



Research Article

Evaluation of polyploidy induced in terms of chlorophyll content and catalase activity in Borage (*Echium amoenum* Glowacka)

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ABSTRACT

In this work, the effect of polyploidy was evaluated through catalase activity, chlorophyll a, chlorophyll b, carotenoid and carbohydrate. This study was used in different levels of colchicine (0, 0.25, 0.5, 0.75 and 1% w/v) in a different time period (24, 36 and 48h) in a completely randomized design with 3 replications. Cytogenetic results showed that chromosome number changed in 0.75 and 1% Colchicine concentration levels and 24, 36 and 48h time period. Analyze of variance showed that the effect of colchicine levels, time duration and interaction for all traits, except the effect of time duration for carotenoid were significant ($P < 0.01$). Results showed that the highest catalase, chlorophyll a, chlorophyll b and carotenoid were attained from 0.25% colchicine with 24 and 36 h time duration conditions. Therefore, colchicine polyploidy induction in current and other studies can be used in plant breeding to produce superior varieties of plants.

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Introduction

Echium amoenum is an herb indigenous to the narrow zone of the northern part of Iran and Caucasus, where it grows at an altitude ranging from 60 to 2200m (Heidari et al. 2006). Petals of *Echium amoenum* have been advocated for a variety of effects such as demulcent, anti-inflammatory and analgesic, especially for common cold, anxiolytic and sedative in folk medicine of Iran (Shafaghi 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 (Rubuluza 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). Determination of karyotype has been great value for the

understanding of the evolutionary Polyploidy content (Heidari et al. 2013).

Chlorophyll loss is associated with environmental stress and the variation in total chlorophyll/carotenoids ratio may be a good indicator of stress in plants (Hendry and Price 1993). In addition, measuring gas exchange and chlorophyll content repeatedly on the same leaves in the field may provide useful information on the relationship between these parameters (Tabatabaei 2013). The ratio between chlorophyll and carotenoids has been much less widely used diagnostically, although this ratio is said to be a sensitive marker distinguishing natural full-term senescence and senescence due to environmental stresses (Mohammed et al. 1995).

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 antimetabolic agent on catalase activity, chlorophyll a, chlorophyll b, carbohydrate and protein of Iranian borage leaves. an 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 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 (Shafaghi 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 (Rubuluzza 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). Determination of karyotype has been great value for the understanding of the evolutionary Polyploidy content (Heidari et al. 2013).

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Materials 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 the tray and then the germinated seeds were transferred to Hoagland solution.

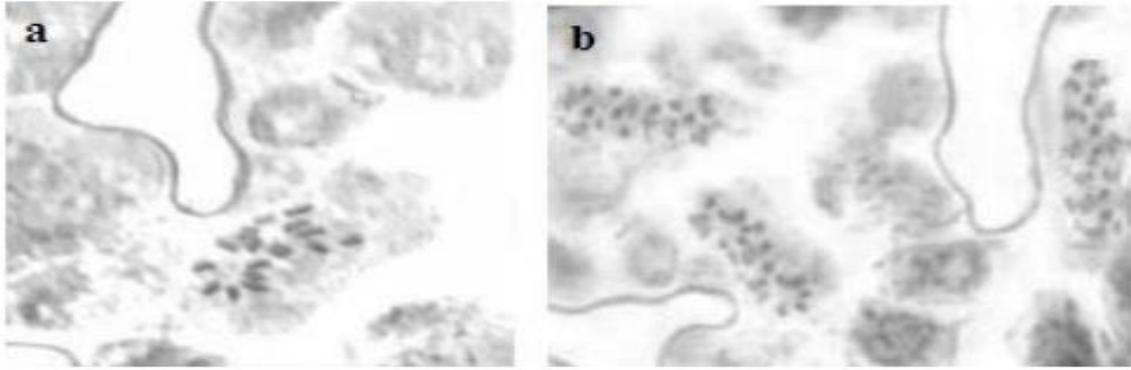


Figure 1. polyploid induction in Iranian borage, A: diploid ($2n=2x=16$), B: triploid $2n=4x=32$)

The experiment had a factorial arrangement with two factors (colchicine concentration and time duration) in a completely randomized design with 3 replications. After two weeks and 5-6 leaves of the 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.

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 (Heidari et al, 2013). 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. *Melissa officinalis* cells in mitotic metaphases were analyzed to establish the chromosome number.

Chlorophyll

To determine the chlorophyll *a*, *b* and *carotenoideid* concentration, 100 mL of the sample was filtered and immersed in acetone (90% V/V) for 20 h in darkness and cold. The absorbance of the solvent was determined at 630, 645 and 665 nm in a spectrophotometer (Cecil CE 2000 model 2041). The chlorophyll concentration was calculated through the Richards and Thompson (1952).

Catalase activity (CAT)

The sample (2 ml) was concentrated by centrifugation at 3500 rpm for 15 min. A blank (2.9 ml sodium phosphate buffer pH 7.0 and 100 ml of supernatant) was used. The results are expressed as U/106 cells (Li et al. 2006). The enzymatic activity of catalase was determined; by spectrophotometry following the hydrogen peroxide degradation rates for 1 min every 15 sec, at 240 nm which is accelerated in the presence of enzyme (Cargnelutti et al. 2006; Hidalgo et al. 2006). The results were analyzed through the analysis of variance with the SAS 9 software for each treatment three replicates were considered.

