Genetic stability of regenerated plants via indirect somatic embryogenesis and indirect shoot regeneration of *Carum coticum* L.

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Ajowan (*Carum coticum* L.) is an important and endangered industrial medicinal plant that growing in some parts of Iran. Two efficient protocols, without somaclonal variation induction, were developed for indirect somatic embryogenesis and indirect shoot regeneration of three Iranian ecotypes of ajowan. In the first experiment, higher concentration of auxin than cytokinin (1, 1.5 and 2 mg/L of 2,4-dichlorophenoxyacetic acid along with 0.25, 0.5 and 0.75 mg/L kinetin) was used for callus induction in 5, 10 and 15 days old hypocotyl explants. In the second experiment, higher concentration of cytokinin than auxin (1 mg/L of kinetin along with 0.5 mg/L of 2,4-dichlorophenoxyacetic acid) was used for callus induction in 15-0 day hypocotyl explants. The higher frequency of somatic embryos was achieved from 15 days old hypocotyl explants of Goom ecotype with 28.33 embryos when induced calli from MS medium supplemented with 1.5 mg/L 2,4-dichlorophenoxyacetic acid in combination with 0.5 mg/L kinetin transferred to free plant growth regulator MS medium. Momentary removing of 2,4-dichlorophenoxyacetic acid was successful for somatic embryogenesis in Iranian ecotypes of ajowan that it significantly reduce the time of the culture and thus reduce the risk of somaclonal variation. Maximum number of initiated shoots per explant was related to Shiraz ecotype with average 18.33 shoots per callus in MS medium supplemented with 1.5 mg/L of specify type of cytokinin (3-methoxy-[6-benzylamino-9-tetrahydropryan-2-yl]purine) plus 0.25 mg/L naphthalene acetic acid. The survival rate of rooted plantlets was 60.86% and 58.33% for indirect somatic embryogenesis and indirect shoot regeneration derived plants, respectively. The genetic stability of regenerated plants via indirect somatic embryogenesis and indirect shoot regeneration was proved through flow cytometry analysis.

**1. Introduction**

Nowadays medicinal plants are used in raw or processed forms in traditional medicine or modern pharmaceutical industry. Ajowan (*Carum coticum* L.) is an economically important medici-

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**Abbreviations:**
ANOVA, Analysis of variance; BA, Benzyl adenine; BAP, 6-Benzylaminopurine; CV, Coefficient of Variation; DSE, Direct somatic embryogenesis; FCM, Flow cytometry; ISE, Indirect somatic embryogenesis; ISR, Indirect shoot regeneration; KIN, Kinetin; LSD, Least significant difference; MS, Murashige and Skoog medium; NAA, Naphthalene acetic acid; PGR, Plant Growth Regulator; PPPD, Photosynthetic photon flux density; TDZ, Thidiazuron; 2,4-D, 2,4-Dichlorophenoxyacetic acid; 3MeOBA9THPP, 3-methoxy-[6-benzylamino-9-
tetrahydropryan-2-yl] purine.

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that is widely used for mass propagation of plants. Somatic embryogenesis can happen in direct and/or indirect ways in plants. In DSE, embryogenesis usually happens without callus induction. While in ISE, callus induction usually happens before the embryogenesis (Quiroz-Figueroa et al., 2006). Indirect somatic embryogenesis has been reported in number of medicinal plants belonging to various families (Hassani et al., 2008; Martin, 2004; Paramageetham et al., 2004). Another aspect of tissue culture is indirect organogenesis via callus induction that has been used in some of the medicinal plants (Irvani et al., 2010; Parveen and Shahzad, 2014; Thomas and Hoshinao, 2015). To our knowledge up today, only two works on ISE (Jasrai et al., 1992; Sehgal and Abbas, 1994) and one work on DSE of ajowan have been reported (Purohit and Kothari, 2007). For indirect shoot regeneration, there is just one recent study published (Koca and Aasim, 2015). One of the major problems that usually happen in regenerated plants via callus induction is that regenerated plants exhibit somaclonal variation as polyploidization, aneuploidy, chromosome aberrations, and sequence changes (Muzamli et al., 2016). Increase the culture time and also the presence of high levels of plant growth regulators such as 2,4-D, NAA and BA in medium can severe this somaclonal variation in in vitro regenerated plants (Clarindo et al., 2008). In recent years plant tissue culture techniques provide a good situation for multiplication, conservation and propagation of several medicinal plants (Li et al., 2012; Parveen and Shahzad, 2014; Shahzad et al., 2011). Ajowan is one of the important medicinal plants that has little area under cultivation in a country like Iran that one of the major habits of this plant and also direct and indiscriminate use can putting this plant in risk on future. The aim of this study was to establish efficient protocols for indirect somatic embryogenesis and indirect shoot regeneration of three Iranian ecotypes of ajowan that supply genetic stability of regenerated plants.

