

## RESPONSE OF SINGLE NODE EXPLANTS OF FIGS (*FICUS CARRICA* L.) SHINGALI CV. TO DIFFERENT PLANT GROWTH REGULATORS AND CULTURE MEDIA

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### ABSTRACT

An efficient micropropagation protocol was developed for the fig tree Shingali cultivar from single node explants grown on MS and WPM enriched with different growth regulators. Healthy and aseptic cultures were achieved from the disinfection with 1.5% mercuric chloride for 5 minutes. At initiation stage, the addition of 1.5 mg.l<sup>-1</sup> BA was superior upon the rest of treatments by giving the highest number of shoots (4.00 shoots/ explant), the highest number of leaves (9.00 leaves/ explant) and the longest shoots (3.65 cm). The best multiplication parameters were recorded from the addition of 2.5 mg.l<sup>-1</sup> BA to woody plant medium (WPM) enriched with 1.5 mg.l<sup>-1</sup> GA<sub>3</sub> by giving 7.09 shoots/ explant, 25.09 leaves/ explant and 8.44 cm as the longest shoots. On the other hand, the best rooting parameters were recorded when 0.2 mg.l<sup>-1</sup> NAA was added to MS medium enriched with 1.5 mg.l<sup>-1</sup> GA<sub>3</sub> by reaching 100% rooting, 26.80 roots/ explant and 8.40 cm as the longest roots. At the acclimatization stage, the whole tested culture mixtures were able to achieve 100% survival rates of plantlets acclimatization. And using peatmoss alone was the superior culture medium at this stage by recording the highest values of plantlet height (9.50 cm), number of leaves (36.00 leaves/ plantlet) and the highest number of shoots (9.00 shoots/ plantlet) after four weeks from transplanting under greenhouse conditions. In general, this micropropagation protocol is very convenient to be used for any future commercial propagation for this important fig tree cultivar in the area.

**KEYWORDS:** Fig tree, *Ficus carica* L., Cytokinins, Auxins, MS, WPM, Micropropagation

### INTRODUCTION

Fig tree (*Ficus carica* L.) belongs to Moraceae family, its fruits can be consumed fresh, dried or as jam. Fig is a deciduous tree that originated in south-west Asia and the eastern Mediterranean area (Zohary and Spiegel-Roy, 1975). Fig fruits are a source of dietary fibers and minerals, an abundant source of amino acids, antioxidants and several vitamins and its free of cholesterol, fat and sodium. Micropropagation of Fig tree can be done through shoot multiplication or by direct and indirect organogenesis ways to provide high numbers of true- to- type genotypes. Conventional propagation under field condition methods suffers from many biotic and abiotic stresses (Toma and Tamer, 2015). Plant tissue culture could offer practical means for rooting of hard to-root plants, disease-free plant production, year-round propagation and germplasm preservation (Shibli *et al.*, 2006).

*Ficus carica* L. is conventionally propagated through cuttings, grafting and budding in addition to sexual propagation by using seeds which is not the preferred method because fig seeds are nonviable. In cuttings propagation, 20-30% of the cuttings usually survive due to poor rooting (Toma and Tamer, 2015).

Plant tissue culture technique is an alternative means for propagation whereby cells, tissues, or organs can be cultured under sterilized and controlled conditions. Thus, PTC is an efficient and widely used tool for the micropropagation of various fruit trees of high economic importance (Shatnawi *et al.*, 2011a). This effective propagation method can be manipulated to continuously and consistently propagate high-quality pathogen-free planting genotypes at a cost-effective price for mass propagation scales. Recent studies on micropropagation of fig trees have shown the use of shoot-tip explants (Shatnawi *et al.*, 2011b), nodal explants (Shibli *et al.*, 1999), leaves (Shibli *et al.*, 2006) apical meristems, and axillary buds (Toma and Tamer,

2015) that have successfully micro propagated fig trees from different cultivars. Up to the present, there have been no tissue culture studies and micropropagation methods being reported for the variety of Shingali. This study aims to develop an efficient micropropagation protocol for this most important fig cultivar to be used in near future as a means toward mass propagation in Duhok Governorate and the whole of Iraq by testing various concentrations of cytokinins including benzyl adenine and kinetin and auxins including Naphthalene acetic acid and butyric acetic acid.

