Induced Colchitetraploids in *Allium cepa* L.

Ramesh Ahirwar*, Sapna Goyal and Rakesh Chandra Verma

School of Studies in Botany, Vikram University, Ujjain (M.P.) 456010, India

Received December 28, 2014; accepted June 30, 2015

Summary Polyploidy was induced by colchicine in *Allium cepa* L. Five plants of *Allium cepa* (AC1–AC5) were isolated from the 0.2% colchicine treated inflorescences. The meiotic stages were analyzed in treated buds. Various cytological features like chromosomal associations (quadrivalents, bivalents and univalents), chiasmata frequency and terminalization coefficient were recorded at diakinesis/metaphase-I. Anaphase/telophase-I/II were studied in all the colchitetraploids. In all these plants, the anaphase/telophase-I were abnormal showing unequal distribution, laggards, micronuclei and multipolar at telophase-II. Pollen mitosis was studied in these plants. The chromosomes ($n=16$) were found at metaphase. Pollen fertility was low (40.9 to 56.9%) in all colchitetraploids.

Key words Induced colchitetraploid, *Allium cepa* L., Pollen fertility, Colchicine, Chromosome.

The importance of polyploid plants in agriculture is well known. Colchicine has been largely used to alter ploidy level frequencies in cell populations to obtain tetraploid plants, starting with diploid material. In fact, polyploid plants usually have larger and thicker leaves, stems or roots, and also flowers, fruits and seeds, and then, a greater yield (Gao *et al.* 1996).

The genus *Allium* consists of 700 species in the world (Friesen *et al.* 2005). *Allium cepa* L. is one of the most important plant species which was cultivated thousands of years ago as an edible plant (Jamila *et al.* 2009). Its chromosome number is low ($2n=16$), and it has a relatively large size and is susceptible to cytological manipulations (Mercykutty and Stephen 1980). The present investigation was planned to induce polyploidy in *Allium cepa* by colchicine treatment and identify the ploidy level by counting the chromosome number.

Materials and methods

Colchicine treatment

Young inflorescences were treated with 0.2% colchicine solution for 6, 12, 18, or 24 h also by using the “cotton swab method”. After treatment inflorescences were thoroughly washed with water.

Study of meiosis

For meiotic studies, young flower buds of appropriate size were collected between 10 to 11 AM and fixed in freshly prepared Carnoy's fluid (absolute alcohol and acetic acid in 3 : 1 ratio). Anthers were separated, teased in a drop of 2% iron acetocarmine on a clean slide and squashed under a cover glass. Pollen mother cells (PMCs) were analyzed for suitable stages of meiosis. Temporary slides were analyzed for different stages of meiosis like diakinesis, metaphase-I, anaphase-I, telophase-I and -II. Chromosomal associations, chiasmata frequency, distribution of chromosomes and other features were recorded. Chiasma frequency was calculated according to Ward (1979).

---

*Corresponding author, e-mail: rameshbotvu@gmail.com

DOI: 10.1508/cytologia.80.419

The preparations were observed under a Leica microscope under oil immersion and magnified to X1000 and photomicrographs were taken from temporary slides with the help of a digital camera (Sony Cyber-shot DSC-W360).

Results

The results indicated that colchicine treatment was very effective for producing tetraploid plants.

Morphological study

Generally, flowers, capsules and seeds increased in size but their number decreased in colchitetraploids. The flowers in colchitetraploids were bigger and fleshier. The flower diameter of colchitetraploids was about 1.5 times more as compared to control. Capsules in colchitetraploids ranged from 42 to 67 as compared to 235 in control. The total number of seeds per plant ranged from 62 to 112 as compared to its diploid counterpart which gives 534 seeds (Table 4). Stomata and pollen grains sizes were also bigger as compared to diploid.

