

SOME MORPHOLOGICAL, CYTOLOGICAL AND QUALITY FACTORS
ASSOCIATED WITH GENETIC MALE STERILITY
IN WATERMELON CITRULLUS LANATUS (THUNBERG) MATSUMURA

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GALE L. FULLER

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Approved by:


Major Professor

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INTRODUCTION

Watermelons, Citrullus lanatus (Thunberg) Matsumura, are produced as an important commercial truck crop and as a popular home garden fruit in the United States. The crop is grown as an annual with plants being produced from seed each year. Therefore, seed production has become a significant part of the industry. Seed of inbred commercial varieties is produced by harvesting from isolated growing fields planted to one variety. Great quantities of seed can be produced economically for most inbred varieties.

However, there is increasing interest in F_1 hybrid watermelons following the trend established in hybrid cantaloupe, cucumbers and other cucurbits which are producing greater yields of higher quality fruits. Hybrid watermelon seed can not be produced without a system of pollen control. The plants are monoecious. Honeybees and other insects transfer pollen from the anthers of staminate flowers to the stigma of pistillate flowers. Obviously, the insects do not confine their activities to single plants so that natural pollination is a random process. Natural pollination is adequate for an isolated inbred variety but to produce F_1 hybrids, protection from this natural process must be exerted for prevention of self-pollination and introduction of the desired pollen source.

Hand pollination is one answer to pollen control where each cross is made manually. Some hybrid seed is now available for production of hybrid watermelons but the seed is very expensive due to

high cost for hand labor. Producers must transplant the seedlings, further increasing the costs, as direct seeding would require too much of this costly seed. Many producers find it difficult to raise these hybrids economically so that the hybrids are not receiving wide acceptance.

Utilization of male sterility would be an important alternative to the problem of hand cross-pollination. If male sterility can be incorporated in a high quality maternal parent line then no contaminating pollen would be produced by that line. All seed produced would be naturally cross-pollinated from the pollen parent line planted in proximity to the seed parent line. F_1 hybrid seed could be produced by this method greatly reducing the cost of the seed.

A gene for male sterility has been induced by irradiation in watermelon (34). Breeding lines have been established which carry this mutation. This paper combined the results of a study of some of the morphological and cytological characteristics of plants carrying the male sterility gene and the evaluation of factors relating to the quality of the fruits from male sterile breeding lines. Consideration was given to the application of genetic male sterility and the value of the breeding lines used in this study.

REVIEW OF LITERATURE

Expression of Male Sterility

Plant sterility is an abnormality characterized by non-functional gametes. A male sterile plant either does not produce male gametes or the gametes are physically prevented from normal dehiscence, germination and resultant fertilization. Male sterile plants appear sporadically in populations as a result of mutation at any one of many loci governing vital steps in the formation of pollen (1). Male sterility enables the plant breeder to avoid emasculation procedures, and presents the possibility of introducing heterosis with increased vigor, yields and earliness into the cultural pattern of crops (2).

The practical utilization of heterosis in crop plants has been greatly facilitated by the use of male sterility for low cost, large scale emasculation of the seed parents of hybrids. There are three basic types of mechanisms of male sterility (26). The first is sterility depending on a single genetic factor which is ordinarily recessive. Lines carrying genetic male sterility are maintained by crossing with heterozygous male fertile plants resulting in progeny at a one to one ratio of sterile to fertile plants (1). Genes for male sterility have been utilized in barley (33), sugar beets (19) and other crops.

A second type is cytoplasmic male sterility. Cytoplasmic factors carried by certain plants may result in male sterility but will produce seed if pollinators are present. The F_1 seed from these plants will also be male sterile because the factor is transmitted by the maternal

cytoplasm. "Wide" crosses in cross-pollinated species or interspecific hybridization may induce pollen sterility cytoplasmically (7).

The third type is cytoplasmic-genetic male sterility which has the same cytoplasmic sterility except certain plants may carry genes which can restore pollen-producing ability to the male sterile cytoplasm (26). The identification of restorer genes have made cytoplasmic sterility factors useful. The first successful use of male sterility for hybrid plant production was with the onion (5). Jones and Clark (13) in 1943 published a scheme to utilize cytoplasmic male sterility to emasculate the seed parent for large scale hybridization.

Seed for hybrid corn can be produced without detasselling by use of cytoplasmic male sterility (12, 23). Genetic restoration of fertility to the hybrids has enabled use of male sterility to successfully supersede other methods of hybrid seed corn production (3, 6). Cytoplasmic male sterility with fertility restorer genes has made hybrid grain sorghum a reality (32). Improvements in sugar beets by use of hybrids was made possible by male sterility (18).

Pollen abortion is a plastic phenomenon subject to changes by the environment. If pollen abortion occurs near the termination of microsporogenesis (a few days to a few hours before anthesis), then any change delaying the breakdown and abortion would result in more viable pollen. Conditions both of the environment and within the plant favor increased pollen viability near the end of the growing season. Successful utilization of a male sterile mutant depends on its sterility under a wide range of growing conditions (7).

Inducing Male Sterility

Male sterility may be the result of spontaneous natural occurring mutations or may be artificially induced. Genes limiting pollen fertility are numerous and selections for these mutants may be undertaken in breeding populations (27). Methods of inducing male sterility artificially but leaving the female gametes fertile are useful. Treatment with chemicals such as growth hormones or hormone-like chemicals may reduce fertility. Maleic hydrazide, naphthaleneacetic acid, 2,4-dichlorophenoxyacetic acid (22), triiodobenzoic acid (39), and sodium alpha, beta-dichloroisobutyrate (FW 450) (9) adversely affected pollen fertility in the cucurbits. Pollen production may be reduced by irradiation using X-rays, gamma rays, ultra violet radiation or other energy sources (40).

A gene for male sterility was reported by Dr. V. M. Watts, former Head, Department of Horticulture, University of Arkansas (34). A seedlot of the "Sugar Baby" cultivar was subjected to gamma radiation. In the second generation a male sterile mutant was found with a distinctive seedling marker. Phenotypic expression of this mutation lies in a completely glabrous condition (no pubescence on the stems or leaves), coupled with male sterility. The glabrous character can be recognized with a hand lens at emergence and is easily detected at thinning time. Early in the season male flowers fail to open. Later after fruit set, staminate flowers open but the anthers do not dehisce (34).

These characters are controlled by a recessive gene and as no evidence has been found of separation of the glabrous character and

male sterility, a pleiotropic gene effect is proposed by Dr. Watts; the symbol for the gene being "ms_g" (34). Rigorous studies indicate that practical male sterility may be expected under ordinary field conditions although small quantities of viable pollen may be produced by the male flowers late in the growing season. The breeding lines are maintained by crossing glabrous recessives with heterozygous siblings (35).

Utilization of Male Sterility

With the availability of a gene for male sterility in watermelons, new interest has been aroused in hybrid watermelons. F₁ hybrid watermelons have been available in the seed trade for several years but the cost-merit relationship has restricted wide acceptance. Hybrid watermelon seed by hand pollination is 15 times more expensive than open pollinated seed (9). This present high cost of F₁ hybrid seed is primarily due to the technical labor involved in blossom isolation, hand pollination, and the identification and selective harvesting of crossed melons (34).

F₁ hybrids must show definite advantages over standard varieties to be accepted (24). Little is known of heterosis in watermelons, but hybrids have been shown to be superior in one or more factors in each of the cucurbits (17). Evidence of the significant improvement using hybrids was demonstrated with cantaloupe and cucumbers in the 1969 Vine Crop Variety Trials at Kansas State University (8). Out of 17 commercial cantaloupe varieties in the trials, the top 9 varieties were F₁ hybrids. These hybrids exceeded the standard varieties in

yield and quality. Slicer and pickler cucumber varieties also demonstrate the dominance of hybrids in these crops.

In watermelons, generally no loss of vigor is observed with inbreeding for 6 generations (21). However, heterosis might influence earliness, yields, fruit qualities and range of adaptability (34). Hybrid watermelons could speed up the release of desirable new characters such as seedless melons. Seed to produce plants with seedless fruits can be the result of an F_1 hybrid between a tetraploid and a diploid which results in sterile triploid (14).