2. Materials and methods

2.1. Plant materials

Three different ecotypes of ajowan (Shahedye-Yazd, Ghoom and Shiraz) from different regions of Iran were used in this study. The seeds were procured from the Research Institute of Forests and Rangelands of Iran and then for multiplication, in 2014–2015 growing season, cultivated in the educational and research farm of College of Aburahmian, University of Tehran, in isolated plots. In the end of growing season, self pollinated seeds were gathered and used for tissue culture experiments.

2.2. Sterilization of seeds and preparation of in vitro explants

Attached seeds were split to single seed and then one drop of Tween 20 was added to them and washed under running tap water. Subsequently, seeds were surface-sterilized by submerging in ethanol (70%, v/v) for 3 min followed by one time washing with sterile distilled water and submerging in sodium hypochlorite (1.5%, v/v) for 5 min and finally rinsing three times with sterile distilled water. Sterilized seeds germinated on half strength MS medium solidified with 0.38% (w/v) phytagel. For growing and preparation of in vitro explants, germinating seeds were located in phytotrons with 21 °C under a 16/8 h (light/dark) photoperiod with a PPFD of 40 μmol m−2 s−1 provided by cool white fluorescent lamps. The hypocotyls were excised from 5-, 10- and 15-d-old seedlings and used as explants.

2.3. Culture media and conditions

For both experiments full strength MS medium was supplemented with 3% (w/v) sucrose and 1 mg/L myoinositol. The medium’s pH was adjusted to 5.8 ± 0.2 with 4 mM NaOH before adding phytagel (0.38% w/v). Culture media were autoclaved at 121 °C for 20 min. All cultures were maintained at 21 °C under a 16/8 h (light/dark) photoperiod with a PPFD of 40 μmol m−2 s−1 provided by cool white fluorescent lamps with 60–65% relative humidity.

2.4. Somatic embryogenesis

For induction of somatic embryogenesis, hypocotyls segments of 5-, 10- and 15-d-old seedlings were used. The experiment was carried out as factorial using a completely randomized design with four factors and three replications (as petri dishes). Five explants segments were placed in each petri dish containing 30 mL of MS medium. Considered factors were included:

A: Age of explants (5-, 10- and 15-d-old hypocotyls)
B: Ecotype (Shahedye-Yazd, Ghoom and Shiraz)
C: 2,4-D concentration (1, 1.5 and 2 mg/L)
D: Kin concentration (0.25, 0.5 and 1 mg/L)

Subcultures were done each two weeks and after 7 weeks, part (~200 mg) of each proliferated callus was transferred to PGR-free medium for one week and same medium was also used as static culture for somatic embryogenesis. Emerged embryos were counted under a binocular microscope. Numbers of the differentiated embryos in the various stages of the development were recorded.

2.4.1. Histology of embryogenic callus explants

7-weeks old embryogenic calli were immersed in fixative solution (63% ethanol, 5% acetic acid, 2% formaldehyde) and evaporated in a desiccator for one hour to remove air bubbles. To remove fixator solution, samples were washed with three subsequent washes (63% ethanol, 5% glacial acetic acid), each for 10 min. Samples were dried on filter paper, submerged in molten 5% agarose and kept in 4 °C overnight for embedding (Athman et al., 2014). Sectioning was done with a rotary microtome. Sections were stained with 0.1% toluidine blue. Finally, observation and documentation of fixed sections on slides were done with a light microscope equipped with camera (Olympus DP70).

