A GENETIC AND MORPHOLOGICAL STUDY OF THE DIPLOID-TETRAPLOID CROSS IN THE POINSETTIA, EUPHORBIA PULCHERRIMA KLOTZSCH

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ABSTRACT

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By Daniel C. Milbocker

The diploid-tetraploid cross in poinsettia as reported by Ewart (1957) and Pai (1960) produced only diploids and tetraploids which were associated with a high frequency of ovary abscission. This research was conducted in an effort to determine by morphological techniques the factors involved in ovary abscission of this cross. Also, genetic analysis by the use of bract color was used to determine the reproductive behavior of the diploid-tetraploid cross which resulted in tetraploid seedlings.

As a result of this research, the triploid poinsettia with 42 chromosomes is reported for the first time. A diploid female, Ecke White, crossed with the tetraploid male, Barbara Ecke Supreme, were the parents. The occurrence of a triploid endosperm among the regularly tetraploid endosperms is hypothesized to produce a small frequency of viable seeds from which triploids were found.

A single tetraploid progeny from the same diploid-tetraploid cross was testcrossed to a homozygous white tetraploid cultivar. This seedling tetraploid had obtained one-half of its four genomes from its diploid maternal parent. Duplication of an embryo sac nucleus without

nuclear division is hypothesized as producing a diploid egg and a single polar nucleus. Gametic union produced a tetraploid embryo and a triploid endosperm which developed into a viable seed.

The failure to form a normal endosperm was found to be the cause of ovary abscission in the poinsettia.

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I. INTRODUCTION

In the vascular plants, especially the angiosperms, there is a widespread prevalence of polyploids among predominantly diploid populations (Swanson, 1957). Successful crosses between diploids and tetraploids may unite the haploid and diploid gamete to produce triploid progeny. The occurrence of diploid and tetraploid offspring in crosses between diploid and tetraploids is not in accord with expectations from the union of a haploid and a diploid gamete. These chromosome types have occurred in a wide variety of plants. The diploid and tetraploid offspring of these crosses generally occur in low frequencies, being associated with a high frequency of ovary abscission, triploids, non-viable seed production or a combination of these (Bremer, 1962; Cooper and Brink, 1945; Wangenheim, Peloquin and Hougas, 1960). This cross in the poinsettia, Euphorbia pulcherrima Klotzsch was reported by Ewart (1957) and Pai (1960) to produce only diploids and tetraploids which were associated with a high frequency of ovary abscission.

During the poinsettia flowering season of 1963, a study was instituted to determine the source of the extra set of chromosomes in the tetraploid progeny from the diploid-tetraploid cross in the poinsettia. Bract color was used as a genetic marker for chromosomes in this study. Barbara Ecke Supreme, a tetrapolid, is homozygous dominant for red bract color; Ecke White, a diploid, is homozygous recessive for white bract color as reported by Stewart (1960). He found that bract color in the

poinsettia was inherited as a single gene controlled difference with white being recessive. A tetraploid progeny resulting from a cross with Ecke White as the female and Barbara Ecke Supreme as the male parent could be testcrossed with a tetraploid homozygous recessive white cultivar to reveal the number of genomes received from each parent.

Six progeny were obtained from the cross made in 1963 between Ecke White and Barbara Ecke Supreme. Five of the seedlings were normal and one had malformed and curled leaves. These six progeny and other cultivars were used in the present research which was started in 1964.

The primary purpose of this research was to determine morphologically the factors involved in ovary abscission, and to investigate genetically the reproductive system in the poinsettia. This purpose was accomplished by:

1. Counting the chromosome numbers in the progeny of the diploid-tetraploid cross.

2. Determining the ratio of white and red plants in a testcross of a tetraploid from the diploid-tetraploid cross.

3. Examining histologically abscissed ovules from the diploid parent.

4. Chromosome countings of embryos from the diploid-tetraploid cross.

5. Studying ovary abscission on inbred cultivars.

The poinsettia is a desirable species to use in this study because:

1. The ovules are relatively large, thus, making cytological

study less difficult.

2. The ovaries absciss providing a marker for determination of the time when seed formation ceases.

3. Bract color provides a distinct genetic marker.

4. A generation from seedling to seed production occurs within a year.

5. Cytological and genetic studies of the diploid-tetraploid cross have been previously reported.

II. LITERATURE REVIEW

The Diploid-Tetraploid Cross in the Poinsettia.

The poinsettia has a basic chromosome number of 14 with a somatic number of 28 (Moyer, 1934; Perry, 1943; Darlington, 1955). Darlington (1955) accepted Moyer's (1934) count; Perry (1943) added 14, and 56 for the poinsettia. A somatic chromosome number of 14 has not been reported nor found since among commercial cultivars. Somatic chromosome numbers of 56 were first reported for several commercial cultivars by Ewart (1957). The cultivars originated as somatic mutations of the diploid, Oak Leaf, or as somatic mutations of Oak Leaf mutants; the oldest recorded being the cultivar, Mrs. Paul Ecke, found as a somatic mutation in 1929 (Stewart, 1960).

Crosses using a diploid (28 chromosomes) as the female parent and the tetraploid (56 chromosomes) as the male parent produced progeny with either 28 or 56 somatic chromosomes and the reciprocal cross produced no progeny as reported by Ewart and Walker (1960) and Sink (1963). A cross between Ecke White (diploid) and a seedling from Barbara Ecke Supreme, a tetraploid, produced a diploid cultivar, Paul Mikkelsen, which was introduced in 1960 to the floral industry, and with its subsequent somatic mutations is now the most popular cultivar among poinsettia growers in the United States (Mikkelsen, 1966).

