Autopolyploids of *Helianthus annuus* L. var. *morden*

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Summary Autopolyploid plants of *Helianthus annuus* var. *morden* were obtained by treating seedlings (2–5 d old) with 0.4%, 0.5% and 0.6% colchicine for 12 h. The autopolyploids exhibited reduction in the height, girth, leaves and flowers of the plants but increase in the size of stomata, pollen grains and meiocytes. Pollen and seed fertility were reduced. There was increase in seed weight from diploids to C₁ and then to C₂ generations in tetraploids, but increase in C₁ and decrease in C₂ generation in octoploids. Low seed setting in C₁ generation which, however improved in C₂ generation only in tetraploids. C₂ generation was absent in hexa-decaploids. The chromosome number in diploids, tetraploids, octoploids and hexa-decaploids were found to be 2n=34, 68, 136 and 272, respectively. Meiotic observations showed various associations of chromosomes such as univalents, bivalents and quadrivalents and abnormalities such as laggards, unequal separation of chromosomes, bridges, stickiness in C₁ generation which lessened in C₂ generation in tetraploids but increase in octoploids. These irregularities appeared to be the major factors responsible for high pollen sterility in autopolyploids. High or number of bivalents and lower frequency of abnormalities at metaphase in tetraploids in C₂ generation led to an improvement in pollen and plant fertility.

Key words *Helianthus annuus*, Colchicine, Induced autopolyploids.

The genus *Helianthus* belongs to the subdivision Tubeflorae and the tribe Heliantheae of the family Compositae. It is commonly known as sunflower. It is extensively grown for its ornamental value, for dyes, vegetable, seeds and as a forage crop. But recently it has gained importance as an oil seed crop. Extensive work is going on various aspect of this plant but comparatively little work has been done on the induction of polyploidy. Dhesi and Saini (1973), Gupta and Roy (1979), Downes and Marshall (1983) and Singh (1992, 1994) successfully induced autotetraploid plants. Present investigation was undertaken to autopolyploids and make a comparative study of chromosomal behaviour and morphological features of autopolyploids with an aim of improving its qualitative and quantitative characters. Induced tetraploids may prove better than diploids in both adaptability and oil yield but octoploids and hexa-decaploids better than diploids only in adaptability.

Materials and methods

Seeds of *H. annuus* var. *morden* were collected from Directorate of Oilseeds Research, Rajendra Nagar, Hyderabad. Seedlings were treated with colchicine at non-toxic level *i.e.* 0.1 to 0.8% concentration, 3 times within the duration of 12 h along with controlled plants. Morphological observations were recorded on different parameters from randomly chosen plants from each row. The leaves were macerated and mounted in glycerine jelly for measurement of stomata. Pollen grains were acetolysed and mounted in glycerine jelly for measurement of their size. Sterility of pollen grains was tested with acetocarmine.

For cytological investigations, young flower buds were fixed in freshly prepared Carnoy's fixative. Squash preparations of anthers were made in 2% aceto-carmine. Best stages of division were photographed. The seeds were counted for each capitulum to determine fertility.

Table 1. Efficiency and effectiveness of colchicine on *H. annuus* var. *morden* in C_1 and C_2 generations

Autopolyploids	No. of seeds sowing	No. of seeds germinated and treated	Plants survived	Reverted	Died	Matured
Control (2n)	100	—	95	—	—	95
C_1						
Tetraploids (4n)	100	93	63 (68)	20 (32)	16 (25)	27 (43)
Octoploids (8n)	100	92	58 (63)	25 (43)	23 (40)	10 (17)
Hexa-decaploids (16n)	100	95	52 (55)	23 (44)	12 (23)	17 (33)
C_2						
Tetraploids (4n)	25	20 (80)	—	—	—	12 (60)
Octoploids (8n)	25	6 (24)	—	—	—	3 (50)
Hexa-decaploids (16n)	25	5 (20)	—	—	—	0 (0)

Numerals mentioned in parenthesis represent in percent value. C=colchicine.

