Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
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Asian Journal of Plant Sciences 7 (2): 177-182, 2008
ISSN 1682-3974

Effects of Bud Position and Culture Medium on Shoot Proliferation from Nodal Culture of Two Mature Guava Cultivars

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Abstract: In this research, the rate of shoot proliferation from different nodes in different positions on shoot [uppermost node (N_1), the second node (N_2) and the third node (N_3)] from two mature guava cultivars, white fleshed Local-1 and red fleshed Local-2 on different culture media (MS and WPM) with different BA concentrations was investigated. The results showed that in both cultivars, the best BA concentration was 1.5 mg L^{-1}. There was a significant interaction between bud positions, culture media and cultivars on shoot proliferation and shoot length. In Local-1 maximum shoot number (2.86) was recorded from buds in N_2 position on MS medium followed by 2.43 shoots/explant from buds in N_2 positions on WPM medium. In Local-2 on both medium the buds in N_1 position had significantly a higher number of shoots/explant (1.57 on MS and 1.86 on WPM) than the buds in N_3 positions (0.43 on MS and 0.71 on WPM). Shoot length, had a similar trend.

Key words: Bud position, node, shoot proliferation, culture media

INTRODUCTION

Guava (*Psidium guajava* L.), a member of the Myrtaceae, commonly known as the apple of the tropics is a tropical fruit tree of great economical importance. The guava tree is native to tropical America, but it is cultivated in every tropical and subtropical country of the world (Samson, 1986; Yadava, 1996; Bose and Mitra, 1993) including Southern parts of Iran, in the provinces of Hormozgan, Systan and Bluchestan.

Guava plant is cultivated successfully in a wide range of growing conditions and also in regions which are not suitable for cultivation the other tropical plants, because it is hardy, drought tolerant and withstand soil pHs ranging from 4.5 to 8.5 (Khattak *et al*., 1999).

For extending the guava cultivation in these areas a rapid and efficient method for clonal propagation of elite mature genotype is necessary. During two past decades, emerging biotechenique for tissue culture and micropropagation of superior guava cultivars have been discussed by several researchers (Amin and Jaiswell, 1987, 1988; Ali *et al*., 2003; Fuenmayor and Montero, 1997; Joshee *et al*., 2004; Khattak, *et al*., 2002; Loh and Rao, 1989; Yasseen *et al*., 1995; Papadatau *et al*., 1990; Canhoto and Cruz, 2000; Meghwal *et al*., 2001, 2003). These researchers investigated the effects of different factors such as the kinds and various concentrations of growth regulators, culture media and explant types for improving the efficiency of shoot performance and proliferation.

In this research, for optimization the rate of shoot proliferation, the effects of bud positions on shoot in relations to culture media and different BA concentrations on nodal segments culture of two elite mature cultivars of guava were investigated.

MATERIALS AND METHODS

Current season shoots (12-20 cm) from field grown adult trees of guava (*Psidium guajava* L.), white-fleshed cultivar Local-1 and red-fleshed cultivar Local-2 in Minab Agricultural Research Center in Hormozgan province of Iran were collected in March 2006. The shoots were transferred to Laboratory of Department of Horticultural Science, Siraz University. Nodal segments of 2-2.5 cm long consisting of two lateral buds were used as explants. They were washed for at least 45 min with tap water containing a few drop of dish washer detergent and then they were treated in 4 g L^{-1} benelate for 30-45 min and then rinsed 2 times with sterilized distilled water. For controlling the phenolic exudation, the nodal segments were placed in solutions of 100 mg L^{-1} ascorbic acid and citric acid each shaken using an orbital shaker (60 g) for 45 min. Then the nodal segments were treated in 200 mg L^{-1} Mercuric Chloride (MC) in vacuum for 2 min. For controlling bacteria contamination the explants soaked in a 500 mg L^{-1} gentamycin solution for 2 h followed by 15% Golrang (a commercial detergent with 5.25% NaClO) for 15 min. After 4 rinses with sterilezed water, they were used in different experiments.

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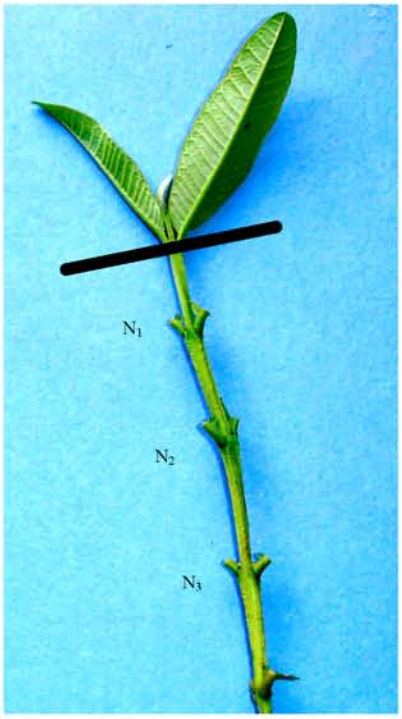

Fig. 1: Designation of different nodes on a shoot

To study the effect of Benzyl Adenine (BA) on shoot proliferation of two mentioned guava cultivars, (Murashige and Skoog, 1962) with different concentrations of BA (0, 0.5, 1 and 1.5 mg L^{-1}) were tested.

