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Cytomorphology of induced octoploid Chili pepper (*Capsicum annuum* L.)

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Summary. Octoploidy was induced in Chili pepper (*Capsicum annuum* cultivar 'cerasiformis') through the application of colchicine and the cytomorphological features of two octoploid plants were described. In general, the octoploids did not exhibit gigas characters when compared to the tetraploids; on the contrary they were less vigorous, suggesting that the optimum and desirable ploidy level for *Capsicum* is probably tetraploid. Chromosome associations such as octovalents and hexavalents, in addition to IVs, IIIs, IIs and Is, were recorded at diakinesis and metaphase I. Meiosis was highly irregular and the pollen and seed fertility was very low. Cytological features of octoploid Chili peppers are compared with octoploids of *Physalis* and *Petunia.*

Key words: *Capsicum annuum* – Colchicine – Octoploid – Cytomorphology

Introduction

Auto-octoploid is a polyploid in which each chromosome of the haploid set has eight homologues in the genome. Reports of such naturally occurring octoploids are rare in the plant kingdom except in a few taxa: *Bromus inermis* (Armstrong 1980) and *Chrysanthemum ornatum* (Watanabe 1981a). However, in a few instances octoploidy has been obtained either spontaneously (*Coffea*) or induced through colchicine treatment (*Petunia, Triticale*). All the octoploids are not gigas in their exomorphic features and sometimes are less vigorous and usually less fertile than their diploid and tetraploid progenitors. Nevertheless, studies on octoploidy will throw considerable light on chromosome pairing, which is of importance in understanding the organisation and distribution of the genetic material in such highly polyploid organisms during the meiotic cycle. In addition, they highlight the evolutionary trends which still remains a matter of interest.

A survey of the literature does not reveal any earlier report of polyploidy higher than tetraploidy in the genus *Capsicum.* Auto-octoploid has been obtained in Chili pepper (*Capsicum annuum* cultivar 'cerasiformis') for the first time. Hence the present study reports on the cytomorphological behaviour of the induced octoploid.

Materials and methods

With a view to obtain tetraploids in *Capsicum annuum* cultivar 'cerasiformis', a diploid with $2n=24$, three lots of seedlings at the 4-leaf stage were treated with 0.3% aqueous colchicine using cotton plugs for 12, 18 and 24 h respectively. The first lot was treated continuously on a single day whereas the second and third lots were treated for two and three days for 9 and 8 h per day, respectively. In addition to obtaining tetraploids in all three treatments, two octoploid plants were obtained in the last treatment. These two were designated 24-8 and 24-10.

The data on morphological parameters were statistically analysed. Buds were fixed in a 1:3 acetic acid and alcohol mixture and squashes were made with standard 2% aceto-carmine to study meiosis.

Results

The two octoploid plants could be easily distinguished from the diploid progenitors as they were more gigas in their morphological features, a feature also observed in the sib autotetraploid plants. In general, both tetraploids and octoploids resembled each other. In most of

Table 1. Comparison of morphological parameters of tetraploids and octoploids in *C. annuum* L

Parameters	Mean ± SE tetraploid	Octoploid	t	df	*P*	8n/4n
Height (cm)	70.16 ± 1.18	66.5 ± 1.06	1.476	6	0.3 – 0.1	0.95
Stem diameter (cm)	4.33 ± 0.25	3.4 ± 0.07	2.079	6	0.1 – 0.05	0.78
Internode length (cm)	3.04 ± 0.08	2.18 ± 0.19	3.68	18	> 0.01	0.72
Leaf length (cm)	9.19 ± 0.82	5.3 ± 0.77	4.77	18	> 0.001	0.58
Leaf width (cm)	3.92 ± 0.40	2.17 ± 0.23	4.23	18	> 0.001	0.55
Leaf area (sq. cm)	18.13 ± 3.03	6.4 ± 1.49	3.80	18	> 0.01	0.35
Flower diameter (mm)	28.1 ± 1.36	17.9 ± 0.67	6.73	18	> 0.001	0.64
Anther length (mm)	2.0	2.21 ± 0.06	3.16	18	> 0.01	1.105
Style length (mm)	4.65 ± 0.16	4.1 ± 0.12	2.79	18	> 0.05	0.88
Fruit length (mm)	29.5 ± 0.83	24.5 ± 0.16	2.455	10	> 0.05	0.83
Fruit diameter (mm)	44.5 ± 2.82	16.5 ± 0.47	4.046	10	> 0.01	0.37

Table 2. Mean chromosome associations and chiasma frequency at Metaphase I in the octoploid *Capsicum annum* L. (Range indicated in parentheses)

Plant no.	Cells	VIII	VI	IV	III	II	I	Chiasma frequencies
24-8	75	0.493 ± 0.09 (1 – 3)	0.747 ± 0.12 (1 – 4)	7.653 ± 0.44 (2 – 15)	1.04 ± 0.19 (2 – 4)	23.466 ± 0.76 (10 – 44)	6.91 ± 1.27 (6 – 36)	67.2
24-10	55	0.44 ± 0.09 (1 – 2)	0.78 ± 0.14 (1 – 4)	7.04 ± 0.53 (2 – 15)	1.22 ± 0.25 (3 – 6)	23.82 ± 0.93 (10 – 40)	8.38 ± 1.55 (6 – 36)	65.9

