

In vitro regeneration of the isolated shoot apical meristem of two commercial fig cultivars ‘Sabz’ and ‘Jaami-e-Kan’



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ABSTRACT

Meristem-tip of two important Iranian fig (*Ficus carica* L.) cultivars (‘Jaami-e-Kan’ and ‘Sabz’) were cultured under *in vitro* conditions to optimize the best condition for their shoot proliferation, root induction, and subsequent plantlet regeneration. Effects of explant size, culture medium (MS and B5) and different levels of BA (6-benzyladenine) in combination with 0.1 mg l⁻¹ NAA (naphthalene acetic acid) were analyzed in the establishment of meristem cultures. The survival percentages of meristems were recorded after five weeks. Murashige and Skoog (MS) basal medium supplemented with different concentrations of BA (1, 1.5 and 2 mg l⁻¹) and NAA (0, 0.1 and 0.5 mg l⁻¹) was used for shoot regenerating. Half strength MS medium containing four concentrations of IBA (indole-3-butyric acid) was used for rooting. The regeneration and survival rate of cultures were significantly affected by different concentrations of BA, explant size and cultivars. The highest meristem survival was recorded from ‘Sabz’ cv., explants with the size of 0.5–0.7 mm, and culture media supplemented with 0.5 mg l⁻¹ BA. The highest proliferation rate (2.4 shoots per micro shoot) was observed in the medium containing 2 mg l⁻¹ BA, while the lowest length of shoots was obtained in this concentration. Furthermore, cv. ‘Sabz’ showed a higher proliferation rate (2.3 shoots per micro shoot) than cv. ‘Jaami-e-Kan’. The highest numbers of rooted micro-shoots were obtained from 1.5 mg l⁻¹ IBA (88.3%) followed by 2 mg l⁻¹ IBA (78.5%). Also, 2 mg l⁻¹ IBA showed the maximum number of roots per micro shoot (14.6). These data show that the presence of plant growth regulators, besides meristem size, plays an important role in meristem culture and micropropagation of fig trees. Moreover, results of this investigation can be applied practically for true to type as well as virus free plantlets regeneration from these important Iranian fig cultivars in a short time.

1. Introduction

The common fig (*Ficus carica* L.), belongs to the family Moraceae, is one of the oldest edible fruit species that is widespread in many regions including Iranian plateau and Mediterranean basin countries (Aradhya et al., 2010). Iran is one of the biggest producers of dried and freshly consumed fig in the world. ‘Sabz’ and ‘Jaami-e-Kan’ are among the most popular dry and freshly used fig cultivars of Iran, respectively. Cultivars ‘Sabz’ and ‘Jaami-e-Kan’ belong to Smyrna and common fig types, respectively. The fruit is very popular for its taste, high nutritional values as well as medicinal properties (Barolo et al., 2014) and play an important role for supplying nutritional requirements of local peoples in Iran conditions.

The fig tree can be vegetatively propagated by grafting, cuttings, air

layering and micropropagation (Pasqual and Ferreira, 2007). The conventional methods for clonal propagation of fruit trees (such as cutting and grafting) are time-consuming and limited to the specific plant materials and may be resulted in poor rooting. It is reported that the survival rate of plant regeneration from cutting varies from 20% to 30% (Kumarm et al., 1998). Also, this method requires a large volume of planting materials and sacrifice of mother plants. During the last decades, the *in vitro* techniques opened their way in different aspects of plant studies and proved their efficiency for rapid and large-scale multiplication of different fruit tree species (Moham Jain and Haggman, 2007; Boudabous et al., 2010; Sahraroo et al., 2018; Hassan and Zayed, 2018). Micropropagation and plantlet regeneration from common fig have been attempted through different types of explant including nodal segments (Brum, 2001; Fraguas et al., 2004; Ferreira and Pasqual,

Abbreviations: AC, (activated charcoal); BA, (benzyl adenine); GA₃, (gibberellic acid); IBA, (indole-3-butyric acid); masl, (meters above sea level); MS, (Murashige and Skoog); NAA, (1-naphthalene acetic acid); PVP, (Polyvinyl pyrrolidone); RCBD, (randomized complete block design)

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2005; Hepaksoy and Aksoy, 2006; Pasqual and Ferreira, 2007), and meristem and shoot tips (Demiralay et al., 1998; Comlekcioglu et al., 2007; Rania et al., 2013; Danial et al., 2014; Shahcheraghi and Shekafandeh, 2016; Al-Shomali et al., 2017) under *in vitro* condition. Besides the direct advantages of micropropagation, and virus free plantlets regeneration in some methods, optimization of *in vitro* culture condition can accelerate and facilitate the research on transgenic plants through genetic transformation technology.

There is no information about micropropagation of 'Sabz' cultivar as the most important Iranian dried fig as well as 'Jaami-e-Kan' as one of the main cultivars freshly used fig in Iran. The objective of this experiment was to optimize the best conditions for *in vitro* micropropagation of two Iranian fig cultivars (cvs. 'Sabz' and 'Jaami-e-Kan').

2. Materials and methods

2.1. Plant materials

One-year-old hardwood cuttings of 'Sabz' cultivar was collected from fig research station of Estahban city (29°8' N; 59°3' E; 1767 masl). The cuttings of freshly used cultivar, 'Jaami-e-Kan' was prepared from agricultural research center of Varamin city (35°19' N; 51°38' E; 918 masl). The cuttings were rooted and grown in pots under greenhouse conditions and then used as source plants for *in vitro* propagation. To facilitate excision of apical meristems, apical shoots with the length of 15–20 mm were taken from plantlets and were washed completely under running water for about 60 min. After that, the explants were immersed in 70% ethanol (30 s), followed by surface-sterilization with 0.1% mercuric chloride (4 min) and were rinsed (three to four times) with sterile distilled water. Different meristem sizes 0.2–0.4 mm (including apical meristem dome along with 1 or 2 leaf primordia) and 0.5–0.7 mm (including apical meristem dome along with 2–4 leaf primordia) were excised from explants under a binocular microscope (Fig. 1). Four meristems were cultured in a petri dish containing 30 ml culture medium (Fig. 1).