In our preliminary study using shoot tips and nodal segments of two Iranian guava cultivars as explants, we noticed that in both cultivars, shoot tip and the nodes from different parts of the shoot responded differently for contamination, exudation of phenolic compound, starting to grow and shoot proliferation. To investigate the effect of bud position on shoot proliferation, after removing shoot terminal buds of Local-1 the nodes were labeled N_1, N_2 and N_3 [uppermost node (N_1), the second node (N_2) and the third node (N_3)]. The distance between two nodes was at least 3 cm (Fig. 1). The explants were cultured on MS or WPM (woody plant medium) (Lloyd and McCown, 1980) supplemented with different concentrations of BA (0, 0.5, 1, 1.5 and 2 mg L^{-1}).

To study the effect of bud positions, culture media and cultivars on shoot proliferation rate, different nodes (N_1, N_2 and N_3) of two cultivars were cultured on MS and WPM media containing 1 mg L^{-1} BA.

All media were supplemented with 3% sucrose (MERCK, LGaA 64271 Darmstadt, Germany) and solidified by 0.7% agar-agar (MERCK, LGaA 64271 Darmstadt, Germany). The pH of the media was adjusted to 5.7±0.05 prior to autoclaving at 1.2 atm pressure, 121°C temperature for 20 min. All cultures were maintained at 25±2°C with 16 h photoperiod of 35-40 µmol m^{-2} sec^{-1} provided by cool white fluorescent lamps.

Each experiment was carried out as a factorial in a completely randomized design with different replications in different experiments and number of explants per treatment specified in the bottom of each table. Data were collected after 6 weeks and then analyzed using SPSS statistical software (SPSS Inc., Chicago. USA). The means were compared using Duncan's multiple range tests (DMRT).

RESULTS

In both cultivars with increasing BA in culture media the number of shoots/explant were increased. In all BA concentrations the number of shoots/explant produced in Local-1 were significantly higher than those produced in Local-2. Best shoot proliferation rate (3.55 shoots/expant) was obtained in 1.5 mg L^{-1} BA in Local-1 which was significantly higher than 1.45 shoots/explant in Local-2 (Table 1).

Interaction between different BA concentrations and two cultivars showed that in both cultivars the highest shoot length was obtained in 0.5 mg L^{-1} BA (3.17 cm in Local-1 and 2.47 cm in Local-2) and with increasing the BA concentration the shoot length was decreased (Table 1).

The effects of bud positions and different BA concentrations on nodal segments behavior of Local-1 on WPM medium is shown in Table 2. The main effect of bud positions regardless of BA concentrations showed that shoot-forming nodes in N_2 positions were 65.1% which were significantly higher than 33.38% nodes in N_1 positions and 48.5% in N_3 positions. The nodes in N_2 positions also proliferated more shoots (2.1 shoots/ explant) than nodes in N_1 (1.2 shoots/explant) and N_3 (1.5 shoots/explant) positions. These shoots obtained an average length of 7.2 cm versus 5.8 and 5.5 cm in N_1 and N_3 positions, respectively. Generally, addition of different BA concentrations in culture media increased percentage of shoot-forming nodes and shoot proliferation rate (Table 2).

Interaction between bud positions and different BA concentrations showed the percentage of shoot-forming buds (100%) in media containing 0.5 and 1.5 mg L^{-1} BA which was significantly higher than control (19.2%). The buds in N_2 positions and 1.5 mg L^{-1} BA formed 3 shoots/explant which was significantly higher than

Table 1: Effects of different concentrations of BA on shoot number and shoot length from nodal segments of two mature guava cultivars Local-1 and Local-2†

	Cultivars		
BA (mg L^{-1})	Local-2	Local-1	Mean
Average shoot No. per explant			
0.0	0.60cd	0.50d	0.55C
0.5	0.80cd	1.55bc	1.18B
1.0	1.05bcd	1.90b	1.48B
1.5	1.45bcd	3.50a	2.48A
Mean	0.98B	1.86A	
Average shoot length per explant (cm)			
0.0	1.87ab	1.70b	1.79B
0.5	2.47ab	3.17a	2.82A
1.0	1.94ab	2.34ab	2.14AB
1.5	1.52b	1.96ab	1.74B
Mean	1.95B	2.29A	

