Cytomorphological Studies in Triploid ($2n=3x=39$) Plants in Cultivated Tetraploid ($2n=4x=52$) Cottons

S. S. Mehetre and M. V. Thombre

All India Co-ordinated Cotton Improvement Project,
Mahatma Phule Krishi Vidyapeeth, Rahuri,
Agricultural University, Rahuri 413 722
Dist. Ahmendnagar, Maharashtra State, India

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Triploids are the individuals with three basic genomes. Triploids in various crop species have been reported since 1908. Gates (1908) reported triploid for the first time in *Oenothera*, while Skovsted (1933) found a triploid plant in Asiatic cotton with various chromosomal abnormalities.

Triploids from diploids are commonly derived through the failure of reduction in meiosis at either pole leading to fertilization of unreduced gamete. Amin (1940) and Iyengar (1944) reported formation of unreduced eggs in self sterile hybrids of *Hirsuta* and *Herbacea* species of *Gossypium*. Unreduced gamete thus formed when fertilized with normal reduced gamete would give rise to triploid ($3x=39$) in diploid ($2x=26$) cottons but hexaploid ($6x=78$) in tetraploid ($4x=52$) cotton. This possibility has however, been eliminated from the occurrence of $2n=3x=39$ chromosomes in these triploids. The detailed observations on cytological and morphological characters in triploid plants were recorded and the probable origin of these plants is discussed.

Materials and methods

For cytological analysis young flower buds of all these plants and their respective parents were fixed in Carnoy's fluid (6:3:1) and squashed in 1% acetocarmine. Pollen sterility was tested with differential strain (Alexander 1969) cytological analysis was made from temporary preparations. The pollen germination tests were carried out as per Iyengar (1939).

The detailed observations on various morphological characters in both the parents and F_1 hybrid were recorded and are given in Table 1.

Results and discussion

Morphological characters

The means and coefficients of variation for characters like plant height, number of sympodia, internodal length, leaf area, petiole length, stomata number, boll diameter, boll weight, lint and seed weight, mote number and seed cotton yield were worked out. Considerable reduction for these characters was observed as compared to their tetraploid parents. The characters like internodal length, leaf area and lint

Table 1. Mean values and coefficients of variation studied in tetraploid ($2n=4x=52$) and triploid ($2n=3x=39$) plants of *G. hirsutum* and *G. barbadense* cottons

