DOI: https://doi.org/10.26682/sjuod.2020.23.1.19
Journal of University of Duhok, Vol. 23, No.1 (Pure and Eng. Sciences), Pp 178-190, 2020

THE RESPONSE OF *IN VITRO* PROPAGATION OF MARUMI KUMQUAT (*FORTUNELLA JAPONICA* THUNB.) TO DIFFERENT CULTURE MEDIA, PLANT GROWTH REGULATORS AND DIFFERENT FRUCTOSE CONCENTRATION

JEILLAN **H**. **H**USSEIN* and **D**IAA **A**. **I**BRAHIM**
*Dept. of Biology, College of Sciences, University of Duhok, Kurdistan Region-Iraq
**Scientific Research Center, College of Science, University of Duhok, Kurdistan Region-Iraq

(Received: February 3, 2020; Accepted for Publication: March 10, 2020)

ABSTRACT

Marumi kumquat (*Fortunella Japonica*) is culture for its valuable nutritional value and medicinal importance in many regions of the world. The current study aimed to evaluate the effect of two types of media enriched with different concentrations of fructose and different plant growth regulators and different fructose concentration on *in vitro* propagation of *Fortunella Japonica*. The findings showed that the most effective treatment for explant surface sterilization was by using 0.1% $HgCl_2$ for ten minutes which give best results for production contamination-free explants at the initiation cultures. At multiplication stage, WPM medium gave better results at all tested BA levels as compared with MS medium. No significant differences were showed by using BA alone or in combination with GA3 in the measured parameters. It has been observed that WPM medium supplemented with 0.5mgl^{-1} BA with the presence of 30mgl^{-1} fructose was able to give the highest shoot length (1.56cm) with maximum shoots number/explant 9.0 and highest leaves number/explant (21.0). The proliferated shoots were exposed to full strength MS medium salts supplemented with 2mgl^{-1} NAA which showed the highest ratio of rooting. *In vitro* rooted plantlets were gradually acclimatized and transferred to open air conditions, which recorded a high survive rate reached to 92%.

KEYWORDS: *Fortunella japonica*; Micropropagation; Shoot multiplication; Fructose; Explant; Plant Growth Regulator.

INTRODUCTION

Kumquat is one of small fruit-bearing tree belongs to the family Rutaceae. The fruits are orange colored edible with thick sweet-flavored skin and sour pulp ("Kumquat". *Collins Dictionary*). Kumquat is stronger than the rest of other citrus species because they can tolerate the temperatures drop below 13˚C. But in general, it prefers growing in a climate where the temperatures do not fall lower 4˚C because of fruits ripening and become sweeter in warm conditions. (Nguyen and Doan, 1989). Kumquat has many medicinal uses such as, full of antioxidants (Nouri and Shafaghat, 2015) and help in healthy looking of skin (Satyal *et al*., 2012). *Fortunella japonica* which is also called Marumi kumquat produces edible round shaped fruit with golden yellow color. It is typically eaten raw because the peel has a sweet flavor but the fruit has a distinctly sour center (Gmitter *et al*., 2007).

In vitro propagation of the plants "vegetatively" by tissue culture is meant to produce pathogen-free plants (Thorpe, 1990), the capability to tear out viruses, bacteria, and fungi by culturing any part of plant (Amgai *et al*., 2016). The products of tissue culture are named clones (Shohael, 2008), which have the same genotype except of those exposed to mutation during culture (Van, 2009). Most of the citrus rootstock are recalcitrant via seed propagation due to lose their germination and viability within a short period (Barman *et al*., 2006).

Thus, micropropagation is an efficient tool for mass production. *Fortunella Japonica* is common species in Japan due to its ancient cultivation there, and very little work has been carried out on the micropropagation of this plant. Accordingly, this study was conducted to

jeillan.hassan3@gmail.com

identify the best type of explant from *in vitro* propagation, better plant growth regulator protocol, the appropriate media type for growth and shoot multiplication, and effect of different concentration of fructose, in addition to rooting of regenerated shoots.

MATERIALS AND METHODS

The current study was conducted in Biotechnology labs at Scientific Research Center, College of Science, University of Duhok in the duration from February 2019 to January 2020.

Both MS medium (Murashige and Skoog 1962) and woody plant medium WPM (McCown and Lloyd 1981) were used in all experiments. Each media was prepared by adding a specific amount of carbon source, growth regulators according to the studied protocols. The pH was adjusted to 5.7 by pH meter by adding few drops of 0.1 N NaOH or HCl. The medium was dispensed in equal sizes (25ml) into culture vessels and capped with polyethylene covers, followed by autoclaving at 121°C and 1.04 kg/m^2 for 15 minutes. The culture room incubation conditions were adjusted at 24± 2°C and 16/8 photoperiod by white lead Lamps at 1000Lux.

