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Perpetuation of *Psidium* species through stooling and assessment of growth behaviour of clonal rootstocks under subtropical conditions

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ABSTRACT

The investigation was carried out to standardize vegetative means of mass perpetuation of *Psidium* species through stooling technique and to assess growth behaviour of clonal rootstocks under subtropical conditions. The coppicing capacity among *Psidium* species varied with maximum number of desirable shoots/ plant in *Psidium chinensis* (27.33) and minimum in *P. friedrichsthalianum* (9.12). All the *Psidium* species under investigation, viz *Psidium cujavillis*, *P. molle*, *P. chinensis* and *P. cattleianum* except *P. friedrichsthalianum* can be successfully perpetuated by stooling with the combined treatment of IBA+NAA (7500 ppm). The rooting success varied between 9.53 and 100.00%, and field establishment of rooted shoots between 0.0-97.13%. The *P. chinensis gave cent per cent* rooting with 93.13% field establishment while *P. friedrichsthalianum* gave only 9.53 % rooting and no plants were established under field conditions. The remaining species, viz *P. cujavillis, P. cattleianum* and *P. molle* gave more than 91.69% rooting and more than 84.03% establishment of rooted shoots. The correlation between rooting versus biochemical and anatomical parameters revealed that higher phenol content and C:N ratio of stooled plants as well as more intercellular space and breadth of sclerenchymatous cells have better impact on rooting while higher protein and longer length of sclerenchymatous cells did not make any impact. Under uniform management, maximum plant height (106.90 cm) was obtained in *P. cattleianum* while minimum in *P. chinensis* (55.80 cm) after six months of transplanting. The stock girth was also maximum in *P. cattleianum* (3.91 cm), followed by *P. molle* (3.69 cm), *P. cujavillis* (3.32 cm) and minimum in *P. chinensis* (2.89 cm). The number of branches, intermodal length (cm), number of leaves / plant, average leaf area (cm^2) and total leaf area / plant (cm^2) also varied significantly. Based on initial growth behaviour, these *Psidium* species can be relatively categorized as dwarf (*P. chinensis*), semidwarf (*P. molle*) and vigourous species (*P. cattleianum* and *P. cujavillis*).

Key words: Clonal rootstocks, Growth behavior, *Psidium* species, Stooling

Though, guava (*Psidium guajava* L.) is an exotic fruit plant but naturalized so much in India that today not only wide range of genetic diversity exists but best quality of guavas are produced in the country. Because of its wider edaphoclimatic adoptability, precocious and prolific bearing, delicious taste, high nutritive value and better economic returns; the guava fruits are very popular and regarded as common mans' fruit. At present guava occupies 2.04 lakh ha area in the country with annual production of 22.70 lakh tonnes (Anonymous, 2009). It is pertinent to mention that the area and production of guava in India is increasing continuously but productivity remained constant in between 10.40 and 11.70 tones/ha since last several years. Apart from various constraints in guava cultivation, problem of guava wilt is very serious. Various causal organisms and management factors have been associated with wilting problem of guava orchards. No chemical measures have been found foolproof to control the problem of guava wilt so far. The alternative lies in use of wilt-tolerant/resistant rootstocks for scion cultivars coupled with scientific management of orchard.

Even today, guava orchards are being established on rootstocks of mixed seedlings, which is not a desirable practice. In fact, desired plant vigour and canopy architecture are the vital factors for optimum quality crop load, mechanization and longevity of the healthy tree. In general, besides other factors, rootstock is the key component in controlling vigour of the scion cultivar (Saroj *et al*. 1997). Therefore, use of clonal rootstock as quality planting material is utmost essential. Some preliminary attempts were made by using seedlings of different *Psidium* species and varieties which are field tolerant against wilt to see the growth, yield, quality and biotic reactions of component plants but responses were highly variable. This necessitates mass multiplication of clonal rootstocks of *Psidium* species through vegetative

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means, which are hardy against various biotic and abiotic stresses as well as impart dwarf canopy structure of component trees. The appropriate strategy may be *In vitro* multiplication of selected rootstocks and some attempts were made to propagate *P. guajava* with little success (Bajpai *et al*. 2007 and Chandra *et al*. 2007). Thus, traditional technique of multiplication, i e stooling or stool/mound layering still seems to be appropriate, which is least cumbersome, practically feasible and economically viable under field conditions. By this technique, both rootstocks and scion cultivars can be multiplied with high degree of success (Lal *et al*. 2007, Mishra *et al*. 2007). Therefore, present investigation was undertaken to perpetuate *Psidium* species through stooling and assess growth behaviour of clonal rootstocks under subtropical conditions.