Results and Discussion

The result of polyploid induced by colchicine treatment showed that in karyotyping technique produced tetraploid ($2n=4x=32$) using two levels of 0.75 and 1% (w/v) and a 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. Colchicine treatment in concentrations of 1% on 48 hours, was produced the highest rate tetraploid plants. Colchicine treatments 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. The concentration of colchicine, and time duration, influenced the level of polyploidisation (Rubuluza et al. 2007; Shao et al. 2003).

Table 1. Analysis of variance of studied traits

SOV	MS				
	catalase	Chlorophyll a	Chlorophyll b	cartnoeid	carbohydrat
Time duration	0.016**	4.13**	1.81**	0.036ns	41.98**
Concentration	0.052**	15.72**	1.84**	0.52**	4.23**
Time* Concentration	0.0088**	7.23**	0.71**	0.24**	3.73**
Error	0.0008	0.66	0.099	0.055	0.46

** And ns respectively significant at the 0.1 level and non-significant

Table 2. comparative of means for studied traits

Time (h)	Concentratio n%	catalase	Chlorophyll a	Chlorophyll b	cartnoeid	carbohydrat
6	0	0.142 h	4.292 abcde	1.163 defgh	1.059 abcd	4.586def
6	0.25	0.323 abc	4.107 bcde	1.478 cdefgh	1.446 abcd	2.999 fgh
6	0.5	0.340 ab	2.409 def	1.011 efgh	1.127 abcd	3.986 ef
6	0.75	0.146 gh	2.187 def	0.792 gh	1.165 abcd	1.764 h
6	1	0.142 h	1.783 ef	0.809 gh	0.996 bcd	1.811 gh
12	0	0.142 h	4.292 abcde	1.163 defgh	1.059 abcd	4.586 def
12	0.25	0.203 efgh	6.715 a	1.880 abcdef	1.679 ab	3.293 fgh
12	0.5	0.259 bcdef	1.120 f	0.621 h	0.831 d	3.563 fgh
12	0.75	0.192 efgh	4.654 abcd	1.664 bcdefg	1.360 abcd	4.516 def
12	1	0.183 fgh	1.783 ef	0.809 gh	1.278 abcd	3.916 efg
24	0	0.142 h	4.292 abcde	1.163 defgh	1.059 abcd	4.586 def
24	0.25	0.322 abc	3.440 cdef	1.881 abcdef	1.772 a	7.138 ab
24	0.5	0.211 defgh	1.216 ab	0.920 fgh	0.847 d	8.443 a
24	0.75	0.283 abcde	6.070 f	2.341 abc	1.772 a	8.079 ab
24	1	0.196 efgh	2.491 def	1.007 efgh	0.983 bcd	6.515 abcd
36	0	0.142 h	4.292 abcde	1.163 defgh	1.059 abcd	4.586 def
36	0.25	0.258 bcdef	5.738 abc	2.667 a	1.163 abcd	6.738 abc
36	0.5	0.239 cdefg	1.815 ef	1.250 defgh	0.871 cd	6.279 bcd
36	0.75	0.329 abc	4.707 abcd	2.029 abcd	1.335 abcd	6.032 bcde
36	1	0.188 fgh	4.274 abcde	2.000 abcde	1.513 abcd	4.857 cdef
48	0	0.142 h	4.292 abcde	1.163 defgh	1.059 abcd	4.586 def
48	0.25	0.356 a	5.212 abc	2.270 abc	1.455 abcd	7.103 ab
48	0.5	0.297 abcd	5.847 abc	2.536 ab	1.611 abc	7.338 ab
48	0.75	0.310 abc	1.386 f	1.142 defgh	0.816 d	6.421 abcd
48	1	0.326 abc	4.471 abcd	2.008 abcd	1.097 abcd	7.008 ab

Means that do not share a letter are significantly different.

Analyze of variance showed that the effect of colchicines levels, time duration and interaction for all traits except the effect of time duration for

cartnoeid were significant ($P < 0.01$) (Table 1). The result of comparing means for interaction between time duration and colchicine

concentration, are presented in Table 2. Results showed that the highest catalase, chlorophyll a, chlorophyll b and carotenoid were attained from 0.25% colchicine with 24 and 36 h time duration conditions (Table 2). Chlorophyll loss is associated with environmental stress and the variation in total chlorophyll/carotenoids ratio may be a good indicator of stress in plants (Hendry and Price 1993). In addition, measuring gas exchange and chlorophyll content repeatedly on the same leaves in the field may provide useful information on the relationship between these parameters (Schaper and Chacko 1991). Interaction between 0.5% colchicines and 24h were showing the highest carbohydrate (Table 2). The result showed that significant increase in catalase accumulation in high levels of colchicines. When a plant faces harsh conditions,

ROS production will overcome scavenging systems and oxidative stress will burst. In these conditions, ROS attack vital biomolecules and disturb the cell metabolism and ultimately the cell causes its own death (Tabatabaei 2013).

Antioxidant enzymes, including catalase, form the first line of defense against free radicals; therefore their regulation depends mainly upon the oxidant status of the cell. Artificial polyploidy generally enhances the vigour of determinate plant parts and may be favourable where vegetative organs and biomass constitute the economic product. In our study, we were able to enhance the catalase, Chlorophyll a, Chlorophyll b and carotenoid rate by testing medium colchicine concentrations with long times and high colchicine concentrations with short times.

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