

## ***In vitro* Propagation of Guava (*Psidium guajava* L.): Effect of Antioxidant, Nutrient Media and Growth Regulators**

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**Abstract:** The current research was carried out during (2016-2017) season to develop an *in vitro* propagation protocol for guava explants from mature trees growing in the experimental orchard of Pomology Department, Faculty of Agriculture, Cairo University. The experiment was carried out to investigate the effects of citric and ascorbic acids combination and PVP on media browning during establishment stage. In addition, the effect of nutrient media composition ( $\frac{1}{2}$ MS, WPM and  $\frac{1}{2}$ DKW) and BAP concentration at 1, 2 or 3 mg L<sup>-1</sup> on multiplication rate and shoot growth was examined. The most effective method to control browning problem involved supplementation of culture media with citric and ascorbic acids; this method enhanced shoot induction up to 50.90% with 1.3 shoots per explant. The highest multiplication rate was recorded on  $\frac{1}{2}$ MS medium; while  $\frac{1}{2}$  DKW medium recorded the highest shoot length. BAP at 3 mg L<sup>-1</sup> recorded the highest value for number of shoots/explant, shoot length and number of leaves/shoot. Guava micro-shoots were successfully rooted on  $\frac{1}{2}$ MS medium supplemented with different auxin types. IBA+NAA recorded the highest rooting percentage as well as the highest root length. Dipping shoot base in IBA solution recorded the highest root number.

**Abbreviations:** Murashige and Skoog medium (MS) • Woody plant medium (WPM) • Driver and Kuniyuki walnut medium (DKW) • 6-benzylaminopurine (BAP) • Indole-3-butyric acid (IBA) • Naphthaleneacetic acid (NAA) and polyvinylpyrrolidone (PVP)

**Key words:** *Psidium guajava* • Micropropagation • Anti-browning • Growth regulators

### **INTRDUCTION**

Guava tree (*Psidium guajava* L.) belongs to the Myrtaceae family, considered as an important fruit crop of tropical and subtropical areas [1, 2]. Guava fruit is rich with carbohydrates, vitamins (C and A), Ca, P, Fe and pectin [3]. It also has high antioxidants content [4]. Guava usually propagated by seeds, therefore the originated seedlings often do not maintain the genetic purity of the propagated cultivars [5]. Different clonal propagation methods including cutting, grafting and air-layering did not provide satisfied results for guava propagation; moreover micropropagation allows rapid multiplication and production of pathogen-free plants [6]. Micropropagation of guava has been achieved by explants from both juvenile seedling and mature trees [5, 7]. However, proliferation of mature tissue is more difficult than tissues from juvenile sources [8]. Leaching of phenolic compounds and media browning are the major problems associated with *in vitro* culture of explants from

mature trees [9-13]. Several methods have been used to overcome the browning problem including pretreatment with citric and ascorbic acid solution, adding citric and ascorbic acid, activated charcoal or PVP in culture media and incubation of cultured explants under dark conditions [14-18].

Media composition has been reported to affect all types of morphogenic responses, including axillary bud proliferation and shoot growth [19-20]. MS medium [21] with full-strength or half-strength has been successfully used for guava micropropagation [13, 22]. Moreover, a satisfactory result have been reported with Rugini olive medium [7] and WPM medium [14-15]. Concerning the effect of growth regulators, BAP is commonly used for guava micropropagation; it produces high proliferation rate and improves shoot growth [7]. Rooting in guava was obtained with IBA or NAA at different concentrations but *in vitro* rooting percentage still low [23 - 24]. Moreover, Zamir *et al.* [12] and Xiaomei and Yang [13] indicated that guava rooting may be induced by dipping in (IBA)

solution and culture in auxin free medium. Hence, the aim of the present study was to establishment an efficient micropropagation protocol for guava explants from mature trees through overcome phenolic browning problem as well as increasing the proliferation and rooting capacities.