Meiotic and pollen mitotic study in control

Meiotic study was done in young flower buds. Each of the PMCs analyzed at diakinesis/metaphase-I had eight ring bivalents (Table 1, Fig. 1). Chromosomes were equally distributed at each pole at anaphase-I (Fig. 2). The number of chiasmata per cell ranged from 23 to 28, mean number being 25.5, out of which 13.9 were terminalized giving a terminalization coefficient of 0.83, and 11.6 chiasmata were unterminalized (Table 2).

Pollen mitosis was observed in the same plant. The chromosomes ($n=8$) were found at metaphase (Fig. 3). The chromosomes had equal distribution at anaphase. Pollen fertility was 93.2% (Table 4).

Meiotic study in colchitetraploids

Five plants of *Allium cepa* L. (designated as AC1, AC2, ..., AC5) were isolated from the 0.2% colchicine treated inflorescences. The meiotic studies were done in treated buds. Various cytological features were recorded at diakinesis/metaphase-I like chromosomal associations (quadrivalent, bivalent, univalent), chiasmata frequency per cell, chiasmata terminalization, and terminalization coefficient.

The average number of quadrivalents in all colchitetraploids was 1.2 per cell, ranging from 0.6 to 2.0 in AC2 and AC5, respectively. The average number of bivalents was 13.3 in all colchitetraploids, ranging from 11.5 in AC5 to 14.4 in AC2 compared with 8.0 in control (Table 1, Figs. 3,

Table 1. Chromosomal associations at diakinesis/metaphase-I of induced colchitetraploids in *Allium cepa* L.

Material	Number of cells analyzed	Quadrivalents		Bivalents		Univalents	
		Mean	Range	Mean	Range	Mean	Range
Control	25	—	—	8.0	—	—	—
4× AC1	25	0.9	0–3	14.2	12–16	—	—
4× AC2	25	0.6	0–1	14.4	13–16	0.8	0–2
4× AC3	25	0.9	0–1	14.2	12–16	—	—
4× AC4	25	1.6	0–5	12.4	6–16	0.8	0–4
4× AC5	25	2.0	0–4	11.5	8–16	1.0	0–4
Average		1.2		13.3		0.5	

AC (*Allium colchitetraploids*)

Figs. 1–12. Meiotic and pollen mitotic division in control and induced colchitetraploids in *Allium cepa* L. 1–3 Meiosis in control. 1, 8II at diakinesis. 2, 8:8 chromosomes at anaphase-I. 3, Pollen mitosis, eight chromosomes. 4–12 Meiosis in colchitetraploids. 4, 16II at diakinesis. 5, 4IV+8II at diakinesis. 6, 16:16 chromosome at anaphase-I. 7, Micronucleus at telophase-I. 8, Tripolar with three micronuclei at telophase-II. 9, 4 Laggards at telophase-II. 10, Pentapolar at telophase-II. 11, Hexapolar with bridge and five laggards at telophase-II. 12, Pollens mitosis, 16 chromosomes (Scale bar=4 μ m).

4). The average number of univalents was 0.5 in all colchitetraploids, ranging from 0 in AC1 and AC3 to 1.0 in AC5. The range of the chiasmata was 27–43 in all the colchitetraploids. The minimum average number of chiasmata per cell was 31.8, out of which 20.5 were terminalized giving a terminalization coefficient of 0.55 and 11.3 unterminalized in AC5, and the maximum average number of chiasmata per cell was observed in AC3 in which out of 36.8 average number of chiasmata per cell, 20.6 were terminalized giving a terminalization coefficient of 0.78 and 16.2 chias-

Table 2. Mean, range and terminalization coefficient of chiasmata at diakinesis/metaphase-I of induced colchitetraploids in *Allium cepa* L.

Material	Number of cells analyzed	Chiasmata per cell		Terminalization		Terminalization coefficient
		Mean	Range	Terminalized	Unterminalized	
Control	10	25.5	23–28	13.9	11.6	0.83
4× AC1	10	35.4	31–40	23.6	11.8	0.50
4× AC2	10	35.8	33–41	22.4	13.4	0.59
4× AC3	10	36.8	34–42	20.6	16.2	0.78
4× AC4	10	36.6	27–43	23.6	10.0	0.42
4× AC5	10	31.8	29–35	20.5	11.3	0.55

Table 3. Various types of chromosomal configurations at anaphase/telophase-I/II of induced colchitetraploids in *Allium cepa* L.

