Ex Vitro Rooting and Survival of Regenerated Shoots from three Fig (*Ficus carica* L.) Genotypes

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Summary

The aim of this study was to optimize the rooting of regenerated shoots from nodal segments of three genotypes of fig named: 'Bargchenari', 'Dehdez' and 'Runu'. For that purpose, the bottom of shoots (about 5 mm) was dipped in Indole-3-butyric acid (IBA) solution of different concentrations: 500, 1000, 1500, 2000 mg L^{-1} and control for 5 seconds and then cultured in sand medium. The test was performed in a completely randomized design with at least 5 replications. In 'Runu', the highest rooting percentage and the number of roots per shoot (100% and 15.67, respectively) were observed in concentration of 2000 mg L^{-1} IBA, whereas, 'Bargchenari' and 'Dehdez' produced the highest rooting percentage and the root number per shoot at concentrations of 1000 and 1500 mg L^{-1} IBA respectively. Three months after transferring rooted shoots to soil mixtures, 'Bargchenari' genotype showed the highest survival rate (80%), shoot length (2.5 cm) and leaf number (4.65 leaves per shoot). In relation to survival percentage, the best medium was perlite, but plantlets showed better growth on perlite + peat medium.

Key words

Ficus carica, IBA, regenerated shoot, rooting, soil mixture

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Introduction

Fig (*Ficus carica* L.) is a fruit tree native to Asia and the eastern Mediterranean region and is a member of the Moraceae family. It is one of the first plants that were cultivated by humans for its dry and fresh consumption. Figs (fruits) are an important source of vitamins, minerals, carbohydrates, sugars, organic acids, and phenolic compounds (Veberic et al., 2008). Iran stands 5th in annual fig fruit production after Turkey, Egypt, Algeria and Morocco (FAO, 2014).

Fig trees can be propagated by vegetative and sexual methods. Due to heterozygosity in sexual reproduction, this method is used in breeding program (Hackett, 1985). The most common method of fig propagation is hard wood stem cutting (Faghih and Sabetesarvestani, 2001). Nowadays, micropropagation is an effective alternative method for meeting the need for vegetative clonal production.

The development of plantlets *in vitro* involves several steps such as establishment stage followed by shoot multiplication stage and multiplied shoots go to rooting stage. One of the main problems in the mass production of plants through micropropagation is the induction of rooting of the *in vitro*-produced shoots (Németh, 1986; Annapurna and Rathore, 2010, Shahcheraghi and Shekafandeh, 2016). In addition, when rooted shoots are transferring in a conventional substrate, usually they stop to grow. They only restart to grow after development of a new root system. Besides, because the plantlets derived from *in vitro* culture have a subtle texture, they may be more susceptible to bacterial and fungal infections (Maene and Debergh, 1983). Therefore, a successful acclimatization is another factor limiting commercial tissue culture production systems (Hartmann et al., 1991). *In vitro* rooting may accounted for 30-75% of the total cost of micropropagation process (Debergh et al., 1992). The *in vitro* derived shoots can be manipulated as cuttings and rooting is more appropriated under *ex vitro* conditions (Maene and Debergh, 1983). *Ex vitro* rooting is a one-step method comprising of both *in vitro* rooting and acclimatization (Ahuja, 1991). Therefore, this method is not only helpful in the hardening of plantlets but also reduces labor and the cost of micropropagation and saving the time (Phulwaria et al., 2012).

In vitro shoot multiplication followed by *ex vitro* rooting method provides an opportunity to produce large number of plants in a short period of time by using a minimum of plant material (Engelmann, 2011; Thiyagarajan and Venkatachalam, 2012; Bhojwani and Dantu, 2013). Plantlets obtained from *ex vitro* rooting have many potential advantages in comparison to plantlets developed from *in vitro* rooting, e.g. better root structure, higher root number, successful acclimatization and higher survival rate (Yan et al., 2010; Benmahioul et al., 2012). In many plant species, *ex vitro* rooting has been investigated, including rose and gerbera (Podwyszynska and Gabryszewska, 2003), papaya (Kataoka and Inoue, 1991), jasmine (Apter et al., 1993), *Carallumae dulis* (Patel et al., 2014), tea (Ranaweera et al., 2013) and *Rotula aquatic* (Martin, 2003). To the best of our knowledge, there are a few reports available for the *ex vitro* rooting of *F. carica* shoots. In this research, we report an improved and efficient rooting by *ex vitro* technique for large-scale plant production.

