Cytomorphological Studies of *Trigonella foenum-graecum* Autotetraploids in Three (C_1 , C_2 , C_3) Generation

I. D. Arya¹, S. Rama Rao² and S. N. Raina²

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Successful induction of polyploidy using colchicine is a well established phenomenon and still remains a potential cytological technique to induce numerical variations in plants. Since the discovery of Blakslee and Avery in 1937 about the chromosome doubling property of colchicine, enormous number of papers have been published on colchicine induced polyploidy. However, this technique was found to be more promising in popular ornamentals and vegetatively propagated plants. But, for some unknown reasons, induction of polyploidy in legumes was found rather difficult. A review of literature reveals that very few reports on successful induction of polyploidy in legumes (Sen and Chedda 1958, Biswas and Bhattacharya 1971, Singh and Roy 1971, Gupta and Gupta 1975, 1976, Raghuvanshi and Singh 1977, Singh and Gopal 1979, Srivastav and Raina 1982) are available in comparison to large number of reports on popular ornamentals Jalil *et al.* 1974, Arora 1975, Zadoo *et al.* 1975, Srivastav and Raina 1981, Rao *et al.* 1982).

Trigonella foenum-graecum (commonly known as “Methi” or “Fenugreek”) of family Leguminosae and subfamily Papilionaceae, is extensively grown in tropical and subtropical regions of India for its vegetable value and seeds which are medicinally important. The present study was undertaken in a view to study the cytomorphological features of *T. foenum-graecum* polyploids in successive generations and to explore the possibility of evolving a high yielding variety in terms of vegetable value and improved seed-set.

Material and methods

Seeds of *T. foenum-graecum* used for colchicization were obtained from Central Arid Zone Research Institute, Jodhpur.

For colchicization three methods viz. seed treatment, cotton-swab and cotyledonary leaves immersion methods were tried. The treatment was carried out for 12 or 18 hrs spread over 2 or 3 days with 0.15% colchicine (Sigma). For meiotic analysis buds were collected and fixed in iron acetic-alcohol overnight. Anthers stained in leucobasic-fuchsin were squashed in 1% aceto-carmin. Microphotographs were taken from temporary preparations.

Observations

Of the all the three methods employed, successful induction of polyploidy was achieved mainly by cotton swab method and partially by cotyledonary leaves immersion method (Table 1). Seed germinated after the treatment. Out of seventy seedlings treated by cotton-swab method with 0.15% colchicine for 12 or 18 hrs equally spread over three days, six were cytologically confirmed as autotetraploids. Only one out of 22 seedlings treated for 10 hrs by cotyledonary leaves immersion method was found to be autotetraploid on cytological analysis.

Swelling of the apical region of the seedlings was the immediate effect of colchicization,

¹ Department of Botany, University of Rajasthan, Jaipur-302004, India (Address for communication).

² Department of Botany, University of Delhi, Delhi-110007, India.

that was observed. In the expected autotetraploids the growth rate was drastically reduced in comparison to their diploids and the flowering was delayed by 2–3 weeks in the colchipooids.

Morphology, Cell Size and Growth Rate

A comparative morphological data of diploid and autotetraploids (C_0 , C_1 , C_2 and C_3) have been summarized in Table 2. Gigantism, a common feature exhibited by all the synthesized autotetraploids, is traced to increase in the cell size. This was reflected in significant

Table 1. Efficiency of colchicine (0.15%) in inducing polyploidy by seed treatment, cotton-swab and cotyledonary leaves immersion methods

Method	No. of days	Duration of treatment (in hours)	No. of seeds/seedlings treated	No. of seedlings survived	No. of colchit-tetraploids	Percentage colchit-tetraploids
Seedtreatment method	1	10	50	—	—	—
Cotton-swab method	3	18	50	39	4	12.90
	3	12	20	16	2	12.5
Cotyledonary leaves immersion method	1	10	22	16	1	6.25

Figs. 1–3. Comparison of autotetraploid (C_0) and diploid *T. foenum-graecum*. Tetraploid (left) and diploid (right) leaves (Fig. 1), flowers (Fig. 2) and floral parts (Fig. 3).

increase of the size of stomata and pollen grains. Plant height was reduced by 24.75 per cent. However, in subsequent generations the height increased and attained near normalcy in C_3 generation. Other parameters such as length and breadth of leaflets, stem thickness, size of flower etc. (Figs. 1–3) had pronounced effect after colchicine treatment. But some characters like number of seeds per plant, length of pod etc. have shown a sharp decline in number and size. But all the synthesized autotetraploids showed enhancement of their vegetative growth as expressed by increased number of branches (Table 2, Fig. 16), thick light green and bigger size leaves (less amount of leaf chlorophylls, Table 2), and of thick stem.