Material	Number of cells analyzed	Anaphase/Telophase-I/II						%
		Normal	Unequal distribution	Laggard	Bridge	Micronuclei	Multi-polar	
Control	100	—	—	—	—	—	—	—
4× AC1	100	66	2	14	3	8	7	34
4× AC2	100	72	3	10	1	6	8	28
4× AC3	100	68	3	13	2	7	7	32
4× AC4	100	63	2	16	1	6	12	37
4× AC5	100	63	2	16	2	8	10	37

Table 4. Pollen fertility and seed set in control and induced colchitetraploids in *Allium cepa* L.

Material	No. of sterile pollens	No. of fertile pollens	(%)	Total No. of capsules	No. of fertile capsules	No. of sterile capsules	No. of seeds
Control	35	480	93.2	235	207	28	534
4× AC1	232	307	56.9	67	50	17	96
4× AC2	224	195	46.5	42	36	6	75
4× AC3	239	201	45.6	57	55	2	112
4× AC4	330	229	40.9	43	34	9	68
4× AC5	299	228	43.2	63	49	14	62

mata were unterminalized (Table 2).

In all these colchitetraploids plants, the anaphase/telophase-I were abnormal showing various types of abnormalities such as unequal distribution, laggard, and micronucleus. Tripolar, pentapolar, hexapolar, heptapolar and octapolar were observed at telophase-II. Minimum abnormalities (28%) in AC2 and maximum (37%) in AC4 and AC5 were observed (Table 3, Figs. 7–11).

Pollen mitosis in colchitetraploids

Pollen mitosis was observed in the same plants. The chromosomes were found as $n=16$ at metaphase (Fig. 12). Pollen fertility ranged from 40.9% in AC4 to 56.9% in AC1 (Table 4). Pollen size also varied in colchitetraploids. In diploid, pollen grains were of equal size, whereas in the colchitetraploids, slight variation was seen in shape and size; the largest pollen grains were 1.5 larger as compared to control.

Discussion

Polyploidy is very common in flowering plants, including the major crops (Leitch and Leitch 2008). It is estimated that 30–70% of the angiosperms are of polyploid origin (Masterson 1994, Hegarty and Hiscock 2007). Polyploidization is known to affect various aspects of morphology, anatomy, physiology and genetics. The most immediate effect is on morphological characters, particularly in the leaf and flower size (Verma *et al.* 2011). One of the most useful approaches for the induction of artificial polyploidy is treating plants with a chemical known as colchicine. Colchicine inhibits the formation of spindle fibers and effectively arrests mitosis at the anaphase stage. At this point, the chromosomes have multiplied but cell division has not yet taken place resulting in polyploid cells (Ramsey and Schemske 1998).

Polyploidy has been induced through colchicine, and comparative study of morphological and cytological characters with diploid by various workers was reported as in *Cajanus cajan* and *Vigna unguiculata* (Patel and Verma 1999); *Phlox drummondii* (Verma and Raina 1991, Verma *et al.* 2011); and *Vicia faba* (Joshi and Verma 2004). Meiosis in tetraploids (4 $\times$) was irregular, while it was normal in control populations (2 $\times$). At diakinesis/metaphase-I, quadrivalents, bivalents and univalents were observed. The frequency of quadrivalents, bivalents and univalents varied among the tetraploids. The frequency of quadrivalents and univalents was found to be low as compared to bivalents. The range of quadrivalents was 0–3, 0–1, 0–1, 0–5 and 0–4 in AC1, AC2, AC3, AC4 and AC5 with an average of 0.9, 0.6, 0.9, 1.6 and 2.0, respectively. The range of bivalents was 12–16, 13–16, 12–16, 6–16 and 8–16 with an average of 14.2, 14.4, 14.2, 12.4 and 11.5, respectively.