MATERIALS AND METHODS

The experiment was carried out at Sardar Vallabh Bhai Patel University of Agriculture and Technology, Modipuram, Meerut (Uttar Pradesh) during 2007–08 to perpetuate *Psidium* species through stooling and assess growth behaviour of clonal rootstocks under subtropical conditions. In the present investigation, five years old stooled plants of different *Psidium* species were headed back in middle of February from the ground level, followed by irrigation. Profuse suckering took place in all species, which were counted before stooling operation. Five *Psidium* species, viz *Psidium cujavillis*, *P. molle*, *P. chinensis*, *P. cattleianum* and *P. friedrichsthalianum* were considered for the study. Before stooling, the plant samples were taken for biochemical (protein, phenol and C:N ratio) and anatomical (length, breadth and intercellular space of sclerenchymatus cells) studies by applying standard methods. The 15 uniform plants planted at 1.5 m apart were headed back in the middle of February, followed by irrigation and weeding at desired intervals were considered as treatment unit. The number of shoots per stool plant was recorded. The under and oversized shoots were thinned out from the stool plants and only desirable shoots (approximately 45–60 cm length and about pencil thickness) were used for stooling purpose. The stooling operation was performed in the month of July. In selected shoots, a complete ring of bark about 2–2.5 cm wide was taken out from the base of shoots and lanolin paste containing 7500 ppm IBA+NAA was smeared thoroughly on and around the portion just above the ring. After 3–4 hr, the shoots were spread gently on the mound and treated portion earthed with well pulverized soil followed by irrigation. After 15 days, second earthing was repeated in order to avoid washing of soils due to heavy rains.

After 45 days of stooling, rooted shoots were taken out carefully from the mother plants with the help of secateurs and observation on percentage rooting was recorded. Thereafter, rooted shoots were planted under field conditions to assess the initial establishment. The meteorological observations at monthly intervals during experimental period were also recorded and depicted as Fig 1. After a month of establishment, ten uniform clonal rootstocks from each species were further planted in earthen pots of 15" size filled with FYM and soil (1:1) for recording observations on linear

