# Cytogenetical Investigations in Colchicine-induced Tetraploids of *Cyamopsis tetragonoloba* L.

Sangeeta BEWAL[1], Jigyasa PUROHIT[1], Arun KUMAR[1], Rajkumari KHEDASANA[2] and Satyawada Rama RAO[2]

[1]Cytogenetics and Molecular Biology Laboratory, Department of Botany, Jai Narain Vyas University, Jodhpur, Rajasthan, India; [2]Department of Biotechnology and Bioinformatics, North-Eastern Hill University, Shillong, Meghalaya, India

**Abstract**: Successful induction of autotetraploidy has been achieved in five accessions of *Cyamopsis tetragonoloba* L. The diploid seedlings of these accessions were treated with different concentrations of aqueous colchicine using the cotton-swab method for 10–18 hours within 2–3 days. The highest percentage of success was recorded when the seedlings were treated with 0.2% colchicine for 10 h within two days. The synthesized plants showed remarkable enhancement in several morphological and floral characters making them more robust and better plants from the food and feed aspect. Cytologically, quadrivalent frequency ranging from 3.18 to 5.45 and univalent frequency ranging from 0.08 to 1.10 were characteristic of the colchicine-induced tetraploids. Among all the associations, bivalent chromosome associations were observed more frequently (2.95 to 6.04). The anaphase I and II disjunction of bivalents/chromosomes was leading more or less regularly and equally to the formation of at least few seeds from some of the synthesized plants. Significant enhancement in morphological traits as revealed in colchicine-induced tetraploid plants having a better food and feed value and normal meiotic behaviour of synthesized autotetraploids leading to a good seed set may ultimately result in the genetic improvement of *Cyamopsis tetragonoloba*.

**Keywords**: cluster bean; genetic improvement; induced polyploidy; meiotic behaviour; quadrivalents

*Cyamopsis tetragonoloba* (cluster bean or guar) of the family *Fabaceae* is a multipurpose crop adapted well to arid/semiarid soils and preferring hot dry climatic conditions (Singh *et al.* 2002). It is a native of India and is grown principally for its green leafy fodder and pods that are used as food and feed besides as a soil-enriching crop legume. It is considered as a viable alternative crop for moth bean (*Vigna aconitifolia*) in the arid and semi-arid areas where it is grown. Guar is a coarse, upright, bushy, drought-resistant summer annual plant, ranging from 2 to 9 feet in height (both dwarf and tall cultivars are grown). Guar is a self-pollinated crop. Guar as a legume also helps to increase soil fertility, fixing atmospheric nitrogen for its own need and also acts as green manure for subsequent crop(s). Guar has attained the status of commercial crop since it supplies animal feed, industrial gum and recently it has also become popular as a new source of proteins to meet the nutritional requirement of the human population. Various industrial applications of guar powder are food, pet food, nutritional products and pharmaceuticals, personal care products, household products, paints, textiles and carpets, mining and flocculation, oils, gas and

other deep well operations, manufacture of paper, building and construction products, explosives, foundries and ceramics, industrial cleaners and related formulations, agricultural formulations and applications. However, the productivity of guar gum remained torpid by and large, since in the last two decades due to erratic rainfall and its distribution in cultivated areas, considerably affecting guar productivity and its overall yield. Attempts to increase the yield have been made by conventional breeding methods involving polyploids (Biswas & Bhattacharyya 1971) and induced mutations (Singh *et al*. 2002). However, there are very few reports about the successful induction of polyploidy in legumes (Sen & Chedda 1958; Biswas & Bhattacharya 1971, 1976; Singh & Roy 1971; Phadnis & Narkhade 1972; Gupta & Gupta 1975, 1976; Raghuvanshi & Singh 1977). Therefore the present investigations were conducted with the primary objective of production of autotetraploids in different accessions, their cyto-morphological characterization and isolation of high gum yielding progeny from synthesized autotetraploids for future exploitation.

## MATERIAL AND METHODS

The seeds of different accessions of *Cyamopsis tetragonoloba* were obtained from the Division of Plant Improvement and Biotechnology, Central Arid Zone Research Institute, Jodhpur. The description of the investigated accessions was given elsewhere (Singh *et al*. 2002).

The seeds were directly sown in small plastic pots, and after colchicine treatment seedlings were transplanted to larger pots in the field. Three methods were employed for colchicinization, i.e. seed treatment, cotton swab method and method of immersion of cotyledonary leaves. For the cotton swab method sterilized cotton swabs immersed either in 0.15 or 0.20% aqueous colchicine were placed on the emerging apical tip between two cotyledonary leaves. To avoid an increase in the concentration, colchicine was added drop by drop at regular intervals on the cotton swabs with the help of sterilized syringe. Such treatment was done for two or three days of three, four or five hours each day. A total of 14 colchicine-induced tetraploids belonging to four different accessions were synthesized. These plants are numbered as P1, P2, P3, ... etc. of respective accessions.

The morphological data on plant height, length of rachis and breadth of leaflets and petals of both diploid and colchicine-induced tetraploid plants were recorded by physical measurements either with a centimetre scale or micrometer as the case may be.

For meiotic studies, flower buds of appropriate size were collected from selected mature plants and fixed on the spot in a freshly prepared 1:3 mixture of glacial acetic acid and 95% ethanol for a minimum of 24 h at room temperature and later stored in 70% ethanol at 10°C. Anthers were squashed in 1% acetocarmine solution with ferric chloride solution as mordant. On average 25–30 pollen mother cells (PMC) were analyzed at diplotene/diakinesis/metaphase I to estimate the range of chromosome associations and recombinational frequencies by chiasma analysis. At anaphase I/II on average 15–20 cells were analyzed to study the distributional pattern of chromosomes and chromatids.

## RESULTS

### Efficiency of colchicine treatment

Four different accessions of *Cyamopsis tetragonoloba* PLG 600, PLG 520, PLG 747, IC 368961 were treated with colchicine (0.15% and 0.20% v/v) for 12 h, 15 h, 18 h distributed up to three days and for 10 h, 12 h distributed up to two days, respectively.

The highest induction percentage (16.66%) (counted as the number of colchicine-induced tetraploids recovered from total number of seedlings treated with colchicine) was achieved in PLG 600 and PLG 747 followed by 14.28% in PLG 520 and IC 368961. The maximum numbers of colchicine-induced tetraploids were recovered in PLG 747 (Table 1).

### Morphology

All the 14 colchicine-induced tetraploid plants were robust from the initial stages of development and it was maintained till maturity although different responses to colchicine were evident in all the four accessions. At maturity, the diploid plants and corresponding colchicine-induced tetraploids were apparently distinct in all the accessions (Table 2).