Genetic, morphological and cytological studies have yielded information on the sexual reproductive behavior of the poinsettia, but

have not revealed sufficient information to determine the means by which diploid and tetraploid progeny were produced in the diploid-tetraploid cross. Ewart (1957) proposed that anomalous meiosis of the megaspore mother cells was a possible explanation for the results he obtained. Ewart and Walker (1960) published two explanations, both of which were unsatisfactory in fully explaining their results. The first, apomixis, did not explain progeny segregation or the presence of tetraploid progeny. The second, non-reduction or normal reduction followed by doubling of the chromosomes explained the tetraploid, the double reduction of the pollen mother cell explained the diploid progeny. They did not explain why triploids were not observed in the progenies. Bremer (1962) proposed endo-duplication as a basis for the results obtained by Ewart (1957) with pseudogamy accounting for the presence of diploids and segregation during meiosis I. Tetraploids are the naturally expected result of endo-duplication through formation of diploid egg cells. Sink (1963) proposed a similar endo-duplication mechanism operative either at the meiotic level or egg nucleus level. His embryo sac study ruled out adventitious embryony, since pollination was required for embryo development, and no evidence of apomixis was found. Sink (1963) also revealed the presence of degenerating material tissue in the ovule which led him to believe that there was an embryo-endosperm-maternal tissue incompatibility factor, involving the chromosome number, causing the degeneration and subsequent failure of seed development in this cross.

He proposed that reduction division during sporogenesis in both

parents would produce a 5n endosperm with either a 3n or 5n embryo and endo-duplication would produce a 9n endosperm with either a 4n or a 5n embryo. The feasibility of this proposal was supported by the possible occurrence of the chromosome number in the embryo being the same as the progeny that has been observed.

III. MATERIALS AND METHODS

Propagation and Cultural Practices

The cultivars used in this research are listed in Tables 1 and 2. To propagate these plants, terminal cuttings three to four inches in length were taken from vegetative stock plants. Intermittent mist and a sand propagation bench were used consistently. Soluble fertilizer (12-31-14) was applied to the cuttings at the rate of one-half ounce per gallon of water every four days starting four days after the cuttings were placed in the propagation bench. This procedure was used whenever accelerated growth was desired on the newly rooted cuttings, or when slow rooting cultivars were being propagated.

Rooted cuttings were potted in steam sterilized three or four inch clay pots using a potting medium of three parts clay loam soil, one part sand, one part sedge peat and one part German peat. The plants were potted without packing the soil, thus permitting adequate water retention and good drainage. The plants were covered with two layers of cheese cloth and drenched twice daily for four days. The cloth was removed and the pots spaced to one per square foot.

Plants grown for flowering were later transplanted to five inch clay pots using the same soil mix. These plants were fertilized twice a week using a soluble fertilizer (12-31-14). The stock solution contained one pound of fertilizer per three gallons of water and was applied with a Hozon.[1] Plants with fully developed bracts were fertilized once

[1] Hozon, Plant Products, New Point, Long Island, New York.

Table 1. The characteristics and source of poinsettia cultivars.

Cultivars	Abbreviation	Chromosome No.	Bract Color	Source
White Ecke	EW	28	white	Ecke, Inc.
Oak Leaf	OL	28	red	Ecke, Inc.
St. Louis	SL	28	red	Ecke, Inc.
Ruth Ecke	RE	28	red	Ecke, Inc.
Barbara Ecke Supreme	BES	56	red	Ecke, Inc.
Indianapolis Red	IR	56	red	Ecke, Inc.
Elizabeth Ecke	EE	56	red	Ecke, Inc.
New Ecke White	IEW (Chimera)	56	white	Ecke, Inc.
Paul Mikkelsen	PM	28	red	Mikkelsen
Mikkelpink	MP	28	pink	Mikkelsen
Mikkeldawn	MD	28	pink-white	Mikkelsen
Spotlight	P-128	28	red	U.S.D.A.
60-530-2	P-130	28	red	U.S.D.A.
Snow Cap	P-131	28	white	U.S.D.A.
61-71-1	P-132	56	white	U.S.D.A.
61-302-1	P-134	28	red	U.S.D.A.
White Cloud	P-135	28	white	U.S.D.A.

Table 2. The characteristics and parentage of cultivars developed at Michigan State University.

Cultivar	Parents	Ploidy	Bract Color
64-2	EW X BES	tetraploid	red
64-4	EW X BES	triploid	red
64-5	EW X BES	triploid	red
64-7	EW X BES	triploid	red
64-8	EW X BES	triploid	red
64-13	EW X BES	triploid	red
65-10	P-128 ♀	diploid	red
65-11	P-128 ♀	diploid	red
65-13	P-128 ♀	diploid	red
65-14	P-128 ♀	diploid	red
65-15	P-128 ♀	diploid	red
65-23	EW ♀	diploid	white
65-24	EW ♀	diploid	white
65-25	EW ♀	diploid	white

a week. A Soiltex Testing Kit[1] was used as a convenient way to check soil pH. No measures were taken to lower soil pH, unless it exceeded

[1]Soil Science Department, Michigan State University, East Lansing, Michigan.

seven; then weak sulfuric acid was applied with a Hozon every four days until the pH was lower than seven. The plants were watered daily as needed and on flowering plants more care was taken since slight wilting resulted in termination of the new cyme and premature abscission of lower leaves.

The greenhouse was thermostatically set at a minimum 21°C. Sudden temperature change is a mutagenic factor for a variety of plants including wheat and rye (Dorsey, 1936), corn (Randolph, 1932), barley and wheat (Peto, 1938a and b) and onions (Huskins and Cheng, 1950). Thus, the greenhouse was vented conservatively to prevent sudden temperature changes from affecting the seed set.

Pollination Technique

Ovaries were hand pollinated by transferring the stamen with tweezers to the stigma of the cyathium to be pollinated. Pollination was done only once on each stigma. Ninety-five percent ethyl alcohol was used to clean the tweezers between crosses. Each ovary was tagged with the identification of the parents, and the date of pollination for later use in laboratory work, or for seed collection data. Non-female cyathia and terminated cymes with no ovaries were removed to facilitate pollination and prevent accidental selfing.