Three kinds of explants (shoot tips, nodes and seeds) were taken from *Fortunella Japonica* field plants. The explants were washed three times with dish washing liquid detergent followed by rinsing them under running tap water for 30 minutes, then the explants were transferred to the laminar cabinet to perform the sterilization procedure protocols as mentioned below:

A. 2.5% NaOCl+ three drops of tween-20 for 30 minutes.
B. 3.75%NaOCl + three drops of tween-20 for 30 minutes.
C. 0.1% $HgCl_2$ + three drops of tween-20 for 10 minutes.

The explants were rinsed with sterilized distilled water four times, three minutes for each; the ends of explants that exposed to disinfectant materials were removed. Finally, the explants were cultured in jars containing 25 ml of MS medium. After four weeks, the levels of contamination were recorded.

To obtain an *in vitro* sterilized plant material for later experimental use, fresh seeds were isolated from mature fruits cultured in a commercial nursery, then sterilized and cultured in three types of media MS, MS+B5 and WPM. The seed germination percentage, plant height average, leaf number average and root length average parameters were recorded after five weeks in culture media.

The sterilized *in vivo* explants (shoot tips and nodes) and *in vitro* explants (shoot tips and cotyledon) were cultured on MS media supplemented with 0.25mgl^{-1}, 0.5mgl^{-1} Benzyle Adinine (BA) as an initiated medium. After four weeks, the ratio of initiated explants and their average shoot length were recorded.

The healthy produced shoots from the initiation stage were subjected to six different combinations of multiplication protocols to study the effects of different growth regulators cultured in two types of media and different fructose concentrations as shown below.

A. MS medium supplemented with BA alone at (0.0, 0.25, 0.5 and 1 mgl^{-1}).
B. WPM medium augmented with BA alone at (0.0, 0.25, 0.5 and 1 mgl^{-1}).
C. MS medium supplemented with 0.5 mgl^{-1} BA combined with different concentration of GA_3 (0.0, 0.25, 0.5 and 0.75mgl^{-1}).
D. WPM medium supplemented with 0.5mgl^{-1} BA combined with different concentrations of GA_3 (0.0, 0.25, 0.5 and 0.75mgl^{-1}).
E. WPM medium supplemented with 0.5mgl^{-1} BA combined with different concentration of IAA (0.0, 0.1, 0.2, 0.3 and 0.4mgl^{-1}).
F. To test the most appropriate concentration of fructose on multiple shoot induction, WPM medium was supplemented with 0.5mgl^{-1} BA combined with different concentration of fructose (20, 30, 40, 50 and 60 gl^{-1}).

To determine the effects of the above combinations, the following parameters were measured after six weeks in culture: the highest length of shoots, shoots number/ explants, mean length of shoots/explant, and leaves number.

The best proliferated shoots were splitted and cultured for rooting by using two types of media MS and WPM. Four different concentrations of NAA were used to test the best root induction protocols as shown below:

A. Full strength MS salts medium supplemented with different concentrations of NAA at (0.0, 1.0, 2.0, 3.0, and 4.0mgl^{-1}).
B. Half strength MS salts medium supplemented with different concentrations of NAA at (0.0, 1.0, 2.0, 3.0, and 4.0mgl^{-1}).
C. Full strength WPM salts medium supplemented with different concentrations of NAA at (0.0, 1.0, 2.0, 3.0, and 4.0 mgl^{-1}).

jeillan.hassan3@gmail.com

D. Half strength WPM salts medium supplemented with different concentrations of NAA at (0.0, 1.0, 2.0, 3.0, and 4.0 mgl^{-1}).

Rooting percentage, number of roots and roots length average were recorded after six weeks in culture in growth room at 24± 2°Cwith 16/8 hours of photoperiods.

The rooted shoots were taken from the *in vitro* rooting medium after washing thoroughly with tap water to remove the adhering agar and illuminate the sources of contamination by bacteria or fungi. The plantlets were immersed in Benlate fungicide solution (0.1%) for 10 minutes before planting in small pots containing sterilized soil mixture of peatmoss, loam and Styrofoam in ratio of (1:1:0.5) (v:v:v:). The small pots were put in sterile boxes and covered by polyethylene bag to maintain high humidity and lighting.

The experiments were designed according to Completely Random Design (C.R.D.) system using three replicates with three plants in each treatment. Substantial differences between the means values were compared with each other in the columns using Duncan's multiple range test at 5% level. The all statistical data were analyzed by using SAS computerized program (SAS, 2001).

