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EFFICIENT PRODUCTION OF TETRAPLOID BARLEY (*Hordeum vulgare* L.) BY COLCHICINE TREATMENT OF DIPLOID BARLEY

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KEYWORDS

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Ttetraploid

ABSTRACT

An experiment was conducted to induce tetraploidy in three diploid barley varieties (Martin, Rihane and Manel) through different colchicines treatments. Colchicine was added for three different concentrations at three different stages of plant development i.e. on seed (0.05% for 48 hours), on pre-germinated seeds (0.1% for 2 hours) and on three leaf stage (0.1% for 16 hours). Colchicine application reduced significantly germination percentage and viability of plants. Seed germination was completely inhibited in Martin, while a reduction of 20% and 30% for germination percentage compared to control was recorded in varieties Manel and Rihane, respectively at 0.1% colchicine concentration. Ploidy evaluation showed no tetraploidy in all the three tested varieties by colchicine application of 0.05% for 48 hours on seeds and 0.1% for 2 hours on pre-germinated seeds. However, tetraploid plants were produced only by treatment with 0.1% for 16 hours of seedlings. The percentages of plants were 40%, 44% and 100% for Rihane, Manel and Martin, respectively. Cytological analyses showed the increase of chromosome numbers from 2n=2x=14 to 2n=4x=28. The increase of ploidy levels caused major changes in some morphological traits. In fact, the induced tetraploids in barley was accompanied by significant ($P<0.01$) decrease in plant height, tiller height, leaf number and leaf length compared to diploid control plants. colchicine treatment induce successfully the production of tetraploid barley plants and could be used in breeding programs.

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1 Introduction

Polyploidy is widely acknowledged as a major mechanism of adaptation and speciation in plants (Osborn et al. 2003). In cereals, chromosome doubling is a critical step in producing polyploïds. Colchicine is the most frequently used chemical to produce autotetraploids in economically important crop species (Reinbergs and Shebeski 1958). The drug inhibits the formation of microtubules by binding to tubulin, the protein subunit of microtubules. With the inactivation of spindle, which is formed by microtubules, the polar migration of chromosome is inhibited producing 'restitution' nuclei, thus resulting in a cell with a doubled chromosome number (Wan et al. 1991). The colchicine concentrations usually applied ranged from 0.1% to 0.8% (Adaniya et al. 2001). The higher doses (0.5-0.8%) often produce malformed seedling and decrease the number of tetraploid plants obtained (Pirkoohi et al. 2011).

There are several methods for polyploidy induction by colchicines treatments in plants such as seed (Hanzelka and kobza 2001; Quan et al. 2004), flower bud (Wu et al. 2007), apical meristem (Lavania and Srivastava 1991; Saharkhiz 2007) and root treatments (Taira et al. 1991). Amongst all tested methods, application of chochicine at the time of tissue culture was reported most efficient methods for polyploidy induction (Roy et al. 2001).

The improvement in crop variety by artificially induced autotetraploids was successfully reported from sugar beet, fodder beet, ryegrass, red clover and *Anthurium andraeanum* (Adaniya and Shira 2001; Chen et al. 2011), with this several varieties of tetraploid rye are in commercial production. By contrast, all attempts to improve the fertility of tetraploid barley sufficiently to warrant commercial production have failed. Several authors reported that fertility in tetraploid barley varies with the varieties and environment (Mrintzing 1948; Oto 1949).

Mrinntzing (1948) expressed the belief that the gap in fertility between diploids and tetraploids could be decreased. In the most plants, artificial polyploidy is often accompanied by increased cell size, leading to larger reproductive and vegetative organs (Adaniya and Shira 2001). The purpose of this study was to induce tetraploidy in three diploid barley varieties by colchicine treatments.

2 Materials and Methods

2.1 Plant materials

The experiment was conducted in the Laboratory of Genetics and Plant Breeding of Tunisia. Three varieties (Martin, Rihane and Manel) of six-rowed diploid (2n=14) barley (*Hordeum vulgare* L.) were used in this study.