2.5. Indirect shoot regeneration

For callus induction, hypocotyl explants from 15-days-old seedlings of three ecotypes (Shahedye-Yazd, Ghoom and Shiraz) were cultured on sterile MS medium fortified with 0.5 mg/L 2,4-D in combination with 1 mg/L Kin. Subcultures were done each two weeks on same medium. After five weeks, parts of induced calli (~200 mg) were used in factorial experiment for shoot regeneration as follow:

A: Ecotype (Shahedye-Yazd, Ghoom and Shiraz)
B: 3MeOBA9THPP concentration (1, 1.5 and 2 mg/L)
C: NAA concentration (0.25, 0.5 and 0.75 mg/L)

After two weeks, samples were examined with a binocular microscope for percentage of regenerated plants and number of regenerated plants per callus.

2.6. In vitro rooting and acclimatization

In both experiments well-developed shoots (~2–3 cm height) were transferred to half strength MS medium supplemented with 0.1 mg/L NAA. The rooted shoots were washed in distilled water to remove all traces of agar and seedlings were planted in compost soil mixed with sand (2:1) in plastic pots and covered with transparent plastic cups. After 7–10 days, cups were partially lifted to allow the plantlets adjust fully to green house conditions with average
temperature around 22 °C. The survival rate of hardened plantlets was calculated.

2.7. Genetic stability analysis by flow cytometry

Flow cytometry was used for determination of the ploidy level of samples prepared from leaves of the regenerated plants through somatic embryogenesis and indirect shoot regeneration along with control plants in Shiraz ecotype. The 0.5 cm² of leaf tissue were chooped with a razor blade for 25 s in 500 mL of modified Galbrith’s nuclei isolation buffer (200 mM Tris, 4 mM MgCl₂, 6H₂O, pH 7.5, 0.5% Triton X-100) (Galbrith et al., 1983). Then, for DNA staining, 500 mL of staining solution 4.6-diamino-2-phenylindole was added to samples. Samples were incubated in this solution for 2 min and then nuclei were passed through a 30-μm nylon filter to eliminate cell debris. A flow cytometer (PA-I; Partec) was used for samples analysis. The genetic stability was determined by comparing nuclear DNA content of regenerated plants via somatic embryogenesis and indirect shoot regeneration with seed grown plants (control).

2.8. Statistical analysis

Statistical analyses were done using Excel 2010 and SAS Ver. 9.1 (Cary, 2004) software. In ISE, ANOVA for number of somatic embryos was conducted with age of explant (5-, 10- and 15-d-old hypocotyls), ecotype (Shahedeye-Yazdi, Ghoom and Shiraz), 2,4-D concentration (1, 1.5 and 2 mg/L) and Kin concentration (0.25, 0.5 and 1 mg/L) factors. Means comparison was performed using LSD test at 0.05 probability level after ANOVA. Utilization of specific analyses were noted in the appropriate figure and table footnotes and in the text.

3. Results and discussion

3.1. Indirect somatic embryogenesis

3.1.1. Callus induction and somatic embryogenesis

Callus induction started from the edges of explants 2 weeks after transfer of hypocotyl explants to medium supplemented with 2,4-D and Kin. Globular somatic embryos emerged in surface of induced calli after 7 weeks of culture (Fig. 1a). Subsequently, embryogenic calli were transferred to PGR-free MS medium to develop to somatic embryos. Heart-shaped and torpedo somatic embryos developed from globular somatic embryos within 15-20 days after maintenance on PGR-free medium. In next 7 days somatic embryo was grew and cotyledonary embryos with secondary leaves started to appear (Fig. 1b and c). At this point, number of cotyledonary-stage embryos was counted.

Analysis of variance for number of somatic embryos showed significant effect of investigated treatments and their interactions on number of somatic embryos (Table 1). Mean comparisons of number of somatic embryos using LSD test at 0.05 probability level showed that the maximum number of somatic embryo was related to 15-d-old explants × Ghoom ecotype × 1.5 mg/L 2,4-D × 0.5 mg/L Kin with average number of 28.33 embryo (Table 2).

The importance of explant age on DSE of ajowan has been reported previously. In that study, 3-, 10-, 15-, and 20-d-old hypocotyls were used for DSE of ajowan in liquid MS medium supplemented with different concentration of 2,4-D (Purohit and Kothari, 2007). They concluded that explant age strongly affect the number of somatic embryos and their ability to further development into plants. They reported that cotyledons from 3-d-old seedlings curled and did not form somatic embryos and the highest number of DSE was achieved with 10-d-old explants. In present study also the effect of explant age on somatic embryogenesis was obvious. With contrary to DSE in liquid culture, older explants gave better results in DSE on solid MS media. This might be due to differences in levels of endogenous PGR and activation of secondary metabolite synthesis in various ecotypes of this medicinal plant. Auxin and cytokinin play fairly important roles in many aspects of plant growth and development. This PGRs control events of major cell specification during embryogenesis (Su et al., 2011).

