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Research Article

In Vitro Micropropagation Using Nodal Segments from Germinated Seeds of Guava

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Abstract:

A study to micro-propagate guava (*Pisidium guajava*) was carried out in 2020 and 2021 at the Tissue Culture Laboratory, Horticulture Department, Faculty of Agriculture, Tanta University. Nodal segments from in vitro germinated seeds of guava were used as explants. Nodal segments (from germinated seeds) were cultured on MS media supplemented with different concentrations of benzyl adenine (BA), kinetin (KIN), or thidiazuron (TDZ) (0, 1 and 2 mg/L). 2 mg/L BA was the most favorable treatment for multiplication of shoots (21.13 shoots/explant) compared to the other treatments and the control (plant growth regulators free medium). Shoots of suitable length were transferred to the rooting media containing (0, 0.5, or 1 mg/L) IBA. The medium containing 0.5 mg/L IBA gave the highest number of roots/plantlets and the longest roots. Under greenhouse for acclimatization, the plantlets of guava derived from the medium supplemented with 0.5 or 1 mg/L indole butyric acid recorded the highest survival percentage (80%). It can be concluded that added 2 mg/L of BA in the medium gave the highest number of shoots/explant during the multiplication stage of guava shoots. In addition, added 0.5 or 1 mg/L IBA during the rooting stage increased the number of roots/plantlets with recorded a highest survival percentage after five weeks of acclimatization stage.

1. Introduction

Guava (*Psidium guajava* L.) belongs to Myrtaceae family. It is grown in tropical and subtropical regions (Khattak et al., 1999). Guava originated mostly in America, Asia, and Australia and more recently in the European Mediterranean region. Wide varieties of meteorological and edaphic conditions are suitable for guava growth (Tzatzani et al., 2023). The total area under cultivation for guava in Egypt is around 39326 feddans, of which 33883 feddans are fruiting areas producing 304378 tonnes with an average of 16.703 tonnes per feddan. (FAO, 2021), Guava is a very valuable fruit with proven nutritional and medical benefits (Tzatzani et al., 2023). While air layering, shoot and root cuttings, grafting and budding have also been found to successfully propagate guava, seeds are still the most common method (Jaiswal and Amin, 1992). In Egypt, the primary means of guava multiplication is sexual propagation; consequently, this primary source of genetic variation causes degradation and the emergence of undesirable cultivars (Ali et al., 2003 and Salama, 2015). For guava clonal propagation, in vitro propagation is an effective technique when combined with traditional methods (Shah et al., 2008). However, several issues with guava explant in vitro cultures have been documented, such as microbial contamination and browning or blacking of the culture media as a result of phenolic compounds leaching, low proliferation phase multiplication rate, and cultural decline marked by necrotic spots and yellowing of the leaves (Amin and Jaiswal, 1987, 1988 and Idris and Mahdi, 1996). As a result, we turn to micropropagation and the current study aimed to develop a protocol for the quick clonal propagation of guava from in vitro germinated seeds.

2. Materials and Methods

2.1. Plant material

The present study was conducted using nodal segments from *in vitro* germinated guava seeds during the two seasons (2020 and 2021) at the Tissue Culture Laboratory, Horticulture Department, Faculty of Agriculture, Tanta University.

2.2. Treatments and Experimental design

The effect of three cytokines benzyl adenine (BA), kinetin (KIN), or thidiazuron (TDZ) at two concentrations (1 or 2 mg/L) compared to the control (plant growth regulators free medium) was studied in a complete randomized design. Eight replicates were used of each treatment and four nodal segments were cultured in each replicate.

2.3 Establishment of non-contaminated cultures

Mature guava fruits were collected to separate

the seeds, which were then carefully cleaned, and surface sterilized for five minutes using 70% ethanol. Seeds were cultured aseptically into 300 ml sterilized glass jars, containing 20 ml of half-strength MS medium (Murashige and Skoog, 1962) supplemented with sucrose 30 g/l and solidified with 7 g/l agar agar. The pH of the media was adjusted to 5.7 before autoclaving at 121ºC and 1.2 kg/cm² for 20 min. All manipulations of sterilization were carried out under laminar airflow. Cultures were incubated at the incubator at 25±2 ºC and 16/8 photoperiod.

2.4 Multiplication stage

Nodal segments (3-4 cm) were aseptically excised from 30-day-old seedlings and cultured on full-strength MS medium containing 30 g/l sucrose and solidified with 7 g/l agar agar. Before the media were autoclaved at 121º C and 1.2 kg/cm² for 20 minutes, its pH was adjusted to 5.7 and growth regulators were added. Cultured jars were kept at 25±2ºC with a 16-hour photoperiod and 1500 lux of light intensity from cool white fluorescent lamps.

Benzyl adenine (BA), kinetin (KIN), or thidiazuron (TDZ) at 1 mg/L or 2 mg/L were added to the multiplication media in addition to the control (plant growth regulators free medium). The Number of shoots/explant and average shoot length were recorded 30 days after culturing the explants.

2.5 Rooting stage

The shoots formed on the multiplication medium were excised and moved to the root induction media, which contained MS at half strength (50%) enhanced with 20 g/L sucrose, 7 g/L agar, and varying indole butyric acid (IBA) concentrations (0, 0.5, 1 or 1.5 mg/L). Eight replicates of each treatment, with three shoots per replicate, were used. Cultured jars were incubated according to the previously stated culture conditions. The count of roots per shoot was recorded 30- days following the culture date.

2.6 Acclimatization stage

Roots were thoroughly cleaned with distilled water to get rid of any medium residue. Then, rooted shoots were cultured in small pots filled with a potting mixture of peat moss, perlite and sand (1:1:1 v/v). Pots were transferred to the greenhouse conditions (30±2°C and 75±5% relative humidity) for hardening. Survival percentage was recorded after five weeks.

