INDUCTION OF POLYPLOIDY IN WATERMELON

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Introduction

The development of seedless watermelon (*Citrullus vulgaris* Sehrad) in Japan by crossing induced tetraploids with the parent diploids is a good example of the successful exploitation of the technique of chromosome doubling through colchicine application in modern plant breeding. Watermelon is cultivated widely in India and its fruits are very popular particularly during summer. Studies on the production of triploid seedless watermelon were commenced at the Indian Agricultural Research Institute in 1956 and the results of this study are summarised in this report.

Material and Methods

Seeds of the watermelon varieties Asahi Yamato and Farrukhabadi maintained by the Plant Introduction Section of the Botany Division of the Indian Agricultural Research Institute were sown in the field for treatment with colchicine. The methods of colchicine application were:—

(*a*) Germinating seeds in 0·1, 0·2, 0·3 and 0·4% colchicine solution.

(*b*) Treatment of the growing point in the cotyledonary stage with colchicine applied either as an aqueous solution or in the following three media.

(1) Colchicine in glycerine (0·2 and 0·4% colchicine in 10% glycerine).

(2) Colchicine emulsion suggested by Warmke and Blakeslee (1939) for induction of polyploidy in *Nicotiana*. The colchicine concentrations used by us were 0·2 and 0·4%.

(3) Colchicine in agar (1% colchicine and 2% agar, mixed in equal parts).

Experimental Results

Effect of treatments.—The treatment of sprouted seeds proved to be too drastic at all the concentrations tried. The percentage of mixoploids and tetraploids obtained from various treatments of the growing point is given in Table I. It would be observed from the data that the maximum frequency of polyploids (total and mixoploids) in both Asahi Yamato and Farrukhabadi, were obtained by treating growing points with 0·2% colchicine emulsion. The next best treatment was the application of 0·4% colchicine solution, as drops, to the growing point for one hour daily on four consecutive days.

TABLE I

Results of different colchicine treatments

Variety	Concentration (%) and medium of application	Method of treatment	No. of plants treated	Tetraploid or mixoploid	% Success
Farrukhabadi	0·4 col. solution	Drop	39	14	35·9
	0·2 col. solution	Drop	45	4	8·66
	0·4 col. emulsion	Drop	36	All died	..
	0·2 col. emulsion	Drop	48	19	39·59
	0·5 col. agar	Smeared on growing point	46	1	2·17
Asahi Yamato	0·4 col. solution	Drop	62	10	16·1
	0·2 col. solution	Drop	41	3	7·3
	0·4 col. emulsion	Drop	38	All died	..
	0·2 col. emulsion	Drop	43	7	16·29
	0·5 col. agar	Smeared on growing point	29	1	3·45

Morphological Characteristics

The tetraploid plants exhibited the usual gigas features characteristic of autopolyploids. Thus these plants, in general, showed greater growth and vigour as compared to the diploids (Figs. 1 and 2). They had thicker, more prominently ribbed stems with shorter internodes; larger, thicker and more deeply lobed leaves of a dark-green colour having a lower length/breadth index of the terminal lobe and larger anther lobes in the male and larger ovaries in the female flowers. The flowering in tetraploid Farrukhabadi was delayed by 10–15 days. The seeds in tetraploid fruits were larger but fewer, more round and characterized by fissures and wrinkles on the seed-coat (Figs. 3, 4 and 5). Tetraploids of Asahi Yamato were very late and since they became heavily infested with beetles and damaged by rains no fruit set could be obtained. Consequently seed setting studies had to be confined only to Farrukhabadi.

Meiotic behaviour

Meiosis was studied during microsporogenesis in two tetraploid plants. In diploids, 11 bivalents were invariably seen at first metaphase. Ring bivalents with chiasmata in both the arms of the chromosomes were most common (Fig. 6). In the tetraploids various combinations of univalents, bivalents, trivalents and quadrivalents, making up a total of 44 were observed. The results of pairing analysis and multivalent frequency at metaphase I and the types of quadrivalents that occurred are given in Tables II, III and IV.

