ROOTING ABILITY OF CUTTINGS OF GUAVA (*PSIDIUM GUAJAVA* LINN.) AS INFLUENCED BY ETIOLATION OF STOCKPLANTS

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Abstract

The study describes the effect of etiolation of stockplants on rooting ability to cuttings of Guava (*Psidium guajava* Linn.). Fifteen months old sun grown containerized stockplants of guava were etiolated in a darkroom. Cuttings from the etiolated and control stockplants were tested for rooting ability. The study revealed that the cuttings of the stockplants etiolated or controlled rooted well. However, etiolation or duration of etiolation does not significantly affect the rooting ability of cuttings of the species. During propagation, cutting morphology is not significantly changed due to the etiolation of the stockplants.

Introduction

Guava (*Psidium guajava* Linn.) is a common fruit tree species grown everywhere particularly in homestead areas of Bangladesh (Banik, 1992). Raising plantation of guava by seedling is time consuming and it is almost impossible with the seedless high yielding hybrid varieties maintaining the fruit quality. To have fruit within a short period of time with maximum duplication of characteristics of mother tree, several vegetative methods are employed. Grafting, budding and their layering are the common methods used to propagate guava (Bailey, 1960. These techniques have several disadvantages like a low rate of multiplication, a high requirement of a skilled labour. These problem have been solved by recently developed technique of propagation by stem cuttings. Propagation by stem cuttings may be a promising technique of vegetative propagation of guava at operational scale. This technique may allow an adequate supply of planting/genetic material and help to maintain the desirable characteristics of trees (Kamaluddin *et al.*, 1996).

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Rooting success in clonal propagation is dependent upon optimizing many endogenous and exogenous factors concerned with inherent capacity for propagation, collection of source materials, preparation and treatment of cuttings, selection of propagation system, adjustment of optimum condition for rooting, treatment of stockplants and their management (Kamaluddin, 1988). The stockplants management involves several preconditioning like girdling, banding, photoperiod, etc.

A considerable body of evidence suggests that etiolation of stockplants enhances rooting. The practice of etiolation has been known to increase adventitious root formation in stem tissue. Cutting from etiolated plants were found to have a high level of endogenous auzin (IAA) at the site of etiolation (Kawase, 1965) which prevents the appearance of rooting inhibitors (Ooyama, 1962). Etiolated stems have been shown to have less mechanical strengthening tissue and lower starch content and cell deposits. Cell wall thickness is also less and there is more of parenchyma tissue which is less differentiated.

Several scientists have studied the effects of etiolation on rooting of cuttings following different methods. For instance, etiolation for stem cuttings of 5-year-old, potted *Carpinus betulus* vs., achieved by covering with a black cloth for 7 days improved rooting of cuttings reported by Richards (1992). Etiolation in 2 shading studies, stock plants were grown in a greenhouse under 0%, 50%, 75% or 95% shade until cuttings were taken, or initially etiolated (100% shade) for 15 days. Percentage rooting increased in proportion to the degree of shading, with a maximum response at 95% shade (Maynard and Bassuk, 1992). Marczynski and Joustra (1993) etiolated two-year-old stock plants of *B. utilis* and *C. maxima* cultivar Purpurea in a darkroom or grown in a greenhouse under 3 different daylengths (8, 15 or 24 h).

In the present study etiolation was done keeping the stock plants in darkroom (100% shade) to explore the effect of etiolation on rooting ability of cuttings.

Materials and Methods

Stockplant etiolation

Stockplants of guava wee kept in a dark room and cuttings taken from the stockplants were put into rooting trials in August and September 1998. Stockplants serving as controls were kept in open sun. Before keeping them in darkroom, they were grown in open sun. The stockplants were the seedlings raised from the seeds of same mother tree and grown in polybags filled with soil and cow-dung 3:1 by volume. When the stockplants were 15 months old, they

were put into the treatment of etiolation. For the purpose, 50 stockplants were kept in darkroom.