†Results based on 3 replications each with 4 explants. ††Means in each column or row with the similar letter(s) are not significant at 5% level of probability using DMRT

Table 2: Effect of bud position and different concentrations of BA on the nodal segments proliferation from mature guava tree Local-1 on WPM medium†

	BA (mg L^{-1})					
	Bud position					
Node	0.0	0.5	1.0	1.5	2.0	Means
Percentage of shoot-forming explants						
N_1	28.2ab	42.0ab	39.1ab	34.1ab	35.8ab	35.38B
N_2	19.2b	100a	82.1ab	100a	62.7ab	65.1A
N_3	28.3ab	51.8ab	66.1a	58.6ab	47.44ab	48.5AB
Means	24.5B	61.9A	61.9A	58.8A	48.5AB	
Average shoot No./explant						
N_1	0.8de	1.4bcd	1.3bcd	1.2cde	1.3cde	1.2B
N_2	0.7e	2.9ab	2.2abc	3.0a	2.0abcd	2.1A
N_3	0.8de	1.6bcd	1.8bcde	1.6bcd	1.5bcde	1.5B
Means	0.8B	1.9A	1.8A	1.9A	1.6A	
Average shoot length/explant (cm)						
N_1	9.1ab	5.0ab	6.0ab	3.2ab	5.0ab	5.8B
N_2	5.2ab	9.0ab	8.3ab	9.5a	5.0ab	7.2A
N_3	3.0b	5.6ab	5.7ab	6.0ab	7.2ab	5.5B
Mean	5.8A	6.5A	6.7A	6.0A	5.8A	

†Results based on 4 replications each with 3 explants. ††Means in each column or row with the same small or capital letter(s) are not significant at 5% level of probability using DMRT

1.2 and 1.6 shoots/explant obtained in N_1 and N_3, respectively (Fig. 2a). Although, in other concentrations of BA buds in N_2 positions had higher shoots/explant, they were not significantly different from buds in positions N_1 and N_3. On MS medium, the main effect of bud positions on explant growth revealed that the nodes in N_1 and N_2 position produced significantly higher percentage of shoot-forming buds (75.2 and 87.9, respectively) than nodes in N_3 positions (43.1) (Table 3). Regardless of the position of buds on shoots, with increasing the BA concentration from 0 to 2 mg L^{-1}, the percentage of shoot-forming nodes increased and reached at maximum 81.4% in 2 mg L^{-1} which was significantly higher than control (44.4). In the same manner, the number of shoots/explant produced in N_1 and N_2 positions (2.18 and 2.43, respectively) were higher than in N_3

Table 3: Effects of bud position and different concentrations of BA on the nodal segments proliferation from mature guava tree cultivar Local-1 on MS medium†

	BA (mg L^{-1})					
	Bud position					
Node	0.0	0.5	1.0	1.5	2.0	Mean
Percentage of shoot-forming explants						
N_1	39.2abc	89.13ab	89.13ab	100a	89.13ab	75.2A
N_2	47.1abc	100a	100a	100a	100a	87.9A
N_3	47.1abc	18.9c	54.3abc	28.7bc	54.3abc	43.1B
Mean	44.4B	71.8AB	71.8AB	78.7A	81.4A	
Average shoot No./explant						
N_1	0.8d	2.abcd	3.0a	3.0a	2.10ab	2.18A
N_2	1.1cd	3.0a	3.0a	2.43ab	2.60a	2.43A
N_3	1.2bcd	0.8d	2.43ab	0.9d	1.8abcd	1.45B
Mean	1.0C	2.16B	2.84A	2.16B	2.17B	
Average shoot length/explant (cm)						
N_1	6.1abc	10.0a	7.8abc	9.4a	9.2ab	8.5A
N_2	7.7abc	10.9a	6.0abc	7.8ab	7.8abc	8.2A
N_3	6.0abc	4.1bc	7.8abc	3.3c	6.1abc	5.6B
Mean	6.6A	9.2A	7.2A	7.0A	7.8A	

†Results based on 4 replications each with 3 explants. ††Means in each column or row with the same small or capital letter(s) are not significant at 5% level of probability using DMRT

position. From N_1 downward to N_3, the average shoot length/explant decreased significantly from 8.5 to 5.6 cm.

