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EFFECT OF SOME METHODS OF PLANTING IN GUAVA (*PSIDIUM GUAJAVA* L.) SEED GERMINATION

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Abstract

The research was carried out to determine the best method of guava seeds germination. The results of the study showed that the seeds cultured in the glass tubes containing the MS medium have recorded the highest percentage of guava seed germination of 100%. The method of the bioreactor has a high germination rate but less than the above-mentioned method (90%). Also, the method of culture in the Petri dishes gave high seed germination percentage reached 70%. But the planting method in the Peat-Moss pots recorded the lowest percentage of seed germination (10%). The treatment of the culture tubes recorded the least time period for germination, which was significantly higher than the other experimental treatments at 40.3 days, while the bioreactor, Petri dishes and Peat-Moss pots methods were recorded at 60.0, 90.7 and 121.3 days respectively. The results of the study in a separate experiment also indicated the superior significantly of the treatment of the MS medium supplemented with 1.5 mg L^{-1} of both the growth regulators BA and Kn in the percentage response to shoot multiplication and the number of shoots with 99% and 9.33 shoots per explant, respectively when shoot tips of guava seedlings were cultured by *in vitro* culture technique. While the MS medium, with a concentration of 1.5 mg L^{-1} of NAA, recorded the highest mean length of the shoots resulting from multiplication at 7.33 cm. The two treatments with concentrations 1.5 mg L^{-1} NAA or 1.5 mg L^{-1} for BA, Kn and NAA were also significantly higher in the mean number of roots, which reached 8.00 and 9.67 roots per shoot respectively, and these two treatments did not significantly differ between them.

Key words : bioreactor, in vitro culture, MS medium, Peat-Moss, seedling.

Introduction

Guava (*P. guajava* L.), belongs to the family Myrtaceae, a tropical and subtropical fruit. South America is its native country and then spread to many areas such as India, South Africa and Egypt (El-Sherif *et al.,* 2011). This plant is one of the most important food crops and medicinal plants in these regions. It is used as food and traditional medical treatment (Kamle *et al.,* 2013). It can be cultivated in all types of soils and preferably sandy soils. And their trees are early in fruiting, yielding in the third year of cultivation in the permanent place. It is a fruit of high nutritional value, as well as fruit in the autumn season, where most fruits are low. The fruit is used to make juices and jams and contains good sources of energy, fibers and vitamins such as vitamin A, niacin, vitamin B6, thiamine and riboflavin. It also contains basic minerals such as calcium, phosphorus, magnesium, iron and potassium. Its natural content of vitamin C is four times higher than its content in oranges. It contains a high percentage of pectin, phenolic compounds, polyphenols, flavonoids and antioxidants, free in the body, causing serious diseases such as cancer, cardiovascular disease, prostate diseases, rheumatism, lung problems, etc. They are also low in calories so it benefits as a food diet (Chen *et al.,* 2010; Luiz *et al.,* 2011; Ryu *et al.,* 2012; Bajpai *et al.,* 2016). Guava is sexually common in seeds and is the most common method. The seed is used directly in culture to produce seedlings that are used as stock for budding and grafting or new varieties (Zamir *et al.,* 2003). The seeds of this plant need a long time to germinate and the seedlings are weak and unequal in height (Doijode, 2001). Therefore, the seeds have some treatments to overcome

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this problem by sowing seeds with hot water or soaking acid such as sulfuric or hydrochloric acid or using plant growth regulators (Kalyani *et al.,* 2014), and sand and mechanical scarification by sand pads (Brijwal and Kumar, 2013). These treatments are carried out with the aim of increasing the percentage of germination of seeds and reducing the length of time for the seedling to emerge. The guava plant also propagates through budding, layering, stem and root cuttings methods (Jamal and Alsusu, 2009). However, vegetative reproduction is practised on a limited scale and its success rate is low for the difficulty of rooting the cuttings. Researchers have also been able to cultivate guava by in vitro technique. The micropropagation is one of the main methods of biotechnology for regeneration of plants and preserving to plant genetic resources (Liu and Yang, 2011). Micropropagation techniques have been used as a widely effective method for a number of commercially important plants. Tissue culture has many advantages compared to traditional propagation methods, such as mass propagation in a short period of time without adherence to the planting season, production of disease-free plants and conservation of species (George and Debergh, 2008). Hassanen *et al.*, (2017) succeeded in obtaining well-acclimatized growth plants using a simple and effective protocol for the micropropagation of guava by culturing the nodal segments of adult guava trees. Zamir, *et al.*, (2007) was able to produce shoots by culturing explants in MS media containing different combinations of glutamine and benzyl adenine. These shoots were developed and rooted using indole acetic acid (IAA) and indole butyric acid (IBA). The study aims to determine the best method to germination of guava seeds for the purpose of spreading its cultivation in Iraq on a large scale to compensate for the loss of fruit trees in the middle and south of the country.