Several reasons have been put forward to explain the low/high quadrivalent frequency in tetraploids. Kostoff (1939) suggested that the quadrivalent frequency depends on the size of chromosome, as a smaller size of the chromosomes shows a lower number of quadrivalents. On the other hand, Armstrong and Robertson (1956) observed more multivalents in the tetraploids with small chromosomes. However, in the present study, the chromosome size is large but a low frequency of quadrivalents was observed.

Sybenga (1975) suggested that the frequency of bivalents and quadrivalents in tetraploids is dependent on the points of pairing initiation. When there is a single point of pairing initiation, bivalents are formed; when there are two points of initiation, both quadrivalents and bivalents are formed in equal frequencies; and when the initiation points are more than two, then quadrivalents are formed in high frequency as compared to bivalents. Chiasmata localization considerably reduces the formation of multivalents in tetraploids and thus plays a role in the diploidization of polyploids (Koul and Gohil 1970). According to Loidl (1990), the formation of quadrivalents also depends on the number of synaptonemal complex initiation sites and the conversion of these into partner switches and the formation of univalents or trivalents in autotetraploids.

The average number of bivalents also varied from cell to cell and plant to plant. The lowest mean of 11.5 was observed in AC5, with the highest being 14.4 in AC2. The chiasmata were randomly distributed in the tetraploids and thus did not show perfect ring or chain configurations at diakinesis/metaphase-I, and probably due to this they did not disjunction normally (Sybenga 1975).

The chiasma frequency of the induced tetraploids was never exactly double than their controls. The lowest mean of chiasma frequency (31.8) was found in AS5 and the highest (36.8) was found in AS3. The low chiasma frequency observed in all the tetraploids was probably due to the presence of univalents and chain quadrivalents.

Gohil and Koul (1981) studied the autotetraploid *Allium tuberosum* and found that anaphase segregation is slightly irregular. They suggested that multivalents were main cause of segregation irregularity at anaphase. In addition, another cause of irregular division of chromosomes at anaphase-I may be due to abnormal behavior of univalents.

In the present investigation, several abnormalities were observed at anaphase/telophase-I like

unequal distribution, laggards, bridges, and micronuclei and multipolar at telophase-II. According to Darlington (1963), tetraploids always show irregularities and these irregularities are considered to be due to the formation of multivalent associations. It was the common feature in colchitetraploids. The cytological causes, resulting in unviable gametes, include non-disjunction of multivalents, lagging chromosomes, univalents, non-viability and other abnormalities.

In induced tetraploids, pollen grains were not of uniform size as in diploid. Large sized pollen grains resulted as a consequence of either direct fusion of two or more pollen grains or the end products of fused PMCs (syncytes). The fusion between pollen grains resulting in large sized pollen grains has been observed in the present investigation.

Pollen mitosis was also observed in tetraploids in which 16 chromosomes were present at metaphase. It was the confirmation of diploid gamete. Malik and Srivastava (2009) demonstrated that treatment with colchicine in *Carthamus* incited appreciable changes in the diameter of pollen grains, decreased the number of pollen grains per anther and increased pollen sterility. Bragdo (1955) recorded that in the tetraploid of red clover, there was a much greater variation in pollen size and fertility. Pollen fertility in tetraploids was ascribed to the formation of multivalent leading to irregular disjunction of chromosomes (Rana *et al.* 2013). Sterility observed in the present investigation could be explained on the basis of post-meiotic irregularities.