Table 1. Efficiency of colchicine treatment in inducing polyploidy by the cotton swab method in *Cyamopsis tetragonoloba* accessions

| Sample No. | Accession No. | Colchicine concentration (%) | Duration of treatment (h) | No. of days of treatment | No. of seedlings treated | No. of seedlings survived | No. of colchicine-induced tetraploids | Percentage of tetraploids |
|---|---|---|---|---|---|---|---|---|
| | | 0.15 | 12 | 3 | 33 | 22 | 1 | 4.45 |
| | | | 15 | 3 | 15 | 8 | – | 0.0 |
| 1 | PLG 600 | | 18 | 3 | 10 | 2 | – | 0.0 |
| | | 0.20 | 10 | 2 | 10 | 6 | 1 | 16.66 |
| | | | 12 | 2 | 12 | 8 | 1 | 12.50 |
| | | 0.15 | 12 | 3 | 30 | 26 | – | 0.0 |
| | | | 15 | 3 | 20 | 11 | – | 0.0 |
| 2 | PLG 520 | | 18 | 3 | 12 | 7 | 1 | 14.28 |
| | | 0.20 | 10 | 2 | 10 | 7 | – | 0.0 |
| | | | 12 | 2 | 12 | 1 | 1 | 12.50 |
| | | 0.15 | 12 | 3 | 25 | 18 | 1 | 5.55 |
| | | | 15 | 3 | 10 | 9 | 1 | 11.11 |
| 3 | PLG 747 | | 18 | 3 | 10 | 4 | 1 | 25.0 |
| | | 0.20 | 10 | 2 | 10 | 5 | 2 | 40.0 |
| | | | 12 | 2 | 10 | 6 | 1 | 16.66 |
| | | 0.15 | 12 | 3 | 12 | 10 | – | 0.0 |
| | | | 15 | 3 | 10 | 9 | 1 | 11.11 |
| 4 | IC 368961 | | 18 | 3 | 10 | 3 | – | 0.0 |
| | | 0.20 | 10 | 2 | – | – | – | 0.0 |
| | | | 12 | 2 | 10 | 7 | 1 | 14.28 |

Plant height, length of rachis, length and breadth of leaflet of the colchicine-induced tetraploids plants showed a reduction from the corresponding diploids, however, internodal length, length and breadth of standard petal, width of wing petal, length of pods showed a considerable increase in comparison with the corresponding diploids in all the accessions. The number of seeds per pod decreased in the synthesized colchicine-induced tetraploids in all the accessions (Table 2).

## Cytology

PLG 600: In diploid, seven bivalents were observed in all the cells analyzed. On average 6.80 ring and 0.20 rod bivalents were observed in this accession whereas univalents were found to be completely absent. The mean number of chiasmata per cell was 14.04. The equal distribution of chromosomes (7:7) was observed in all cells analyzed at anaphase I (Tables 3–6 and Figures 1–3).

PLG 600/P1: The genomic formula determined for this plant was 3.18 IV + 6.04 II + 0.72 I per cell, their range being 2–7, 0–10, 0–2, respectively. Out of 25 cells analyzed, 13 cells were observed to consist of four quadrivalents. The chiasmata per cell ranged from 19 to 29, their mean being 25.48, out of which 17.68 were terminalized giving the terminalization coefficient of 0.44. Four out of 15 cells analyzed at anaphase I had the unequal distribution (13:15, 12:16) of chromosomes without any lagging univalent or bivalent. The remaining cells had the equal distribution of chromosomes (14:14) indicating that the course of meiosis was normal (Tables 3–6 and Figures 4 and 5).

PLG 600/P2: All the cells analyzed had the average association in the form of 5.36 IV + 3.08 II + 0.24 I per cell and their range was 3–7, 0–8, 0–2, respectively. Out of 25, 10 cells had six quadrivalents, which shows the maximum mean number of multivalents per cell in this plant. The chiasmata ranged from 24 to 30, their mean being 27.32, in which 19.84 were terminalized giving the terminalization coefficient of 0.37. Quadrivalents outnumbered the other associations. 13 out of 15 cells analyzed at anaphase I had the equal distribution of chromosomes (14:14). Other two cells had un-

Table 2. Morphological features of diploids and colchicine-induced tetraploids of *Cyamopsis tetragonoloba* accessions

| Sample No | Character | PLG 600 | | PLG 520 | | PLG 747 | | IC 368961 | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2*x* | 4*x* | 2*x* | 4*x* | 2*x* | 4*x* | 2*x* | 4*x* |
| 1 | plant height (cm) | 83 | 40 | 63 | 43 | 78 | 56 | 81 | 48 |
| 2 | length of rachis (cm) | 9 | 6 | 8 | 5 | 10 | 5 | 9 | 6 |
| 3 | length of odd leaflet (cm) | 9 | 10 | 9 | 5 | 9 | 9 | 8 | 9 |
| 4 | breadth of odd leaflet (cm) | 6 | 5 | 5 | 4 | 5 | 4 | 6 | 6 |
| 5 | internodal length (cm) | 2.5 | 3 | 2 | 3 | 2.5 | 4 | 3 | 3 |
| 6 | length of standard petal (cm) | 0.5 | 0.9 | 0.6 | 0.7 | 0.6 | 0.8 | 0.6 | 0.9 |
| 7 | breadth of standard petal (cm) | 0.4 | 0.3 | 0.3 | 0.2 | 0.2 | 0.3 | 0.4 | 0.5 |
| 8 | length of wing petal (cm) | 0.5 | 0.8 | 0.6 | 0.8 | 0.6 | 0.8 | 0.5 | 0.7 |
| 9 | breadth of wing petal (cm) | 0.3 | 0.5 | 0.3 | 0.6 | 0.4 | 0.6 | 0.5 | 0.5 |
| 10 | length of pod (cm) | 7 | 4.5 | 6.5 | 6.0 | 7.1 | 4.9 | 6 | 3.7 |
| 11 | seeds per pod | 8 | 3 | 7 | 3 | 8 | 4 | 7 | 3 |

equal distribution with the presence of lagging univalents (Tables 3–6 and Figures 6 and 9).

PLG 600/P3: In this plant the mean number of quadrivalents and bivalents was 4.56 and that of univalents was 0.56 giving an average of 4.56 IV + 4.56 II + 0.56I (Figures 7 and 8). The chiasma frequency per cell ranged from 26 to 31 and their mean number was 27.52. Maximum numbers of four quadrivalents were observed in 11 cells. The mean number of terminalized chiasmata ranged from 12 to 26 giving the terminalization coefficient of 0.41. The distribution of chromosomes at anaphase I was found to be equal in nine cells and the remaining cells had the unequal distribution