Characters	Acala glandless		Empire glandless		MCU-5		Laxmi		<i>G. barbadense</i>	
	4x=52	3x=39	4x=52	3x=39	4x=52	3x=39	4x=52	3x=39	4x=52	3x=39
Plant height	122.00 (20.13)	17.00 (—)	115.00 (18.19)	69.00 (—)	113.00 (21.16)	69.50 (—)	139.00 (19.85)	79.00 (—)	151.00 (21.18)	71.00 (—)
No. of sympodia	6.40 (9.17)	— (—)	9.80 (13.12)	— (—)	6.20 (20.13)	— (—)	5.20 (7.18)	7.00 (—)	4.2 (6.39)	7.00 (—)
No. of monopodia	3.00 (10.19)	— (—)	26.0 (17.19)	— (—)	6.90 (9.17)	— (—)	6.10 (12.20)	4.00 (—)	3.00 (7.17)	1.00 (—)
Internodal length, cm/internode	4.30 (15.05)	5.98 (26.74)	6.72 (12.67)	5.80 (13.21)	5.80 (11.62)	4.13 (32.58)	6.21 (12.10)	4.80 (27.10)	5.22 (17.0)	3.10 (22.89)
Leaf area, s. cm/leaf	133.65 (11.56)	68.59 (23.94)	178.65 (8.73)	104.65 (17.71)	119.63 (5.40)	89.53 (21.56)	175.68 (13.39)	99.12 (2.11)	212.10 (14.28)	14.11 (12.17)
Petiole length, cm/petiole	10.43 (14.13)	12.25 (9.46)	12.50 (4.96)	10.70 (10.65)	12.80 (2.03)	10.65 (12.76)	12.10 (18.17)	7.22 (7.11)	13.20 (7.19)	9.81 (10.12)
Stomata size, microns/stoma	39.16 (8.83)	39.32 (3.16)	34.26 (13.85)	34.48 (7.18)	32.33 (18.70)	29.16 (7.18)	36.12 (7.89)	34.20 (4.11)	39.30 (16.27)	32.11 (11.19)
Boll diameter (cm)	3.65 (2.25)	2.71 (3.56)	3.60 (2.10)	2.10 (2.61)	3.77 (4.50)	2.81 (13.13)	3.78 (1.89)	2.98 (6.27)	3.50 (1.41)	2.12 (3.12)
Boll number	24.20 (11.32)	10.00 (—)	27.30 (10.19)	7.00 (—)	34.10 (9.89)	11.00 (—)	29.15 (12.82)	4.00 (—)	21.00 (13.11)	3.00 (—)
Boll weight (g/boll)	3.47 (8.17)	2.52 (—)	3.49 (7.19)	2.83 (—)	— (10.13)	1.89 (—)	3.50 (7.89)	1.80 (—)	3.00 (10.12)	1.12 (—)
Seed weight (g/boll)	1.66 (3.31)	0.61 (14.18)	1.61 (10.55)	0.81 (16.15)	1.74 (11.79)	0.71 (25.19)	1.20 (1.89)	0.78 (—)	1.09 (2.11)	0.30 (—)
Lint weight (g/boll)	2.8 (7.32)	1.91 (—)	2.88 (9.17)	2.02 (—)	— (8.19)	— (8.17)	2.30 (8.12)	1.02 (—)	1.91 (9.27)	0.60 (—)
No. of seeds/boll	27.20 (6.19)	10.90 (—)	26.90 (5.12)	9.50 (—)	28.20 (7.19)	9.56 (—)	26.30 (7.10)	8.02 (—)	24.20 (6.89)	5.50 (—)
No. of motes/boll	— (—)	5.10 (4.12)	— (—)	8.20 (—)	— (—)	7.20 (3.11)	— (—)	20.12 (—)	— (—)	18.00 (—)
Yield per plant (gm)	135.00	20.00	80.10	15.00	160.00	20.00	165.00	6.5	69.00	4.00

weight per boll and boll diameter were found to be more variable in triploids. While petiole length and number of stomata per unit area in triploids were relatively less variable as compared to their parents (Table 1). All the plants were peculiar due to their smaller leaves, nondehiscent anthers, slower growth and reduced vigour.

It is obvious that loss of thirteen chromosomes led to changes in gross morphology of the plant, inducing partial sterility and reduction in plant vigour. These characters appear to be governed by polygenes present in *G. hirsutum* and *G. barbadense*. Abrupt changes in gross morphology of cotton haploids have been reported (Chaudhari 1976) due to loss of one complete set of chromosomes.

Cytological observation

All the triploid plants isolated from field populations of commercial varieties of *G. hirsutum* and *G. barbadense* cottons and X-ray treated material were studied cytologically and observations on behaviour of chromosomes during meiosis are given in Table 2.

Table 2 indicates that the parents showed normal meiosis and 26^{II} at diakinesis and metaphase I and latter stages upto pollen formation were completely normal. On an average $19.0^{II} + 1.0^I$, $2.3^{III} + 16.0^{II} + 0.1^I$ and $0.9^{III} + 18^{II} + 0.3^I$ with 1.17, 1.77 and 1.67 chiasmata were observed in triploids e. g. Acala glandless, Empire glandless and MCU-5 glandless respectively at M-I. While in Giza-45 (*G. barbadense*) and Laxmi (*G. hirsutum*) mean chromosome configurations of $0.2^I + 18.2^{II} + 0.8^{III}$ and 0.8^I , $0.17^{II} + 14^{III}$ with 1.57 and 1.49 chiasmata respectively while at metaphase I it was $0.2^I + 18.5^{II} + 0.6^{III}$ and $1.1^I + 17.3^{II} + 2.11^{III}$ with 1.47 and 1.46 chiasmata respectively. Only one PMC had at metaphase I one hexa and one octavalent in Empire glandless triploid (Fig. 14) which might be due to chromosome stickiness as it did not persist further. Chromosome separation at anaphase I in all the triploids were normal, no bridges, fragments or laggards were observed except unequal separation of chromosomes at each pole (Table 3). Only bipolar spindles were observed. Univalents did not divide. Second meiotic division was normal however, tetrads and in a few cases triads were observed (Fig. 17). Pollens formed were variable in size (Table 3). The pollen fertility and pollen germination in these plants were very low as compared to normal plants.