RESULTS AND DISCUSSION

Both sodium hypochlorite and Mercuric chloride (Table 1) were used to perform the ideal surface sterilization condition of seeds, shoot tips and nodes. Maximum healthy percentage (100%, 85%, and 90%) were respectively observed for seeds, shoot tips and nodes with the use of Mercuric chloride solution 0.1% (w/v) for 10 minutes, followed by sodium hypochlorite (70%) v/v for 30 minutes. Sodium hypochlorite (50%) v/v showed the lowest healthy percentage (70%, 60%, and 65%) for seeds, shoot tips and nodes respectively. Mercuric chloride ($HgCl_2$) is a broad range of disinfectant. Mercuric is a highly hazardous chemical element which has the ability to form toxic volatile compounds and chlorine is electronegative therefore can oxidizes the peptide bonds and denatures the protein of microbes (Barrette *et al.*, 1989). Although mercuric chloride is the most commonly used disinfectant to kill the microbes on the explants but it has sever effect on other organism such as irritation to eyes, skin and respiratory tract. It will cause undesirable effects in the environment if the used mercuric chloride was discarded from the laboratory (Kumar *et al*., 2009).

Table (1): The effects of different disinfectant agents on contamination of Marumi kumquat explants

Type	Disinfectant agent	Concentration	Duration of exposure (min)	Percentage of contamination %	Percentage of healthy explants %
Seed	NaOCl	2.5%	30	30	70
	NaOCl	3.75%	30	2	98
	$HgCl_2$	0.1%	10	0	100
Shoot tip	NaOCl	2.5%	30	40	60
	NaOCl	3.75%	30	30	70
	$HgCl_2$	0.1%	10	15	85
Nodes	NaOCl	2.5%	30	35	65
	NaOCl	3.75%	30	5	95
	$HgCl_2$	0.1%	10	10	90

The results in Table (2) reveals that 100% of seeds were germinated on MS medium while the ratio reached 90% on MS supplemented with B5 vitamin. Moreover, the least ratio was achieved by using WPM medium. On the other hand, the highest plant height and leaves number (3.65 cm, 3.11) were recorded in the plantlets grown in MS respectively which were significantly higher than the rest of treatments. Meanwhile, the highest

roots length achieved 5.96 cm and was recorded in the plantlets cultured in MS medium supplemented with B5 vitamin. Previous studies on the micropropagation of citrus species showed that the cytokinin concentration and various explant types have critical effects on shoot regeneration (Duran-Villa 1989; Silva *et al*. 2006). Both *in vivo* and *in vitro* shoot tips showed best response than nodes and cotyledons (Table 3). The maximum shoot length were recorded in plantlets grown in MS medium

supplemented with 0.25 mgl^{-1} BA using *in vivo* shoot tips (1.3 cm), nodes (0.6 cm) and cotyledon (0.48 cm). Meanwhile, *in vitro* shoot tips recorded the highest initiation percentage (100%) but the highest shoot length was found in MS medium augmented with 0.5 mgl^{-1} (Ahmed *et al*., 2011).

Table (2): Effect of different types of media on seed germination percentage, plant height, leaf number and root length average of *Fortunella japonica* after five weeks in culture

Type of culture media	Seed germination percentage (%)	Plant height average (cm)	Leaves number average	Roots length average (cm)
MS	100 a	3.65 a	3.11 a	5.23 a
MS+B5	90 b	1.04 b	2.44 b	5.96 a
WPM	65 c	0.62 b	2.22 b	2.52 b

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

Table (3): Response of different explant types to growth of *Fortunella japonica* cultured on MS

Explant type	BA concentrations mgl^{-1}	Initiation percentage	Mean length of shoots (cm)
***in vivo* shoot tips**	0.25	80.000 b	1.03 a
	0.5	40.000 d	0.78 b
***in vitro* shoot tips**	0.25	80.000 b	0.51 c
	0.5	100.000 a	0.71 b
***in vivo* nodes**	0.25	50.000 c	0.65 bc
	0.5	30.000 e	0.48 c
***in vitro* cotyledons**	0.25	30.000 e	0.63 bc
	0.50	20.000 f	0.48 c

media supplemented with different concentrations of BA after 6 weeks in culture
Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

Significant increases were obtained in shoots and leaves numbers. The highest shoots length was obtained using WPM medium as compared to MS medium (Table 4). However, among the different concentrations of BA, 0.5mgl^{-1} BA produced more shoots number and longer shoots than 0.0, 0.25 and 0.5 mgl^{-1} BA in both media. The data showed that the MS medium supplemented with 0.25 mgl^{-1} BA gave not-significant difference in both shoots and leaves number parameters (2.05, 4.33) respectively

jeillan.hassan3@gmail.com

when compared to the control treatment (2.16, 4.66) respectively.

The findings also revealed that the WPM medium augmented with 0.5mgl^{-1} of BA increased the highest shoots length, shoots number, the mean shoots length and leaves number significantly and recorded the highest values when compared with the other treatments. These results matched the findings of Ahmed *et al.* (2011) who reported that 0.5 mgl^{-1} of BA increased the number of leaves.

