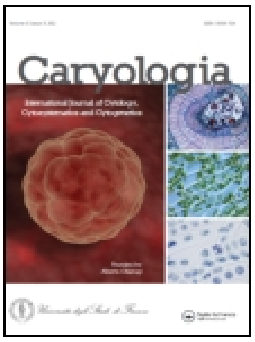

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Production of colchicine induced tetraploids in *Vicia villosa* roth

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Abstract — Seeds *V. villosa* (hairy vetch) were treated with 0.005% colchicine and 12% tetraploid plants (2n=4x=28) were obtained. Besides two chimeric plants, one of them consisted of diploid, triploid, tetraploid, hexaploid, heptaploid, octoploid roots and the other diploid and triploid roots were also obtained in hairy vetch.

Tetraploid plants were determined by the chromosome numbers in the root tips which were confirmed by studies conducted in the pollen mother cells (PMCs). Chromosome irregularities in the PMCs of diploids were not observed, however normal and abnormal chromosomal behavior were detected in tetraploids.

The morphological observations pointed out those tetraploids were shorter, surface of leaves were greater, and size of stomata were bigger but the number of stomata in per area were less than diploids. Flowering was delayed in tetraploids, the number of flowers was less, but forming a few larger flowers than diploids. It was also observed that the colour of leaves was darker, and amount of total chlorophyll in the leaves was higher in tetraploids.

Pollen grains also showed some differences in diploids and tetraploids. While diploid pollen grains had tricolporate, numbers of pore and colpi showed variations in tetraploids; pollen grains with 4 pores and 4 colpi, 5 pores and 5 colpi and 6 pores and 6 colpi were identified. The pollen size in the tetraploids was defined bigger than others. There were differences in the shapes of pollen grains; they were elliptical in diploids, triangular in polyploids. Pollen viability was measured as 99% in diploids, and this rate lowered to 81% in tetraploids.

Key words: chimeric plants, colchicine, hairy vetch, pollen, polyploidy, stomata, *Vicia villosa.*

INTRODUCTION

High yield and quality crop production are very significant in agriculture. Thus; the main issue that must be considered first is plant breeding.

Polyploid plant production is commonly used in traditional plant breeding, and produced polyploid fruits and vegetables have been used as human and animal food for years.

Polyploids either arise spontaneously or produced artificially. It is estimated that 47-70% of the angiosperm species are polyploid (Grant 1981; Masterson 1994). Differences in ploidy have been observed among related congeners and even within populations of taxonomic species (Clausen *et al.* 1945; Stebbins 1947; Darlington 1963; Lewis 1980; Grant 1981) and there is an evidence that individual polyploidy taxon may have multiple orgins (Soltis and Soltis 1992). These observations suggest that polyploidy is not only a rare macroevaluation event but also ongoing process (Ramsey and Schemske 1998).

Nowadays, most common chemical agent used for obtaining polyploid plant is colchicine, an alkalloidal toxic, extracted from seeds and corms of *Liliaceae* family member of *Colchicum autumnale* L.

The characteristic of being bigger increases their commercial interest, and it attracts agricultural production (Klug and Cummings 2003). This situation was interested not only by researchers who work on plants utilized for vegetative parts but also researchers specialised on

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ornamental plant breeding (Rose and Tobutt 2000; Vainöle and Repo 2000). Obtaining polyploid plant is widely used to increase the cold resisting and bigger flowering species in ornamental breeding. For medicinal plants, polyploids are usually more valuable because they exhibit increased biomass and content of effective compounds (Gao *et al.* 1996). Their dark green color is the result of bigger cells and more chlorophyll content. Photosynthesis potential is even higher than diploids (Molin *et al.* 1982; Ilarslan 1990). Tolerance of polyploids to environmental stress and genetic adaptations is generally higher than diploids (Estilai and Shannon 1993).

Vegetative parts of *Vicia villosa* Roth. which is inclusive of *Leguminosae* family are utilized as fresh fodder and hay furthermore the seeds are used as livestock fodder. *Rhizobium* bacteria is established inside the root nodules of legumes fix nitrogen from the atmosphere into the plant usable form, therefore the plants of those family provide significant contributions to nature as green manure. *Vicia villosa* Roth. are selected and carried out in this study due to these properties and has not been experimented before. The purpose of this study is to obtain polyploid plants of *Vicia villosa* via colchicine treatment determined by cytological and morphological criteria.

MATERIALS AND METHOD

Vicia villosa Roth. (Menemen -79) certified seeds obtained from Aegean Agricultural Research Institute were used. Experiment was arranged as two groups. Plants from first group were used as control, and second group plants were treated with colchicine. 100 seeds were used per each group.

The seeds were surface-sterilised by immersion in 0.01% NaOCl solution for 5 minutes. Then they rinsed with distilled water. Soaking the seeds in distilled water for less than 20 hours proved is more effective in inducing the polyploidy (Joshi and Verma 2004) seeds compared to soaked seeds in distilled water for 24 h.

Thereafter, the seeds were soaked in 0.02% and 0.005% colchicine solution for 8 hours at room temperature. All test tubes were covered with aluminum foil to make colchicine effective and to avoid light exposure. They were taken out of the solution, rinsed in distilled water and placed in petri dishes on moisten filter papers. After 5 days, seeds of control groups and colchicine treated groups were sown in the pots.

Cytological Methods - The chromosome number in the root tips of *V. villosa* were evaluated through Feulgen method. Primary root tips were pre-treated with α-monobromonaftaline, fixed in acetic-alcohol (1:3, v/v) and stored in 70% alcohol at 4°C. Then they were cut as ½ length and hidrolised at 1 N HCl for 12 minutes at 60°C. Root tips were rinsed in cold HCl for 1-2 minutes, and after basic fuchsine treatment these were kept in the dark for 1.5-2 hours at room temperature. Stained root tips with slide over 2% dropped in aceto-orcein were squashed.

Chromosome counts were conducted to evaluate polyploidy level in the roots of *V. villosa* colchicine treated plantlets for 12 weeks. Squashed preparations stained by 2% aceto-orcein from anthers were prepared to investigate meiosis in the PMCs of control and treated group.