TABLE II

Pairing frequency in diploid and tetraploid watermelon at metaphase I

Plant No.	Chromosome No.	No. of PMC's studied	Mean frequency per nucleus of			
			Uni-valents	Bi-valents	Tri-valents	Quadri-valents
1	22	20	..	11	..	..
2	44	31	0·225	9·1	0·225	6·23
3	44	18	0·11	10·78	0·11	5·5

TABLE III

Frequency of multivalents in tetraploid watermelon at metaphase I

Plant No.	No. of cells with quadrivalent + trivalent frequency of												Multivalents	
	0	1	2	3	4	5	6	7	8	9	10	11	Mean/cell	Coefficient of realization
1	0	0	0	1	5	4	5	6	8	0	1	1	6·45	0·586
2	0	0	0	2	2	4	5	3	2	0	0	0	5·61	0·51

It will be observed from Table III that 11 quadrivalents, which is the maximum number possible, was observed in one cell (Fig. 7). The coefficient of realization of multivalents ranged from 0·51–0·56. Among the quadrivalents the symmetrical types were the most prevalent. The mean chiasma frequencies per nucleus and per bivalent are given in Table V. It is interesting that in spite of the small size of the chromosomes and the low frequency of chiasmata, the coefficient of realization of multivalents is fairly high.

TABLE IV

Frequency of different types of quadrivalents in tetraploid watermelon at metaphase I

Plant No.	No. of quadrivalents of type: 3 X ta			4 X ta	Total No. of quadrivalents studied	Quadrivalents with symmetrical configuration (%)
	X	Y	|	[ ]		
1	10	10	14	159	193	89·1
2	1	5	4	89	99	92·8

TABLE V

Chiasma frequency in diploid and tetraploid watermelon at metaphase I

Material	No. of PMC's studied	No. of config. with X ta: 0	1	2	3	4	Chiasmata: Total	Per cell	Per bivalent
Diploid ..	20	..	..	220	..	..	440	22·00	2·0
Tetraploid	49	9	49	436	44	284	2045	41·73	1·8

In diploids the anaphase disjunction was regular. In the tetraploids, there were a few lagging univalents in some cells. At metaphase II, 22 chromosomes were most commonly seen at each pole (Fig. 8). In a few cells, however, there was an unequal number of chromosomes at the two poles probably due to the non-inclusion of lagging chromosomes into either of the groups. Regular equational division of the chromosomes took place at second anaphase. Tetrads were formed after the completion of the second division and were of the tetrahedral type.

Fruit and seed setting

Data on seed setting in diploid and tetraploid fruits developed under open pollinated condition and in selfed tetraploid fruits are given in Tables VI and VII. As indicated by the data, the mean seed set per fruit in the tetraploid following selfing is considerably lower than the seed set obtained in open pollinated tetraploid and diploid fruits. Further, the percentage of shrivelled seeds in tetraploid is 17·73% higher than that of the diploid. Only one fruit could be obtained from 72 selfed tetraploid flowers. In other cases, the fruits developed normally for about a week, but later started shrivelling and finally dropped off.

TABLE VI

Seed setting in diploid and tetraploid watermelon

Material	Total No. of seeds per fruit	No. of shrivelled seeds	% shrivelled seeds
Diploid ..	681	157	23·06
Tetraploid ..	446	182	40·79

TABLE VII

Seed setting in tetraploids

Pollination	No. of fruits obtained	Mean No. of seeds per fruit
Open pollinated ..	4	526
Selfed ..	1	364

Sucrose content of 2x and 4x fruits

The sugar content, determined by a hand refractometer, was 8% in the fruits of both diploid and tetraploid Farrukhabadi. Chromosome doubling therefore did not result in any alteration in this character.

Triploid seeds

One fruit obtained from crossing tetraploid and diploid Farrukhabadi yielded 67 seeds. The seeds on dissection showed a very poor embryo development and they germinated only when the seed coats were removed.

Triploid plant

Three triploid plants of the variety Farrukhabadi were raised during 1958. The seedlings were abnormal and poor in growth. There were several rudimentary seeds in a fruit set in one plant. These seeds were soft and the fruit could be eaten without removing the seeds. However the formation of a large number of ex-embryonate seeds may detract the value of a triploid Farrukhabadi fruit. Owing to unfavourable weather conditions and heavy infestation with pumpkin beetle, the plants were unhealthy and fruit setting very poor.

DISCUSSION

Success in the commercial production of triploid watermelons would depend upon (*a*) the availability of a suitable method of chromosome doubling, (*b*) the ease with which crosses between tetraploids and diploids can be made, (*c*) germinability of $3x$ seeds, (*d*) shape, size and yield of fruits in the triploid, and (*e*) the absence of seeds in the fruits set on the triploid plant. The results obtained by the Japanese workers (Kihara, 1951) and during the present study indicate that chromosome doubling can be induced in watermelon without much difficulty by applying colchicine on young seedlings, either as an emulsion comprising colchicine (0·2%), stearic acid, morpholine and lanoline or as drops (0·4%). The tetraploids breed true for chromosome number and there is hence no difficulty in maintaining them. Crosses between $4x$ and $2x$ plants seem to yield variable results and seed setting in such crosses is apparently conditioned by environmental conditions (Kihara, 1951). While we could not get any fruit setting in $2x \times 4x$ crosses one fruit was obtained in crosses between $4x \times 2x$ plants (the number of flowers pollinated was 29). Kihara (1951) found that in three sets of crosses made between June 14 and July 15, July 16 and August 4 and August 5 onwards, the percentage of fruit setting was 92·3, 25·0 and 10·7 respectively. It should therefore be possible to obtain a high percentage of success in $4x \times 2x$ crosses by fixing the optimum period for hybridization work.