Cytology

The most common situation in the diploid was 8 II (Fig. 4, Table 3). However, some cells had 0–2 univalents. The average number of associations per cell was $7.8 \text{ II} + 0.36 \text{ I}$. The number of chiasmata per cell ranged from 9–16 and the mean number was 12.81 out of which 10.81 were terminalized giving terminalization coefficient as 0.84.

Cotton-swab method was found more useful for successful induction of polyploidy, though one autotetraploid was isolated from the plants treated by cotyledonary leaves immersion method (Table 1). All the six synthesized autotetraploids (C_0) were characterized by the presence of a mixture of quadrivalents, trivalents, bivalents and univalents. However, the average bivalent number was higher in the colchipooids. The average number of associations per cell was $0.55 \text{ IV} + 0.44 \text{ III} + 13.77 \text{ II} + 0.88 \text{ I}$, range being 0–2, 0–2, 10–16 and 0–4 respectively (Fig. 7, Table 3). The quadrivalents were either ring or chain type. Out of 40 cells analysed 32 (80%) had quadrivalents and 21 (52.5%) had trivalents. Number of chiasmata ranged from 18–26, mean being 22.77 out of which 18.80 were terminalized giving

Table 2. Morphological features of diploids and corresponding tetraploids

	2x	C_0	C_1	C_2	C_3
Germination (%)	98.00	—	83.8	88.4	91.3
Height of the plant (cm)	40.18	30.12	38.72	39.01	40.21
Number of branches/plant	5	2	4	8	8
Leaf texture	Thin	Thick	Thick	Thick	Thick
Length of odd leaflet (cm)	2.67	2.94	3.32	3.12	3.20
Breadth of odd leaflet (cm)	1.82	1.85	2.39	2.45	2.45
Number of stomata/unit area	7	4	4	5	5
Length of stomata (μ)	31.04	35.72	34.01	33.98	34.01
Breadth of stomata (μ)	23.71	22.45	20.14	21.58	21.64
Pollen stainability (%)	92.67	67.10	71.22	76.31	79.01
Length of pod (cm)	10.05	4.10	4.81	4.72	5.52
Seed/Pod	15.51	1.66	2.18	2.50	3.02
Total leaf chlorophyll (mg/gm fresh wt.)	1.641	—	—	1.445	1.509
Leaf chlorophyll a (mg/gm fresh wt.)	1.268	—	—	1.140	1.147
Leaf chlorophyll b (mg/gm fresh wt.)	.3737	—	—	.36	.3624
Total leaf proteinins (mg/gm fresh wt.)	24.00	—	—	26.00	26.00

terminalization coefficient of 0.82 (Table 3). At anaphase I, 11 out of 25 cells (44.0%) had equal (16: 16) distribution of chromosomes (Fig. 10) followed by 15: 17, 14: 18, 13: 19 in 6, 3 and 2 cells, respectively (Table 4). One cell (4%) each had 14: 17 and 15: 16 distribution with one univalent lagging behind and another one cell (4%) had 14: 16 with one bivalent lagging behind (Fig. 10, Table 4).

