regulators may have direct effect on sprouting which needs to be explore. Similar results were also examined by other researchers (Khattak *et al.,* 1983; Debnath and Maiti, 1990).

Conclusion

Guava is one of the most important fruit species. Clonal multiplication through stem cutting might be one of the efficient methods of true-to-type propagation to avoid segregation of genetic variety for the species. Data presented in this study indicates that, the highest number of roots per cutting, root length, number of leaves and number of sprouts per cutting was observed in the cuttings treated with 400 mg kg^{-1} IBA solution while the maximum survival percentage was achieved in cuttings treated with 200 mg kg^{-1} IBA solution. Silt media performed better than sand and top soil regarding different parameters. Therefore, considering the above results, guava clonal propagation by soft wood cutting with 400 mg kg^{-1} IBA solution in low-plastic tunnels proves to be the best for speedy multiplication.

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