Results

Nine hundred seeds of variety *morden* were sown. 2–5 d old seedlings, 100 each, were treated with 0.1 to 0.8% dose of aqueous solution of colchicine. Out of this range, 0.4, 0.5 and 0.6% doses were found to be effective for induction of polyploidy. As initially evident by morphological variations plants were observed constantly and continuously from the date of sowing till harvesting. Simultaneously the same number of untreated seeds were also grown for the comparison as control in C_1 generation. Effectiveness and efficiency of colchicine have been presented in Table 1. Seeds obtained from C_1 generation of autopolyploids by selfing were collected and sown in fields for raising plants of C_2 generation on being cytologically investigated. Tetraploids and octoploids were raised in C_2 generation but hexa-decaploids were not. The comparative recordings of various morphological features (qualitative and quantitative) of control (diploid) as well as induced (polyploid) plants of C_1 generation have been presented in Tables 2 and 3. On observation plant height, girth, size of leaves, flower diameter, pollen fertility, seed setting were found to be reduced in autopolyploids in comparison with control (diploid) plants while, size of stomata, pollen grains, meiocytes and weight of seeds were found to be increased in tetraploids in C_1 and C_2 generations, in octoploids increase in C_1 and decrease in C_2 generation.

Plants were analysed for cytological studies and it was found that in many of them chromosome number changed. They became 4, 8 and 16n (Figs. 2, 3, 4) in C_1 and C_2 generation.

The controlled (diploid) plants revealed 17 bivalents and normal tetrads at diakinesis and metaphase I (Fig. 1). Cytological studies of colchicine treated plants revealed that some plants were tetraploids, octoploids and hexa-decaploids in C_1 generation. This was confirmed by the number of bivalents and chromosome numbers $X=34$, $X=68$ and $X=136$ (Figs. 2, 3, 4). Meiosis in autopolyploid plants of C_1 and C_2 generations indicated high degree of irregularities which improved in C_2 generation only in tetraploids.

In treated plants the chromosome associations were variable with an average of (8.0–31.6) univalents, (28.4–104.4) bivalents and (5.6–9.3) quadrivalents (Table 4) in all the autopolyploids.

Abnormalities such as lagging, bridges, stickiness and unequal separation of chromosomes were common in all the autopolyploids. This resulted in the formation of large number of sterile pollen grains. Pollen sterility was found to be proportional to the percentage of doses (Table 3).

Table 2. Comparative analysis of qualitative characters of diploid (control) and all the 3 autopolyploids of *H. annuus* var. *morden* of C_1 generation

Variables	Diploid	Tetraploids	Octoploids	Hexa-decaploids
Stem				
Colour	Dark green	Medium green	Medium green	Dark/medium green
Leaves				
At early stages	Non-variegated	Variegated	Non-variegated	Variegated
Colour	Dark green	Medium green	Medium green	Dark/medium green
Apex	Acute	Slightly acuminate	Acute/slightly acuminate	Acute/acuminate
Size	Large	Medium	Medium	Medium
Shape	Cordate	Ovate/ovate oblong	Ovate/oblong	Ovate/ovate oblong
Surface of leaves	Covered with stiff hairs	Covered with soft hairs	Covered with soft hairs	Covered with soft hairs
Flower				
Colour	Yellow	Pale yellow	Orangish yellow	Pale yellow
Bracts	Dark green	Medium green	Medium green	Medium green
Flowering	Early	Delayed	Delayed	Delayed
Growth rate	Fast	Slow	Slow	Slow

Table 3. Comparative morphology of autopolyploids of *Helianthus annuus* var. *morden* of C_1 generation

Variables	Diploids	Tetraploids	Octoploids	Hexa-decaploids
Height (cm)	75–96(87±4.2)	28–38(31±1.5)*	25–45(36±3.9)*	15–50(34±4.2)*
Girth (cm ²)	6–8(7±0.2)	2.4–3.5(2.8±0.02)*	2–3(1.5±0.2)*	2.5–6(3.7±0.61)
No. of internodes	5–8(6±0.6)	6–9(7±0.5)	5–8(6±0.6)	6–9(7±0.6)*
Length of internodes (cm)	12–16(14±0.7)	5–7(6±0.3)*	6–8(7±0.5)*	7–11(9±0.7)
Length of leaves (cm)	11–14(12±0.4)	6–8(6.5±0.15)	7–9(8±0.2)*	9–11(10±0.2)*
Breadth of leaves (cm)	8–13(10±0.7)	4–6(4.4±0.2)*	5–6(5.5±0.1)	6–7(6.5±0.1)*
Length/breadth ratio	1.4–1.6(1.5±0.03)	1.4–1.8(1.6±0.2)	1.4–4.4(2.2±0.6)	1.4–1.6(1.5±0.3)
Appearance of buds (days)	30–40(35±2.0)	40–55(48±2.5)*	40–55(48±3.0)	45–60(53±2.3)
Blooming period (days)	12–20(15±1.8)	15–20(17±1.02)*	19–25(21±1.0)*	16–27(21±1.87)*
Flower diameter (cm)	15–18(16±0.6)	6–8(7±0.34)	6–8(7±0.3)*	1.5–9(6±0.3)*
No. of seeds	990–1200(1097±3.6)	32–400(168±0.7)	16–200(90±3.3)*	13–500(242±1.3)*
Weight of 50 seeds	2.0–2.4(2.2±0.03)	2–3(2.5±0.1)	2.7–3.0(2.8±0.02)*	3–3.3(3.1±0.04)*
Seed viability	85–92(88±1.3)	10–65(40±0.8)*	9–50(30±0.8)*	30–65(51±0.7)*
Seed setting	76–92(86±2.2)	3–55(29±1.0)*	10–45(26±0.9)*	60–84(69±1.6)*
Length of stomata (μm)	20–22(21±0.4)	31–38(35±1.5)*	40–44(43±0.6)*	43–51(48±1.5)*
Breadth of stomata (μm)	14–15(13±0.06)	20–32(27±2.3)*	21–29(26±1.5)*	26–30(28±0.7)*
Size of pollen grains (μm)	28–30(29±0.4)	44–49(47±0.1)*	46–49(47±0.5)*	50–56(53±0.9)*
Pollen sterility (%)	5–21(16±0.4)	50–79(62±3.4)*	49–76(64±5.0)*	81–85(83±0.67)*
Life span (months)	3–4(3.6±0.6)	4.5–5(4.8±0.1) [#]	5–5(5±0.0)* [#]	3.5–5(4.6±0.3)* [#]

