RESPONSE OF GUAVA (*PSIDIUM GUAJAVA* L.) SOFTWOOD CUTTINGS TO PACLOBUTRAZOL APPLICATION IN DIFFERENT ROOTING MEDIA

Rashad Qadri, M Tahir Akram, Imran Khan^{*1,4}, Muhammad Azam, Numrah Nisar, M Awais Ghani, Mohsin Tanveer^{1,2} and MM Khan⁴

Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan

Keywords: Paclobutrazol, *Psidium guajava*, Rooting capability, Rooting media, Vegetative propagation

Abstract

The scope of clonal propagation of guava (*Psidium guajava* L.) by soft wood cuttings collected from mature plant is described. The most successful media among those traditionally used and to identify promising alternatives was determined. Soft wood cuttings were done to determine the most successful medium and Paclobutrazol (PBZ) hormone concentration. Guava soft wood cuttings were treated with 0, 200, 400 and 600 mg/l PBZ solution and planted in three different rooting media (sand, silt and top soil) and rooted in low-tunnel. The cuttings were allowed to grow under low-tunnel for three months to assess the rooting capability and shooting competency. The study revealed that the species has a great potential for clonal propagation through soft wood cuttings.

Introduction

Guava (*Psidium guajava* L.) is one of the most important fruits and also used as a traditionally medicinal plant for treatment of diarrhoea, gastroenteritis and analgesic properties. Guava clonal propagation is considered to avoid genetic variety segregation, to maintain fruits quality and have maximum potential for economically crucial trees improvement within less time (Singh *et al.* 2004, Giri *et al.* 2004). Efforts were made to grow guava by layering and cuttings. Layering was successful for guava but is a laborious work. The grafting method is more successful in guava than budding (Khattak *et al.* 2002). Rooting by vegetative means of propagation is no doubt the most expanded and efficient method. Plant growth regulators were also used to increase the growth and shooting capacity. Fruit formation was controlled by the balance between endogenous gibberellic acid and the fruit development to occur (Hedden and Graebe 1985). The level of endogenous gibberellins can be moderated by application of anti-gibberellin biosynthesis such as paclobutrazol to reduce foliage growth (Williams *et al.* 2003).

Paclobutrazol is known to inhibit gibberellin biosynthesis and can cause several physiological changes in plants including increased photosynthetic pigments, improved nutrient uptake, senescence retardation and enhanced flowering and seed yields (Davis *et al.* 1988). PBZ reduces inter-nodal growth of stems but it increases root growth and early fruit set in plants like tomato (Berova and Zalatev 2000). Hafeez *et al.* (1988) obtained 90.11 to 94.44% success in leaf tip cuttings treated with PBZ concentration of 3, 6 and 12 ppm for 24 hrs. Because of several positive effects of PBZ on several tree crops as well as certain annuals, the proposed study was planned to investigate the effects of PBZ on shooting, rooting and survival percentage of softwood cuttings of guava when planted in different rooting media.

^{*}Author for correspondence: <drimran@uaf.edu.pk>. ¹Department of Agronomy, University of Agriculture, Faisalabad, Pakistan. ²School of Land and Food, University of Tasmania, Hobart, Tasmania, Australia. ³Department of Environmental Sciences, Lahore College for Women University Lahore, Lahore, Pakistan. ⁴Department Crop Sciences, College of Agriculture and Marine Sciences, Sultan Qaboos University, PO. box 34, AL-Khod 123, Sultanate of Oman.