Morphological Methods - Leaves of control group and polyploid plants were collected 26 weeks after sowing from the same nodes of the growth parts. Leaves that stuck onto white paper were copied, and perspective drawing was prepared. Leaflets were cut from perspective point, scanned and transferred to computer. Leaflet area was measured as cm^2 via Leaf Hunter program.

Thin sections were taken from the lower surface of leaves to evaluate stomata size. They were examined in Olympus-BH-2 light microscope with x40 objective and x10 ocular, images had been taken by Image pro-express program and Evolution LC color camera and then stomata lengths and breadths had been calculated.

Stomata counts on lower epidermis of leaves were conducted to determine stomata density of plants. Thus; colourless nail varnish was coated on to lower leaf surface and kept for dry. This dry layer was carefully removed from the surface, and placed on to microscope slide, 1 drop of distilled water was dropped on it, and the lamel was covered. Thus; perspectives of the stomata cells on varnish were prepared, and stoma cells within 1 mm^2 area were counted.

Determination of Chlorophyll Amount - Determine the total amount of chlorophyll, polyploid plants and the control group 0.5 g of plant leaves, a quantity of calcium carbonate ($CaCO_3$) powder and 15 ml by adding 80% acetone, placed on ice cold muller and disintegrated within it. Then the tissue fragments (homogenate) 3000g centrifuged in +4°C, for 10 minutes. After centrifugation the result, top liquid (supernatant) was measured by volume and included in total chlorophyll content was determined by 645 and 663 nm wavelength absorbances in spectrophotometer. Samples by 1 liter of total chlorophyll content denominated in mg with the following formula were calculated according to ARNON (1949).

Total chlorophyll (C) = $20.2 \times D_{645} + 8.02 \times D_{663}$.

Palynological Methods - Control and polyploid plants of flowering, pollen shapes and diameters were calculated. Hence, the flowers bloom after the spill, the petal was opened and exploited under stereo microscopes pollen grains were collected around anthers with the help of needle tip. These pollens were examined via WODEHOUSE (1935) method.

Pollen shape according to AYTUG (1967), apparently based equatorial P/E (P: polar axis, E: equatorial diameter) were determined according to the rate. Obtained for each feature and control plants as well as 50 measurements were made for polyploid plants, was evaluated using biometric methods, arithmetic mean and standard deviation were calculated.

Pollen grains of control group and polyploid plants were stained with Cotton blue prepared in lactophenol to evaluate the percentage of pollen viability. Each group was counted for 5500 pollen grains.

RESULTS AND DISCUSSION

Cytological observations - The seeds of *V. villosa* were pretreated with 0.02% and 0.005% colchicine. After one week of mortality, all seedlings in first concentration died while seedlings growths in second concentration were observed. At the end of 7 weeks, seedlings growth rates were; 44% of colchicine applied hairy vetch and 90% in the control group was detected.

In the control group, the diploid number of chromosomes in hairy vetch 2n = 14 (Fig. 1a) was detected. 0.005% and 0.02% to preliminary study, seeds were performed and 0.02% colchicine for 8 hours' development of the plants did not increase concentration, lower concentration of working with have proved to be appropriate.

In 1940 DERMEN, application time and concentration of colchicine course of polyploid plant that has reported a remarkable gain. DERMEN (1940) has reported that the role of application time and concentration of colchicine in the process of polyploid plants obtaining is significant. It was pointed that the duration and concentration of the colchicine differed from the plant's growth period and treated parts (ELCI 1966; SAGSOZ 1974 and 1982). LANDIZINKY and SHEFER (1982) applied 0.02% colchicine solution for 8 hours to young *V.sativa* shoots and reached positive results. JOSHI and VERMA (2004) obtained 50% tetraploid *V.faba* plants when applied 0.005% colchicine for 8 hours, while waiting 24 hours at these concentrations the results could not obtain from seeds.

0.005% increase was obtained as a result of colchicine treatment in hairy vetch, 12 tetraploid hairy vetch plants and 2% the chimeric plants, and chromosome number 2n = 4x = 28 were identified as in Fig. 1b.

Moreover, in the colchicine applied, mitotic chromosome counts in hairy vetch plants, one, diploid (2n = 14) and triploid (2n = 3x = 21) (Fig. 2a) and one with different ploidy levels, diploid (2n = 14), tetraploid (2n = 4x = 28), hexaploid (2n = 6x = 42) (Fig. 2b), heptaploid (2n = 7x = 49) (Fig. 2c) and octoploid (2n = 8x = 56) (Fig. 2d) where a combination of roots mixoploid (chimeric) plants were detected. After the emergence of chimeric plants colchicine treatment, the different methods applied have been reported by many investigators (DERMEN and SCOTT 1938; FELTZ 1953; ELCI 1982; COHEN and YAO 1996). OZER and SAGSOZ (1994) have been pregerminated in perennial rye plants after colchicine treatment process, reviewing their chromosomes at a rate of 81.75% in diploid, tetraploid at a rate of 5.84%, 2.19% at the rate of diploid and tetraploid roots that coexist with mixoploid and at a rate of 7.30% with different ploidy levels at the same root tissues of the chimeric plants were observed. TEPE *et al.* (2002) *in vitro* with colchicine treatment to mint, observed triploid, tetraploid, and hekzaploid as well as a few consisting different ploidy level of mixoploid plants were obtained.

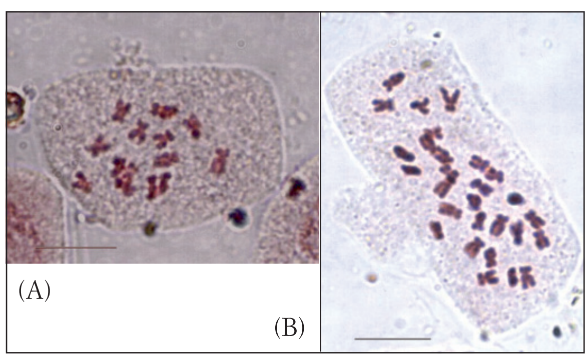

Fig. 1 — Metaphase chromosomes in the root tips of hairy vetch. (A) In the control group (2n = 14); (B) In the colchicine treated group (2n = 4x = 28) (bar = 10 μm).