There was a significant interaction between bud positions and different concentrations of BA on percentage of shoot-forming explants, proliferation rate and shoot length/explant. In 0.5 mg L^{-1} BA, the percentage of shoot-forming explant in N_1 and N_2 positions (89.13 and 100, respectively) was significantly higher than N_3 (18.9). In 1.5 mg L^{-1} BA, all buds in N_1 and N_2 positions produced shoot which was significantly higher than the number of buds forming shoot in N_3 positions (28.75%). Similarly, in 0.5 and 1.5 mg L^{-1} BA, the number of shoots produced in N_1 and N_2 positions (from 2-3 shoots/explant) was higher than those produced in N_3 position (0.8 to 0.9 shoots/explant) and also in the same BA concentrations and in N_1 and N_2 positions the shoot length was higher (7.8 to 10.9 cm) than in N_3 position (4.1 and 3.3 cm).

The results showed significant interaction between bud position, culture medium and cultivar on shoot proliferation and shoot length (Table 4). In Local-1 maximum shoot number (2.86) was recorded from buds in N_2 position on MS medium followed by 2.43 shoots/explant from buds in N_2 positions on WPM medium and they were significantly higher than the shoots produced from the buds in N_1 and N_3 positions in MS and WPM media. Shoot length had the same trend. In Local-2 the buds in N_1 position obtained significantly higher number of shoots/explant (1.57 on MS and 1.86 on WPM) than the buds in N_3 positions (0.43 on MS and 0.71 on WPM) on both medium. In the same manner they had higher shoot length.

Fig. 2: Different stages of guava micropropagation (a) shoot proliferation from bud in N_2 position of guava Local-1 (b) rooting and (c) an acclimatized plant in pot

Table 4: Effects of bud position, culture medium and cultivar on shoot proliferation and length of guava mature tree nodal segments†

	Local-1		Local-2		
Node	MS	WPM	MS	WPM	Mean
Average shoot No. per explant					
N_1	1.43cde	1.29cdef	1.57bcde	1.86bc	1.54B
N_2	2.86a	2.43ab	0.86def	1.71bcd	1.96A
N_3	1.29cdef	1.00cdef	0.43f	0.71ef	0.86B
Mean	1.86A	1.57A	0.952B	1.43AB	
Average shoot length per explant (cm)					
N_1	1.43d	4.30bcd	6.64bc	7.21ab	4.89A
N_2	8.43a	8.14a	0.78d	3.14bcd	5.13A
N_3	2.43bcd	2.14cd	1.79cd	1.43d	1.95B
Mean	4.10A	4.90A	3.10A	3.93A	

†Results based on 7 replications and 2 explants per culture vessel. ††Means in each column or row with the similar letter(s) are not significant at 5% level of probability using DMRT

Plantlets obtained in all experiments were successfully rooted, acclimatized and transferred to the soil (Fig. 2b, c).

DISCUSSION

In both cultivars, with increasing BA concentrations the number of shoot/explant increased and shoot length decreased. This may be due to competition among shoots in absorption of growth regulator and nutrient from the medium (Ali *et al*., 2003).

In Local-1 and in all BA concentrations, shoot number and length were higher than those in Local-2. This might be explained in term of genetic variation between the two cultivars. The effects of different cultivars on shoot proliferation was reported by others researchers (Majumder and Mukherjee, 1972; Yasseen *et al*., 1995; Singh *et al*., 2001, 2002). Shoot proliferation in guava has been proposed with application of a single cytokinin with varied success (Amin and Jaiswal, 1987, 1988; Loh and Rao, 1989; Papadatau *et al*., 1990). In this

research, the addition of BA in culture medium induced buds to start regeneration in different positions. In Local-1 and on WPM medium, the buds in N_2 positions obtained the best proliferation rate in all BA concentrations, while on MS medium the buds in N_1 position regenerate shoots as well as buds in N_2 position. Comparing the effects of different bud positions of two cultivars (Local-1 and Local-2) on two culture medium and on proliferation showed that in Local-1 the best proliferation obtained from buds in N_2 position whereas in Local-2 the buds in N_1 position produced the best proliferation rate (Table 4). The contradictory results was probably due to the diverse physiological conditions prevailing in the explants used (e.g., endogenous levels of plant growth regulators and different kind and concentrations of nutrient in culture media) (Yadav *et al*., 1990). In previous researches in guava micropropagation the effects of different factors such as explant, genotype, culture medium compositions and different growth regulator concentrations were demonstrated by others (Raziuddin *et al*., 2004; Singh *et al*., 2001, 2002; Zamiri *et al*., 2003). Therefore, the bud positions on shoot is another factor that might be considered for increasing the efficiency of guava micropropagation.

CONCLUSION

Both guava cultivars under investigation were successfully micropropagated on WPM medium with different concentrations of growth regulators. It was found that there was a significant interaction between bud positions, culture media and cultivars for shoot proliferation and shoot length.

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