Jasrai et al. (1992) suggested that for induction of somatic embryogenesis on calli, gradually reducing of 2,4-D in medium is more important than momentary removing of 2,4-D. They decreased 2,4-D concentration in several subsequent subculture steps while keeping the initial concentration of Kin to induce somatic embryogenesis in ajowan. Herein to get high efficiency of somatic embryo formation, we transfer induced calli directly to PGR-free MS medium. Hence, this simplified protocol suits better Iranian ecotypes of ajowan than protocol developed earlier for Indian ecotypes (Jasrai et al., 1992). It seems that brief treatment of 2,4-D is essential for extensive somatic embryogenesis but it not crucial for further development of somatic embryos (Abdin and Ilah, 2007). Tawfik and Noga (2002) used combination of 2,4-D and Kin for ISE in cumin (Cuminum cuminum L.), medicinal plant from Apiaceae family. For formation of somatic embryos in induced calli derived from cumin hypocotyls segments, they transfer induced calli to PGR-free MS medium and report that the presence of kinetin in the callus induction medium along with 2,4-D enhanced both the callus proliferation and the later differentiation of the embryos on the PGR-free medium. In contrast to the cytokinin induced callus, utilization of higher 2,4-D concentration usually establish a long term culture of embryogenic calli that maybe used for induction and screening of somaclonal cell lines (Tawfik and Noga, 2002).

3.1.2. Histological analysis

Histological observations of embryogenic calli proved that in the induction phase of embryogenesis, proembryogenic masses of cells were formed in their surface. In cross-section of embryogenic calli, dense cytoplasm and mitotic activity in marginal cells, where formation of globular somatic embryo occurs, was evident (Fig. 1d–f).

3.2. Direct shoot regeneration

3.2.1. Callus induction and ISR

Callus induction initiated on hypocotyls segments cultivated on MS medium supplemented with 0.5 mg/L 2,4-D + 1 mg/L Kin within 15 days (Fig. 2a). Subcultures were done for next two weeks in the same medium. In sixth weeks, the calli derived from 15 days old

<table>
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<th>Source of variation</th>
<th>dfa</th>
<th>Mean squares</th>
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<tr>
<td>2,4-D</td>
<td>2</td>
<td>519.18</td>
</tr>
<tr>
<td>Kin</td>
<td>2</td>
<td>135.13</td>
</tr>
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</tr>
<tr>
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</tr>
<tr>
<td>Age of Explant × Kin</td>
<td>4</td>
<td>62.03</td>
</tr>
<tr>
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</tr>
<tr>
<td>Ecotype × Kin</td>
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</tr>
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</tr>
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<td>Ecotype × 2,4-D × Kin</td>
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<tr>
<td>Age of Explant × Ecotype × 2,4-D × Kin</td>
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<td>55.13</td>
</tr>
<tr>
<td>Coefficient of Variation (%)</td>
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<td></td>
</tr>
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2,4-D: 2,4-Dichlorophenoxyacetic acid. Kin: Kinetin.

*a degree of freedom.

*b Significant at 1% probability level.

Table 1

Analysis of variance for number of somatic embryos in ajowan.
hypocotyls were subcultured on MS medium supplemented with 3MeOBA9THPP (1, 1.5 and 2 mg/L) alone or in combination with NAA (0.25, 0.5 and 0.75 mg/L) for shoot induction. Three rounds of subcultures were done on these media and shoot regeneration from individual induced calli has been observed in 8th weeks of culture (Fig. 2b and c). Numbers of shoots initiated per explant were counted in this step. Indirect shoot regeneration was firstly visible on medium supplemented with 1.5 mg/L 3MeOBA9THPP (Fig. 2d).