Table 3. Average number and range of associations in diploid (2*x*) and synthesized colchicine-induced tetraploids of different accessions of *Cyamopsis tetragonoloba*

| Accession No. | Ploidy | No. of cells analyzed | Quadrivalents | | Bivalents | | Univalents | |
|---|---|---|---|---|---|---|---|---|
| | | | mean | range | mean | range | mean | range |
| PLG 600 | 2*x* | 25 | – | – | 7.00 ± 0.00 | – | 0.00 | – |
| PLG 600/P1 | 4*x* | 25 | 3.18 ± 1.16 | 2–7 | 6.04 ± 2.34 | 0–10 | 0.72 ± 0.96 | 0–2 |
| PLG 600/P2 | 4*x* | 25 | 5.36 ± 1.22 | 3–7 | 3.08 ± 2.28 | 0–8 | 0.24 ± 0.64 | 0–2 |
| PLG 600/P3 | 4*x* | 25 | 4.56 ± 1.35 | 1–7 | 4.56 ±2.56 | 0–10 | 0.56 ± 0.89 | 0–2 |
| PLG 520 | 2*x* | 25 | – | – | 6.88 ± 0.32 | 6–7 | 0.24 ± 0.64 | 0–2 |
| PLG 520/P1 | 4*x* | 25 | 4.08 ± 1.32 | 1–7 | 5.36 ± 2.54 | 0–12 | 0.48 ± 1.26 | 0–6 |
| PLG 520/P2 | 4*x* | 25 | 5.32 ± 0.96 | 3–7 | 3.24 ± 1.94 | 0–7 | 0.08 ± 0.33 | 0–2 |
| PLG 747 | 2*x* | 25 | – | – | 7.00 ± 0.00 | – | 0.00 | – |
| PLG 747/P1 | 4*x* | 25 | 4.80 ± 1.46 | 1–7 | 4.28 ± 2.94 | 0–12 | 0.16 ± 0.54 | 0–2 |
| PLG 747/P2 | 4*x* | 25 | 4.52 ± 1.23 | 2–6 | 4.56 ± 2.65 | 0–10 | 0.40 ± 0.97 | 0–4 |
| PLG 747/P3 | 4*x* | 20 | 3.90 ± 2.21 | 0–7 | 5.40 ± 3.86 | 0–14 | 1.10 ± 3.12 | 0–14 |
| PLG 747/P4 | 4*x* | 20 | 5.45 ± 1.11 | 2–7 | 2.95 ± 1.98 | 0–9 | 0.20 ± 0.60 | 0–2 |
| PLG 747/P5 | 4*x* | 20 | 4.20 ± 1.69 | 0–6 | 5.25 ± 2.80 | 0–11 | 0.60 ± 1.42 | 0–4 |
| PLG 747/P6 | 4*x* | 20 | 5.15 ± 1.49 | 3–7 | 3.60 ± 3.00 | 0–10 | 0.00 | – |
| IC 368961 | 2*x* | 25 | – | – | 7.00 ± 0.00 | | 0.00 ± 0.00 | – |
| IC 368961/P1 | 4*x* | 15 | 4.93 ± 1.56 | 0–6 | 4.06 ± 3.19 | 1–14 | 0.13 ± 0.49 | 0–2 |
| IC 368961/P2 | 4*x* | 20 | 4.65 ± 1.31 | 2–7 | 4.40 ± 2.63 | 0–10 | 0.10 ± 0.43 | 0–2 |
| IC 368961/P3 | 4*x* | 25 | 5.16 ± 0.96 | 4–7 | 3.24 ± 2.10 | 0–6 | 0.16 ± 0.54 | 0–2 |

Table 4. Average number and frequency of multivalents in various accessions of *C. tetragonoloba*

| Sample No. | Accession No. | No. of cells analyzed | No. of cells with multivalent frequency of | | | | | | | | Mean number of multivalents per cell |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| 1 | PLG 600/P1 | 25 | – | – | 3 | 6 | 13 | – | 2 | 1 | 3.80 |
| 2 | PLG 600/P2 | 25 | – | – | – | 3 | 3 | 5 | 10 | 4 | 5.36 |
| 3 | PLG 600/P3 | 25 | – | 1 | 1 | 1 | 11 | 3 | 7 | 1 | 4.56 |
| 4 | PLG 520/P1 | 25 | – | 1 | 2 | 4 | 9 | 6 | 2 | 1 | 4.08 |
| 5 | PLG 520/P2 | 25 | – | – | – | 1 | 5 | 5 | 13 | 1 | 5.32 |
| 6 | PLG 747/P1 | 25 | – | 1 | 1 | 1 | 7 | 8 | 3 | 4 | 4.80 |
| 7 | PLG 747/P2 | 25 | – | – | 2 | 3 | 7 | 6 | 7 | – | 4.52 |
| 8 | PLG 747/P3 | 20 | 3 | 1 | 1 | 1 | 6 | 2 | 4 | 2 | 3.90 |
| 9 | PLG 747/P4 | 20 | – | – | 1 | – | 2 | 5 | 10 | 2 | 5.40 |
| 10 | PLG 747/P5 | 20 | 1 | – | 2 | 3 | 5 | 5 | 2 | 2 | 4.10 |
| 11 | PLG 747/P6 | 20 | – | – | 1 | 2 | 4 | 4 | 4 | 5 | 5.15 |
| 12 | IC 368961/P1 | 15 | 1 | – | – | – | 4 | 2 | 8 | – | 4.93 |
| 13 | IC 368961/P2 | 20 | – | – | 2 | 1 | 5 | 8 | 2 | 2 | 4.65 |
| 14 | IC 368961/P3 | 25 | – | – | – | – | 8 | 7 | 8 | 2 | 5.16 |

of 13:15, 12:16, 11:17, however one cell had two lagging univalents (Tables 3–6 and Figure 10).