Chromosome Counting

The counting of chromosomes was accomplished through a modification of Ewart's (1957) root tip smear technique. Dividing shoot meristems were excised and stripped of sheath leaves. The end section of

primordia not exceeding one eighth inch in length was immediately placed in modified Carnoy's solution (Bladwin, 1938) composed of one part glacial acetic acid; two parts 95 percent ethyl alcohol and three parts chloroform. Shoot tips were more convenient to collect than root tips, and shoot cells tended to flatten into a polar view with a greater frequency than did root cells. Latex and chloroplasts were not a problem in rapidly growing material. The tip remained in the modified Carnoy's solution at least 15 minutes, but could be stored several days at room temperature before removal and emmersion into a mixture of 95 percent ethyl alcohol. The tips were removed from this solution after ten minutes, and placed in distilled water for 10 to 15 minutes. Each tip was cut into approximately four pieces, with one piece being placed on a clean slide and covered with a drop of aceto-orcein stain (La Cour, 1941). The tissue was spread using mashing type motions with the flat side of a knife blade, exercising care not to roll the tissue. Another drop of aceto-orcein and a cover slip were added. After five minutes, the slide was placed in the fold of a paper towel, both placed on a flat surface and pressed with the thumb. The edges of the cover slip were sealed with vaseline or colorless fingernail polish to prevent evaporation. The chromosomes were clearest when examined immediately, but slide preparations kept well for three days in refrigeration at $2^{o}C$. A modification of this method was used to count the chromosomes of embryos. The embryo sac was removed from the ovule and fixed using the same procedure for shoot tips. The embryo sac was spread only by pressure on the cover slip after staining.

Ovule Morphology Technique

Morphological investigations of the ovule were made according to the procedure of Sink (1963). The ovules were removed from the ovaries and cut transversely at the middle. The placental end was fixed in Navashin's fluid (Conn, 1960) under vacuum of 20-25 millimeters of mercury. After 48 hours the ovules were washed in running tap water for 24 hours, and dehydrated with tertiary butyl and ethyl alcohol (Johansen, 1940). The dehydrated ovules were imbedded in Tissuemat.[1] Sectioning was done on a rotary microtome at 10 microns. The sections were placed and spread in a drop of Haupt's adhesive and three drops of four percent formalin and allowed to dry on a warming tray at 43°C. The paraffin was removed with xylene, a drop of Permount applied and a cover slip added. The sections were observed under a phase microscope at X500 and photomicrographs were taken at X50 and X100. Older material was observed with a light microscope at X25 and X50.

Pollen Viability Test

Pollen grains were collected from freshly dehisced anthers and placed on a slide. A drop of cotton blue stain was added which consisted of equal parts of phenol crystals, lactic acid, glycerine and distilled water after a modification of Johansen (1940). A cover slip was placed over this according to the method of Sink (1963). After five minutes,

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[1] A 56.5°C. melting point paraffin produced by Fisher Scientific Company, Pittsburgh, Pennsylvania.

all pollen grains were counted in a representative scan of the slide with a microscope at X430. Plump, blue stained pollen grains were counted as viable and shrunken, or light stained grains were counted as non-viable. Three counts were made with a minimum of 100 grains evaluated for each count.

IV. RESULTS

Genetic Study of the Tetraploid Seedling

Cytological examination of the six offspring from the diploid-tetraploid cross showed that 64-2, a seedling with deformed foliage, was the only tetraploid. It was testcrossed with the tetraploid cultivar P-132 from the U.S.D.A. which is recessive for white bract color. The cross produced a ratio of 3.5 red to 1.0 white plants (Table 3). Color was determined by petiole evaluation. White poinsettias invariably have green petioles and red or pink poinsettias have red pigmented petioles.

Table 3. The distribution of red and white plants from the testcross.

	P-132 X 64-2	64-2 X P-132	Total
No. of pollinations	164	105	269
No. of seed	88	74	162
No. of plants	75	55	130
No. of white plants	18	11	29
No. of red plants	57	44	101

Triploid Progeny

The remaining progeny from the diploid-tetraploid cross were identified as 64-4, 64-5, 64-7, 64-8 and 64-13. Chromosome counts

revealed that all five were triploid 3N = 42 (Figure 1). All five progeny were red for bract color and segregated three bright and two dark red. The dark red progeny also had the broader oval type bract of the Ecke White parent, while the other three had either an intermediate or the longer narrow bract of Barbara Ecke Supreme. All progeny had sinuate leaves of the Barbara Ecke Supreme parent.

Although no data were recorded, variation in plant characteristics occurred between the triploids and commercial cultivars. Two hundred plants of commercial cultivars and 400 triploids were rooted and flowered to observe that 64-5 was easiest to root and 64-13 was the earliest to initiate bracts. The triploid poinsettia as demonstrated by these offspring and others is a vigorous grower.

Repetition of the Diploid-Tetraploid Cross

A repetition of the diploid-tetraploid cross using the same parents produced three seedlings. All were triploids with sinuate leaves, and bright red bracts with segregation for bract type and initiation time. These progenies were 65-1, 65-2, and 65-3. The progeny 65-1 was similar to Barbara Ecke Supreme in bract formation, and initiated bracts within 60 days from the date the cuttings were taken, as compared to 90 days for Barbara Ecke Supreme. Seedling 65-2 had a bright red color with the bract shape and initiation time similar to Ecke White.

Ovule Morphology

Ovules for morphological investigation were collected from abscissed ovaries of the Ecke White-Barbara Ecke Supreme cross at daily

Figure 1. Somatic chromosomes of the triploid poinsettia, 3n = 42.

A. Microphotograph of somatic triploid cell. X1250.

B. Interprative drawing taken from Figure 1-A. Approximately X4400.

Figure 2. Endosperm development in the diploid and the diploid-traploid crosses.

A. Diploid cross ovule 30 days after pollination with the endosperm consisting of closely packed spherical cells. X100.

B. Diploid cross ovule 44 days after pollination with endosperm consisting of cubical cells. X100.

C. Diploid-tetraploid cross ovule 38 days after pollination with endosperm consisting of closely packed spherical cells and endosperm degeneration. X50.

D. Diploid-tetraploid cross ovule 50 days after pollination with integument shrinkage and remnants of the endosperm and embryo. X50.

