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Protocol for Micropropagation of Two *Ficus carica* Cultivars

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Abstract: Shoot tips of two fig cultivars (Conadria and Black Mission) proliferated *in vitro*, were cultured on modified MS medium supplemented with 0.5 mg/l 6-benzylaminopurine (BAP). The present investigation aimed to investigate the effect of different medium salt strengths (Double, full, half and quarter) and different sources of carbon (sucrose and fructose) at different concentrations (0.1 and 0.2 mol/l) supplemented in MS medium on shoot multiplication and rooting stage of studied fig cultivars. Obtained results showed that Black Mission cultivar surpassed Conadria cultivar in shoot number while, Conadria gave higher leaves number and shoot length than Black Mission. Full and double MS medium strengths enhanced both of shoots number and shoot length, significantly. Fructose as a carbon source was better than sucrose for multiplication. The best shoot length and shoot number were obtained when fructose was added to the medium at 0.2 mol/l. In rooting stage, MS medium with half strength increased markedly rooting percentage in comparison with other medium strengths. Rooting percentage, root number, root length and plantlet length of fig shoots were better on MS medium contained fructose (0.1M) as a carbon source compared with those on medium contained sucrose.

Key words: Black Mission · Carbon sources · Conadria · Fig cultivar · Medium strength · Shoot proliferation

INTRODUCTION

Low water required crops are an important need concerning shortage in water resources in arid and semi arid zones. One of these crops is *Ficus carica* L. There is a major interest in increasing fig-cultivated area in Egypt since it is one of the traditional Mediterranean crops has a high nutrient value. In order to achieve this target there is a need to get rapid plant cloning for producing sufficient number of fig seedlings. Biotechnology tools can play this role and provide a rapid method for plant mass propagation [1]. However, *in vitro* growth and multiplication of *Ficus carica* are affected by many factors [2], these factors included culture medium strength, type and concentration of carbon source. The composition of the medium is a determining factor for growth. The cells of most plant species can be grown on completely defined media, the wide use of MS-medium [3] or its modifications. Andreu and Marín [4] stated that culture medium composition influenced the multiplication rate of Prunus rootstock, whereas, MS produced a significantly higher number of shoots than other media tested. In addition, Tang *et al*. [5] found that average shoot regeneration was obtained on leaf section of *Pyrus communis* "Bartlett" when cultured on Murashige and Skoog full medium containing 6 mg/l BA and 0.1mg/l NAA. Meanwhile, Jain *et al.* [6] showed that among the different media tested, one half-strength MS medium reduced shoot-tip necrosis significantly and gave higher shoot multiplication and growth in H. procumbens. Regarding to carbon sources, Vespasiano and Otoni [7] reported that, plant cell, tissue and organ cultures require a carbohydrate supply in order to satisfy energy demands. Although there are many available carbon sources, sucrose is the major one [8, 9]. In addition, Jain *et al*. [6] showed that, sucrose at 0.086 M (3%) was the preferred carbon source in terms of both growth and preventing shoot-tip necrosis of H. procumbens compared to glucose, maltose and fructose at equimolar concentrations. Moreover, Abdel-Gawad *et al*. [10] indicated that all sucrose treatments (20, 30 and 40 g/l) enhanced the proliferation percentage and shoot number of pineapple (*Ananas comosus*) compared with mannitol and fructose treatments.

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This study aimed to investigate the effect of different medium strength and carbon sources concentrations on micropropagation of two fig cultivars (Conadria and Black Mission).

MATERIALS AND METHODS

This study was carried out at the Biotechnology Laboratory of Pomology Department, National Research Centre, Dokki, Giza, Egypt during the period from 2012 to 2014. Shoot tip of two fig cultivars (Conadria and Black Mission) were collected in plastic bags and presented to the laboratory. Explants were washed with tap water and sterilized into the Laminar Flow Hood and cultured individually on MS medium (1962) as a basal medium supplemented with 0.5 mg\l 6-benzylaminopurine (BAP), 30 g/l sucrose and 6 g/l Difco Bacto agar [11] during the establishment stage. The pH of the medium was adjusted to 5.7 and autoclaved at 121°C and 15 Ib\in^2 for 20 minutes. The cultured explants were incubated under 16 hours of artificial light (fluorescent light at 30 µM\sec) and 8 hours of darkness at average temperature 23±2°C. *In vitro* raised clusters were selected with three shoots for each and cultured on the tested modified media. This investigation was carried out as follows:

Multiplication Stage: Effect of MS Medium Salts Strength: Different medium salts strengths (double, full, half and quarter) supplemented with BAP at 0.5 mg\l were tested to find out the best medium strength that encourages the highest multiplication rate.