Table (4): The effect of different BA concentrations and media types on shoot multiplication of *Fortunella japonica* after 6 weeks in culture

Media types	BA concentrations mgl^{-1}	Highest shoots lengths (cm)	Shoots number/ explants	Mean length of shoots cm	Leaves number/ explants
MS	0.00	0.75 d	2.16 b	0.65 c	4.66 c
	0.25	1.03 b-d	2.05 b	0.75 bc	4.33 c
	0.50	1.36 ab	3.50 ab	1.13 ab	7.00 bc
	1	0.70 d	2.66 b	0.53 c	6.00 bc
WPM	0.00	0.95 cd	2.16 b	0.63 c	3.66 c
	0.25	1.03 b-d	3.16 ab	0.73 bc	7.33 bc
	0.50	1.56 a	4.50 a	1.26 a	10.83 a
	1	1.20 a-c	4.16 a	1.11 ab	9.16 ab

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

Table (5) have illustrate the effect of 0.5 mgl^{-1} BA combined with different levels of GA_3 using two types of media, the result clarifies that MS medium supplemented with 0.5mgl^{-1} BA combined 0.5 mgl^{-1} GA_3 recorded the highest values of studied parameters (shoots length, shoots number per explant, mean length of shoots and leaves number per explant) which recorded 1.40, 4.33, 1.13 and 10.16 respectively. At the same time, those values were decreased significantly when the concentration of GA_3 was reduced to 0.75 mgl^{-1} and BA alone.

Moreover, the plantlets grown in WPM showed similar response using the same protocols but the parameters were less than those of MS media. Gibberlic acid (0.5, 0.75mgl^{-1}) is the most commonly used growth regulator for internodes elongation and meristem growth. The previous results are disagree with those of Chawla, (2004) who mentioned that the presence of GA_3 lead to produce longer shoots than those cultured without it, while Henderson, (1958) disclosed the inhibitory effects of gibberelline in micropropagation of Scorzonera, Daucus, Rubus, Helianthus tuberosus, and Helianthus aownins.

jeillan.hassan3@gmail.com

Table (5): The effect of BA+GA_3 and media type on shoot multiplication of *Fortunela japonica* after six weeks in culture media

Media type	BA+GA_3 concentration mgl^{-1}	Highest shoots length (cm)	Shoots number/ explant	Mean length of shoots (cm)	Leaves number/explant
MS	0.5+0.0	1.30 a	2.83 ab	1.00 a	6.66 a
	0.5+0.25	1.21 a	4.16 ab	0.90 a	9.00 a
	0.5+0.5	1.40 a	4.33 ab	1.13 a	10.16 a
	0.5+0.75	1.18 a	3.00 ab	1.03 a	6.66 a
WPM	0.5+0.0	1.46 a	4.66 a	1.21 a	9.33 a
	0.5+0.25	1.45 a	3.83 ab	1.30 a	5.50 a
	0.5+0.5	1.31 a	3.16 ab	1.06 a	7.83 a
	0.5+0.75	1.16 a	2.66 b	0.96 a	5.33 a

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

Table (6) evaluates the effect of 0.5 mgl^{-1} BA combined with different concentrations of IAA (0.0, 0.1, 0.2, 0.3 and 0.4) in WPM medium which was the best medium for multiplication, the results showed that non-significant differences was found in studied parameters by increasing the concentration of IAA. Moreover, the highest values were recorded in the WPM medium supplemented with 0.5 mgl^{-1} BA combined with 0.3 mgl^{-1} IAA (1.31, 6.44, 1.1 and 14.66) of highest shoot length, shoot number per explant, mean length of shoots and leaves number per explant respectively, which start to decrease when the levels of IAA increased to 0.4 mgl^{-1}. These results correspond with the findings of (Eklöf *et al*., 1997) who explained that cytokinin and auxin ratio effects the plant morphogenesis and organogenesis.

jeillan.hassan3@gmail.com

Table (6): The effects of interaction between 0.5 mgl^{-1} BA and different concentration of IAA on shoot multiplication of *Fortunella japonica* after six weeks in WPM culture medium.

BA+IAA concentrations (mgl^{-1})	Highest shoots length (cm)	Shoots number/ explant	Mean length of shoots cm	Leaves number/explant
0.5+0.0	1.30 a	5.22 ab	1.15 a	10.33 bc
0.5+0.1	1.11 a	6.11 a	0.90 a	12.44 ab
0.5+0.2	1.18 a	6.66 a	1.02 a	14.11 a
0.5+0.3	1.31 a	6.44 a	1.10 a	14.66 a
0.5+0.4	0.63 b	4.33 b	0.53 b	9.00 c

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

Table (7) estimates the effects of carbon source in media culture, the data of studied parameters showed that shoot multiplication traits were influenced by different level of fructose added to WPM enriched with 0.5 mgl^{-1} BA. Adding 30 gl^{-1} of fructose concentration caused valuable effect which was significantly better than the other levels in all shoot multiplication parameters among all other tested treatments. The highest shoot number per explant reached 9.00 recorded at 30 gl^{-1} of fructose, was better when compared to 40, 50, and 60 gl^{-1} and not differs significantly from 20 gl^{-1}. The highest shoot length (1.56 cm) was also noticed at 30gl^{-1} which showed obviously higher than 20, 40, 50 and 60gl^{-1}. The maximum numbers of leaves per explant was obtained when WPM medium supplemented with 30 gl^{-1} of fructose (21.0), and the lowest number recorded on 60 gl^{-1} fructose (10.0). Meanwhile, fructose levels lead to decrease shoot length gradually, The above results outcomes harmonize the findings of (Agnieszka *et al*., 2013) who found that fructose at 30 gl^{-1} produce highest number of shoots and shoot length during his study on the *in vitro* propagation of (*physocarpus opulifolius* L).

