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Shoot pruning affect micropropagation of *P. guajava* L. and *P. friedrichsthalianum* (O.Berg) Nied.

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ABSTRACT

The present experiment has been designed in order to see the effect of pruning on explants collection and it's *in vitro* performance on shoot initiation, shoot multiplication and root initiation. For this purpose, two species of guava namely, *Psidium guajava* L. and *P. friedrichsthalianum* (O.Berg) Nied. (Chinese guava) were selected. Healthy shoots were pruned at 50% and 75% level along with the unpruned shoots (control). Nodal segments of new growth were used as explants. Best MS media along with plant growth hormones obtained in our previous work were used for culturing explants. Explants collected from pruned shoots (50% and 75%) gave better shoot regeneration and shoot multiplication over the unpruned (control) shoots in case of *P. guajava* L cv. L-49. The highest numbers of shoot regeneration (64.2%) were recorded in explants obtained from 50% pruned shoots of *P. friedrichsthalianum*. Lowest shoot regeneration was recorded in explants collected from unpruned (39.3%) shoots in highest number of days (21.3). The highest number of root formation was obtained in explants obtained from 75% pruned shoots in both guava species over 50% pruned shoots with 66.3% in cv. L-49 and 64.3% in *P. friedrichsthalianum*. Hence, the explants when collected after pruning gave better results for *in vitro* shoot initiation, shoot multiplication and root formation over the explants collected from unpruned shoots (control).

Key words: Lucknow 49, Chinese guava, rootstock, *In vitro* propagation.

INTRODUCTION

Guava, originated from tropical America is the fourth most important fruit crop of India. It is hardy in nature, prolific bearer and highly remunerative crop even without much care. There are 150 species reported in *Psidium* species, most of which are fruit bearer. Fruit of guava (*P. guajava* L.) has high antioxidants like lycopene, polyphenol and carotenoids. It is also rich in key nutrients like vitamins A, vitamin C, potassium, fiber, calcium and iron. Fruits of guava are very rich in pectin so best jelly is made out of it. The guava has medicinal value as fruits, roots, bark and leaves are used for curing gastrointestinal problems, diarrhea and dysentery (Rathore, 10). Another species of guava i.e. *P. friedrichsthalianum* is mainly used to impart dwarfness when used as rootstock. The fruits and leaves of Chinese guava are small in size. Fruit is aromatic and acidic in nature. Fruits are commonly used to prepare a sour and refreshing drink. Fruit is highly rich in pectin and make good firm jelly even with fully ripe fruit. Mostly it is mainly propagated through seeds, but due to segregation and mixing of genetic character during sexual propagation the plants do not maintain the genetic purity. Guava cv. Lucknow-49 (L-49) and *P. friedrichsthalianum* are commercially used as rootstock in guava owing to its resistance to wilt. Rootstocks exhibit great effect on plant architecture, yield, and quality and impart resistance to biotic and abiotic stresses in fruit crops.

Guava is generally propagated through asexual means of propagation like cutting, budding, stooling etc. Although these asexual means are successful and give uniformity to the plants but requires larger space and time. Micro propagation of woody trees is difficult and its success largely depends on the explants condition. If explants will be collected from a healthy shoots from a microbe free environment then the success for its *in vitro* establishment will be high. Pruning helps to give new shoots which are microbe free and young. Mishra *et al*. (8) also observed that severely pruned guava encourage new vegetative growth which served as a reliable source for explants culture. So, thinking that juvenile nature explants will give less phenol exudation and better *in vitro* performance when cultured, the present experiment has been conducted.

MATERIALS AND METHODS

The present experiment was carried out in the Department of Horticulture, College of Agriculture, CCS Haryana Agricultural University, Hisar, India. The aim of the present study was to see the effect of pruning of shoots for collecting nodal segment

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explants for successful development of protocol for guava micro propagation. Two independent experiments were conducted on two species of guava. The trees of *Psidium guajava* cv. Lucknow-49 and *P. friedricsthalianum* were grown in the horticulture orchard on which pruning was performed on selected shoots. The trees selected for explants collection were physiologically mature and grown in a healthy soil and environmental condition.