## MATERIALS AND METHODS

**Plant Material:** The plant materials for the current research were collected from a 30 year old seeded guava tree, which was previously selected and characterized in the experimental orchard of Pomology Department, Faculty of Agriculture, Cairo University [25]. Active growing shoots from a single tree were collected in the morning during July and August of 2016-2017 season. Guava shoots were immediately brought to the laboratory; shoots were stripped of leaves, washed with tap water and divided into nodal cuttings. Surface sterilization performed with commercial bleach (5.25% sodium hypochlorite) for 10 min, followed by mercury chloride at 1000 mg L<sup>-1</sup> for 5 min, then washed with sterile distilled water.

**Starting Stage and Antioxidant Treatments:** Nodal explants were cultured on ½MS medium supplemented with 30g L<sup>-1</sup> sucrose, 1mg L<sup>-1</sup> BAP and 6.5g L<sup>-1</sup> agar. The culture media was supplemented with one of the following antioxidants (control (without antioxidant), 0.5% PVP or a mixture of 150 mg L<sup>-1</sup> citric acid and 100 mg L<sup>-1</sup> ascorbic acid). Four weeks later the percentage of explants developing new shoots, number of shoots per each explant, length of new shoots (cm) and number of leaves per explant were recorded.

**Multiplication Stage:** To examine the effect of media composition and BAP concentration on proliferation rate, the sprouted buds were transferred to one of the following media; ½MS [21], WPM medium [26] or ½DKW medium [27]. Each medium was supplemented with three different concentration of BAP (1, 2 or 3 mg L<sup>-1</sup>). Sub-culture was performed every four weeks. All culture media combinations supplemented with 30 g L<sup>-1</sup> sucrose and 6.5 g agar L<sup>-1</sup>, media pH was adjusted to 5.8 before adding agar and autoclaved at 121°C for 15 min. All cultures were maintained in growth chamber at 25°C and 16h photoperiod (provided with 40-60µmol m<sup>-2</sup> s<sup>-1</sup> cool-white fluorescent lamps). Multiplication rate, shoot length (cm) and number of leaves per shoot were recorded at the end of 3<sup>rd</sup> sub-culture.

**Rooting Stage:** Shoots of the 3<sup>rd</sup> sub-culture (2-3 cm) were used for rooting treatments. Two auxin types (IBA and

NAA) and two rooting methods were examined. The single phase rooting method consisted of culturing guava shoots on ½MS medium supplemented with one of the following auxin treatments; 2mg L<sup>-1</sup> of IBA, NAA, combination of IBA+NAA. The two phase rooting method proposed by Liu and Pijut [28] consisted of dipping shoot base in 250mg L<sup>-1</sup> solution of IBA or NAA for 30s, then transferred to ½MS medium without growth regulators. Darkening of rooting media was performed with 1g L<sup>-1</sup> active charcoal. Two months later; rooting percentage, number of roots and root length were recorded.

**Statistical Analysis:** The treatments were arranged in a complete randomized design with three replicates for each treatment, data were subjected to variance analysis according to Snedecor and Cochran [29] and means were compared according to Duncan's multiple range tests at 1% level [30].

## RESULTS AND DISCUSSION

**Starting Stage:** The data illustrated in Table (1) indicated that antioxidants treatments were effective for controlling the problem of phenolic compounds and its toxicity to growing explants. Among the tested antioxidants, citric and ascorbic acids combination had significantly increased survival percentage (50.90%), followed by PVP (42.30%), while control treatment recorded the lowest survival rate (39.50%). Moreover, both antioxidant treatments improved number of shoot per explant, shoot length and number of leaves per shoot compared with control.

As reported previously, media browning due to phenolic compounds is the major problem of guava micropropagation. Media browning results from the oxidation of phenolic compounds, released from the cut ends of the explants by polyphenoloxidase (PPO) [23]. Several methods have been proposed to overcome browning problem, e.g. treatment with citric acid, ascorbic acid and PVP [9, 13, 16]. Browning can be avoided by inactivation of PPO; antioxidants such as citric and ascorbic acids prevent enzymatic browning by inhibit PPO activity [31].