jeillan.hassan3@gmail.com

Table (7): Effect of different concentrations of fructose on *Fortunella japonica* multiplication cultured on WPM medium supplemented with 0.5 mgl^{-1} BA after six weeks in culture.

Fructose concentrations gl^{-1}	Highest shoots length (cm)	Shoots number/ explant	Mean length of shoots cm	Leaves number/explant
20	1.15 b	7.83 a	0.93 ab	16.60 b
30	1.56 a	9.00 a	1.16 a	21.00 a
40	1.13 b	5.66 b	0.90 ab	10.50 c
50	1.02 b	4.00 b	0.63 bc	11.62 c
60	0.99 b	5.33 b	0.53 c	10.00 c

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

Table (8) shows the results for roots formation, four combinations were tested to evaluate the microshoots rooting. The results revealed that the four media types full and half MS salts strength, full and half WPM salts strength at different concentrations of NAA (0.0, 1, 2, 3 and 4 mgl^{-1}). Full and half MS strength showed highest rooting percentage (100% and 80%) respectively at 2 mgl^{-1} NAA. On the time that both full and half WPM strength media gave the highest rooting ratio 80% at both 1 and 4 mgl^{-1} NAA in full WPM strength and at 3mgl^{-1} NAA in half strength.

Reducing MS salts strength to the half decrease the number of roots per explant and root length average at all NAA levels. These findings revealed that the maximum roots number per explant (8.33) and root length average (5.54 cm) at 2 mgl^{-1} grown in full MS strength compared with (5.44) and (2.29 cm) in half MS strength.

Table (8) also illustrates that the optimal concentration of NAA on full strength WPM for rooting initiation was 4mgl^{-1} (9.44) roots which gave the highest number as compared with control treatment. Moreover, the best root length was achieved at 1mgl^{-1} (3.23 cm) using full strength WPM media.

It can be concluded that the full strength MS and WPM were more suitable for root induction in Marumi plants. The current results are not corresponded with Suneel *et al*. (2009) who denoted that during citrus rootstocks grow in half strength MS media was better for root formation than full strength MS media. These outcomes showed that the NAA has an optimal role on rhizogenesis under *in vitro* conditions in Marumi plant due to its effects on roots regeneration and stimulating cell elongation (Denial *et al*., 2009).

Table (8): The effects of different concentrations of NAA and media type on *in vitro* rooting of *Fortunella japonica* after 6 weeks in culture medium

Media type	NAA Concentration mgl^{-1}	Rooting percentage (%)	Roots number/ explant	Roots length average (cm)
MS	0	30.000 fg	1.00 d	1.81 b-e
	1	80.000 b	4.33 b-d	2.73 bc
	2	100.000 a	8.33 ab	5.54 a
	3	60.000 cd	3.22 cd	1.54 c-e
	4	40.000 ef	0.77 d	1.27 c-e
1/2 MS	0	70.000 bc	0.88 d	1.51 c-e

jeillan.hassan3@gmail.com

	1	50.000 de	1.22 cd	0.87 de
	2	80.000 b	5.44 a-d	2.29 b-d
	3	40.000 ef	3.11 cd	0.89 de
	4	30.000 fg	0.55 d	0.47 e
WPM	0	40.000 ef	1.00 d	0.87 de
	1	80.000 b	3.88 b-d	3.23 b
	2	70.000 bc	6.11 a-c	1.27 c-e
	3	50.000 de	4.22 b-d	0.68 de
	4	80.000 b	9.44 a	2.14 b-e
1/2 WPM	0	20.000 g	0.44 d	0.42 e
	1	40.000 ef	1.22 cd	0.81 de
	2	70.000 bc	3.66 b-d	0.95 de
	3	80.000 b	4.33 b-d	1.12 c-e
	4	70.000 bc	1.88 cd	0.43 e

Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

At the end of the investigation, 92% of the rooted plantlets were survived after six weeks by gradually hardening and transferring to pots containing sterilized mixture of (peatmoss + loam + styrofoam) 1:1:0.5 (v: v: v). Following these steps of gradually hardening agree with what has been suggested by many researches in citrus plants which finally transferred to open air field condition (Awatef, 2017; Goswami *et al*., 2013).

