Cytogenetics of *Cucumis* L.

IV. Comparative study of natural and induced polyploids

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The phenomenon of polyploidy has been recognized as one of the most outstanding features in evolution of plants (Stebbins 1971, Jackson 1976) and has also been exploited in crop improvement through polyploid breeding (Müntzing 1951, Randolph 1932). In Cucurbitaceae success of polyploidy has been debated both from evolutionary and agronomic points of view. Recent reports in this family on a number of genera with polyploid forms, *Trichosanthes*, *Melothria*, *Momordica*, and *Cucumis* (Singh 1974, Singh and Roy 1979, Roy *et al.* 1966, Agrawal *et al.* 1976, Shimotsu 1965, Dane and Tsuchiya 1979), on success of polyploids in *Citrullus vulgaris* (Kihara 1951, Andrus *et al.* 1971), and on positive response to polyploid induction for fruit character in *Luffa* and *Cucumis* (Roy and Ghosh 1971, Singh and Roy 1975) do not support the old generalization on the role of polyploidy in speciation and in breeding in Cucurbitaceae (Singh 1979) and thus have generated new interest.

The present communication discusses cytogenetic behaviour of four natural polyploid taxa of *Cucumis* ($2n=48$), *C. pustulatus*, *C. membranifolius*, *C. melleus* and *C. heptadactylis*, and of two colchicoids of *C. melo* var. *momordica* and *C. prophetarum* with a view to understand the polyploidy in them and cytological stability and agronomic potential of the induced polyploids.

Materials and methods

Identities and sources of the taxa investigated are listed in Table 1.

Healthy seeds of *C. melo* var. *momordica* and *C. prophetarum* were used for colchicine treatment. Three concentrations of colchicine, 0.2%, 0.3%, and 0.4% were applied in three different ways:

a) Apical bud treatment

A small cotton web soaked in aqueous colchicine solution of a concentration was kept on exposed apical buds of young seedlings for different durations spread over to 2-3 days between 6.10 a.m. to 2.30 p.m. in order to synchronise the treatment with division cycle (Table 2). Cotton was constantly kept wet by dropping the solution at frequent intervals. At the end of the treatment, the apical buds

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were washed thoroughly with water.

b) *Seed treatment*

Seeds soaked on moist filter paper for 12 hours, wiped between the folds of blotting paper were immersed in colchicine solution of different concentrations between 6.30 a.m. to 2.30 p.m. for different periods (Table 2). Treated seeds were washed in running water for half an hour and sown in pots.

c) *Seedling treatment*

The cotyledonary leaves of young seedlings were immersed in different concentrations of aqueous colchicine solution. The roots were kept wet by wrapping them in moist cotton. After a thorough wash, seedlings were planted in pots. In *C. prophetarum* this treatment was followed by apical bud treatment (Table 2).

All the secondary shoot arising from treated plants were removed, so as to boost the growth of the treated shoot and to ensure higher ploidy level.

Table 1. Identity and source of *Cucumis* Species

Name	2n	PI Number	Source
<i>C. pustulatus</i> Hook. F. (syn. <i>C. aculeatus</i> Cogn.)	48	273649	Georgia, U.S.A.
<i>C. membranifolius</i>	48	273571	" "
<i>C. meeusei</i>	48	376068	" "
<i>C. heptadactylis</i>	48	282446	" "
<i>C. prophetarum</i>	24		Jodhpur, India
<i>C. melo</i> var. <i>momordica</i>	24		" "

For meiotic analysis young flower buds were fixed in acetic alcohol (1 : 3) between 7.00 a.m. to 18.30 a.m. A few drops of 5% ferric chloride were added to the fixative. Anthers were squashed in 1% acetocarmine. Temporary preparations were used for detailed meiotic analysis and photography. Slides were made permanent by n-butylalcohol schedule (Celarier 1956).

Results

Table 2 shows that colchicine was most effective at 0.3% or 0.4% and also that treatment of apical buds was better than any other.

Morphology

Morphologically as well as anatomically both the colchiploids were vigorous in leaf, flower, fruit and the seed sizes compared to their respective diploid counterparts (Figs. 1a, b, c, d, e, f). Stomata size was also large but the number per unit area was reduced. Similarly the seed size were larger, although number of seeds per fruit was less (Table 3). A comparative account of morphological details of diploid species and their respective autotetraploids is summarised in Table 3.