Development of a fruit shape uniformly intermediate between the elongate cylinder and the spherical types would be highly desirable. The two shapes are controlled by a single gene pair that lacks dominance. Therefore, the intermediate shape is the result of a heterozygous condition according to Weetman (36). The intermediate shaped melons are generally less subject to white heart than the spherical types and are free from the gourd-neck type of fruit malformation that is a common problem in cylindrical varieties. The intermediate shape is considered nearly ideal for shipping and handling and therefore, is in great demand commercially. This shape can be produced as an F_1 hybrid between spherical and cylindrical parent lines (34).

If the male sterile line is used as the seed parent, then no contaminating pollen will be produced by these plants. However, since the male sterile line cannot be selfed, it is maintained by crossing to heterozygous siblings and 50% of the offspring will be

male fertile. These must be rogued from the seed parent line. The glabrous seedling marker indicates male sterile plants so the normal male fertile plants can be rogued at an early stage, such as at thinning time, without extra cost (35).

A homozygous male sterile line of high quality is the greatest problem to overcome before commercial scale production can commence. The original plants carrying the male sterile gene were highly susceptible to diseases, non-vigorous and of low fruit quality, with low soluble solids, irregular rind thickness, white heart, flesh cavities, and angularity of fruit shape due to very low to almost no seed production. Outcrossing with commercial varieties and fixed breeding lines has developed resistance to fusarium wilt Fusarium oxysporum f. niveum and anthracnose Colletotrichum lagenarium. The male sterile lines now carry improved seed production and improved vigor (35). Combination of high flesh quality and increased seed production with the other desirable characters already present in these lines constitutes the major remaining developmental task in this breeding program.

Morphology and Anatomy

The stem structure of the watermelon is an herbaceous vine with discrete vascular bundles. The vascular region is delimited on the outside by a broad zone of parenchyma. Outside this lies a band of sclerenchyma, then a narrow band of chlorenchyma and a discontinuous band of collenchyma just below the epidermis (10). The epidermis is regular with a thin, smooth cuticle and stomata. Numerous rigid, multicellular hairs are produced on raised bases on stems and leaves (38).

The floral parts of the staminate flower are initiated in the following sequence: sepal lobes, petal lobes, then stamen lobes (16). The primordia of the stamens are laid down directly after those of the petal lobes. There are three stamens with two larger than the third. Two conspicuous lobes are present at the upper extremities of the two larger stamens. The smaller stamen has only one lobe. Each of the larger stamens stands opposite the petal lobes, the smaller is midway between sepal and petal lobes (38). Chakravarty (4) presented evidence that the basic number of stamens is 5 in Cucurbitaceae with reductions to 3 by the process of fusion. Each stamen is a bilobed thecum with one microsporangium for each lobe. Ten microsporangia are present in the watermelon.

Cytological Studies

The watermelon has 11 pairs of chromosomes (37). Cytologists have reported no unusual features of microsporogenesis in this species. However, difficulties have been encountered with cytological studies. Pollen mother cells are not amenable to manipulation using conventional cytological techniques. The main difficulty is that the chromosomes are not easily differentiated from the cytoplasm suggesting a cell chemistry in Cucurbitaceae which is different from other plant groups. The chromosomes are also relatively small and usually not well separated from each other (38).

Whitaker (37) stated that meiotic divisions take place in a normal manner. During the second or homotypic division the spindles are

sometimes formed at right angles to each other while in other cases they are parallel. Passmore (20) also found regular behavior of the chromosomes of C. lanatus. Microspores develop their characteristic markings while still in the sporocyte walls.

Shimotsuma has made extensive investigations into crosses between C. lanatus and C. colocynthis (28). Irregularities in normal bivalents occur in the interspecific crosses. Three types of meiotic irregularities were found in autotetraploid C. lanatus and C. colocynthis: (1) the members of the quadrivalents frequently disjoin unequally at the first meiotic anaphase, (2) there were incomplete disjunctions of the members of the quadrivalents resulting in lagging and dividing univalents at first metaphase that tend to lag and divide equationally at first anaphase and were left in the cytoplasm at first or second telophase (29).

Doctors Kihara and Shimotsuma have gained much fundamental information on the sterility of translocation heterozygotes in their work toward development of seedless watermelons by chromosomal translocations (15). Chromosomal interchange heterozygotes were obtained by gamma irradiation forming ④ and 9_{II} or larger ring configurations at diakinesis (30). By studying the tetrads from sporocytes with ring chromosomes, it was possible to detect single crossovers and whether alternate or adjacent disjunction (15). Functional pollen grains were always found to be single although pollen quartet association continues for a short time after dehiscence. Sterile pollen in quartets may not separate. A high degree of sterility was encountered in pollen and seed production as a result of the reciprocal translocations (30).

METHODS AND MATERIALS

Breeding Line Pedigrees

Breeding lines carrying the "ms_g" gene, which was discovered by Dr. V. M. Watts, were used in this study. Seed for the 1969 planting was obtained from selfed, heterozygous, high quality fruits from the 1968 growing season. The 1969 breeding lines were designated MS-9-2 through MS-9-10, the notation referring to Male Sterile (MS) - 1969 (9) - line number (2 through 10).

Pedigrees of these breeding lines are given in Table 1. Notations for the breeding lines identify the year, line number and hill number. Ark-67-5N-1 indicates: Arkansas breeding line - year 1967 - line number 5 - normal plant number 1. The MS prefix designates male sterile lines developed at Kansas State University from the Arkansas parent lines. "N" denotes a normal plant and "J" denoted a glabrous, male sterile plant. Figures 1a through 1f show factors related to quality of parental fruits of the 1969 breeding lines. Discrete breeding lines were maintained throughout this study.

Field and Greenhouse Procedures

Seeds were planted in Jiffy-7 peat pellets in the greenhouse in early May 1969, then transplanted in the cotyledon stage into the growing field at the Ashland Horticultural Research Farm. Each line population consisted of 36 plants of which approximately 1/3 or more were glabrous where available with the remainder being normal. The

Table 1. Pedigree of breeding lines.

1967	Line numbers for:	
	1968	1969
Ark-67-5N-1*	MS-8-1-7N	MS-9-2
Ark-67-13N-1-4	MS-8-3-5N	MS-9-3
Ark-67-13N-2-4	MS-8-4-1N	MS-9-4
Ark-67-5N-9	MS-8-2-13N	MS-9-5
Ark-67-17J-2-1 x N-1	MS-8-6-13N	MS-9-6
Ark-67-17J-2-1 x N-1	MS-8-7-16N	MS-9-7
KMS-67-1-3N (C.S.)**	MS-8-8-16N	MS-9-8
KMS-67-8-11	MS-8-9-9N	MS-9-9
Ark-67-15N-6	MS-8-10-6N	MS-9-10

* Ark. lines were developed by Dr. V. M. Watts, former Head, Department of Horticulture, University of Arkansas.

** KMS. lines are Kansas Male Sterile lines developed at Kansas State University from a 1964 cross of Ark-63-24-2N-3 x Crimson Sweet.

Fig. 1. Quality factors in pedigree of 1969 breeding lines.

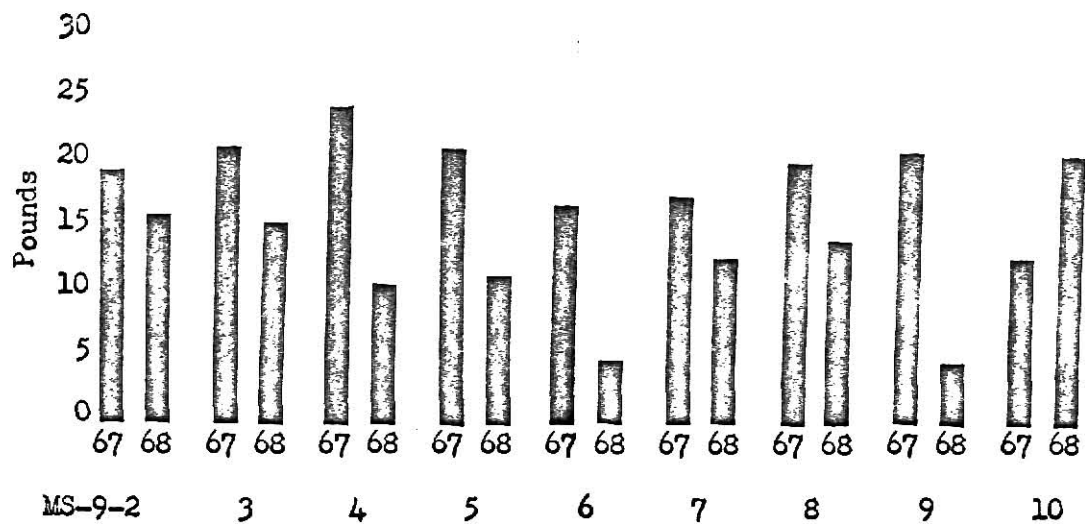


Fig. 1a. Parental fruit weights by years.