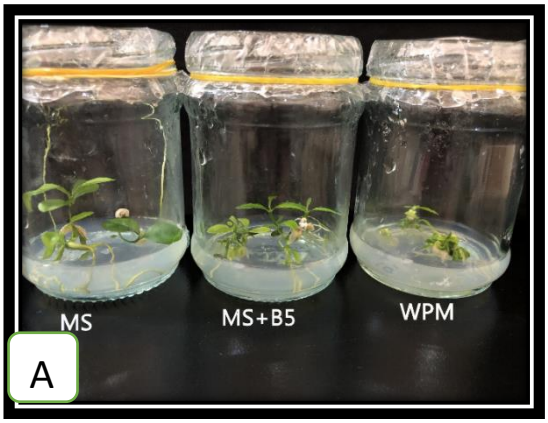
CONCLUSION

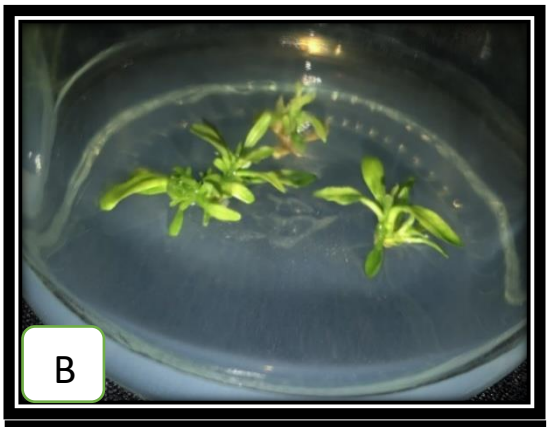
Depend on the results gained from the present investigation concerning the effect of different factors included in micropropagation of Marumi (*F. japonica*), the following conclusions can be arrived: Treating the explants with 0.1% $HgCl_2$ + three drops of tween-20 is the most effective protocol for obtaining the highest healthy ratio of uncontaminated explant. In initiation stage, *in vitro* shoot tips was the most effective explant type which showed the highest percentage of initiation response when cultured on MS medium supplemented with 0.5mgl^{-1} BA for *F. japonica*. Valuable multiplication was observed via using WPM medium containing 0.5mgl^{-1} BA + 0.3mgl^{-1} IAA. It was clear at multiplication stage, adding fructose sugar in amount of 30gl^{-1} in WPM medium enriched with 0.5 mgl^{-1} BA stimulate the mass production and record better values for all tested parameters. For root development, full strength of WPM medium salts supplemented with 4mgl^{-1} NAA increase the root number.

RECOMMENDATIONS

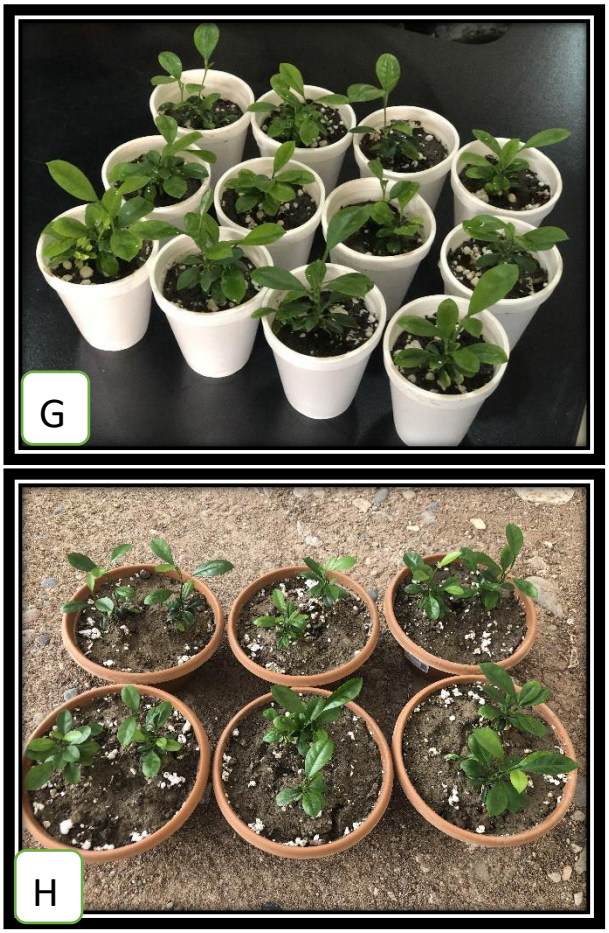
Use the obtained typical protocol in this study to increase the mass production via *in vitro* propagation in Kurdistan region and make channels for cooperation with other institutions for commercial production. Furthermore, Analyzing plant parts extracts and compared to natural plants to identify the effective materials which can be used as medical materials can be recommended. Finally, the current recommended protocol for mass production opens the door to start studying the genetic transformation to enhance traits of this tree.

jeillan.hassan3@gmail.com





jeillan.hassan3@gmail.com



A
: seedling on three types of media.
B: shoot tip *in vivo* on MS medium supplemented with 0.25 mgl^{-1} BA.
C: multiplication stage on WPM medium supplemented with 0.5 mgl^{-1} BA.
D: multiplication stage on WPM medium supplemented with 0.5 mgl^{-1} BA with 30 gl^{-1} fructose concentration.
E: rooting stage on the full strength MS medium supplemented with 2 mgl^{-1} NAA.
F: rooting stage on the full strength WPM medium supplemented with 4 mgl^{-1} NAA.
G: acclimatized plantlet.
H: well established plants grown in the greenhouse.