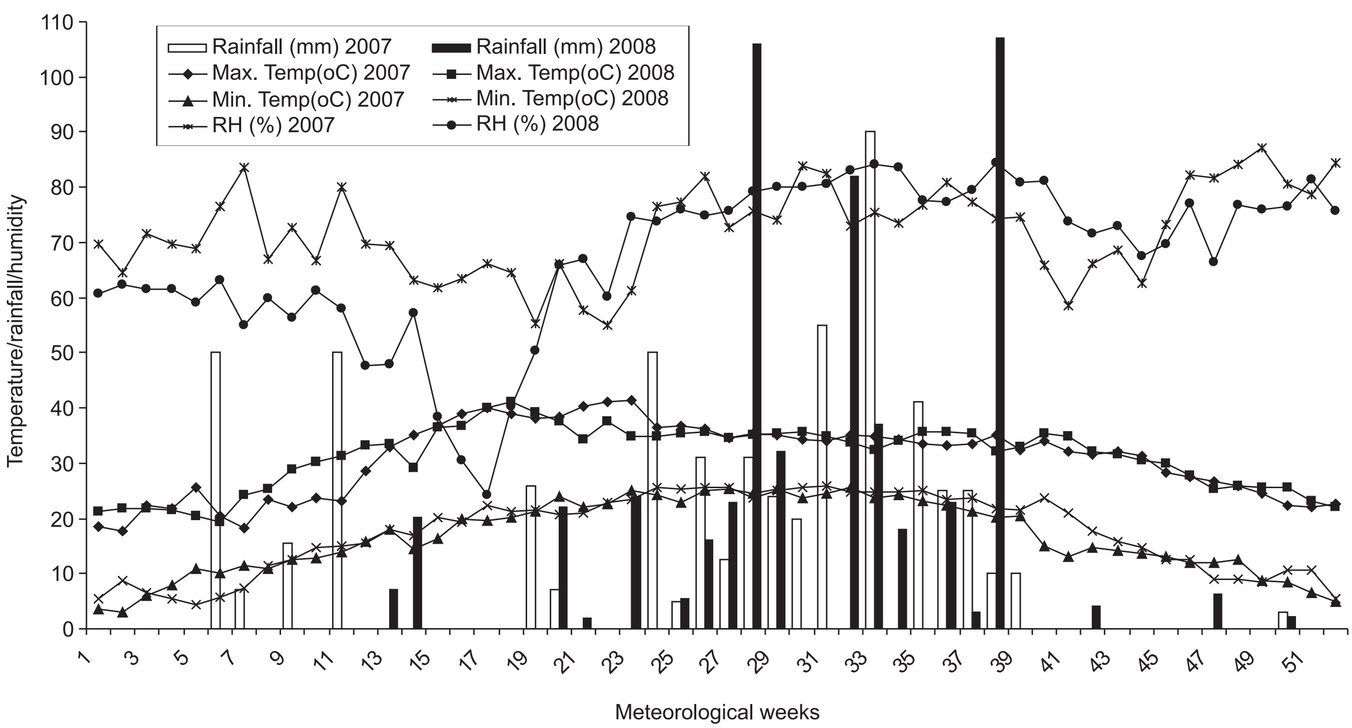

Fig. 1 Meteorological observations during experimental period (2007–08)

plant height and stock girth at monthly intervals continued up to six months while other morphometric parameters such as number of secondary branches, intermodal length, number of leaves/plant, average leaf area and total leaf area/plant were recorded at the termination of the experiment. The data on growth increment of linear plant height and stock girth was computed as expressed in Fig 2. The experiment was laid out in RBD for rooting and field establishment studies while to assess growth behaviour in pot experiment it was in CRD with three replications. Being large variations in various parameters among *Psidium* species, the data were subjected to statistical transformation where ever necessary as suggested by Snedecor (1950).

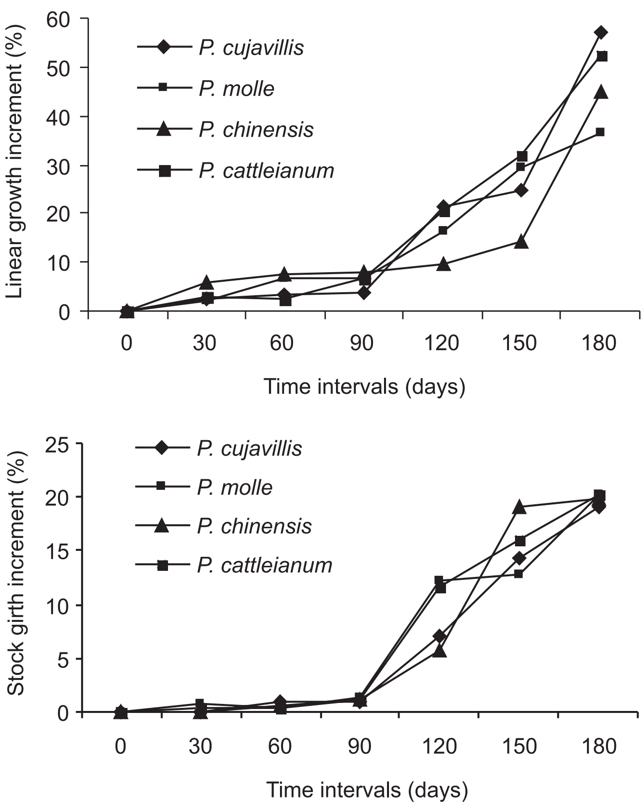

Fig. 2 Linear growth and stock girth increment of different *Psidium* rootstocks at monthly intervals.