2.2. Media preparation and experimental design

The micropropagation of two Iranian fig cultivars was performed as an experiment with three stages (George et al., 2008) as follow:

2.2.1. Stage I: Initiating a culture

MS (Murashige and Skoog, 1962) and B5 (Gamborgm et al., 1968) basal media each supplemented with 30 g l⁻¹ sucrose, 2 g l⁻¹ activated charcoal (AC) and 0.1 mg l⁻¹ NAA were used in this study. All prepared media were adjusted to pH 5.8 with 1 N KOH or 1 N HCl before autoclaving at 121 °C, 101.325 kPa for 25 min. Effects of two culture media (MS and B5), two sizes of meristem tips (0.2–0.4 mm and 0.5–0.7 mm), and three concentrations of BA (0.5, 1 and 1.5 mg l⁻¹) were investigated in a factorial experiment with five replicates. The treatments were set up in a randomized complete block design (RCBD) of 120 Petri dishes each contained four meristems. Due to the time-consuming nature of meristems excising, a set of 24 Petri dishes was cultured on each day (replicate one from each treatment). Therefore, the blocks were defined by day to reduce any error caused by this time gap. Plant growth regulators were added before autoclaving and media were gelled with 0.75% Difco bacto agar. After ten weeks, developed meristems were used for the following stage.

All cultured media were kept in the dark for ten days then followed by maintaining at 25 ± 2 °C under cool-white fluorescent light (16 h photoperiod, 40 μE m⁻² s⁻¹). Survival rate was calculated as a percentage of the total number of meristems that showed a green color at the end of 5th week. Then survived meristems were transferred onto MS medium supplemented with 0.5 mg l⁻¹ BA, 0.1 mg l⁻¹ NAA, and 0.05% PVP. The explants were subcultured in the same media at three weeks intervals.

2.2.2. Stage II: Increasing propagules

Well-developed micro-shoots (Fig. 1B) that were obtained from the initiation stage were divided into two-node segments and were used as the explants for proliferation in the basal MS medium supplemented with different concentrations of BA (1, 1.5 and 2 mg l⁻¹), NAA (0, 0.1 and 0.5 mg l⁻¹) and 0.05% PVP. The explants were subcultured every two weeks on the fresh media, and the data were recorded after six weeks. The experiment was conducted as an RCBD in a factorial arrangement with ten replicates (petri dish) each contained two micro shoots. Similar to the first stage, the blocks were defined by day.

2.2.3. Stage III: Rooting and acclimatization

Regenerated shoots were used for induction of roots on half-strength solid MS medium supplemented with various concentrations of IBA (0.5, 1, 1.5 and 2 mg l⁻¹) and 0.05% PVP. Percentage of rooted plants, the number of roots per plant and length of roots were measured after five weeks in culture. The experiment was conducted as a completely randomized design in a factorial arrangement with three replicates. Each replicate contained ten plantlets. The regenerated plants were transferred into sterile Perlite after washing with sterile distilled water. They were then maintained in a growth chamber and were sprayed with sterile distilled water during the first week. After that, the plants were irrigated at weekly intervals with a half-strength Coic solution (Coic et al., 1975) and when sufficiently matured, were transferred into the soil-Perlite (1:1) and ultimately to the soil in greenhouse condition.

2.3. Statistical analysis

The data were subjected to analysis of variance (ANOVA). Statistical analyses were performed using SAS (SAS Institute Inc, 2002). Mean comparisons was conducted using Duncan's multiple range test (Duncan, 1955).

3. Results

3.1. Initiating a culture

Results indicated that the survival rates of meristems were affected by fig cultivars, explant sizes and IBA concentrations but not culture media (Table 1). The survival rate of 'Sabz' meristem was no significantly affected by their sizes. However, small sized meristems of 'Jaami-e-Kan' significantly had a lower survival rate than the large sized ones (Fig. 2). Some of the explants showed abnormal shape (hook-shape), and some tended to induce callus.

The survival rates of small-sized meristems were also significantly different between the two studied cultivars and the small-sized meristems of fig cv. 'Sabz' showed a higher survival rate than the same explants of 'Jaami-e-Kan'. On the other hand, a large number of small-sized meristems from 'Jaami-e-Kan' cv. frustrated and failed to grow, while, those of 'Sabz' cv. have been grown and developed to the microshoots. However, there were no statistically significant differences between the large-sized meristems of two studied cultivars (Fig. 2). There were no significant differences between meristem survival rates in two different culture media. However, the survival percentage of excised meristem-tips was affected by different concentrations of BA ($p \leq 0.01$). The media supplemented with 0.5 mg l⁻¹ BA showed the highest survival rate (77%) in both culture media (Fig. 2). Nearly all of the explants that had been cultured in control (BA-less) medium were failed to grow. After 12 weeks, the percentage of survival rate in the small- and large-sized meristems were 37% and 58% in 'Sabz' cv., while it was 29% and 41% in 'Jaami-e-Kan' cv., respectively.

3.2. Increasing propagules

Two studied cultivars showed significant differences in the proliferation rate ($p \leq 0.01$). Particularly, 'Sabz' had a higher proliferation