Materials and Methods

An experiment was conducted during 2014 at Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Highly productive and phenotypically superior healthy mother plants of local guava cultivar Gola (6 - 7 years old) having excellent bearing and fruit quality were selected from Post-graduate Agricultural Research Station (PARS), Faisalabad. Apical shoot cutting at juvenile stage of 12 cm long, each with 2 - 4 nodes carrying 2 - 4 pairs of leaves, were taken during the study. After collection, cuttings were thoroughly washed and their bark was cut off on the lower portion with budding knife to facilitate callusing process. After preparation basal portion of cuttings was quickly dipped in copper oxychloride (1 g per litre of water) solution for 30 sec to avoid any fungal infection and kept in open air for 5 min. The cuttings were then treated with 0, 200, 400 and 600 mg/l PBZ. 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 lowtunnel. Before using, all rooting media were sun sterilized under the polyethylene for 2 weeks in order to dry and kill harmful pathogens. The plastic tunnel had a covering of 0.10 mm thick poly sheet. A total of 432 cuttings were planted in 36 plots, 9 plots for each replication (0, 200, 400 and 600 mg/l PBZ 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-tunnel. A light spray was done every morning with a hand spray till the transfer of rooted cuttings from the low-tunnel.

The root capability of cutting was measured by examining development of number of roots per cutting, root length, root fresh weight, and root dry weight at 90 days after planting (DAP). The shooting competency of guava cuttings was examined through measuring number of sprouts per cutting, sprouting length, shoot height and number of leaves per cutting at 90 DAP. The experiment was laid out according to completely randomized block design (CRBD) with factorial arrangement. The data collected was analyzed statistically using analysis of variance (ANOVA) and difference among the treatments means were compared by applying Tukey's HSD test at 5% level of probability (Abdi and Williams 2010). Graphs were made using Sigma plot v.10. Error bars in graph are representing standard error values for each treatment.

Results and Discussion

A significant effect of different rooting media and PBZ application treatments was observed on rooting capability of cutting as illustrated by measuring number of roots per cutting, root length, root fresh weight and root dry weight (Table 1). Among rooting media, highest roots per cutting were in silt and lowest roots were observed in sand. Among PBZ treatments, the highest number of roots per cutting was observed in the cuttings treated with 600 mg/l PBZ solution and the lowest was in cuttings without PBZ treatment (Fig. 1). No root formation was observed in the sand medium with 600 mg/l PBZ and control treatment. Interaction between rooting media and PBZ concentrations revealed the highest root per cutting was observed in cutting plated in silt with 600 mg/l PBZ concentration. Furthermore, longest root length was obtained in sand with 200 mg/l PBZ treatment followed by with 400 mg/l PBZ solution in the silt medium. Root fresh and root dry weight was also significantly influenced due to different rooting media and PBZ concentration treatments. Maximum root fresh and dry weight per cutting was observed in cutting treated with 600 mg/l PBZ solution and in silt medium, while statistically similar results were observed when cutting were treated with other two concentrations of PBZ as compared with control (Fig. 1).

Treatments	Number of	Root length	Root fresh weight	Root dry weight
	roots	(cm)	(mg)	(mg)
Rooting media (RM)				
Sand	$1.95 \pm 0.27c$	$3.49 \pm 0.33c$	$351.58\pm8.82c$	$139.04 \pm 8.53c$
Silt	$12.67 \pm 0.96a$	$5.97\pm0.40b$	$737.97 \pm 28.66b$	$301.77 \pm 17.99b$
Top soil	$6.87\pm0.50~b$	$6.47\pm0.23a$	858.91 ± 33.94a	$372.08 \pm 17.57a$
LSD value at 5%	0.87	0.43	36.19	18.36
Paclobutrazol (mg/l)				
0	$3.08 \pm 0.13c$	$4.18\pm0.12b$	334.61 ± 33.21d	$143.39 \pm 14.74c$
200	$6.45 \pm 0.43b$	$6.39 \pm 0.51a$	$674.74 \pm 27.38c$	$285.43 \pm 13.39b$
400	$6.83 \pm 0.74 b$	$6.63\pm0.48a$	$753.33 \pm 15.43b$	$331.61 \pm 19.30a$
600	$10.72 \pm 0.99a$	$4.04\pm0.19b$	835.28 ± 19.21a	$323.44 \pm 11.36a$
LSD value at 5%	1.12	0.54	46.20	23.44
Interaction $RM \times PBZ$	* Fig. 1	* Fig. 1	* Fig. 1	* Fig. 1

Table 1. Effect of different rooting media and paclobutrazol concentrations on different parameters related to rooting capacity of guava cuttings.