Effect of Carbon Source and Concentration: Sucrose and fructose were added at 0.1 and 0.2 mol/l in MS medium supplemented with BAP at 0.5 mg/l. *In vitro* raised clusters with two shoots were cultured on the previous media. After four subcultures, data concerned with average shoot number, average leaf number per shoot and average shoot length (cm) per shoot were recorded.

Rooting Stage:

Effect of MS Salts Strength: Different salt strengths (double, full, half and quarter) were tested to find out the best medium strength that encourages the highest rooting rate. Individual shoots were cultured on the previous media supplemented with 1.0 mg/l NAA and 0.5 mg/l BAP [11].

Effect of Carbon Source and Concentration: Sucrose and fructose were added at 0.1 and 0.2 mol/l in MS medium supplemented with 1.0 mg/l NAA+0.5 mg/l BAP to find out the best carbon source and concentration for rooting.

Statistical Analysis: Treatments were arranged in complete randomized design, each treatment was replicated three times, each replicate involved three jars and each contained three clusters (in multiplication) or three plants (in rooting). Means were compared according to the method described by Snedecor and Cochran [12].

RESULTS AND DISCUSSION

Effect of Medium Salts Strength on Shoot Multiplication and Rooting: In regard to shoot multiplication of two fig cultivars (Black Mission and Conadria), data in Table 1 and Fig.1 revealed that culture medium with full strength recorded the highest values of both shoot and leaf number while, culture medium with double strength gave the highest shoot length without significant difference with full strength. Besides, Black Mission cultivar surpassed Conadria cultivar in shoot number. However, Conadria produced higher number of leaves and shoot length in comparison with Black Mission. In addition, data indicated that shoots of Conadria cv. produced the highest values of leaves number and shoot length when cultured on medium with double strength in comparison with other studied treatments. On the other side, shoots proliferation rate of Black Mission cv. was markedly higher than other treatment. With respect to

Table 1: Effect of MS- power on multiplication of fig cultivars (Black Mission and Conadria).

	Shoot number			Leaf number			Shoot length		
Treatment	Black Mission	Conadria	Mean	Black Mission	Conadria	Mean	Black Mission	Conadria	Mean
¼ MS	18.0c	10.0g	14.0c	2.20g	2.78d	2.45d	0.69g	1.68e	1.19c
½ MS	19.67b	11.5f	15.59b	2.33f	3.76b	3.05c	1.15f	2.14c	1.65b
Full MS	25.67a	13.75d	19.71a	3.65c	5.12a	4.39a	2.12d	4.06b	3.09a
Double MS	12.67e	10.75g	11.71d	2.76e	5.21a	3.99b	1.5e	4.90a	3.20a
Mean	19.00A	11.5B	--	2.74B	4.22A	--	1.37B	3.20A	--

Table 2: Effect of MS- power on rooting of fig cultivars (Black Mission and Conadria).

	Rooting %			Root number			Root length			Plantlet length		
Treatment	Black Mission	Conadria	Mean	Black Mission	Conadria	Mean	Black Mission	Conadria	Mean	Black Mission	Conadria	Mean
¼ MS	100	66.67	83.34	10.11a	6.52c	8.32a	5.8a	1.67d	3.74b	10.42a	5.72c	8.07a
½ MS	100	77.78	88.89	5.23d	9.15b	7.19b	4.2b	4.33b	4.27a	6.33b	5.42d	5.88b
Full MS	100	55.56	77.78	3.14e	2.23f	2.69c	1.85c	2.0c	1.93c	6.22b	5.33d	5.78b
Double MS	100	33.33	66.67	1.24g	1.11g	1.18d	0.5f	1.0e	0.75d	5.45d	4.12e	4.79c
Mean	100.0	58.34	--	4.93A	4.75B	--	3.09A	2.25B	--	7.11A	5.15B	--

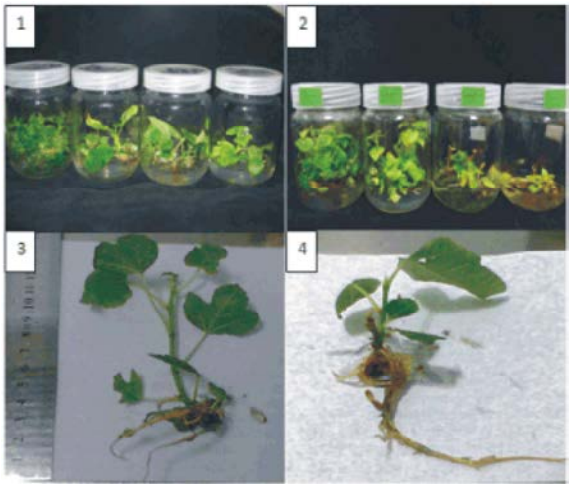

Fig 1: Fig shoot micropropagation: 1- Effect of MS power on multiplication of Black Mission cv. from the right: ¼ MS, ½ MS, MS and 2MS. 2- Effect of MS power on multiplication of Conadria cv. from the right: ¼ MS, ½ MS, MS and 2MS. 3- Black Mission plantlet rooted on ¼ MS 4- Conadria plantlet rooted on ½ MS