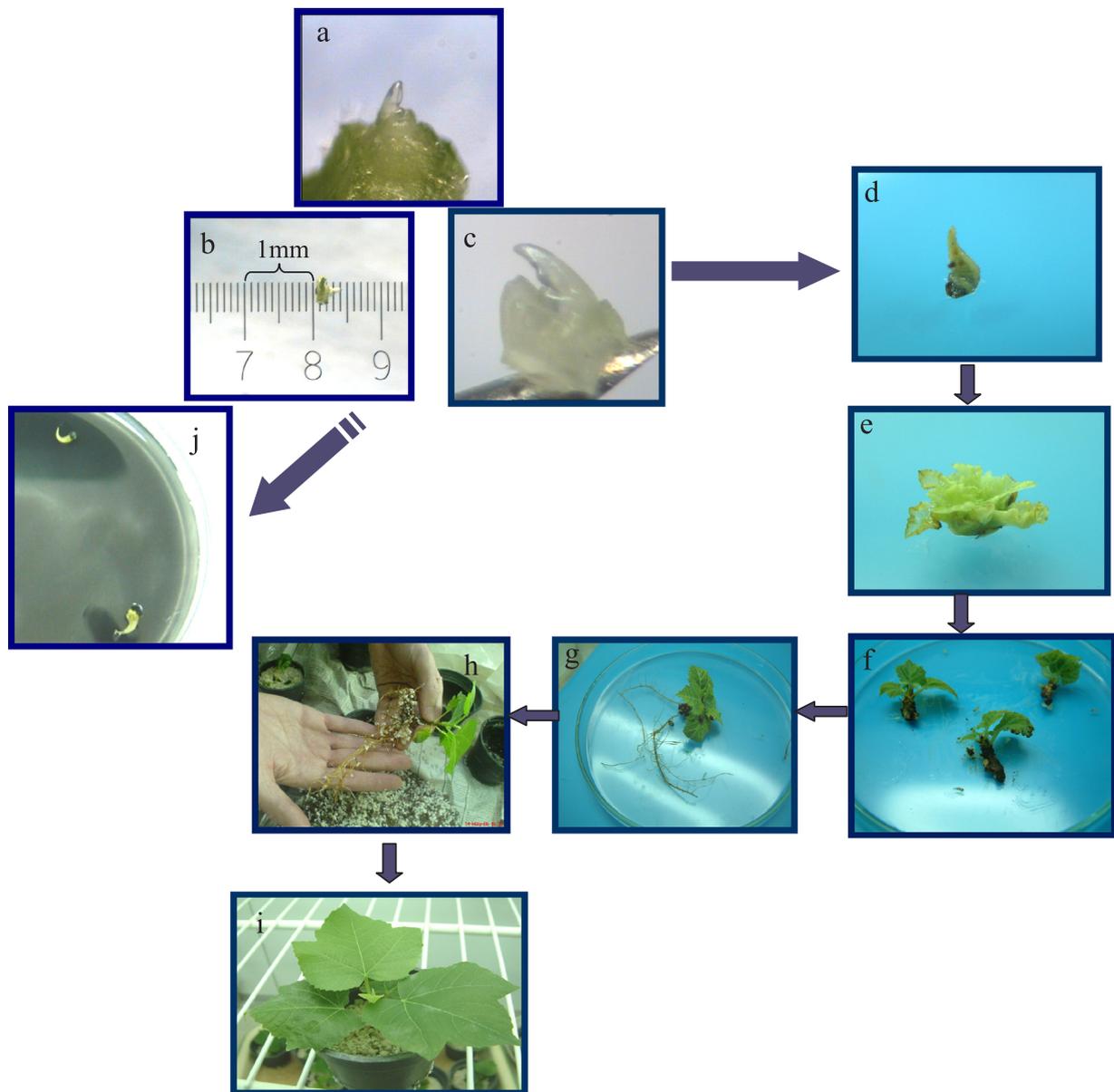


Fig. 1. Different stage of fig meristem tip culture from meristem excision to potted plants. a-c) small isolated meristem tip; d) meristematic explant in 6th week; e) developed explant before using for proliferation (12th week); f) regenerated nodal explants (6th week); g) rooted plantlets; h) transplanting the rooted plant; i) potted plant; j) hook-shape explants.

Table 1
Analysis of variance (ANOVA) of the effects of different factors on survival percentage of excised meristems.

Source	DF	Mean square
Cultivar	1	33,333.33**
Culture media	1	20.833 ^{ns}
Explant size	1	31,687.5**
Cultivar × Explant size	1	24,083.33**
BA	2	1187.5**
Error	92	237.432
(CV)%		18.96

**, * and ^{ns} respectively significantly different for the 0.01 and 0.05 levels and non-significant effect.

rate (2.3 shoots per micro shoot) than ‘Jaami-e-Kan’ (1.7 shoots per micro shoot) (Fig. 3). The maximum proliferation rate was obtained on the medium containing 2 mg l⁻¹ BA (2.4 shoots per micro shoot).

Results of analysis of variance showed that different treatments

significantly affected the length of internodes ($p \leq 0.01$) (Table 2). NAA concentration had significant effects on the shoot development ($p < 0.01$) (Table 2). The shoot length increased as NAA concentration increased from 0 to 0.5 mg l⁻¹ (Fig. 3). The interaction between BA concentrations and cultivars was significant on the shoot proliferation and elongation ($p \leq 0.01$) (Table 2). Therefore, curve analyzing was used to investigate the response of two cultivars to different BA concentrations. Ideally, the curve must go past through the origin (0, 0) because no proliferation was obtained in BA less medium (control). Results showed that the quadratic polynomial fits data well (Fig. 4). For both genotypes, the highest shoot length was observed in the medium supplemented with 1 mg l⁻¹ BA. Length of shoots was decreased as BA concentration was increased from 1 to 2 mg l⁻¹ and the lowest shoot elongation was recorded in the highest BA concentration (2 mg l⁻¹) for both cultivars (Fig. 4). The reduction rate was higher for ‘Jamm-e-kan’ cultivar compared with the ‘Sabz’ cultivar. However, freshly consumed cultivar (‘Jaami-e-Kan’) showed higher shoot elongation, than dry type (‘Sabz’). The explants cultured in the control medium failed to show any

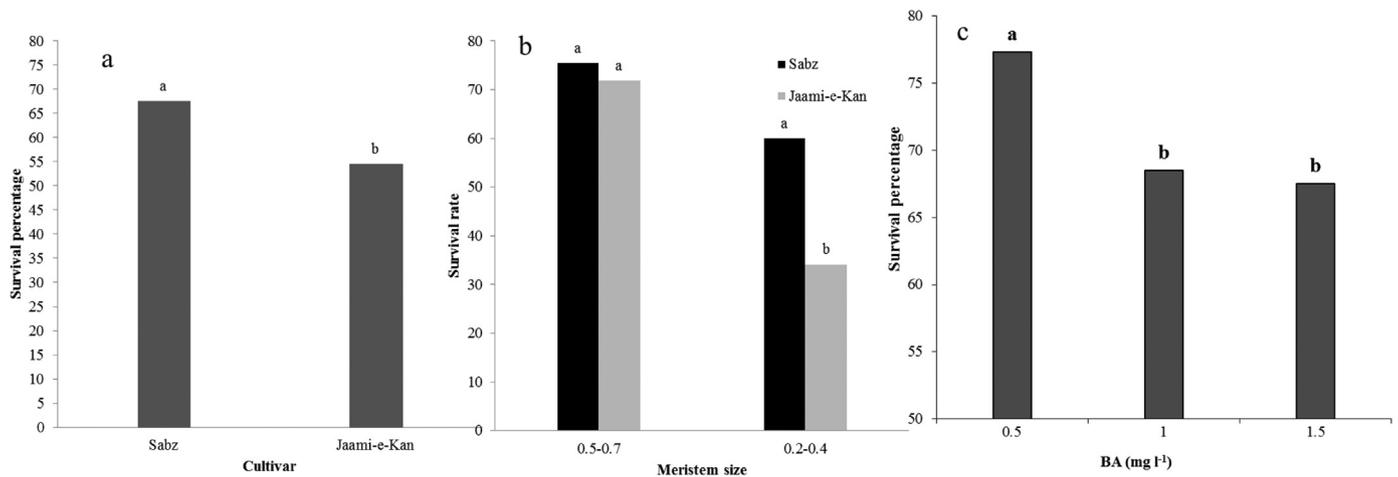


Fig. 2. Effects of cultivar (a) meristem size (b) and BA concentrations (c) on the survival percentage of excised meristems from two fig cultivars. Almost all meristems that were cultured on the control died. Means followed by the same letter are not significantly different as indicated by the Duncan multiple range test at $P \leq 0.01$.