Complete loss of chromosome complement from normal tetraploids or diploids resulting in haploids have been reported in various crop species (Kimber and Riley 1963). Such reduction in chromosome number may occur due to parthenogenesis. However, loss of complement of thirteen chromosomes from tetraploid species ($2n=52$) as has been reported here appears to be rare in *G. hirsutum* and *G.*

Figs. 1-17. 1, metaphase I with 26^{II} and 2, normal fertile pollen (Acala glandless, ($4x=52$). 2-7: Meiosis in Acala glandless triploid ($3x=39$). 3, diakinesis with $19^{II} + 1^I$. 4, metaphase I with $19^{II} + 1^I$. 5, late metaphase showing precocious separation of univalents. 6, anaphase I with unequal separation. 7, fertile and sterile pollen grains. 8-11: Meiosis in MCU-5 triploid ($3x=39$). 8, diakinesis with one trivalent. 9, metaphase I with $18^{II} + 3^I$. 10, metaphase I with $18^{II} + 3^I$. 11, fertile pollens with size variations. 12-17: Meiosis in Empire glandless triploid ($3x=39$). 12, late diakinesis with $17^{II} + 5^I$. 13, precocious separation. 14, metaphase I with occasional hexa and octavalent. 15, anaphase I with unequal chromosomes. 16, anaphase II with unequal separation. 17, abnormal sporads with three and normal sporads four microspores.

Figs 18–28. 18–25: Meiosis in tetraploid ($4x=52$) and triploid ($3x=39$) Laxmi. 18, metaphase with 26^{II} (tetraploid Laxmi). 19, diakinesis showing $18^{II}+1^I$. 20, meiosis in triploid Laxmi. Late diakinesis with $3x=39$ chromosomes. 21, metaphase I with $3^{III}+11^I+8^I$ (polar view). 22, metaphase I $1^{III}+8^{II}$ (side view). 23, early anaphase with two laggards. 24, anaphase showing normal separation of chromosomes. 25, sterile and fertile pollens with size variations. 26–28: Meiosis in *G. barbadense* tetraploid ($4x=52$) var. Giza-45. 26, metaphase I with 26^{II} . 27, anaphase I with normal separation. 28, normal fertile pollens.

barbadense plants.

The triploid plants ($2n=39$) are commonly derived through the failure of reduction in meiosis, leading to formation of $2n$ gamete. Amin (1940) and Iyengar (1944) reported formation of unreduced eggs in self sterile hybrid of *Hirsuta* and

Figs. 29–37. 29–32: Meiosis in triploid ($3x=39$) *G. barbadense* var. Giza-45. 29, diakinesis with paired and unpaired chromosomes. 30, metaphase I with $2.0^{III}+16.0^{II}+1.0^I$. 31, anaphase I with unequal separation of univalents. 32, sterile and fertile pollens. 33, metaphase I with $19.0^{III}+1.0^I$ (Laxmi triploid). 34, sterile and fertile pollens (Laxmi triploid). 35, diakinesis with paired chromosomes ($2n=39$, Giza-45). 36, anaphase I with unequal separation ($2n=39$, Giza-45). 37, sterile and fertile pollen ($2n=39$, Giza-45). All chromosomes magnified at $950\times$, sporads $450\times$ and pollen grains at $450\times$.

Herbacea species of *Gossypium*. Unreduced gamete thus formed when fertilized with normal (reduced) 'n' gamete should give rise to triploid ($3x=39$) in diploid, ($2n=26$) but hexaploids ($6x=78$) in tetraploid ($4x=52$), cottons. This possibility has, however, been eliminated in the present studies. These triploids might have originated from splitting of spindles resulting in super-reduction (Darlington 1937).