RESULTS AND DISCUSSION

Coppicing capacity

High coppicing capacity is desirable character of plants propagated through stooling. The maximum number of shoots/ plant was recorded in *Psidium chinensis* (38.26), followed by *P. cujavillis* (31.35), *P. cattleianum* (31.34), *P. molle* (25.13) and minimum in *P. friedrichsthaleianum* (23.13). The differences among various species with respect to number of shoots/plant varied significantly except in between *P. cujavillis* and *P. cattleianum* (Table 1). However, all shoots were not desirable for stooling operation, thus very thin, small and over sized shoots were removed and only desirable shoots of about 45–60 cm length and about pencil thickness were left for the purpose. It is obvious from the data presented in Table 1 that maximum number of desirable shoots were also recorded in *P. chinensis* (27.33), followed by *P. cujavillis* (18.21), *P. cattleianum* (17.17), *P. molle* (14.52) and minimum in *P. friedrichsthaleianum* (9.12). This indicated that *P. friedrichsthaleianum* is a shy coppicer as compared to other *P.* species. The differences among themselves were highly significant except in between *P. cujavillis* and *P. cattleianum*.

Rooting and establishment

Data presented in Table 1 reveal that profuse rooting was observed in all the *Psidium* species except in *P. friedrichsthaleianum* after treating with IBA+NAA at 7500 ppm. The *P. chinensis* gave cent per cent rooting while in *P. friedrichsthaleianum* only 9.53% rooting was observed. In *P. cujavillis*, *P. cattleianum* and *P. molle* more than 92.72% rooting was recorded. The findings of present investigation indicated that combined application of IBA+NAA is very useful for multiplication of these clonal rootstocks.

The variation in rooting response was further correlated with some biochemical and anatomical parameters (Table 2). Among biochemical parameters, higher total phenol and C:N ratio favoured rooting while total protein content of stooled shoots did not make any impact. In fact, higher level of phenol content combines with endogenous and exogenous level of auxin and makes indol-phenol-complex, which acted at the base of the shoots just above the girdled portion along with enzymes leading to adventitious root formation.

Table 1 Effect of IBA+NAA (7500 ppm) on coppicing capacity, rooting and establishment in *Psidium* species through stooling

Species	Parameters			
	Total number of shoots/ plant	No. of desirable shoots/ plant	Rooting (%)	Establish-ment (%)
Psidium cujavillis	31.35 (5.61)	18.21 (4.33)	96.72 (82.13)	91.17 (72.46)
Psidium molle	23.13 (4.85)	14.52 (3.88)	91.69 (75.19)	84.03 (66.19)
Psidium chinensis	38.26 (6.23)	27.33 (5.28)	100.00 (89.43)	97.13 (83.86)
Psidium friedrichsthalianum	14.10 (3.82)	09.12 (3.10)	09.53 (13.97)	00.00 (0.57)
Psidium cattleianum	31.34 (5.64)	17.17 (4.20)	92.72 (73.20)	87.00 (68.46)
SE (±)	1.32	1.21	0.30	0.27
CD (*P*=0.05)	2.93	2.76	0.83	0.76

Figures in parentheses are transformed values

Table 2 Correlation of rooting versus biochemical and anatomical parameters

Parameter	Rooting (r value)
Biochemical parameters	
Protein content	–0.6690
Phenol content	0.8952*
C:N ratio	0.9935*
Anatomical parameters	
Length of sclerenchymatous cell	–0.6450
Breadth of sclerenchymatous cell	0.4580*
Intercellular space of sclerenchymatous cell	0.9980*

*Significant at *P*=0.05 level

Similarly, higher level of C:N ratio caused by nitrogen starvation favoured the root formation. The beneficial effect of C:N ratio on rooting has already been reported by Mitra and Bose (2001). Moreover, protein is the derivatives of nitrogenous compound which is mainly responsible for vegetative growth, thereby it did not play vital role in the rooting of *Psidium* species.