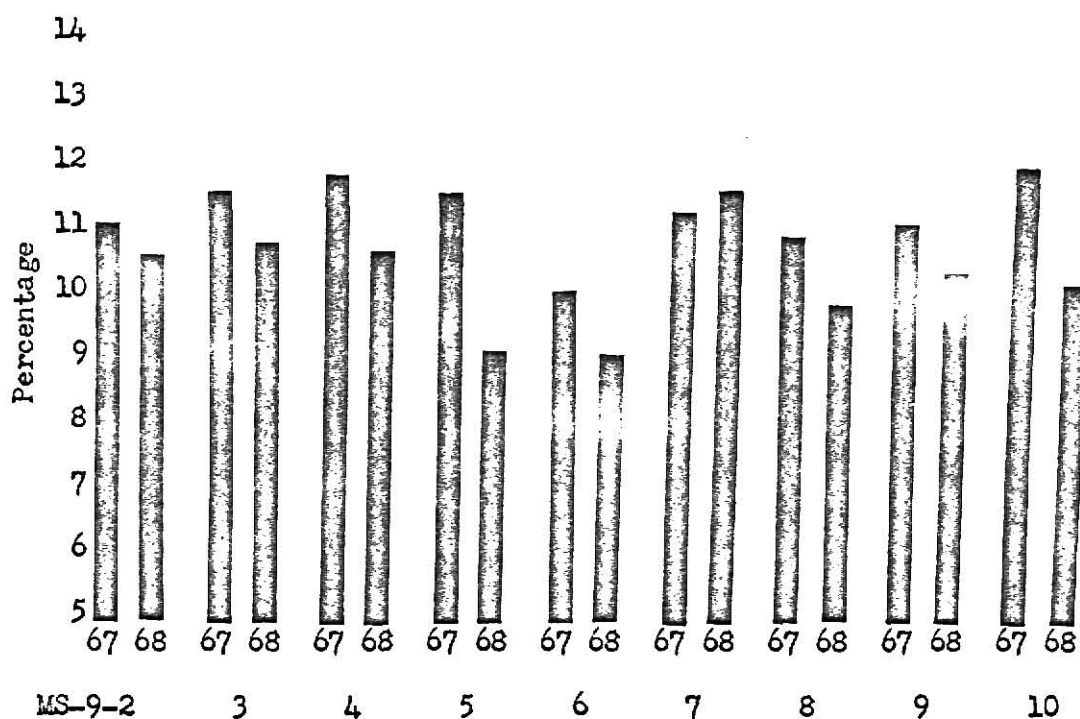


Fig. 1b. Total soluble solids of parental fruits by years.

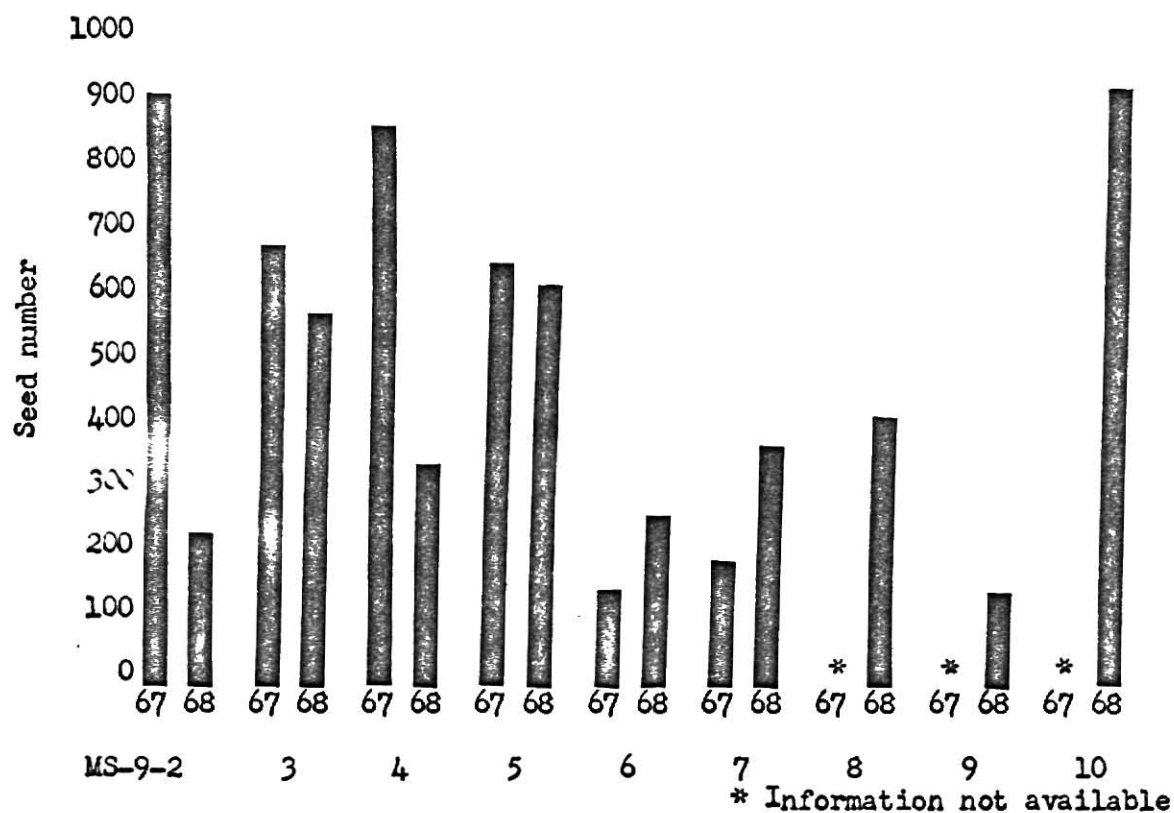


Fig. 1c. Seed numbers in parental fruits by years.

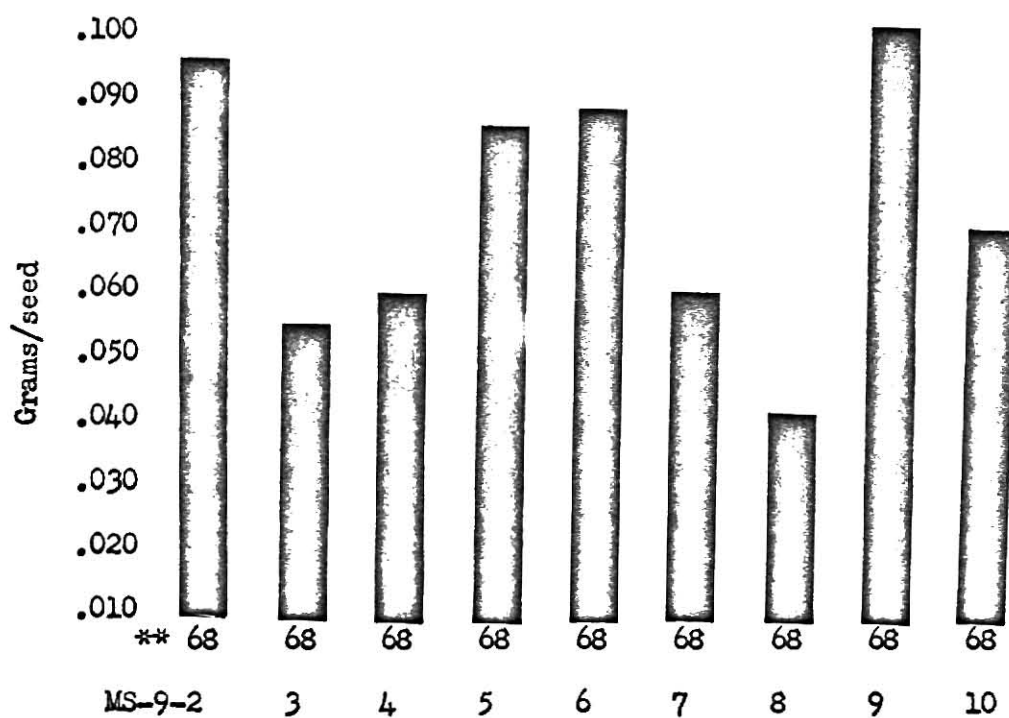


Fig. 1d. Seed weight of parental lines.

