Observations on Sterility of Induced Autotetraploid Watermelons[1]

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Abstract. Autotetraploid watermelons were produced by treating the growing points of diploid seedlings with aqueous colchicine solution or by soaking diploid seeds in colchicine solution. The soaking method was more effective.

Diploid pollen was apparently more viable than tetraploid pollen. Examination of germinating tetraploid pollen revealed that while over two-thirds germinated normally a small percentage sent forth 2, 3 or 4 normal-sized pollen tubes or a single bifurcated tube. Germinating diploid pollen always put forth only one unbranched pollen tube. Tetraploid plants did not set fruit when pollinated with pollen from tetraploids; however, they did set fruit when pollinated with pollen from diploids.

Cytological observations were made of pollen mother cells of both diploid and tetraploid plants. Meiosis in diploids was regular; however, irregularities were observed in tetraploids. Examination of some pollen mother cells of tetraploids at the quartette stage revealed microsporocytes in addition to the 4 microspores. The abnormal quartette formations and lower pollen viability of tetraploids were believed associated with irregularities observed at meiosis. Preliminary investigations of megaspore formation revealed no apparent differences between the diploid and tetraploid megaspores. Although meiotic irregularities were found, a sufficient amount of apparently viable pollen was observed. A physiological basis for the self-sterility is suggested.

Introduction

The feasibility of utilizing triploid sterility in producing seedless watermelons was shown by Kihara (4) in 1951. Triploids were obtained by crossing tetraploids by diploids (2N = 22). Poor fruit set was observed in selfed tetraploids, while in the tetraploid-by-diploid crosses fruit set was more frequent. The number of viable seeds formed in the tetraploids was always few. Using advanced generations of the original tetraploids induced by Kihara and Nishiyama (5), Shimotsuma (9) has shown that seed fertility can be increased with proper selection. The increased seed fertility was associated with a reduction in multivalent associations.

Stoner and Johnson (10) reported that autosterility of autotetraploid watermelons could be partially overcome by applying 1% naphthaleneacetamide.

Preliminary investigations with colchicine-induced tetraploids at the Indiana station indicated a surprisingly high degree of self-sterility in certain tetraploid lines that was not apparent in other lines. In tetraploid lines of 'Purdue Hawkesbury' and 'Charleston Gray' it was not possible to obtain fruit set upon selfing, while in other tetraploid lines such as 'Early Canada' and 'Peacock' the number of fruit set was fairly high although few viable seeds were set. Furthermore, the lines which showed complete self-sterility were all wilt resistant while the others were wilt susceptible. The following studies were made to learn something of the basis for this complete self-sterility. A method of increasing the efficiency of tetraploid induction with colchicine was developed.

Materials and Methods

Autotetraploid watermelons were produced from diploids by 2 methods of colchicine treatment. In the first method 1 drop of 0.3% aqueous colchicine solution was applied to cotton covering the growing point of diploid seedlings of the cultivars 'Charleston Gray 133' and 'Princeton'. The frequency and duration of treatment varied from 2 drops of colchicine in a 24-hr period to 8 drops in an 80-hr period. In the second method diploid seeds were soaked from 6 to 24 hr (at 6-hr intervals) in either 0.1% or 0.2% colchicine solution just prior to planting. All treated plants were compared regularly with diploid check plants for morphological evidences of polyploidy. Treated plants displaying morphological characters and growth patterns similar to the diploid check plants were discarded.

Pollen size was used as the primary criterion for determining tetraploidy of individual plants. Pollen grains were stained with a drop of 2% acetocarmine and microscopically examined for stainability and size. Pollen diameter was measured with a micrometer eyepiece. Pollen germination was also studied using the techniques described by Eigsti (2).

Male flowers to be used for observations of microsporogenesis were collected and held in a fixing solution of 3 parts absolute alcohol and 1 part glacial acetic acid for 12 to 24 hr. They were then rinsed with distilled H_2O, preserved in 70% alcohol, and stored at 40°F until examined. Acetocarmine and/or propionocarmine stains were used. Slides showing evidence of meiotic divisions

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daylength. Comparable results were found by Lane, et al. (3) when investigating the flowering response in several long-day plants. They found that incandescent light or light rich in the far-red spectrum promoted flowering. Fluorescent light was very ineffective when used to extend the daylength for promotion of flowering in the long-day plants investigated.

Failure of 1 hr incandescent light, following 15 hr fluorescent light, to promote bulbing and 1 hr of fluorescent light following 15 hr of incandescent, to entirely prevent bulbing can only be speculated upon at this point. Further study would be needed to evaluate these onion varieties critically for their sensitivity to duration and intensity of light quality in fully promoting or inhibiting bulb formation under long-day conditions.

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were made permanent by the quick-freezing technique described by Bowen (1).