2.2 Seeds treatment

Ten seeds of each barley variety were initially sterilized with 12% sodium hypochlorite solution for 10 min and washed 3 times with sterilized water. Seeds were then transferred to Petri dishes (10 seeds per Petri dish with three replications) containing two filters paper. Petri dishes were placed in a germination chamber in the dark condition at 25 ± 2°C. In this study, two colchicine levels and two treatment durations were used respectively on seeds and pre-germinated seeds and compared with untreated control: i) 2 hours in colchicine solution at 0.1% concentration, ii) 48 hours in colchicine solution at 0.05% concentration. Germination counts were made daily and were considered to have germinated when the radical emerged.

Table 1Variance analysis of viability of plants for three barley varieties.

Variance sources	df	Viability of plants
variety	2	4.38^{ns}
Treatment	1	193.38^{**}
variety x treatment	2	3.72^{ns}

ns-Non significant at the 0.05 probability level and **- Significant at the 0.01 probability level.

Table 2 Percentage of viable plants after cochicine treatment (0.1% for 16 hours).

	Martin		Manel		Rihane	
	Control	Treated	Control	Treated	Control	treated
Number of viable plants	9	2	9	9	7	5
Percentage of viable plants (%)	90	6.6	90	30	70	16.6

2.3 Seedling treatment

Seeds of each variety were sown in mixture of sand and peat (1:2) in small pot (13 cm of diameter) in a growth room at 30±2°C day/night temperature and at 16 h photoperiod. When the plants were at 4-6 leaf stage, plant carefully pulled out and then washed the roots of plants by running water, 2-3 cm of root tips was immersed in colchicine solutions (0.1%) for 16 h. The treated seedling were rinsed overnight in running tap water and then placed in pots of soil at a density of 10 plants/ pot (three replications) for growth to maturity.

2.4 Data collection

Two months after treatment, the treated seedlings were examined morphologically and also screened for any ploidy changes. Data were recorded on percentage of fertile plants, length of leaf, plant height, height of tillers and number of leaf.

2.5 Ploidy evaluation

The ploidy level was estimated by chromosome counting of root tips from colchicine treated plants. Average two root tips from each seedling were cut to a length of 1 cm, kept in distilled water at 0°C for 24 hours, fixed in 3:1 absolute ethanol: glacial acetic acid, then hydrolysed in 1 HCl for 12 min. Then about 1 mm the root of tips were cut and transferred a drop of 1% acetocarmine acetic acid on a slide for 3-5 min and squashed in beneath a cover slip. The preparations were observed with an optical microscope at a magnification 1000X.

2.6 Statistic analysis

The obtained data were statistically analysed using one way ANOVA and subsequent comparison of means was performed using the LSD at 1% and 5% probability. Statistical analysis was carried out using computer software (SPSS for Windows, 18.0).

3 Results and Discussion

3.1 Effect of colchicine application on seed germination

Germination percentage of treated seeds of different varieties and untreated control were demonstrated in figures 1, 2 and 3. A significant difference was noted between control and treated cultivar (Figure 4). Rihane showed 100% germination after 3 days for control. However, after 5 days, the percentage of germination for the parents Martin and Manel were 20% and 50%, respectively.

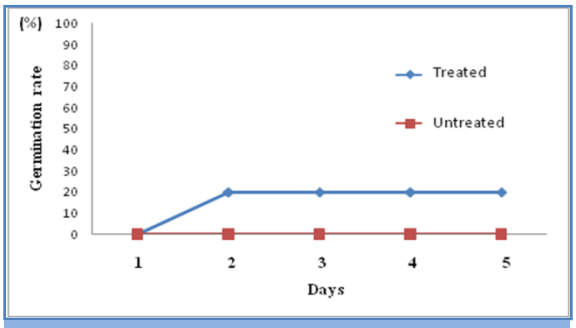

Figure 1 Evalution of seed germination of Martin cultivar with and without colchicine treatment.