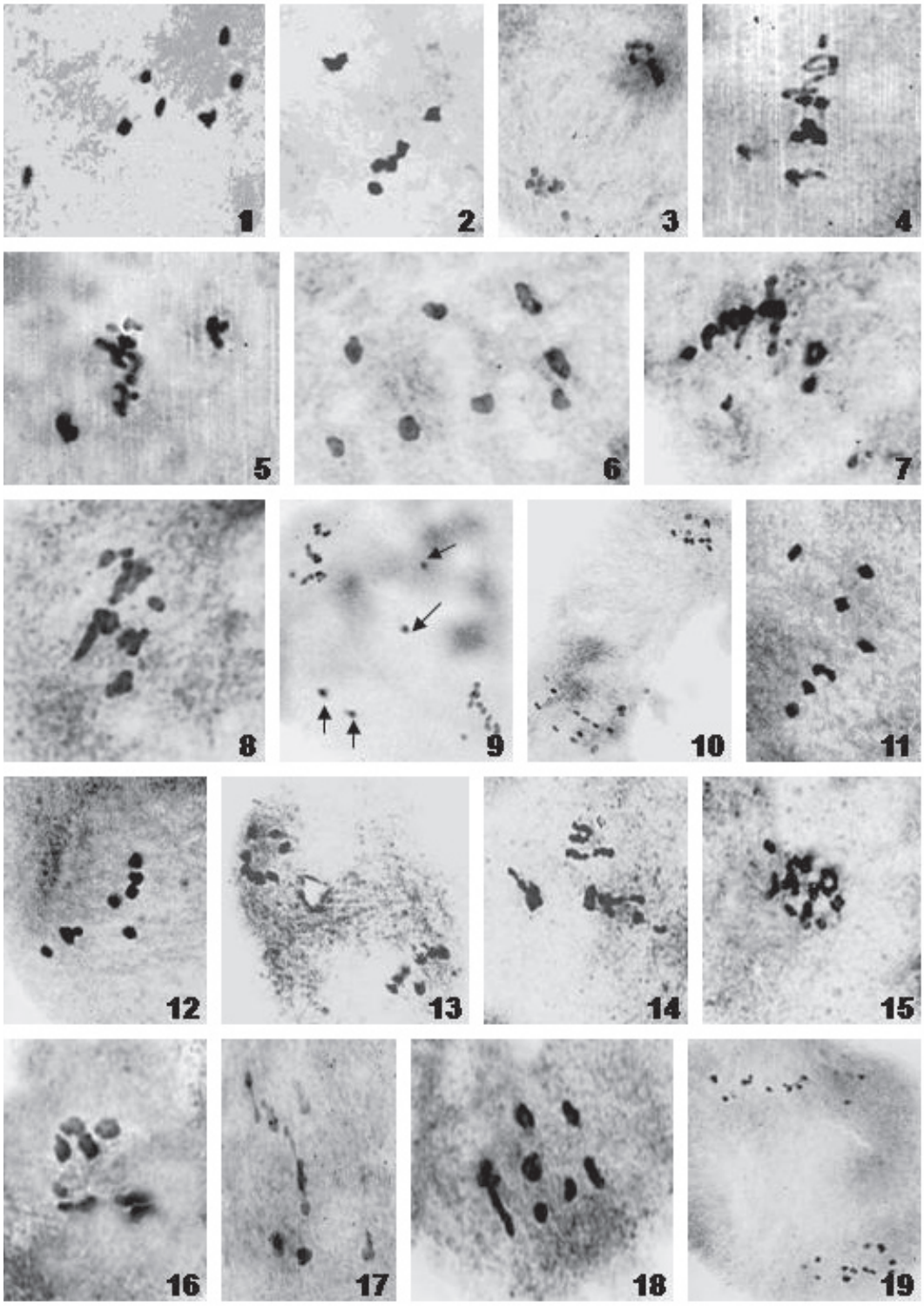
PLG 520: The average number of bivalents was 6.88 and that of univalents was 0.24. The bivalents were present in the form of either ring or rod (Figures 11 and 12). The average number of chiasmata per cell was 14.16, ranging from 12 to 16. The normal distribution of chromosomes (7:7) was observed at anaphase I (Tables 3–6 and Figure 13).

PLG 520/P1: The average association was observed as 4.08 IV + 5.36 II + 0.48 I per cell, which was in the range of 1–7, 0–12, 0–6, respectively. Out of 25, nine cells had four quadrivalents. The average number of chiasmata per cell was 26.88 and it ranged from 21 to 28, in which 16.48 were terminalized, giving the terminalization coefficient of 0.63. Except one cell, which had a univalent as laggard (13:1U:14), all the other cells had the equal distribution of chromosomes (14:14) analyzed at anaphase I (Tables 3–6 and Figures 14 and 15).

PLG 520/P2: The average chromosome association for 25 cells analyzed at metaphase I was 5.32 IV + 3.24 II + 0.08 I per cell, in the range of 3–7, 0–7, 0–2, respectively. 13 cells showed the presence of six quadrivalents giving the mean number of multivalents per cell as 5.32. The total chiasma frequency per cell was 27.16, ranging from 24 to 28, out of which 20.80 were terminalized giving the terminalization coefficient of 0.30. All cell analyzed had the equal distribution of chromosomes (14:14) at anaphase I, except one cell which showed the unequal distribution of chromosomes (13:15) without any lagging univalent or bivalent (Tables 3–6 and Figures 16–20 and 29 and 30).

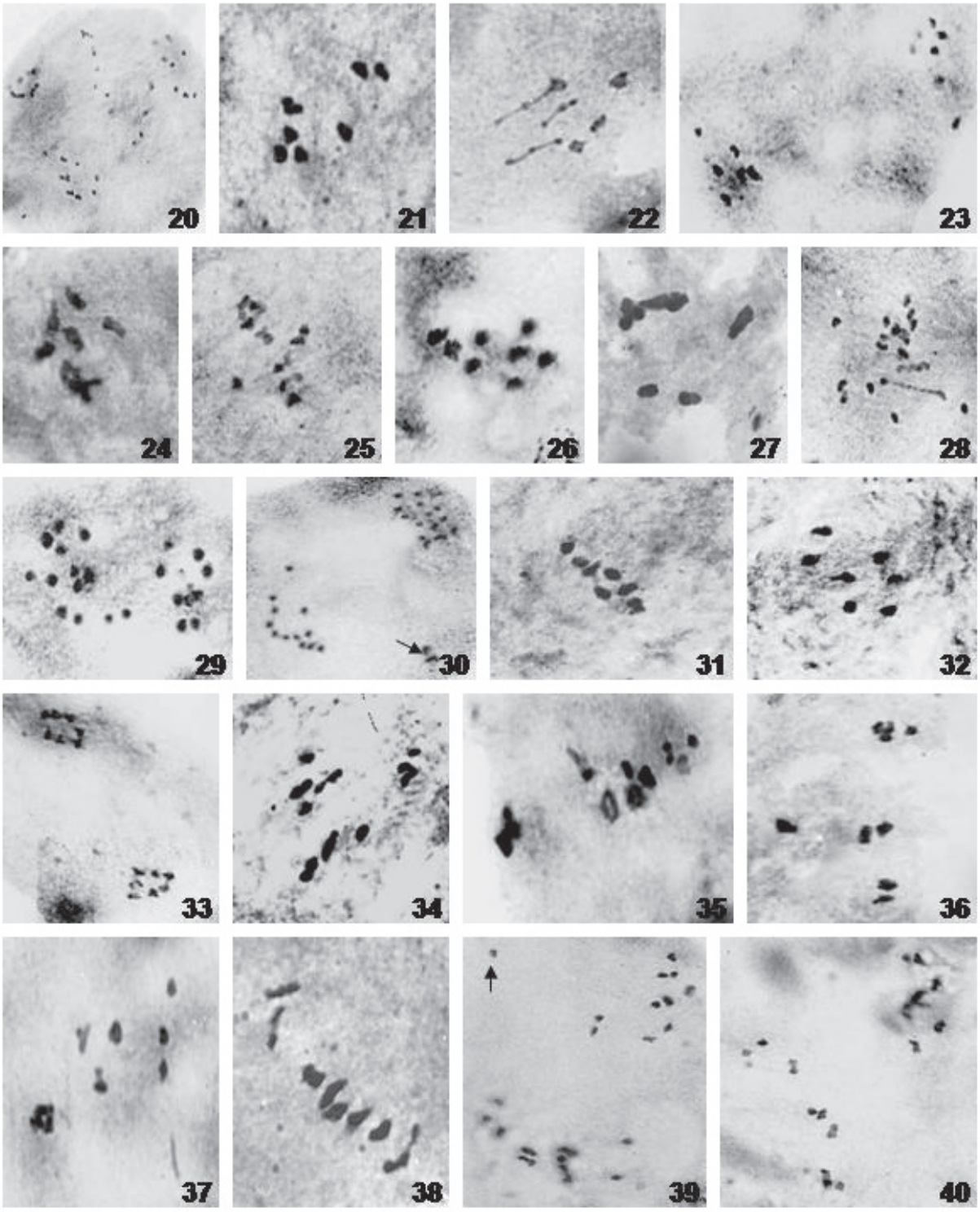
PLG 747: Seven bivalents were observed in all the cells analyzed at metaphase I. An average of 7.00 bivalents was observed in this accession whereas univalents were found to be completely absent. The mean number of chiasmata per cell was 13.68. All cells analyzed at anaphase I had the equal distribution of chromosomes (7:7) (Tables 3–6 and Figures 21–23).