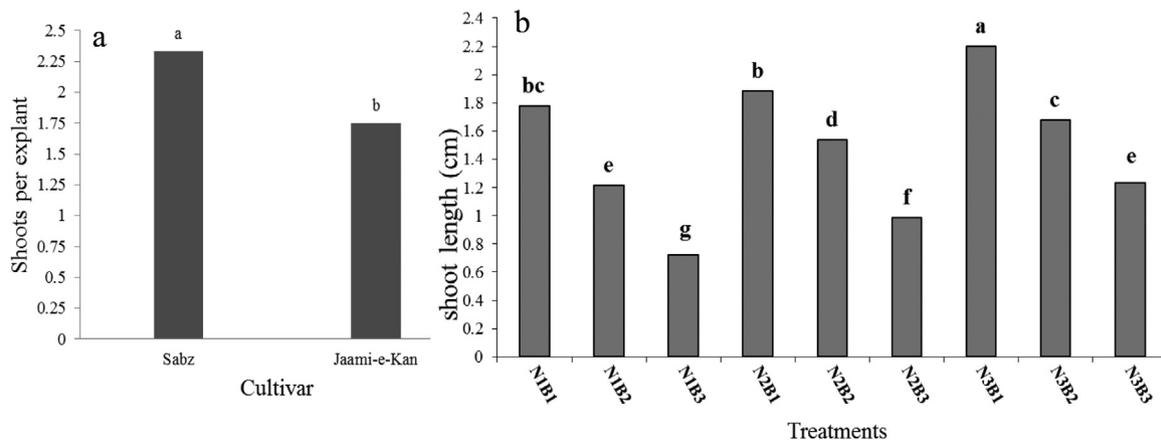


Fig. 3. Effects of cultivar on the shoot proliferation (a); effects of NAA and BA levels on the shoot length (b). N1, N2 and N3 represent 0.0 mg l^{-1} , 0.1 mg l^{-1} and 0.5 mg l^{-1} NAA levels, respectively. B1, B2 and B3 represent 1 mg l^{-1} , 1.5 mg l^{-1} and 2.0 mg l^{-1} BA concentrations, respectively.

Table 2
Analysis of variance for effects of different factors on the shoot proliferation and elongation.

Source	DF	Mean square	
		Elongation	Proliferation
NAA	2	0.4753**	0.186 ^{ns}
BA	2	28.52**	19.477**
NAA × BA	4	3.172**	0.2069 ^{ns}
Cultivar	1	32.041**	30.625**
BA × Cultivar	2	1.521**	0.3 ^{ns}
Sampling error	153	0.0535	0.553
(CV)%		10.08	36.69

** : Significant differences at 0.01 level of probability; ^{ns}: no significant differences.

response.

3.3. Rooting and acclimatization

According to the analysis of variance, different cultivars had no significant effect on the percentage of rooted micro-shoots, whereas the effect of cultivars and IBA levels was significant ($p \leq 0.01$) on the root elongation and root number (Table 3). The highest percentage of rooted micro-shoots (88.3%) was obtained from medium containing 1.5 mg l^{-1} IBA, while the lowest percentage of rooted plantlets was

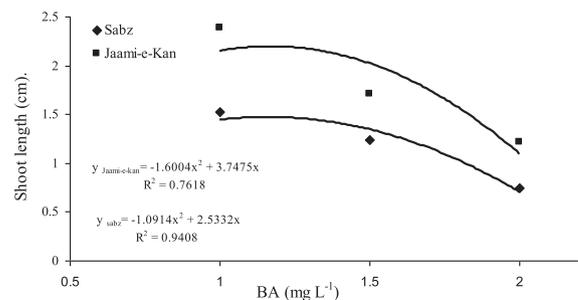


Fig. 4. The plot of shoot elongation against different BA (6-benzyladenine) concentrations for ‘Sabz’ and ‘Jaami-e-Kan’ fig cultivars under *in vitro* condition. Two-node segments on the MS basal medium supplemented with BA, NAA and 0.05% PVP, subcultured every two weeks and data were recorded after six weeks.

attained using 0.5 mg l^{-1} IBA treatment (Fig. 5). IBA-less medium did not result in any root formation on the base of micro-shoots. Accordingly, ‘Sabz’ cultivar, which had a higher survival rate of meristem and shoots length in the previous steps, produced longer roots (5.08 cm) than ‘Jaami-e-Kan’ cv. (4.45 cm) (Fig. 5). The maximum of root length was obtained in the medium containing 1.5 mg l^{-1} IBA followed by 2 mg l^{-1} IBA, with average root length of 6.3 and 6.11 cm, respectively, while the lowest root length (1.9 cm) was observed in the medium containing 0.5 mg l^{-1} IBA (Fig. 5).

Table 3

Analysis of variance for effects of different IBA levels and cultivars on the root related parameters.

Source	DF	Number of roots per plant	Mean square	
			Root length (cm)	Rooted plants (%)
IBA	3	160.11**	24.78**	1527.77**
Cultivar	1	37.5**	2.041**	16.66 ^{ns}
IBA × Cultivar	3	2.277 ^{ns}	0.149 ^{ns}	16.67 ^{ns}
Error	16	1.291	0.084	45.83
CV (%)		11.75	6.10	9.55

* *: significant differences at 0.01 level of probability; ^{ns}: no significant differences.

Similarly to other studied factors, ‘Sabz’ cultivar produced a higher number of roots per micro shoot (10.9) compared with ‘Jaami-e-Kan’ (8.1). Also, the highest number of roots per micro shoot were obtained from the medium containing 2 mg l⁻¹ IBA (14.6) (Table 4).

4. Discussion

Plant regeneration through apical meristem is considered to be one of the most promising procedures not only for virus elimination in plant tissue culture but also for multiplying a selected plant material true to its type showing the same agronomic characteristics (Orlikowska et al., 2000). Direct shoot development from the apical meristem bypass the callus formation phase, hence minimize the somaclonal variation and ensuring the genetic stability of *in vitro* raised plantlets (Kaushal et al., 2014). We were able to successfully produce fig plantlets from meristem culture of two well-known Iranian fig cultivars under *in vitro* condition. However, a significant difference was observed between the two studied cultivars for most of the studied factors under the same conditions. Recommendation for optimum tissue culture conditions including media ingredients are highly dependents on the plant species, genotype as well as explant types and should be optimized for each circumstance (Bhojwani and Razdan, 1996). The behavior of plant tissues under *in vitro* conditions often seems to be under an over-riding genetic control, with other factors exerting only a minor effect (George et al., 2008). However, it is highly accepted that age, genotype, explant types, plant growth regulators as well as environmental conditions are among the main factors that affect the prosperity of plant tissue cultures (Al-Ramamneh et al., 2017). The differences in the response of two studied cultivars could be attributed to the indigenous physiological

Table 4

Simple effects of IBA (indole-3-butyric acid) and cultivars on the number of roots per micro shoot and root length.