The microtomic sectioning of rooting zone showed that higher length of sclerenchymatous cells in continuous ring probably the main reason of shy rooting of in *P. friedrichsthaleianum.* Whereas, in all other species, the intercellular space of sclerenchymatous cells was more, this showed discontinuity of rings. Therefore, the anatomical characters like higher intercellular space and breadth of sclerenchymatous cells showed positive correlations in all the *Psidium* species while the longer length of sclerenchymatous showed negative correlation with respect to rooting.

The ultimate success is judged by field establishment of rooted shoots. Profuse and fibrous root system coupled with optimum environmental conditions are prerequisite for field establishment of plants. In present investigation, profuse and fibrous root system was observed in all the *Psidium* species except *P. friedrichsthaleianum*. Therefore, no rooted shoots were established in field conditions in *P. friedrichsthaleianum* while in all other species percentage field establishment was more than 87.00% (Table 1). The highest percentage of field establishment was recorded in *P. chinensis* (97.13), followed by *P. cujavillis* (91.17), *P. cattleianum* (87.00) and *P. molle* (84.03) respectively and differences among themselves were highly significant. The similar results were reported by Jain *et al*. (2003) in litchi and Singh *et al.* (2007) in guava.

Linear growth and stock girth

The observation on vegetative vigour parameters were taken into account only for four *Psidium* species, i e *P. chinensis*, *P. cujavillis*, *P. cattleianum* and *P. molle*, as none of the plants were established in *P. friedrichsthaleianum* under field conditions. Marked differences in linear growth of stooled plants have been recorded at monthly intervals. It is evident from the data presented in Table 3 that there was normal cessation of growth at initial stages due to partial damage of roots of stooled shoots during uprooting from the field and low temperature immediately after transplanting in the pots. However, after 120 days onwards, (after February), the linear growth was accelerated with the commencement of spring temperature (Table 3, Fig 1). The trend of linear growth increment in various species at different intervals was depicted in Fig. 2. At final observation, i e 180 days after

Table 3 Linear growth (cm) and stock girth (cm) of clonal rootstocks of *Psidium* species at different intervals

Interval (days)	*Psidium* species				SEm	CD
	Psidium cujavillis	*Psidium molle*	*Psidium chinensis*	*Psidium cattleianum*	(±)	(*P*=0.05)
Linear growth (cm)						
0	34.59	29.93	25.07	38.60	0.02	0.04
30	35.61	30.52	26.50	39.68	0.09	0.20
60	37.00	32.51	28.50	41.00	0.03	0.07
90	38.40	34.76	30.81	43.89	0.07	0.16
120	46.63	40.44	33.76	53.15	0.15	0.31
150	58.16	52.34	38.53	70.20	0.20	0.42
180	91.35	71.46	55.80	106.90	0.23	0.49
Stock girth (cm)						
0	2.24	2.38	1.85	2.46	0.03	0.07
30	2.24	2.40	1.85	2.47	0.04	0.08
60	2.26	2.41	1.86	2.48	0.04	0.08
90	2.28	2.44	1.89	2.51	0.04	0.08
120	2.44	2.74	2.00	2.80	0.04	0.08
150	2.79	3.09	2.38	3.25	0.03	0.07
180	3.32	3.69	2.89	3.91	0.05	0.10

planting, maximum linear growth was recorded in *P. cattleianum* (106.90 cm), followed by *P. cujavillis* (91.35 cm), *P. molle* (71.46 cm) and minimum in *P. chinensis* (55.80 cm). The differences in linear growth among various *Psidium* species were significant, thus it can be used as an index for assessing vigour of clonal stocks.

The girth of stooled plants was also measured at monthly interval. Significantly higher stock girth was recorded in *P. cattleianum* (3.91 cm), followed by *P. molle* (3.69 cm), *P. cujavillis*(3.32 cm) and minimum in *P. chinensis* (2.89 cm) at final stage of observation. However, the girth increment (Fig 2) was almost nil at initial stages and it was also increased with increasing spring temperature but there was no definite pattern in stock girth at succeeding intervals. Though, higher girth is better for anchorage and health of plant but only stock girth cannot be used as a criterion for assessing vigour of plants. As in present investigation, the stock girth of *P. molle* was more than *P. cujavillis* but plant vigour in term of height was more in *P. cujavillis*. Thereby, vegetative vigour measured in term of stock diameter has little significance in predicting ultimate vigour of plants.