Cytology

At MI both the colchiploids showed univalents and multivalents along with

normal bivalents (Figs. 1g, h). Ranges and means of univalents were 0–4 and 1.2, and 0–4 and 0.88, respectively in *C. melo* var. *momordica* and *C. prophetarum*. In natural polyploid taxa, *C. pustulatus* and *C. membranifolius* showed regular bivalents only (Figs. 2a, b) while in *C. meeusei* and *C. heptadactylis* occasional quadri-valents (means 0.12 and 0.2, respectively) were observed (Table 4, Figs. 2d, 2e).

Table 2. Details of colchicine treatments in *Cucumis* species

Species	No. of plants treated	Colchicine concentration %	Duration in hrs.	No. of surviving plants	2×	4×
<i>C. melo</i> var. <i>momordica</i>	15	0.2	Seedling treatment 16–18	10	8	2, mixoploid
	15	0.3	14–15	9	6	3, mixoploid
	15	0.4	16–17	7	5	2, tetraploid
<i>C. prophetarum</i>	10	0.4	Seed treatment 8 hrs.	8	7	1, tetraploid
	10	0.3	8 hrs. seed treatment + 8 hrs. seedling treatment	7	6	1, tetraploid

Table 3. Comparative morphology of diploids and induced autotetraploids in *Cucumis* species

Characters studied	<i>C. melo</i> var. <i>momordica</i>		<i>C. prophetarum</i>	
	Diploid	Tetraploid	Diploid	Tetraploid
Thickness of stem	0.3 cm	0.4 cm	0.2 cm	0.3 cm
Length of internode	8.17 cm	6.06 cm	4.12 cm	5.0 cm
Length of petiole	7.06 cm	7.62 cm	5.86 cm	6.75 cm
Length and breadth of leaf	6.09 × 7.7 cm	6.16 × 10.36 cm	3.8 × 3.12 cm	4.08 × 5.08 cm
Colour of leaf	Light green	Dark green	Light green	Dark green
Length of calyx tube	0.56 cm	0.63 cm	0.45 cm	0.52 cm
Length and breadth of corolla tube	0.47 × 0.39 cm	0.74 × 0.58 cm	0.6 × 0.55 cm	0.8 × 0.7 cm
Size of fruit	3.4 × 3.2 cm	3.8 × 3.5 cm	3.9 × 2.5 cm	4.2 × 2.5 cm
No. of seeds/fruit	180	85*	120	15*
Seed size	4.2 × 2.5 mm	6.0 × 2.83 mm	4.34 × 2.14 mm	4.85 × 2.5 mm
No. of stomata/field	10.8 (10 × 45)	6.4 (10 × 45)	11 (10 × 45)	6.7 (10 × 45)
Length and breadth of guard cells	4.5 × 1.1	4.83 × 1.25	3.8 × 0.8	4.22 × 1.1
Length and breadth of stomatal aperture	3.2 × 0.95	3.5 × 1.16	3.5 × 0.8	4.0 × 1.05
Pollen size	3.75 μm	4.38 μm	2.38 μm	3.0 μm

* Including shrivelled seeds.

The mean chiasma frequency was almost identical in all the four natural polyploids. The details of chromosome associations are summarised in Table 4.

At AI both the colchiploids have almost similar percentages of PMCs with regular distribution of chromosomes (48 and 50) and high pollen fertility (76% and

Table 4. Mean chromosomal association and chiasma frequency in induced and natural polyploids of *Cucumis*

Species	Univalents		Bivalents		Trivalents		Quadrivalents		Chiasma frequency		Terminalization of chiasma		Terminalization coefficient
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	
Natural polyploids													
<i>C. pustulatus</i>	0	0	24	24	0	0	0	0	44-48	46.1	37-46	43.0	0.93
<i>C. membranifolius</i>	0	0	24	24	0	0	0	0	46-48	47.0	37-47	42.5	0.90
<i>C. meusei</i>	0	0	22-24	23.5	0	0	0-1	0.12	45-48	46.9	37-46	43.0	0.92
<i>C. heptadactylis</i>	0	0	20-24	23.5	0	0	0-2	0.20	44-48	46.0	39-45	44.4	0.97
Colchipooids													
<i>C. prophetarum</i>	0-4	0.9	13-24	20.8	0-3	0.4	0-4	1.2					
<i>C. melo</i> var. <i>momordica</i>	0-4	0.5	16-24	21.2	0-2	0.13	0-5	1.7

Figs. 1a-j. a, leaves of *C. prophetarum*, diploid left and tetraploid right; *C. melo* var. *momordica*. b, diploid pollen. c, tetraploid pollen. d, diploid flower left and tetraploid right. e, diploid fruits up and tetraploid down. f, diploid seeds up and tetraploid down. Pollen mother cells of tetraploid *C. prophetarum* showing: g, 24 II. h, 20 II + 2 IV at MI. i, and j, laggards at AI and AII. $\times 1200$.