Fig. 2 — Metaphase chromosomes in the root tips of chimeric plants with different ploidy level in colchicine treated hairy vetch plants. (A) Triploid (2n = 3x = 21); (B) Hexaploid (2n = 6x = 42); (C) Heptaploid (2n = 7x = 49); (D) Octoploid (2n = 8x = 56) (bar = 10 µm).

Chromosome counts, obtained at the root tips, were confirmed by the studies of the PMCs, and tetraploid plants were detected. Detected chimeric plants in root tips analysis have showed the same chromosome numbers as diploid plants (2n = 14) by studies of PMCs. The formation of chimeric plants is often a situation that can be found in studies of polyploidy plant root tips chromosome counts, meiotic chromosomes in PMCs absolutely must be investigated in during polyploid plant obtaining.

Prophase I in diploids undergoes normally. Leptoten and 7 bivalents in diakinesis can be seen in Fig. 3a. Bivalents lined up at equatorial plate during metaphase I (Fig. 3b). Spindles are generally in parallel arrangement in anaphase II and chromosomes move towards respective poles (Fig. 3c,d). At the end of the telophase II microspore tetrads occur by simultaneous cytokinesis. The tetrads are mostly isobilateral and tetrahedral. Diploid plants of any disorder in the cytoplasm of the pollen mother cell (PMC) were encountered.

Control hairy vetch plants were counted in 165 microspore tetrads, and triads at a rate of 3.03%, 96.36% at a rate of normal tetrads, and 0.61% at a rate of pentads have been found.

Meiosis in PMCs were regular in additional to the irregularities which have been found in tetraploid plants. It was noticed that clustering of chromosomes as unit into separated groups formed in leptotene (Fig. 4a,b). Degenerative signs such as deep cleavages in the cytoplasm of the PMCs were noted in tetraploids (Fig. 4b). Although 14 bivalents were counted in each cell during diakinesis, univalents and tetravalents were observed in some cells (Fig. 4c). Single chromosome or chromosome groups were located outside of equatorial plate in metaphase I. Chromosome bridges and fragments were observed in anaphase I and in some the two chromosome groups were so close to each other that they seemed as one group (Fig. 4d).

14 chromosomes were significantly observed in two equatorial plate during metaphase II (Fig. 4e). Chromosome bridges, lagging chromosomes and chromosome fragments, different orientation of spindle were observed as irregularities in anaphase II (Fig. 4f-h). The two chromosome groups became close to each other and merged (Fig. 4g). This explains the occurrence of triads and higher volume of one spore compared to another. As a result of irregularities, in addition to 4 spores, 5, 6, 7, 8 and 9 which are smaller than normal spores were encountered (polyspory) (Fig. 5 a,b). 165 microspore tetrads were counted in tetraploid plants and 3.64% triads, 59.36% tetrads and 16.97% pentads, 13.33% in 6 spores, 2.42% in 7 spores, 3.64% in 8 spores, 0.61% in 9 spores were monitored.

Spores equal to the volume as well as in different sizes, 3 large and 1 small volumes, were detected in tetrads. It can be observed on figure 4 that these chromosomes were not separating from each other, and conjoined microspores were formed. Microspore tetrads produced at the end of meiosis were mostly in isobilateral and tetrahedral form.

Morphological observations - According to morphological results, the stem length of the 26-week hairy vetch plantlets were measured as 147 ± 10.1 cm in diploids and 93.13 ± 26.56 in tetraploids (Table 1). Shorter stem length was observed in tetraploids compared to diploids. This indicated slower stem growth in tetraploids. JOSHI and VERMA (2004) measured the *V.*

TABLE 1 — Comparison of morphological and physiological characteristics of the diploid and tetraploid hairy vetch plants.

Character	Diploid	Tetraploid
Plant height (cm)	147 ± 10.1	93.13 ± 26.56
Leaflet area (cm2)	0.443 ± 0.05	0.596 ± 0.07*
Stomata breadth (µm)	18.07 ± 2.04	20.95 ± 1.62*
Stomata length (µm)	25.54 ± 2.76	31.61 ± 3.26*
Stomatal density (number / mm2)	55.87 ± 3.83	41.50 ± 6.77
First inflorescene (days)	98 ± 14	121.8 ± 6.3
Total number of flowers	58.67 ± 10.07	22.40 ± 5.59
Total chlorophyll content (mg / l)	62.25 ± 0.72	66.26 ± 1.11
Pollen stainability (%)	98.7	80.97

Values are means ± standard error. *Statistically significant different at $p < 0.01$ as determined by t-test.