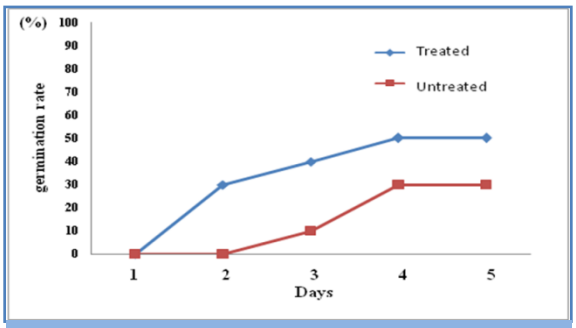

Figure 2 Evalution of seed germination of Manel cultivar with and without colchicine treatment.

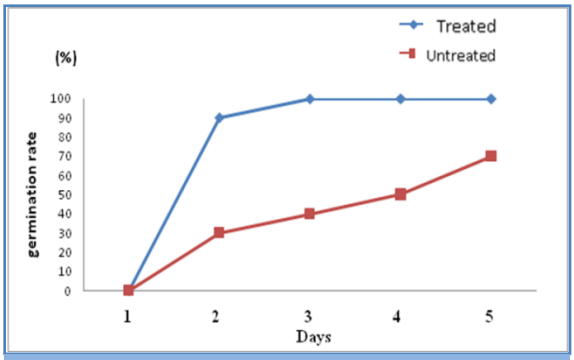

Figure 3 Evalution of seed germination of Rihane cultivar with and without colchicine treatment.

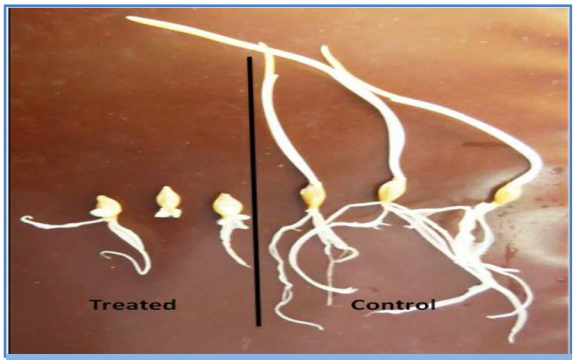

Figure 4 Effect of colchicine treatment on germination after 5 days.

Figure 5 deformed seed after colchicine treatment.

