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RESEARCH ARTICLE

MICROPROPAGATION FOR DROUGHT RESISTANCE OF FIG (*FICUSCARICA*) *IN VITRO*

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ABSTRACT

Ficuscarica L. fam. Moraceae is one of the most important horticulture crops in the arid and semi-arid regions in the world. In order to study the drought resistance in two fig varieties (Soltani and Abiad), experiment has been conducted to achieve a method for multiple-shoot induction under drought stress using polyethylene glycol with apical buds collected from mature trees of *Ficuscarica L.*, using Morashige and Skoog's (MS) medium supplemented with different growth regulators benzyl adenine (BA). Growth factors were affected by presence of PEG in media and there were negative relationship in survival percent, shootlets number, shootlets length, fresh weight, dry weight and rooting percent with 8% PEG concentration in media, while the root length was longer and thick at the high concentration of PEG in media. Proline content as drought genes expression increased positively with PEG. The shootlets number decreased to 19.88% and 20.18% in soltani and abiad respectively at 8% compared with control. Plantlets that generated from study can tolerate drought with healthy growth to 4% PEG.

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INTRODUCTION

Ficuscarica L. great fruit crop, which is belonged the family (Moraceae), was commonly known as Fig plant which considered as one of the traditional Mediterranean crops. Fig is deciduous tree or large shrub, growing height up to7 meters (23ft). Fig grew as rain- fed in the Mediterranean conditions. Through last few years tins plant had exposed to drought (an abiotic) stress caused by the decrease in the amount of rains and water. It is the time to produce plants that can tolerate drought via *in vitro* propagation. To increase the cultivated area of fig (*Ficuscarica L.*) and that will not be available, thus tissue culture has become an important technique and an alternative method to overcome such problems for propagation and breeding of fig and other plants. Polyethylene glycol (PEG) widely used to induce water stress, and is a nonionic water polymer, which is not expected to penetrate into plant tissue rapidly (Abd El Rahman*et al* 2007). The application of tissue culture methods offers new prospects for rapid multiplication of many plants (Mustafa and Taha, 2012). Hadeer *et al* (2014) found in their study that (BA) at the rate of 5 mg/L and (GA3) at 1.0 mg/L enhanced shoot multiplication. The best number of leaves was obtained from medium supplemented with 3 mg/L (BA) combined with (GA3) at 1 mg/L. In addition, plant length increased when (GA3) was added alone at 1 mg/L, followed by (GA3) combined with either (Kin) at 5 mg/L or (BA) at 3 mg/L.Fig has wide variety of chemical constituents, its use in traditional medicine as remedies for many health problems, and its biological activities. The plant has been used traditionally to treat various ailments such as gastric problems, inflammation, and cancer. Some of the most interesting therapeutic effects include anticancer, hepato protective, hypoglycemic, hypo lipidemic, and antimicrobial activities (Shukranul *et al*., 2013). Abiotic stress limits crop productivity, and plays a major role in de-terminating the distribution of plants in the world and receiving an increasing attention. Water deficit is one of the major abiotic tress which adversely effects on the plant growth (Jaleel*et al*., 2008, and Anitha *et al.*, 2011). Shekafandeh and Hojati (2012).Their results showed in Sabz and Siah cultivars, that shoot length, shoot fresh and dry weights lower than control in drought treatments caused by 4% PEG, while the