References

- Armstrong, J. M. and Robertson, R. W. 1956. Studies of colchicine-induced tetraploids of *Trifolium hybridum* L. 1. Cross and self-fertility and cytological observations. *Can. J. Agric. Sci.* **36**: 255–256.
- Bragdo, M. 1955. Production of polyploids by colchicine. *Euphytica* **4**: 78–82.
- Darlington, C. D. 1963. *Chromosome Botany and the Origins of Cultivated Plants*. George Allen and Unwin Ltd., London.
- Friesen, N., Fritsch, R. M. and Blattener, F. R. 2005. Phylogeny and new intergeneric classification of *Allium* (Alliaceae) based on nuclear ribosomal DNA ITS sequences. *Aliso* **22**: 372–395.
- Gao, S. L., Zhu, D. N., Cai, Z. H. and Xu, D. R. 1996. Autotetraploid plants from colchicine-treated bud culture of *Salvia miltiorrhiza* Bge. *Plant Cell Tissue Organ Cult.* **47**: 73–77.
- Gohil, R. N. and Koul, R. 1981. Studies on male and female meiosis in Indian *Allium* II. *Allium tuberosum*. *Chromosoma* **82**: 735–739.
- Hegarty, M. and Hiscock, S. 2007. Polyploidy: Doubling up for evolutionary success. *Curr. Biol.* **17**: 927–929.
- Jamila, Z., Guetat, A. and Mahamed, N. 2009. Morpho-phenological characterization of *Allium roseum* L. (Alliaceae) from different bioclimatic zones in Tunisia. *Afr. J. Agric. Res.* **4**: 1004–1014.
- Joshi, P. and Verma, R. C. 2004. High frequency production of colchicine induced autotetraploids in Faba bean (*Vicia faba* L.). *Cytologia* **69**: 141–147.
- Kostoff, D. 1939. Evolutionary significance of chromosome length and chromosome number in plants. *Biodynamics* **51**: 1–14.
- Koul, A. K. and Gohil, R. N. 1970. Cytology of tetraploid *Allium ampeloprasum* with chiasma localization. *Chromosoma* **29**: 12–19.
- Leitch, A. H. and Leitch, I. J. 2008. Genomic plasticity and the diversity of polyploid plants. *Science* **320**: 481–483.
- Loidl, J. 1990. The initiation of meiotic chromosome pairing: the cytological view. *Genome* **33**: 759–778.
- Malik, A. and Srivastava, A. K. 2009. Karyotypic analysis of different populations of *Carthamus tinctorius* L. (Asteraceae). *Comp. Cytogenet.* **3**: 51–55.
- Masterson, J. 1994. Stomatal size in fossil plants: Evidence for polyploidy in majority of angiosperms. *Science* **264**: 421–424.
- Mercykutty, V. C. and Stephen, J. 1980. Adriamycin induced genetic toxicity as demonstrated by *Allium cepa* test. *Cytologia* **45**: 769–777.
- Patel, O. P. and Verma, R. C. 1999. Cytomorphology of induced tetraploidy in *Cajanus cajan* and *Vigna unguiculata*. *Cytologia* **64**: 29–36.
- Ramsey, J. and Schemske, D. W. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annu. Rev. Ecol. Evol. Syst.* **29**: 467–501.
- Rana, P. K., Himshikha, K. P., Singhal, V. K. and Gupta, R. C. 2013. Impact of reciprocal translocations and non-synchronous disjunction of chromosomes on pollen fertility in *Astragalus chlorostachys* from the northwest Hima-

- layas (India). *Cytologia* **77**: 1–7.
- Sybenga, J. 1975. *Meiotic Configurations*. Springer-Verlag, Berlin, Heidelberg, New York.
- Verma, R. C., Ahirwar, R. and Das, P. 2011. Induction of polyploidy in *Phlox drummondii*. *J. Cytol. Genet.* **12**: 1–6.
- Verma, R. C. and Raina, S. N. 1991. Characteristics of colchipsoid *Phlox drummondii*. *Indian J. Genet. Plant Breed.* **51**: 246–251.
- Ward, E. J. 1979. Chiasma frequency and distribution in Maize family segregation for K 10 and trisomy 10. *Genetics* **92**: 223–230.
-