Table 3. Comparison of chromosome size, chiasma frequency and chromosome associations in different octoploids of Solanaceae

Species	Range in the length of somatic chromosomes	Mean frequency of higher associations		Mean chiasmata per bivalent			Source
		Octovalents	Hexavalents	Diploid	Tetraploid	Octoploid	
Physalis pubescens (n = 12)	1.8 – 3.0 μ	–	–	1.44	0.97	0.88	Menzel 1951 Lydia 1982
Petunia axillaris (n = 7)	3.56 – 6.68 μ	0.33 (0 – 2)	0.26 (0 – 1)	1.46	1.32	1.33	Padmaja 1979
Capsicum annuum (n = 12)	3.3 – 7.0 μ	0.49 (0 – 3)	0.75 (1 – 4)	1.79	1.46	1.39	Present study

the morphological features the tetraploids exhibit gigas characters when compared to the octoploids (Figs. 1 and 2), except for the anthers, which are large, thick, sometimes deformed and coalesce with the corolla in the octoploid. A detailed comparison of eleven morphological features between the tetraploids and octoploids is listed in Table 1.

Cytology

In the octoploids all the PMCs were abnormally larger and fewer per anther than those found in the diploid and tetraploid plants. Although chromosome pairing could not be studied in detail at the pachytene stage because of clumping, considerable pairing and the occasional occurrence of partner exchanges could be recognized. In conformity with this, multivalents were observed at diakinesis and metaphase I. At diakinesis, only one nucleolus was observed per PMC. Chromosome associations such as octovalents and hexavalents (0.8 and 1.2 per cell, respectively) were observed; quadrivalents, and bivalents were the most frequent associations (10.2 and 20.2 per cell, respectively). A few trivalents (0.4 per cell) also occurred. The chromosomes in the PMCs appear to be smaller as compared to the diploids and tetraploids.

