COMPARISON ON THE EFFECT OF TREATMENT AND SUBCULTURING ON SHOOT REGENERATION FROM SHOOT TIP SEEDLINGS OF *Psidium guajava* L. var. Beaumont

Tajul A. Abdullah[1], Maheran A. Aziz *[2], Azmi A. Rashid[2], Ghizan Saleh[3], S.M.A. Elhory[2], Syaiful B Panjaitan[2], Hailmi M.S. [1] and Noor Muzamil M.[1]

[1] Department of Biotechnology, Faculty of Agriculture and Biotechnology, Universiti Darul Iman, Kampus Kota, Jalan Sultan Mahmud, 20400 Kuala Terengganu, Terengganu, Malaysia,[2]Department of Agriculture Technology, [3]Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, E-mail*: maheran@agri.upm.edu.my

ABSTRACT

An experiment was carried out in order to study the effect of treatment containing 0.5 and 1.0 mg/L 6-benzylaminopurine (BAP) and effect of subculture on percentage of shoot formation, number of shoot per explant and mean shoot height of shoot tip explant of *Psidium guajava* L. var. Beaumont. MS medium (Murashige and Skoog, 1962) was used with 30 g/L of sucrose and 7g/L agar type 900. Shoot tips of elongated shoots were decapitated to abolish its apical dominance prior to sub-culturing was carried out to a fresh medium every 4 weeks. The increase in mean number of shoots per explant and the shoot height was observed for every subculture. Treatment 1.0 mg/L BAP in comparison to treatment 0.5 mg/L BAP showed higher mean number of shoot per explant per subculture whereas treatment 0.5 mg/L BAP showed higher mean shoot height per subculture. Data were analyzed using T-test until the fifth subculture.

INTRODUCTION

Psidium guajava L. is a plant under the Myrtaceae family. It is now widely distributed throughout the tropic and sub tropic areas and grows up to 7 m high. Its fruits are rich in vitamin C and it is consumed fresh or processed into jam and juice (Morales *et al.,* 1994; Lozoya *et al*., 1994). Guava fruits may be round, oval or pear-shaped. The peel color of the ripe fruit is yellow while the flesh color may be white, pink, yellow, salmon, or carmine, depending on the variety (Chan, 1993). The pulp and peel of *Psidium guajava* L. both showed high content of dietary fiber of 48.55−49.42% and polyphenols antioxidants of 2.62−7.79% (Escrig *et al*., 2001)

Among the major problems encountered in guava cultivation in Malaysia is the occurrence of plant diseases such as scabby canker, stylar-end ring rot, antrachnose and root knot disease caused by nematode (Bourke, 1988; Lim and Khoo, 1990, Nik and Vijaysegaran, 1992). The diseases may cause a reduction in guava productivity thus there is a need to produce new varieties that are resistant to such diseases. However, breeding through conventional method is slow due to the perennial nature of the crop. An alternative approach, which can reduce the time taken to genetically improve the crop is through the non-conventional breeding technique *via* genetic trans-formation. Before such technique could be implemented, an efficient *in vitro* plantlet regeneration protocol needed to

be developed. The aim of this study was to evaluate the effect of BAP and subculture on shoot regeneration from shoot tip explants of *Psidium guajava* var. Beaumont.

MATERIALS AND METHODS

A comparison of T-test between two treatments, 0.5 and 1.0 mg/L BAP, was conducted to determine the best level of BAP for shoot regeneration from shoot tip explant of *P. guajava* L. var. Beaumont. In this experiment, both treatments were performed under a homogenous environment for accurate comparison.

Seeds of *P. guajava* L. var. Beaumont were extracted from the fruit, washed under running tap water for 10 minutes and blotted dry. The seeds were immersed in 37% HCl for 5 minutes to break their dormancy and then rinsed with sterile distilled water for five times. The seeds were immersed in liquid MS medium (Murashige and Skoog, 1962) supplemented with 30 g/L sucrose, 3 mg/L GA3 and 2 mg/L BAP for 5 days to further break their dormancy. Finally the seeds were placed in conical flasks containing MS medium supplemented with 30 g/L sucrose, 7 g/L agar and 2.0 mg/L BAP for germination. Twelve to fifteen seeds were placed in each sterile conical flask containing 50 ml of the germination medium. Seedlings which were eight week old were then transferred on MS medium supplemented with 30 g/L sucrose and 7 g/L agar but without any growth regulators and maintained for another 2 weeks. Shoot tips reaching 0.3 to 0.5 cm in length, were excised from the 10 week-old seedlings and used for the study. The shoot tip were cultured vertically in jars with five explants in a jar. The basal medium employed was MS medium supplemented with 30 g/L sucrose and solidified with 7 g/L agar Type 900. Fifty milliliter of the medium was dispensed into 6 cm x 8 cm jar and was covered with heavy duty aluminum foil. All media were adjusted to pH 5.8$\pm$0.01 before autoclaving at 121°C and 108Kpa for 20 minutes. Cultures were incubated at 25$\pm$2°C with 16 hours photoperiod from cool, white fluorescent light providing an intensity of 25 μmol m-2 s-1. The relative humidity in the culture room was maintained at 50-70%.