77%). Among natural polyploids, *C. pustulatus* and *C. membranifolius* have normal distribution of chromosomes, and high pollen fertility, (75 and 78%, respectively), while in two other taxa, *C. meeusei* and *C. heptadactylis*, 30% and 20% of PMCs respectively, showed irregular distribution of chromosomes in the form of laggards

Figs. 2a-h. Pollen mother cells at MI showing: a, 24 II in *C. pustulatus*. b, in *C. membranifolius*. c, in *C. meeusei*. d, 24 II+1 IV in *C. meeusei* and *C. heptadactylis*. e, f in PMC at AI showing g, unequal distribution. h, laggards. $\times 1200$.

and unequal distribution (Figs. 2g, h), and little lower pollen fertility (52% and 62%, respectively, Table 5).

Discussion

In *Cucumis*, reports of natural polyploids by Shimotsuma (1965) and Dane and Tsuchiya (1979) and induction of polyploid with increased fruit size in some wild and semicultivated species by Singh and Roy (1975) have raised new interest. A comparison of meiotic behaviour of colchipooids and natural polyploids shall provide an insight into the nature of polyploidy for natural polyploids and into future cytological, biological and agronomic stability of colchipooids.

Table 5. Chromosome distribution and pollen fertility in induced and natural polyploids of *Cucumis*

Species	% of cells with normal distribution	% of cells with unequal distribution	% of cells with laggards	% Pollen fertility
Natural polyploids				
<i>C. pustulatus</i>	100	0	0	75
<i>C. membranifolius</i>	100	0	0	78
<i>C. heptadactylis</i>	80	15	5	60
<i>C. meusei</i>	70	20	10	52
Colchipooids				
<i>C. melo</i> var. <i>momordica</i>	50	20	30	77.7
<i>C. prophetarum</i>	48	22	30	76.7

In general, induction of polyploidy increases the cell size (Stebbins 1950), which in turn provides gigantism to the plants over their diploids. In cucurbits it has not been a universal feature especially in relation to fruit character. Earlier reports of Shifriss (1942) indicated that initial increase in size of ovary does not keep pace with further development of plant, thus resulting in small fruits. But in the present investigation, contrary to earlier reports, in both the taxa, the plants with increased ploidy showed vigour after initial stunted growth and later attained a near normal growth producing vigorous plants. The initial increase in the ovary size of female flowers in the present material kept pace with the further development of plants resulting in comparatively larger fruits; the seed size also increased although the number of fruits per plant and the number of seeds per fruit were quite low (Table 3), which is not unexpected keeping in view the new genetic constitution of these autopolyploids, which result in meiotic and physiological instability. However this vigour and prolonged duration of these autotetraploids, if consistently observed in later generations can be exploited for getting fruits over the normal season. These observations reinforce the earlier observations of Singh and Roy (1975) that in wild and semicultivated taxa which have not undergone heavy selection pressure, the polyploid breeding still stands with hope.

In both the autotetraploids a high frequency of bivalents has been recorded, so much so that a high percentage of cells has only bivalents. There can be various

reasons for such a cytological behaviour in a raw autotetraploid. In *Cucumis* species small size and high number of submedian and acrocentric chromosomes can result in reduction of chiasma frequency and/or in their localization which can result in breakdown of a multivalent into bivalents at an early stage of meiotic cycle. Moreover in a raw autotetraploid, chiasma frequency can get reduced because of genetic imbalance and mechanical interference for the availability of partner exchange at the pairing initiation site, which may attract different partners at the same time. John and Henderson (1962) have also observed bivalent formation in autotetraploids with small chromosomes. The other possibility for high bivalent frequency may be genetic control of pairing which requires further investigations.