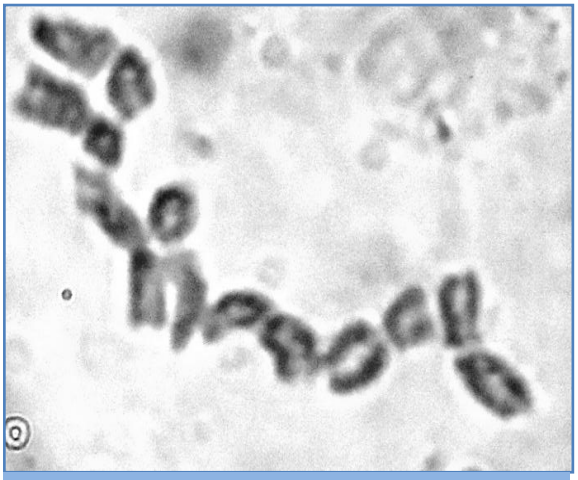

Figure 6 Ploidy level of plant obtained from deformed seed with 7 chromosomes

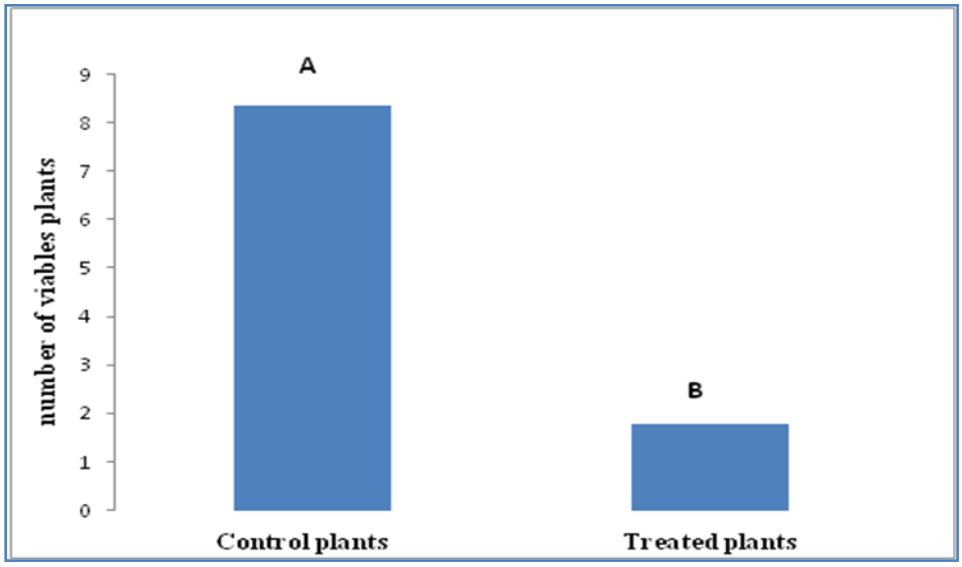

Figure 7 Effect of colchicine treatment on number of viable plants.

Colchicine application reduced germination percentage significantly. In fact, Manel and Rihane showed a reduction of 20% and 30%, respectively, compared to control (Figure 2 and 3). For Martin, colchicine application completely inhibited the seed germination (Figure 1). Similar results were obtained by Lépengué et al. (2012) which showed a reduction of germination percentage about 30.55% and 43.42% for *Zea mays* and *Hibiscus Sabdariffa*, respectively. Similar type of finding was obtained by Hassein et *al*. (2001) and Bakry et *al*. (2007) on *Musa acuminata* and *Vicia narbonensis respectively*. Cytological observations of plants, obtained from colchicines treated seeds, showed that no plants were tetraploid.

After colchicine treatment, a seed deformation was observed particularly on variety Manel (Figure 5). However, Rihane and Martin showed no deformation of seeds. The deformation observed in germination comprise an enlargement of radicle and swelling of the embryo and endosperm. This deformation resulted in a clear aberration in chromosome number of seeds deformed and haploid cell (n = 7) was identified (Figure 6).

3.2 Effect of colchicine application on pregerminated seeds

Pregerminated seeds were treated by colchicine solution (0.05%) during 48 hours continues their normal development and no anomalies were shown compared to control. Ploidy evaluation showed that no tetraploid plant was obtained for the three tested cultivars (Martin, Rihane and Manel). In this aspect the observation of present study are contradictory with, Smith (1995) those who observed 20% of tetraploid plant for four barely genotypes (Awnless, Herta, 40 Day and Wong) comparatively to control.