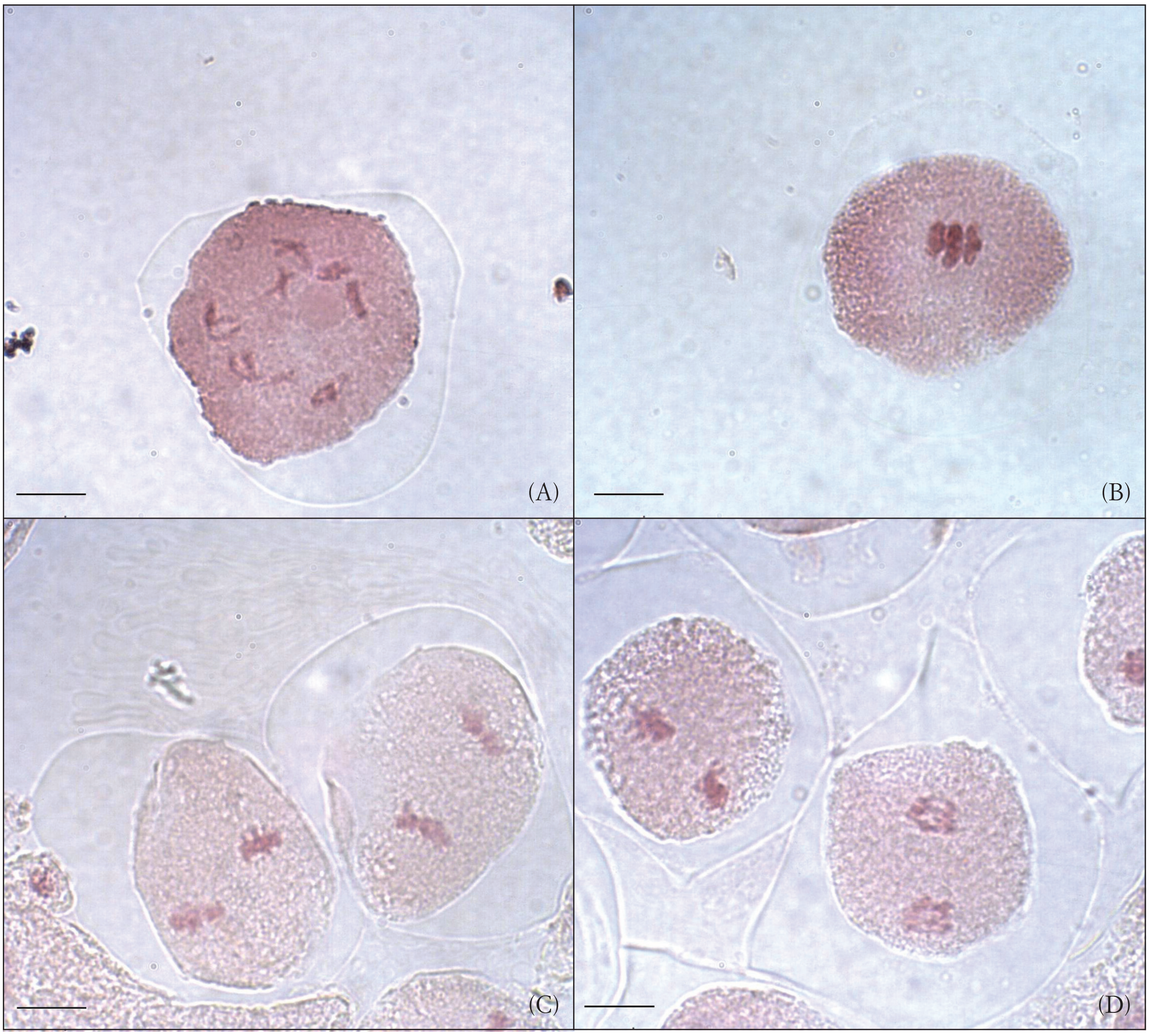

Fig. 3 — Meiosis in the PMCs of diploid hairy vetch. (A) Diakinesis; (B) Metaphase I; (C) Metaphase II and anaphase I; (D) Metaphase II and early anaphase II (x1000, bar = 10 µm).

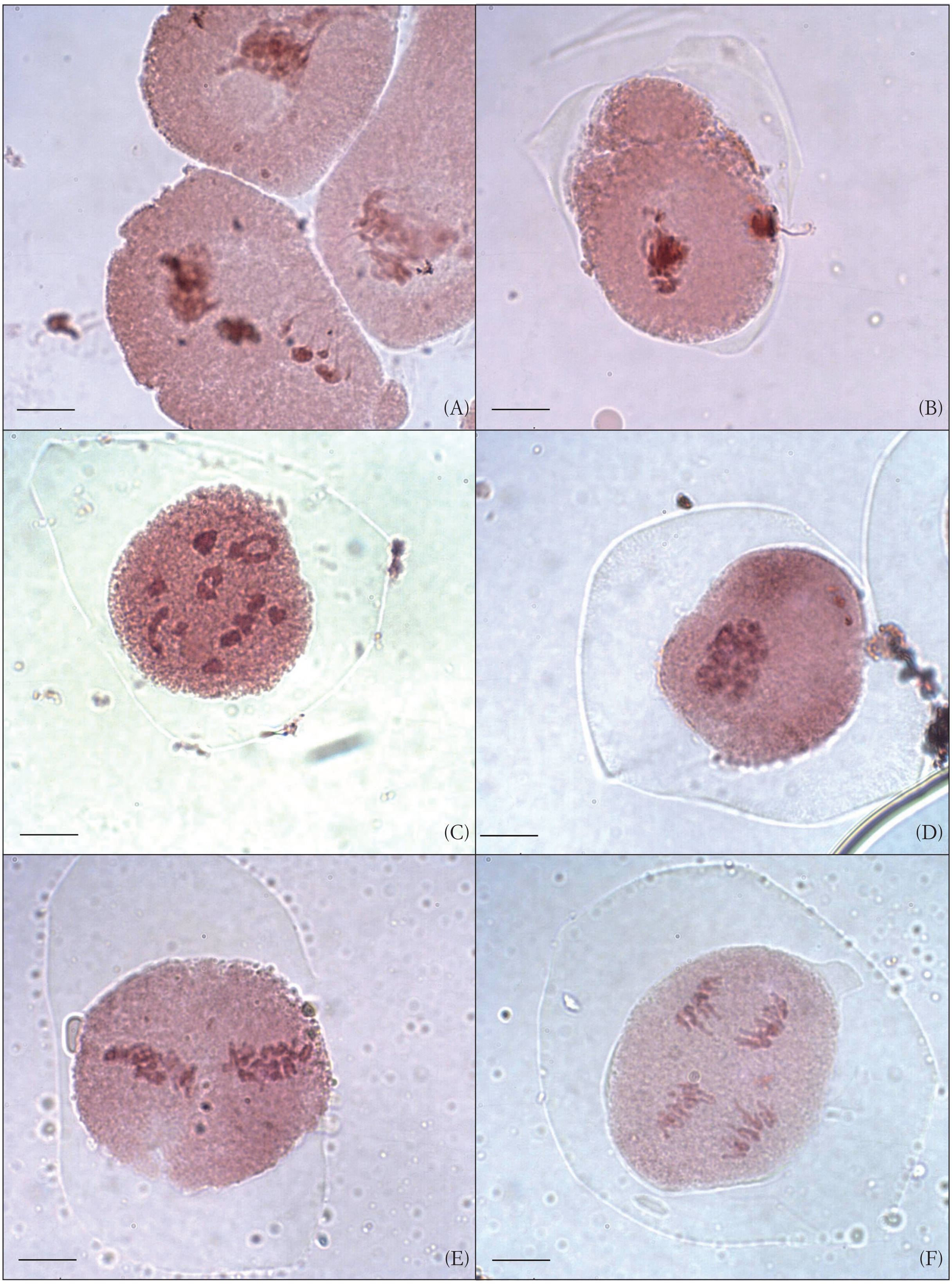

Fig. 4 — Meiosis in the PMCs of tetraploid hairy vetch. (A, B) Leptotene ; chromosome clusters; (C) Chromosome associations at diakinesis; (D) Anaphase I; (E) Metaphase II; (F-H) Different orientation of spindle during anaphase II (x1000, bar = 10 μm).