Materials and Methods

The research was carried out at the Tissue Culture Laboratories of the Marine Science Center and the Date Palm Research Center at the University of Basrah, Basrah government, Iraq. Guava (*P. guajava* L. cv. Green guava) seeds have been used to source one of the Indian cultivars introduced into Iraq. Seeds were grown in four methods:

1. 100 guava seeds were grown in the plastic Petri dishes on medical cotton (Plate 1, C).
2. 10 guava seeds were grown in the plastic pot containing Peat-Moss (Plate 1, D).
3. 100 guava seeds were grown in a bioreactor containing liquid MS medium (Plate 1, A).
4. 5 guava seeds were grown in a culture glass tube containing semi-solid MS medium (Plate 1, B).

The MS medium (Murashige and Skoog, 1962) was obtained from Zist Arman Sabz Company (ZAS) by taking 4.33 g L^{-1} of the MS salts with 40 g L^{-1} sucrose, 170 mg L^{-1} sodium hydrogen Orthophosphate and 100 mg L^{-1} Myo-inositol and 10 ml L^{-1} of the vitamins group, 3 g L^{-1} PVP (polyvinyl pyrrolidone) and plant growth regulators (auxin and cytokinins) by stage of growth. The pH was then adjusted to 5.8. When the semi-solid food medium was prepared, agar was added at a concentration of 7 g L^{-1}. The liquid and semi-solid MS media were autoclave with a temperature of 121°C and a pressure of 1.5 bar and then left to cool and stored in the growth chamber until use. The seeds were sterilized within the laminar air flow cabinet using the 1.05% of sodium hypochlorite with the addition of two drops of the Tween 20 (polysorbate 20) for 20 minutes. Then washed with sterilized distill water to remove residual sterile material from seed surfaces.

Parameters

1. Seed germination: % germination, germination rate (days), % survivor seedlings.
2. Vegetative growth: Shoot number, leaves number, the phenotypic traits of the seedlings.
3. Acclimatization of plants produced by in vitro culture technique.

All plants were transferred after 3 months of seed germination to plastic pots which containing Peat-Moss (Plate 1, E). Plants were weekly irrigated with liquid fertilizer NPK at a concentration of 1 g L^{-1}. Shoot tips of plants were pinched after four months of growing to promote the growth of axillary shoots. Plants were cultured in the orchard at 12 months and pinched the shoot tips of lateral shoots to promote the growth of axillary buds and to be used as a source of the explants in the later tissue culture experiment.

The experiment of guava explants cultures

The shoot tips which excised from the seedlings of the green guava cultivar were placed in an antioxidant solution consisting of citric and ascorbic acid at a concentration of 150 and 100 mg L^{-1} respectively and for 30 minutes (Zaid and Tisserat, 1983). These explants were sterilized as in the sterilization method of the seeds above. Then these explants cultured in the MS medium containing plant growth regulators for the shoot multiplication as follows:

1. 1.5 mg L^{-1} BA
2. 1.5 mg L^{-1} Kn
3. 1.5 mg L^{-1} BA + 1.5 mg L^{-1} Kn (Plate 1, F)

4. 1.5 mg L^{-1} NAA
5. 1.5 mg L^{-1} BA + 1.5 mg L^{-1} Kn + 1.5 mg L^{-1} NAA
6. Without plant growth regulators

The following indicators were studied:

1. The percentage response to shoot multiplication
2. Number of leaves per shoot
3. Shoot length (cm)
4. Number of roots per shoot

Experimental design and statistical analysis

The experiment is designed according to the Complete Randomized Design (CRD). The data was statistically analyzed using the analysis of variance by GenStat program, Version 13. Compare the mean of the treatments using the revised least significant difference RLSD at 5% probability (Al-Rawi and Khalaf Allah, 2000).

Results and Discussion

The results in Fig. 1 and 2 show that the method of seed germination in the culture tubes was significantly superior to the other treatments of the experiment in the percentage and rate of the germination which recorded 100% and 40.3 days, respectively. The bioreactor method followed by the method above, which recorded 90% and 60.0 days, respectively. This method also was significantly superior compared with the treatment of germination in plastic Petri dishes, which recorded 70% and 90.7 days, respectively. This is due to the availability of nutrients in the MS medium for culture tubes and bioreactor methods that stimulated germination and promoted growth in a short period of time. The results of the study were similar to those found during their study on the germination of guava seeds by in vitro culture, which took the explants that were used in the shoot multiplication (Shah *et al.,* 2008).