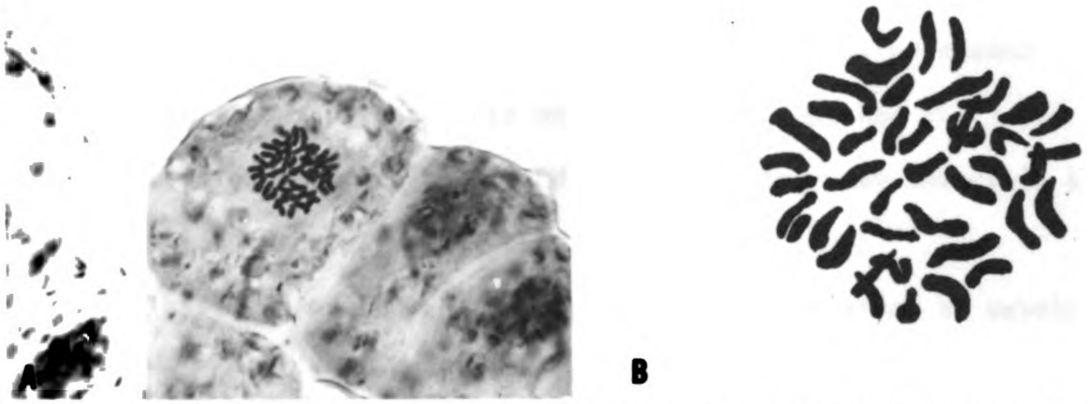

Figure 1. Somatic chromosomes of the triploid poinsettia.

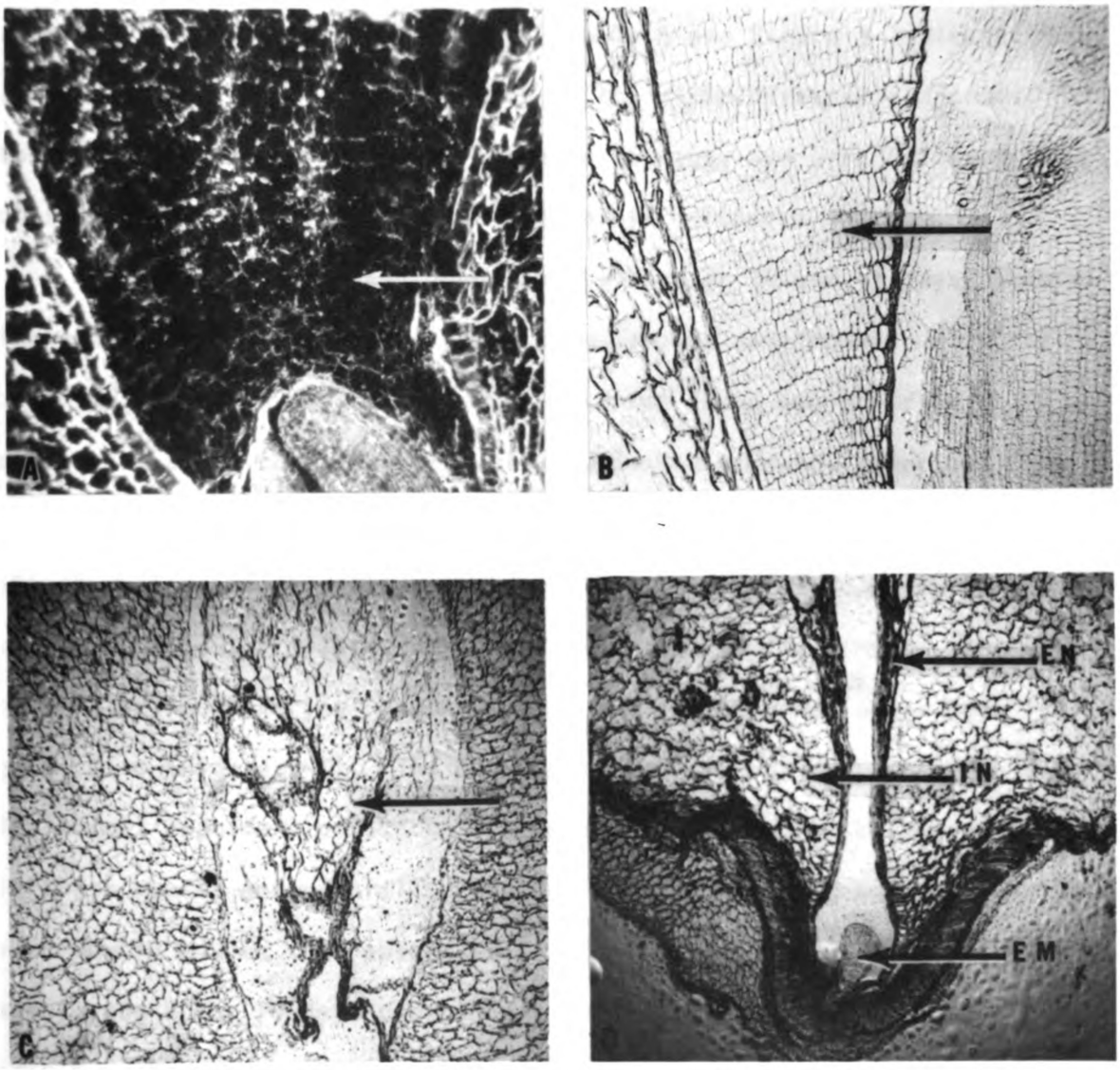

Figure 2. Endosperm development in the diploid and the diploid-tetraploid crosses.

intervals from 30 days after pollination until abscission ceased. Ovules of the cross between Ecke White and Ruth Ecke had the least ovary abscission and were collected at two and four day intervals as the normal for comparative purposes.

At least two ovules were examined for each interval of development in the normal series. The rate of normal embryo and endosperm growth is indicated in Table 4 and Figure 3. Five ovules were examined for each two day interval of the diploid-tetraploid cross, and ten were examined for the longer intervals. Table 5 and Figure 3 indicate the rate of embryo and endosperm growth in the diploid-tetraploid cross. Normal ovules developed to full size in 26 days, and were followed closely by ovules of the diploid-tetraploid crosses.

