Polyploidy induction in Iranian Borage (Echium amoenum L.) by colchicine treatment

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Abstract. Iranian borage (Echium amoenum L.) is medicinal plant that has anti-bacterial properties of its metabolite. This study aimed to investigate the effect of colchicine to induce polyploidy in borage, different levels of colchicine (0, 0.25, 0.5, 0.75 and 1% w/v) was used in a different time period (24, 36 and 48h) in a completely randomized design with 3 repeats. Cytogenetic results showed that chromosome number changed on 0.75 and 1% Colchicine concentration levels and 24, 36 and 48h time period. These results were also confirmed by amplification patterns of ISSR markers. Some examples of this plant cells was observed different aneuploidy. Results indicate that treating ex vitro-seed and Shoot Apical Meristem of borage with colchicine treatment can produce stable tetraploid plants. Variation in the morphological characteristic of stomatal guard cell length was found among the diploid and tetraploid plants under the same growing condition. The polyploids generated in these and other studies can be used in breeding to produce superior varieties of plants in diverse genera.

Key words: Iranian Borage, Colchicine, Polyploidy, ISSR markers.

Introduction

Iranian borage (Echium amoenum L.) is biennial plants that belong to Boraginaceae family, and has many uses in Iranian ethnic medicine (Nooriyan Soroor et al. 2013). Echium amoenum is a herb indigenous to the narrow zone of northern part of Iran and Caucasus, where it grows at an altitude ranging from 60 to 2200m (Heidari et al. 2006). Petals of Echium E. amoenum have been advocated for variety of effects such as demulcent, anti-inflammatory and analgesic, especially for common cold, anxiolytic and sedative in folk medicine of Iran (Shafagh et al. 2002).

The development of polyploid (chromosome doubling) induction protocols offers enormous potential for further improvement in the family, naturally-occurring polyploidy is a phenomenon that has provided an important pathway for evolution and speciation in plants (Hannweg et al. 2013). Polyploids have played an important role in higher plant evolution, as most flowering plants are tetraploids (Rubulza et al. 2007). Polyploidy has been used in horticulture as a breeding tool to enhance ornamental characteristics such as plant size, leaf thickness, increased width-to-length ratio of leaves and flower size (Shao et al. 2003). Polyploids exhibiting valuable new phenotypic traits can occupy new niches and become important agriculturally and horticulturally (Glowacka et al. 2010). Colchicines treatment causes higher morphological and molecular mutation more than other mutagenic substances. This study is aimed at developing an effective polyploidisation system in the Borage using colchicine treatment of ex vitro and was determined the effect of colchicines different concentrations as an antimitotic agent on Iranian borage leaves by karyotype and ISSR markers.

Material and Methods

Plant material

Iranian borage seeds were obtained from Mashhad Research Institute, Iran. After spending 3 months in cold temperature at - 5 °C to break dormancy, seeds were sterilized and cultivated in tray and then the germinated seeds were transferred to Hoagland solution. The experiment had a factorial arrangement with two factors (colchicine concentration and time duration) in a completely randomized design with 3 repeats. Over two weeks and 5-6 leaves of plants were transferred to pots containing soil and perlite. After a week of being compatible with the roots, the plant apex to induce Polyploidy influenced by different concentrations of 0, 0.25, 0.5, 0.75 and 1% (w/v) colchicines at intervals of 6, 12, 24, 36 and 48 h. After almost a month and make new leaves, the plants were sampled to determine polyploidy by karyotyping and ISSR markers.

Preparing karyotype

The seeds that had 0.5 to 1.0 cm long roots were submitted to pretreatments: 0.0029 M 8-hydroxyquinoline (8-HQ) for 2 to 5 h, at room temperature. Next, roots were washed in distilled water for 5 min. The roots tips were then fixed in Carnoy solution (three parts ethyl alcohol: one part acetic acid) for 12 h at room temperature. The material was hydrolyzed in 1 N HCl at 60°C for 8 min, and then stained with the Schiff reagent for 90 min. The material was then squashed on slides containing a drop of 1% acetic carmin. Echium E. amoenum cells in mitotic metaphases were analyzed to establish the chromosome number.