Material and methods

Shoot proliferation and rooting

Single-node segments of three fig genotypes 'Bargchenari', 'Dehdez' and 'Runu' (from mother plants) were cultured on Murashige and Skoog (MS) (1962) medium supplemented with 0.5 mg L^{-1} benzylaminopurine (BA) and 0.2 mg L^{-1} isopentenyladenine (2ip) as multiplication medium. Shoots produced from multiplication stage (at least 1.5 cm in length) were washed with sterile water to remove traces of agar. The basal end of washed shoots were dipped in IBA solution with different concentrations: 500, 1000, 1500, 2000 mg L^{-1} and control (distilled water without IBA) for 5 seconds and planted in plastic culture trays containing sterilized sand and covered with transparent polyethylene films. The cultivated trays were irrigated with ¼ of MS basal salts and placed under the greenhouse conditions at natural light, relative humidity of 40% and temperature of 25±2°C. After two weeks the cover was gradually punctured and after next two weeks it was completely removed. The rooting percentage, numbers of roots per shoot and root length were measured.

Growth and survival

Within the first experiment, for evaluating the growth and survival of plantlets, rooted shoots of three fig genotypes were transferred to the pots containing soil mixture (field soil, leaf mold and sand; 1v: 1v: 1v), and then were placed in the same condition as mentioned in previous paragraph. The percentage of survival (%), shoot length (cm) and the number of leaves per shoot were measured in the intervals of one, two and three months after the transfer. In the second experiment, the effect of different media including peat, perlite and peat + perlite (1V: 1V) on growth performance of 'Bargchenari' rooted shoots was studied. Data were recorded one and two months after potting.

Statistical analysis

The experiments were performed in a completely randomized design with at least 10 replications including a single shoot in each replicate. Data of different experiments were subjected to one-way ANOVA using SAS software, ver. 9.1.3 (SAS institute, Cary, NC, USA). The mean values were compared using Duncan's multiple range test (DMRT) at $P < 0.05$.

Results

The result showed that the shoots of all genotypes, without any IBA treatment (control) planted in sand medium produced the roots. However, they responded differently to different concentrations of IBA (Table 1, Fig 1). In 'Bargchenari', the highest percentage of rooting (73.33%) and the highest number of roots per shoot were obtained in the 1000 mg L^{-1} IBA. However, 100% rooting and the highest number of roots per shoot were achieved in 1500 and 2000 mg L^{-1} IBA in 'Dehdez' and 'Rnue', respectively. That means, 2000 mg L^{-1} IBA, which had a positive effect on shoot rooting in 'Rnue' had an inhibitory effect on shoot rooting of 'Bargchenari' genotype (35.71 %).

In the same manner, the highest number of roots per shoot and root length were obtained in: 'Bargchenari' (8.47, 0.71 cm) on 1000 mg L^{-1} IBA; 'Dehdez' (15.75, 0.66 cm) on 1500 mg L^{-1} IBA,

Table 1. Effect of IBA different concentrations on rooting performance of three fig genotypes

