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 (Aradhy 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 planting 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; Boudabouts 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,
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 (Gamborg 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 Coïc solution (Coïc 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 different used culture media. However, the survival percentage of excised meristem-tips was affected by different concentrations of BA (p ≤ 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 ≤ 0.01). Particularly, ‘Sabz’ had a higher proliferation.
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

Table 1

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

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar</td>
<td>1</td>
<td>33,333.33**</td>
</tr>
<tr>
<td>Culture media</td>
<td>1</td>
<td>20.833 m</td>
</tr>
<tr>
<td>Explant size</td>
<td>1</td>
<td>31,687.5**</td>
</tr>
<tr>
<td>Cultivar × Explant size</td>
<td>1</td>
<td>24,083.33**</td>
</tr>
<tr>
<td>BA</td>
<td>2</td>
<td>1187.5**</td>
</tr>
<tr>
<td>Error</td>
<td>92</td>
<td>237.432</td>
</tr>
<tr>
<td>(CV)%</td>
<td></td>
<td>18.96</td>
</tr>
</tbody>
</table>

**, m and m respectively significantly different for the 0.01 and 0.05 levels and non-significant effect.
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 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 2**

<table>
<thead>
<tr>
<th>Source</th>
<th>Elongation</th>
<th>Proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>BA</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>NAA×BA</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Cultivar</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Sampling error</td>
<td>153</td>
<td>10.08</td>
</tr>
</tbody>
</table>

CV[%]: 10.08 36.69

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

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.

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 were cultured on the MS basal medium supplemented with BA, NAA and 0.05% PVP, subcultured every two weeks and data were recorded after six weeks.
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 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 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 (Nalk 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; Shahcheragh 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 Table 3

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Number of roots per plant</th>
<th>Mean square (cm)</th>
<th>Rooted plants (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBA</td>
<td>3</td>
<td>160.11 **</td>
<td>24.78 **</td>
<td>1527.77 **</td>
</tr>
<tr>
<td>Cultivar</td>
<td>1</td>
<td>37.5 **</td>
<td>2.041 **</td>
<td>16.66 **</td>
</tr>
<tr>
<td>IBA × Cultivar</td>
<td>3</td>
<td>2.277 **</td>
<td>0.149 ns</td>
<td>16.67 ns</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>1.291</td>
<td>0.084</td>
<td>45.83</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>11.75</td>
<td>6.10</td>
<td>9.55</td>
</tr>
</tbody>
</table>

* #: significant differences at 0.01 level of probability; ns: no significant differences.

Table 4

<table>
<thead>
<tr>
<th>IBA (mg l(^{-1}))</th>
<th>Cultivar</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>Sabz</td>
</tr>
<tr>
<td>1.5</td>
<td>Jaami-e-Kan</td>
</tr>
<tr>
<td>2</td>
<td>Sabz</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Number of roots per micro shoot</th>
<th>Root length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabz</td>
<td>2.6d*</td>
<td>1.9c</td>
</tr>
<tr>
<td>Jaami-e-Kan</td>
<td>8.2c</td>
<td>4.75b</td>
</tr>
<tr>
<td>Sabz</td>
<td>12.5b</td>
<td>6.3a</td>
</tr>
<tr>
<td>Jaami-e-Kan</td>
<td>14.6a</td>
<td>6.1a</td>
</tr>
<tr>
<td>Sabz</td>
<td>10.9a</td>
<td>5.08a</td>
</tr>
<tr>
<td>Jaami-e-Kan</td>
<td>8.1b</td>
<td>4.45b</td>
</tr>
</tbody>
</table>

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

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 ≤ 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 (Demiray et al., 1998; Nobre et al., 1998; Kumarm et al., 1997; Fraguas et al., 2004; Shahcheragi and Shekafandeh, 2016). Apart from the cytokinin activity, different cytokinins may be a modulator of the endogenous auxins (Singh and Agarwal, 2016). According to Demiray 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. Comlekiciglu 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 (1mg 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 (Kharwar 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 (Rzepek-Plevnes and Kurek, 2000), Ficus anastasia (Al Malik and Elmee, 2010) and Ficus carica (Shahcheragi 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 meristem cv. ‘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 sp. (Kalinaia 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; Shahcheragi 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 Shahcheragi 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 cultivars. 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 μM IBA (< 0.5 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 2–3 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.

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of common fig (Ficus carica L.) and carryover effect. Jordan. J. Biol. Sci. 10 (1), 13-18.