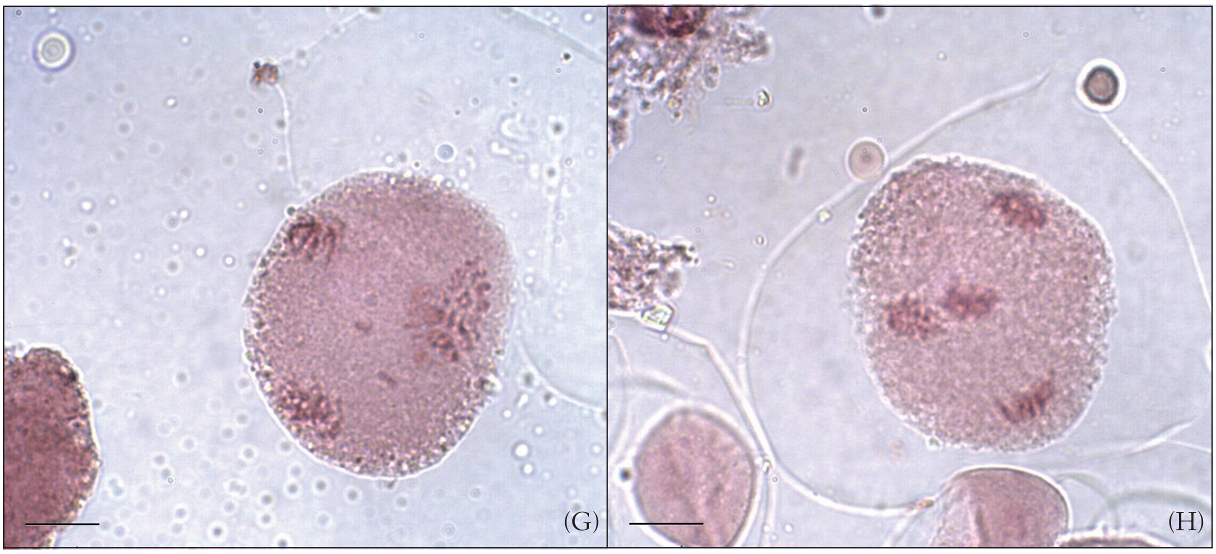

Fig. 4 — Condt.

faba stem length, and the results were 75 ± 1.89 cm for diploids and 51 ± 10.05 for tetraploids. The growths of polyploids were slower than diploids. It was indicated that the slower growth rate could be attributed to the reduced rate of cell division (EIGSTI 1947), less growth hormones (AVERY and POTTORF 1945; LARSEN and MINTUNG 1950) and the low activity of metabolites in tetraploids (JOSHI and VERMA 2004). JOSHI and VERMA (2004) recorded that polyploid plants were slower in growth initially, but later showed gigantism considering economic importance with respect to high yield.

Leaflet area in hairy vetch was 0.443 ± 0.05 cm^2 in diploids and leaftlets of tetraploids were larger in the area with 0.596 ± 0.07 cm^2 (Table 1, Fig. 6a). Leaflets areas, *t-test* results indicate a statistically significant difference at $p < 0.01$. Tetraploids leaflet areas larger than diploid plants have been reported by several investigators (BHIRAVAMURTY and RETHY 1984; DENIZ 1985; OZER and SAGSOZ 1991; CHAKRABORTI *et al.* 1998; JOSHI and VERMA 2004; JASKANI *et al.* 2005a).

Stomata breadth of diploids was 18.07 ± 2.04 µm, while it was 20.95 ± 1.62 µm in tetraploids.

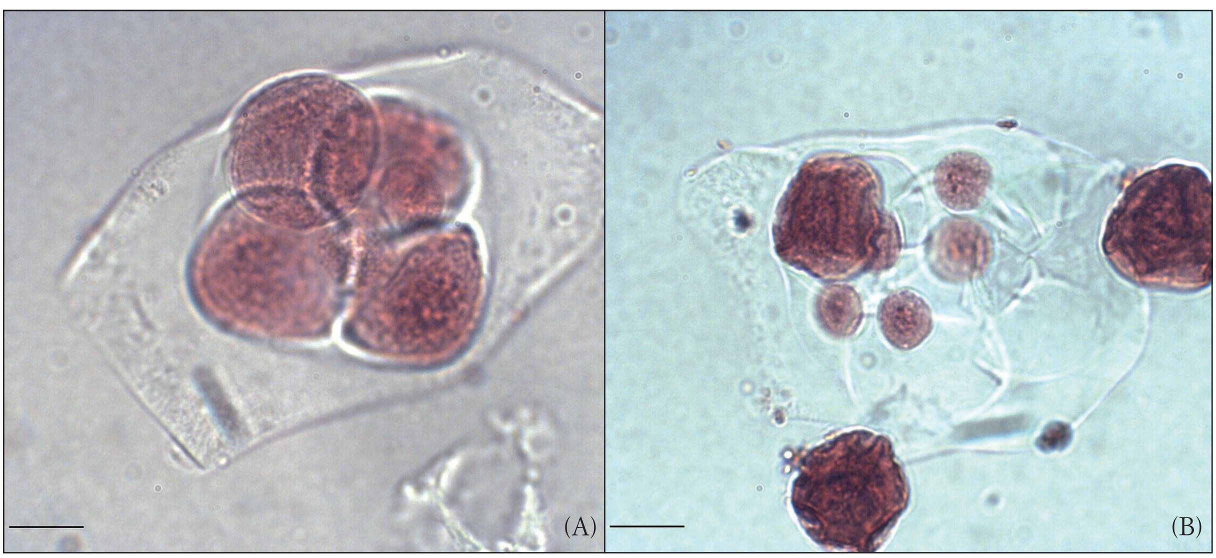

Fig. 5 — Polyspory in tetraploid hairy vetch. (A) Hexad; (B) 9 microspores (x1000, bar = 10 µm).