Examinations of megasporogenesis were also made. The female flowers were fixed and held in a solution of 4 parts absolute alcohol and 1 part glacial acetic acid for 24 hr. The material was dehydrated, infiltrated, sectioned and mounted for staining. The stains used were safranine and fast green.

Fertility of diploids, of tetraploids and of crosses within and between these 2 types was studied by artificial self- and cross-pollinations and by natural pollinations by bees in cages.

Results and Discussion

Treating the initial growing point of seedlings of 2 cultivars with a 0.3% aqueous colchicine solution resulted in a range of induced tetraploidy from 0–6% (Table 1). Treatments B and C, 8 drops of 0.3% colchicine solution over a 52- or 80-hr period respectively, appeared to be more effective in inducing polyploidy than the lower-dosage treatment A, 2 drops over a 24-hr period. Possibly the percentage of induced polyploidy would have been further increased by applying colchicine at more frequent intervals or by keeping a constant supply on the seedlings for a longer time.

Table 1. Effects of treating diploid seedlings with aqueous colchicine.

Cultivar	Treatment[a]	Number of plants treated	Number of induced tetraploids
Charleston Gray 133	A	72	0
	B	48	2
	C	115	5
Princeton	A	65	2
	B	49	3
	C	117	7

[a]A—2 drops of a 0.3% colchicine solution over 24-hr period,
B—8 drops of a 0.3% colchicine solution over 52-hr period,
C—8 drops of a 0.3% colchicine solution over 80-hr period.

Table 2. Effect of soaking diploid seeds of 12 varieties in 2 different colchicine solutions.

Cultivar	Treatment[a]	Per cent of germination[b]	Per cent of tetraploidy
Charleston Gray 133	A	72	8
	B	60	20
Princeton	A	64	4
	B	72	8
Wilt-Resistant Congo	A	94	3
	B	90	4
Chris-Cross	A	49	0
	B	52	3
Klondike R-7	A	43	6
	B	7	2
Purdue Hawkesbury	A	70	0
	B	64	2
P.H. × KR-7	A	99	2
	B	89	14
P.H. × La. #1	A	73	0
	B	80	3
Spalding	A	88	1
	B	45	4
Summit	A	67	2
	B	27	3
Whitehope	A	85	0
	B	84	3
Dixie Queen	A	89	6
	B	57	8

[a]A—seeds soaked in 0.1% colchicine solution for 24 hr,
B—seeds soaked in 0.2% colchicine solution for 18 hr.
[b]Germination percentage of all variety check plants was in excess of 90%.

Seeds of the same cultivars soaked in a 0.1% solution for 24 hr or in a 0.2% solution for 18 hr resulted in a range of induced tetraploidy of 0–20%. Because of the higher percentage of tetraploid induction, the seed-soaking method was used on the 12 cultivars listed in Table 2. Different responses to given colchicine treatments were evident. The range of induced tetraploidy was from 0 to 8% at the 0.1% treatment and from 2 to 20% at the 0.2% treatment. The per cent of germination varied from 43% to 99% in the 0.1% treatment and from 7% to 90% in the 0.2% treatment.

Morphological differences between the tetraploids and the diploids were very evident. Tetraploid plants produced larger leaves, tendrils and flowers and were more pubescent than diploid plants. Determination of the exact chromosome number of reproductive tissue proved difficult.

Ten seeds from a self-pollinated tetraploid watermelon grown in a previous year were planted, and pollen grains from these plants were measured. The average diameter was 78 microns. A diploid plant of the same cultivar had an average pollen grain diameter of 64 microns. The pollen grain diameters of the tetraploid check plants were similar to those from the induced tetraploids and the morphological appearance of the plants was also similar. Pollen grains from all tetraploids were larger than from diploids (Table 3). Size differences were clear-cut with no overlapping of measurements between ploidy levels. On the basis of pollen diameter and plant morphological characteristics, the successfully treated plants were considered to be tetraploids.

Table 3. Measurements of pollen diameter at 2 different ploidy levels.[a]

Cultivar	Pollen diameter in microns			
	Range of means		Average of means	
	Diploid	Tetraploid	Diploid	Tetraploid
Charleston Gray 133	60–66	71–82	64	76
Princeton	61–66	74–82	64	76
Wilt-Resistant Congo	61–65	73–78	63	77
Klondike R-7	62–68	70–79	65	75
Chris-Cross	62–63	71–73	62	72
Purdue Hawkesbury	60–69	75–78	63	76
P.H. × KR-7	61–66	72–80	63	76
P.H. × La. #1	62–64	71–77	63	74
Spalding	63–64	74–81	63	77
Summit	64–67	74–82	65	76
Whitehope	60–63	74–76	62	75
Dixie Queen	60–64	69–81	62	75

[a]Averages are unweighted.