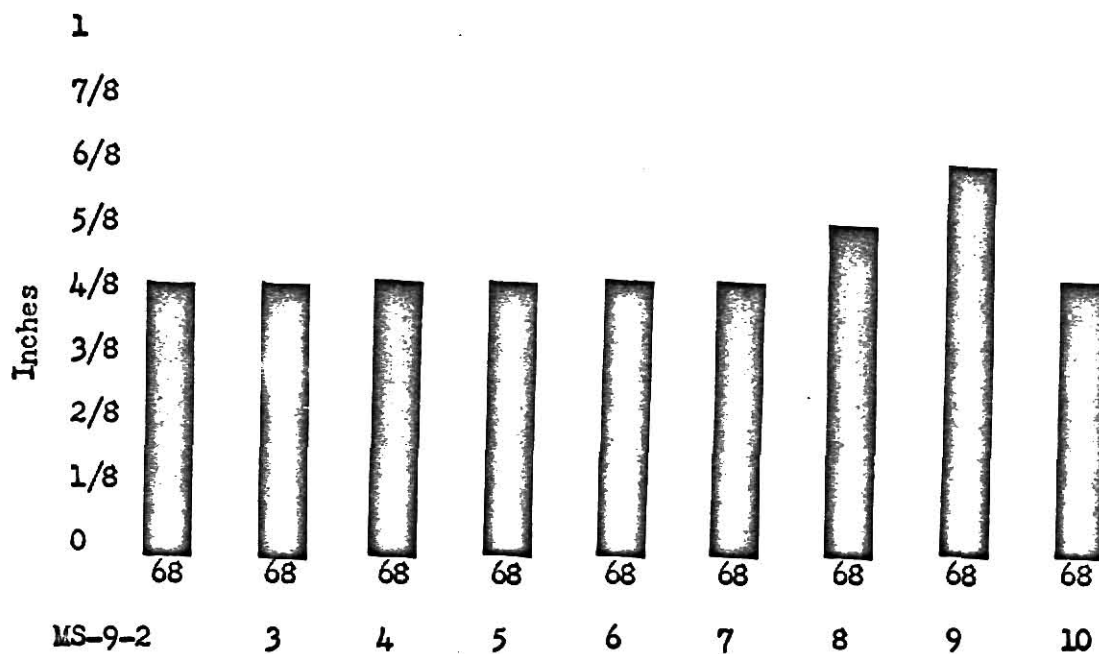


Fig. 1e. Rind thickness of 1968 parental fruits.

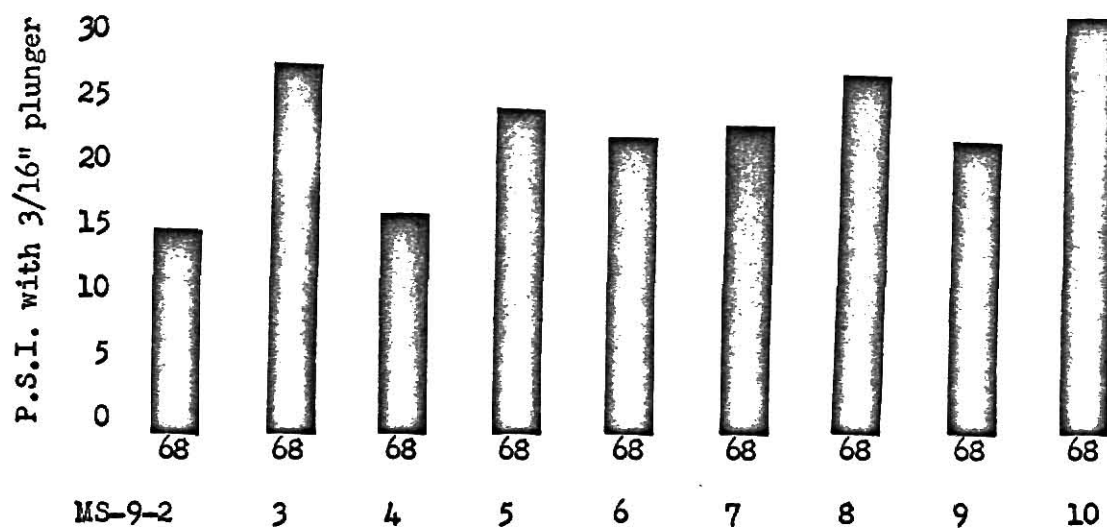


Fig. 1f. Rind toughness of 1968 parental fruits.

lines were transplanted through black plastic mulch with a spacing of 12 feet between rows and 5 feet within the row. Lines MS-9-2, 6, 7, 8, 9 and 10 were transplanted May 22, 1969, line MS-9-3 on May 30, and lines MS-9-4 and 5 on June 11.

Fertilizer was placed under the plastic before planting at the rate of 150 lbs./acre of 10-10-10 analysis. A side dressing of 33 1/2 lbs. of nitrogen per acre was applied one month after planting. Spraying for control of insects and diseases followed a weekly schedule and after each rain using Thiodan insecticide and Dithane M-45 alternated with Z-78 fungicides. Soil between the plastic strips was cultivated and Alanap herbicide was applied July 2, at the rate of 2 lbs. of N-1-naphthylphthalamic acid per acre.

Controlled pollinations were made during July and early August. Staminate and pistillate flowers were selected and the corolla was sealed with cellophane tape the afternoon prior to opening. The following morning the tape was removed, the pistillate flowers were pollinated and then retaped and tagged for identification. Characteristics of the stems and flowers of the normal and glabrous plants are shown in Plate I. The field layout showing fruits with flagged stakes and tags is shown in Plate II.

Normal plants were self-pollinated and the male sterile plants were either sib-mated or outcrossed. The pollen sources used for outcrossing were other KSU breeding lines 69-1, 69-24, 69-29: varieties, Crimson Sweet and Charleston Gray; and a Crimson Sweet mutant induced by irradiation. The mutant was from Crimson Sweet seed that was

EXPLANATION OF PLATE I

Top. Stem of normal, pubescent watermelon plant on left and glabrous, male sterile plant on right.

Bottom. Pistillate flowers top and staminate flowers bottom of glabrous watermelon plant on left and normal plant on right.