REFERENCES

Agnieszka I.; Katarzyna J. K. & Ewelina J. (2013). The effect of carbon source in culture medium on micropropagation of common ninebark (Physocarpus opulifolius (L.) maxim.) 'Diable D'or'.

Ahmed, M. E.; Begum, H.; Sivaramakrishnan, S.; Teixeira da Silva, J. A.; Reddy, S. A. & Al-gabal, A. Q. (2011). Rapid protocol for *in vitro* multiplication of *Citrus limonia* Osbeck Rootstock. Citrus grandis L. Qsbeck). In Vitro Cell. Dev. Biol. Plant 36: 511–516.

Amgai, R.B.; H. K. Prasai and Y. R. Pandey. (2016). Hormonal effect on mandarin orange (*Citrus reticulata* Blanco) micro-propagation. *Nepal Journal of Biotechnology*, 4(1), 33-36.

Awatef, M. E. Badr-Elden. (2017). Establishment of in Direct Propagation of Mandarin (*citrus reticulata L.*) Using Tissue Culture. Vol. 57, No.3, pp.405-416.

Barman P.; G. S. K. Swami, C. P. Patil and N. Thammaiah. (2006). Response of Rangpur lime seeds to various chemicals and bio-control agents for germination

jeillan.hassan3@gmail.com

and viability of seeds. *Journal of Asian Horticulture* 3(1), 22-26.

Barrette, W. C.; D. M. Hannum; W. D. Wheeler and J. K. Hurst. (1989). Biochemistry. 28, 9172-9178.

Chawla, H. S. (2004). *Introduction to Plant Biotechnology,* Oxford IBH publishing Co., pvt. Ltd, New Delhi, India, pp 39-41.

Denial, G. H., A. N., Yousif and M. S. Omar. (2009). Clonal propagation of Dianthus caryophyllus L. the 2ed Kurdistan Conference on Biological Sciences, 6-8 May. Journal of Duhok University (12)1:91-95.

Duran-Villa N., Ortega V., Navarro L. (1989). Morphogenesis and tissue culture of three Citrus species. Plant Cell Tissue Organ Cult. 16: 123–133.

Eklöf, S.; Åstot, C.; Moritz, T.; Blackwell, J.; Olsson, O.; and Sandberg, G. (1997). Auxin-cytokinin interactions in wild type and transgenic tobacco. Plant Cell Physiol.33**,** 225–235.

Gmitter, F.G., J. Soneji, M.N. Rao, and S. Huang. Citrus spp. (2007). Eds. Janick, J. and R.E. Paull. The Encyclopedia of Fruit and Nuts. 773-784.

Goswami, K.; R. Sharma, P. K. Singh and G. Singh. (2013). Micropropagation of seedless lemon (*citrus limon L.* cv. Kaghzi Kalan) and assessment of genetic fidelity of micropropagated plants using RAPD markers. 19(1):137-145.

Henderson, J. H. M. (1958). Effect of gibberellin on sunflower tissue culture. Nature 182:880.

Kumar, K. J.; Xing, M. Z. C.; Azzoz, M.; Jaleel, C. A. Plant Omics. (2009). 2(3), 120-126.

"**Kumquat**". *Collins Dictionary. n.d*. Retrieved 25 September 2014.

McCown, B. H. and Lloyd, G. (1981) Woody Plant Medium (WPM)—A Mineral Nutrient Formulation for Microculture of Woody Plant Species. HortScience, 16, 453-453.

Murashige, T. and Skoog, F. (1962).A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plantar. 15:478-487.

Nguyen, V. D. and Doan, Th. N. (1989).Medicinal Plants in Vietnam.ISBN 92 9061 101 4.

Nouri A. and A. Shafaghat. (2015). Chemical constituents and antioxidant activity of essential oil and organic extract from the peel and kernel parts of Citrus japonica Thunb. (kumquat). University of Urmia.

SAS. (2001). SAS/STAT Vser's Guide for personal Computers. Release 6.12 SAS Institute Inc. Cary, NC, USA.

Satyal, P.; Paudel, P.; limbu, K. & Setzer, W. N. (2012). Leaf essential oil composition\n of Citrus japonica from Nepal. J Essent Oil bear Plants. 15:357–359.

Shohael, A. M. (2008). Advantages of plant tissue. JSPS Fellow at gene Research Center, PhD. Tsukuba University, JAPAN.

Silva R.P., Almeida W.A.B., Souza E.S., Filho F.A.A.M. (2006). In vitro organogenesis from adult tissue of 'Bahia' sweet orange (Citrus sinensis). Fruits 61: 367–371.