Rooting trials

Sixty one-node cuttings with two leaves trimmed to half from etiolated or control stockplants were put into a non-mist propagator. Before setting the cuttings into propagator the cuttings were immersed briefly in a solution of fungicide, Diathane M45 (Rohm & Co. Ltd., France; 2 g per litre of water) to avoid fungal infection. They were rinsed and kept under shade for 10 minutes in open air. The cuttings were then planted into a non-mist propagator in completely randomized blocks. The cuttings were planted into perforated plastic trays (12 cm depth) filled with coarse sand mixed with fine gravel. Each tray contains 10 cuttings and severed as a plot. Thus the number of replicate cuttings per treatment was 30. The cuttings were watered once only just after the setting into the propagator and no watering was done till the transfer of rooted cuttings from the propagator. Rooting trials were conducted with cuttings of stockplants three days, six days or nine days after putting them in etiolation treatment. At the same time cuttings from the stockplants serving as control were also set into the rooting trials.

To examine the change in leaf weight during the etiolation, 10 leaf discs, each 0.5 cm^2, were taken from five stockplants at three days interval from the day of setting the experiment. The samples wee dried in oven at 70°C for 48 hours and dry weights were recorded.

Maintenance of propagator environment for rooting

85-90% humidity may maintained within the propagator. Every day the propagator was opened briefly in the morning and in the late afternoon to facilitate gas diffusion. The propagator was kept under bamboo shed to avoid excessive heat accumulation. Further shading was achieved by putting jute mate over the roof of the shed. In this way, photosynthetic photon flux inside the propagator was reduced to about 12% full sun. During the experiment, mean maximum temperature was 32°C and the mean minimum temperature 25°C.

Weaning

The cuttings in the propagator rooted four weeks after setting them into the propagator. The cuttings were weaning before transferring them from the propagator particularly towards the end of rooting period to facilitate easy and undisturbed separation of rooted cuttings from the rooting media. For weaning, the propagator was kept open at night for three days and then at day and night for another three days.

Data collection and statistical analysis

The rooted cuttings were measured for length, diameter and leaf area. Number of roots developed in each cutting was recorded. Roots of each cutting wee separated and dried in an oven at 70°C for 48 hours for dry weight assessment. Leaves and internodes were also dried into to oven. Possible treatment differences were explored by analysis of variance and Duncan's Multiple Range Test (DMRT) by running SPSS and Excell software under IBM PC based Windows environment. DMRT was used to make specific treatment comparison that permits to make decision as to which differences are significantly and which are not. It uses significant ranges; each range depends upon the number of means in the comparison. Rooting percentage values were adjusted accordingly using following arc sign transformation formula before putting the data into analysis of variance since the percentages cutting rooted were distributed between the range of 70 to 1000 and proportions were based on equal denominator.

$$Y = \text{Sin}^{-1} (X)^{½}$$

Where,

Y = Arc sign transformed value, X = Proportion of number of cuttings rooted to the number of cutting planted. And the value 1000 percent were submitted 100–¼n) where 'n' is the number of units upon which the percentage data is based i.e., the denominator used in computing the percentages.

Results

Effect of etiolation on rooting ability of cuttings of Guava

The cuttings of the stockplants etiolated or controlled rooted well (Rooting percentage ranged from 93.33 to 100). But etiolation or duration of etiolation did not affect significantly on rooting of cuttings (Table 1). Rooting percentage was not significantly affected by etiolation. Number of roots produced per cutting significantly increased with the time of rooting trials which was not due to the duration of etiolation. In etiolated or control cuttings, number of roots per cutting increased with the time of rooting trials. Cuttings taken after nine days produced maximum number of roots per cutting than those set three or six days after the start of etiolation. The same trend was observed in mean root dry weight. Root dry weight per cutting was significantly highest in etiolated or control cuttings at the trials set nine days after the start of the experiment.

Table 1. Effect of etiolation on rooting ability of cuttings of guava.

Variables	Etiolation			Control			P	
	3 days	6 days	9 days	3 days	6 days	9 days	Etiolation	Duration
Rooting percentage	96.67[a]	96.67[a]	93.33[a]	96.67[a]	96.67[a]	100[a]	NS	NS
Root No. per cutting	1213[b]	14.65[b]	2023[a]	14.76[b]	14.11[b]	20.33[a]	NS	**
Root dry weight per cutting (mg)	60.04[b]	65.5[ab]	68.52	65.07[ab]	65.96[ab]	72.31[a]	NS	*

Significant: ***** indicates $p < .0005$, **** $p < .001$, *** $p < .005$, ** $p < .01$, * $p <. 05$, NS: Not significant at $P < .05$ (ANOVA and DMRT).

