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CLONAL MULTIPLICATION OF GUAVA THROUGH SOFTWOOD CUTTINGS UNDER MIST CONDITIONS

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Guava (*Psidium guajava* L.) is a luscious and important tropical fruit crop. The objective of the study was to develop vegetative propagation system to avoid clonal degradation in guava fruit plants. Softwood cuttings from five year old gola accession were prepared from the shoot tops of current season growth, measuring 12 cm in length and carrying 2 to 4 nodes. IBA and NAA (0.2, 0.4, 0.6 and 0.8g/100g talcum powder) were selected to treat cuttings for root induction. Cuttings were planted under mist conditions by maintaining temperature at 25˚C and 85% relative humidity for 25 days. Maximum survival percentage (92.17%) of plants at transplanting was noted with 0.4g concentration followed by 0.2g (85.50%). In general IBA 0.4g concentration performed better as compared to NAA for all parameters studied. This study revealed the potential of clonal propagation of guava through softwood cuttings treated with auxins.
Keywords: Guava, *Psidium guajava*, propagation, rooting, softwood cuttings, IBA and NAA

INTRODUCTION

Worldwide Pakistan is the 2nd largest producer of guava after India (Jagtiani *et al.,* 1998). Guava, along with mango and mangosteens, reached a combined global production of 39984576 MT (FAO, 2010-2011). The other largest guava producing countries are Mexico, Brazil and Thailand (Padilla *et al.,* 2003). Venezuela, Cuba, and Colombia are important Latin-American countries cultivating guava (Morton, 2000; Swain and Padhi, 2012). In Pakistan it ranks fifth with respect to area and production, and is grown almost in all provinces, predominately Punjab. Major growing areas include Jhang, Kasur, Lahore, Gujranwala, Sheikhupura, Sahiwal and Faisalabad. In Sindh province, an excellent pear-shaped guava with a smaller seed core is grown mostly in the districts of Larkana, Dadu, Shikarpur and Hyderabad. In Khyber Pukhtunkhwa, the Mardan and Kohat districts and Hazara Valley are famous for production of good quality guava.

Guavas are commercially propagated sexually through seeds. During sexual propagation plants cannot maintain the genetic purity of the variety due to the segregation and recombination of characters, and has given rise to selection of several promising landraces. 'Sharakpur' guava is the result of such seedling selections in Pakistan. For these reasons standardization of cultivars is not possible (Singh, 1996). Clonal propagation of guava can be considered to avoid the segregation of genetic variety, maintain the quality of fruits and have considerable potential for the improvement of economically important trees within a limited time frame (Giri *et al.,* 2004; Singh *et al.,* 2004).

Several methods of propagation have been proposed for the guava both sexual (by seed) (Zamir *et al.,* 2003) and asexual methods (by cutting, layering, budding and grafting) (Singh, 1985; Chandra *et al.,* 2004, Mortan, 1987). Rooting among the vegetative methods of propagation is undoubtedly the most evolved and expanded method (Manica *et al.,* 2000; Awan *et al.,* 2012) but the information regarding the rooting ability of the cuttings in guava is very scarce. Propagation by cuttings has significant advantage, since, in addition to obtaining plants with the same type of tree, will ensure production of economically important tree in just one growing season (Tavares, 1994). Plant species and cultivars vary markedly in their rooting behavior. Cuttings from some kind of plants root easily while cuttings from some other kind still have never been rooted and guava is kind of specie hard to root. However, there is convincing evidences that auxins are essential for root development Hartman, 1981. Therefore to overcome some of these inherent difficulties encountered by the cuttings to root, auxins are helpful (Reddy and Singh, 1998).

Keeping in view the gravity of the problems ascertained with guava fruit crop, the researchers intended research activities to cope with inherent problems of guava. The objective of the present investigation is to develop asexual propagation system to avoid clonal degradation in guava crop.