Means in a vertical column sharing a common letter do not differ significantly at 5% probability level.

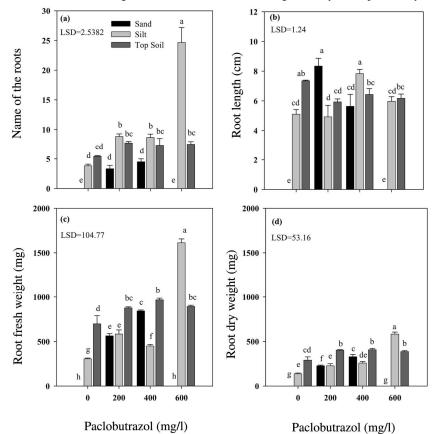


Fig. 1. a-b: Effect of different growth media on number and length of roots of guava. c-d: Effect of different paclobutrazol concentrations on rooting competency (fresh and dry weight) of guava softwood seedlings under different growth media.

Similar results were also reported by Hafeez et al. (1988) who indicated that root length increased at low concentration of PBZ while root length significantly reduced under high concentration of PBZ. The results of this study are in accord with those of Darwesh et al. (2013) who reported that growth regulators help in stimulating the roots from cuttings when the endogenous hormone levels and climatic factors are favorable. An increase in fine roots as a result of PBZ application may improve plants to recover after root injury (Watson 2004). PBZ induced increase in number of roots per plant and fresh and dry weight might be due to: (1) the inhibition of gibberellin production and (2) increase in increased carbohydrate supply to roots resulting from reduced demand for growth above ground. Besides the application of PBZ, rooting medium also influenced the rooting capability, as obtained during this study indicated highest number of roots observed in silt media followed by top soil while highest fresh and dry weight and root length was noted in top soil followed by silt medium. Least rooting capability of guava was noted in sand medium. Better root development in top soil might be due to better soil microenvironment to the roots, better availability of nutrients and proper aeration. On other hand interactive effects of PBZ and rooting media gave very surprising results, showing that dose dependent effect of PBZ under different rooting medium e.g. higher root length was in sand media when treated with 200 mg/l PBZ while higher number of roots and root fresh weight were under top soil medium and PBZ of 600 mg/l.

According to ANOVA (Table 1), rooting media and PBZ treatments showed significant effects on number of sprouts per cutting, sprouting length, shoot height and number of leaves. It was noted that PBZ treatment gave highest number of leaves per cutting grown in silt. However, the cutting treated with 0 and 600 mg/l PBZ solution and grown in sand medium did not produce any leaf (Fig. 2). On other hand, highest number of sprouts per cutting was observed where cuttings treated with 400 mg/l PBZ solution and grown on top soil and sand (Table 4). However, no sprouts were observed on cuttings grown in sand medium and treated with 0 and 600 mg/l PBZ

Treatments	Number of leaves	Number of sprouts	Sprouting length (cm)	Shoot height (cm)
Rooting media (RM)				
Sand	$2.87 \pm 0.37c$	$0.78 \pm 0.07c$	$2.39\pm0.05b$	$12.60 \pm 0.28c$
Silt	$8.19 \pm 0.52a$	$1.43\pm0.40b$	$2.74 \pm 0.21a$	$19.95 \pm 0.43a$
Top Soil	$5.54\pm0.61b$	$1.79 \pm 0.36a$	$1.99 \pm 0.16c$	$17.72\pm0.49b$
LSD Value at 5%	0.62	0.35	0.19	0.49
Paclobutrazol, PBZ (mg/l)				
0	$3.66 \pm 0.50c$	$1.00 \pm 0.19c$	1.13±0.21c	$11.86 \pm 0.28d$
200	$6.64 \pm 0.61a$	$1.51\pm0.26b$	3.21±0.20a	$20.12\pm0.60b$
400	$6.17 \pm 0.55 ab$	$2.05 \pm 0.48a$	3.39±0.06a	$22.15 \pm 0.36a$
600	$5.67\pm0.35b$	$0.77 \pm 0.19c$	1.77±0.09b	$12.90 \pm 0.36c$
LSD value at 5%	0.79	0.45	0.24	0.63
Interaction $RM \times PBZ$	* Fig. 2	* Fig. 2	* Fig. 3	* Fig. 2