PLATE I



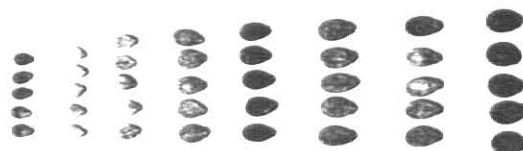
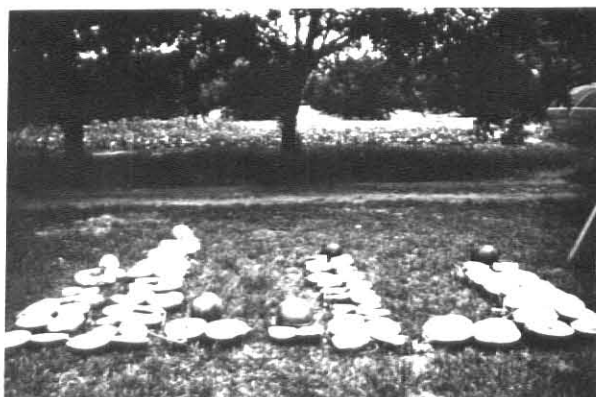
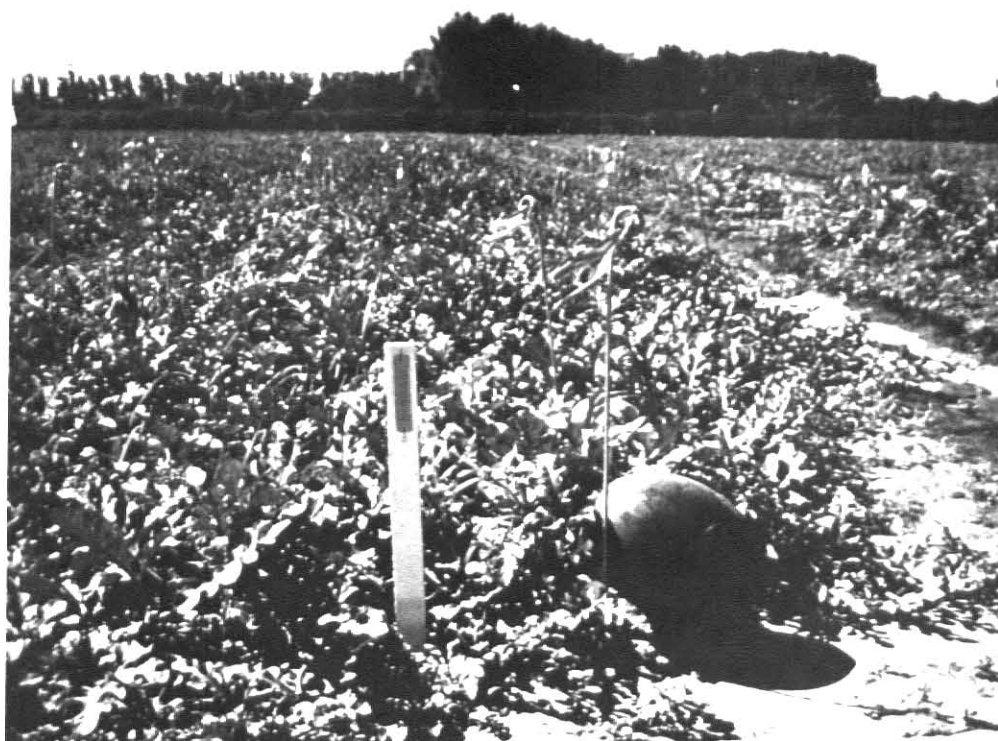
EXPLANATION OF PLATE II

Top. Field layout showing line stake and metal marking stakes for the pollinated fruits.

Bottom left. Fruits prepared for seed removal from one harvest after fruit records were taken.

Bottom right. Seeds varied in size and weight among the lines. The average individual seed weights in grams of the pictured groups are from left to right: 0.023, 0.051, 0.062, 0.080, 0.085, 0.098, 0.114 and 0.123 grams.

PLATE II



subjected to 30,000 r. of gamma radiation from a Cobalt 60 source at the Kansas State University nuclear reactor. The radiation treatment was part of a class project for Horticultural Crop Breeding. The irradiated seeds were planted in peat pellets in the greenhouse, then transplanted to the field in late April 1969. Several mutations were observed in the M_1 generation but one was of particular interest; a glabrous plant very closely resembling the male sterile glabrous plants. There was no pubescence on the stems or leaves. The male flowers were reduced in size with small greenish, crinkled petals. Self-pollinations were made on this plant and pollen was transferred to male sterile, glabrous plants.

Irrigation of the planting was performed by the furrow flooding method using gated pipe. The plants in each line were numbered 1 through 36 so that each plant could be identified. Each plant was rated on a scale of 0 to 5 for disease injury caused primarily by anthracnose. A rating of 0 represented no evidence of disease injury on leaves or stems up the scale to a 5 for plants that had died apparently of disease injury. Another rating for the general vigor and productivity of the plant was made on a 0 to 5 scale. Five represented an excellent plant which was very vigorous and highly productive grading down to 0 for a plant that failed to develop to maturity.

Each plant was classified whether normal "N" or glabrous "J" and whether male sterile or fertile. Fruit rind pattern (Plate III) was recorded for each plant whether splashed (irregular shaped blotches of dark green pigment scattered over the fruit usually more concentrated

EXPLANATION OF PLATE III

Watermelon Rind Patterns

Top left. Splashed with light green background (Spl LGB).

Top right. Splashed with dark green background (Spl DGB).

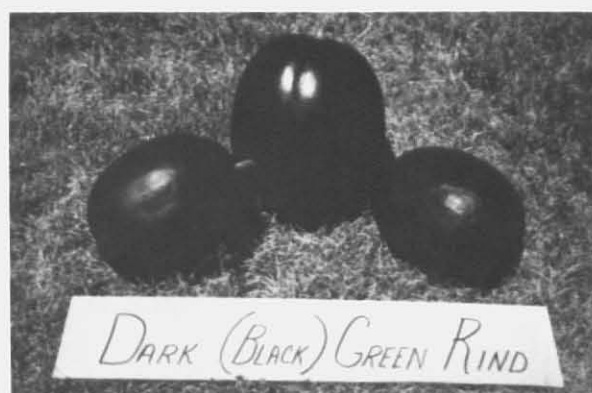
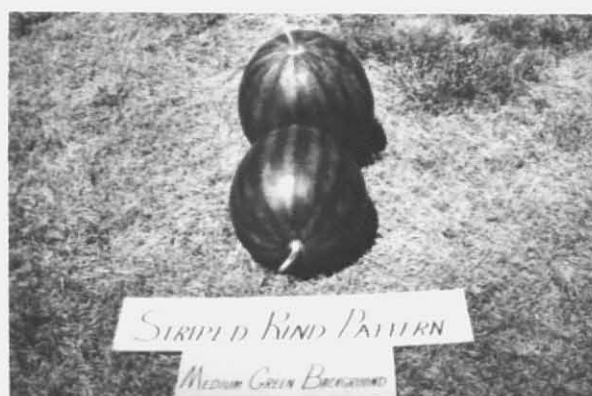
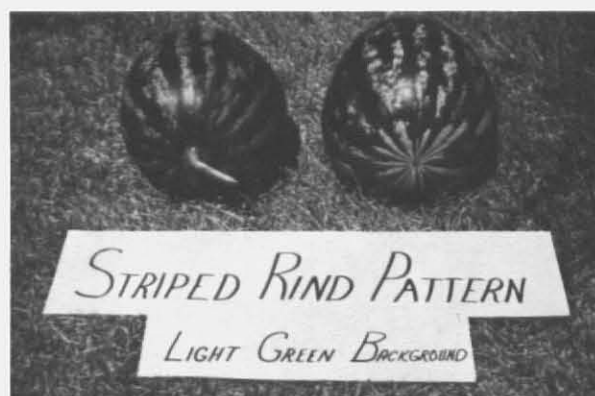
Center left. Striped with light green background (Str LGB).

Center right. Striped with medium green background (Str MGB).

Bottom left. Striped with dark green background (Str DGB).

Bottom right. Dark green (Dk Gr).

PLATE III



near the stem end) or striped in pattern. The background color pattern was recorded either as light green or dark green or solid dark green without stripes or splashing. Notation symbols for each of the different rind patterns were as follows: Spl LGB - Splashed with light green background, Spl DGB - Splashed with dark green background, Str LGB - Striped with light green background, Str MGB - Striped with medium green background, Str DGB - Striped with dark green background, and Dk Gr - Solid dark green.

Harvest season was from August 11 to October 1. The fruits required 35 to 40 days to mature from pollination to harvest. The following data were recorded on the harvested fruits: weight, rind toughness (measured with a standard fruit pressure tester with a 3/16 inch tip plunger), rind thickness (in eighths of an inch), and percent total soluble solids (measured by a hand refractometer from the center of the fruit). Plate 11 depicts fruits prepared for seed removal from one harvest after fruit records were taken. Seeds were removed from each fruit and dried. The seeds were counted and a sample of 100 seed was weighed to find the average seed weight for each melon. Plate 11 shows variation in seed sizes.

A sample of 100 seed from each selfed fruit from normal plants was planted in flats in the greenhouse 1969-70. Two weeks after germination the seedlings were counted with the number of glabrous and normal seedlings recorded. Seedlings from the best quality heterozygotes of each line were planted in the greenhouse bench for further breeding work. One thousand seeds were planted from fruits of

homozygous recessive plants sibmated with pollen from heterozygous plants. The seeds were selected in lots of 25 from lines which produced sufficient seed on the male sterile plants. Two hundred seed were also germinated from the crosses between glabrous male sterile plants and the glabrous Crimson Sweet mutant. These seedlings were also transplanted into the greenhouse bench.