DNA extraction and ISSR amplification

Genomic DNA was isolated from young leaves using the method of Doyle and Doyle (1990). Purified total DNA was quantified using 0.8% agarose gel. Three ISSR primers, as shown in Table 1, were selected. Individual ISSR PCRs were carried out in a 20 mL reaction volume containing 20 ng DNA, 2.5 mM MgCl2, 0.4 mM primers, 300 mM dNTPs, 0.5 U Taq DNA polymerase, and 2 mL of 10 x PCR reaction buffer (Mg2+ free). All amplifications were performed using a TC-512 PCR thermocycler with initial denaturing at 94 °C for 5 min, followed by 40 cycles of 94 °C for 30 s, then specific temperature for particular primer (Table 1) for 70 sec, and 72°C for 3 min, followed by a final extension at 72°C for 10 min. PCR products were resolved on 1.5% agarose gel in 1xTAE buffer. The DNA was stained with 0.5 mg/mL ethidium bromide, visualized and photographed under a UV transilluminator. A sample without template DNA was included as a negative control in each experiment to check contamination. Electrophoretic profile was visualized under UV radiation and photographed with a UV transilluminator. The sizes of DNA fragments

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer Sequence</th>
<th>Annealing Temperature (°C)</th>
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</thead>
<tbody>
<tr>
<td>ISSR-8</td>
<td>CTCCTCTCCTCCTCTCTGT</td>
<td>54</td>
</tr>
<tr>
<td>ISSR-11</td>
<td>GAGAGAGAGAGAGAGAC</td>
<td>52</td>
</tr>
<tr>
<td>ISSR-22</td>
<td>TCTCCTCCTCCTCCTCCC</td>
<td>52</td>
</tr>
</tbody>
</table>
were estimated by comparison with standard ladder (1kb; fermentase, Germany).

**Results and Discussion**

The result of polyploid induction by colchicine treatment showed that in karyotyping technique produced tetraploid (2n=4x=32) using two levels of 0.75 and 1% (w/v) and period of 24, 36 and 48 hours (Fig. 1b) and other treatments did not produce tetraploid plants (Fig. 1a). 0.75 and 1% Colchicines concentration levels and 48h the highest rate tetraploid and aneuploidy plants (Figs 2 and 3). Colchicine treatment in concentrations of 1% on 48 hours, was produced the highest rate tetraploid plants (Fig. 3). Colchicine concentrations for 48 h at concentration 0.75% and 1% (w/v) resulted in 30% and 40% tetraploids respectively while the 24 h treatment was less effective (Figs 2 and 3). The concentration of colchicine, and time duration, influenced the level of polyploidisation (Rubuluza et al. 2007, Shao et al. 2003). To develop an effective polyploidisation system, the effects of colchicine concentration and exposure time on genotypes that differed in species and ploidy were examined (Glowacka et al. 2010). High concentrations of colchicine in some plants did not survive. Blakesley et al. (2002) and Rubuluza et al. (2007) also reported the negative effect of high colchicine concentrations.

Each of the diploid and tetraploid plants showed a strictly defined range of stomatal guard cell length variability (Fig. 4). The stomatal guard cell length alone cannot be an absolute criterion for polyploidy identification of *Echium amoenum* L. In previous research (Glowacka et al. 2010, Borrino & Powell 1988) the effect of polyploidy on stomatal guard cell length increase was reported. These results indicate that stomata length measurements can be useful not only for diploid and polyploid but also for species with different ploidy level if they are very closely related to each other (Joachimiak & Grabowska-Joachimiak 2000).

The DNA profiles for ISSR markers are presented in Figure 5. In all, 3 primers showed 100% polymorphism. The concentration of colchicine, and time duration, influenced the level of polyploidisation (Rubuluza et al. 2007, Shao et al. 2003). To develop an effective polyploidisation system, the effects of colchicine concentration and exposure time on genotypes that differed in species and ploidy were examined (Glowacka et al. 2010). High concentrations of colchicine in some plants did not survive. Blakesley et al. (2002) and Rubuluza et al. (2007) also reported the negative effect of high colchicine concentrations.
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A.

B.

Figure 5. PCR amplified with ISSR primers, (a) ISSR-18 (b) ISSR-22. The numbers in the figure correspond to the numbers listed in Table 2.

amount of total polymorphic bands generated by ISSR markers in high concentrations of colchicine (0.75% and 1% w/v) was more than that generated using low concentrations of colchicine (Fig. 5). The introduction of molecular markers in plant breeding has presented a valuable tool for the characterization of genetic materials (Heidari et al. 2013). ISSR-22 marker was showed more polymorphism than ISSR-18 marker.

Artificial polyploidy generally enhances the vigour of determinate plant parts and may be favourable where vegetative organs and biomass constitute the economic product (Dhawan & Lavanía 1997). In our study, we were able to enhance the polyploidisation rate (20%) by testing medium colchicine concentrations with long times and high colchicine concentrations with short times. Giving suitable examples, it is shown that genomic multiplication can confer enhanced production and/or qualitative improvement in the biochemical profile of secondary metabolites. A plea is made to utilise the induced polyploidy approach as a rapid means to attain enhanced production of secondary metabolites: pharmaceuticals, aroma chemicals, etc.

Reference