PLG 747/P1: All the cells analyzed had the average association in the form of 4.80 IV + 4.28 II + 0.16 I per cell, their range being 1–7, 0–12, 0–2, respectively. The chiasmata ranged from 26 to 29, their mean being 27.56, in which 19.04 were terminalized giving the terminalization coefficient of 0.44. 12 out of 15 cells analyzed at anaphase I had the equal distribution of chromosomes (14:14). Two cells had unequal distribution with the presence of lagging univalents and one cell also had unequal distribution with or without any laggard (Tables 3–6 and Figures 24 and 25).




Figures 1–40. Documentation of cytological examinations in different *Cyamopsis tetragonoloba* accessions/plants

1–2 – PLG 600, Metaphase I, 7 II; 3 – PLG 600, Anaphase I, 7:7; 4 – PLG 600/P1, Metaphase I, 3IV + 7II + 2I; 5 – PLG 600/P1, Metaphase I, 4IV + 5II + 2I; 6 – PLG 600/P2, Metaphase I, 6IV + 2II; 7 – PLG 600/P3, Metaphase I, 4IV + 5II + 2I; 8 – PLG 600/P3, Metaphase I, 4IV + 6II; 9 – PLG 600/P2, Anaphase I, 13:4I:11; 10 – PLG 600/P3, Anaphase I, 15:13; 11–12 – PLG 520, Metaphase I, 7 II; 13 – PLG 520, Anaphase I, 7:7; Fig. 14 – PLG 520/P1, Metaphase I, 2IV + 10II; 15 – PLG 520/P1, Metaphase I, 4IV + 6II; 16 – PLG 520/P2, Metaphase I, 6IV + 2II; 17 – PLG 520/P2, Metaphase I, 6IV + 2II; 18 – PLG 520/P2, Metaphase I, 6IV + 2II; 19 – PLG 520/P2, Anaphase I, 13:15




20 – PLG 520/P2, Anaphase II; 21–22 – PLG 747, Metaphase I, 7 II; 23 – PLG 747, Anaphase I, 7:7; 24 – PLG 747/P1, Metaphase I, 7IV; 25 – PLG 747/P1, Metaphase I, 2IV + 10II; 26 – PLG 747/P2, Metaphase I, 6IV + 2II; 27 – PLG 747/P3, Metaphase I, 6IV + 2II; 28 – PLG 747/P4, Metaphase I, 11II + 4I; 29 – PLG 520/P2, Anaphase I, 13:2I:13; 30 – PLG 520/P2, Anaphase I, 12:2I:14; 31–32 – IC 368961, Metaphase I, 7 II; 33 – IC 368961, Anaphase I, 7:7; 34 – IC 368961/P1, Metaphase I, 4IV + 6II; 35 – IC 368961/P2, Metaphase I, 5IV + 4II; 36 – IC 368961/P3, Metaphase I, 5IV + 2II ($2n = 24$); 37 – IC 368961/P3, Metaphase I, 6IV + 2II; 38 – IC 368961/P3, Metaphase I, 5IV + 4II; 39–40 – IC 368961/P2, Anaphase I, 13:1I:14, 15:13

Scale bar = 10 μm (arrow head showing quadrivalents)

Table 5. Mean number, range of chiasmata and terminalization coefficient in *Cyamopsis tetragonoloba* accessions

| Sample No. | Accession No. | No. of cells analyzed | Chiasmata | | | | | | Terminalization coefficient |
|---|---|---|---|---|---|---|---|---|---|
| | | | total | | terminalized | | unterminalized | | |
| | | | mean | range | mean | range | mean | range | |
| 1 | PLG 600/P1 | 25 | 25.48 ± 2.19 | 19–29 | 17.68 ± 2.72 | 14–23 | 7.80 ± 2.81 | 1–12 | 0.44 |
| 2 | PLG 600/P2 | 25 | 27.32 ± 1.40 | 24–30 | 19.84 ± 1.91 | 15–22 | 7.48 ± 2.00 | 4–12 | 0.37 |
| 3 | PLG 600/P3 | 25 | 27.52 ± 1.74 | 26–31 | 19.52 ± 3.09 | 12–26 | 8.08± 2.97 | 2–14 | 0.41 |
| 4 | PLG 520/P1 | 25 | 26.88 ± 1.81 | 21–28 | 16.48 ± 2.91 | 7–20 | 10.40 ± 2.84 | 7–21 | 0.63 |
| 5 | PLG 520/P2 | 25 | 27.16 ± 1.04 | 24–28 | 20.80 ± 1.57 | 18–23 | 6.44 ± 2.00 | 3–10 | 0.30 |
| 6 | PLG 747/P1 | 25 | 27.56 ± 0.75 | 26–29 | 19.04 ± 3.61 | 15–24 | 8.52 ± 3.64 | 4–18 | 0.44 |
| 7 | PLG 747/P2 | 25 | 27.42 ± 1.44 | 24–29 | 18.48 ± 3.52 | 6–25 | 8.00± 2.48 | 2–12 | 0.43 |
| 8 | PLG 747/P3 | 20 | 26.00 ± 3.67 | 13–28 | 16.60 ± 4.06 | 5–22 | 9.40 ± 2.92 | 6–13 | 0.56 |
| 9 | PLG 747/P4 | 20 | 27.90 ± 1.44 | 24–31 | 20.30 ± 3.56 | 11–25 | 7.60 ± 2.85 | 3–15 | 0.37 |
| 10 | PLG 747/P5 | 20 | 27.00 ± 1.42 | 22–28 | 17.65 ± 4.44 | 9–26 | 9.35 ± 3.69 | 2–16 | 0.52 |
| 11 | PLG 747/P6 | 20 | 27.60 ± 1.20 | 23–29 | 17.75 ± 2.92 | 13–23 | 9.85 ± 3.30 | 4–16 | 0.55 |
| 12 | IC 368961/P1 | 15 | 28.06 ± 0.77 | 26–30 | 16.13 ± 4.12 | 7–22 | 11.80 ± 4.00 | 6–22 | 0.73 |
| 13 | IC 368961/P2 | 20 | 27.15 ± 1.38 | 24–29 | 17.90 ± 2.23 | 14–23 | 9.25 ± 2.44 | 4–13 | 0.51 |
| 14 | IC 368961/P3 | 25 | 27.08 ± 1.32 | 24–28 | 17.92 ± 3.40 | 9–23 | 9.24 ± 3.82 | 4–19 | 0.51 |