While the treatment of seed germination in the Peat-Moss pots recorded the lowest percentage and rate of germination which reached 10% and 121.3 days, respectively, (Fig. 1 and 2). The results of the study agree with what Doijode (2001) and the results found by Brijwal and Kumar (2013) indicate the low rate of seed germination when cultured in the traditional method.

The low percentage of germination in the planting method in the Peat-Moss method may be due to watering the seeds without adding nutrients. High seed content of phenolic compounds may also inhibit seed germination and thus reduce germination percentage (Singh and Singh, 2018). This problem has been overcome in both culture tubes and bioreactors methods through the addition of PVP, which prevents the oxidation of phenolic substances that may play a role in inhibiting germination (Singh *et al.,* 2001; Elezaby and Abd Allatif, 2017; Hassanen *et al.,* 2017).

The results from Fig. 3 show that both culture tubes and bioreactors were significantly different from the other two methods in the percentage of survivor plants that reached 86.33% and 88.00%, respectively. The increase in the percentage of survivor plants in the two methods of culturing in glass tubes and bioreactors is due to the availability of nutrients, which in turn produced strongly and healthy plants that kept them alive (Idris *et al.,* 2006).

The treatment of plastic Petri dishes differed significantly from the treatment of planting in the Peat-Moss pots, with 25.67% of survivor plants recorded. While the method of planting in the Peat-Moss pots recorded the lowest percentage of survivor plants amounted to 11.00%.

The results from table 1 indicate that the method of seed germination in the bioreactor was significantly different from the other methods in the number of lateral shoots and the number of leaves. This method recorded the highest values of 5.0 lateral shoots per the main shoot and 11.33 leaves per shoot, respectively. While the method of planting in the Peat-Moss pots gave the lowest value in the number of lateral shoots and the number of leaves that reached 1.00 shoot and 3.33 leaves per shoot, respectively. The phenotypic characteristics of table 1 indicate that the growth was good and rapid and the leaf size was significant in the shoots of the guava plant that were germinated in a bioreactor container. While growth was slow and leaf size was small in the shoots of germination treatments in Petri dishes and Peat-Moss pots. The growth of shoots was good and rapid in the treatment of germination in the culture tubes, but the size of the leaves was medium. That the reason for the significant superiority of the germination method in the bioreactor in the number of lateral shoots and the leaves formed is the complete food components provided by the MS medium as well as being a liquid food, ready and easy to absorb by developing seedlings. As for the small number of shoots and leaves in seedlings growing in the method of cultivation in the culture tubes, the reason for the narrowing of the space of the culture tube has caused the reduction of the absorption of nutrients from the components of the MS medium as a result of competition for food.

***In vitro* culture of guava shoot tip explants**

The results from table 2 indicate that there are significant differences between the means of growth regulator treatments in shoot multiplication and their

Table 1: Effect of the planting method on some vegetative characteristics of guava seedlings.

Planting Method	Number of shoots	Number of leaves	Phenotypic characteristics
Bioreactor	5.00	11.33	Shoot growth is good and fast and the size of the leaves is large.
Culture tubes	1.00	4.67	Shoot growth is good and fast and the size of the leaves is medium.
Petri dishes	1.00	4.00	Shoot growth is limited and weak and the size of leaves is small.
Peat-Moss pots	1.00	3.33	Shoot growth is very slow and the size of the leaves is very small.
R-LSD (P≥0.05)	1.883	3.766	

Table 2: Effect of plant growth regulators on shoot growth, development and rooting of guava plant by in vitro culture technique.

Treatment (mg L^{-1})	Response to shoot multiplication	Number of shoots per explant	Shoot length (cm)	Number of roots per shoot
1.5 BA	90%	4.76	2.33	0.00
1.5 Kn	75%	3.33	3.33	0.00
1.5 BA + 1.5 Kn	99%	9.33	5.33	5.00
1.5 NAA	50%	2.33	7.33	8.00
1.5 BA+ 1.5 Kn+ 1.5 NAA	90%	4.33	2.33	9.67
Without growth regulators	-	1.00	2.33	2.67
R-LSD (P≥0.05)	4.551	1.729	1.027	2.011