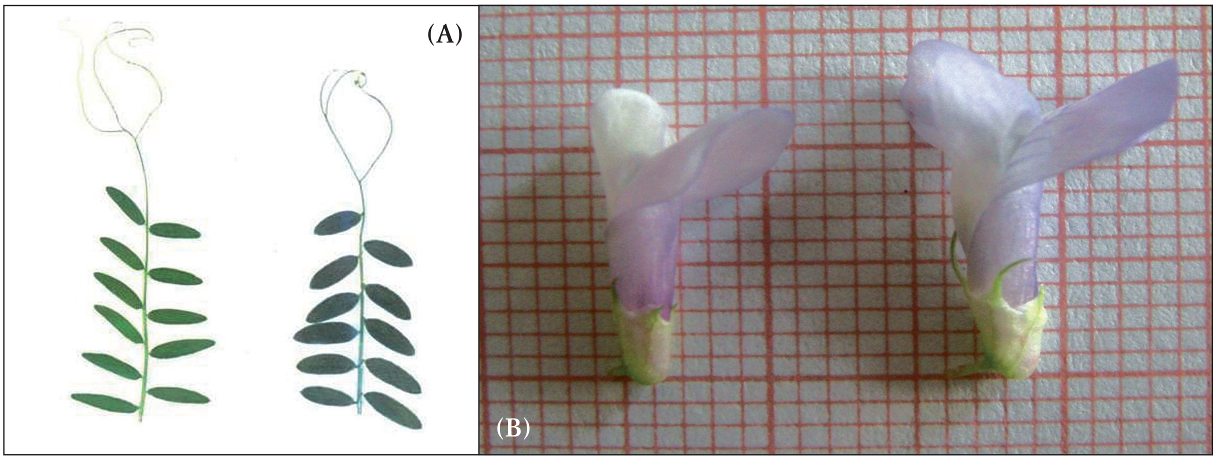

Fig. 6 — Leaves (A) and flowers (B) of diploid and tetraploid hairy vetch. Diploid (left), tetraploid (right).

Stoma length was determined as 25.54 ± 2.76 µm for diploids and 31.61 ± 3.26 µm for tetraploids (Table 1, Fig. 7a,b). *t-test* results of stoma length, and breadth in diploids and tetraploids indicate statistically significant differences at $p < 0.01$.

The counted stomata number unit area was 55.87 ± 3.83 in diploid hairy vetchs; this number was 41.50 ± 6.77 in tetraploids (Table 1). These results in the unit area stomata numbers were less in tetraploid hairy vetch leaves than diploids (Fig. 7c,d).

Besides bigger stomata cells and a decrease in stomata number per unit area were observed in tetraploids. In recent year studies, most researchers (TAN and DUN 1973; BORRINO and POWELL 1988; PRZYWARA *et al.* 1988; JOSHI and VERMA 2004) examined the size of the stomata cells, and reported bigger cells in polyploids. COHEN and YAO (1996) produced tetraploid *Zantedecshia* via *in vitro* colchicine teratment. They estimated tetraploid plants by measuring stoma cell size before chromosome number count and putative tetraploids were selected with the help of the chromosome counts, and they confirmed that 91% of these plants were tetraploid. Stomata size was identified as the most effective chriteria determination polyploidy. Due to easier and echonomical application, this method has been used for years in determination of polyploid plants (COHEN and YAO 1996). Many researches indicated that when polyploidy level increased, plant cells became greater and the number of stoma per area decreased (MUKHERJEE 1986; HÖMMÖ and VALANNE 1987; ILARSLAN 1990). TEPE *et al.* (2002) obtained triploid, tetraploid and hexaploid mint plants by *in vitro* colchicine treatment. They also counted 34-37 stomata in diploids, 24-27 stomata in triploids, 14-17 stomata in tetraploids and 12-13 stomata in hexaploids. Researchers reported a high rate of correlation between chromosome and stoma numbers of the leaves.

First flowering day were 98 ± 14 days in diploids, whereas it was 121.8 ± 6.3 days in tetraploids (Table 1). Approximately a delay of 23 days has been observed in the flowering of the tetraploid plants.

Total number of flowers in diploids was 58.67 ± 10.07 while it was 22.40 ± 5.59 in tetraploids. Our results indicated a delay in the first flowering, less but larger flowers in tetraploid according to diploids (Fig. 6b). JOSHI and VERMA (2004) observed that in first flowering day in diploid, *Vicia faba* plants were 53.2 ± 1.09 and 92.66 ± 5.67 in tetraploids. Comparing tetraploids to diploids, a delay of 40 days in the flowering in tetraploids was reported. Total number of *V. faba* flowers was 57.8 ± 1.64 in diploids and 40.8 ± 8.47 in tetraploids were reported.

Darker leaves in tetraploid plants were noticed, and after total chlorophyll investigations, higher chlorophyll content was detected in tetraploids. Total chlorophyll content in diploids was 62.25 ± 0.72 mg/l in diploids, and 66.26 ± 1.11 mg/l was determined in tetraploids (Table 1). Our results indicate that polyploid plants leaves are darker than diploid plants (Fig. 6a) and they contain more chlorophyll in tetraploids. Researchers reported that a bigger amount of total chlorophyll remained in polyploid plants. JASKANI *et al.* (2005b) investigated some characteristics of diploid and tetraploid watermelon plants, and reported that tetraploid plants contain more chlorophyll (55.6 ± 0.6) than diploids (52.3 ± 0.7).