leaf area and specific leaf area were not significantly (P> 0.05) affected. In Siah cultivar, shoot length, fresh and dry weights were lower in stressed media in comparison to control. In contrast to Sabz cultivar, leaf area and specific leaf area of Siah cultivar were significantly reduced by addition of 6% PEG. Naturally, the amount of proline in ' Sabz' was higher than in 'Siah'. However, in both cultivars, with addition of PEG in culture media, leaf proline content was increased, in comparison to control. With increasing PEG% in culture media, the amount of leaf soluble sugare content increased and the amount of starch decreased. Their results showed that 'Siah' is more sensitive to drought than 'Sabz' and that in vitro culture can be used to evaluate drought tolerance of cultivar. Rania (2013) proliferated shoot tips of two fig cultivars (Conadria and Black Mission) in vitro, which were cultured on modified MS medium supplemented with 0.5 mg/l 6-benzylaminopurine (BAP). The investigators 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. The 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 at0.2 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. Salam (2014) found in here study on potato that all measurements were affected and decreased by increasing of polyethylene glycol concentration in media, the survival percent and shoot lets number was decreased through multiplication stage, and the same observation was noticed on leaves number and shoot lets length. Eman *et al*. (2016) used nodal stem segments with lateral buds as explant. Five concentrations (i.e. 0, 4, 6, 8 and 10 %) of PEG were tested, among which the optimum concentration for screening was determined as 8 %. Analysis of variance showed highly significant variation among the tested landraces, concentrations of PEG and the interaction between them in the percentage of response (%R), number of shoot per explant (NSE) and drought susceptibility index. The average percentage of reduction due to PEG treatment was 48.30 and 52.57 % for the %R and NSE, respectively. Soliman *et al.* (2016) they found when shoots of fig plants were sub-cultured on MS medium supplemented with 3 mg L-1 BAP and 0.5 mg L-1 2iP at different concentrations of manitol (0.0, 50, 100, 150, 200, 250, and 300 mM) under in-vitro culture conditions, that their results showed that increasing manitol concentration in the medium causes a gradual decrease in all growth parameters and plant regeneration efficiency. The results indicated that manitol can be used as water stress creating agent under in vitro conditions and Black Mission cultivar was relatively tolerant to drought stress as compared to Brown Turkey and Brunswick. Bundig *et al.* (2017) reported that both, under in vitro and in vivo conditions, proline displayed an increase under osmotic stress conditions in nearly all potatoes tested, but no direct correlations were found to stress tolerance. However, a genotype classified as tolerant displayed earlier proline accumulation. Proline is thought of as one factor for plants to withstand stressful conditions, but cannot be used to distinguish potato genotypes for their stress tolerance to osmotic stress in vitro. Nadia *et al* (2017) screened durum wheat (Triticum durum Desf.) genotypes with three levels of drought tolerance, in order to evaluate their response to water stress at callus induction and plant regeneration levels. Significant differences were observed among the genotypes, and polyethylene glycol (PEG) levels used, and their interactions were however, significant for all the studied characters. Increase in PEG concentration increased the time required for callus initiation and reduced the number of calli frequency of embryogenic structures and number of plants regenerated, showing the adverse effect of PEGon the somatic embryogenesis developmental under invitro conditions tested. The present study aimed to produce drought resistance micro propagated plantlets of *Ficuscarica* L.

MATERIALS AND METHODS

This study was carried out in the tissue culture lab of North Sinai research station Desert Research Centre (DRC) in period from 2011:2015. The study aimed to propagate *Ficuscarica* explants after exposing explants directly to drought stress using polyethylene glycol *in vitro*. Plant materials were collected from the farms at the West North Coast of Egypt and were kept in an ice box. These cuttings were prepared into small explants each contained one node and then were washed well under running water with one drop of Tween 20. Surface sterilization was accomplished by soaking explants in Ethanol 70% for 3 min., then in 40% Sodium hypochlorite (5.2% active ingredient) for 25 min. They were washed well five times with distilled sterilized water (d.s.w) and then were soaked in sterilized antioxidant (150mg citric acid +200mg ascorbic acid/L) solution and were kept at 4˚c until using. The surface sterilized explants were placed vertically in culture jars of Morashige and Stooge (MS) medium (1968) supplemented with 30.0 gm /l sucrose, vitamins at 0.5 mg /l of (Thiamine HCl, Pyridoxine HCl and Nicotinic acid) and 100 mg /l Myo-inositol. Media was fortified with 2.0mg BA and 2.0mg 2ip was applied in the establishment and the multiplied shootlets were cultured on the previous media of Morashinge and Skog (1968) for the drought stress by adding Polyethylene Glycol (PEG) to the media at 2.0%, 4.0%, 6.0% and 8.0%. The pH value was adjusted at 5.6 – 5.7 by adding H Cl (0.1M) or Na OH (0.1M). Medium was gelled with 2.0g/l phytagel and then was autoclaved at 121°C for 20 min. For rooting experiments, MS medium was supplemented with 1.0 mg NAA and 1.5mg IBA/L in addition to the tested concentrations of PEG. Leaves samples of each PEG concentration were weighed twice weight fresh and dry weight for the two fig varieties and the water content was determined. Proline content was estimated in leaves according to the method of Bates *et al.,* (1973).

RESULTS AND DISCUSSION

The sterilized explants of *Ficuscarica L.* varieties (Soltani and Abiad) were cultured on establishment media which was supplemented with 2.0mg /L of each of Benzyl adenine and isopentyle adenine to produce desirable number of shootlets in order to get an aseptic culture. Data in table (1) showed that survival percent of the explants was 70 % with soltani and 60% with abiad. The mean no. of the proliferated shootlets/explant was 2.14 with soltani and 2.11with abiad.