Each treatment was replicated three times and each treatment per replication contained 10 explants. Data were recorded at every subculture of four weeks interval whereby the shoot tips were decapitated and transferred onto fresh medium of the same composition. The experiments were carried out until the 20th week. The percentage of explants with shoot, number of shoots produced per explant and shoot height attained (cm) were determined.

RESULTS AND DISCUSSION

Shoot tip explants of guava cultivar Beaumont placed on MS medium supplemented with 0.5 and 1.0 mg/L BAP started to elongate after two weeks of culture. By the third week of culture, the first pair of leaves opened while callus emerged at the basal cut end of the shoot. On the sixth week, axillary branching occurred in some of the cultures.

T-test carried out indicated a significant difference between 0.5 mg/L BAP and 1.0 mg/L BAP on percentage of explant producing shoot, mean number of shoots produced per explant and shoot height from the first till the fifth subculture (Figure 1 A-C). The

results revealed that MS medium supplemented with 0.5mg/L and 1.0mg/L BAP showed 100% shoot formation from the first subculture (week 4) until the fourth subculture (week 16). However, in the fifth subculture (week 20), treatment 1.0 mg/L BAP exhibited a reduction to 93.33% shoot formation while treatment 0.5mg/L BAP maintained 100% shoot formation.

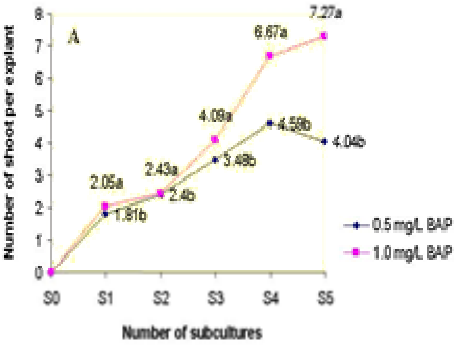
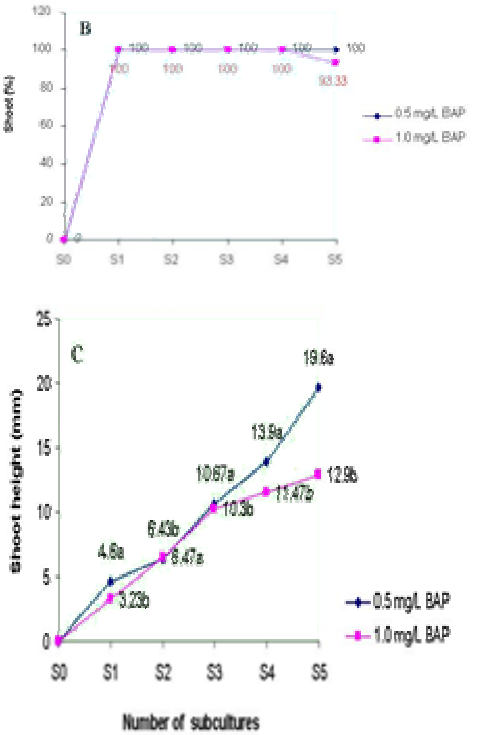


Figure-1: Effect of 0.5 mg/L and 1.0 mg/L BAP on **(A)** percentage of shoot formation, **(B)** mean number of shoot produced per explant and (**C**) mean height (mm) of shoot formed from shoot tip of *Psidium guajava* L. var. Beaumont **from the** first until thel fifth subculture. Note: Values are means of 3 replications. Means with the same alphabet are not statistically different at P≤0.05 based on the T-test analysis.

Referring to Figure 1B, at week 4 (first subculture), treatment 1.0 mg/L BAP produced 2.05 mean number of shoots per explant and differed significantly from treatment 0.5 mg/L BAP which produced 1.81 mean number of shoots per explant. However, at subculture 1 treatment 0.5mg/L BAP produced significantly higher shoot height (4.6 mm) in comparison to treatment 1.0mg/L BAP (3.23mm) (Fig. 1C). At week 8 (second subculture), treatment 1.0 mg/L BAP produced significantly higher mean number of shoot (2.43) and shoot height (6.47mm) in comparison to treatment 0.5mg/L BAP which produced 2.40 mean number of shoot per explant and 6.43 mm shoot height (Figures 1 B & C).



In the third subculture (week 12) both treatments 0.5 mg/L and 1.0 mg/L BAP showed an increasing trend from the previous week on both number of shoots produced per explant and shoot height attained. Treatment 0.5 mg/L BAP produced 3.48 mean number of shoots per explant and 10.67mm of shoot height while treatment 1.0 mg/L BAP produced 4.09 mean number of shoot per explant and attained 10.30 mm shoot height (Figures 1 B & C). Figures 2 A & B show shoot formation from shoot tip

explant of *P. guajava* L. var. Beaumont on 0.5 and 1.0 mg/L BAP respectively, in the third subculture.