2.7 Statistical analysis

The obtained data of all treatments were statistically analyzed by analysis of variance (ANOVA) using the MSTAT-C statistical package. Then means were compared using Tukey's honestly significant difference (HSD) test at probability ≤ 0.05 (Tukey, 1949).

3. Results and Discussion

3.1. Multiplication stage

Effect of benzyl adenine (BA), kinetin (KIN) and thidiazuron (TDZ) on the number of shoots/explant

Table 1 indicates that there were significant differences in the number of shoots/explant between the tested treatments. The highest number of shoots/explant after 30 days was obtained on the medium supplemented with 2 mg/L BA followed by 1 mg/L BA (21.13 and 15.50 shoots/explant, respectively). While medium supplemented with 1 or 2 mg/L TDZ recorded 9.13 and 9.63 shoots/explant, respectively. On the other hand, the lowest number of shoots/explant was obtained on the medium supplemented with 1 mg/L Kin (5.50 shoots/explant). In accordance with current results, Mohamed et al. (2022) tested different concentrations of BA (0, 2, 4, 6, 8 and 10 mg/L) during the multiplication stage of Paulownia species and they found that the number of shoots/explant significantly increased with increasing BA concentration.

Regarding the average length of shoots, the highest average length of shoots was obtained on the medium supplemented with 1 mg/L Kin (4.68 cm) followed by the plant growth regulators free medium (4 cm) followed by the medium supplemented with 2 mg/L Kin (3.26 cm). While the lowest average length of shoots was obtained on the medium supplemented with 2 mg/L TDZ (1.43 cm). Increasing the concentrations of cytokinin has negative effects on the average length of shoots. Habib et al., (2016) revealed that medium supplemented with 3 or 5 mg/L benzyl amino purine increased number of shoots and shoot length in liquid and solid mediums compared to control during multiplication stage. BA showed significant negative effects on shoot length as reported by Ali et al. (2003) who indicated that increasing BA concentration has led to a reduction in the shoot length of guava which in agreement with current results.

Table (1): Effect of benzyl adenine (BA), kinetin (KIN), or thidiazuron (TDZ) on the number of shoots/explant and average shoot length after 30 days culturing the explants

Treatments	Number of shoots/explant	Average length of shoots (cm)
Control	7.25 fg	4.00 b
1 mg/L BA	15.50 b	2.78 cd
2 mg/L BA	21.13 a	2.34 de
1 mg/L Kin	5.50 gh	4.68 a
2 mg/L Kin	6.50 fg	3.26 c
1 mg/L TDZ	9.13 de	2.15 e
2 mg/L TDZ	9.63 c	1.44 f

Means followed by the same uppercase letters are not significantly different at level P≤0.05 according to Tukey's HSD test.

3.2 Rooting stage

Effect of different concentrations of indole butyric acid (IBA) on number of roots/plantlet

Statistical analysis for the number of roots/plantlet for guava shoots cultured *in vitro* on

MS medium supplemented with different concentrations of IBA showed highly significant differences between the treatments. It is clear from data in Table 2 that the average number of roots/plantlet decreased with increasing the IBA concentration. IBA at 0.5 mg/L had a remarkable average number of roots (3 roots/plantlet) followed by the medium supplemented with 1 mg/L IBA (2.88 roots/plantlet) compared to the control. These results are in the same line with those of Ayad (2012) who stated that 0.5 mg/L IBA+2.0 mg/L gibberellin (GA_3) increased shoot length and reduced the number of shoots of peach (*Prunus persica* L). Also, Harb et al. (2015) observed that the medium containing 1.0 mg /l of both IBA and NAA gave the highest root and shoot numbers in jackfruit.

Table (2): Effect of different concentrations of indole butyric acid (IBA) on the number of roots/plantlet 30- days following the culture date

Treatments	Number of roots/plantlet
Control	1.00 c
0.5 mg/L IBA	3.00 a
1 mg/L IBA	2.88 a
1.5 mg/L IBA	2.00 b

Means followed by the same uppercase letters are not significantly different at level P≤0.05 according to Tukey's HSD test.

3.3 Acclimatization stage:

Five weeks following acclimation, the survival percentage was calculated. The greatest percentage of 80% is found in the plantlets derived from the medium supplemented with 0.5 mg/L indole butyric acid (IBA) and the medium supplemented with 1 mg/L IBA. (Table 3). Harb et al. (2015) obtained similar results with rooted jackfruit plantlets when it was transplanted into plastic pots with sand and peat for hardening. The plantlets were covered with plastic bags and kept in a chamber at 80% relative humidity, 32±2°C under a 12-hour photoperiod for acclimatization and the survival percentage (85%) was recorded. Also, Anju et al. (2016) obtained an 85% survival percentage with rooted guava shoots after transferring them to pots containing soil, sand and FYM (1:1:1 v/v) and kept under plastic bags which were removed gradually.

Table (3): Success percentage after five weeks of acclimatization stage

Treatments	Survival (%)
Control	60
0.5 mg/L IBA	80
1 mg/L IBA	80
1.5 mg/L IBA	40

Conclusion

From the results of this study, we can conclude that the best medium during the multiplication stage of guava shoots through shoot proliferation on nodal segments was the MS medium supplemented with 2 mg/L BA where it gives the highest number of shoots/explant. The obtained shoots were of suitable length for the next stage. During the rooting stage, the half-strength MS medium supplemented with 0.5 mg/L IBA was the best medium for the rooting of shoots. Rooted shoots were successfully acclimatized to ex-vitro conditions. Plantlets obtained from the medium supplemented with 0.5 mg/L IBA and the medium supplemented with 1 mg/L IBA showed the highest survival percentage (80%).

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