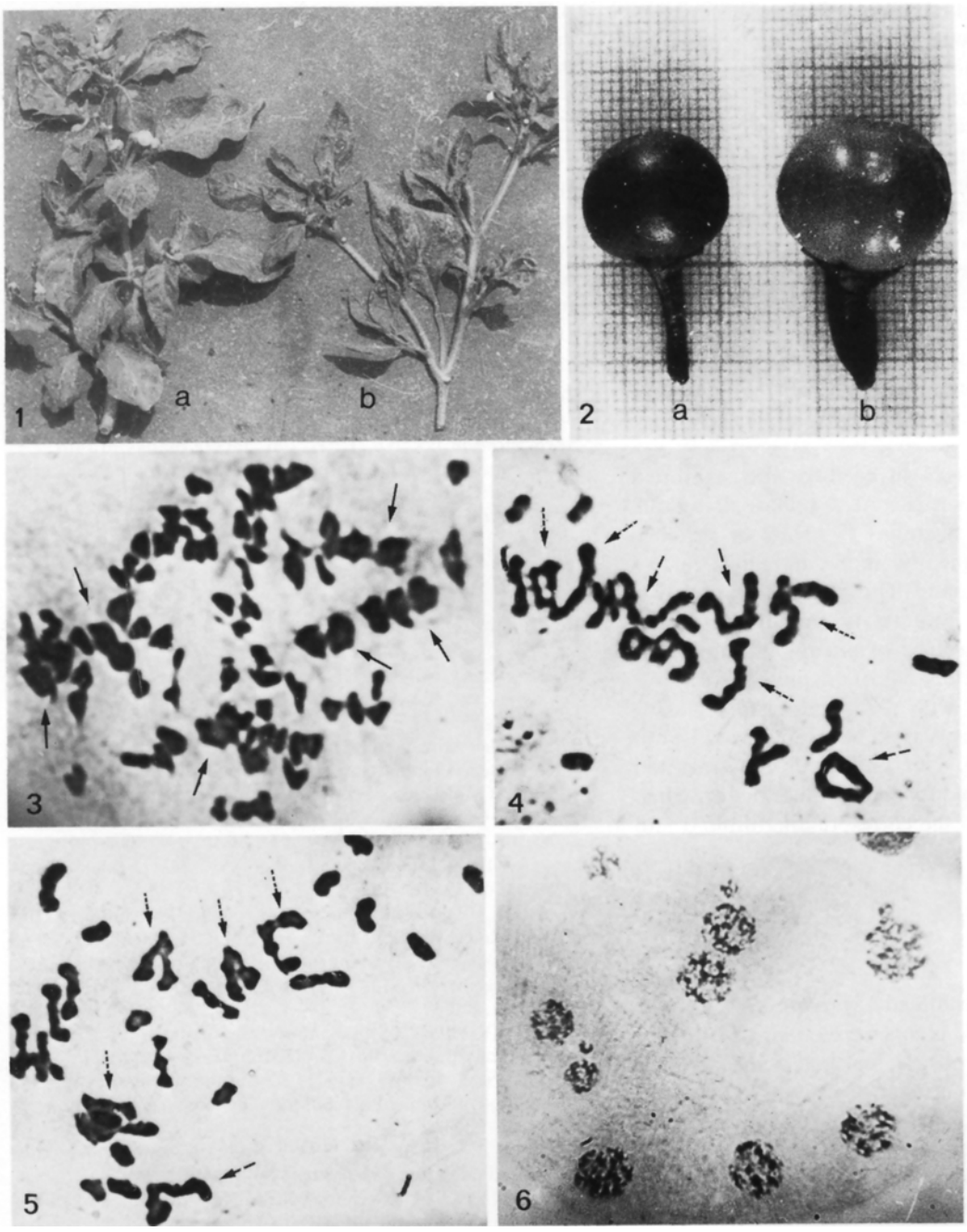

Fig. 1. Twigs of (*a*) tetraploid and (*b*) octoploid Chili pepper

Fig. 2. Fruits of (*a*) octoploid and (*b*) tetraploid plants of Chili pepper

Fig. 3. Late diakinesis showing higher associations (↑) in octoploid Chili pepper (× 1500)

Fig. 4. Metaphase I showing 9II + 2III + 6IV + 4VI (↑) + 3VIII (↑) associations in octoploid Chili pepper (× 1100)

Fig. 5. Metaphase I showing 14II + 4III + 6IV + 4VI (↑) + 1VIII (↑) associations in octoploid Chili pepper (× 1100)

Fig. 6. Telophase II showing 10 groups of chromosomes in octoploid Chili pepper

At metaphase I chromosome pairing was similar to that at diakinesis except for the formation of a few univalents (Table 2 and Figs. 3–5). The data on octovalent frequency conform to the binomial expectation whereas hexavalent frequency does not conform to it. A maximum of 79.2% of the chromosomes per PMC were involved in multivalent formation. The mean chiasma frequencies in the two plants are less than twice that in the corresponding autotetraploids and less than four times that in the corresponding diploids (67.2 and 65.9 for octoploids, 35.12 for tetraploids and 21.53 for diploids).

Linear co-orientation of multivalents predominates (90%) over the remaining kinds which largely contributed to the irregular disjunction of the chromosomes at anaphase I.

The subsequent stages of meiosis also exhibited high degrees of irregularities. At telophase II all the PMCs had more than 4 groups (5 to 22) of chromosomes (Fig. 6). Subsequently, more than four microspores per PMC were found. This clearly indicates that production of euploid gametes is rare, if not totally absent, while the occurrence of aneuploid gametes is more probable in the octoploids of the present study.

Pollen stainability with 2% acetocarmine as a measure of pollen fertility varied between 3 to 12% with a mean of 5.18 ± 0.85. Not one seed was obtained either upon selfing, upon open pollination or when mated with pollen from the corresponding diploid and tetraploid plants.

Discussion

Production of octoploids directly from diploids is a rare event. The two octoploid plants were obtained from the 24 h treatment; it is probable that colchicine arrested the spindle formation during two cell division cycles ensuring the formation of octoploids.

The octoploid Chili pepper plants showed a significant reduction in the size and vigour of morphological features in comparison to corresponding tetraploids which indicates that increase in ploidy level is not always accompanied by gigas features. Each species has a ploidy tolerance capacity and when the optimum level is achieved, subsequent increase in the level of ploidy is not beneficial to the organism – rather, a retarding effect is produced, as in the present case.

A similar situation was reported in the octoploids of *Petunia* (Levan 1939), *Solanum tuberosum* (Muntzing and Lunquist 1939) and *Coffea* (Chinnappa 1969) where the plants were fragile and deformed and had reduced viability. However, a reverse situation was recorded in *Bromus* (Armstrong 1980), *Chrysanthemum ornatum* (Watanabe 1981a), *Solanum aculae* (Lam 1943; Sinha 1953), Strawberry (Mok and Evans 1971; Byrne and Jelenkovic 1976) and *Triticale* (Riley et al. 1961; Riley and Chapman 1958) where the plants were quite stable and vigorous at the octoploid level, a situation also common even in the decaploid *Chrysanthemum crassum* (Watanabe 1981b). From the present study it appears that the optimum and desirable ploidy level for vigorous growth in Chili peppers is tetraploid.

The comparison of octoploids of *Capsicum, Petunia* and *Physalis* (Table 3) reveals a positive correlation between chromosome length, mean chiasma frequency per bivalent and mean frequency of higher associations. Also, chromosome associations higher than the quadrivalents were present in *Capsicum annuum* and *Petunia axillaris* and absent in *P. pubescens,* probably since the length of the somatic chromosomes is greater in them than in *P. pubescens.*

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