	IBA (mg l ⁻¹)				Cultivar	
	0.5	1	1.5	2	Sabz	Jaami-e-Kan
Number of roots per micro shoot	2.6d [†]	8.2c	12.5b	14.6a	10.9a	8.1b
Root length (cm)	1.9c	4.75b	6.3a	6.11a	5.08a	4.45b

* Means followed the same letter have no significant differences with Duncan Multiple Range Test (DMRT) at 5% probability.

properties and their evolutionary differences. ‘Sabz’ and ‘Jaami-e-Kan’ cultivars belong to Smyrna and Common fig type, respectively. Also, ‘Sabz’ is well known as a dried fig and it has been cultivated in semi-arid lands with low irrigation. This cultivar has been adapted to such hard conditions (as a dry farming crop). Therefore, the higher survivability of ‘Sabz’ meristems in comparison with the other cultivar, which has been grown in orchards with flood irrigation, is probably due to the higher adaptability of this cultivar with unfavorable conditions that may have been affected its genetic rearrangement through an evolutionary process. Previous studies demonstrated that explants survival under *in vitro* culture is somewhat dependent on the genotype of mother plants (George et al., 2008). Significant effects of genotypes on the shoot proliferation and rooting were reports in other plant species (Naik et al., 1999; Ning et al., 2007; Karimi Alavijeh et al., 2016; Mittal et al., 2016; Scalzo et al., 2016). It is reported that genotypes had significant effects on the produced shoot and root numbers under *in vitro* condition in *F. carica* (Pasqual and Ferreira, 2007; Shahcheraghi and Shekafandeh, 2016). In accordance to our results, Taha et al. (2013), observed significant differences between shoot number, shoot length and leaf numbers from two fig cultivars ‘Conadria’ and ‘Black Mission’ under *in vitro* condition.

In our study, the survival rate of excised meristem-tips was affected by their initial size. It is well established that the size of the explant determines its survival capacity under tissue culture conditions (Manganaris et al., 2003; Salami et al., 2005; Nhut et al., 2007; Lassois et al., 2013). In general, the larger the explant size, the higher the chance of its survival is (Smith, 2013). Furthermore, it is reported that the presence of leaf primordium appears to determine the capability of a meristem explant to develop (Wang and Hu, 1980). Sha Valli Khan

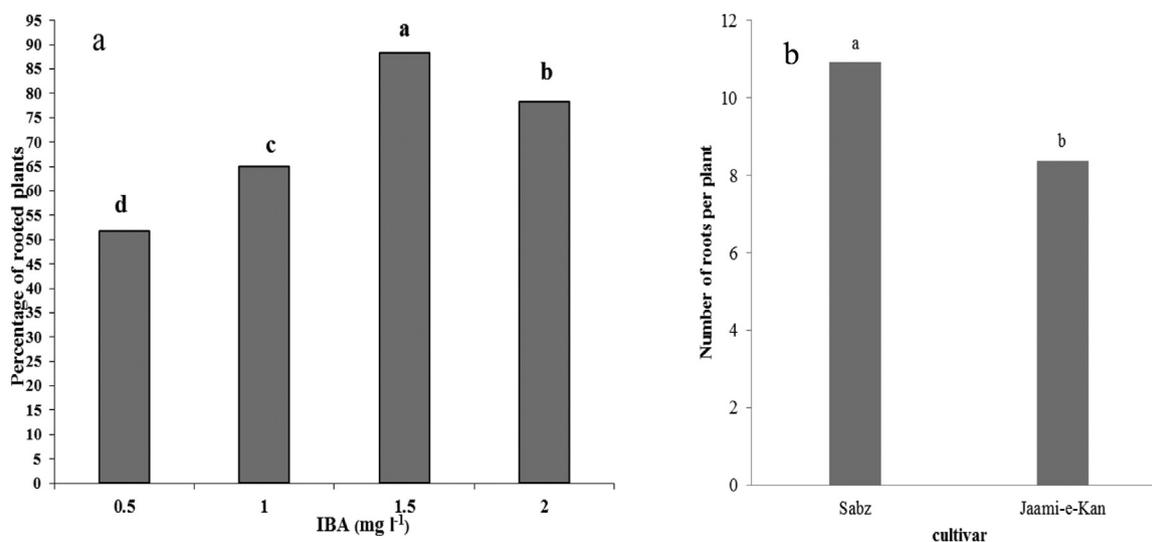


Fig. 5. a) Effect of different levels of IBA on the root induction of *in vitro* raised micro-shoots (nearly no micro-shoots rooted on control). B) Effect of cultivar on the number of roots per plant. Means followed by the same letter are not significantly different as indicated by the Duncan multiple range test at $P \leq 0.01$.

et al. (1997) observed that explants with two axillary meristems gave a better response to regeneration than shoot tip explants with single apical meristems.

In the present research, the inclusion of BA in the culture media was necessary for growing of meristem. Growing body of evidence indicate that cytokinins are required in culture media for shoot induction and proliferation, thought its effective type and optimal concentration varies with the system (Park et al., 2008). Other researchers reported the presence of cytokinins for the proliferation of fig trees (Demiralay et al., 1998; Nobre et al., 1998; Kumarm et al., 1998; Fraguas et al., 2004; Shahcheraghi and Shekafandeh, 2016). Apart from the cytokinin activity, different cytokinins may be a modulator of the endogenous auxins (Singh and Agarwal, 2016). According to Demiralay et al. (1998) medium supplemented with 0.5 mg l^{-1} BA and 0.1 mg l^{-1} IBA resulted in the maximum establishment of fig meristem. Comlekcioglu et al. (2007) reported the highest culture establishment in the medium containing 0.2 mg l^{-1} GA3 and 0.5 mg l^{-1} BA. However, Gunver et al. (1998) acquired the optimal response in the higher concentrations of cytokinin and auxin (1 mg l^{-1} BA and 1 mg l^{-1} NAA) for the establishment of fig meristem culture. Biochemical complexity of an explant (both genetic and physiological status) in combination with the compounds of culture media such as types and concentrations of plant growth regulators have substantial effects on the callus induction, proliferation and adventitious shoot formation under *in vitro* condition (Khawar et al., 2005). It is well documented that effect of BA, which is a synthetic cytokinin, is stronger than the other cytokinins on the shoot regeneration (Torresm, 2013). Also, previous reports indicated that BA resulted in the better shoot proliferation rates on *Ficus benjamina* (Rzepka-Plevnes and Kurek, 2000), *Ficus anastasia* (Al Malki and Elmeer, 2010) and *Ficus carica* (Shahcheraghi and Shekafandeh, 2016) than other sources of cytokinins. However, Nobre et al. (1998) reported that addition of cytokinin in the culture medium is not necessary for the establishment stage of fig meristems cvs. 'Berbera' and 'Lampa Branca'. Furthermore, Hepaksoy and Aksoy (2006) obtained the highest regeneration from medium supplemented with 5 mg l^{-1} BA in addition of 1 mg l^{-1} IBA. Cytokinin has been employed occasionally to enhance the growth of isolated meristem tips, but high levels of cytokinin limit the growth of explants (George et al., 2008).