 Table 2. Effect of different rooting media and paclobutrazol concentrations on several parameters related to shooting capacity of guava cuttings.

Means in a vertical column sharing a common letter do not differ significantly at 5% level.

solution. Interactive effect of rooting media and PBZ concentration further showed that highest sprout length was obtained in the sand with 200 mg/l PBZ treatment followed by with 400 mg/l PBZ solution in the silt medium (Fig. 2). No root formation was observed in the sand medium with 600 ppm PBZ and control treatment. Shoot height per cutting was also influenced by rooting media and PBZ treatments (Table 1). Maximum and highest number of shoot was observed where cuttings treated with 200 mg/l PBZ solution, respectively when grown in sand (Fig. 2).

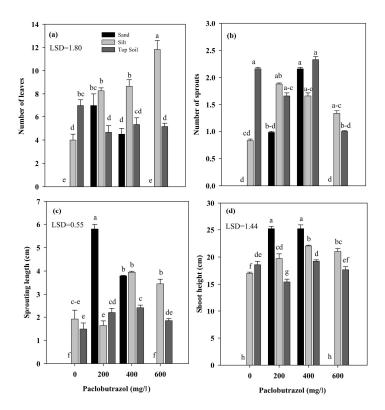


Fig. 2. a-b: Effect of different growth media on number of leaves and number of sprouts of guava. c-d: Effect of different paclobutrazol concentrations on sprouting length and shoot height of guava softwood seedling..

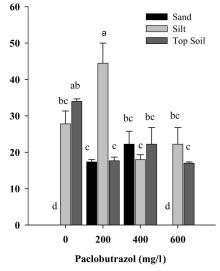


Fig. 3. Effect of different paclobutrazol concentrations on sprouting percentage of guava softwood seedling under different growth media.

Lower concentration of PBZ increased shoot growth. This might be due to previous reported effects of PBZ such as (1) increase in cell division, (2) increase in number of chloroplast with concomitant higher photosynthetic activity, and (3) greater metabolic activity. Further PBZ suppressed gibberellin biosynthesis associated with alteration in other hormones important for axillary bud initiation. It was also indicated that PBZ application at high concentration induced reduction in shoot height this was due to the thickening of cell wall in phloem fiber caps, decrease in xylem ring width and inter-fascicular supporting tissues (Burrows *et al.* 1992). Limited effects of PBZ on shoot were also reported in apple (Ma *et al.* 1990), peach (Erez 1986) apricot (Jacyna *et al.* 1989) and citrus (Aron *et al.* 1985).

Rooting media differ significantly for the survival percentage of guava soft wood cuttings. The highest survival percentage of guava cutting was achieved in silt when applied PBZ significantly improved the survival percentage of guava cutting. The interactive effect of both factors also had pronounced effect on the survival percentage of guava cuttings. Moreover, no cutting was survived when the cuttings were grown on sand and treated with 0 and 600 mg/l of PBZ solution. Likewise sprouting percentage was higher under low level of PBZ and phenomenon behind this might be associated with PBZ induced more translocation of carbohydrates to sprouting buds, while reduced cell proliferation due to PBZ treatment was also found to be responsible for restricted shoot growth (Baloch 1994). After inducing roots these growth regulators may have direct effect on sprouting which needs to be explored (Rahman *et al.* 2004).