PLG 747/P2: In this plant the mean number of quadrivalents and bivalents was 4.52 and 4.56, respectively, and that of univalents was 0.40 giving an average of 4.52 IV + 4.56 II + 0.56 I per cell. The chiasma frequency per cell ranged from 24 to 29 and the mean number was 27.42. The mean number of terminalized chiasmata ranged from 6 to 25 giving the terminalization coefficient of 0.43. The distribution of chromosomes (14:14) at anaphase I was found to be equal in 12 cells and two cells had unequal distribution with the presence of lagging univalents, however, one cell also had unequal distribution but without any laggard (Tables 3–6 and Figure 26).

PLG 747/P3: All the cells analyzed had the average association in the form of 3.90 IV + 5.40 II + 1.10 I per cell, their range being 0–7, 0–14, 0–14, respectively. Three cells in this plant were found to lack multivalent frequency. The chiasmata ranged from 13 to 28, their mean being 26.00, in which 16.60 were terminalized giving the terminalization coefficient of 0.56. Univalents outnumbered the other associations in comparison with the other plants. 10 out of 15 cells analyzed at anaphase I had the equal distribution of chromosomes (14:14). Two cells had unequal distribution (13:15, 12:16) without any laggard; however, other three cells had unequal distribution with the presence of two lagging univalents (Tables 3–6 and Figure 27).

PLG 747/P4: The average chromosome association for 25 cells analyzed at metaphase I was 5.45IV + 2.95II + 0.20I per cell, in the range of 2–7, 0–9, 0–2, respectively. 10 out of 25 cells showed the presence of six quadrivalents giving the mean number of multivalents per cell as 5.40. The total chiasma frequency per cell was 27.90, ranging from 24 to 31, out of which 20.30 were terminalized giving the terminalization coefficient of 0.37. All cells analyzed had the equal distribution of chromosomes (14:14) at anaphase I (Tables 3–6 and Figure 28).

PLG 747/P5: In this plant the mean number of quadrivalents and bivalents was 4.20 and 5.52, respectively, and that of univalents was 0.60 giving an average of 4.20IV + 5.52II + 0.60I per cell. The chiasma frequency per cell ranged from 22 to 28 and the mean number was 27.00. The mean number of terminalized chiasmata ranged from 9 to 26 giving the terminalization coefficient of 0.52. The distribution of chromosomes (14:14) at anaphase I was found to be equal in 11 cells and two cells had unequal distribution with the presence of 1 or 4 lagging univalents, however,

Table 6. Anaphases I distribution in colchicine-induced tetraploids of various accessions of *Cyamopsis tetragonoloba*

| Sample No. | Accession No. | 2*n* | No. of cells analyzed | Chromosome distribution | No. of cells | Percentage |
|---|---|---|---|---|---|---|
| 1 | PLG 600/P1 | 28 | 15 | 14:14 | 11 | 73.30 |
| | | | | 13:15 | 2 | 13.30 |
| | | | | 12:16 | 2 | 13.30 |
| 2 | PLG 600/P2 | 28 | 15 | 14:14 | 13 | 86.60 |
| | | | | 12:16 | 1 | 6.60 |
| | | | | 11:4U:13 | 1 | 6.60 |
| 3 | PLG 600/P3 | 28 | 15 | 14:14 | 9 | 60.00 |
| | | | | 13:15 | 2 | 13.30 |
| | | | | 12:16 | 2 | 13.30 |
| | | | | 11:17 | 1 | 6.60 |
| | | | | 10:2U:16 | 1 | 6.60 |
| 4 | PLG 520/P1 | 28 | 15 | 14:14 | 14 | 93.60 |
| | | | | 13:1U:14 | 1 | 6.60 |
| 5 | PLG 520/P2 | 28 | 15 | 14:14 | 14 | 93.60 |
| | | | | 13:15 | 1 | 6.60 |
| 6 | PLG 747/P1 | 28 | 15 | 14:14 | 12 | 80.00 |
| | | | | 13:15 | 1 | 6.60 |
| | | | | 13:2U:14 | 1 | 6.60 |
| | | | | 13:1U:13 | 1 | 6.60 |
| 7 | PLG 747/P2 | 28 | 15 | 14:14 | 12 | 80.00 |
| | | | | 13:15 | 1 | 6.60 |
| | | | | 13:1U:14 | 1 | 6.60 |
| | | | | 12:2U:14 | 1 | 6.60 |
| 8 | PLG 747/P3 | 28 | 15 | 14:14 | 10 | 66.60 |
| | | | | 13:15 | 1 | 6.60 |
| | | | | 12:16 | 1 | 6.60 |
| | | | | 13:2U:13 | 2 | 13.30 |
| | | | | 11:2U:15 | 1 | 6.60 |
| 9 | PLG 747/P4 | 28 | 15 | 14:14 | 15 | 100 |
| 10 | PLG 747/P5 | 28 | 15 | 14:14 | 11 | 73.30 |
| | | | | 13:15 | 1 | 6.60 |
| | | | | 12:16 | 1 | 6.60 |
| | | | | 11:4U:13 | 1 | 6.60 |
| | | | | 13:1U:14 | 1 | 6.60 |
| 11 | PLG 747/P6 | 28 | 15 | 14:14 | 10 | 66.60 |
| | | | | 13:15 | 2 | 13.30 |
| | | | | 12:16 | 1 | 6.60 |
| | | | | 13:1U:14 | 2 | 2.20 |
| 12 | IC 368961/P1 | 28 | 15 | 14:14 | 8 | 53.30 |
| | | | | 13:15 | 3 | 20.00 |
| | | | | 12:16 | 3 | 20.00 |
| | | | | 13:1U:14 | 1 | 6.60 |
| 13 | IC 368961/P2 | 28 | 15 | 14:14 | 11 | 73.30 |
| | | | | 13:15 | 3 | 20.00 |
| | | | | 13:1U:14 | 1 | 6.60 |
| 14 | IC 368961/P3 | 28 | 15 | 14:14 | 12 | 80.00 |
| | | | | 13:15 | 1 | 6.60 |
| | | | | 12:16 | 1 | 6.60 |
| | | | | 11:17 | 1 | 6.60 |

other two cells also had unequal distribution but without any laggard (Tables 3–6).