Pruning of shoots and explants collection: Shoots of selected trees were pruned at different level *Viz*, 50% and 75%. The vegetative shoots of 20 cm length were collected from pruned shoots as well as from the unpruned shoots of *Psidium guajava* cv. L-49 and *P. friedricsthalianum* in the morning hours. The excised shoots were brought to the laboratory and washed under running tap water. The nodal segments from first and second node were taken as explants.

Surface sterilization and anti-phenolic treatment: The explants were further washed with detergent (Teepol) and then the explants were dipped in 0.2% k-cycline for 30 minutes. Washing of explants with double distilled water was done after every step. Again the explants were treated with 0.2% carbendazim for 30 minutes followed by 4-5 washing with double distilled water. After that the nodal segments were agitated in 0.2% ascorbic acid and 0.4% citric acid solution for 12 minutes in order to control phenol exudation. Sub culturing of explants after 24 hours of inoculation was done to control phenol exudation and thus better culture establishment. Sterilization of explants were carried out under laminar air flow chamber where explants were dipped for 30 seconds in 70% ethanol followed by rinsing and then dipped for 8 minutes in 0.1% Mercuric chloride followed by rinsing with sterilized double distilled water in order to remove all the traces of sterilizing agents .

Culture condition: After sterilization the explants were inoculated into jam bottles containing suitable Murashige and Skoog's media (9) and the pH of the media was adjusted to 5.7. Inoculated bottles were maintained at 25±1°C under 16/8 hour photoperiod (2000 lux florescent tubes) in the culture room.

Culture establishment and shoot proliferation: Our previous work indicated the appropriate growth medium for culture establishment, shoot multiplication and rooting (Kala *et al*. 5) which was used in the present study to see the response of shoot pruning on explants performance under *in vitro* propagation. Here, the MS supplemented with 2.0 mg/l BAP+0.5 mg/l NAA was used as establishment media. For multiplication media MS supplemented with 1.5mg/l BAP+0.5mg/l Kinetin+0.1mg/l NAA along with 25.0mg/l Adenine Sulphate was used and for rooting of the micro shoots half strength of MS containing 2.0 mg/l IBA+0.5mg/l NAA was used in *Psidium guajava* cv. L-49 and *P. friedricsthalianum*.

Statistical analysis: The Completely Randomized Design was used. Critical difference at 5% significance was calculated. The data in parentheses indicated angular transformed values.

RESULTS AND DISCUSSION

When the nodal explants of guava (*P. guajava* L. and *P. friedrichsthalianum*) were collected from pruned shoots a significantly higher shoots were regenerated in minimum number of days in both the *Psidium* species during their culture establishment. Here, pruning of shoots at 50% and 75% were found better over unpruned (control) shoots (Table 1 & 2). This shows that healthy, young and soft explants (actively growing shoots) are more suitable for micro propagation than old woody tissues (Amin and Jaiswal 2, Jaiswal and Amin, 4, Singh *et al*., 13 and Singh ***et al***., 14). Similarly, Aekaterini and Maria (1) also reported that culture establishment was very low if explants were collected from adult tree as compare to seedling plant. In the present experiment, the role

Table 1: Effect of pruning on micropropagation of Lucknow 49 guava cultured through nodal segment explants collected from pruned tree.

Pruning intensity	Shoot formation			Root formation		
	(%) Regeneration*	Days taken for shoot formation*	No. of shoots per explant**	(%) Root formation	Days taken for root initiation	Roots per micro shoot
Control	30.2±1.9 (33.3)	23.0±1.0	3.2±0.1	76.4±1.5 (60.9)	28.0±1.1	3.7±0.1
50%	76.6±1.6 (61.0)	16.0±1.1	7.0±0.3	79.7±1.5 (63.2)	28.9±0.8	3.8±0.1
75%	78.6±1.1 (62.4)	18.8±1.0	7.0±0.1	83.9±1.2 (66.3)	27.0±0.8	3.9±0.1
CD at 5%	(2.13)	2.06	0.44	(1.99)	N.S.	N.S.

Figures in parentheses indicate angular transformed values. ± SE (mean)
* MS+2.0 mg/l BAP+0.5 mg/l NAA
** MS+1.5mg/lBAP+0.5mg/lKinetin+0.1mg/lNAA along with 25.0mg/l Adenine Sulphate
Rooting media used: 1/2MS+2.0 mg/l IBA+0.5mg/l NAA

Table 2: Effect of pruning on micro propagation of Chinese guava cultured through nodal segment explants collected from pruned tree.