## MATERIALS AND METHODS

Single node explants were collected from mature fig trees grown in Duhok Governorate to be used as explants at establishment stage to get stock plant cultures. The explants were disinfested by surface sterilization by using 1.5% mercuric chloride for five minutes. Healthy cultures were achieved and maintained for the next micropropagation stages. At the initiation stage, the explants were inoculated in MS medium enriched with 1.5 mg.l<sup>-1</sup> GA<sub>3</sub> with testing different concentrations of BA (0.0, 1.5, 2.0 and 2.5 mg.l<sup>-1</sup>). Ten glass culture vessels were used for each experimental unit by planting three explants in each culture vessel which contained 25 ml of the culture medium. The cultures were kept in the primary growth room at the plant tissue culture lab belonging to the Department of Horticulture, College of Agricultural Engineering Sciences, University of Duhok under fully controlled conditions of 25±2°C, 16 hours of lighting with cold-white-light and 8 hours of darkness as a photoperiod and 1000 lux of light intensity. After four weeks in culture, the number of shoots per explant, number of leaves per explant and mean length of shoots were recorded as initiation stage parameters.

At the shoot multiplication stage, two types of culture media were tested, Morishige and Skoog medium (MS) and woody plant medium (WPM) both enriched with 1.5 mg.l<sup>-1</sup> GA<sub>3</sub>. Two kinds of cytokinins including benzyl adenine and kinetin were tested separately to enhance the micro-shoots produced at the initiation stage to be multiplied to the required number for the next stages. Different concentrations of BA (0.0, 1.5, 2.5, and 3.5 mg.l<sup>-1</sup>) and Kinetin (0.0, 2.5, 3.5, and 4.5 mg.l<sup>-1</sup>) separately. After four weeks in culture, the number of shoots per explant,

number of leaves per explant and mean length of shoots were recorded as multiplication stage parameters.

At the root formation stage, two kinds of auxins were tested to enable the multiplied microshoots to form roots including IAA, IBA and NAA at 0.0, 1.0, 2.0 and 3.0 mg.l<sup>-1</sup>. After four weeks in culture, the number of roots per explant, mean length of roots and rooting percentage were recorded as rooting parameters.

For the acclimatization stage, the well-rooted plantlets were carefully removed from the culture vessels and were thoroughly washed with tap water and treated with Benlate fungicide (1%) for 10 minutes before transplanting into various culture mixtures in plastic pots under polyethylene. The culture mixtures included: peatmoss, peatmoss+ sand (1:1), peatmoss+ perlite (1:1), peatmoss+ vermiculite (1:1) and peatmoss+ perlite+ vermiculite (1:1:1). The plantlets were regularly watered and sprayed with ¼ MS salt strength to enhance them to reproduce the cuticle layer and perform better under greenhouse conditions. After two weeks, the polyethylene was removed totally and the plantlets were continued their growth and development in the greenhouse. After four weeks in culture, the survival rate, plant height, number of leaves per plantlet and number of shoots per plantlet were recorded as acclimatization parameters.

The whole experiments were arranged according to a Completely Randomized Design (CRD) and the comparison among means was done according to the Duncan multiple range test at 0.05% and the whole data were statistically analyzed by SAS computerized program (SAS, 2010).

## RESULTS AND DISCUSSION

Generally, the results of this experiment were very successful in improving a reliable and effective micropropagation protocol for Shingali figs. After testing several disinfestation treatments including sodium hypochlorite (NaOCl) with different concentrations and durations, unfortunately, one hundred percent of contamination was occurred. That's why, mercuric chloride was finally used at 1.5% for 5 minutes which gave ideal results in producing healthy and aseptic cultures from the inoculation of single nodes explants on MS medium (Fig. 1: A). These results confirm that NaOCl sometimes is not sufficient to get rid of contamination.

That's why, mercuric chloride is a good alternative means for disinfestations (Maheshwaran *et al.*, 2000). These results are in agreement with those recorded by Hameed (1994) and AL-obaidi (1999). The use of mercuric chloride and sodium hypochlorite is common in explant sterilization in tissue culture techniques and each of them has advantages and disadvantages (Reeves *et al.*, 1983).

At the initiation stage, Table (1) declares that the addition of 1.5 mg.l<sup>-1</sup> BA was superior to the rest of the treatments by giving the highest number of shoots (4.00 shoots/ explant), the highest number of leaves (9.00 leaves/ explant)

and the longest shoots (3.65 cm). This may be due to the important role of cytokinins in increasing the synthesis of RNA, proteins and enzymes inside the cell which enhance bud growth as well (Al-Rifae'e and Al-Shobaki, 2002). In addition, BA is the most effective cytokinin in cell division as compared to other cytokinins (Zhu *et al.*, 2005 and Al-Ansary *et al.*, 2007). The produced microshoots at the initiation stage were successfully transferred to the shoot multiplication medium by testing various BA and Kinetin concentrations on WPM and MS media.