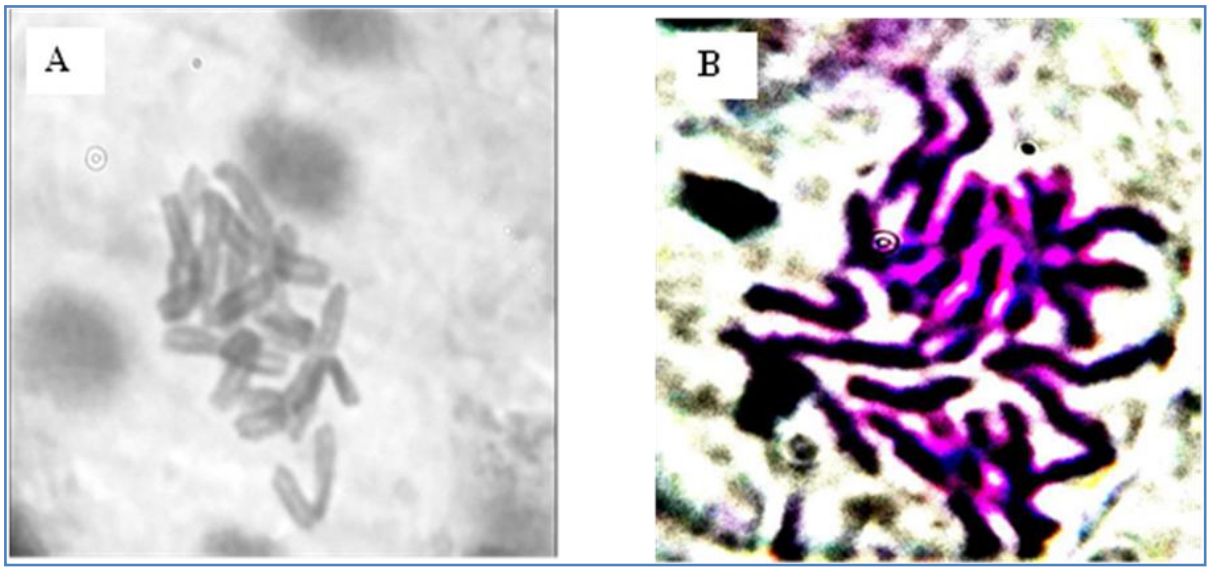

Figure 8 Mitotic metaphase showing diploid barley (A) and tetraploid barley (B).

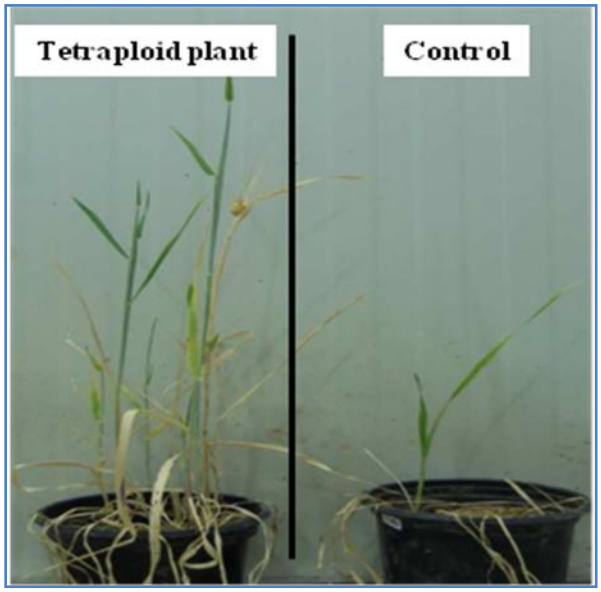

Figure 9 comparison of plant development of tetraploid plant and control diploid plant.

3.3 Effect of colchicine application on seedling

LSD analysis showed a significant reduction of plants viability after colchicine treatment (0.1% for 16 h) for the three tested cultivars (Figure 7). A significant difference was observed between all genotypes (Table 1).Amongst all tested genotype, Manel was showing the most highest percentage of viable (Table 2).

Chromosome counts were made when the plant was in the seedling stage (2-3 leaf) after colchicine treatment. The result of the cytological studies showed that the percentages of tetraploid plant were 100%, 44% and 40% for Martin, Manel and Rihane, respectively. Results showed that all the plants had the normal chromosome number (2n=4x=28). In fact, twenty eight and fourteen chromosomes, were found in every cell examined for teraploid and diploid plant, respectively (Figure 8).

Tetraploids seedlings induced by colchicines treatment were visibly detected, being shorter and having broader stems than diploid (control) seedling grown at the same time (figure 9). Tetraploid plants showed reduced growth and can be easily characterized by plant height , tillers height,leaves number and leaves length than control plants (Figure 10). Similar type of observation was reported Nigel et al., 2007 on *Lavandula angustifolia*).Plants with increased ploidy levels are sometimes apparent by their distinct morphology. Increasing ploidy often results in increased cell size that in turn results in thicker, broader leaves and larger flowers and fruit.