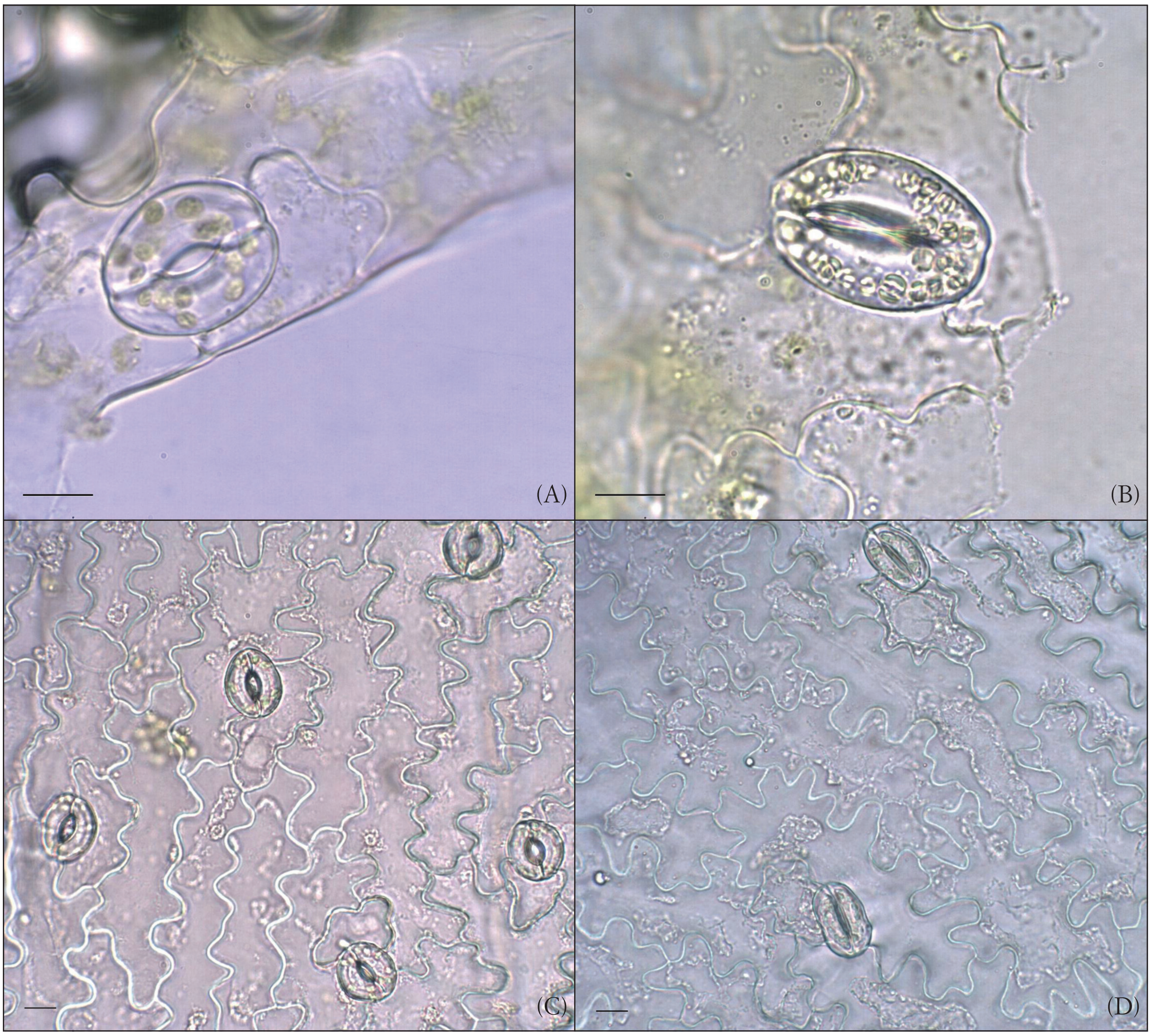

Fig. 7 — Stoma cells of diploid (A, C) and tetraploid (B, D) hairy vetch plants. (A,B x1000, C,D x400, bar = 10 μm).

Palynological Observations - Pollen grains of diploid hairy vetch plants were tricolporates with 3 pores and 3 colpi (Fig. 8a). There was found the increase in the number of pollen grains pore and colpus in tetraploids. Colpus and pore numbers of 100 pollen grains of diploids and tetraploids were analyzed. Tricolporate; with 3 pores and 3 colpi (2%), tetracolporate; with 4 pores

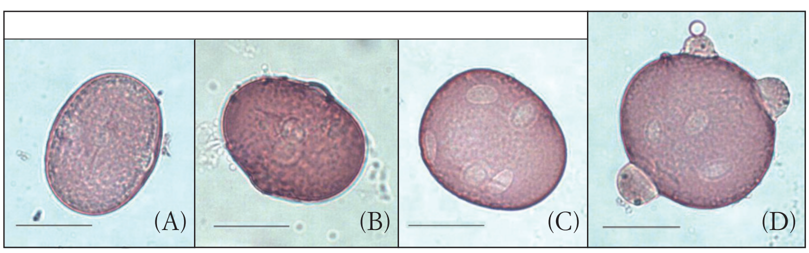

Fig. 8 — Pollen grains of diploid and tetraploid hairy vetch. (A) Tricolporate pollen of diploids with 3 pores and colpi. (B-D) Pollen of tetraploids with different number of pores and colpi; (B) Tetracolporate; (C) Pentacolporate; (D) Hexacolporate pollen grains.