rooting stage, data in Table 2 showed that MS medium with half strength increased markedly rooting percentage in comparison with other medium strengths. In addition, it encouraged root growth whereas produced the highest root length. However, fig shoots when cultured on ¼ MS produced the highest number of roots and plantlet length. In respect to differences between two fig cultivars, it was found that Black Mission was surpassed conadria in all studied parameters. Moreover, it was noticed that shoots of Black Mission gave the highest values of rooting percentage, root number, root length and plantlet length when subcultured on MS with quarter strength in comparison with other treatment.

Generally, it is clear that Black Mission cultivar surpassed Conadria cultivar in all studied parameters. In addition, it could be recommended that medium with full strength was the best than other tested strengths with multiplication; this conclusion was in the same line with that found by Mamiya and Sakamoto [13]. They studied the effect of strength of basal medium on producing plants from somatic embryos in Asparagus and they found that strength of basal medium had significant effects on shoot growth. Whereas, the weights of shoots increased by increasing strength of medium from half, full, or twice, the fresh weight of shoots were increased 8.9, 31.0, or 60.0 mg per plant, respectively. Nower [14] stated that, mixture of half- strength MS + half strength B5 medium produced high yield of somatic embryos of mango cv. Sdeek and Zebda. Moreover, this mixture was suitable to develop these embryos into plantlets. Meanwhile, Soumendra *et al.* [15] stated that sterilized pomegranate seeds were germinated well on half-strength MS medium and 6 g/l agar. In addition, they used half strength MS medium for further *in vitro* elongation of pomegranate plantlets. Moreover, Dantas *et al*. [16] stated that the highest shoot proliferation rate of pear cultivars was observed when cultured on ¾ strength MS medium with 1.6 µM/l BAP. Zaied [17] stated that one-fourth strength of MS medium was more effective for establishment of apricot and one- fourth modified medium strength of MS medium was efficient for peach and almond plants. Meanwhile, root formation of fig shoots was better on quarter and half MS strength than full strength. Obtained results were in the same line with what found by Paudyal and Haq [18]. They found that more than 75% of the shoots of *Citrus grandis* rooted when subcultured on half-strength MS medium with NAA. Meanwhile, pummel shoots on 0.25x-MS did not promote root formation. In addition, they noticed that 0.5x-MS with 1.3 mM NAA induced the highest root length. However, medium strength supplemented with NAA decreased the root length. It could be concluded that, nutrient requirements differed from plant to another and from stage to another. In addition, the mineral concentration considered critical factor for shoot and root proliferation in plants. Several studies emphasized this role and attributed the efficiency of *in vitro* technique to supply cultured tissues with all nutrient elements at optimum level in needed time [19].

Table 3: Effect of carbon source and concentration on multiplication of fig cultivars (Black Mission and Conadria).

	Shoot number			Leaf number			Shoot length		
Treatment	Black Mission	Conadria	Mean	Black Mission	Conadria	Mean	Black Mission	Conadria	Mean
Sucrose 0.1 M	28.67d	17.50f	23.09c	4.38c	5.93a	5.16a	3.45f	4.17d	3.81c
Sucrose 0.2 M	11.00g	10.50h	10.75d	3.39e	3.14e	3.27d	1.59h	2.54g	2.07d
Fructose 0.1 M	37.67b	45.00a	41.34a	4.15d	5.86a	5.01b	4.92b	5.43a	5.18a
Fructose 0.2 M	21.33e	36.33c	28.83b	4.66b	2.64f	3.65c	4.59c	4.04e	4.32b
Mean	24.67b	27.33a	--	4.15b	4.39a	--	3.64b	4.05a	--

Table 4: Effect of carbon source and concentration on rooting of fig cultivars (Black Mission and Conadria).

	Rooting %			Root number			Root length			Plantlet length		
Treatment	Black Mission	Conadria	Mean	Black Mission	Conadria	Mean	Black Mission	Conadria	Mean	Black Mission	Conadria	Mean
Sucrose 0.1 M	100.0	16.67	58.34	4.17c	3.00d	3.59c	5.94a	3.33d	4.64b	5.92c	4.08e	5.00b
Sucrose 0.2 M	16.67	16.67	58.34	1.00f	1.00f	1.0d	5.00b	3.00e	4.00c	5.33d	3.00g	4.17c
Fructose 0.1 M	100.0	100.0	100.0	4.33b	4.00c	4.17a	5.64a	4.17c	4.91a	7.67a	6.17b	6.92a
Fructose 0.2 M	100.0	66.67	83.34	5.17a	2.75e	3.96b	4.10c	1.65f	2.88d	6.33b	3.83f	5.08b
Mean	79.17	50.00	--	3.67A	2.69B	--	5.17A	3.04B	--	6.31A	4.27B	--