MATERIAL AND METHODS

Gola accession of guava was selected as plant material for clonal propagation, which was collected from Square No. 9, University of Agriculture, Faisalabad. Five year old trees were selected for taking softwood cuttings. Softwood

cuttings measuring 12 cm long with 2 to 4 nodes and carrying at least 4 leaves were prepared from apical portions of shoots. The bark on lower portion of these cuttings was injured with knife to facilitate callusing process. IBA (0.2, 0.4, 0.6 and 0.8g/100g talcum powder) and NAA (0.2, 0.4, 0.6 and 0.8g/100g talcum powder) was used to treat cuttings for root induction using quick dip method. Before treating the cuttings basal portion with IBA and NAA, the cuttings were quickly immersed in copperoxychlorde solution to avoid any fungal attack. The basal portion of cuttings (50 per treatment) was quickly inserted in auxin and talcum powder mixture. The treated cuttings were 1/3 inserted into the sand medium under mist propagation structure. Cuttings were planted during mid-August to mid-September (2011) under mist conditions by maintaining 25˚C temperature and 85% relative humidity for 25 days. Fig.1 indicates the different steps for clonal propagation. The rooted cuttings were transplanted in-to 6x6 inch polythene bags filled with coconut husk and silt 3:1. The transplanted plantlets were then covered with polythene sheet for acclimatization. After one week of transplanting the polythene sheet was removed and plantlets were allowed to grow under normal environment. Data was recorded on percent rooted cuttings, number of roots per cutting, root length (cm) and survival percent of guava plantlets.

The experiment was laid out according to Completely Randomized Design (CRD) with two factors layout. There were five treatments in the experiment which were replicated thrice with 50 cuttings per treatment. Data collected was analyzed for Analysis of Variance and significance among treatment means was compared using Duncan's Multiple Range (DMR) test (Steel *et al.*, 1997).