Microtechnique

For the morphological study of bud development, seedlings were collected when the first true leaf had expanded (approximately two weeks after germination), then every 5 to 7 days until flowering. Both pubescent and glabrous seedlings were collected. The seedlings were killed and fixed in FAA (10 parts 95% ethanol - 1 part glacial acetic acid - 1 part formalin - 8 parts water), dehydrated and cleared in an ethanol and tert-butyl alcohol series (25), and embedded in paraffin. Serial sections 12 microns thick were cut on a rotary microtome. Longitudinal sections of the apical meristematic region and cross-sections of stem tissue were cut and mounted on slides using Haupt's adhesive. The slides were stained using hemalum (Mayer's modified), safranin and fast green counterstain in schedules outlined by Sass (25). The stained sections were mounted in permount. Photomicrographs were taken of the meristems, developing buds and cross-sectioned tissue using an Olympus MG inverted microscope with Adox KB-17 film.

Cytological studies were made of meiotic divisions of the pollen mother cells. Staminate buds of normal and glabrous plants were

collected every half hour throughout the day and night. The buds were killed and fixed in freshly prepared Carnoy's fluid. The anthers were dissected out of the buds and squashed in acetocarmine. The smear was mounted in Hoyer's medium. Photomicrographs were taken of the division figures using a Bausch and Lomb dynoptic research microscope with Adox KB-17 film.

For an indication of pollen viability, a staining technique was employed. Aniline blue in lactophenol (11) was used to stain pollen of normal and male sterile anthers prepared by a smear technique. Pollen grains assumed to be viable by this chemical test stain a dark blue while non-functional pollen grains did not stain. Percentage pollen viability was calculated from 100-grain pollen counts taken randomly from different areas of each of 40 slides prepared. Photomicrographs were made of the normal and male sterile pollen using the Olympus microscope.

EXPERIMENTAL RESULTS

Evaluation of Breeding Lines

The 1969 breeding lines MS-9-2 through MS-9-10 used in this study were heterozygous for the glabrous character. In Table 2 heterozygosity for the " ms_g " gene is shown by the occurrence of normal and glabrous seedlings. The percentage of glabrous plants was generally slightly less than 25%. The transplanted seedlings grew comparably to those of other breeding lines and varieties. The glabrous plants were less vigorous than pubescent siblings. Pubescent

Table 2. Test for 'ms_g' gene heterozygosity of parental fruits by progeny segregation.

1969 Line number	1968 Fruit		Percent seed germination	Number of normal seedlings	Glabrous seedlings	
	Number	Rind pattern			Number	Percent
MS-9-2	MS-8-1-7	SpI DGB	94	74	20	21.3
MS-9-3	MS-8-3-5	SpI DGB	91	75	16	17.6
MS-9-4	MS-8-4-1	Dk Gr	87	68	19	21.8
MS-9-5	MS-8-2-13	SpI DGB	89	66	23	25.9
MS-9-6	MS-8-6-13	Dk Gr	90	69	21	23.4
MS-9-7	MS-8-7-16	SpI DGB	88	70	18	20.5
MS-9-8	MS-8-8-16	Str MGB	96	74	22	22.9
MS-9-9	MS-8-9-9	SpI DGB	83	64	19	22.9
MS-9-10	MS-8-10-6	SpI DGB	91	67	24	26.4

plants were first to produce staminate and pistillate flowers. Glabrous plants were one to 2 weeks later in producing the first pistillate flowers. The staminate flowers were small and did not open. Later, after 2 to 3 pistillate flowers had been produced, staminate flowers began to open but the anthers were shrivelled and non-dehiscent. All glabrous plants in the field had these male sterile characteristics and all pubescent plants appeared to have normal functional male flowers.

The individual plant ratings of disease resistance taken after fruit set were averaged for each line and the means are compared in Fig. 2. The ratings of growth pattern considering vigor and productivity of the vines are compared in Fig. 3. Fruit rind patterns were compared. All glabrous plants had dark green rinds or dark green background with lighter green striping (refer to Plate II). The pubescent plants were carrying genes for the splashed pattern in all lines except line MS-9-8 which had a striped pattern from its *Crimson Sweet* parentage. Lines MS-9-4 and 6 had some dark green fruits on the pubescent plants. Table 2 shows the parental fruits were dark green.

The rind background color was compared in fruits of the pubescent plants. Considering possible linkage of the dark green rind color with male sterility, the number of plants with fruits carrying genes for light green and dark green backgrounds were compared with the number expected in each category for linkage of dark green rind color with male sterility. One light green to 2 dark green would be expected. The pooled-data chi-square test reveals that rind colors approach a 1:2 ratio of light green to dark green with a probability of 0.90.

Table 3. Rind colors of fruit of normal plants in 1969 lines.

Line number	Total number of normal plants	Observed numbers of plants with fruits having:		Expected numbers of plants with fruits having:		χ^2 values
		Light green background	Dark green background	Light green background	Dark green background	
MS-9-2	20	5	15	7	13	0.878
MS-9-3	14	4	10	5	9	0.311
MS-9-4	11	4	7	4	7	0.000
MS-9-5	19	8	11	6	13	0.973
MS-9-6	18	3	15	6	13	2.192
MS-9-7	21	10	11	7	14	1.927
MS-9-8	15	5	10	5	10	0.000
MS-9-9	26	9	17	9	17	0.000
MS-9-10	13	6	7	4	9	1.444
Totals	157	54	103	53	104	7.725

Sum of χ^2 values = 7.725, with 9 DF
Pooled-data $\chi^2 = 0.029$, with 1 DF
Heterogeneity $\chi^2 = 7.696$, with 8 DF

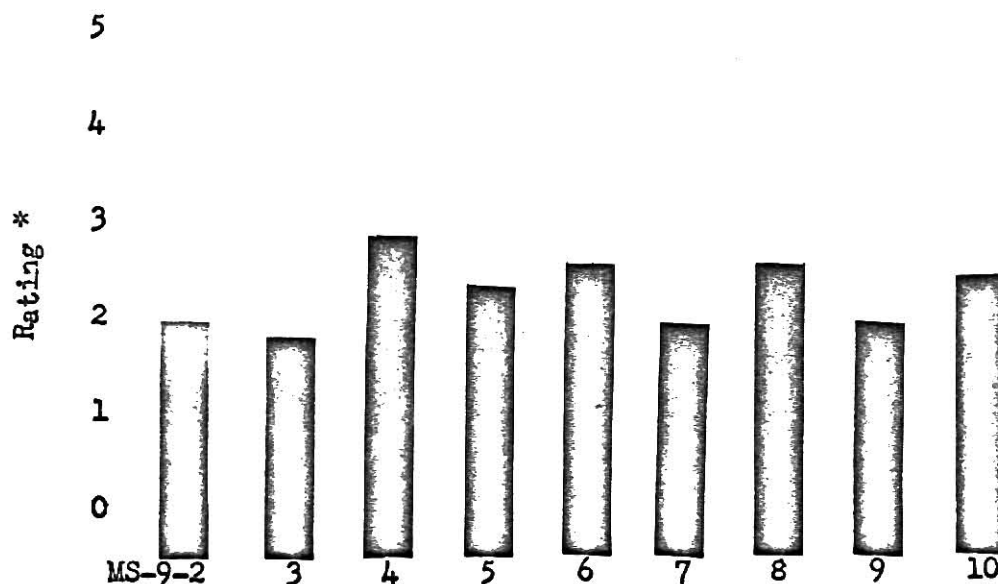


Fig. 2. Disease injury ratings of breeding lines in 1969.