The production of triploid seeds through artificially pollinating tetraploids with diploids will involve considerable labour and expense. Stebbins (1956) has expressed the view that the large amount of labour involved in this work militates against the success of these triploids in the United States. By growing diploids and tetraploids near each other and by using suitable marker genes, the need to carry out artificial hand-pollination can be obviated. A single gene controlling dark parallel striping on the fruit serves as a suitable genetic marker. If this dominant gene is present in the diploid pollinator, fruits of triploids will be striped while tetraploids will not show striping; thus the two types of plants derived from a tetraploid can be readily distinguished.

Since the triploid seeds needed for raising seedless watermelons are obtained from $4x \times 2x$ crosses, $4x$ seeds resulting from self-pollination tend to get mixed up with $3x$ seeds. Yamashita *et al.* (1957) have found $2x$ seeds also in the commercial $3x$-seed samples. It is thus necessary to have means for separating the $4x$, $3x$ and $2x$ seeds in a mixture. Fortunately, this can be easily accomplished since $3x$ seeds occupy an intermediate position between those of $2x$ and $4x$ in the length, width, thickness and weight of seeds (Yamashita *et al.*, 1957; Shimotsuma and Matsumoto, 1957). The $3x$ seeds, however, show a poor germination. The testa of the $3x$ seeds is thick and rugged and in many seeds the embryos are shrivelled. A probable reason for these abnormalities is the deviation from the normal 2:3:2 chromosome number relationship among the embryo, endosperm and maternal tissue. Thus, the chromosome numbers in these three organs in a $3x$ seed would be 33, 55 and 44 respectively as against 44, 66 and 44 in the tetraploid or 22, 33 and 22 in the diploids. Such a deviation from the chromosome number proportions in these tissues may lead to various types of somatoplastic sterility (Brink and Cooper, 1947). We found that the germination of the $3x$ seeds is vastly improved when the seedcoat is partially removed. Also, Yamashita *et al.* (1957) observed that complete germination can be obtained by a careful selection of healthy seeds and by maintaining the temperature of the hot-bed in which the seeds are sown at 30° C.

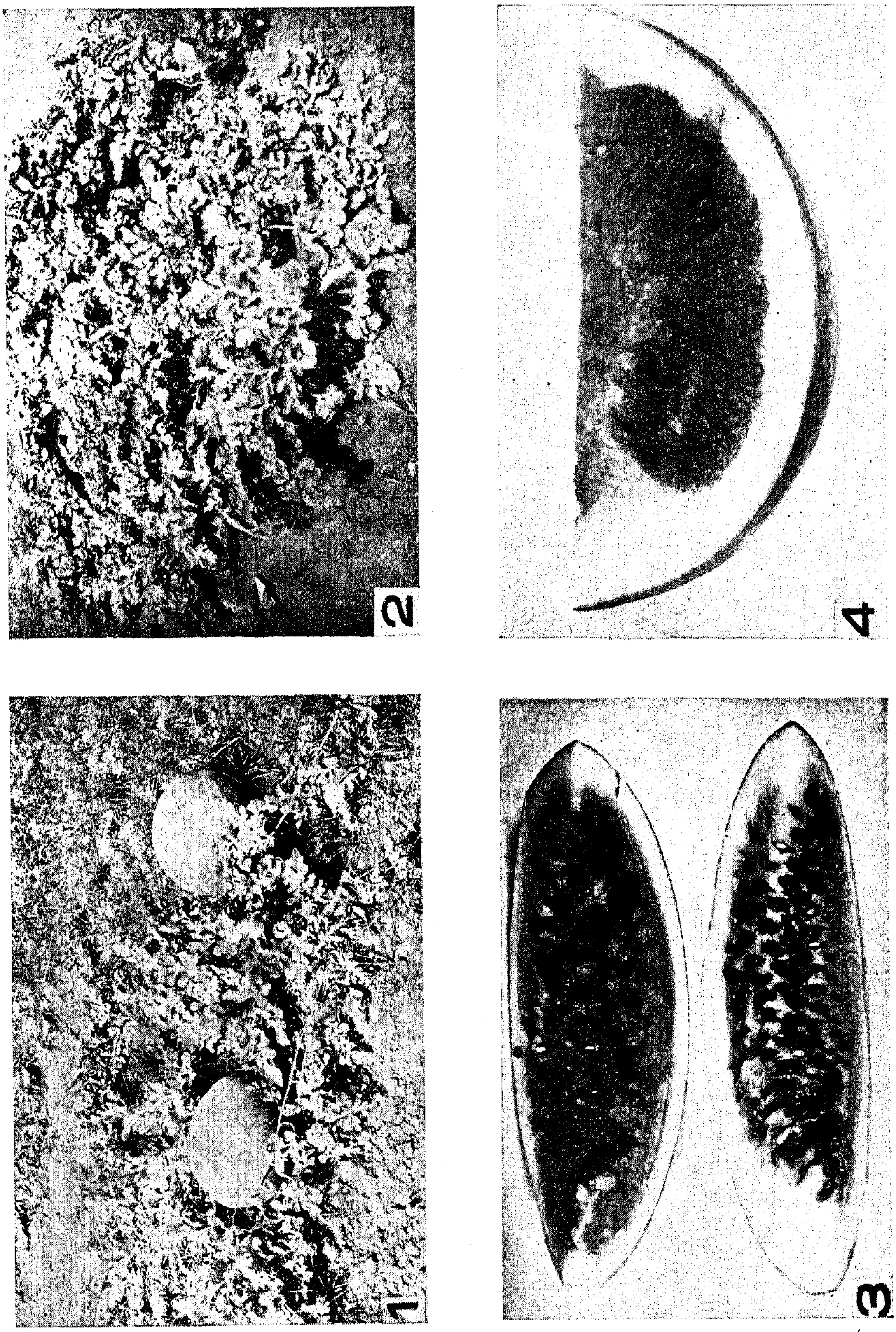
The yield of fruits in the triploid plants is directly related to the extent of stimulation provided by pollination. Diploid plants are therefore sown near triploids to act as pollen donors. Tetraploids can also be grown for this purpose but diploids are preferable in view of the high degree of pollen sterility found in the tetraploids. Colocynth is grown in Japan as pollinator with satisfactory results (Yamashita *et al.*, 1957).