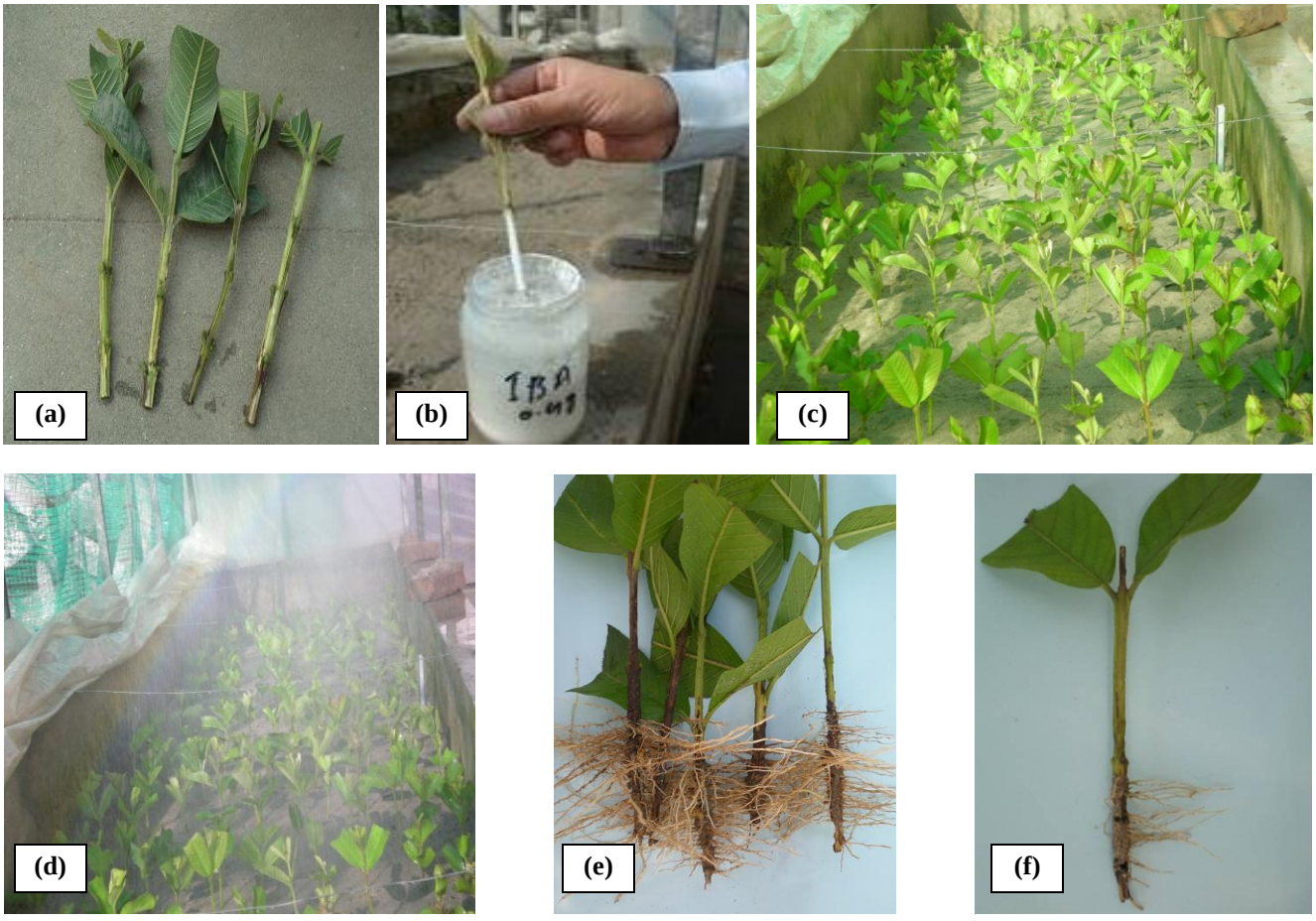

Figure 1. Clonal propagation of guava through softwood cuttings: a) softwood cuttings, b) cuttings treatment with auxins, c) planting of cuttings in sterile sand, d) cuttings growing under conditions, e) rooting of cuttings treated with 0.2g NAA, and f) 0.4g IBA.

RESULTS AND DISCUSSION

***Percent rooted cuttings*:** Comparison of two growth regulators indicated that cuttings were significantly affected by growth regulators, concentrations and their interaction for percent rooted cuttings (Table 1). Overall comparison of growth regulators proved the superiority of IBA (45.73%) over NAA (17.47%) for rooting of cuttings. Among different IBA and NAA concentrations, 0.4g (56.67%) was statistically the best followed by 0.2g (39.33%) which was at par with 0.6g. The maximum rooted cuttings (87.33%) were recorded in concentration 0.4g of IBA as compared to NAA (26%). The possible reason may be the antagonistic effect of IBA with endogenous gibberellin and with other factors that has promoting effect on root formation. These findings also cope with results of Wahab *et al.* (2001) who investigated the influence of IBA, IAA and NAA with concentrations 1000, 2000, 3000, 4000, 5000 and 6000 ppm and observed significant increase in rooting of cuttings of guava with IBA (1000 or 2000 ppm) and NAA (2000 ppm) (78.84, 75.96 and 76.59%, respectively). Paclobutrazol is also effective for rooting of cuttings. Rahman *et al.* (2004) observed 71.22% rooting in softwood cuttings of guava treated with paclobutrazol at 100 ppm solution as compared to NAA (1000 ppm).

***Number of roots per cutting*:** Significantly positive results were obtained when comparison of growth regulators, concentration and interaction was statistically analyzed for number of roots per cutting (Table 2). Overall maximum number of roots per cutting was noted in softwood cuttings treated with IBA (19.20) as compared to NAA (9.80). Among the concentrations of IBA and NAA, 0.4g induced the maximum number (25.50) of roots followed by 0.2 g with 20.49 roots per cuttings whereas control did not induce any roots. The combination of growth regulators and concentrations showed that IBA (0.4g) had the highest (38.00) number of roots per cutting (Fig.1f) followed by 0.6g (24.67). There are reports that auxins like IBA enhance rooting in cuttings. Cuttings treated with 500 to 1000 ppm of IBA increased the rooting percentage, number of roots per cutting as well as length of the roots per cutting (Noor *et al.*, 2004). The formation of high number of roots per cutting may be the fact that the cambial activity is involved in root induction (Rahman *et al.*, 1991).

***Root length (cm)*:** Analysis of variance for average root length (cm) showed highly significant results for both group of growth regulators, concentration and interaction (Table 3). The comparison of growth regulators showed the superiority of IBA (4.43 cm) over NAA (3.33 cm) for long roots. The means of concentrations of IBA and NAA were also compared and concentration 0.2g induced the longest roots (6.33 cm) in guava softwood cuttings followed by 0.4g (6.17 cm). The results indicates that the longest roots were recorded in softwood cuttings that were treated with 0.4g IBA (8.00 cm) as compared to cuttings treated with NAA (6.00 cm with 0.2g) (Fig.1e,f). The possible reason may be amount of food reserves in cuttings (Jain *et al.*, 1999).