* 0-none, 1-slight, 2-moderate, 3-severe, 4-very severe, 5-dead

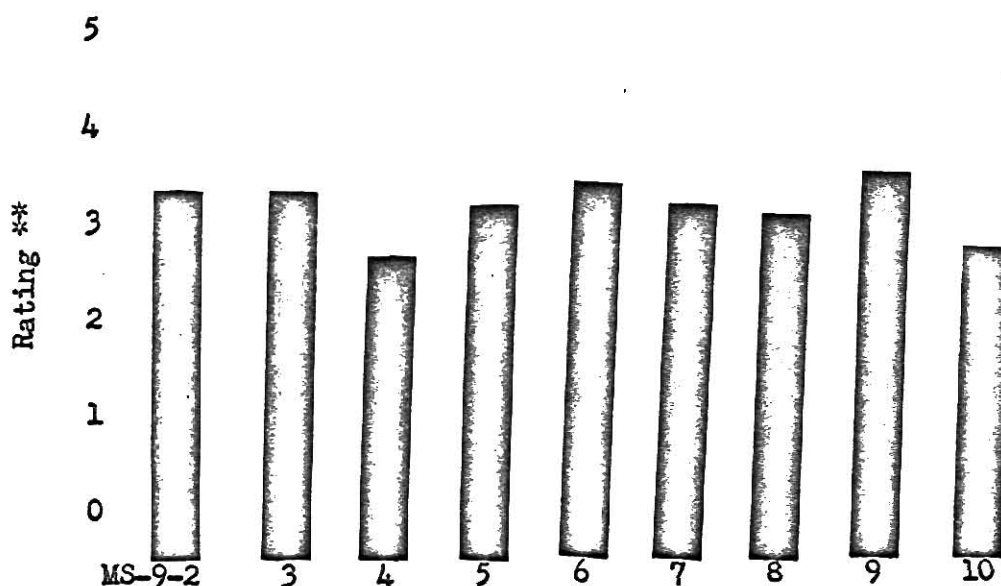


Fig. 3. Rating of vigor and productivity of breeding lines in 1969.

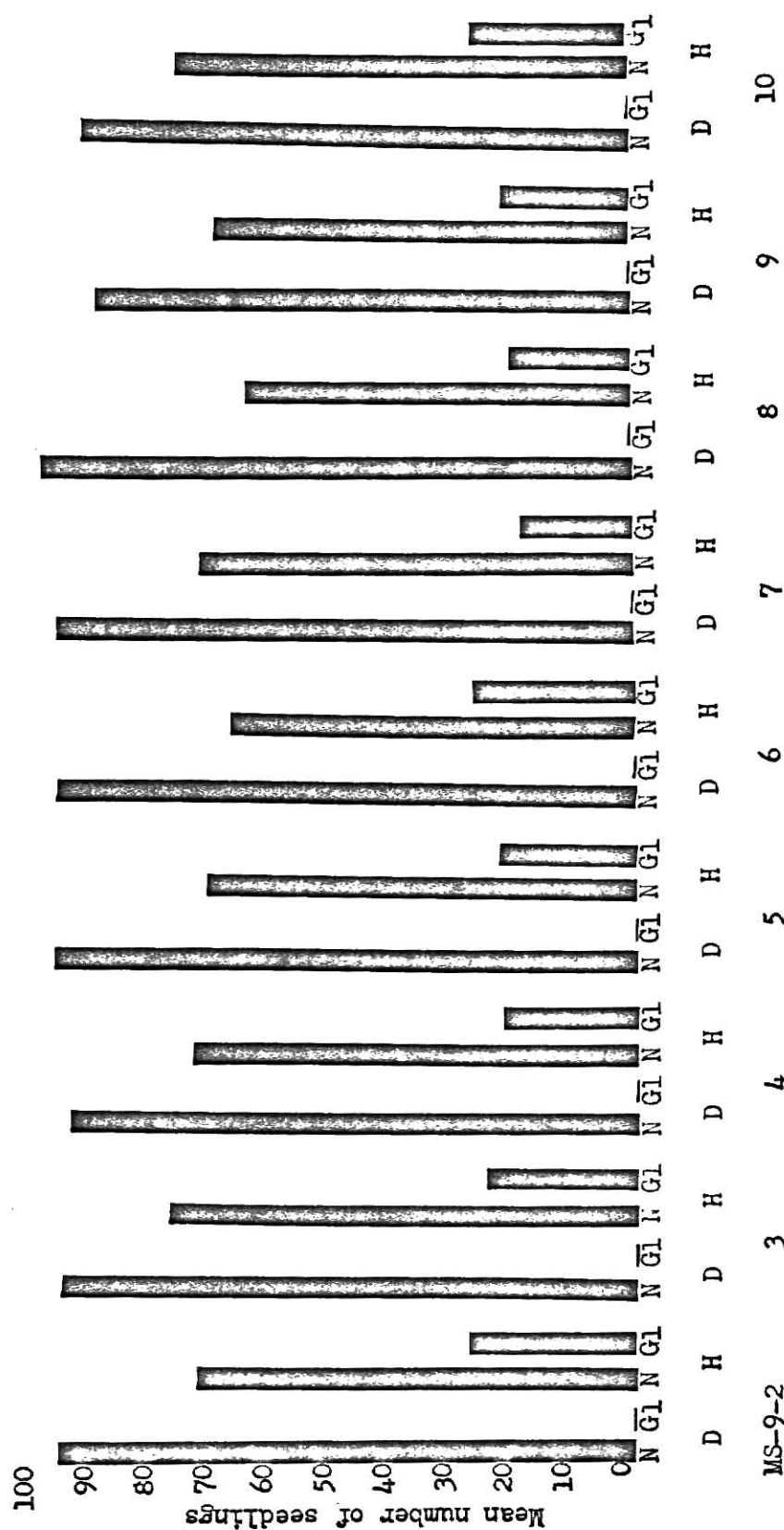
** 0-plant did not mature, 1-very weak plant, 2-weak, little productivity
3-moderate, 4-vigorous, 5-very vigorous and productive

Table 3 gives the observed and expected numbers in each category for each line.

Hand pollination resulted in 288 fruits set (134 fruits on male sterile plants and 154 successfully self-pollinated on normal plants). These fruits were harvested and evaluated. In order to present these data in meaningful relationships, it was helpful to know the genetic constitution of each plant in reference to the " ms_g " gene. The homozygous recessives ($ms_g ms_g$) were identified by their glabrous, male sterile expression. The heterozygous ($Ms_g ms_g$) plants and the homozygous dominant ($Ms_g Ms_g$) were pubescent and normal which required analysis of progeny segregation to differentiate the two. Germination of seed samples from each selfed, normal fruit separated the segregating heterozygous lines from the homozygous dominants.

Figure 4 shows the mean number of normal seedlings from each fruit which had no glabrous segregates and was classified homozygous dominant ($Ms_g Ms_g$). All of these fruits had a light green background rind color except line MS-9-6 where plants 1, 24, and 29 had dark green rinds. Plants producing the segregating populations were classified heterozygous. Figure 4 also shows the mean number of pubescent and glabrous seedlings for the heterozygotes. The chi-square test was applied to determine if the segregation ratios were following the 3:1 expected if the " ms_g " is a simply inherited recessive gene. The chi-square values given in Table 4 indicate segregation ratios close to the expected values. All the fruits which produced the segregating populations had dark green background rinds except plant 35 of MS-9-7 and plant 21 of MS-9-9. These two had

Fig. 4. Determination of heterozygotes by progeny segregation.



N - Normal seedling
 Gl - Glabrous seedling
 D - Homozygous dominant ($Ms_g Ms_g$)
 H - Heterozygous ($Ms_g ms_g$)

Table 4. Segregation ratios of seedlings from heterozygous fruits.

Line number	Observed number of plants		Expected numbers under 3:1 ratio hypothesized		χ^2 values
	Normal	Glabrous	Normal	Glabrous	
MS-9-2	703	233	702	234	.005
MS-9-3	296	89	280	96	.680
MS-9-4	217	56	205	68	2.819
MS-9-5	275	91	274	92	.015
MS-9-6	579	220	599	200	2.667
MS-9-7	362	93	341	114	4.709
MS-9-8	570	164	550	184	2.627
MS-9-9	953	309	947	315	.142
MS-9-10	294	86	285	95	1.137
Totals	4249	1341	4194	1396	14.801

Sum of χ^2 values = 14.801, with 9 DFPooled-data χ^2 = 2.898, with 1 DFHeterogeneity χ^2 = 11.903, with 8 DF

a splashed rind pattern with a light green background. Table 5 compares seedling segregation ratios with fruit rind color.

Table 5. Comparison of seedling segregation ratios with fruit rind color.

Rind color of parental fruits	Number of fruits	Number of seedlings		Totals
		Normal	Glabrous	
Light green	38	3406	42	3448
Dark green	63	4536	1341	5877
Totals	101	7942	1383	9325

contingency $\chi^2 = 802.542$

Seedlings produced from crossing homozygous recessive plants to heterozygous siblings were expected to have a 1:1 ratio of normal to glabrous. From 1000 seeds planted, 892 germinated with 473 normal and 417 glabrous. The goodness of fit to the 1:1 expected was tested giving a chi-square value of 3.270.