Such cases have been reported in *Festuca-Lolium* derivatives (Darlington and Thomas 1937) and raw polyploid species and polyploid endosperm of *Lilium* (Brock 1954). Clark (1940) reported that spindle splitting can be induced by UV treatment.

Super-reduced gametes arise either through formation of three nuclei with $n+n+2n$ chromosome at the end of first meiotic division due to the formation of tripolar spindles and misdivision at anaphase I and II leading to four haploid and two diploid microspores or through $n+3n$ misdivision at either of two nuclei during anaphase I resulting in one haploid ($n=13$) and one triploid ($3n=39$) and two diploid ($2n=26$) microspores (Fig. 1). Haploid ($n=13$) microspores thus formed by either

Diagram 1. Possible origin of triploid ($2n=3x=39$) in normal tetraploid ($2n=4x=42$) *G. hirsutum* and *G. barbadense* cottons due to abnormal chromosomal separation at anaphase I and II. Fertilization of normal ($2n=26$) gamete by exceptional gamete ($n=13$) may result in plants with $2n=3x=39$ chromosomes. A, occurrence of three nuclei with $n+n+2n$ chromosomes at the end of the first meiotic division due to tripolar spindle formation. This misdivision at anaphase I and II lead to four haploid ($n=13$) and two diploid ($2n=26$) microspores. B, first meiotic division is normal. Misdistribution of $n+3n$ in one of two nuclei in anaphase II results in one haploid, ($n=13$) one triploid ($3n=39$) and two diploid ($2n=26$) microspores.

or both the process when fertilize with normal diploid ($2n=26$), gamete, triploid ($3n=39$) embryo is formed. The triploids under discussion thus might have been formed from fertilization of reduced ($n=2x=26$) gamete by super-reduced gamete ($n=x=13$). In the PMCs of the parental varieties of these triploids twenty six perfect bivalents were observed and meiosis was normal. In *G. hirsutum* triploids viz., Acala, Empire glandless, MCU-5 and Laxmi, the average chromosome associations of 1^I+19^{II} , $0.3^I+18.0^{II}+0.9^{III}$, $1.6^I+16.0^{II}+1.8^{III}$, $0.3^I+18.0^{II}+0.9^{III}$ and $1.1^I+17.3^{II}+0.11^{III}$ were observed respectively, while *G. barbadense* triploid formed $0.2^I+18.5^{II}+0.6^{III}$. Thus it is observed that there was normal bivalent formation

in all these cases. More than one univalent observed in these cases might be due to the precocious separation. The occasional trivalent observed indicated segmental homology of the chromosomes.

The unequal separation of univalents ranging from 19–20 and 11–28 at each pole might be due to precocious separation or trivalents as a whole passing to either of poles in resulting on abnormal pollen with low fertility.

In the triploids of Asiatic cotton ($3x=39$) reported by Skovsted (1933) number of multivalents were observed which were expected due to addition of thirteen chromosomes to the normal complement of twenty six chromosomes. The present case of triploids in *G. hirsutum* and *G. barbadense* cotton however, appears to be different.

Summary

Morphologically distinct plants were noted in M_2 generation of *G. hirsutum* varieties Acala glandless, Empire glandless, MCU-5 glandless and Laxmi and one in *G. barbadense* var. Giza-45. Meiosis in these plants indicated that the PMCs contained ($2n=3x=39$) chromosomes. From the behaviour of $3x=39$ chromosomes, it is suggested that the loss of 13 chromosomes in these plants is not only from A or D genome alone but few chromosomes from both the genomes. Splitting of spindles resulting in super-reduction might have caused triploidy in these plants. The pollen grains formed showed high size variation and low fertility. Pollen germination test indicated pollen sterility. Significant differences were observed in triploids for all morphological characters when compared with their parents which might be due to the deletion of thirteen chromosomes. Thus, these characters appeared to be governed by polygenes present in both A and D genomes.

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