Effect of Carbon Sources on Shoot and Root Formation: In respect of effect of carbon source on fig shoots proliferation, data in Table 3 showed that fructose at 0.1M markedly encouraged shoot proliferation whereas this carbon source leaded to produce the highest number of shoots and shoot length meanwhile sucrose at 0.1M gave the highest number of leaves in comparison with other treatments. Moreover, Conadria cultivars surpassed Black mission in all measured parameters. In addition, shoots of conadria cultivar produced the highest number and length of shoots when cultured on medium contained Fructose at 0.1M in comparison with the other treatments. However, medium contained sucrose at 0.1M encouraged shoots of Conadria cultivar to produce the highest number of leaves and fructose at 0.1 M came in second rank without significant differences with sucrose. In respect to influenced of root formation with carbon source. Data in Table 4 showed that rooting percentage, root number, root length and plantlet length of fig shoots were better on MS medium contained fructose 0.1M as carbon source verses those on MS medium contained sucrose. In addition, it was found that Black Mission produced the highest value of root formation parameters in comparison with conadria cultivar. Moreover, Black Mission shoots produced the highest rooting percentage, root length and plantlet length when cultured on MS medium contained fructose at 0.1M as carbon source meanwhile, when fructose concentration raise to 0.2M encouraged root formation whereas Black Mission shoots produced high rate of root number in comparison with other treatment. Generally, data indicated that fructose at 0.1M resulted in the best shoot growth than sucrose fir shoots proliferation rate and encouraged root formation from fig shoots. Moreover, Black Mission was more response to fructose than Conadria cultivar.

An important factor in plant tissue culture media is the carbon source because they act as source of energy to the plants especially when they are not enable to photosynthesize their own carbohydrates during the first stage of tissue culture [20]. In addition, Akter *et al*. [21] reported that plants growth is affected by the existence of carbon source. It is importance to supply cultured plant tissues with permanent carbohydrates from the medium to enhance *in vitro* growth, under *in vitro* conditions of low light intensity, low gas exchange and high level of humidity had adverse effect on photosynthesis rate which reach to low level [22]. Therefore, cultured explants need to permanent sources for sugar to recover the decreasing in carbon source levels. In addition, Thrope [23] showed that root formation and root growth need high level of energy and carbohydrates metabolic considered the main process to provide this energy. Moreover, Traore [24] showed that during germination, shoot and root meristem has an increasing in metabolic activity and needed a continuously energy sources to build new block of tissues. Several authors studied effects of carbon source on *in vitro* developing of explant and concluded that these effects differed by varying carbon source, concentration, type of plant and cultured explants [13, 25-28]. Whereas, some authors found sucrose is more suitable for wide spectrum of plants [24]. However, other studies concluded that glucose or fructose was effective than sucrose [29-32]. Both of sucrose and glucose were used widely in tissue culture

technique. However, other carbon sources also have been reported to be efficient to promote embryogenesis in plants [30]. Elhag *et al.* [32] indicated that glucose or fructose surpassed sucrose in promoting cacao somatic embryos using calli induced from zygotic tissues. Traore [24] stated that fructose encouraged high percentage of embryogenesis in cacao than sucrose. Nambiar *et al.* [33] showed that addition of sucrose at 3% to culture medium raised osmotic stress to the cultured tissues.

In addition, it was found that enzymes that required for carbohydrate metabolic considered a critical factor for efficiency of carbon source in tissue culture. For example, Daigny *et al*. [34] indicated that sorbitol is effective with apple tissues since it can be converted into fructose by sorbitol dehydrogenase or into glucose by sorbitol oxidase. However, results obtained by Treora [24] showed that sorbitol inefficient with cacao and attributed this result to absence of these enzymes. In addition, Bahmani *et al*. [35] showed that type and concentration of sugars had a markedly effect on root formation of apple shoots of (MM106), whereas, sucrose at 90 M surpassed other carbon sources (fructose, glucose, sorbitol and maltose). In addition, they stated that both of fructose and maltose failed to promote root formation due to absence or inhibition of required enzyme for metabolic processes of these carbon sources. Moreover, in light of the fact that sucrose used to storage disaccharide meanwhile both of fructose and glucose used as a quick source for energy in plant cells and both of them derived from sucrose. Our study suggested that, fructose surpassed sucrose in encouraging shoot multiplication and root formation of two fig cultivars, may be attributed to existence low amount of required enzymes to convert sucrose into fructose and glucose which considered as a quick sources for energy that needed in high amount during shoot proliferation and root formation process.

CONCLUSION

It could be concluded that fig explants can be micropropagated by using full MS medium in multiplication stage, ½ MS in rooting stage and fructose as a carbon source at 0.1 M/l.

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