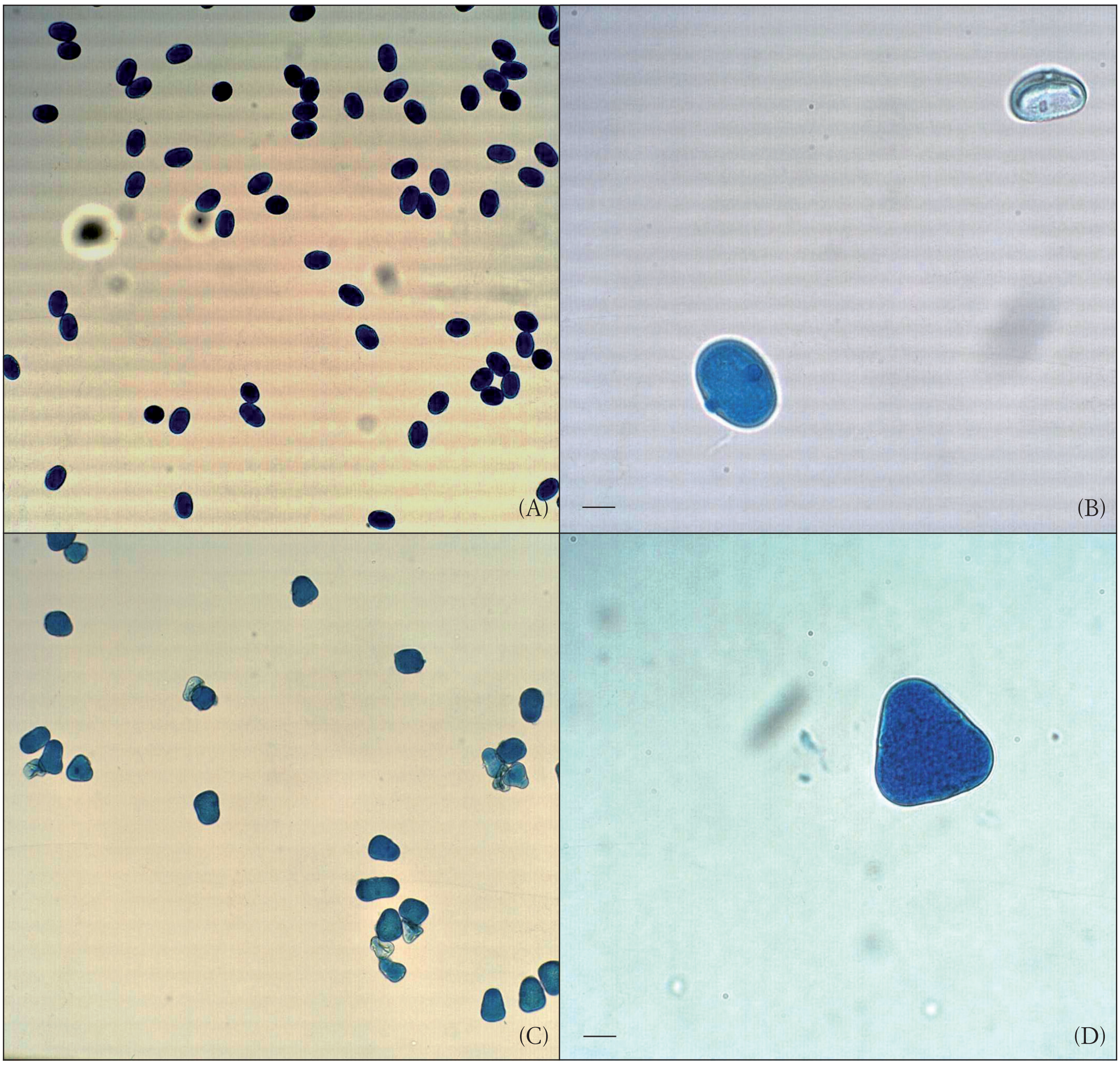

Fig. 9 — Viable and nonviable pollen grains of diploids (A, B) and tetraploids (C, D). Notice haploid pollen grains are elliptical, diploids triangular (A,C x100, B,D x 400, bar = 25 μm).

and 4 colpi (4%), pentacolporate; with 5 pores and 5 colpi (20%) and the hexacolporate with 6 pores and 6 colpi (74%) were counted in diploid pollen grains (Fig. 8b, c, d).

An increase in the numbers of colpi and pores of pollen grains was observed in tetraploids. When pollen grains of diploids were tricolporate, 98% of the pollen grains of tetraploids were tetra-penta-hexacolparate with more colpi content. RAMANA (1974) reported a relation between ploidy levels and germination pores in *Solanum* species. Pollen grains with n chromosome were 3 germinated pores whereas pollen grains with 2n chromosomes were 4 germination pores. According to investigators, in some situations, haploid pollen grains can have 3 or 4 pores and diploid pollen grain can have more than 4 pores. RHODES and ZHANG (1999) reported that

TABLE 2 — Control and tetraploid hairy vetch of pollen polar axis (P) and equatorial diameter (E), pollen shapes.

	Control	Tetraploid
P	38.78 ± 1.02 μm	50.346 ± 3.174 μm
E	28.64 ± 0.954 μm	35.705 ± 2.542 μm
P/E	1.354	1.410
Polen Shape	Prolate	Prolate

evaluation of the tetraploidy was conducted via chromosome counting, pollen grains size and numbers of colpi.

JASKANI *et al.* (2005b) applied colchicine to watermelon and tetraploid plants with bigger leaves size and flowers with 4 pollen colpi arised, diploids in this study had 3 pollen colpi.

Polar axis of diploid hairy vetch was measured as 38.78±1.02 µm and equatorial diameter was 28.64±0.954 µm. Pollen shape was prolate in diploids. Polar axis in tetraploids was 50.346±3.174 µm and equatorial diameter was 35.705±2.542 µm. Pollen shapes were prolate as in diploid plants (Table 2). There were significant increases in the polar and equatorial diameters of the tetraploids.

It can be observed on the Fig. 9 b and d that haploid pollen grains obtained were elliptical and diploids triangular. LANDIZINKY and SHEFER (1982) referred *V. sativa* pollen grains of diploid plants as elliptic and autotetraploid plants induced by colchicine treatment as tetrahedral. JOSHI and VERMA (2004) had pointed that *V. faba* diploid plants had elliptic pollen grains and tetraploid plants induced by colchicine had triangular ones. LANDIZINKY and SHEFFER (1982) stated that separation of diploid and autopolyploid plants can be done by pollen shape.

5500 tetraploid and diploid plant pollen grains were counted to evaluate pollen viability. Viable pollen rate was measured 98.7% in diploid, 80.97% in tetraploids (Fig 9a, c). We observed higher pollen viability in diploids. BHIRAVAMURTY and RETHY (1984) detected that pollen viability in diploid *S. nigrum* was 95.5%, whereas in natural tetraploids it was 93.7% and 17.2% in autotetraploids induced by colchicine treatment. OZER and SAGSOZ (1994) had treated pre germinated rye plants with colchicine and obtained tetraploid plants. The percentage of viable pollen grains in diploid rye was 96% whereas it was 83% in tetraploids. JOSHI and VERMA (2004) issued 100% pollen viability in *V. faba* diploid plants and 44.46±5.20% in tetraploids.

Tetraploid *V. villosa* plants with bigger leaves and flowers were obtained via colchicine as a result of this study. It was proved that polypoids can be detected through examination of stomata size, pollen grain size, and pore and colpi numbers. These were referred in line with litterateurs as distinguishing criteria.

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