A major problem in the production of seedless watermelons is the development of the ovule in some triploid combinations to a hard seedcoat, without embryo. These hardened seeds in the botanically seedless fruits are undesirable from the consumer's point of view, although such seeds are sometimes soft and edible. The Farrukhabadi triploid produced by us had a large number of such seeds. Yamashita *et al.* (1957) have found that there are varietal differences with regard to the tendency to form seeds without embryos. While in many Japanese varieties of watermelon the ovules showed no development in the triploid, some varieties from the United States, Spain and Pakistan gave unsatisfactory results. Thus, it is very important that the correct parents are chosen for the production of seedless fruits. Though it is difficult to draw any generalised inference from the behaviour of the single Indian variety used in the present study, it appears that it may be preferable to use Japanese varieties in such work since the experience of the Japanese workers with varieties from the Indian sub-continent has also been unfavourable and since it has been found by Singh and Joshi (1955) that some Japanese varieties of watermelon are outstanding in their performance when grown in parts of India.

The fruits of triploid watermelon may offer a suitable material for studying the biochemical effects of seedlessness. We could estimate only the sucrose content in $2x$, $3x$ and $4x$ fruits and no significant difference was found. However, quantitative and even occasionally qualitative alterations in biochemical characters following chromosome doubling are not uncommon both in induced (Eigsti and Dustin, 1955) and naturally occurring polyploids (Janaki Ammal and Sundara Raghavan, 1958) and it would be extremely interesting to study the triploid fruits from this view-point since, in addition to changes resulting from the increased chromosome number, there may also be changes arising from seedlessness in such fruits.

SUMMARY

Several colchicine treatment methods were tried in the varieties Farrukhabadi and Asahi Yamato of watermelon in order to induce chromosome doubling in them. Treatment of seedlings by drop method in the cotyledonary stage with an emulsion comprising 0·2% colchicine, stearic acid, morpholine and lanoline gave the highest percentage of affected plants (38·59%). Next in efficiency was the periodic application of drops of 0·4% colchicine on the growing point. The tetraploids had all the usual characteristics associated with autopolyploidy and during meiosis in a microsporocyte 11 quadrivalents were observed. The mean frequency of quadrivalents per cell was 5·91. Seed fertility in tetraploid watermelon was much reduced and from $4x \times 2x$ crosses in Farrukhabadi one fruit containing 67 seeds was obtained. The $3x$ seeds germinated only when the seed coat was partly removed and the fruit set on a $3x$ plant had seeds without embryo. There appears to exist genetic variability with regard to the ability of a strain to



FIGS. 1–4