The synthesized colchitetraploids (C_0) were analysed in further three generations (C_1 , C_2 , C_3). In C_1 plants no significant increase/decrease of quadrivalents, bivalents and univalents average was observed while there is a notable decline in the trivalent average from 0.44 to 0.31 per cell (Table 3). The average number of associations per cell in C_1 plants was $0.55 \text{ IV} + 0.31 \text{ III} + 13.52 \text{ II} + 0.82 \text{ I}$, range being 0–3, 0–1, 8–14 and 0–4 respectively (Fig. 8). Number of chiasmata ranged from 19–29, mean being 23.85 out of which 20.34 were terminalized giving terminalization coefficient of 0.85 (Table 3). At anaphase I majority of the cells (57.1%) had equal (16: 16) distribution of chromosomes (Fig. 11). The remaining cells had 15: 17 (22.8%), 14: 18 (5.7%), 13: 19 (2.8%) distribution of chromosomes. However, 2 cells (5.7%) showed

the presence of 1 lagging univalent and two cells (2.8%) had 1 lagging bivalent (Table 4).

In C_2 generation the quadrivalent average was higher in comparison to both C_0 and C_1 plants (Fig. 9, Table 3), while the trivalent average had shown a significant further decrease

Figs. 4-15. Cytology of diploids and autotetraploids. 4-6: Diploids. 4-5, metaphase I, 8 II. 6, anaphase I, 8: 8. 7-15: Autotetraploids (C_0 , C_1 , C_2 , C_3). 7-9, metaphase I. 7, 3 IV+8 II+4 I. 8, 16 II. 9, 1 IV+2 III+10 II+2 I. 10-14, anaphase I. 10, 16: 16. 11-13, 17: 15. 14, anaphase I showing late disjunction. 15, anaphase II showing laggards.

Table 3. Mean number of associations and chiasmata per cell in diploid, C_0 , C_1 , C_2 and C_3 plants

Ploidy	Genera- tion	Chiasmata					Quadrivalents		Trivalents		Bivalents		Univalents	
		Mean	Range	Termi- nized	Un- termi- nized	Termi- nization coefficient	Mean	Range	Mean	Range	Mean	Range	Mean	Range
2x		12.81	9-16	10.81	2.0	0.84					7.8	7-8	0.36	0-2
4x	C_0	22.77	18-26	18.80	3.97	0.82	0.55	0-2	0.44	0-2	13.77	10-16	0.88	0-4
4x	C_1	23.85	19-29	20.34	3.57	0.85	0.55	0-3	0.31	0-1	13.52	8-14	0.82	0-4
4x	C_2	24.61	20-31	21.46	3.15	0.87	0.63	0-4	0.23	0-2	14.31	7-13	0.81	0-3
4x	C_3	23.92	19-28	20.96	2.96	0.87	0.71	0-4	0.21	0-1	14.36	9-14	0.70	0-2

from 0.31 to 0.23 per cell. The average number of associations was $0.63 \text{ IV} + 0.23 \text{ III} + 14.31 \text{ II} + 0.81 \text{ I}$, range being 0–4, 0–2, 7–13 and 0–3 respectively. The number of chiasmata per cell ranged from 20–31 and the mean was 24.61 out of which 21.46 were terminalized giving

Table 4. Anaphase I distribution of chromosomes

Generation	No. of cells analysed	Chromosome distribution	No. of cells	Percentage
C ₀	25	16: 16	11	44.0
		15: 17	6	24.0
		14: 18	3	12.0
		13: 19	2	8.0
		14: 1U ⁺ : 17	1	4.0
		15: 1U: 16	1	4.0
		14: 1B ⁺⁺ : 16	1	4.0
C ₁	35	16: 16	20	57.1
		15: 17	8	22.8
		14: 18	2	5.7
		13: 19	1	2.8
		14: 1U: 17	2	5.7
		15: 1B: 14	1	2.8
		14: 1B: 16	1	2.8
C ₂	34	16: 16	19	55.8
		15: 17	9	26.4
		14: 18	2	5.8
		15: 1U: 16	3	8.8
		14: 1B: 16	1	2.9
C ₃	24	16: 16	12	50.0
		14: 18	5	20.8
		13: 19	4	16.6
		14: 1U: 17	3	12.8

U⁺ = Univalent

B⁺⁺ = Bivalent

Fig. 16. Gross morphology: *T. foenum graecum* of autotetraploid and diploid plant (2 plants in one pot). Tetraploid C₃ generation (left) and diploid (right).

terminalization coefficient of 0.87 (Table 3). At anaphase I, 19 out of 34 cells analysed had equal (16:16) distribution of chromosomes (Table 4). The remaining cells had either unequal distribution or lagging bivalent or univalent (Table 4).