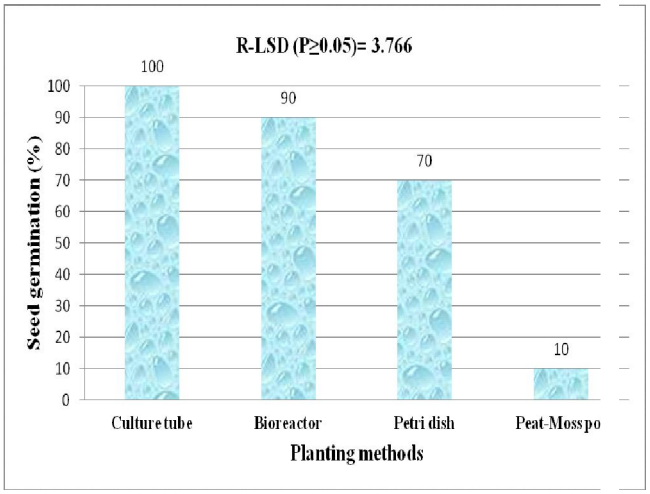

Fig. 1: Effect of the planting methods on the percentage of guava seed germination.

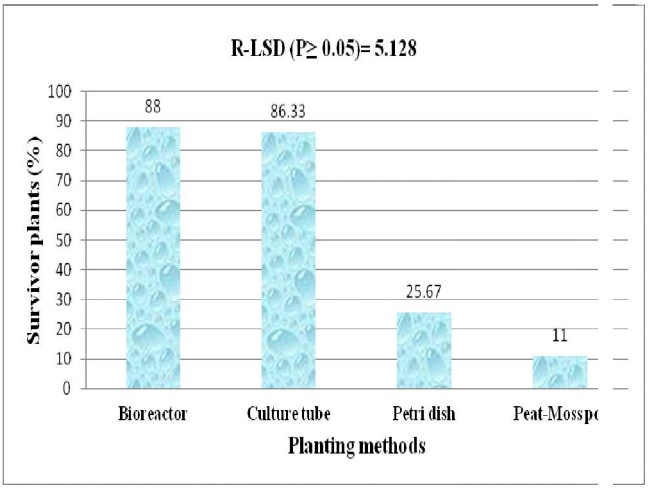

Fig. 1: Effect of the planting methods on the percentage of guava survivor plants.

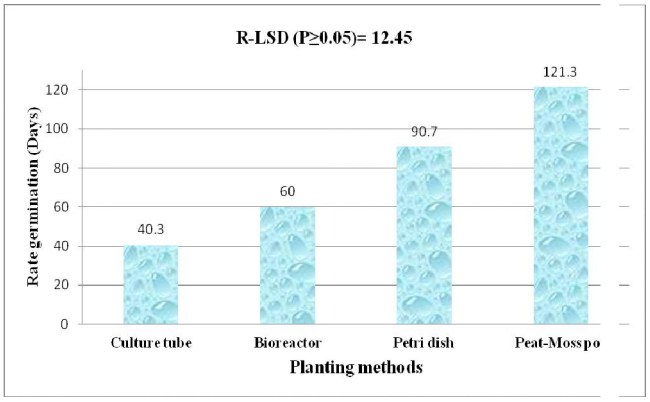

Fig. 1: Effect of the planting methods on the on the rate germination of guava seeds.

rooting in guava plants. As the MS medium supplemented with benzyl adenine (BA) and kinetin (Kn) concentration of 1.5 mg L^{-1} for each of them gave the highest percentage of response to the shoot multiplication and the number of shoots, which reached 99.00% and 9.33 shoots $explant^{-1}$ (Plate 1, G). This is due to the fact that cytokinins promote the division of plant cells, which have a role in breaking the apical dominance and encouraging the growth of lateral shoots (Gaspar *et al.,* 2003; Hassanen *et al.,* 2017). The results of the study were similar to what Shah et al. (2008) found when they studied on the addition of benzyl adenine and kinetin to the MS medium for the guava shoot multiplication. While the MS