* Significant at 0.01 level. [#] Significant at 0.05 level. Range (mean±standard error).

Results indicate that there is a recovery of bivalents and decrease of univalents and quadrivalents per cell and other abnormalities from C_1 to C_2 generations in tetraploids. As a result of decrease in the frequency of abnormalities, the number of bivalents increases in succeeding generation which makes the way for normal separation of chromosomes (Table 4).

Discussion

The seedlings of var. *Morden* of sunflower were treated with different doses of colchicine. They reacted differently. Three types of polyploids were produced, e.g., tetraploids, octoploids and

Figs. 1–4. 1) Control (diploid) showing $2n=34$ chromosomes $\times 2000$. 2) Tetraploid showing $2n=68$ chromosomes $\times 1500$. 3) Octoploid showing $2n=136$ chromosomes $\times 1600$. 4) Hexa-decaploid showing $2n=272$ chromosomes $\times 1000$.

hexa-decaploids. Dhesi and Saini (1973) and Singh (1992, 1994) were successful in producing autotetraploids in sunflower (variety not mentioned).

The most significant change in the autopolyploid plants was the reduction in the height of plants than that of diploid plants. Generally the height of autopolyploid plants was reduced, e.g. *Nicotiana glauca* (Deshmukh and Pal 1950), *Capsicum* L. (Raghuvanshi 1984), *Sesamum indicum* (Mitra and Biswas 1984). However, on the contrary, in some cases, the height of plants was increased, e.g., *Cajanus cajan*, *Vigna unguiculata* cv. GC 82-7 (Patel and Verma 1999). Octoploids of *Datura stramonium* (Avery *et al.* 1959), *Petunia* (Levan 1940) were short stemmed. The reduction in the height of autopolyploids of sunflower might be due to decrease in length of internodes. According to Dhesi and Saini (1973) the height of autotetraploid plants of sunflower was half of the controlled plants. The girth of stem of treated plants was also reduced as compared to diploid plants. This may be due to reduction in the height of plants.

The leaves of these plants were small, thin and light/medium green in colour. This might be due to hormonal disbalance, Dhesi and Saini (1973) have recorded that the leaves of the induced plants were thick and dark green in colour. Length/breadth ratio of leaves was higher in these plants. Similar results were also observed by Dhesi and Saini (1973). Leaves of treated plants ex-

Table 4. Chromosomal abnormalities and associations in autopolyploids of *H. annuus* var. *morden* in C_1 and C_2 generations

Auto polyploids	Abnormalities						Associations			
	No. of PMCs observed	No. of abnormal PMCs	%	Stickiness %	Laggards %	Bridges %	Fragmentation %	Univalents	Bivalents	Quadrivalents
Control	25	—	—	—	—	—	—	—	17	—
C_1 Tetraploids	25	6	24	2 (33)	2 (33)	1 (17)	1 (17)	8.0 (0-20)	28.4 (15-34)	5.6 (0-15)
Octoploids	25	8	32	2 (25)	3 (38)	2 (25)	1 (13)	19.3 (0-60)	41.8 (20-64)	10.5 (0-25)
Hexadecaploids	25	13	52	4 (31)	5 (38)	3 (23)	1 (8)	31.6 (4-90)	104.4 (70-136)	9.3 (0-26)
C_2 Tetraploids	25	4	16	—	2 (50)	1 (25)	1 (25)	—	30.6 (27-34)	1.0 (0-4)
Octoploids	25	12	48	3 (25)	3 (25)	4 (33)	2 (17)	11.3 (0-30)	50.0 (30-64)	8.3 (0-19)
Hexadecaploids	—	—	—	—	—	—	—	—	—	—

Numerals mentioned in parenthesis represent in % value.