**Table (1):** Effect of BA on initiation stage of Shingali Fig tree (*Ficus carica* L.) grown in MS medium enriched with 1.5 mg.l<sup>-1</sup> GA<sub>3</sub> after four weeks in culture

BA (mg.l <sup>-1</sup> )	Number of shoots/ explant	Number of leaves/ explant	Mean length of shoots (cm)
0.0	1.00 d	3.00 d	1.34 c
1.5	4.00 a	9.00 a	3.65 a
2.5	3.00 b	7.00 b	3.64 a
2.5	2.00 c	5.00 c	2.56 b

\*Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (p<0.05).

Table (2) shows that the addition of 2.5 mg.l<sup>-1</sup> BA to WPM was the best in regard to multiplication parameters. Since, it gave 7.09 shoots/ explant, 25.09 leaves/ explant and the longest shoots which reached 8.44 cm which were significantly superior to the rest of BA and Kinetin concentrations (Fig. 1: C). These results agrees with those published by Darwesh (2014) and Danial *et al.* (2014) when they found that BA was better than kinetin for fig trees multiplication

stage. The reasons behind the positive role of cytokinins on the multiplication stage might be

due to their profound role in releasing lateral buds from the dominance of terminal buds without the need to remove the apical bud by promoting formation of xylem tissues of buds which facilitate the transformation of water and nutrient leading to lateral bud growth (Mohammed & Al-Younis, 1991). These results confirm the findings of Metwali *et al.* (2014) that the different plant genotypes (fig cultivars) response variously to the culture medium under *in vitro* conditions.

**Table (2):** Effect of BA and kinetin on shoot multiplication stage of Shingali Fig tree (*Ficus carica* L.) grown in WPM medium enriched with 1.5 mg.l<sup>-1</sup> GA<sub>3</sub> after four weeks in culture

Cytokinins (mg.l <sup>-1</sup> )	Number of shoots/ explant	Number of leaves/ explant	Mean length of shoots (cm)
Control 0.0	2.36 f	4.55 f	3.35 d
BA 1.5	3.64 e	8.91 e	4.73 c
BA 2.5	7.09 a	25.09 a	8.44 a
BA 3.5	6.36 b	19.45 b	7.85 a
Kinetin 2.5	2.00 d	8.40 e	3.87 d
Kinetin 3.5	5.30 c	16.00 c	5.22 b
Kinetin 4.5	4.60 d	12.00 d	3.51 d

\*Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (p<0.05).

On the other hand, Table (3) shows that the addition of 1.5 mg.l<sup>-1</sup> BA to MS medium was significantly the best among the rest of the treatments except 3.5 mg.l<sup>-1</sup> Kinetin in regard of number of shoots per explant. Since, 1.5 mg.l<sup>-1</sup> BA recorded the highest values including 5.10 shoots/ explant, 12.00 leaves/ explant and the longest shoots (5.00 cm) (Fig. 1: B). The

prominent superiority of BA over Kinetin is very well reported in previous studies (Toma and

Tamer, 2015). This might be due to its chemical formula and having the double bonds which makes it very effective. While, kinetin is exclusively activates the flavonoid synthesis gene, benzyl adenine affected more significantly the synthesis of proteins, and enhance photosynthesis and plant tolerance-related genes (Maheshwaran *et al.*, 2000).

**Table (3):** Effect of BA and kinetin on shoot multiplication stage of Shingali Fig tree (*Ficus carica* L.) grown in MS medium enriched with 1.5 mg.l<sup>-1</sup> GA<sub>3</sub> after four weeks in culture

Cytokinins (mg.l <sup>-1</sup> )	Number of shoots/ explant	Number of leaves/ explant	Mean length of shoots (cm)
Control 0.0	3.00 c	4.90 e	1.57 d
BA1.5	5.10 a	12.00 a	5.00 a
BA 2.5	4.00 b	8.00 c	3.04 b
BA 3.5	3.60 b	6.00 d	2.90 c
Kinetin 2.5	4.60 b	10.20 b	3.90 b
Kinetin 3.5	5.30 a	5.90 d	1.98 d
Kinetin 4.5	3.10 c	5.40 d	1.60 d

\*Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (p<0.05).