Suneel Sh.; Atam, P. and Ajinath, T. (2009). *In vitro* Propagation of Citrus Rootstocks. Cluj 37(1):48-88.

Thorpe, T. A. (1990). The current status of plant tissue culture, in Plant Tissue Culture: Applications and Limitations (Bhojwani, S. S., ed.), Elsevier, Amsterdam, pp. 1–33.

Van, L. B. (2009). Plant tissue culture (overview). Plant Biotechnology Vietnam Open Course Ware University of Science.

jeillan.hassan3@gmail.com

پوختە

پرتەقالا ژاپونی ژبەر بهایێ وێ یێ خارنێ و بکارئینانێت وێ یێت پزیشکی ل ژمارەکا ناڤچا ل سەرانسەری جیهانێ دهێتە چاندن. ئارمانجا ڤێ توێژینێ هەلسەنگاندنا کارتێکرنا دوو جورێت ناڤەندا و رێکخستییێت گەشەکرنا ڕوەکی یێت جیاوازە لسەر زێدەکرنا هویر یا ڤی ڕوەکی. هاتە تێبینی کرن کو باشترین سەرەدەری بۆ تافیرکرنا سەرڤە ب رێکا HgCl2 0.1% ب خەستییا هاتە ئەنجامدان بۆ ماوێ دەە خولەکا کو بلندترین رێژا ڕوەکێت نە پیسبووی د ناڤەندێ دەستپێکێ دا تومارکر. د قوناغا زێدەبونێ دا، هاتە دیتن کو ناڤەندێ WPM ئەنجامێت باشتر تومارکرن ژ ناڤەندێ MS ل هەمی خەستییێت BA ئەوێت هاتینە بکارئینان. چ جیاوازییێت وەیی یێت بەرچاڤ لدەمێ بکارئینانا BA بتنێ یان بزێدەکرنا GA3د تایبەتمەندییێت هاتینە پیڤان دا نینن. هاتە تێبینی کرن کو ناڤەندێ WPM یێ ڕەق یێ زێدەکری ب زێدەکرنا 30 گم/لتر ژ فرەکتوزی ب هەبوونا 0.5 ملگم/لتر یێت BA مەزنترین درێژی دا چەقێت ڕووەکی (1.56سم) و زێدەترین ژمارێت چەقا بۆ هەر پارچەکا ڕووەکی (9.0) و زێدەترین ژمارا بەلگا دا هەر ڕوەکەکی (21.0). باشترین ئەنجامێت قوناغا ڕەگا هاتنە دیارکرن د ناڤەندێ هێزا تمام یا خوێ یێ MS ڕەق یێ زێدەکری ب زێدەکرنا 2 ملگم/لتر ژ NAA ب رێکا تومارکرنا بلندترین رێژا چێبوونا ڕەگا. ڕوەکێت ڕەگ دایین ب رێکا زێدەکرنا هویر پلە پلە هاتنە گونجاندن و هاتنە ڤەگوهاستن بۆ بارودوخێت خانییێت شویشەی و رێژەکا بلند یا مانێ تومارکر کو گەهشتە 92%.

الخلاصة

يعتبر البرتقال الياباني مهماً لقيمته الغذائية واستعمالاته الطبية في العديدمن المناطق في العالم. تهدف الدراسة الحالية الى تقييم تأثير نوعين من الأوساط الغذائية ومنظمات النمو النباتية المختلفة على الإكثار الدقيق لهذا النبات. لوحظ بأن أفضل معاملة للتعقيم السطحي تمت بإستعمال كلوريد الزئبق بتركيز 0.1% لمدة عشر دقائق، الذي سجل أعلى نسبة نباتات سليمة غير ملوثة في وسط النشوء. ووجد في مرحلة التضاعف، بأن وسط WPM سجل أفضل النتائج مقارنةً بوسط MS عند جميع تراكيز BA المستعملة. كما لوحظ عدم وجود إختلاف معنوي عند إستعمال BA لوحده أو بإضافة GA3 في الصفات المدروسة. وتبين بأن الوسط WPM الصلب المدعم بإضافة 30 غم/لتر من الفروكتوز بوجود 0.5 ملغم/لتر BA أعطى أعلى طول لتفرعات النبيتات (1.56 سم) وأكثر عدد أفرع لكل قطعة نباتية (9.0) وأعطى أكثر عدد من الأوراق لكل نبتة (21.0). وأظهرت نتائج التجذير تفوقاً واضحاً MS في وسط كامل القوة الملحية شبه الصلب المدعم بإضافة 2ملغم/لتر NAA من خلال تسجيله أعلى نسبة تجذير. تم أقلمة المبيتات المتمايزة تدريجيا ونقلها إلى ظروف البيت الزجاجي وسجلت نسبة بقاء عالية وصلت إلى 92%.

jeillan.hassan3@gmail.com