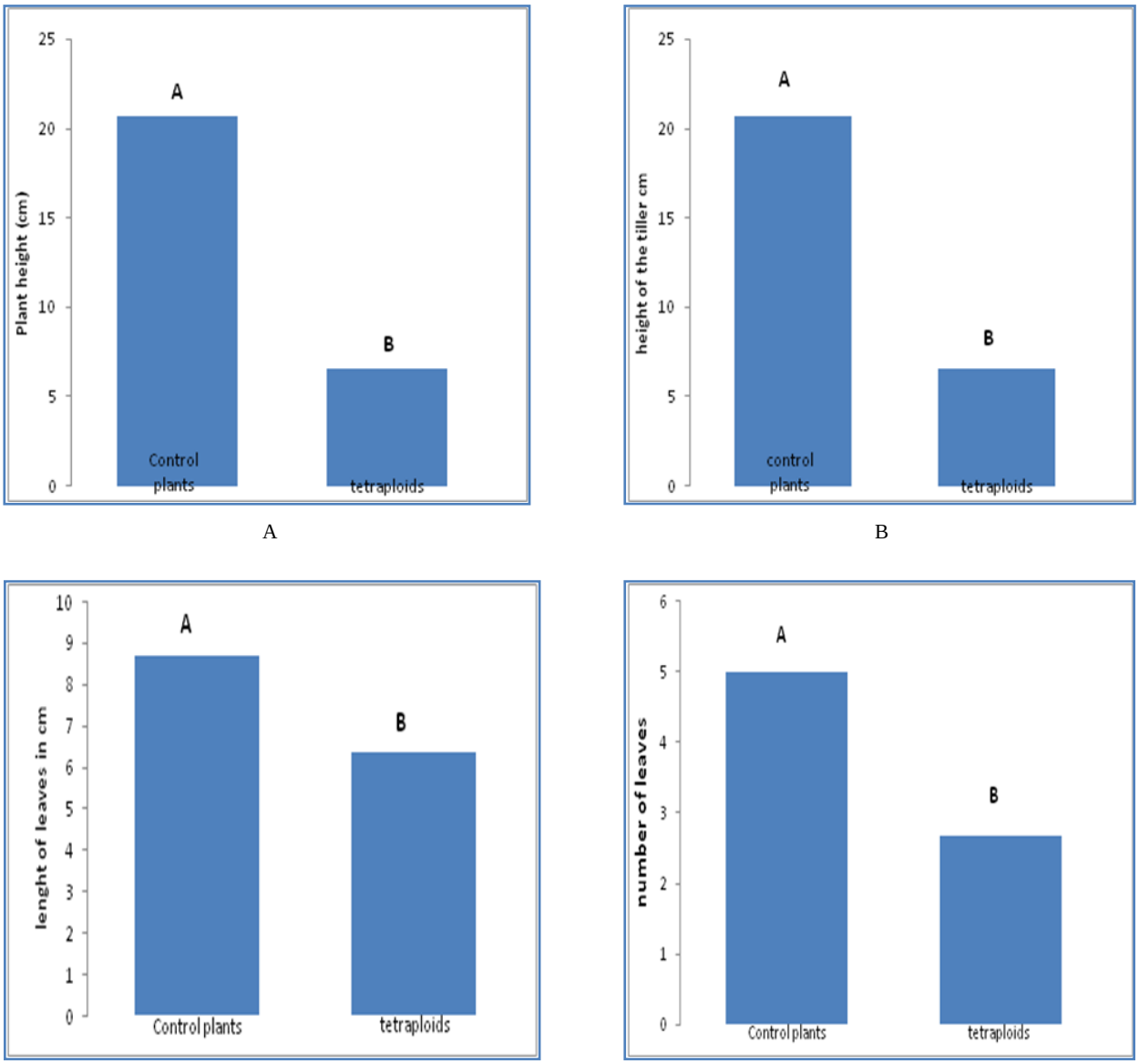

Figure 10 Comparison of plant height (a), height of tillers (b), length of leaves (c) and number of leaves (d) of the tetraploid and controls plants.

Shoots are often thicker and can have shortened internodes and wider crotch angles (Hosseini Grouh et al. 2011). According to Omidbaigi et al. (2010), the leaves of the tetraploid plants were dark green, more dissected and dentated at their margins and more thickness. Indded, the tetraploids plants were sturdier and had a little larger seeds than diploids.

In this study, the most efficient treatment method for chromosome doubling in barley was seedling treatment in concentration 0.1% of drug treatment for 16 h on seedling. This method appeared to be effective on the production of tetraploids and could be further used in breeding programs.

References

Adaniya S, Shira D (2001) *In vitro* induction of tetraploid ginger (*Zingiber officinalis* Roscoe) and its pollen fertility and germinability. Scientia Horticulturae 88: 277-287.

Bakry F, Paulo RN, Pichot S and Jenny C (2007) In liquid medium colchicines treatment induces non chimeral doubled-diploids in a wide range of mono-and interspecific diploid banana clones. Fruits 62: 3-12.