Results of mean comparison for number of shoot induction by LSD test at 0.05 probability level is given in Table 3. Murashige and Skoog medium supplemented with 1.5 mg/L 3MeOBA9THPP and 0.25 mg/L NAA produced the highest frequency of shoot regeneration (18.33) in Shiraz ecotype (Table 3). Traditional approaches of plant in vitro cultivation by manipulating the relative ratio of auxin to cytokinin have been successfully used to regenerate several medicinal plants from callus tissue (Irvani et al., 2010;
and thus 3MeOBA9THPP seems to have better properties for in vitro culturing than standardly used classical cytokinins such as BAP or Kin. Recently, direct comparison of classical cytokinins TDZ and BAP with 3MeOBA9THPP was done on micropropagation experiment with South African medicinal plants Merwilla plumbea, Aloe arborescens and Harpagophyllum procumbens (Amoo et al., 2014; Amoo et al., 2015). Authors concluded that explants treated with 3MeOBA9THPP gave unambiguously highest number of adventitious shoots and have highest rooting frequency than explants treated with classical cytokinins.

Removal of NAA from shoot inducing medium was found ineffective and maintenance of low NAA concentration even during shoot regeneration was favorable for high number of regenerants and more over facilitates better rooting in later phase of in vitro culture of ajowan (Table 3). Interestingly, it is obvious that decision of cell fate in specific tissues depends on the ratio between auxin and cytokinin as well as on type of PGR used. Both factors are then important for maintenance of cell proliferation and stimulation of cell differentiation to form new organs, such as shoots or roots.

Table 3
Effect of different concentrations of 3MeOBA9THPP alone or in combination with NAA on indirect shoot regeneration of ajowan.

<table>
<thead>
<tr>
<th>Plant growth regulator (mg/L)</th>
<th>Ecotype</th>
<th>3MeOBA9THPP</th>
<th>NAA</th>
<th>Shahedye-Yazd</th>
<th>Ghoom</th>
<th>Shiraz</th>
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<tr>
<td>1.00</td>
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<tr>
<td>1.00</td>
<td>0.75</td>
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<tr>
<td>1.50</td>
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<td>13.00</td>
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</tr>
<tr>
<td>1.50</td>
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<td>4.00</td>
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<td>11.00</td>
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<td>0.25</td>
<td>3.00</td>
<td>6.66</td>
<td>12.00</td>
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<tr>
<td>2.00</td>
<td>0.50</td>
<td>2.66</td>
<td>6.00</td>
<td>11.33</td>
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<td>1.00</td>
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<td>1.50</td>
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<tr>
<td>2.00</td>
<td>2.66</td>
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<td>4.33</td>
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<td>LSD 5%</td>
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3MeOBA9THPP: 3-methoxy (-6-benzylamino-9-tetrahydropyran-2-yl) purine. NAA: Naphthalene acetic acid. LSD: Least significant difference.

Thomas and Maseena, 2006). In present study, we have found that combination of low NAA concentration (0.25 mg/L) with novel synthetic cytokinin 3MeOBA9THPP gave good results for indirect shoot induction of ajowan. Tetrahydropranyl substitution at N9 position of a cytokinin purine ring has positive effect on 3MeOBA9THPP translocation via plant tissue and hinders conversion and accumulation of non-active cytokinin N9-glycosides (Podlešáková et al., 2012). 3-methoxy substitution also protects cytokinin better from irreversible degradation (Szućová et al., 2009) and thus 3MeOBA9THPP seems to have better properties for in vitro culturing than standardly used classical cytokinins such as BAP or Kin. Recently, direct comparison of classical cytokinins TDZ and BAP with 3MeOBA9THPP was done on micropropagation experiment with South African medicinal plants Merwilla plumbea, Aloe arborescens and Harpagophyllum procumbens (Amoo et al., 2014; Amoo et al., 2015). Authors concluded that explants treated with 3MeOBA9THPP gave unambiguously highest number of adventitious shoots and have highest rooting frequency than explants treated with classical cytokinins.

Removal of NAA from shoot inducing medium was found ineffective and maintenance of low NAA concentration even during shoot regeneration was favorable for high number of regenerants and more over facilitates better rooting in later phase of in vitro culture of ajowan (Table 3). Interestingly, it is obvious that decision of cell fate in specific tissues depends on the ratio between auxin and cytokinin as well as on type of PGR used. Both factors are then important for maintenance of cell proliferation and stimulation of cell differentiation to form new organs, such as shoots or roots.