The fertility of diploid and tetraploid plants was tested by making both self- and cross-pollinations. Forty-five self-pollinations were made on diploid plants and 25 fruit were set. Cross-pollinations between diploid plants also resulted in a high percentage of fruit set. On the other hand, no fruit were set from 131 self-pollinations on tetraploid plants. Further, no fruit set resulted from 48 cross-pollinations between tetraploid plants. Fifty-five crosses between tetraploid plants and diploid pollinators resulted in 12 fruit set. Ten tetraploid and 3 diploid plants were isolated individually in nylon-mesh cages when flowering began. A hive of bees was placed in each cage for over 50 days. The diploid plants set fruit which contained viable diploid seed. There was no fruit set on any of the 10 tetraploid plants. The cages were removed from the tetraploids and all plants set fruit when open-pollinated. Seeds from these fruit were presumably triploid and were planted the following year in a field with diploids; the fruit of these plants was seedless.

Pollen viability was estimated by its stainability. In all 12 varieties the mean percentage of stainable pollen was

higher at the diploid level than at the tetraploid level (Table 4). However, the lowest tetraploid viability measurement, 70% in the variety 'Wilt-Resistant Congo', would still appear to be high enough to insure adequate pollen for pollination. Thus, the level of apparent pollen viability was not considered low enough to be a prime factor in the sterility exhibited by the tetraploid plants.

Table 4. Percentages of stainable pollen of 12 cultivars at 2 different ploidy levels.[a]

Cultivar	Range of means		Average of means	
	Diploid	Tetraploid	Diploid	Tetraploid
Charleston Gray 133	64–98	40–98	88	79
Princeton	86–100	68–98	97	88
Wilt-Resistant Congo	74–96	44–88	86	70
Klondike R-7	70–84	64–90	77	76
Chris-Cross	98	74–82	98	78
Purdue Hawkesbury	96–100	96	97	96
P.H. × KR-7	80–100	64–92	93	84
P.H. × La. #1	98	92–94	98	93
Spalding	94–98	80–88	97	84
Summit	96–100	78–84	98	81
Whitehope	100	80	100	80
Dixie Queen	90–98	78–92	94	85

[a]Averages are unweighted.

Pollen of tetraploids and diploids was germinated in the laboratory. A small percentage of pollen grains from tetraploids put forth as many as 4 pollen tubes. Each of these tubes appeared to be the same size as the normal single tubes from pollen of diploids. Other pollen from tetraploids put forth pollen tubes that forked. Bifurcated pollen tubes and tubes from pollen grains which developed more than 1 tube did not grow as rapidly nor attain the same length as pollen tubes from diploids. Germinating pollen from diploids never produced more than 1 tube per grain. These pollen tubes were never forked. It is probable that the abnormal pollen tubes would not bring about fertilization since their growth appeared too short to reach the embryo sac. It is also probable that where there was more than 1 tube or the tube was forked, a complete chromosome complement would not be present in each tube or branch of a tube, and it was doubtful that fertilization could take place.

Cytological observations of pollen mother cells from several cultivars showed that diploid meiosis was regular, while some irregularities were observed in the induced tetraploids. It was noted that quadrivalents were not usually formed at prophase and metaphase I in tetraploids as was found by Kihara (4). As many as 21 chromosome configurations were found at some metaphase I plates possibly suggesting bivalent pairing in some varieties. Eleven configurations would be expected with complete quadrivalent formations, while 22 configurations would be expected with bivalent pairing. Metaphase I chromosomes were not always oriented on the equatorial plate but were sometimes dispersed in the spindle or elsewhere in the cytoplasm. At anaphase I bridges and lagging chromosomes were found in some cells. In many metaphase II figures no distinct plate appeared to be formed, and at second anaphase lagging chromosomes and bridges were sometimes evident. Such abnormalities may be the basis for considerable pollen sterility.

Examination of microspore quartettes of diploids and tetraploids revealed that 99% of the diploid quartettes were apparently normal, while only 87% of the tetraploid quartettes appeared normal. Some tetraploid quartettes contained as many as 3 microsporocytes in addition to 4 microspores. Abnormal quartettes probably lead to nonviable pollen.

Preliminary investigations revealed that there were no apparent differences between the formation of megaspores in diploid and tetraploid material. At both ploidy levels the basal megaspores appeared to develop normally while the other 3 megaspores failed as described by Kirkwood (6).

The abnormal quartettes and nonstaining pollen of the tetraploid material are believed to be associated with the irregular chromosome segregations observed in meiotic figures. In spite of these irregularities more than two-thirds of the tetraploid pollen germinated in a normal fashion in the laboratory; however, such pollen did not bring about fruit set. Further, since pollen from diploids brought about fruit and subsequent seed set, it appears that the tetraploid ovaries did contain some ovules that were developed and in a receptive state. Thus, the lack of fruit set from self- and cross-pollinations of tetraploid plants can be explained neither by a lack of normal-appearing pollen nor by nonfunctional ovules.

The sterility is suggested to be of a partially physiological nature brought about by the doubling of the chromosome number. Sterility of this nature has also been reported by other workers (3, 7, 8).

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