The C_3 plants had shown a sharp increase in quadrivalent average, even when compared to the control (C_0) plants (Table 3), while the trivalent average was further decreased to 0.21 per cell. The average number of associations was $0.71 \text{ IV} + 0.21 \text{ III} + 14.36 \text{ II} + 0.70 \text{ I}$, range being 0–4, 0–1, 9–14 and 0–2 respectively. The chiasmata per cell ranged from 19–28 and the mean was 23.92 out of which 20.96 were terminalized giving terminalization coefficient of 0.87 (Table 3). At anaphase I, 12 out of 24 cells (50%) analysed had equal (16:16) distribution of chromosomes. The remaining cells had either 14:18 or 13:19 distribution. 3 cells showed the presence of 1 univalent lagging behind (Table 4).

Precocious disjunction of quadrivalent or bivalents or of both might have resulted in the occurrence of trivalents and univalents, which were observed in the present investigation.

Pollen stainability: A drastic reduction of pollen stainability from 92.67% in diploids to 67.10% in C_0 plants was recorded. But it showed an upward trend in C_1 (71.22%), C_2 (76.31%) and C_3 (79.01%) generations (Table 2).

Seed set: Only 5 seeds were recovered from C_0 plant against 100–150 seeds collected from diploid. However, in further generations the seed set/plant was considerably improved and in C_3 about 80 seeds per plant were collected. The size of the seeds was bigger in autotetraploids in comparison to diploids.

Discussion

A review of earlier literature shows that successful attempts have been made to raise higher polyploids and to study their cytomorphological details in *Trigonella foenum-graecum* (Roy and Singh 1968, Singh and Roy 1971, Raghuvanshi and Singh 1977, Singh and Singh 1973, 1977, Singh and Jai Gopal 1979). The present work aims, not only at the production of polyploids but also to establish them in order to develop a variety which will be of improved economic value. A selected cultivar of *T. foenum-graecum* was colchicized to synthesize an autotetraploid and it was maintained through three successive generations. A detailed cytological analysis has been carried out (Table 3, Figs. 7–15) in each generation and germplasm has been multiplied.

The present data shows (Table 1) that for successful induction of polyploidy, cotton-swab method is most suitable. It has been observed, as reported earlier by Raghuvanshi and Joshi (1965), that the seed treatment was always met with complete failure. Similar observations were made in a number of legumes by different authors (Sen and Vidyabushan 1960, Gupta and Gupta 1975, Srivastav 1983). In this case the present authors are in complete agreement with Bates (1939) and Srivastav (1983) that a check on root development and/or germination results in the failure of seed-treatment method to induce autotetraploidy. Though partial success was achieved by cotyledonary leaves immersion method, it is not much useful because percentage of success is as low as 6.25.

The reduced growth rate and over-all development of the plants in C_0 generation which appeared to gain normalcy through C_1 , C_2 and C_3 generations, is a common feature associated with autopolyploids and is reported in a number of plants (Chandler and Barton 1955, Zadoc *et al.* 1975, Raghuvanshi and Singh 1977, Srivastav 1983). The causative factors are presumed to be the reduced rate of cell division (Wettstein 1924, Eigsti 1947) and reduction in the rate of metabolic activities (Larsen 1943, Schwanitz 1951). A sharp increase in the size of stomata, both in length and breadth, is attributed to the increase in the cell size. However, in successive generations of C_1 , C_2 and C_3 it has shown a slight decrease when compared to C_0 plants but

still they were bigger than their putative diploids. It is generally believed that an increase in the stomatal size will result in the decrease of their number per unit area (Rao *et al.* 1982, Srivastav 1983). This phenomenon was observed in the present material also. The C_0 plants on average had only 4 stomata against 7 in diploids, per unit area. But, interestingly the number raised to 5 in C_2 and C_3 generations.

The *T. foenum-graecum* colchipooids in C_0 generation have shown a pronounced gigantism which was retained up to 70% even in C_3 generation. An overall enhancement of different vegetative characters viz. length and breadth of leaflet, apart from texture and colour of the leaf, must be the reason for gigantism. Such retention of gigantism by the colchipooids even in C_3 generation is viewed as an advantageous phenomenon as *T. foenum-graecum* is more useful for its vegetative parts.