Stock morphometric parameters

To assess the branching pattern of stooled plants, total number of branches were counted and presented in Table 4. The perusal of the data showed significant variation in number of secondary branches/plant. The maximum number of branches was recorded in *P. chinensis* (10.6) which was significantly higher over other *Psidium species.* Whereas, lowest number of branches were recorded in *P. molle* (1.66). More than three branches/plants were recorded in *P. cattleianum* and *P. cujavillis*. This indicated that *P.chinensis* is bushy in nature than others. Regarding inter nodes, shorter inter nodes were recorded in *P. chinensis* (1.46 cm) while more than 4 cm size of inter node were recorded in other *Psidium* species.

The size of leaves in term of average leaf area and number of leaves/plant showed inverse relationship. The *P. molle* showed maximum average leaf area (84.21 cm^2) while *P. chinensis* showed minimum average leaf area (8.11 cm^2) but number of leaves/plant were maximum in *P. chinensis* (807.74) and minimum in *P. molle* (51.83). Whereas, the average leaf area and number of leaves/plant of *P. cattleianum* and *P. cujavillis* were 71.61 cm^2, 69.76 cm^2 and 114.37 and 93.70 respectively. The differences in number of leaves/ plant were largely influenced by number of secondary branches, inter-nodal length and plant height of an individual species. However, total leaf area/plant was highest in *Psidium cattleianum* (8175.29 cm^2), followed by *P. chinensis* (6550.21 cm^2), *P. cujavillis* (6546.62 cm^2) and minimum in *P. molle* (4354.46 cm^2). The highest leaf area in *P. cattleianum* was attributed by relatively larger average leaf area and more number of leaves/plant. Probably, this may be one of the reasons of vigourous nature of this species, where more leaf surface attributed better photosynthetic efficiency. In view of this, linear plant height, number of branches, inter-nodal length and total leaf area may be taken into consideration for assessing vigour of clonal rootstocks of *Psidium* species. Based on relative vigour of stooled plants, these *Psidium* species can be relatively classified as dwarf (*P. chinensis)*, semi-dwarf (*P. molle)* and vigourous (*P. cattleianum* and *P. cujavillis*). Infact, morphological characters are largely govern and influenced by their genetic makeup of an individual species, if grown under uniform conditions. Thus, the variations observed in different morphometric parameters are quite natural but the study facilitated to quantify such relative variations among the species. In conformity to the findings, Prakash *et al*. (2002) have also reported high degree of genetic diversity in guava using RAPD markers.

From this investigation, it can be concluded that *Psidium* species, viz *P. chinensis, P. cujavillis, P. cattleianum* and *P. molle* can be successfully perpetuated through stooling with treatment of 7500 ppm IBA+NAA and these clonal rootstocks based on relative plant vigour can be used for guava scion

Table 4 Morphometric parameters of clonal rootstocks of *Psidium* species

Parameter	*Psidium* species				SEm (±)	CD (*P*=0.05)
	Psidium cujavillis	*Psidium molle*	*Psidium chinensis*	*Psidium cattleianum*		
Number of branches	3.02 (1.88)	1.66 (1.47)	10.06 (3.26)	3.13 (1.90)	0.05	0.11
Internodal length (cm)	4.08 (2.14)	4.22 (2.17)	1.46 (1.40)	4.25 (2.18)	0.05	0.10
Number of leaves/plant	93.70 (9.70)	51.83 (7.23)	807.74 (28.41)	114.37 (10.71)	0.35	0.73
Average leaf area (cm^2)	69.76 (8.37)	84.21 (9.20)	8.11 (2.92)	71.61 (8.48)	0.19	0.40
Total leaf area/plant (cm^2)	6546.62 (80.92)	4358.46 (66.02)	6550.21 (80.94)	8175.29 (19.93)	0.28	0.59

Figures in parentheses are transformed values cultivars. However, there is a need to assess the component plant of desired guava variety for ascertaining various parameters like plant vigour, precocity, bearing behaviour, productivity, quality and other biotic and abiotic influences on long-term basis.

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