Table 1. The effect of sterilization treatment on the survival and growth of two fig varieties explants

variety	Explant No.	Survival%		Shootlet no.	Mean shootlet/explnt	Shoot length	
		No.	%			total	Mean
Soltani	30	21	70	45	2.14	135	3.0
Abiad	30	18	60	38	2.11	101.8	2.68

Table 2. The effect of polyethylene glycol on shootlet growth of Fig explants on multiplication stage

PEG concentrations	Survival %		Shootlets No /explant (mean)		Average of shoot length cm/shoot	
	soltani	Abiad	Soltani	Abiad	Soltani	Abiad
Control+(2mgBA+2mg2IP	100	100	16.2	16.8	8.46	7.8
2%+(2mgBA+2mg2IP	93	93	11.37	11.05	6.17	6.0
4%+(2mgBA+2mg2IP	93	80	8.85	11.05	6.17	6.0
6% +(2mgBA+2mg2IP	93	80	5.17	5.17	4.3	4.5
8% +(2mgBA+2mg2IP	66.6	53.3	3.22	3.39	3.33	3.63

Table 3. The effect of polyethylene glycol on the rooting of fig explants in vitro

PEGconcentrations	Root formation %		Root No / shootlet		root length / shootlet cm (mean)	
	soltani	Abiad	Soltani	Abiad	Soltani	Abiad
Control	100	100	4.02	3.93	7.13	6.53
2%+1.5NAA+3.0IBA	100	93.33	3.6	2.15	7.2	6.99
4%+1.5NAA+3.0IBA	80.0	73.33	2.92	3.0	7.625	6.84
6%+1.5NAA+3.0IBA	66.66	60.0	2.5	2.55	8.55	8.33
8%+1.5NAA+3.0IBA	53.3	46.66	7.72	2.55	9.42	8.42

Table 4. The effect of PEG on the endogenous proline in response to drought in Fig explants

cultivars	Proline content in control µm/g f.w	PEG(2%)	PEG(4%)	PEG (6%)	PEG (8%)
		Proline content µm/g f.w			
Soltani	19.1	88.1	139.6	166.5	172.1
Abiade	17.6	78.7	127.8	145.0	151.7
differences	1.5	10.4	11.8	21.5	20.4

Figure 1. Establishment stage of fig soltani and abiad on Morashige and Skoog media

Fig. 2.The effect of PEG in media (2,4and6%) on *Ficuscarica L*. shootlets

The mean shootlets length was 3.0 cm/shootlet with soltani and 2.68cm/shootlet with abiad. To activate the drought genes ability to express, the produced shootlets were cultured to grow on the drought stress media which made by the addition of polyethylene glycol (MW6000) which subsequently produce drought resistant shootlets and finally plantlets. The drought stress media were contained 2.0mg /L of each of BA and 2IP. The recorded data in table (2) showed that the survival percent was 100% with soltani and abiad, while it decreased with all the used PEG treatments. The explant produced different numbers of shootlets according to the variety and the components of media. The highest mean of shootlets/explant was obtained when explants were culture on the control media (2.0mg BA/L+2.0mg2ip/L) in both varieties and also the highest mean of shootlets length were obtained with both varieties. The presence of 2.0% of the drought reagent polyethylene glycol in the culture media resulted in 11.37 shootlets /explant with variety soltani and 11.05 shootlets /explant with the variety abiad and also resulted in 6.17cm/shootlets length with variety soltani and was 6.0 cm /shootlet with abiad. The increasing of PEG in the media will increase the drought stress effects on the shootlets growth and their multiplication. Shootlets number and the average of shootlet length were affected when media was supplemented with 4.0 % of PEG. The shootlet number was 8.85/explant with soltani and was 7.05 shootlets / explant with abiad, while the average of shootlet length was5.37 cm with the variety soltani and was4.9 cm with the variety abiad. The same observation was noticed at 6.0% and 8.0% of PEG but it was more effective on the shootlets number and also shootlet length in both varieties. The shootlets number was 5.17shootlets/explant with both the soltani and abiad at 6.0% PEG in the media, while it was 3.22 shootlets/explant in soltani and it was3.39 shootlets/explant with abiad at 8.0% of PEG. The shootlets length at 6%PEG was 4.3cm with soltani and it was 4.5 cm with abiad, while at 8% PEG the shootlet length was3.33cm with soltani variety and was 3.63cm with abiad. It was observed from table (2)and figure(2) that all traits such as shootlets number and shootlet length, also fresh weight, dry weight and water content in the multiplied were more affected by the presence of PEG in media. The increasing of PEG concentration decrease the shootlets number, shootlet length, fresh weight and water content all these traits were affected by water stress.