**Multiplication Stage:** Data presented in Table (2) and Figure (2) showed that nutrient media and BAP concentration had an obvious effect on multiplication rate of guava. Guava shoots grown on ½MS medium recorded the highest multiplication rate (3.94) compared with both of WPM and ½DKW media. However, ½DKW recorded

Table 1: Effect of anti-oxidant treatments on survival percentage and growth of guava explants during starting stage

Treatments	Survival%	No. of shoots per explant	Shoot length (cm)	No. of leaves/ shoot
Control	39.50 b	1.00 c	2.56 b	3.10 b
Ascorbic + citric acid	50.90 a	1.30 a	4.10 a	5.80 a
PVP	42.30 b	1.10 b	3.10 ab	2.90 b

Means followed by the same letter within each column are not significantly different at  $p < 1\%$

Table 2: Effect of nutrient media and BAP concentration on multiplication rate, shoot length and leaf number/shoot of guava

Nutrient media	BAP (mg L <sup>-1</sup> )			Mean
	1	2	3	
<b>Multiplication rate</b>				
½ MS	2.85 d	4.21 b	4.76 a	3.94 A
WPM	2.11 e	2.91 cd	3.33 c	2.78 B
½ DKW	1.52 f	1.82 ef	3.36 c	2.23 B
Mean	2.16 C	2.98 B	3.82 A	
<b>Shoot length (cm)</b>				
½ MS	2.10 e	3.20 cd	3.40 c	2.90 B
WPM	1.90 e	3.10 cd	2.90 d	2.63 B
½ DKW	4.10 b	4.50 a	4.80 a	4.46 A
Mean	2.70 B	3.60 A	3.70 A	
<b>Leaf number/shoot</b>				
½ MS	4.80 d	7.10 b	8.50 a	6.80 A
WPM	3.90 e	4.00 e	5.00 d	4.30 C
½ DKW	6.00 c	7.00 b	5.00 d	6.00 B
Mean	4.90 B	6.03 A	6.16 A	

Means followed by the same letter within each column are not significantly different at  $p < 1\%$

the highest shoot length (4.46 cm). ½ MS medium recorded the highest number of leaves per shoot (6.80) followed by ½DKW medium (6.00), while WPM medium recorded the lowest value (4.30). Increasing BAP concentration in the growth medium increased multiplication rate and shoot length. The highest multiplication rate was recorded with 3 mg L<sup>-1</sup> BAP (3.82) compared with the other two concentrations.

As previously reported, nutrient media composition has a direct effect on proliferation rate [19-20]. The obtained results showed that, ½MS medium has been successfully used for guava micropropagation as reported by Xiaomei and Yang [13] and Usman *et al.* [22]. Shoot growth on ½DKW medium proved to be superior to WPM. This could be due to the higher level of calcium, nitrogen and sulfur in the ½DKW medium [32]. BAP is the most common cytokinin used for guava propagation, BAP improved percentage of bud sprouting and stimulating the formation of axillary shoots [12]. The differences in BAP effects on multiplication rate and shoot growth on different media types might be related to the effect of mineral composition on sensitivity of explants to plant growth regulators [33]. The hypothesis that minerals affect cell sensitivity to plant growth regulators is supported by several studies [34-36].

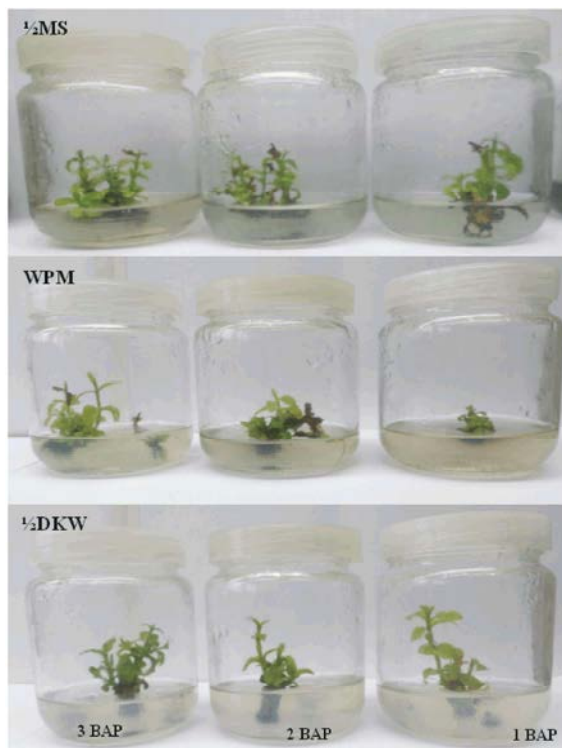


Fig. 1: Effect of nutrient media and BAP on multiplication rate and shoot length of guava shoots