Table 5. Comparative study of tetraploid plants of C_1 generation produced by other workers

Variables	Presented observations (4n)	Observations of Dhesi and Saini (1973) (4n)	Observations of Singh (1992, 1994) (4n)
Genus	<i>Helianthus</i>	<i>Helianthus</i>	<i>Helianthus</i>
Species	<i>annuus</i>	<i>annuus</i>	<i>annuus</i>
Variety	<i>morden</i> (cultivated)	not mentioned (wild)	not mentioned (wild)
Conc. used	0.4–0.6%	0.25%	0.1–0.5%
Method of treatment	three times a day after the gap of 4 h	continuously for 6 h	8 h per day for 3 consecutive days
Frequency of tetraploid plants	2.86%	—	20%
Height	reduced to two third (2/3)	reduced to half (1/2)	—
Girth	reduced to two third (2/3)	reduced to half (1/2)	—
Length, breadth of leaves	reduced to two third (2/3)	reduced to half (1/2)	—
l/b ratio	little higher	little higher	—
Flower size	smaller	smaller	larger
Stomata	larger	larger	—
Stomata/field	less stomata	more	—
Pollen grains	larger	same as diploid	larger
Pollen fertility	reduced	reduced	reduced
No. of seeds/head	less	less	less
Seeds	larger and healthier	—	larger and healthier
Seed weight	more than diploids	—	more than diploids
Plant fertility	less	—	less
Maturity period	delayed	diploids and tetraploids mature at the same time	—

hibited certain peculiarities and deformities as well. The changes in leaves and increase in size of stomata could be due to chromosomal aberrations, disturbances in phytohormones and hormonal disturbances. This resulted in decrease in size of leaves. Frequency of stomata per field was increased. According to Dhesi and Saini (1973) stomata was larger in size and frequency of stomata per field was more in autotetraploids. In the present study, autopolyploid plants showed higher length/breadth ratio, bigger stomata and increased frequency of stomata per microscopic field, signifying the closeness of stomata. There was a significant increase in size of stomata, indicating a favorable response towards polyploidy.

The flowering was delayed. It took 3–5 months whereas controlled plants matured in 2–3 months. It may be due to change in metabolic activities reported by Biswas and Bhattacharya (1971). Lewis (1977) observed that slower growth generally brings later flowering, size of flowers was also reduced but Singh (1992, 1994) found that the size of flowers and its parts were large. The colour of flowers was also changed and it may be due to the physiological factors. Size of pollen grains was increased in all the autopolyploids. Singh (1992, 1994) also observed that the size of pollen grains was increased. However, Dhesi and Saini (1973) found no difference in the size of pollen grains of both control (diploid) and tetraploid plants.

In autopolyploids, seed setting and seed fertility were decreased but there was significant increase in seed weight. Genetic factors might be playing an important role in determining seed fertility in autopolyploids. It was also recorded by Singh (1992, 1994) that the seeds obtained from synthesized plants were slightly bigger and heavier than the seeds obtained from normal plants but the seed setting was reduced which showed considerable promise for improvement in their ornamental value through polyploid breeding. Autopolyploids took more time to mature due to slow growth. But according to Dhesi and Saini (1973) both diploids and autotetraploids matured almost at the same time.

The analysis of t-test has shown that the effect of colchicine on these characters was significant

(Table 3).

The autopolyploids exhibited slower growth rate than their respective diploids. Slower rate is a common feature in autopolyploid plants and has been attributed to number of factors such as reduced rate of cell division, low amount of growth hormones and lower rate of metabolic activities.

Cytological abnormalities such as stickiness, laggards, bridges unequal separation of chromosomes were more frequent. Stickiness might be due to disturbances in cytochemical balanced reaction as reported by Rao and Lakshmi (1980). Observed multivalents resulting from stickiness have been observed by Ghatnekar (1964) and others. According to him it might be due to reciprocal translocation. Lagging of single univalent was the most common phenomenon. The occurrence of more than 1 lagging chromosomes might be attributed in meiosis to hindrance of the prometaphase chromosome movement accompanied by adhesion of the centromeric to the adjacent inner surface of the plasma (Barthelmess 1957). Formation of bridges was primarily attributed to fusion between broken chromosomes rather than broken chromatids (Coldecott and Smith 1952).