Normal endosperm developed rapidly between 30 and 45 days, obtaining mature size in 50 days. Cellular endosperm development was first observed at 18 days and a change in cellular shape was observed at 26 days. The cells changed from a closely packed somewhat spherical form (Figure 2-A) to a cubical form (Figure 2-B). The cubical cells were in planes running transversely across the endosperm tissue. Endosperm cells of the diploid-tetraploid cross were never observed to change from a closely packed spherical conformation, but remained as larger more fragile cells (Figure 2-C). The endosperm also ceased to increase in size. The normal endosperm breakdown that precedes the embryo became extensive in these ovules and generally the entire endosperm degenerated leaving a hollow in ovules that abscissed after 40 days. A few ovules in ovaries abscissing after 70 days contained a callus type growth of undetermined origin. The maternal tissue began to shrink after 40 days to increase

Figure 3. Embryo, endosperm and ovule growth of the diploid and the diploid-tetraploid crosses.

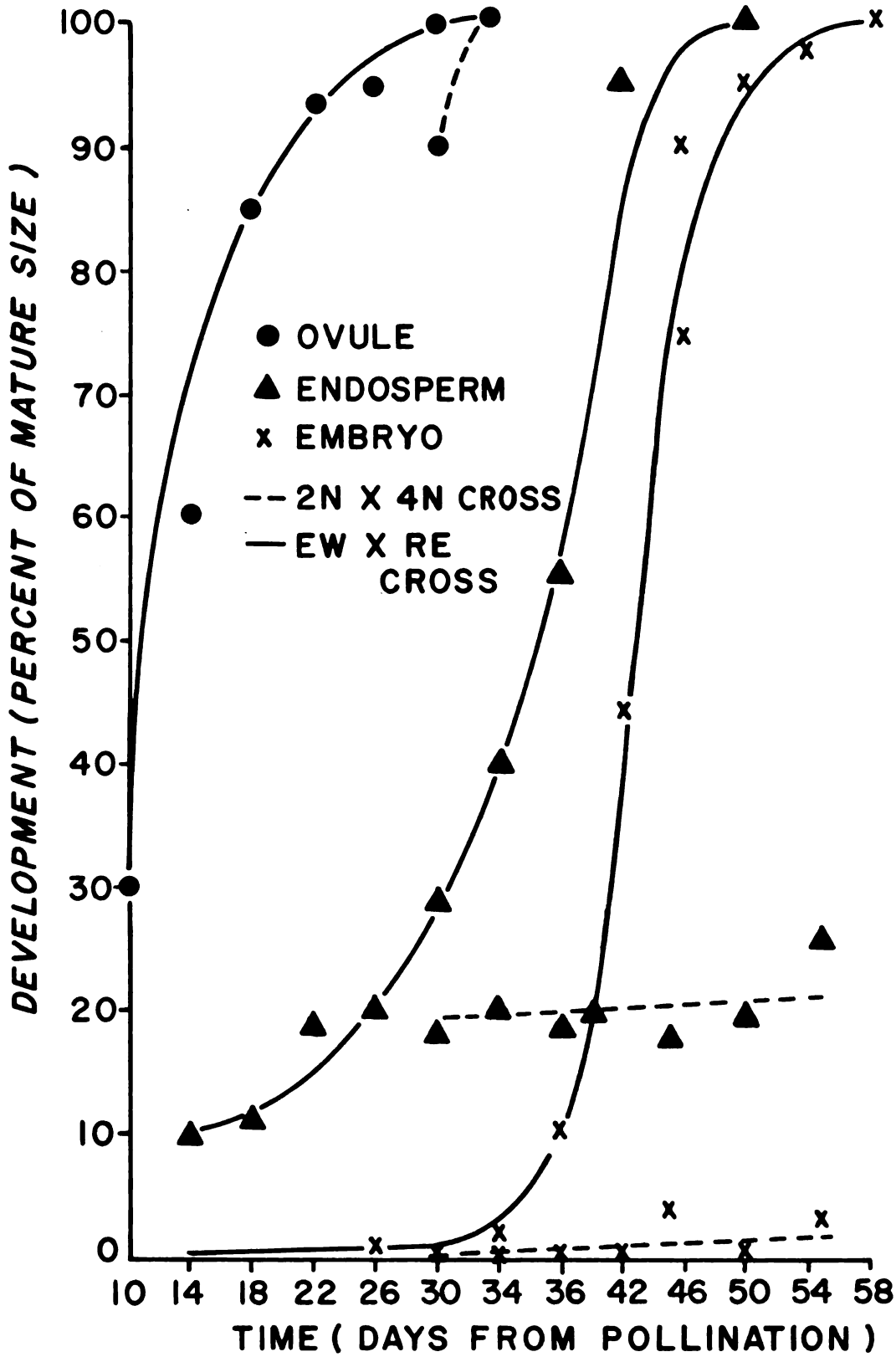
DEVELOPMENT (PERCENT OF MATURE SIZE)
100
90
80
70
60
50
40
30
20
10
0
OVULE
ENDOSPERM
EMBRYO
2N X 4N CROSS
EW X RE CROSS
10 14 18 22 26 30 34 36 42 46 50 54 58
TIME (DAYS FROM POLLINATION)

Table 4. Normal ovule development in percent of mature size.

Days from pollination	Percent of ovule diameter	Percent of endosperm diameter	Percent of embryo volume
10	30		
14	60	10	<1
18	85	12	<1
22	93	18	<1
24	95	22	<1
26	95	20	1
28	100	30	1
30	100	28	2
32	100	25	2
34	100	40	2
36	100	43	8
38	100	55	10
40	100	61	22
42	100	95	44
44	100	90	55
46	100	90	75
50	100	100	95
54	100	100	98
58	100	100	100
62	100	100	100
66	100	100	100
70	100	100	100

Table 5. Diploid-tetraploid cross ovule development in percent of mature size.

Days from pollination	Percent of ovule diameter	Percent of endosperm diameter	Percent of embryo volume
30	90	17	< 1
32	100	20	<1
34		20	<1
36		18	<1
38		18	<1
40		20	<1
45		17	4
50		20	<1
55		26	3
60		33	2
64		20	20
68		33	1
70		30	12
77		37	14

the size of the cavity occupied by the endosperm. Degeneration of the type found in the endosperm was rarely found in the integuments. Endosperm degeneration consisted of advancing cellular destruction (Figure 2-C); integument degeneration was characterized by a general shrinking of the cells (Figure 2-D).