The increasing trend which occurred in the third subculture continued into the fourth subculture for both BAP treatments (Figures 1 B & C). Treatment 0.5 mg/L BAP showed an increase to 4.59 mean number of shoots per explant and 13.90 mm shoot height at subculture 4, while Treatment 1.0 mg/L BAP produced significantly higher values of 6.67 mean number of shoots per explant and 11.47 mm shoot height. Figure 2C and D shows shoot formation from shoot tip explant of *P. guajava* L. var. Beaumont on 0.5 mg/L and 1.0 mg/L BAP respectively in the fourth subculture.

In the fifth subculture (week 20) treatment 1.0mg/L BAP produced the highest number of shoots per explant (7.27) compared to treatment 0.5 mg/L BAP (4.04). However, treatment 0.5 mg/L BAP produced the highest shoot height (19.6 mm) in comparison to 12.93mm shoot height attained in treatment 1.0 mg/L BAP (Figures 1B & C). This study is in agreement with a study of Shekafandeh and Khosh-Khui (2008) who achieved the highest shoot height of *Psidium guajava* L. var. Local-1 and Local-2 using nodal explants showed highest shoot length on MS and WPM medium supplemented with 0.5 mg/L BAP. Figure 2E and F show shoot formation from shoot tip explant of *P.*

In comparing the effect of 0.5 mg/L and 1.0 mg/L BAP with subculture on and 1.0mg/L BAP respectively, in the fifth subculture. Raziudin *et al*., 2004). The effect of subculture on mean number of shoot produced per explant and the shoot height, as carried out in this study , is another factor that could be considered in increasing the shoot multiplication of *P. guajava* L. var. Beaumont, it was observed that treatment containing 1.0mg/L BAP produced significantly higher mean number of shoot per explants, whereas treatment 0.5 mg/L BAP, however the difference are non significant. However, treatment 0.5mg/L BAP produced longer shoots. Shoot tips were decapitated at each subculture and this could have accounted for the increase in number of shoots produced per explant. Orlikowska *et al*., (1999) stated that the removal of leaf and apex and the correct shoot orientation of *Codiaeum variegatum* cv. Excellent increased the number of axillary shoots produced. Our result on the effect of sub- culture on shoot regeneration of *Psidium guajava* L. var. Beaumont indicated the increasing trend in the mean number of shoots produced per explants and shoot height was proportional to the number of subculture. This is in parallel with the reports of Santarem and Astarita (2003) on *Hypericum perforatum* L. where by the subculture of shoot-forming explants on to a proliferation medium containing 4.5mmol/L BAP promoted organogenic response, regardless of the treatment used for shoot induction. In previous researches on micropropagation of guava, the effects of different factors such as genotype, explant type, culture medium composition and different growth regulator concentrations were demonstrated by others (Singh *et al*., 2001, 2002; Zamiri *et al*., 2003 and efficiency of *in vitro* regeneration of guava.

In this study no blackening of the media was experienced when shoot tip explants derived from *in vitro* seedlings were cultured. Yasseen *et al.* (1995) reported that stem nodes derived from

seedlings of *P. guajava* L. do contain some phenols that caused browning but showed higher regeneration capacity compared to explants derived from mature trees.

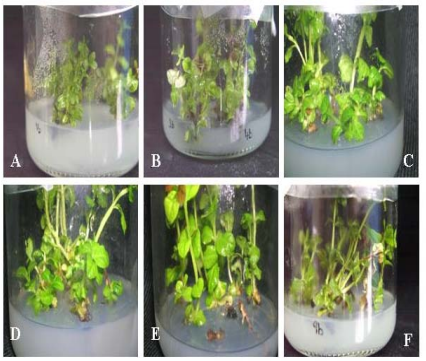


Figure-2: Shoot proliferation from shoot tip explant of P. *guajava* L. var. Beaumont on MS medium + 0.5 mg/L BAP **(A) a**nd on MS medium + 1.0mg/L BAP **(B)** at subculture 3; Massive shoot proliferation on MS medium + 0.5mg/LBAP **(C)** and MS medium + 1.0mg/L BAP **(D)** at sub-culture 4;Massive shoot proliferation on MS medium + 0.5 mg/L BAP **(E)** and MS medium + 1.0 mg/L BAP **(F)** at subculture 5.

CONCLUSION

Massive shoot proliferation could be induced from shoot tips of P. *guajava* L. var. Beaumont. Treatment 1.0mg/L BAP was more effective in producing higher shoot number per explant, while treatment 0.5mg/L BAP resulted in improved shoot height, with subculture. In addition, decapitation of the shoot tips prior to each subculture enhanced axillary shoot proliferation.

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