Pruning intensity	Shoot formation			Root formation		
	(%) Regeneration*	Days taken for shoot formation*	No. of shoots per explant**	(%) Root formation	Days taken for root initiation	Roots per micro shoot
Control	40.1±0.9 (39.3)	21.3±0.6	3.4±0.1	70.0±2.0 (57.2)	27.0±1.0	2.1±0.1
50%	81.2±1.3 (64.2)	17.2±0.7	5.9±0.1	74.0±1.2 (59.3)	26.6±0.5	2.1±0.1
75%	79.3±1.1 (62.9)	19.0±0.8	6.0±0.2	81.3±1.1 (64.3)	25.7±0.3	2.5±0.2
CD at 5%	(1.58)	1.45	0.26	(1.39)	N.S.	0.28

Figures in parentheses indicate angular transformed values. ± SE (mean)
*MS+2.0 mg/l BAP+0.5 mg/l NAA
**MS+1.5mg/lBAP+0.5mg/lKinetin+0.1mg/lNAA along with 25.0mg/l Adenine Sulphate
Rooting media used: 1/2MS+2.0 mg/l IBA+0.5mg/l NAA

of establishment media was kept constant for all the cultures of shoot regeneration in both the guava species. So, as the explants collected after pruning of shoots were found to be more responsive in shoot regeneration, this practice can be exploited for better protocol establishment.

The response of pruning on explant multiplication was also reported in the present experiment. However, when the regenerated shoots of both the *Psidium* spp. were transferred to multiplication media, no visible results were obtained among the different level of pruning (50% and 75%). Although maximum number of shoots were obtained per explants collected from pruned shoots and the minimum shoots per nodal explants was observed in unpruned (control) shoots. In past a successful regeneration was observed mostly from seedling explants (Shah *et al*., 12 and Aekaterini and Maria 1). The selection of explants at a specific responsive stage of a mature tree's life cycle is of great importance to overcome recalcitrance (Krishna and Singh, 6). In the present experiment also, new vegetative shoots were used to break the woody and recalcitrant behaviour of explants. Amin and Jaiswal (2) also found that new vegetative growth that occurs from the base of the main stem (off-shoots) serves as a reliable source of shoot tip and nodal segments for guava tissue culture. Also, Greenwood (3) proposed that micro propagation of adult plants could be facilitated using rejuvenation methods, which are supposed to return tissue explants from the mature to juvenile phase and include application of cytokinin either during or immediately after explants are placed in culture, serial grafting, propagation of stump sprouts or severe pruning.

Effect of pruning on root multiplication was not much noticed. During the experiment, when the multiplied micro shoots were transferred on a rooting media (found best in the previous work), in order to see the pruning effect on root initiation in *P. guajava* L. and *P. friedrichsthalianum*. The percent root formation in micro shoots obtained from pruned explants was found better as compared to unpruned one. However, non significant results were obtained for number of days taken for root initiation and roots regenerated per micro shoot in both the species. Similarly, Aekaterini and Maria (1) also found low rooting in × *Malosorbus florentina*. This shows rooting of micro shoots are largely dependent on PGRs rather that explants origin. The requirement of PGRs is necessary and it has become a necessity to standardise when dealing with tree species. The levels and PGRs included in the culture medium largely determine the success of tissue culture work (Kumar *et al*., 7 and Rout *et al*., 11).

The success of tissue culture of woody plant depends on the origin of explants. In the present experiment, pruning of shoots at different levels were found good for *in vitro* shoot initiation and shoot multiplication along with the good root formation in the explants. In both the *Psidium* spp. the shoot regeneration was almost double in explants collected from 50% and 75% pruned shoot in comparison to explants collected from unpruned shoots. Similarly, pruning significantly affected the *in vitro* root formation where 75% shoot pruning was found better over unpruned and 50% pruned shoots for *in vitro* propagation. So, pruning of shoots can be used as for explants collection for the successful micro propagation of *Psidium guajava* L. cv. L-49 and *P. friedrichsthalianum* (Chinese guava).

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