IBA mg L^{-1}	Genotype								
	'Bargchenari'			'Dehdez'			'Runu'		
	Rooting %	Root number	Root length (cm)	Rooting %	Root number	Root length (cm)	Rooting %	Root number	Root length (cm)
Control	33.33	2.13 ^{c}	0.41 ^{bc}	66.67	0.67 ^{b}	0.20 ^{b}	50.00	0.75 ^{b}	0.13 ^{b}
500	69.23	5.85 ^{ab}	0.69 ^{ab}	66.67	1.33 ^{b}	0.37 ^{ab}	0.00	0.00 ^{b}	0.00 ^{b}
1000	73.33	8.47 ^{a}	0.71 ^{a}	50.00	7.00 ^{ab}	0.24 ^{ab}	50.00	7.00 ^{ab}	0.20 ^{b}
1500	37.50	4.12 ^{abc}	0.21 ^{c}	100.00	15.75 ^{a}	0.66 ^{a}	60.00	1.60 ^{b}	0.14 ^{b}
2000	35.71	2.21 ^{bc}	0.09 ^{c}	85.71	5.29 ^{ab}	0.34 ^{ab}	100.00	15.67 ^{a}	0.47 ^{a}

In each column, means with the same letters are not significantly different using Duncan's multiple range test (DMRT) at $p< 0.05$.

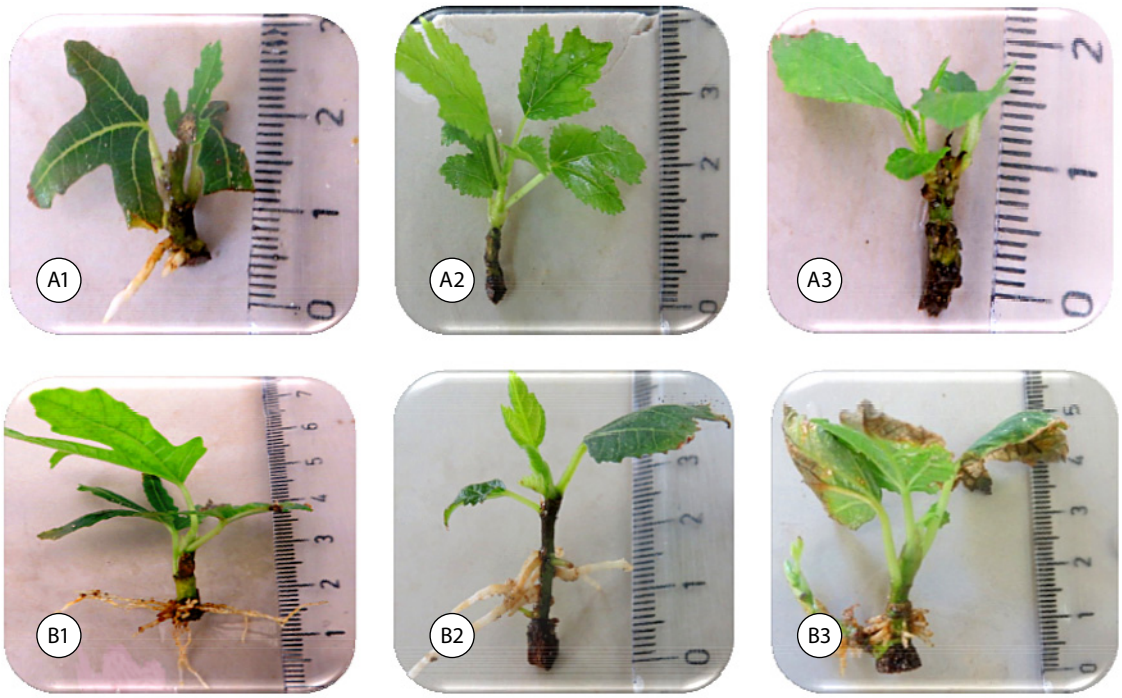

Figure 1. Effect of IBA on *ex vitro* micro-shoots rooting in fig genotypes. A: control (A1 - 'Bargchenari', A2 - Dehdez, A3 - Runu); B1 - Bargchenari, (1000 mg L^{-1}), B2 - Dehdez (1500 mg L^{-1}), B3 - Runu (2000 mg L^{-1})

and 'Runu' (15.67, 0.47 cm) on 2000 mg L^{-1} IBA, and those values were significantly higher than the values for their controls.