The embryo in normal ovules differentiated to form cotyledons and a radicle at 26 days. Rapid growth of the embryo began after 34 days and followed endosperm growth by five days, reaching maturity in approximately 54 days. Volume measurements were computed using the embryo length as the diameter and the radicle diameter as the thickness to compute the volume of a disc which is the shape that the embryo approximates in the seed. The endosperm is not completely absorbed by the embryo and is positioned as a hemisphere on each side of the cotyledons forming a pea-sized seed. The integuments form two seed coats as they become compressed by the growing endosperm.

In ovules of the diploid-tetraploid cross, the embryo was first observed to differentiate at 36 days. In most ovules little or no differentiation or growth was observed. A small portion of the ovules examined had no embryo. These were either non-existent or lost during sectioning. The embryos did not show any degeneration until the ovules approached 60 days of age. Ovules older than 60 days appeared as shrunken mature seed, but contained only a thick spongy inner integument and remnants of the endosperm and embryo (Figure 2-D). A few contained a yellow callus growth.

Chromosome Counts of Embryos

Ecke White ovaries pollinated by Barbara Ecke Supreme were removed at four day intervals and the embryos were examined cytologically. Cell division figures were difficult to find and the chromosomes were lightly stained. The endosperm was not observed dividing on material as young as 16 days after pollination and up to 34 days. Younger embryo sacs were not examined because they were watery, fragile and difficult to remove from the ovule. The embryo was easy to observe in the endosperm material but was infrequently found in active division. The cells of the embryo were smaller, stained more darkly and remained in a group. The embryos contained between 38 and approximately three hundred cells on most slides at 21 to 24 days after pollination. At this age a few embryos were found in active cell division during mornings that were not cloudy. Thirty-one embryos were examined of which seven were found in division stages. One was diploid, three were triploid, two were tetraploid, and one was pentaploid (70 chromosomes). The pentaploid had five dividing cells that were counted. One triploid and one tetraploid were difficult to count. Five of the seven embryos contained division figures which were spread well enough to determine if any were aneuploids, and none were found. The ovaries from which this material was taken were destined to absciss, since the endosperm mass was less than one millimeter in diameter as was found in ovules of abscissed ovaries. Ovaries left on a few more days abscissed at about 30 days from pollination.

Cross- Versus Self-Pollination

Crosses were made between five diploid cultivars; in addition, each of the cultivars was also self-pollinated. The percent ovary abscission in Table 6 is compiled for a comparison between cross- and self-pollination.

Table 6. The cultivar and percent ovary abscission in self- and cross-pollinated cultivars.

Self	Percent ovary abscission	Cross	Percent ovary abscission
EW	66	EW X P-131	100
P-131	87	P-131 X EW	70
RE	60	EW X RE	12
P-128	40	EW X P-128	40
P-135	95	EW X P-135	47
		P-135 X EW	10

Cross-pollination increased the amount of ovary retention, with one exception, (EW X P-131), in all crosses made when compared to self-pollination. The average ovary abscission of self-pollinated Ecke White and Ruth Ecke was 63 percent. The cross had 12 percent ovary abscission or 51 percent less abscission than the self-pollinated parents. The average of self-pollinated Ecke White and P-128 was 53 percent. The average of self-pollinated Ecke White and P-135 was 80.5 percent.

The cross using Ecke White as the female parent was 47 percent and the reciprocal was 10 percent with an improvement of 33.5 and 70.5 percent, respectively. The average of self-pollinated Ecke White and P-131 was 76.5 percent. The cross in which Ecke White was used as the male parent abscissed 70 percent. The reciprocal cross abscissed 100 percent.

Increased Level of Homozygosity

Progenies produced by one generation of self-pollinated Ecke White, numbered 65-23, 65-24 and 65-25 and of P-128 numbered 65-10, 65-11, 65-13, 65-14 and 65-15 were cross pollinated with 65-23, 65-24 and 65-25 as the female parent. The rate of ovary abscission was compared to the cross between P-128 and Ecke White, the latter employed as the female parent. The total pollinations between the Ecke White selfed progenies and the P-128 selfed progenies were evaluated as a single cross (Table 7). Forty percent abscission was observed when thirty-five Ecke White ovaries were pollinated with P-128. Ovary abscission for the composite of all the inbred crosses was 27 percent or 13 percent less than the cross between Ecke White and P-128.

Time of Pollination

Selected cultivars were self-pollinated to determine the effect of different pollinating times on ovary abscission. Pollinations were made as follows: early -- before the stigma fully opened in a star-like shape; medium -- stigma star-like, but not recurved and; late -- stigma

recurved toward the style and ovary protruding from the cyathium.

Table 7. The number and direction of progeny crosses.

Cross with 65-23	Number of pollinations	Cross with 65-24	Number of pollinations	Cross with 65-25	Number of pollinations
65-10	2	65-10	5	65-10	2
65-11	2	65-11	5	65-11	1
65-13	4	65-13	4	65-13	1
65-14	3	65-14	3	65-14	1
65-15	4	65-15	3	65-15	1
	15		20		6

The four cultivars which responded to early pollination were Ecke White, Improved Ecke White, Elizabeth Ecke and Indianapolis Red with 39, 29, 20 and 19 percent less ovary abscission, respectively, than late pollination (Table 8). The four cultivars which responded to late pollination were Ruth Ecke, Oak Leaf, P-128 and P-135 with 27, 9, 9 and 8 percent less ovary abscission, respectively, than early pollination. Early pollination produced less abscission of the ovaries among more cultivars tested than did late pollination.

Pollen Viability

Three or more pollen counts were taken at different times and averaged to yield the percent pollen viability for each cultivar as

Table 8. The effect of self-pollination time on ovary abscission.