At the rooting stage, the results showed that plant tissue culture is an effective means to be used instead of the conventional propagation methods in figs. Table (4) shows the effects of three auxins (IAA, IBA and NAA) concentrations

on Shingali figs microshoots grown in MS medium. It is clear that the addition of 0.2 mg.l<sup>-1</sup> NAA was significantly the best treatment by recording the best root formation parameters. Since, it produced 26.80 roots/ explant, 8.40 cm as the longest roots and 100% of rooting

(Fig. 1: D). Achieving such a high rooting percentage for fig explants is very beneficial compared with conventional cutting which records a very low (20- 30%) rooting percentage

(Mustafa and Taha, 2012). these results are similar to those reported by Soliman *et al.* (2010) and Dainal *et al.* (2014) when they found that IBA and NAA were effective in improving fig rooting formation.

The current results assured auxins roles in enhancing the rooting process by promoting adventitious roots initiation in the bases of cultured shoots (Sharma *et al.*, 2007). The differences in the potency of IAA, IBA and NAA in promoting rooting might be due to the structure of the different auxins under testing, the endogenous hormone concentration, also the genetic makeup of species under consideration (Toma, 2019).

**Table (4):** Effect of IAA, IBA and NAA on root formation stage of Shingali Fig tree (*Ficus carica* L.) grown in MS medium after four weeks in culture

Auxins (mg.l <sup>-1</sup> )	Number of roots/ explant	Mean length of roots (cm)	Rooting Percentage (%)
Control 0.0	4.20 h	1.60 e	60 e
IAA 0.1	17.60 d	5.00 d	85 d
IAA 0.2	19.60 c	7.20 b	90 c
IAA 0.3	6.80 g	4.60 d	80 e
IBA 0.1	20.60 b	6.20 c	90 c
IBA 0.2	21.40 b	7.80 b	95 b
IBA 0.3	8.40 f	5.78 c	85 d
NAA 0.1	21.20 b	7.08 b	95 b
NAA 0.2	26.80 a	8.40 a	100 a
NAA 0.3	10.20 e	6.08 c	90 c

\*Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (p<0.05).

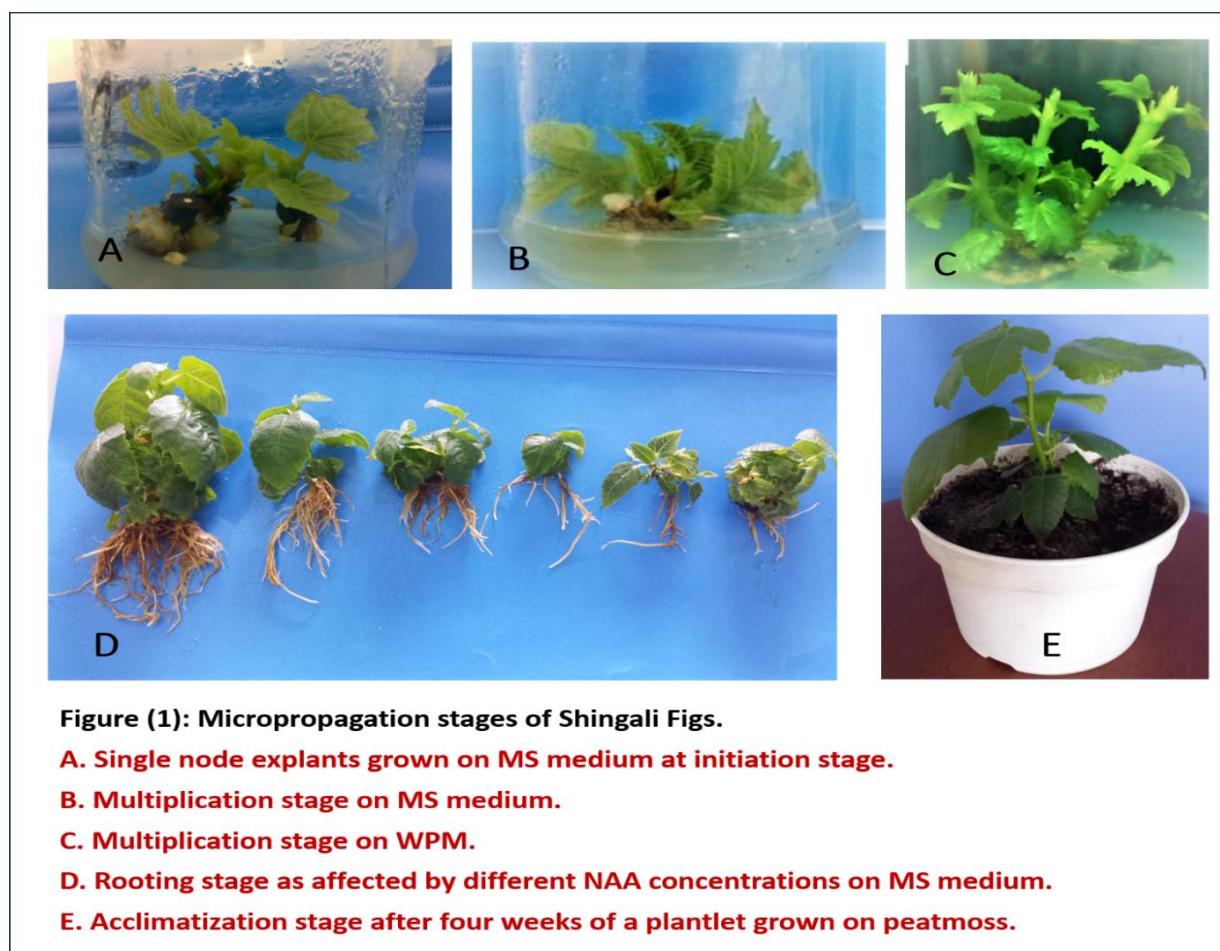
The Shingali fig plantlets of well-developed roots were successfully transplanted from lab conditions to greenhouse conditions by gradually moving in different culture media mixtures. Table (5) shows that planting in pots containing peatmoss only was physiologically and morphologically superior upon the rest of culture media mixtures by providing 100% of survival and the highest plantlet height (9.50 cm), the highest number of leaves (36.00 leaves/ plantlet) and the highest number of shoots (9.00 shoots/