The results of growth and plantlet survival after one, two and three months in soil condition are presented in Table 2. After one month, maximum survival percentage was obtained in 'Bargchenari' (88%) and minimum in 'Runu' (61.54%). Maximum shoot length was observed in 'Dehdez' (1.30 cm); although the differences in shoot length between three genotypes were not significant. 'Dehdez' also produced a significantly higher leaf number (4.28) than 'Runu' (1.25). In the second month, the most plantlets of 'Runu' were not able to survive, and it was not possible to analyze the data on remainder, so they were eliminated. After two months, shoot length of 'Bargchenari' and 'Dehdez' plantlets was 2.35 and 2.37 cm, respectively, and the number of their leaves was increased by 11 and 9%, respectively. After three months, the shoot length of both genotypes was more than two folds greater in comparison to one month growth period. It seems that 'Dehdez' lost some of their leaves, although there were no significant differences between two genotypes.

In the assessment of three pot media: peat, perlite and peat + perlite on the survival of 'Bargchenari' genotype plantlets, it was observed that peat alone was not suitable and all the plantlets gradually died. Therefore, Table 3 showed the results of perlite and peat + perlite mixture on plantlets growth, one and two months after transfer. After one month, the plantlets survived better in perlite (77.78%) than in peat + perlite (30%). However, there was no significant difference between two media in relation to shoot length and leaf number, while in perlite + peat the number of leaves per shoot was higher than in perlite alone.

Table 2. Effect of genotype on survival percentage, shoot length and leaf number after transfer to soil mixture

Genotype	Survival %	Shoot length (cm)	Leaf number
		First month	
Bargchenari	88	1.17 [a]	3.95 [a]
Dehdez	70	1.30 [a]	4.28 [a]
Runu	61.54	1.19 [a]	1.25 [b]
Statistical significance		ns	**
		Second month	
Bargchenari	80	2.35 [a]	4.40 [a]
Dehdez	60	2.37 [a]	4.67 [a]
Statistical significance		ns	ns
		Third month	
Bargchenari	80	2.50 [a]	4.65 [a]
Dehdez	60	2.63 [a]	3.00 [a]
Statistical significance		ns	ns

In each month and in each column, mean values followed by the same letters are not significantly different using Duncan's multiple range test (DMRT) at $p< 0.05$.

Table 3. Effect of different soil media on survival, shoot length and leaf number in Bargchenari genotype

Soil mixture	Survival %	Shoot length (cm)	Leaf number
		First month	
Perlite	77.78	1.73 [a]	3.00 [a]
Perlite + Peat	30	1.27 [a]	4.00 [a]
Statistical significance		ns	ns
		Second month	
Perlite	66.67	1.22 [b]	3.00 [a]
Perlite + Peat	30	4.73 [a]	5.33 [a]
Statistical significance		**	ns

In each month and in each column, mean values followed by the same letters are not significantly different using Duncan's multiple range test (DMRT) at $p< 0.05$.

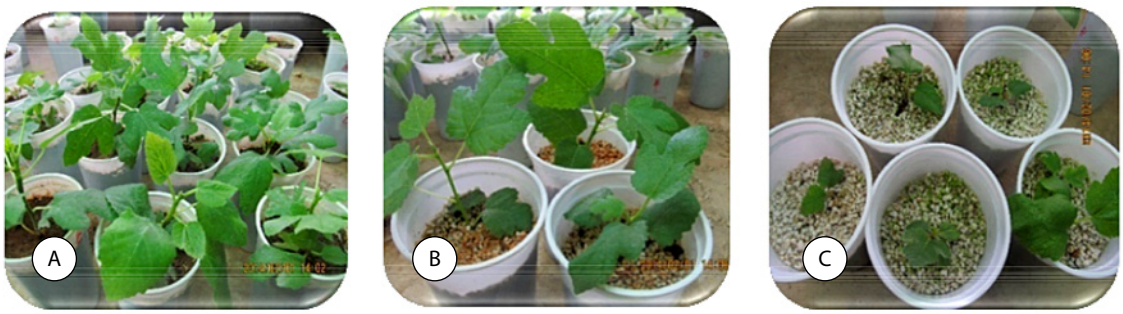

Figure 2. *Ex-vitro* rooting of 'Bargchenari'; A - three months after transfer to soil, B - two month after transfer to perlite + peat, C - two months after transfer to perlite.