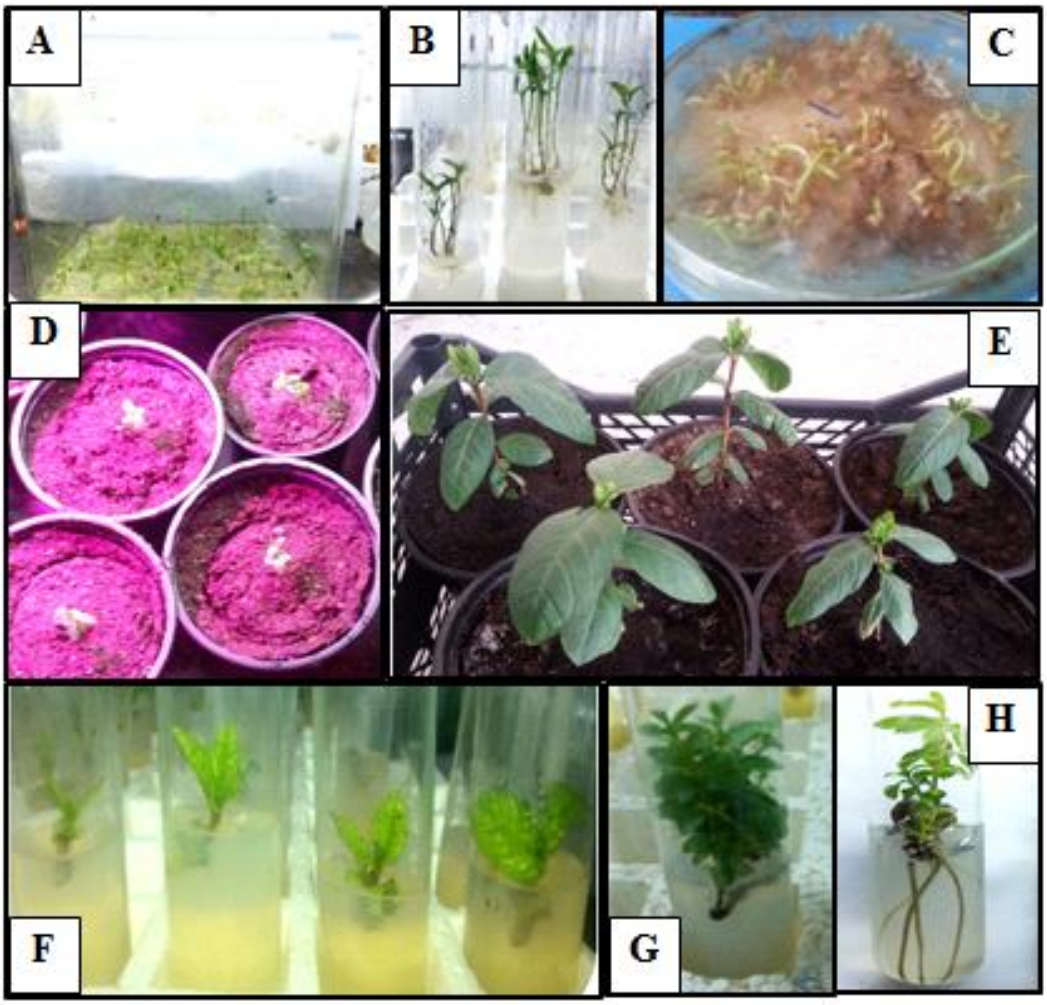

Plate 1: Propagation of guava (*Psidium guajava* L.) plant. Seed germination by bioreactors method (A); by culture tubes method (B); by Petri dishes method (C); by Peat-Moss pots method (D); Guava seedlings (E); Shoot tips cultured in MS medium (F); Shoot multiplication in MS medium supplemented with Benzyl adenine and kinetin at 1.5 mg L^{-1} for each of them (G); Root formation in shoot that cultured on MS medium supplemented with NAA at 1.5 mg L^{-1}.

medium, which is not supplemented with growth regulators, did not result in a multiplying of the shoots. The results of table 2 also showed that the MS medium, which was supplied with naphthalene acetic acid (NAA) at a concentration of 1.5 mg L^{-1}, had the highest mean length of the shoot which reached 7.33 cm, significantly different from other growth regulator treatments. The results of the same table indicate that the MS medium supplemented with naphthalene acetic acid (NAA) or combination of BA + Kn + NAA, at a concentration of 1.5 mg L^{-1}, were significantly different from other treatments in the number of roots formed (Plate 1, H). The two treatments recorded the highest mean number of roots, which reached 8.00 and 9.67 roots $shoot^{-1}$, respectively. While the MS medium, which is not supplemented with growth regulators gave the lowest mean of the number of roots which reached 2.67 roots $shoot^{-1}$. The MS medium supplemented with 1.5 mg L^{-1} BA or 1.5 mg L^{-1} Kn did not result in rooting of the shoots.

Conclusion

Planting guava seeds with a culture tubes or bioreactor are the best method to get large numbers of seedlings. A large number of guava and similar plants can also be obtained from culturing shoot tips with tissue culture technology. The combination of benzyl adenine and kinetin added to the MS medium gave the highest response to the shoot multiplication. The addition of naphthalene acetic acid to the MS medium of rooting led to an increase in the mean number of roots formed at the shoot base.

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