plantlet) after four weeks in culture (Fig. 1: E). These results approve that peatmoss is always the best choice for acclimatizing plantlets produced through the tissue culture technique. Peatmoss has many advantageous characteristics like high water absorbing capacity, preventing soil compaction, holding soil nutrients, free of bacteria, fungi and weed seeds, being perfect for acid-loving plants, and availability and cheapness (Arodin, 2022).

**Table (5):** Acclimatization stage of Shingali Fig (*Ficus carica* L.) plantlets grown in different culture mixtures after four weeks in culture

Culture mixture	Survival rate (%)	Plantlet height (cm)	Number of leaves/ plantlet	Number of shoots/plantlet
Peatmoss	100	9.50 a	36.00 a	9.00 a
Peatmoss+ Sand (1:1)	100	8.80 b	29.00 b	8.00 a
Peatmoss+ Perlite (1:1)	100	7.90 c	23.00 c	6.00 b
Peatmoss+ Vermiculite (1:1)	100	6.00 d	18.00 d	5.00 b
Peatmoss+ Perlite+ Vermiculite (1:1:1)	100	5.30 e	12.00 e	4.00 b

\*Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test ( $p < 0.05$ ).



As a general conclusion, this study approved that this economically important fig cultivar “Shingali” could be clonally microperforated by tissue culture technique using this reliable protocol with WPM but for better rooting MS culture medium enriched with some available plant growth regulators. This would certainly hand the local figs growers to use it for any future mass propagation commercial projects in addition to other previous studies.

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زیده کرنا هویر یا هژیرا شنگالی ب چاندنا گرکیت نیکانه ل ژیر تیراتییت جیاواژ ژریکخهریت شینوبونا رووهکی وناقهنندیت چاندنی