3.3. Rooting and hardening

Regenerated plantlets from ISE were separated before being transferred to rooting medium (Fig. 3a). In ISR, after removal of the remaining callus, regenerated plantlets were transferred to rooting medium (Fig. 3b). Nevertheless, regenerated shoots from both ISR and ISE experiments massively proliferate roots when transferred
to half-strength MS medium supplemented with 0.1 mg/L NAA. Fully developed root system was observed within 2 weeks. Plantlets having well-developed roots (≈3 cm) were used for hardening and transfer to pot. Pots were covered with transparent plastic cups for 7–10 days (Fig. 3c) and then cups were lifted to allow the plantlets adjust fully to green house conditions (Fig. 3d). The survival rate of rooted plantlets was 60.86% and 58.33% for ISE and ISR derived plants, respectively.

3.4. Genetic stability by flow cytometry

Ploidy stability of regenerated plants from both ISE and ISR was determined by chromosome counting and flow cytometry. Flow cytometry histogram revealed that the ploidy level of plants regenerated via ISE was the same as that of control plants produced from mature seed culture (Fig. 4a and b). The ploidy level of plants regenerated through ISR was also the same as that of control plants (Fig. 4a and c). The 2C DNA content of seed-derived plants of Shiraz ecotype was estimated to 21.47 pg. Also the 2C DNA content of regenerated plants obtained from ISE and ISR were 31.72 and 30 pg, respectively (Table 4), that confirm similarity in genome size of regenerated plant via ISE and ISR with control plant. Slightly high coefficient of variation for FCM (Table 4) revealed it that ajowan is one of the difficult species that precision measurement of its DNA content (CVs below 5%) is not easily attainable and replicate measurements can helps to its reproducibility DNA content estimation (Dloezel and Bartos, 2005). One of the major factors that responsible for somaclonal variation in tissue culture studies is the synthetic auxin 2,4-D, especially when this PGR used for callus formation (Stanišić et al., 2015). Synthetic auxin 2,4-D can lead to DNA synthesis and endoreduplication through stimulation of unorganized cell growth and disturbing cell cycle (Neelakandan and Wang, 2012). It is obvious that in vitro regeneration of ajowan plants via ISE and ISR did not induce quantitative changes in DNA content, and genome size stability of regenerated plants was assured via ISE and ISR protocols. To the best of our knowledge this is the first report on the genetic stability of in vitro regenerated plants trough ISE and ISR reported in ajowan medicinal plant.

4. Conclusion

From the results of the ISE, it is obvious that higher level of auxin than cytokinin was lead to promote somatic embryogenesis in induced calli of ajowan. Contrariwise, higher level of cytokinin to auxin was lead to indirect regeneration from induced calli in ISR. Herein it showed that appropriate formation of somatic embryos from induced calli can be achieved by transfer of induced calli to medium devoid of 2,4-D and Kin, that it can significantly reduce the time of the culture and thus reduce the risk of somaclonal variation. Using of synthetic cytokinin 3MeOBA9THPP for the first time gave good results for indirect shoot induction of ajowan. Hypocotyls segments with 15-d old age were significantly better

Table 4
2C DNA content obtained from seed regenerated plants, indirect somatic embryogenesis and indirect shoot regenerated plants in ajowan.

<table>
<thead>
<tr>
<th>Source of cells</th>
<th>DNA content (pg*)</th>
<th>Coefficient of variation (%)</th>
</tr>
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<tbody>
<tr>
<td>Seed obtained plant (control)</td>
<td>21.47</td>
<td>8.83</td>
</tr>
<tr>
<td>Indirect somatic embryogenesis</td>
<td>31.72</td>
<td>8.67</td>
</tr>
<tr>
<td>Indirect shoot regeneration</td>
<td>30.00</td>
<td>7.50</td>
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* pictograms.
than younger hypocotyls for ISE, that this might be due to differences in endogenous PCR levels and/or due to the activation of secondary metabolite synthesis. Ghoom and Shiraz ecotypes were more powerful in vitro regeneration than Shahedye-Yazd ecotype. Although synthetic auxin 2,4-D was used for callus induction in both experiment, but flow cytometry analysis prove ploidy stability of regenerated plants via ISE and ISR protocols and regenerated plant were similar to seed grown plants.

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References


Fig. 4. Flow cytometry analysis and chromosome counts of ajowan. (a) Histograms of relative 2C DNA content of nuclei isolated from seed germinated plants. (b) Flow cytometry profiles of plants regenerated by somatic embryogenesis. (c) Flow cytometry profiles of plants regenerated via indirect shoot regeneration.