The natural polyploids also had a very high bivalent frequency, so much so that in *C. membranifolius* and *C. pustulatus* only regular bivalents were observed, which is indicative of allopolyploid nature of these two species. High pollen fertility of these taxa further strengthens this conclusion. But regular bivalent formation can be observed even in autotetraploid as a consequence of attainment of diplontic behaviour or on account of genetic control of pairing. Hence conclusive evidence on nature of polypoidy in these taxa can be obtained from crossing of these taxa with ancestral diploid taxa, as has already been evidenced in case of *C. pustulatus* by Dane *et al.* (1980), or through evidences from other lines of investigations.

In two other natural polyploid species *C. heptadactylis* and *C. meeusei*, the recorded multivalent associations are indicative of their autopolyploid nature. Though such a frequency of multivalents can also be possible in segmental allopolyploids but the comparable multivalent frequency observed in artificial autotetraploids studied in present investigation reinforce the autotetraploid nature of these two species. Earlier Shimotsuma (1965) and Dane and Tsuchiya (1979) in *C. heptadactylis* observed a higher frequency of multivalents than observed in present investigation. This suggests that presently observed low frequency of multivalents in these taxa is due to the progressive attainment of diplontic behaviour in subsequent generations. Probably, either selection of desirable mutant ensuring bivalent formation, or zygote resulting from fusion of gametes from PMCs with regular bivalents has conferred upon the tetraploids the advantage for the progression to diplontic nature. The low pollen fertility observed in these two natural polyploid species compared to the two preceding polyploid taxa suggest that multivalent formation leads to irregular distribution of chromosomes, and lowers the pollen fertility. A comparable situation in the colchipooids studied in present investigation further suggests that they can also regain diplontic behaviour and fertility with preferential selection of diplontic gametes in subsequent generations.

The seed fertility in the induced autotetraploids, even with a high pollen fertility, was very poor which is suggestive of the fact that for seed fertility the normal meiotic cycle at gametic level is not the only prerequisite, and it is affected by some other factor as well, such as genetic or physiological.

In C_2 generation 20–25% of the plants breed true for their ploidy level which can be differentiated morphologically from their diploid counterparts on account of the vigour. Reversion of a majority of the progenies to the diploid level indicates that monoploid gametes compete better than diploid, on account of their prolonged

genetic stability in nature. Subsequent selection of polyploid progenies may result in stable tetraploidy.

The present observations supporting earlier reports of Roy and Ghosh (1971) and Singh and Roy (1975) indicate a happy situation for polyploid breeding in wild and semicultivated cucurbitaceous taxa. Though conclusive evidences can be obtained by the detailed biological and agronomic assessment of these autotetraploids in subsequent generations, but the comparable observations in natural autotetraploids studied here strongly suggest that through generation advance they should regain diplontic behaviour to result in normal meiotic cycle and fertility keeping the desired features of higher ploidy, as observed in *C. vulgaris* (Andrus *et al.* 1971).

From the basic point of view, the observations in natural polyploid taxa suggest that polyploidy either independently or in combination with hybridization has evolved new population in the genus *Cucumis*. It should be pointed out that all the species recorded with polyploid number so far are perennials. Therefore in nature the process of polyploidy has been successful in species, where perenniality of a taxa can sustain either autotetraploidy or a sterile hybrid for a longer period to achieve spontaneous doubling of chromosomes, and produce allopolyploid to resort back to sexual mode of reproduction.

Summary

Colchipooids were raised in *C. melo* var. *momordica* and *C. prophetarum*. Morphologically autotetraploids were vigorous with a little increase in fruit size, though with decrease in number of fruits per vine and seeds per fruit.

A comparative meiotic analysis of colchipooids and four natural polyploids, *C. pustulatus*, *C. membranifolius*, *C. meeusei* and *C. heptadactylis* revealed that *C. pustulatus* and *C. membranifolius* with regular bivalent association are allopolyploids, while *C. meeusei* and *C. heptadactylis* with a few multivalent associations are autopolyploids, may be segmental allopolyploids. A higher frequency of bivalents in *C. heptadactylis* compared to earlier reports suggests that either selection of mutants ensuring bivalent association or diplontic gametes are evolving these species to diplontic behaviour. A high bivalent frequency comparative to natural polyploids observed in colchipooids suggest that they can attain diplontic behaviour like natural polyploids with generation advance and later may stabilise cytologically, sexually and agronomically.

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