PLG 747/P6: The average chromosome association for 25 cells analyzed at metaphase I was 5.15IV + 3.60II per cell, in the range of 3–7, 0–10, respectively whereas univalents were absent. The chiasma frequency per cell ranged from 23 to 29 and the mean number was 27.60. The mean number of terminalized chiasmata (17.75) ranged from 13 to 23 giving the terminalization coefficient of 0.55. The distribution of chromosomes at anaphase I was found to be equal in 10 cells and three cells had the unequal distribution of 13:15, 12:16, however, one cell had one univalent as laggard (Tables 3–6).

IC 368961: Seven bivalents were observed in all cells analyzed at metaphase I and univalents were absent. The bivalents were present in the form of either ring or rod. The average number of chiasmata per cell was 14.24, ranging from 12 to 17. The normal distribution of chromosomes (7:7) was observed at anaphase I (Tables 3–6 and Figures 31–33).

IC 368961/P1: In this plant the mean number of quadrivalents and bivalents was 4.93 and 4.06, respectively, and that of univalents was 0.13 giving an average of 4.93IV + 4.06II + 0.13I per cell. The chiasma frequency per cell ranged from 26 to 30 and the mean number was 28.06. The mean number of terminalized chiasmata ranged from 7 to 22 giving the terminalization coefficient of 0.73. The distribution of chromosomes (14:14) at anaphase I was found to be equal in eight cells and six cells had the unequal distribution of 13:15, 12:16, however, one cell also had unequal distribution with a single univalent as laggard (Tables 3–6 and Figure 34).

IC 368961/P2: The average chromosome association for 25 cells analyzed at metaphase I was 4.65IV + 4.40II + 0.10I per cell, in the range of 2–7, 0–10, 0–2, respectively. The total chiasma frequency per cell was 27.15, ranging from 24 to 29, out of which 17.90 were terminalized giving the terminalization coefficient of 0.51. 12 out of 15 cells analyzed at anaphase I had the equal distribution of chromosomes (14:14). Two cells had unequal distribution with the presence of either one or two lagging univalents whereas one cell had the 13:15 distribution without any laggard (Tables 3–6 and Figure 35, 39–40).

IC 368961/P3: The average chromosome association of 4.20IV + 5.52II + 0.60I per cell was observed, which was in the range of 4–7, 0–6, 0–2, respectively. The maximum number of cells showed the occurrence of either four or six quadrivalents. The chiasma frequency per cell ranged from 24 to 28 and the mean number was 27.08. The mean number of terminalized chiasmata ranged from 9 to 23 giving the terminalization coefficient of 0.51. The distribution of chromosomes (14:14) at anaphase I was found to be equal in 12 cells and three cells had the unequal distribution of chromosomes with 13:15, 12:16, 11:17 (Tables 3–6 and Figures 36–38).

## DISCUSSION

Induction of autotetraploidy using colchicine (Blakeslee & Avery 1937) and cyto-morphological characterization and the genetic analysis of polyploids have been a subject of immense interest among geneticists and breeders for a long time (Lawton-Rauh 2000; Otto & Whitton 2000; Rieseberg 2001; Wolfe 2001; Ramsey & Schemske 2002; Mable 2003). As already mentioned earlier, some morphological features show an increase viz. internodal length, length and breadth of standard petal, length and breadth of wing petal etc. while few characters show a decrease thereby exhibiting a clear-cut response of *C. tetragonoloba* to colchicine treatment. Such observations were previously demonstrated in a large number of plant species (Sen & Chedda 1958; Sen & Vidyabhusan 1960; Raghuvanshi & Joshi 1965; Gupta & Gupta 1975, 1976; Gupta & Sinha 1978; Srivastava & Raina 1983; Mishra 1986). The role of optimum threshold limits is also attributed to different response to colchicine in various accessions beyond which the polyploidy exhibits a negative effect on morphological features (Singh & Roy 1971). The cultivation history of *C. tetragonoloba* may also have influenced the manifestation of various morphological features (Arora 1975; Srivastava & Raina 1983).

Theoretically in autotetraploidy, each chromosome represents itself four times, due to which mostly the quadrivalent associations would be expected. In earlier literature it was stated that the frequency of quadrivalents in comparison with bivalents shows gradation from high quadrivalent associations to perfect bivalent formations (Dhillon 1970; Somaroo & Grant 1971;

Jalil *et al.* 1974; Arora 1975; Gupta & Gupta 1975, 1976; Zadoo *et al.* 1975; Pal & Khoshoo 1977; Gupta & Sinha 1978; Madhusoodanan & Arora 1979). In the present investigations, a maximum number of quadrivalent associations was observed, which was reported rarely in previous studies. The occurrence of maximum quadrivalent associations may be attributed to maximum homology among the chromosomes, due to which the partners might get a chance to exchange their genetic material. An interesting feature of the meiotic analysis of synthesized tetraploid plants of *C. tetragonoloba* is that PMCs with seven quadrivalents/multivalents were encountered in all the accessions presently investigated and the number of PMCs per accession having quadrivalent association ranged from 1 to 5, barring two accessions, namely PLG 747/P2 and IC 368961/P1. Such high quadrivalent or multivalent frequencies were reported by Morrison and Rajhathy (1960), Sparrow *et al.* (1942), who correlated this phenomenon with that of size genome as well as chromosomes.

The maximum mean value (28.06) of chiasma frequency was observed in IC 388961/P1 and minimum (25.48) was observed in PLG 600/P1. These high values of chiasma frequency indicate that the chiasmata were localized rather than randomized. Due to the presence of localized chiasmata quadrivalents were observed mostly in the form of ring whereas chain quadrivalents were present at low frequencies. Localized chiasmata are considered to reduce the formation of multivalents in autoploids (Levan 1940; Koul & Gohil 1970; Srivastava & Raina 1983), in contrast of which the present authors observed more quadrivalents than bivalents in most PMCs.

In all the accessions, the authors recovered at least one synthesized tetraploid plant, except one accession, i.e. IC 368961, which was totally seed sterile in $C_0$ generation. The highest seed set (21) was observed in PLG 747 and the lowest (5) in PLG 520. The majority of collected seeds were shrivelled and shrunken. Partial or total seed sterility is one of the commonest features in colchicine-induced tetraploids and there are only few reports where the seed set was equivalent or slightly lower than in their respective diploids (Gottschalk 1978; Srivastava & Raina 1983). The production of non-viable and unbalanced gametes which arise due to meiotic anomalies viz. mis-disjunction of multivalents, lagging bivalents or univalents and other abnormalities were found to be the main reasons for partial or total seed sterility (Srivastava & Raina 1983) whereas some authors stated contrary to these views that a cytological cause might be the sole criterion for seed sterility (Einset 1944; Srivastava & Raina 1983).