After two months, the percentage of plant survival decreased to 66.67% in perlite medium. The maximum length of shoots (4.37 cm) was gained in the peat + perlite medium, which was significantly higher than those in perlite medium (1.22 cm). The number of leaves was also higher in the peat + perlite (5.33) medium than in perlite (3) but there were no statistically significant differences between them. Figure 2 shows plants adapted to different soil mixtures.

Discussion

In vitro rooted plantlets require a good acclimatization before transfer to the field conditions. Plantlets in *ex vitro* adaptation deal with gradual transition from the artificial culture conditions to the natural living environment. In the acclimatization stage it is necessary to ensure optimal culture conditions to obtain high survival rates. *Ex vitro* rooting appears better than *in vitro*, as *ex vitro* rooted plants do not require acclimatization before transfer to the field conditions (Yan et al., 2010). It has been reported that in *Rosa hybrid* 'Red Bells' *ex vitro* rooting of the shoots from the multiplication stage and acclimatization took place in one month (Clapa et al., 2013). This method is useful in lowering the cost and saving the time. *Ex vitro* rooting reduced the total cost of a micropropagation protocol up to 70% (Debergh et al., 1992; Yan et al., 2010; Ranaweera et al., 2013).

The degree of *ex vitro* rooting success is genotype dependent. The effect of genotype on microshoots rooting of other plant species such as rose and gerbera varieties have been studied (Hackett, 1985; Podwyszynska and Gabryszewska, 2003).

In this study, the highest percentage of rooting and maximum root number per shoot achieved in 1500 and 2000 mg L^{-1} IBA in 'Dehdez' and 'Rnue', respectively. Clapta et al. (2013) reported that the highest root number per shoot was gained with 200 mg L^{-1} in 'Chester' blackberry microshoots. We also found that increasing concentration of IBA to supra-optimal concentrations had negative effects: reduced root number and shorter roots. Kumar et al. (1998) also indicated that the use of higher or sub-optimal concentrations of IBA *in vitro* condition limits root growth of fig, which is consistent with the results of this study.

Genotipe 'Runo' showed 61.54% survival in the first month, but, it was not able to continue the growth and gradually the most of plantlets died, probably due to low leaf number. In second and

third month, both cultivars ('Bargchenari', 'Dehdez') survived and in means of survival 'Bargchenari' acted better than 'Dehdez'.

Generally, various materials such as peat, bark, perlite, vermiculite, pumice, sand and soil alone or in combination could be used as rooting media (Bonga and Durzan, 1987). Optimal rooting medium is the mixture of some of above mentioned material that produces neutral pH to slightly acidic and large water storage capacity, while the drainage and ventilation are good (Torres, 2012). Peat medium alone was not suitable for 'Bargchenari' and all plants died on this medium. Peat may be highly acidic for rooting of some plant species, such as fig. More plantlets were able to survive on perlite medium than perlite + peat, although, addition of peat seems to improve the growth of plantlets (shoot length and leaf number). Hepaksoy and Aksoy (2005) achieved maximum survival percentage of fig cultivar Sarilop in peat and minimum percentage in perlite, which was not in accordance with results of this research.

Conclusion

Rooting was genotype dependent. In 'Runu', the highest rooting (100%) was observed in the concentration of 2000 mg L^{-1} IBA, whereas, 'Bargchenari' and 'Dehdez' produced the highest rooting percentage in concentrations of 1000 and 1500 mg L^{-1} IBA respectively. Three months after transferring rooted shoots to soil mixtures, 'Bargchenari' genotype showed the highest survival rate (80%). The best rooting medium was perlite from regarding percentage of survival.

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