**Rooting Stage:** According to the data presented in Table (4) and Figure (2), both types of auxin strongly influenced rooting parameters at the end of the rooting period. However, IBA showed better results for rooting of guava shoots; since it produced higher rooting percentage as compared with NAA. While the highest rooting percentage (62.67 %) was observed with IBA+NAA combination treatment. There were significant differences between different auxin treatments regarding the mean number of roots per shoot and the root length. Dipping shoot base in IBA solution recorded the highest number of roots (2.85 roots/shoot), while IBA+NAA treatment recorded the highest root length (4.20 cm).

Data revealed that there were insignificant differences between auxin treatment methods (dipping shoot base in auxin solution *vs.* addition of auxin to culture medium) for both types of auxin. The obtained results are in agreement with Roussos *et al.* [37] who reported that combination of

Table 3: Effect of different auxin type and rooting methods on rooting percentage, number of roots and root length of guava shoots

Auxin treatment	Rooting (%)	Number of roots/ shoot	Roots length (cm)
IBA <sup>1</sup>	60.00 a	2.16 cd	2.90 c
IBA <sup>2</sup>	55.00 a	2.85 a	3.40 b
NAA <sup>1</sup>	19.00 b	2.30 c	2.70 c
NAA <sup>2</sup>	25.00 b	2.00 d	1.60 d
IBA +NAA	62.67 a	2.60 b	4.20 a

Means followed by the same letter within each column are not significantly different at  $p < 1\%$ . <sup>1</sup>(single phase rooting), <sup>2</sup>(two phase rooting).

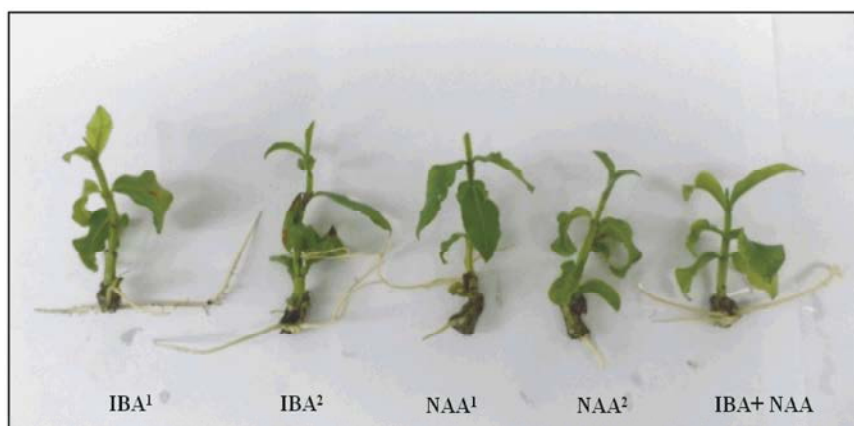


Fig. 2: Effect of auxin type and rooting methods on guava rooting, <sup>1</sup>(single phase rooting), <sup>2</sup>(two phase rooting)

IBA and NAA has proved to be superior over the use of each auxin alone. Moreover, Tanimoto [38] reported that IBA is more effective in rhizogenesis as compared to NAA. These differences may be due to the continuous release of IAA from IBA [39 -40]. Furthermore, the inferior effect of NAA on the root number may be due to the reason that NAA remains present in the tissue and may block further root development [41].

### CONCLUSION

On the basis of the obtained results, it can be concluded that, media browning may be avoided by adding citric and ascorbic acids in the starting medium. The multiplication rate of guava was better on ½MS or ½DKW supplemented with 3 mg L<sup>-1</sup> BAP. Enhanced rooting was obtained in medium supplemented with combination of both IBA and NAA.

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