The highest rooting percentage, root length and the root number has been reported by Vale *et al.* (2008) when applied IBA and NAA at concentration of 3000 mg L^{-1}. (Meahl, 1963) also observed increased root length and number of roots per cutting treated with auxins in guava.

***Survival of plants (%)*:** The results shown in Table 4 regarding percent survival of guava plants shows significant results for overall effect of concentrations but non-significant for growth regulators and interaction of growth regulators and concentrations. The maximum survival percentage (92.17%) was noted with 0.4g concentration of both IBA and NAA followed by 0.2g (85.50%) which was statistically similar with concentration 0.6g (80.50%).

The highest survival percentage of plants was with 0.4g concentration. It might be the reason that the same concentration of auxins induced maximum number of roots and number of roots has direct relationship with plants survival (Rahman *et al.*, 1988). Rahman *et al.* (2004) also examined the highest survival (57.22%) of plants propagated from paclobutrazol treated softwood cuttings followed by 54.97% with IBA. NAA showed the lowest (15.83%) survival of guava plantlets (Rahman *et al.*, 1988). This increase in survival percentage in paclobutrazol and IBA is because of high number of roots. On the other hand potting media can also influence the survival of plants.

Table 1. Effect of IBA and NAA on rooted cuttings (%) of guava

Hormones Conc. (g/100g talc. powder)	Rooted cuttings (%)		Mean
	IBA	NAA	
0.0	0.00±0.00e	0.00±0.00e	0.00±00.00d
0.2	48.67±2.40b	30.00±0.00c	39.33±04.31b
0.4	87.33±1.76a	26.00±0.58c	56.67±13.74a
0.6	49.33±5.70b	18.00±0.58cd	33.67±07.46bc
0.8	43.33±4.37b	13.33±0.33d	28.33±06.99c
Mean	45.73±7.53a	17.47±2.81b	

Means sharing similar letters in a row or in a column are statistically non-significant ($p>0.05$); ± values indicates the standard deviation

Table 2. Effect of IBA and NAA on number of roots per softwood cutting of guava

Hormones Conc. (g/100g talc. powder)	Number of roots		Mean
	IBA	NAA	
0.0	0.00±0.00e	0.00±0.00e	0.00±0.00e
0.2	21.33±0.19c	19.66±0.20d	20.49±0.39b
0.4	38.00±0.19a	13.00±0.29e	25.50±5.59a
0.6	24.67±0.04b	10.00±0.29g	17.33±3.28c
0.8	12.00±0.12f	6.33±0.19h	9.17±1.27d
Mean	19.20±3.40a	9.80±1.76b	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05); ± values indicates the standard deviation

Table 3. Effect of IBA and NAA on root length (cm) in softwood cuttings of guava

Hormones Conc. (g/100g talc. powder)	Root length (cm)		Mean
	IBA	NAA	
0.0	0.00±0.00h	0.00±0.00h	0.00±0.00e
0.2	6.67±0.04b	6.00±0.00c	6.33±0.15a
0.4	8.00±0.12a	4.33±0.01e	6.17±0.82b
0.6	5.00±0.00d	3.33±0.02f	4.17±0.37c
0.8	3.00±0.12g	3.00±0.01g	3.00±0.05d
Mean	4.53±0.75a	3.33±0.53b	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05); ± values indicates the standard deviation

Table 4. Effect of IBA and NAA on survival (%) of plants propagated from softwood cuttings of guava

Hormones Conc. (g/100g talc. powder)	Plants survival (%)		Mean
	IBA	NAA	
0.0	0.00±0.00	0.00±0.00	0.00±0.00d
0.2	85.33±1.45	85.67±1.45	85.50±0.92b
0.4	91.33±0.88	93.00±1.00	92.17±0.70a
0.6	80.00±3.61	81.00±3.79	80.50±2.35bc
0.8	75.33±2.60	73.67±0.67	74.50±1.26c
Mean	66.40±9.02	66.67±9.09	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05); ± values indicates the standard deviation

***Conclusion*:** The study revealed that applications of different plant growth regulators have great potential to induce rooting in difficult to root plants. Among the auxins, IBA with 0.4g per 100g talcum powder mixture induce maximum roots in softwood cuttings. It could be quite safe to recommend that clonal propagation of guava through softwood cutting is reliable for nursery plants production as it is quick, easy and economical method of vegetative propagation.

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