پوخته

پروتوکولهکی زیده کرنا هویر یا هژیرا شنگالی هاته بدهستفهنینان ب ریکا چاندنا گرکیت نیکانه دناف نافهنندیت چاندنی MS و WPM نهویت هاتینه زیده کرن بتیراتییت جیاواژ ژریکخهریت شینوبونا رووهکی. چاندنگههیت پاک و ساخلم هاتنه بدهستفهنینان دهرنهجامی بکارنینانا کهرستنی کلوریدی زیبهق ( ملگم/ لتر) بو ماوی 5 خولهکان. دقوناغا دهستپیکنی دا، سهردهریا 1.5 ملگم/ لتر ژ BA یا بسهرکهفتی بوو ل سهر ههمی سهردهرییت دیتر ب بهرهمینانا مهزترین ژمارا چهقا (4.00 چهق/ پارچا رووهکی) وبلندترین ژمارا بهلگان ( 4.00 بهلک/ پارچا رووهکی) ودریژترین چهق (3.65 سم). سهبارت باشتترین ناستین دووهیندهلیبونی، بکارنینانا 2.5 ملگم/ لتر BA دناف نافهنندیت WPM یی زیده کری ب 1.5 ملگم/ لتر ژ GA3 ب بهرهمینانا 7.09 چهق/ پارچا رووهکی و 25.09 بهلگ/ پارچا رووهکی و 8.44 سم وهک دریژترین چهق. باشتترین ناستیت ریهدانی هاتنه تومارکرن ددهمی بکارنینانا 0.2 ملگم/ لتر NAA دنافهنندیت MS یی بهیزکری ب 1.5 ملگم/ لتر ژ GA3 ب بهرهمینانا ریها ب ریژهیا 100% و 26.80 ریه/ پارچا رووهکی ودریژترین ریه گههشتنه 8.40 سم. دقوناغا قورسکرنی وهگوهاستنی بو خانینی شیشهی، ههمی رووهک ب سهرکهفتیانه هاتنه قهگوهاستن وریژهیا قورتالیوونی گههشته 100%. ددهمی بکارنینانا پیتموسی بتنی وهک نافهنندیت چاندنی باشتترین نهجام هاتنه تومارکرن وهکو دریژایا رووهکی گههشتیه 9.50 سم و 36.00 بهلک/ رووهک و 9.00 چهق/ رووهک پشتی 4 حهفتیا ژفهگوهاستنی. بشیوهکی گشتی، نهف پروتوکوله یی گونجایه ب بکارنینانی دپروژیت بازرگانی بو بهرهمینانا هژیرییت شنگالی نهویت گهلهک دسهرکهفتینه ددهقهری دا.

## استجابة التين (*Ficus carica* L.) صنف شنكالي لزراعة العقد المفردة تحت مستويات مختلفة من منظمات النمو وأوساط الزراعة

### الخلاصة

تم تطوير بروتوكول اكثار دقيق لشجرة التين الصنف الشنكالي المزروع من عقد مفردة النامية في وسطي الزراعة MS و WPM المعززين بمستويات مختلفة من منظمات النمو النباتية. حيث تم الحصول على مزارع معقمة بعد التعقيم بمادة كلوريد الزئبق بتركيز 1.5 ملغم/ لتر لمدة 5 دقائق. في مرحلة النشوء، تفوقت معاملة إضافة 1.5 ملغم/ لتر من البنزيل أدنين على بقية المعاملات وذلك بإنتاج أكبر عدد من الفروع (4,00 فروع/ جزء نباتي) وأكبر عدد من الأوراق (9.00 ورقة/ جزء نباتي) وكذلك أطول الفروع (3.65 سم). أما أفضل مقاييس التضاعف الخضري فقد تم تسجيلها من خلال إضافة 2.5 ملغم/ لتر من البنزيل أدنين الى وسط WPM المعزز بـ1.5 ملغم/ لتر من حامض الجبرليك من خلال انتاج 7.09 فروع/ جزء نباتي و 25.09 ورقة/ جزء نباتي وأطول الفروع (8.44 سم). من جانب آخر، فإن أفضل مقاييس التجذير فقد تم تسجيلها من خلال إضافة 0.2 ملغم/ لتر NAA الى وسط MS المعزز بـ1.5 ملغم/ لتر من حامض الجبرليك حيث تم الحصول على نسبة تجذير 100% و 26.80 جذر/ جزء نباتي وأطول الجذور 8.40 سم. أما في مرحلة الأقلعة، فإن جميع النباتات تم نقلها بنجاح من خلال الزراعة في الأوساط الزراعية المستخدمة وبنسبة بقاء 100%. في حين أن استخدام البيتموس لوحده كوسط زراعي هو المتفوق على غيره من الأوساط من خلال الحصول على 9.50 سم كارتفاع النبات و 36.00 ورقة/ نبات وكذلك أكبر عدد من الفروع (9.00 فروع/ نبات بعد 4 أسابيع من النقل الى البيت الزجاجي. عموماً، فإن هذه البروتوكول الاكثاري يعد مفيداً جداً للاستخدام في اكثار التين بصورة تجارية في المشاريع التجارية وخاصة لهذا الصنف الهام في المنطقة.