In case of autotetraploid plants, the basic genome is represented four times and hence it is generally expected that the chromosomal association in them should be in the form of all quadrivalents. As a result the mean number of quadrivalents per cell must be higher than any other type of associations in the cell. But in the present material, the mean number of quadrivalents is much lower than expected and bivalents outnumbered all other types of associations. The mean number of quadrivalents in C_0 plants was 0.55 and range was 0–2. Similar observations were made in variety No. 2 of *Trigonella foenum-graecum* (Raghuvanshi and Singh 1977), *Tephrosia wallachii* (Srivastav 1983) and in some cereals and grasses (Morrison and Rajhathy 1960). Some authors (Armstrong and Robertson 1956, Morrison and Rajhathy 1960) reported that plants with larger chromosomes have higher quadrivalent frequency, when raised to higher ploidy level, than the plants having smaller chromosomes. Contrary to this, Kostoff (1940) reported that plants with smaller chromosomes have a tendency to form fewer quadrivalents. The present data on *T. foenum-graecum* colchipooids supports the view forwarded by the former authors. This is because a discrete karyotypic analysis reveals that *T. foenum-graecum* has relatively larger chromosomes but only fewer quadrivalents were observed at MI of meiosis. The lower quadrivalent frequency may be due to small heterozygosity in the original material (Srivastav 1983) or localized chiasmata (Levan 1940) or genetic control (Riley and Chapman 1958, Jauhar 1975, Raghuvanshi and Singh 1977) and also the number of points of pairing initiation. In the case of present material, though a clear explanation cannot be given, it is presumed that probably a combined effect of all the above factors might have resulted in low frequency of quadrivalents. In C_0 plants it is observed that the chiasma frequency per cell was always lower and never equalled to twice to that of their putative diploids. The presence of trivalents and univalents in the material (Table 3) is believed to be the reason for the drop in chiasma frequency. Similarly the terminalization coefficient (T.C.) which was 0.84 in diploids was decreased to 0.82 in C_0 plants. But it has shown an upward trend in successive generations. The difference, between C_0 and successive generations, in the terminalization coefficient value is believed to be more pertaining to chromosomal than genic in nature.

Despite the higher bivalent frequency and fewer quadrivalents per cell, the chromosomal distribution at AI was very much affected and in C_0 almost 55% of the cells had unequal distribution (Table 4). However, in further generations (C_1 , C_2 , C_3) more number of cells had equal distribution of chromosomes. Trivalents and univalents, though they are few in number, are much prone for misdisjunction (Srivastav 1983) and they result in the unequal distribution of chromosomes. However, despite the low mean and average of trivalents and univalents, it is not known why C_3 plants had (Table 4) more number of cells with unequal distribution of chromosomes at AI and produced more seeds in comparison to C_1 and C_2 .

The reduced seed set in C_0 plants is believed to be the result of several meiotic errors viz. misdisjunction, unequal distribution of chromosomes at AI etc. The same reasons are expected to have influenced and decreased pollen stainability in C_0 plants.

Though the quadrivalent frequency per cell has shown a nominal increase (from 0.5 to 0.7), the sharp upward trend (from 13.77 to 14.36) in the average of bivalents per cell, from C_0 to C_3 is more significant feature to be noted. This type of "diploidization" in succeeding generations, observed in the present colchitetraploids, is a common phenomenon in most of the plants (Aastveit 1958, Sjodin and Ellerstrom 1969) while a case opposite to this is reported in *Phlox drummondii* by Raghuvanshi and Pathak 1975.

Summary

Induction of autotetraploidy has been achieved in *Trigonella foenum-graecum* ($2n=16$) by cotton-swab method using 0.15% colchicine. The synthesized colchipooids (C_0) were maintained for three successive generations (C_1 , C_2 , C_3). Detailed morphocytological analysis has been carried out in each generation. A distinct enhancement in different vegetative parts was observed in the autotetraploids making them economically more useful. The 5 seeds collected in C_0 generation were multiplied and as many as 200 seeds were collected in C_3 generation.

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