Cultivar	Early		Medium		Late	
	No. pollinations	Percent ovary abscission	No. pollinations	Percent ovary abscission	No. pollinations	Percent ovary abscission
BES	34	71	45	78	24	83
EW	39	46	27	70	20	85
EE	25	80	22	100	14	100
IEW	23	44	22	73	11	73
IR	27	81	25	92	19	100
MD	5	100	5	100	2	100
MP	3	100	7	100	8	88
PM	8	88	7	100	2	100
OL	43	26	22	23	30	17
P-128	25	44	34	26	26	35
P-130	84	77	53	96	42	83
P-132	10	10	6	33	1	00
P-134	35	67	22	86	27	96
P-135	52	98	42	93	10	90
RE	37	62	48	42	60	35
SL	31	100	45	100	30	100

determined by the cotton blue staining method (Table 9). Ecke White and Mikkeldawn had the lowest percent viable pollen among the commercial cultivars with 57 and 41 percent, respectively. Two triploids, 64-4

Table 9. Pollen viability for selected cultivars.

Cultivar	Percent pollen viability	Cultivar	Percent pollen viability
EW	57	64-2	94
BES	90		
IR	73	65-10	69
EE	73	65-11	71
IEW	89	65-13	80
MD	41	65-14	96
MP	69	65-15	70
PM	75	65-23	99
OL	90	65-24	98
RE	87		
SL	94		Triploids
P-128	95	64-4	51
P-130	90	64-5	89
P-131	97	64-7	81
P-132	97	64-8	64
P-134	97	64-13	75
P-135	66	65-1	44
		65-2	76
		65-3	87

and 65-1, had 51 and 44 percent pollen viability. The variation was not great enough to consider pollen viability as a limiting factor in ovary fertilization where any of the cultivars were used as the male parent.

V. DISCUSSION

Genetic Study of the Tetraploid Seedling

Bract color in the poinsettia is controlled by gene differences at a single locus wh, with the gene for red (WH) being dominant over its allele wh which yields white. In the diploid-tetraploid cross, Ecke White and Barbara Ecke Supreme are homozygous for their respective colors (Stewart, 1960).

The progeny, 64-2, was a red tetraploid; therefore, some genes were obtained from Barbara Ecke Supreme, the male parent. It contained either the simplex, duplex, triplex or quadraplex as a genetic constitution for bract color. The testcross results of 64-2 and P-132 were compared with the theoretical segregation ratios of Haldane (1930).

Table 10. Tetraploid segregation ratios from Haldane.

Parental constitution	Chromosome segregation	Chromatid segregation
SSSS	1:0	1:0
SSSs	1:0	27:1
SSss	5:1	3.7:1
Ssss	1:1	13:15

The observed ratio was 3.5 red to one white which is favorable as an explanation for 64-2 having one-half Ecke White parentage. The female

or Ecke White genetic complement either doubled after reduction or failed to reduce as was proposed by Ewart and Walker (1960) and gave 64-2, its progeny, 28 chromosomes represented by two genes for white color. The other 28 chromosomes came from a normal diploid gamete of Barbara Ecke Supreme. These testcross results cannot be explained by accidental selfing, since the red female parent, 64-2, produced seedlings with white bracts.

Triploid Progeny

Three principal observations were made from the study of the progeny from the diploid-tetraploid cross:

1. The triploid poinsettia was found for the first time among reported progenies of the diploid-tetraploid cross.
2. The triploids, through visual observation, were found to be vigorous plants.
3. The triploids segregated for both parental characters.

The sinuate leaf and bract color were paternally inherited in all offspring: the ovoid bract shape was maternally inherited in three. Six offspring from triploid crosses with P-132, a tetraploid white cultivar, were both triploid and tetraploid ranging in color from pink to red.

Ovule Morphology

Brink and Cooper (1947) postulated that the failure of most fertilized zygotes to develop into mature viable seed is due to a failure of endosperm development. This has been found true in members of the Graminaceae, Rosaceae, Solanaceae and others. The poinsettia was found

to require normal endosperm for seed development. The importance of endosperm was shown in two ways:

1. All ovules examined from abscissed ovaries contained abnormal or no endosperm.
2. An ovule with a normal endosperm will develop into a ripe seed with or without a normal embryo. With four exceptions, 200 mature non-viable seeds examined contained either incompletely developed embryos or no embryo.

Chromosome Counts of Embryos

One diploid, three triploid, two tetraploid and one pentaploid embryos were found in active cell division among 31 ovules examined from the diploid-tetraploid cross. The number of triploid (3 in 31 ovules) and tetraploid (2 in 31 ovules) embryos is in excess of the frequency of seedlings (1 in 1230 ovules in the 1966 season, 1 in 630 in 1965 and 1 in 342 in 1964) obtained from this cross. If the number of chromosomes in the embryos is important in preventing abscission, only the pentaploid should have abscissed since the other three chromosome numbers have been found in seedlings of this cross. The size of the embryo sac indicates that all 31 ovules would have abscissed. The embryo chromosome number, therefore, is not an important determinant of the number of progeny obtained from the diploid-tetraploid cross. This was also shown by the presence of seed containing normal endosperm without embryos. Cooper and Brink (1945), while working with tomatoes and Wangenheim, Peloquin and Hougas (1960), while working with the potato, also found the endosperm to be more important than the embryo

in determining seed set. In this research repeated efforts were made to determine the chromosome number of the endosperm, but none were successful. Morphological investigations of abscissed ovaries in which no normal endosperm was found established the necessity for normal endosperm development before seed will form. Brink and Cooper (1947) established that the endosperm chromosome number was the major obstacle of endosperm development in diploid-tetraploid crosses of other plants. Wangenheim, Peloquin and Hougas (1960) found that a tetraploid potato cross produced hexaploid endosperm in normally developing seed. The tetraploid-diploid cross produced an abnormally developing pentaploid endosperm that inhibited seed formation. The few seeds that formed had hexaploid endosperms of an unknown origin. The poinsettia ovule forms similarly to the potato in embryo and endosperm development in the diploid-tetraploid cross. The analogous requirement of a triploid endosperm for normal development in place of an expected tetraploid endosperm was not established because only four viable seeds were obtained from the diploid-tetraploid cross during two seasons of research. Ovules from which successful seeds developed were not numerous enough to make this study possible.