All the synthesized colchicine-induced tetraploids had some cells at anaphase I with unequal distribution and/or lagging bivalents or univalents barring one plant viz. PLG 747/P4 resulting in a high viable seed set. It is obvious that the seed set in the present material is not dependent upon quadrivalent frequency but may be due to chromosomal abnormalities occurring in significant numbers during anaphase I/II.

It is desirable to carry out enhanced appreciation of the effects of polyploidy on the evolution of morphological and reproductive characteristics, metabolic pathways and other features that are considered significant in relation to adaptation and speciation. Newly formed auto- and allopolyploids exhibit considerable meiotic complexity, including multivalent pairing, multisomic inheritance, and production of unbalanced gametes which have been recorded in the present synthesized colchicine-induced tetraploids resulting in variations. Genetic variability is the basis for the production of better plant types which meet the increasing demand for multipurpose plants like *Cyamopsis tetragonaloba*. Thus the colchicine-induced tetraploids produced in the present work are of immense importance to plant breeders as well as to the local population by providing better food and feed plants.

## References

Arora O.P. (1975): Differential response of *Verbena* species to colchitetraploidy. The Nucleus, **18**: 166–171.

Biswas A.K., Bhattacharyya N.K. (1971): Induced polyploidy in legumes I. *Cyamopsis psoraloides* DC. Cytologia, **36**: 469–479.

Biswas A.K., Bhattacharyya N.K. (1976): Induced polyploidy in legumes III. *Phaseolus vulgaris* L. Cytologia, **41**: 105–110.

Blakeslee A.F., Avery A.G. (1937): Methods of inducing chromosome doubling in plants by treating with colchicine. Heredity, **28**: 393–411.

Dhillon T.S. (1970): *Phlox drummondii* I. Cytogenetics of colchicine induced autotetraploids. The Japanese Journal of Genetics, **45**: 305–312.

Einset J. (1944): Cytological basis for sterility in induced autotetraploid of *Lactuca sativa* L. American Journal of Botany., **31**: 28–45.

Gottschalk W. (1978): Open problems in polyploidy research. The Nucleus, **21**: 99–112.

Gupta R., Gupta P.K. (1975): Induced polyploidy in *Crotalaria* L. I. *C. juncea* and *C. retusa*. Journal of Indian Botanical Society, **54**: 175–182.

Gupta R., Gupta P.K. (1976): Induced polyploidy in *Crotalaria* L. II. *C. brownie* and *C. sericea*. Journal of Indian Botanical Society, **55**: 261–266.

Gupta A.K., Sinha R.P. (1978): Cytotaxonomy of artificially induced polyploids of *Crotalaria*. The Nucleus, **21**: 26–28.

Jalil R., Zadoo S.N., Khoshoo T.N. (1974): Colchiploid Balsams. The Nucleus, **17**: 118–124.

Koul A.K., Gohil R.N. (1970): Cytology of the tetraploid *Allium ampeloprasum* with chiasma localization. Chromosoma, **29**: 12–19.

Lawton-Rauh A. (2000): Evolutionary dynamics of duplicated genes in plants. Molecular Phylogenetics and Evolution, **29**: 396–409.

Levan A. (1940). Meiosis in *Allium porrum*, a tetraploids species with chiasma localization. Hereditas, **26**: 454–462.

Mable B.K. (2003): Breaking down taxonomic barriers in polyploidy research. Trends in Plant Science, **8**: 582–590.

Madhusoodanan K.J., Arora O.P. (1979): Induced autotetraploidy in *Matricaria chamomilla* L. Cytologia, **44**: 227–232.

Mishra U. (1986): Cytogenetic studies in some members of *Papilionaceae*. [Ph. D. Thesis.] Patna University, Patna.

Morrison J.W., Rajathy T. (1960): Chromosome behaviour in autotetraploidy cereals and grasses. Chromosoma, **11**: 297–309.

Otto S.P., Whitton J. (2000): Polyploid incidence and evolution. Annual Review of Genetics, **34**: 401–437.

Pal M., Khoshoo T.N. (1977): Evolution and improvement of cultivated *Amaranthus* VIII. Induced autotetraploidy in grain types. Zeitschrift für Pflanzenzüchtung, **78**: 135–148.

Phadnis B.A., Narkhede M.N. (1972): Chromosome behavior in colchicine induced autotetraploids of *Cicer arietinum* L. Cytologia, **37**: 415–421.

Raghuvanshi S.S., Joshi S. (1965): Studies on the comparative effects of certain chemicals on the polyploidising efficiency of colchicine in *Trigonella foenum-graceum* L. Caryologia, **18**: 69–84.

Raghuvanshi S.S., Singh A.K. (1977): Polyploid breeding in *Trigonella foenum-graecum* L. Cytologia, **42**: 5–19.

Ramsey J., Schemske D.W. (2002): Neopolyploidy in flowering plants. Annual Review of Ecology and Systematics, **33**: 589–639.

Rieseberg L.H. (2001): Polyploid evolution: keeping the peace at genomic reunions. Current Biology, **11**: R925–R928.

Sen N.K., Chedda H.R. (1958): Colchicine induced tertaploids of five varieties of black gram. Indian Journal of Genetics, **18**: 238–248.

Sen N.K., Vidyabhusan R.V. (1960). Studies of the induced polyploidy in horse gram. Indian Journal of Genetics and Plant Breeding, **20**: 212–233.

Singh A., Roy R.P. (1971): Studies on the colchicine-induced tetraploids of four species of *Trigonella*. Cytologia, **36**: 133–142.

Singh J., Arora R.N., Saini M.L., Agrawal R.P.S., Yadav B.D. (2002): Guar Production and its Utilization in India. CAZRI (ICAR), New Delhi.

Somaroo B.H., Grant W.F. (1971): Meiotic chromosome behaviour in induced autotetraploidy and amphidiploids in the *Lotus corniculatus* group. Canadian Journal of Genetics and Cytology, **13**: 663–671.

Sparrow A.H., Ruttle M.L., Nebel B.R. (1942): Comparative cytology of sterile intra and fertile intervarietal tetraploids of *Antirrhinum majus*. American Journal of Botany, **29**: 711–715.

Srivastava P.K., Raina S.N. (1983): Cytogenetics of *Tephrosia*. VII – Colchicine induced polyploidy in eleven species. La Cellule, **74**: 79–114.

Wolfe K.H. (2001): Yesterday's polyploids and the mystery of diploidization. Nature Review Genetics, **2**: 333–341.

Zadoo S.N., Roy R.P., Khoshoo T.N. (1975): Cytogenetics of cultivated *Bouganvillea*. V. Induced tetraploidy and restoration of fertility in sterile cultivars. Euphytica, **24**: 517–524.

Received for publication May 30, 2009
Accepted after corrections September 3, 2009

*Corresponding author:*

Prof. Satyawada Rama Rao, North- Eastern Hill University, Department of Biotechnology and Bioinformatics, Shillong 793022, Meghalaya, India
tel.: + 91 364 272 2404/272 2401, e-mail: srrao@nehu.ac.in