Treatment of guava cutting with PBZ suggested that its application could increase the guava growth and survival at lower concentration. Higher PBZ concentration may induce toxic effects on shoot growth and sprouting. PBZ application on silt shows better effects followed by top soil application as compared to sand culture. Thus PBZ application at low concentration on silt as growth medium proved to be effective in increasing guava growth and establishment.

References

- Abdi H and Williams L J 2010. Tukey's Honestly Significant Difference (HSD) Test, Neil Salkind (Ed.), Encyclopedia of Research Design, Thousand Oaks, CA.
- Aron Y, Monselise SP, Goren R and Costo J 1985. Chemical control of vegetative growth in citrus trees by paclobutrazol. Hort. Sci. 20: 96-98.
- Baloch AF 1994. Phases of Plant Growth. In: Malik MN, Horticulture. 2nd edn., National Book Foundation, Islamabad, Pakistan.
- Berova, M and Zlatev, Z 2000. Physiological response and yield of paclobutrazol treated tomato plants (*Lycopersicon esculentum* Mill.). Plant Growth Regul, **30**: 117-123.
- Burrows GE, Boag TS and Stewart WP 1992. Changes in leaf, stem, and root anatomy of *Chrysanthemum* cv. Lillian Hoek following paclobutrazol application. J. Plant Growth Reg. **11**: 189-194.
- Darwesh RS, Madbolly EA and Gadalla EG. 2013. Impact of indole butyric acid and paclobutrazol on rooting of date palm (*Phoenix dactylifera* L.) off-shoots cultivar Zaghloul. J. Hort. Sci. Orn. Plants 5: 145-150.
- Davis TD, Steffens GL and Sankhla N 1988. Triazol plant growth regulators. Hort. Rev. 10: 151-188.
- Erez A 1986. Growth control with paclobutrazol of peaches grown in meadow orchard system. Acta Hort. 160: 217-224.
- Giri C, Shyamkumar B and Anjaneyulu C 2004. Progress in tissue culture, genetic transformation and applications of biotechnology to trees. Trees Struct. Funct. 18: 115-135.
- Hafeez UR, Khan MA, Niazi ZM, Khan DA and Rehman C 1988. Rooting of different types of guava stem cutting using growth regulator. Pak. J. Agri. Res. 9: 363-365.

- Hedden P and Graebe JE 1985. Inhibition of gibberellin biosynthesis by paclobutrazol in cell-free homogenates of *Cucurbita maxima* endosperm and *Malus pumila* embryos. J. Plant Growth Regul. 4: 111-122.
- Jacyna T, Sparrow SM and Dodds KG. 1989. Paclobutrazol in managing mature cropping apricot trees. Acta Hort. 240: 139-142.
- Khattak MS, Inayatullah M and Khan S 2002. Propagation of guava from semi hard wood cuttings. Frontier J. Agri. Res. B. (1): 81-92.
- Ma FW, Wang JC and Rong W 1990. Effect of plant growth regulators on *in vitro* propagation of apple cultivar Fuji. J. Fruit Sci. 7: 201- 206.
- Rahman N, Tehsinullah, Nabi G and Jan T 2004. Effect of different growth-regulators and types of cuttings on rooting of guava (*Psidium guajava* L). Q. Sci. Vision **9**: 1-4.
- Singh M, Jaiswal U and Jaiswal VS 2004. *In vitro* regeneration and improvement in tropical fruit trees. Srivastava, Narula and Srivastava (Eds.). Plant Biotech. and Mol. Markers. p. 228-243. Anamanya Publishers, New Delhi.
- Watson GW 2004. Effect of transplanting and paclobutrazol on root growth of 'green column' black maple and 'summit' green ash. J. Environ. Hort. 22: 209-212.
- Williams DR, Potts BM and Smethurst PJ 2003. Promotion of flowering in *Eucalyptus nitens* by paclobutrazol was enhanced by nitrogen fertilizer. Can. J. For. Res. 33: 74-81.

(Manuscript received on 18 July, 2017; revised on 19 January, 2018)