Morphology of etiolated or non-etiolated cuttings of guava

There was no significant effect of etiolation on cutting morphology of guava (Table 2). Cutting length, diameter, leaf area, volume, leaf dry weight, internode dry weight per cutting, specific internode length (SIL), specific leaf area (SLA) and specific internode volume (SIV) were not significantly changed during the etiolation of stockplants of guava. But leaf weight per unit lef area was significantly lower in etiolated leaves (Figure 1). The decrease in leaf weight per unit leaf area was noticed at six days after the start of etiolation and there was no further decrease as evident from the data taken nine days after etiolation of stockplants.

Table 2. Morphology of etiolated or non-etiolated cuttings of guava

Variables	Etiolation			Control			P	
	3 days	6 days	9 days	3 days	6 days	9 days	Etiolation	Duration
Cutting length (cm)	5.30[a]	4.97[a]	5.51[a]	5.65[a]	5.34[a]	5.39[a]	NS	NS
Cutting diameter (mm)	3.28[a]	3.33[a]	3.54[a]	3.17[a]	3.39[a]	3.69[a]	NS	NS
Leaf area per cutting (cm^2)	36.4[a]	33.5[a]	34.5[a]	31.5[a]	34.5[a]	40.7[a]	NS	NS
Volume per cutting (cm^3)	0.67[a]	0.56[a]	0.73[a]	0.62[a]	0.63[a]	0.76[a]	NS	NS
Leaf dry weight (mg)	326[a]	285[a]	315[a]	289[a]	288[a]	335[a]	NS	NS
Stem dry weight (mg)	291[a]	212[a]	245[a]	200[a]	204[a]	302[a]	NS	NS
Specific leaf area ($cm^2\ g^{-1}$)	111[a]	118[a]	112[a]	110[a]	112[a]	118[a]	NS	NS
Specific internode length (cm^{-1})	20.1[a]	27.0[a]	24.8[a]	29.8[a]	26.2[a]	18.8[a]	NS	NS
Specific internode vol. (cm^3g^{-1})	2.39[a]	2.44[a]	2.50[a]	2.31[a]	2.56[a]	2.58[a]	NS	NS

Significant: ***** indicates $p < .0005$, **** $p < .001$, *** $p < .005$, ** $p < .01$, * $p <. 05$, NS: Not significant at $P < .05$ (ANOVA and DMRT).

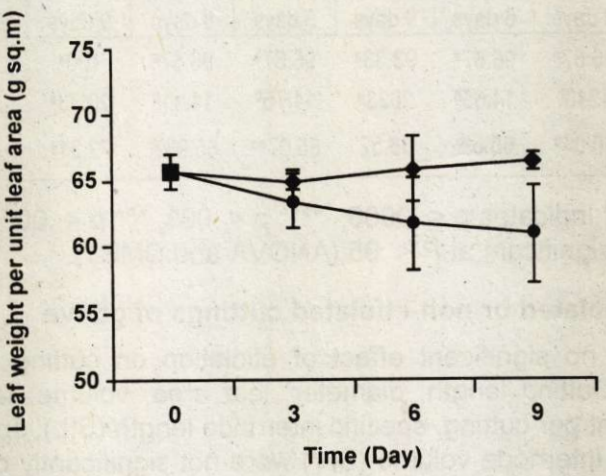

Fig.1. Leaf dry weight per unit leaf area of control (♦) and etiolated (•) leaves of guava over time of etiolation

Discussions

The cuttings of the stockplants etiolated or controlled rooted well (Rooting percentage ranged from 93.33 to 100). Etiolation or duration of etiolation did not significantly affect rooting of cuttings in the species guava (Table 1). Several workers have observed that etiolation significantly increased rooting ability of cuttings of stockplants. For instance, Bassuk *et al.*, (1988) and Schmidt (1981) report that etiolation of stockplants by black adhesive tape or black plastic sheet before bud break significantly increase rooting percentage. Rahmand and Blake (1988) ringed and/or etiolated jackfruit shoots for 20 days before detachment as cuttings were dipped for two minutes in a solution of IBA at 750 ppm. Before insertion in 1:1 compost: vermiculite mixture increased rooting success. Mukherjee and Chaterjee (1978, 1979) reported that rooting of cuttings of jackfruit (*Artocarpus heterophyllus*) was successfully induced through forcing of shoots etiolation and application of Indole Butyric Acid (IBA) under mist spray. The results of the present study different from them. This might be due to the whole plant etiolation.