Analysis of variance showed NAA treatments had no significant effect on the proliferation rate of two studied fig cultivars. In agreement with our results, Nobre et al. (1998) reported that NAA did not affect proliferation of fig 'Berbera' and 'Lampa Branca'. There are some reports indicating that the presence of NAA either with or without BAP reduced the frequency of bud break and multiple shoot formation (Amin and Jasiwal, 1993; Pattnaik and Chand, 1997). However, the opposite records are also available, which indicate that the presence of auxin in the cytokinin-supplemented media significantly improve shoot length (Siril and Dhar, 1997; Salami et al., 2005). These discrepancy results could be attributed to the explant sources and experiment conditions. The indigenous hormonal status of an explant plays an important role in the success of a tissue culture system. On the other hand, when the researchers used explants with a high content of indigenous auxin, the exogenous application of auxin sources reduced the shoot proliferation and *vice versa*. We should bear in mind that auxin is a major obstacle of axillary bud development and it is the main cause of the apical dominance of shoot tip in high concentrations.

Treatments with elevated BA concentrations promoted the shoot numbers per micro shoot but decreased the shoot length and negatively affected shoot development. The same results were reported from other fruit tree species including chokecherry (Pruskim et al., 2000) grapevine (Salami et al., 2005) and *Prunus* spp. (Kalinina and Brown, 2007). The interaction effect was significant for shoot length, and the media supplemented with 1 mg l^{-1} BA and 0.5 mg l^{-1} NAA resulted in the highest shoot elongation. Kumar et al. reported that the longest shoots in fig explants were obtained in the medium containing 2 mg l^{-1} BA.

Adventitious roots formation on the *in vitro* raised shoots have great

importance in a commercial propagation system. Previous results indicated that half strength MS medium is a more suited platform for root induction than MS medium in different fig cultivars (Dhage et al., 2012; Shahcheraghi and Shekafandeh, 2016). The role of auxins in root development was reviewed, and it is a well-established fact that auxins are the main factors involved in the root induction on the base of *in vitro* raised shoots (Némethm, 1986). IBA is considered as one of the best auxin sources for root induction in different plant species (Kaushal et al., 2014; Danial et al., 2014; Shekhawat et al., 2015). Our results indicated that IBA is necessary for root induction in produced fig micro-shoots. The control cultures showed the lowest percentage of rooted micro-shoots, length of roots and number of roots per micro shoot. The results of present study are in agreement with the results of Kumar et al., Nobre et al. (1998), Hepaksoy and Aksoy (2006) and Shahcheraghi and Shekafandeh (2016) who reported that presence of IBA is necessary for *in vitro* root induction in fig micro-shoots. However, our observations do not confirm the results of Brum (2001) and Fraguas et al. (2004) who indicated that there is no need for auxin sources in the culture medium for root induction and growth of 'Roxo de Valinhos' fig cultivar. The highest root induction was recorded in the highest IBA concentration (2 mg l^{-1}), but the maximum root elongation was attained in the lower concentrations of IBA (1.5 mg l^{-1}). The growing body of evidence suggests that exogenous application of auxin sources are required for root induction at the primary stages of root emerging and high concentrations of this plant growth regulator can inhibit root growth and elongation (Pacheco-Villalobos et al., 2016). Similar to our results, Kumar et al. reported that media containing 2 mg l^{-1} IBA showed the highest number of roots per plant. Also, Nobre et al. (1998) observed that $2.5 \mu\text{M}$ IBA ($\approx 0.5 \text{ mg l}^{-1}$ IBA) showed the highest root numbers in *F. carica*. Danial et al. (2014) also reported that the longest roots were obtained in the 0.5 mg l^{-1} IBA concentration and higher levels of this plant growth regulators significantly reduced the root length as $2\text{--}3 \text{ mg l}^{-1}$ IBA completely ceased the root formation in fig. Adventitious root formation through tissue culture systems highly depends on the interaction of many different endogenous and exogenous factors.

5. Conclusion

Collectively our data showed that the presence of plant growth regulators, besides meristem size, could play an important role in meristem culture and micropropagation of fig trees. Also, significant differences were observed between the two studied cultivars for most of the analyzed factors that could be attributed to the differences in their genetic background and or adaptation to different environmental conditions. In conclusion, we recommend MS medium supplemented with cytokinin for future tissue culturing. Results of this study can be used practically for micropropagation of fig trees cvs. 'Sabz' and 'Jaami-e-Kan' as well as eliminating of plant disease by meristem tip culture.

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Declarations of interest

None.

References

- Al Malki, A.A.H., Elmeer, K.M.S., 2010. Influence of auxin and cytokinin on *in vitro* multiplication of *Ficus anastasia*. *Afr. J. Biotech.* 9 (5), 635–639.
- Al-Ramamneh, E.A., Daradkeh, N., Rababah, T., Pacurar, D., Al-Qudah, M., 2017. Effects of explant, media and growth regulators on *in vitro* regeneration and antioxidant activity of *Juniperus phoenicea*. *Aust. J. Crop Sci.* 11 (7), 828–837.
- Al-Shomali, E., Sadler, M.T., Ateyyeha, A., 2017. Culture media comparative assessment