The shootlets number decreased to 19.88% and 20.18% in soltani and abiad respectively at 8% compared with control, but at 4%PEG was 54.63 %with soltani while it 72.93% with abiad which can tolerates drought than soltani. The shootlet length at 6% PEG was 50.83% and 57.69% with soltani and abiad respectively compared with control. These results indicated that soltani and abiad can grow to 6%PEG but at 4% will grow well and also their production after that. The multiplied shootlets were divided into small clusters of 2-3 shootlets each and were cultured on rooting media contained 1.5 mg NAA and 3.0mg IBA/L and were incubated as usual in the presence of different concentrations of PEG in media. Through period about 60days most of shootlets formed roots. Data in table (3) showed that, the rooting percent was between 100 and 46.66%. The highest rate for rooting percent was noticed with the PEG free media and 2.0% of PEG and then decreased to 80.0% and at 4% of PEG in both varieties, with the increasing of PEG concentration (6%) the rooting was 66.66% in soltani and 60.0% in abiad and with the concentration 8% the rooting was 53.3% and 46.66% with soltani and abiad respectively table(3). The rootlet number and length were also affected by the presence of PEG in media. The highest root number was observed with the control media in both varieties soltani and abiad and followed by results with 2% of PEG in which the root number was in decrease until 8% which was the lowest in both varieties. Data in case of root number was in decrease contrasting with PEG concentrations, while the root length was in positive relation with the increasing of PEG concentrations. The control treatment showed to be the lowest in root length (7.13:6.53cm) / root and soltani was the best than abiad table (3). The highest root length was recorded with the high concentration of PEG (8%) it was 9.42: 8.42cm with soltani and abiad respectively table (3). The root length with 6% of PEG was 8.55: 8.33 cm and soltani was the best table (3). Abiotic stresses such as salt and drought enhance plants to increase gene expression (their production of proline) to avoid drought stress and to decrease it`s bad effects. Data in table (4) exinbitedthe proline content in *Ficuscarica* L. varieties soltani and abiad shootlets produced in vitro.

It is clear that proline concentration in shootlets is increased with the increasing of PEG concentration in culture media (19.1:17.6 µm/gf.w) recorded with soltani and abiad in control media and was increased from 88.1 to172.1µm/g. f.w at 8% recorded with soltani and alsowith variety abiad, proline content was 78,7 at 2% PEG and proline contentincreased to 151.7µm/gfw)at 8% PEG. These results were in agreement with that of Jaleel*et al*., 2008, and Anitha *et al.*, 2011 they reported that water deficit is one of the major abiotic stress which adversely effects on the plant growth. Shekafandeh and Hojati (2012). Their results showed that fig (in Sabz) cultivar, shoot length, shoot fresh and dry weights at drought treatments caused by 4% PEG were lower than control, while the leaf area and specific leaf area were not significantly (P> 0.05) affected. In Siah cultivar, shoot length, fresh and dry weights were lower in stressed media in comparison to control. In contrast to Sabz cultivar, leaf area and specific leaf area of Siah cultivar were significantly reduced by addition of 6% PEG. However, the addition of PEG in culture media, leaf proline content was increased, in comparison to control. With increasing PEG% in culture media, the amount of leaf soluble sugare content increased and the amount of starch decreased. That drought in vitro culture can be used to evaluate drought tolerance of cultivar. Bundig *et al.* (2017) reported that both, under in vitro and in vivo conditions, proline displayed an increase under osmotic were stress conditions in nearly all potatoes tested, but no direct correlations found to stress tolerance.

However, a genotype classified as tolerant displayed earlier proline accumulation. Proline is thought of as one factor for plants to withstand stressful conditions, but cannot be used to distinguish potato genotypes for their stress tolerance to osmotic stress in vitro. Finally polyethylene (PEG) the water stress reagent in media enhanced drought genes to produce proline as gene expression in cells to avoid drought effect. all measurements were affected and decreased by increasing of polyethylene glycol concentration in media, the survival percent and shootlets number was decreased through multiplication stage, and the same observation was noticed on leaves number and shoot lets length. Growth factors were decreased by increasing of PEG to 8% and can grew, but the production can also affected, but growth at 4 and 6% was best than8% PEG. Fig plants can tolerate drought effects with healthy growth to 4% of water stress

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