Chen C, Hou X, Zhang H, Wang G and Tian L (2011) Induction of Anthurium andraeanum ‘‘Arizona’’ tetraploid by colchicine *in vitro*. Euphytica 181:137–145.

Hanzelka P, Kobza F (2001) Genome induced mutation in *Challistephus chinensis* Nees. 1. Effect of colchicines application on the early plant development. Zahradnictvi Horticultural Science 28: 15-20.

Hassein H, Combes D and Boussaid M (2001) Premiers essais de polyploïdisation chez Vicia narbonensis par l'utilisation de la colchicine. Ecologia mediterranea 27 : 109-124.

Hosseini Grouh MS, Meftahizade H, Lotfi N, Rahimi V and Baniasadi B (2011) Doubling the chromosome number of *Salvia hains* using colchicine: Evaluation of morphological traits of recovered plants. Journal of Medicinal Plants Research 5: 4892-4898.

Lavania UC, Srivastava S (1991) Enhanced productivity of tropane alkaloids and fertility in artificial autotetraploids of *Hyoscyamus niger* L. Euphytica 52: 73-77.

Lépengué MI, Cherif M, Ake S, M'Batchi B (2012) Amélioration de la croissance du maïs (*Zea mays* L. var. LG 60, Poaceae) par des traitements à la colchicine. Journal of Applied Biosciences 52: 3660– 3668.

Nigel AR, Urwin J, Therese M (2007) Generation and characterization of colchicine autotetraploid *Lavandula angustifolia*. Euphytica 156: 257-266.

Omidbaigi R, Mirzaee M, Hassani ME, Sedghi Moghadam M (2010) Induction and identification of polyploidy in basil (*Ocimum basilicum* L.) medicinal plant by colchicines treatment. International Journal of Plant Production 4: 87-98.

Oto T (1949) Some properties of tetraploid barley, Japanese Journal of Genetics 24:81-82.

Osborn TC, Pires J C, Birchler JA, Auger DL, Chen ZJ, Lee H S, Comai L, Madlung A, Doerge R W, Colot V (2003) Understanding mechanisms of novel gene expression in polyploids. Trends in Genetics, 19: 141-147.

Pirkoohi MH, Keyvanloo M, Hasanpoor M (2011) Colchicine-induced ploidy in mint by seed treatment. International Journal of Argriculture and Crop Sciences 3: 102-104.

Quan K, Guolu L, Qigao G, Xiaolin L (2004) Polyploid induction of *Arctium lappa* by colchicine. Plant Physiology Communications 40: 157-158.

Reinbergs E, Shebeski LH (1958) Fertility of barley autoterraploids. Fertility in successive generations of four autotetraploid barley varieties and the effect of selection for fertility in the O.A.C.21 autotetraploid. Canadian Journal of Plant Science 39: 9-107.

Roy AT, Leggett G, Koutoulis A (2001) *In vitro* tetraploid induction and generation of tetraploids from mixoploids in hop (*Humulus lupulus* L.). Plant Cell Report 20: 489-495.

Saharkhiz MJ (2007) The effects of some environmental factors and ploidy level on morphological and physiological characteristics of feverfew (*Tanacetum parthenium* L.) medicinal ornamental plant. Ph.D. Thesis, Tarbiat Modares University, Iran, pp 173.

Smith WE (1995) Fertility of autotetraploid varieties and hybrids of barley. University of Alberta, Edmonton, Alberta, pp, 434-442.

Taira T, Shao ZZ, Hamawaki H, Larter EN (1991) The effect of colchicine as a chromosome doubling agent for wheat-rye hybrids as influenced by pH, method of application, and post - treatment environment. Plant Breeding 109: 329-333.

Wan Y, Duncan DR, Rayburn AL, Petolino JF and Widholm JH (1991) The use of antimicrotubule herbicides for the production of doubled haploid plants from antherderived maize callus. Theoretical and Applied Genetics 81: 205–211.

Wu HZ, Zheng S, He Y, Yan G, Bi Y, Zhu Y (2007) Diploid female gametes induced by colchicine in oriental Lilies. Scientia Horticulturae 114: 50-53.