- of common fig (*Ficus carica* L.) and carryover effect. *Jordan J. Biol. Sci.* 10 (1), 13–18.
- Amin, M.N., Jasiwal, V.S., 1993. *In vitro* response of apical bud explants from mature tree of jackfruit (*Artocarpus heterophyllus*). *Plant Cell Tissue Organ Cult.* 33, 59–65. <https://doi.org/10.1007/BF01997599>.
- Aradhya, M.K., Stover, E., Velasco, D., Koehmstedt, A., 2010. Genetic structure and differentiation in cultivated fig (*Ficus carica* L.). *Genetica* 138, 681–694. <https://doi.org/10.1007/s10709-010-9442-3>.
- Barolo, M.I., Ruiz Mostacero, N., López, S.N., 2014. *Ficus carica* L. (Moraceae): an ancient source of food and health. *Food Chem.* 164, 119–127. <https://doi.org/10.1016/j.foodchem.2014.04.112>.
- Bhojwani, S.S., Razdan, M.K., 1996. *Plant Tissue Culture: Theory and Practice. A Revised Edition.* Elsevier, Amsterdam, pp. 483–536.
- Boudabous, M., Mars, M., Marzougui, N., Ferchichi, A., 2010. Micropropagation of apple (*Malus domestica* L. cultivar Douce de Djerba) through *in vitro* culture of axillary buds. *Acta Bot. Gall.* 157 (3), 513–524. <https://doi.org/10.1080/12538078.2010.10516227>.
- Brum, G.R., 2001. Micropropagação da figueira (*Ficus carica* L.) 'Roxo de Valinhos' Dissertação (Mestrado em Fitotecnia). Universidade Federal de Lavras, Lavras, MG, pp. 41.
- Coïc, Y., Lesaint, C., 1975. La nutrition et en eau des plantes en horticulture avancee. *Doc. Tech. De. SCPA* 23, 1–22.
- Comlekcioglu, S., Kuden, A.B., Kacar, Y.A., Kamberoglu, M.A., 2007. Meristem culture of two fig cultivars in Turkey. *Fruits* 62, 125–131. <https://doi.org/10.1051/fruits:2007006>.
- Daniyal, G.H., Ibrahim, D.A., Brkat, S.A., Khalil, B.M., 2014. Multiple shoots production from shoot tips of fig tree (*Ficus carica* L.) and callus induction from leaf segments. *Int. J. Pure Appl. Sci. Technol.* 20 (1), 117–124.
- Demiralay, A., Yalcin-Mendi, Y., Aka-Kacar, Y., Centime, S., Aksoy, U., Ferguson, L., Hepaksoy, S., 1998. *In vitro* propagation of *Ficus carica* L. var. Bursa Siyahı through meristem culture. *Acta Hort.* 480, 165–167.
- Dhage, S., Pawar, B., Chimote, V., Jadhav, A., Kale, A., 2012. *In vitro callus induction and plantlet regeneration in fig (Ficus carica L.)*. *J. Cell Tiss. Res.* 12, 3395–3400.
- Ferreira, E.A., Pasqual, M., 2005. *Ficus carica* produced by micropropagation. *Acta Hort.* 738, 437–441.
- Fraguas, C.B., Pasqual, M., Dutra, L.F., Cazzetta, O., 2004. Micropropagation of fig (*Ficus carica* L.) 'Roxo de Valinhos' plants. *Vitr. Cell Dev. Biol. Plant* 40, 471–474. <https://doi.org/10.1079/IVP2004562>.
- Gamborg, O.L., Miller, R.A., Ojima, K., 1968. Nutrient requirements of suspension culture of soybean root cells. *Exp. Cell. Res.* 50, 151–158. [https://doi.org/10.1016/0014-4827\(68\)90403-5](https://doi.org/10.1016/0014-4827(68)90403-5).
- George, E.F., Hall, M.A., Klerck, G.D., 2008. *Plant Propagation by Tissue Culture*, 3rd edition. Springer, Netherlands. <https://doi.org/10.1007/978-1-4020-5005-3>.
- Gunver, G., Ertan, E., Aksoy, U., Ferguson, L., Hepaksoy, S., 1998. A study on the propagation of figs by the tissue culture techniques. *Acta Hort.* 480, 169–172.
- Hassan, S.A.M., Zayed, N.S., 2018. Factor controlling micropropagation of fruit trees: a review. *Sci. Int.* 6, 1–10.
- Hepaksoy, S., Aksoy, U., 2006. Propagation of *Ficus carica* L. clones by *in vitro* culture. *Biol. Plant.* 50, 433–436. <https://doi.org/10.1007/s10535-006-0063-8>.
- Kalinina, A., Brown, D.C.W., 2007. Micropropagation of ornamental *Prunus* spp. and GF305 peach, a *Prunus* viral indicator. *Plant Cell Rep.* 26, 927–935. <https://doi.org/10.1007/s00299-007-0315-x>.
- Karimi Alavijeh, M., Ebadi, A., Zarei, A., Omidi, M., 2016. Somatic embryogenesis from anther, whole flower, and leaf explants of some grapevine cultivars. *Plant Tissue Cult. Biotech.* 26 (2), 219–230. <https://doi.org/10.3329/ptcb.v26i2.30572>.
- Kaushal, S., Sidana, A., Dev, K., 2014. *In vitro* plant production through apical meristem culture of *Gentiana kurroo* Royle. *J. Med. Plants Stud.* 3 (1), 04–09.
- Khawar, K.M., Saryhan, E., Sevımay, C., Cocu, S., Parmaksiz, I., Uranbey, S., Ipek, A., Kaya, M.D., Sancak, C., Ozcan, S., 2005. Adventitious shoot regeneration and micropropagation of *Plantago lanceolata* L. *Period Biol.* 107, 113–116.
- Kumarm, V., Radha, A., Kumar Chitta, S., 1998. *In vitro* plant regeneration of fig (*Ficus carica* L. cv. gular) using apical buds from mature trees. *Plant Cell Rep.* 17, 717–720. <https://doi.org/10.1007/s002990050471>.
- Lassois, L., Lepoivre, P., Swennen, R., van den Houwe, I., Panis, B., 2013. Thermotherapy, chemotherapy, and meristem culture in banana. *Methods Mol. Biol.* 11013, 419–433. https://doi.org/10.1007/978-1-62703-074-8_32.
- Manganaris, G.A., Economou, A.S., Boubourakas, I.N., Katis, N.L., 2003. Elimination of PPV and PNRSV through thermotherapy and meristem-tip culture in nectarine. *Plant Cell Rep.* 22, 195–200. <https://doi.org/10.1007/s00299-003-0681-y>.
- Mittal, M., Devi, R., Gosal, S.S., 2016. Effect of genotypes and activated charcoal on high frequency *in vitro* plant regeneration in sugarcane. *Indian J. Biotechnol.* 15, 261–265.
- Moham Jain, S., Haggman, H., 2007. *Protocols for Micropropagation of Woody Trees and Fruits.* The Netherlands, The Netherlands, pp. 558 (ISBN 978-1-4020-6352-7).
- Murashige, J., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 15, 473–497.