Table 3. Results of ANOVA

Source of variation	DF	Trait studied								
		1			2			3		
		SS	MS	F	SS	MS	F	SS	MS	F
		Rooting percentage			Root number per cutting			Root dry weight per cutting (mg)		
Etiolation	1	75.317	75.317	800	4.414	4.414	.562	26.362	26.362	.967
Duration	2	.000	.000	.000	177.968	88.984	11.340	195.344	97.672	3.581
Etiolation * Duration	2	150.635	75.317	.800	6.764	3.382	.431	15.998	7.999	.293
Residual	12	1129.760	94.147		94.166	7.847		327.296	27.275	
Total	17	1355.712	79.748		283.312	16.665		564.999	33.235	
		Cutting length (cm)			Cutting diameter (mm)			Leaf area per cutting (cm^2)		
Etiolation	1	.181	.181	2.380	.000	.000	.000	.630	.630	.155
Duration	2	.395	.197	2.595	.657	.328	4.837	13.368	6.684	1.640
Etiolation * Duration	2	.241	.120	1.593	.145	.073	1.069	23.012	11.506	2.823
Residual	12	.912	.076		.815	.068		48.906	4.076	
Total	17	1.728	.102		1.161	.095		85.916	5.054	
		Cutting volume (cm^3)			Leaf dry weight per cutting (g)			Internode dry weight		
Etiolation	1	.007	.007	.546	.000	.000	1.126	.001	.001	267
Duration	2	.141	.071	5.356	.002	.001	4.347	.012	.006	2.146
Etiolation * Duration	2	.052	.026	1.969	.002	.001	3.994	.017	.008	2.961
Residual	12	.158	.013		.003	.000		.034	.003	
Total	17	.359	.021		.007	.000		.063	.004	
		Specific leaf area (cm^2g^{-1})			Specific internoe length (cmg^{-1})			Specific internode volume (cm^3g^{-1})		
Etiolation	1	38.824	38.824	1.610	4.364	4.364	.290	.016	.016	.179
Duration	2	65.296	32.648	1.354	71.427	35.713	2.376	.170	.085	.928
Etiolation * Duration	2	16.632	8.316	.345	191.572	95.786	6.373	.015	.007	.080
Residual	12	289.283	24.107		180.354	15.029		1.101	.092	
Total	17	410.035	24.120		447.716	26.336		1.302	.077	
		Leaf weight per unit leaf area (gm^2) of guava								
Etiolation	1	168.033	168.033	5.248						
Duration	2	19.059	9.529	.298						
Etiolation * Duration	2	74.259	37.129	1.160						
Residual	12	768.384	32.016							
Total	17	1029.735	35.508							

The results also supported by the results of Marczynski and Joustra (1993), who found that percentage of rooting of cuttings tended to be reduced if stockplants grown in short photoperiod and less than 10% irradiance.

Average number of roots in both etiolated and controlled. Cuttings of guava were significantly enhanced over time of start of the etiolation experiment. It was not due to the duration of etiolation but might be due to age of shoots or some other environmental factors. In etiolated or control cuttings, number of roots per cutting increased with the time of trials. Etiolation as well as duration of etiolation had no significant effect on almost all the variables like length, diameter, leaf area, volume, leaf dry weight, internode dry weight per cutting, specific internode length (SIL), specific leaf area (SLA) and specific internode volume (SIV) in cuttings of guava (Table 2).

During the etiolation, leaf weight per unit of area was significantly decreased in etiolated (Figure 1). The decrease of leaf weight per unit leaf area was noticed at six days after the start of etiolation and further decrease was minor as evident from the data taken at six days or nine days after etiolation of s tockplants. Kamaluddin and Grace (1993a) supported the decrease of leaf weight per unit leaf area during the etiolation. They have observed that the leaf weight per unit leaf area decreased and increased the specific leaf area when high-light grown seedlings were transferred to the low-low light regime.

Conclusion

Etiolation of stockplants did not significantly increase rooting ability of cuttings of guava. In the present study, whole plant etiolation of juvenile materials did not significantly influence the rooting ability of cuttings. Effect of part etiolation of stem particularly for mature trees may be investigated. Normally, mature materials root poor and hence often a constraint for propagating selected mature genotypes to be used as stockplants. This could be an another area to be explored in future study.

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