These abnormalities in the behaviour of chromosomes may be responsible for reduced fertility and seed setting. Higher pollen sterility may have resulted from disturbed meiosis which is evident by unequal separation of chromosomes at anaphase I and bivalents at metaphase I. The lower fertility can also be anticipated due to environmental, physiological or genetic causes (Morrison and Rajhathy 1960, Gottschalk 1978). It appears that seed and pollen sterility in autopolyploids may be due to a number of factors of very complex nature. Precise causes is till not understood.

From the present investigation it appears that the induced autopolyploids of winter variety of sunflower exhibits improvement in its ornamental value through polyploid breeding. The reduced height of plants might result in better blooming and seed setting in tetraploids and octoploids. All autopolyploids may prove to be stronger than diploid plants.

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References

- Avery, A. J., Satura, S. and Blakeslee, R. J. 1959. The Genus *Detura*, Chronica Botanica, 20 Ronald Press, New York, 289 p.
- Barthelmess, A. 1957. Chemisch induzierte multipolare. Mitosen, Protoplasma **48**: 546–561.
- Biswas, A. K. and Bhattacharya, N. K. 1971. Induced polyploidy in legumes I *Cyamopsis psoraloides* D.C. Cytologia **36**: 469–479.
- Coldecott, R. S. and Smith, L. 1952. A study of X ray induced chromosomal aberrations in barley. Cytologia **17**: 224–242.
- Deshmukh, M. J. and Pal, B. P. 1950. A note on the tetraploid of *Nicotiana glauca*. Indian J. Genet. **10**: 72–77.
- Dhesi, J. S. and Saini, R. G. 1973. Cytology in induced polyploids in sunflower. Nucleus **16**: 49–52.
- Downes, R. W. and Marshall, D. R. 1983. Colchicine induced variants in sunflower. Euphytica **32**: 757–766.
- Ghatnekar, M. V. 1964. Primary effects of different mutagens and the disturbances induced in the meiosis of X_1 and X_2 of *vicia faba*. Caryologia **17**: 219–244.
- Gottschalk, W. 1978. Open problem in polyploid research. Nucleus **21**: 91–112.
- Gupta, S. A. and Roy, S. K. 1979. Colchiploid sunflower. Sci. Culture **46**: 294–295.
- Jackson, R. C. 1983. Colchicine induced quadrivalent formation in *Helianthus*. Evidence of ancient polyploidy. Theor. Appl. Genet. **64**: 219–222.
- Levan, A. 1940. The cytology of *Allium amplexen* and occurrence in nature of its asynapsis. Hereditas **26**: 359–394.
- Lewis, W. H. 1977. Temporal adaptation correlated with ploidy in *Claytonia virginica*. Syst. Bot. **1**: 340–347.
- Mitra, A. K. and Biswas, A. K. 1984. Induced Polyploidy in Sesames, *Sesamum indicum* L. In: Manna, G. K. and Sinha, U. (eds.). Perspectives in Cytology and Genetics **4**: 213–219.
- Morrison, J. W. and Rajhathy, T. 1960. Frequency of quadrivalents in autotetraploid plants. Nature **187**: 528–530.
- Patel, O. P. and Verma, R. C. 1999. Cytomorphology of induced Tetraploidy in *Cajanus cajan* and *Vigna unguiculata*. Cy-

- tologia **64**: 29–36.
- Raghuvanshi, R. K. 1984. Cytomorphological features of colchicine induced tetraploid and aberrants in chilli (*Capsicum* L.). *Perspective in Cytol. and Genet.* **4**: 209–212.
- Rao, N. B. and Lakshmi, N. 1980. Gamma rays induced meiotic abnormalities in *Capsicum annuum* L. *Caryologia* **33**: 509–518.
- Schwanitz, F. 1951. Untersuchungen an polyploiden pflanzen XI. Zur chlorophyllgehalt diploiden and polyploiden Pfl. *Zucht.* **21**: 30–36.
- Singh, R. N. 1992. Chromosomal abnormalities and fertility in induced autotetraploid *H. annuus* in C_1 and C_2 generations. *Cytologia* **57**: 277–282.
- 1994. Morphocytogenetical studies and pollen fertility of induced autotetraploid of *Helianthus annuus*. *Revista Biol (Lisboa)* **15**: 69–75.
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