- Naik, S.K., Pattnaik, S., Chand, P.K., 1999. *In vitro* propagation of pomegranate (*Punica granatum* L. cv. *Ganesh*) through axillary shoot proliferation from nodal segments of mature tree. *Sci. Hort.* 79, 175–183.
- Németh, G., 1986. Induction of rooting (pp. 49–64) In: BAJAJ, y.P.S. (Ed.), *Biotechnology in Agricultural Forestry.* Springer Verlag, Berlin, Germany, pp. 515 (pp. 49–64).
- Nhut, D.T., An, T.T.T., Huong, N.T.D., Don, N.T., Hai, N.T., Thien, N.Q., Vu, N.H., 2007. Effect of genotype, explant size, position, and culture medium on shoot generation of *Gerbera jamesonii* by receptacle transverse thin cell layer culture. *Sci. Hortic.* 111 (2), 146–151.
- Ning, G.G., Fan, X., Huang, W.J., Bao, M.Z., Zhang, J.B., 2007. Micropropagation of six *Prunus* mume cultivars through axillary shoot proliferation, and ISSR analysis of cloned plants. *Acta Biol. Crac. Ser. Bot.* 49, 25–31.
- Nobre, J., Romano, A., Aksoy, U., Ferguson, L., Hepaksoy, S., 1998. *In vitro* cloning of *Ficus carica* L. adult trees. *Acta Hort.* 480, 161–164.
- Orlikowska, T., Sabøa, I., Kucharska, D., 2000. The effect of leaf and shoot tip removal and explant orientation on axillary shoot proliferation of *Codiaeum variegatum* Blume var. pictum Muell. Arg. cv. Excell. *Sci. Hortic.* 85, 103–111.
- Pacheco-Villalobos, D., Díaz-Moreno, S.M., van der Schuren, A., Tamaki, T., Kang, Y.H., Gujas, B., Novak, O., Jaspert, N., Ali, Z., Wolf, S., Oecking, C., Ljung, K., Bulone, V., Hardtke, C.S., 2016. The effects of high steady state auxin levels on root cell elongation in *Brachypodium*. *Plant Cell* 28, 1009–1024. <https://doi.org/10.1105/tpc.15.01057>.
- Park, S.Y., Kim, Y.W., Moon, H.K., Murthy, H.N., Choi, Y.H., Cho, H.M., 2008. *In vitro* propagation of *Salix pseudodoliasigne* from nodal explants. *Plant Cell Tissue Organ Cult.* 93, 341–346.
- Pasqual, B.M., Ferreira, E.A., 2007. Micropropagation of fig tree (*Ficus carica*). In: *Protocols for Micropropagation of Woody Trees and Fruits.* Springer, Netherlands, pp. 409–416. https://doi.org/10.1007/978-1-4020-6352-7_37.
- Pattnaik, S.K., Chand, P.K., 1997. Rapid clonal propagation of three mulberries, *Morus cathayana* Hems L., *M. lhou* Koiz. And *M. serrata* Roxb., through *in vitro* culture of apical shoot buds and nodal explants from mature trees. *Plant Cell Rep.* 16, 503–508. <https://doi.org/10.1007/BF01092774>.
- Pruskin, K.W., Lewis, T., Astatkie, T., Nowak, J., 2000. Micropropagation of chokecherry (*Prunus virginiana* L.) and pincherry (*P. pensylvanica* L.) cultivars. *Plant Cell Tissue Organ Cult.* 63, 93–100.
- Rania, A.T., Mustafa, N.S., Hassan, S.A., 2013. Protocol for Micropropagation of two *Ficus carica* cultivars. *World J. Agric. Sci.* 9 (5), 383–388 (2013).
- Rzepka-Plevnev, D., Kurek, J., 2000. The influence of media composition on the proliferation and morphology of *Ficus benjamina* plantlets. *Acta Hort.* 560, 473–476.
- Sahraroo, A., Mirjalili, M.H., Corchete, P., Babalar, M., Moghadam, M.R.F., Zarei, A., 2018. Enhancement of rosmarinic acid production by *Saturia khuzistanica* cell suspension: effects of phenylalanine and sucrose. *SABRAO J. Breed. Genet.* 50 (1) (25–3).
- Salami, A.R., Ebadi, A., Zamani, Z., Ghasemi, M., 2005. Improvement in apex culture in an Iranian grapevine (*Vitis vinifera* L. 'Bidaneh Sefid') through fragmented shoot apices. *Int. J. Agr. Biol.* 7 (3), 333–336.
- SAS Institute Inc, 2002. *SAS users guide: statistics.* 9th edn. SAS Institute Inc., Cary, NC.
- Scalzo, J., Donno, D., Miller, M., Ghezzi, M., Mellano, M.G., Cerutti, A.K., Beccaro, G.L., 2016. Effect of genotype, medium and light on *in vitro* plant proliferation of *Vaccinium* spp. *N.Z. J. Crop Hort. Sci.* 44 (4), 231–246. <https://doi.org/10.1080/01140671.2016.1206946>.
- Khan, Sha Valli, Prakash, P.S., Rao, K.R. E., 1997. *In vitro* micropropagation of an endemic fruit tree *Syzygium alternifolium* (Wight) Walp. *Plant Cell Rep.* 16, 325–328. <https://doi.org/10.1007/BF01088290>.
- Shahcheraghi, S.T., Shekafandeh, A., 2016. Micropropagation of three endemic and endangered fig (*Ficus carica* L.) genotypes. *Adv. Hort. Sci.* 30 (3), 129–134.
- Shekhawat, M.S., Kannan, N., Manokari, M., Ravindran, C.P., 2015. *In vitro* regeneration of shoots and ex vitro rooting of an important medicinal plant *Passiflora foetida* L. through nodal segment cultures. *J. Genet. Eng. Biotech.* 13 (2), 209–214.
- Singh, A., Agarwal, P.K., 2016. Enhanced micropropagation protocol of ex vitro rooting of a commercially important crop plant *Simmondsia chinensis* (Link) Schneider. *J. For. Sci.* 62 (3), 107–115. <https://doi.org/10.17221/80/2015-JFS>.
- Siril, E.A., Dhar, U., 1997. Micropropagation of mature Chinese tallow tree (*Sapium sebiferum* Roxb.). *Plant Cell Rep.* 16, 637–640. <https://doi.org/10.1007/BF01275506>.
- Smith, R.H., 2013. *Plant Tissue Culture: Techniques and Experiments*, 3rd edition. Elsevier, Academic Press, pp. 208 (ISBN: 9780124159853).
- Taha, R.A., Mustafa, N.S., Hassan, S.A., 2013. Protocol for micropropagation of two *Ficus carica* cultivars. *World J. Agric. Sci.* 9 (5), 383–388.
- Torres, C.K., 2013. *Tissue Culture Techniques for Horticultural Crops.* Van Nostrand Reinhold, New York, USA, pp. 283.
- Wang, P.J., Hu, C.Y., 1980. Regeneration of virus-free plants through *in vitro* culture. *Adv. Biochem. Eng./Biotech.* 18, 61–99.