Cross- Versus Self-Pollination

The poinsettia has colorful bracts, nectaries that exude abundant nectar and sticky pollen; all adaptions for insect cross-pollination in nature. (Brink and Cooper (1947) found that enforced self-pollination of normally cross-pollinated plants is a major source of endosperm failure and ovary abscission. They found that increased homozygosity of the endosperm genes was an impediment to

endosperm formation. Genes in the homozygous state limited the vigor of the endosperm preventing it from successfully competing with the maternal tissue of the ovule for available nutrients. A comparison of ovary abscission in self-pollinated cultivars with cross-pollinated cultivars showed that ovary abscission is reduced in cross-pollinated cultivars.

Increased Level of Homozygosity

Increasing the homozygosity of the parents involved in cross-pollination produced a decrease in ovary abscission. These results were similar to those obtained from the comparison of cross-pollinations. Since ovary abscission is believed to be caused by the failure of the endosperm to develop normally, these results further indicate the importance of the endosperm genetic constitution in controlling ovary abscission.

Time of Pollination

The specificity of some cultivars to pollination timing was sufficient to prevent any seed formation if a favorable pollination time was not used throughout the pollinating period. Pollinations performed at times adverse to stigma receptiveness can be expected to produce unfertilized ovaries that would absciss. No study was undertaken to determine if unfertilized ovaries were producing the observed ovary abscission response.

Endosperm Control of Progeny in the Diploid-Tetraploid Cross

The endosperm was shown to be of primary importance in the development of the poinsettia seed through morphological study of the ovule. Sink (1963) suggested that a chromosome number ratio existed between the embryo, endosperm and maternal tissue. The embryo was discounted from this consideration when seed which should have had the same endosperm maternal tissue ratio formed with either diploid, triploid, tetraploid or no embryo.

Counting chromosome numbers in the endosperm in successfully developing seeds was impossible due to their low frequency of occurrence. In a similar study of the diploid-tetraploid cross in potato, Wangenheim, Peloquin and Hougas (1960) observed that normal endosperm formed only when a hexaploid endosperm formed in a tetraploid maternal plant. The analogous situation in the diploid female poinsettia would be the triploid endosperm. The requirement of one successful chromosome number in the endosperm of the tetraploid potato (Wangenheim, Peloquin and Hougas, 1960) and similarly in other plants used in diploid-tetraploid crosses (Brink and Cooper, 1947) suggests that this could be true in the poinsettia. An hypothesis based upon the specificity of a triploid endosperm for successful seed development in a diploid maternal poinsettia parent would fit the observed results of this research. The diploid-tetraploid cross unites a diploid sperm with two haploid polar nuclei to form a tetraploid endosperm which develops abnormally. The high frequency of ovary abscission in the diploid-tetraploid cross was observed and found to

be dependent upon the formation of abnormal endosperm. This hypothesis permits the embryo chromosome number to be independent of successful seed set and this was observed.

Anomalous meiosis as suggested by Ewart (1957) would produce a polyploid megaspore which would raise the endosperm chromosome level higher than the triploid level. Therefore, anomalous meiosis could not produce successful seed formation and non-reduction or endo-duplication could not operate to produce the observed progeny from this cross. The few successful seed produced would develop from the union of a gamete and a polar body with a total of three genomes to form a triploid. One less genome could be contributed through either a haploid sperm or a missing polar nucleus. The union of a diploid sperm with the haploid egg would produce the nine triploid obtained from this cross.

Endomitosis occurring after megaspore formation and before gametic union would produce 64-2, the tetraploid with one-half of its four genomes from the diploid female parent. Double reduction of the male gamete as suggested by Ewart and Walker (1960) would yield a diploid embryo and a triploid endosperm to produce the diploid progeny they reported but without segregation for bract color of both parents as reported by Pai (1960).

Since triploids and diploids have never been found together in progeny of the diploid-tetraploid cross, the possibility exists that a reduction division of the chromosomes in the zygote destroys the triploids and some tetraploids to leave only diploids with segregation

for both parents as reported by Ewart and Walker (1960) and Pai (1960).

Bremer (1962) used endo-duplication to explain diploid and tetraploid progenies from the diploid-tetraploid cross of several dicotyledonous plants including the poinsettia and the potato. This system is left in doubt among many dicotyledonous plants, because of the absence of diploid progenies produced through endo-duplication when fertilization is not complete as was found in the onion by Hakansson and Levan (1957). This dissimilarity with monocotyledonous plants demonstrates the possibility that the system found in the potato (Wangenheim, Peloquin and Hougas, 1960) and hypothesized for the poinsettia is operative among diploid-tetraploid crosses in dicotyledonous plants in general.

VI. SUMMARY AND CONCLUSIONS

1. The triploid poinsettia with 42 chromosomes is reported. A diploid female, Ecke White, crossed with the tetraploid male, Barbara Ecke Supreme, were the parents. The occurrence of a triploid endosperm among the regularly tetraploid endosperms is hypothesized to produce a small frequency of viable seeds from which triploids were found.

2. A single tetraploid progeny from the same diploid-tetraploid cross was testcrossed to a homozygous white tetraploid cultivar. This seedling tetraploid obtained one-half of its four genomes from its diploid maternal parent. Duplication of an embryo sac nucleus without nuclear division is hypothesized as producing a diploid egg and a single polar nucleus. Gametic union produced a tetraploid embryo and a triploid endosperm which developed into a viable seed.

3. The failure to form a normal endosperm was found to be the cause of ovary abscission in the poinsettia. The failure to develop triploid endosperms in diploid ovules was hypothesized as the cause for inhibited endosperm development in crosses between diploids and tetraploids.

4. Two genetic factors were tested to determine their effect on ovary abscission. The first was the comparison of ovary abscission in cross-pollinations with that in self-pollinations. The second was the comparison of ovary abscission in a cross of two

cultivars with a cross between the self-pollinated progenies of each. Increased heterozygosity in the cross improved ovary retention in both experiments. Increased heterozygosity produces a hybrid vigor in the endosperm which is necessary for retention of the ovary in a plant adapted to cross fertilization.

5. Pollination time was found to affect ovary abscission in some cultivars.

6. Embryo chromosome number was found to be independent of ovary abscission. The failure to develop a vigorous embryo resulted in seed without embryos, incompletely developed embryos or malformed seedlings.

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