Evaluation of Quality Factors

After the homozygous dominant and heterozygous fruits were identified by progeny testing, the qualities of the fruits were compared according to their " ms_g " gene constitution. Fifty-four homozygous dominant fruits, 100 heterozygous and 134 homozygous recessive fruits were evaluated. Means for the fruit weights, total soluble solids, seed numbers, seed weights, rind thickness and rind toughness were compared from each line and presented graphically in Fig. 5.

Fig. 5. Evaluation of quality factors of 1969 fruits.

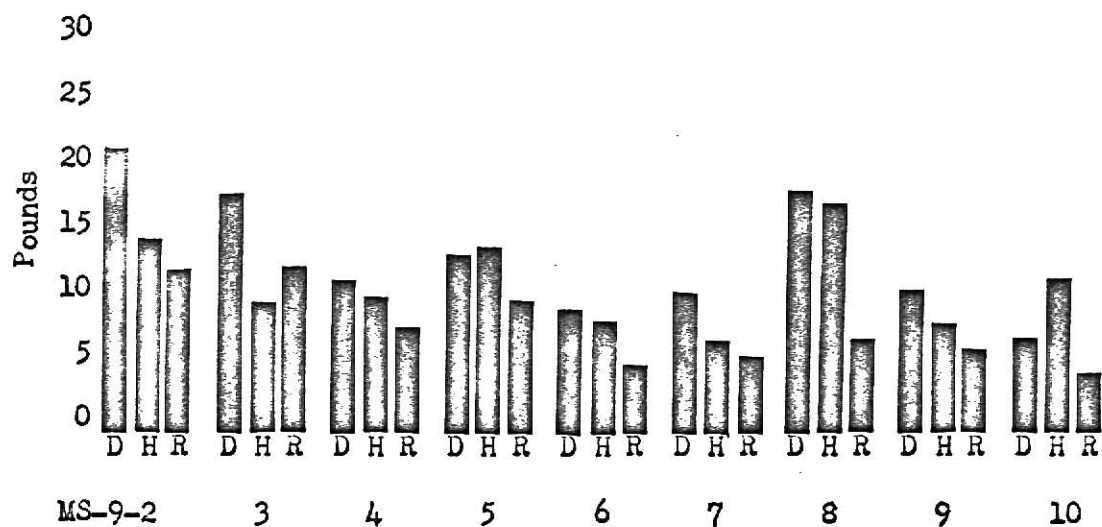


Fig. 5a. Comparison of mean fruit weights.

D - Homozygous dominant
 H - Heterozygous
 R - Homozygous recessive

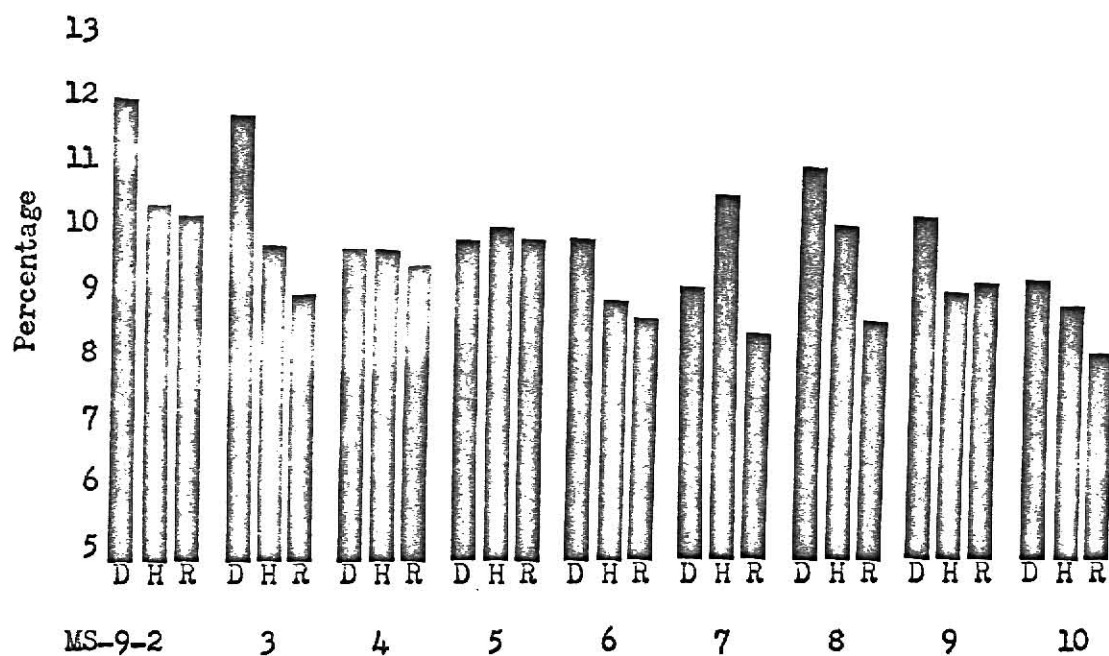


Fig. 5b. Comparison of total soluble solids.

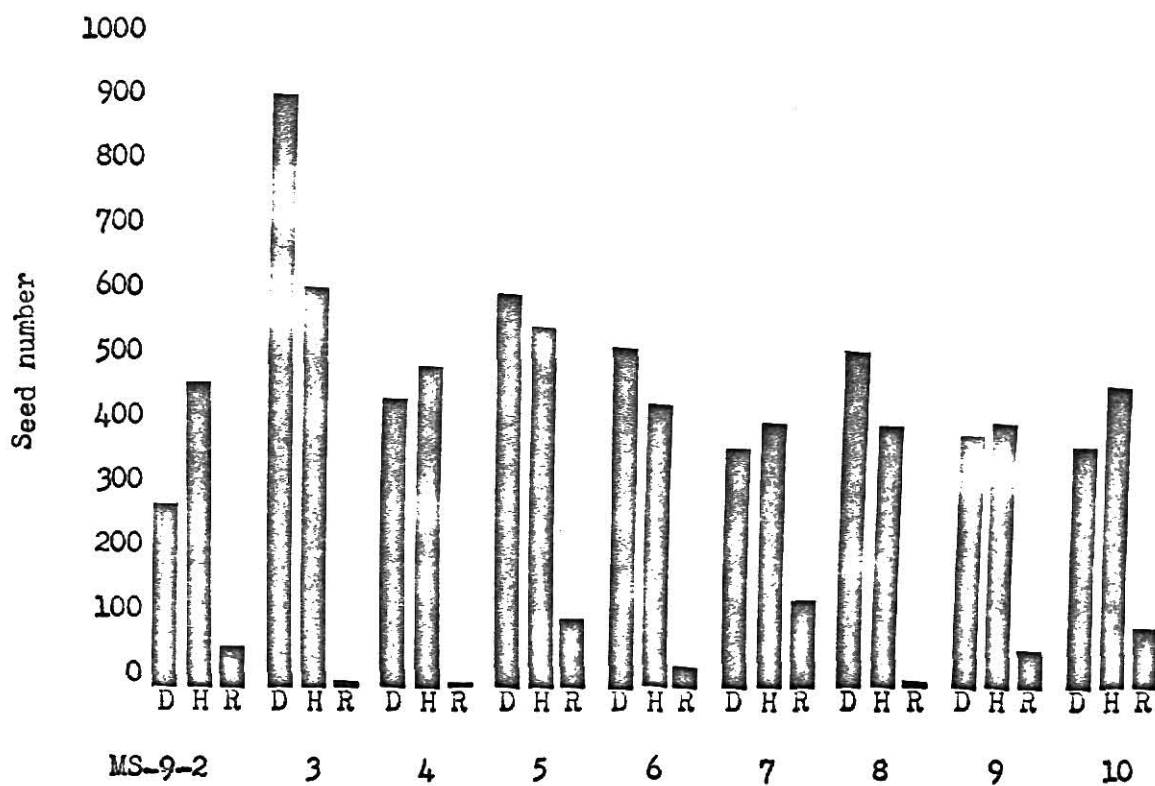


Fig. 5c. Comparison of seed numbers.

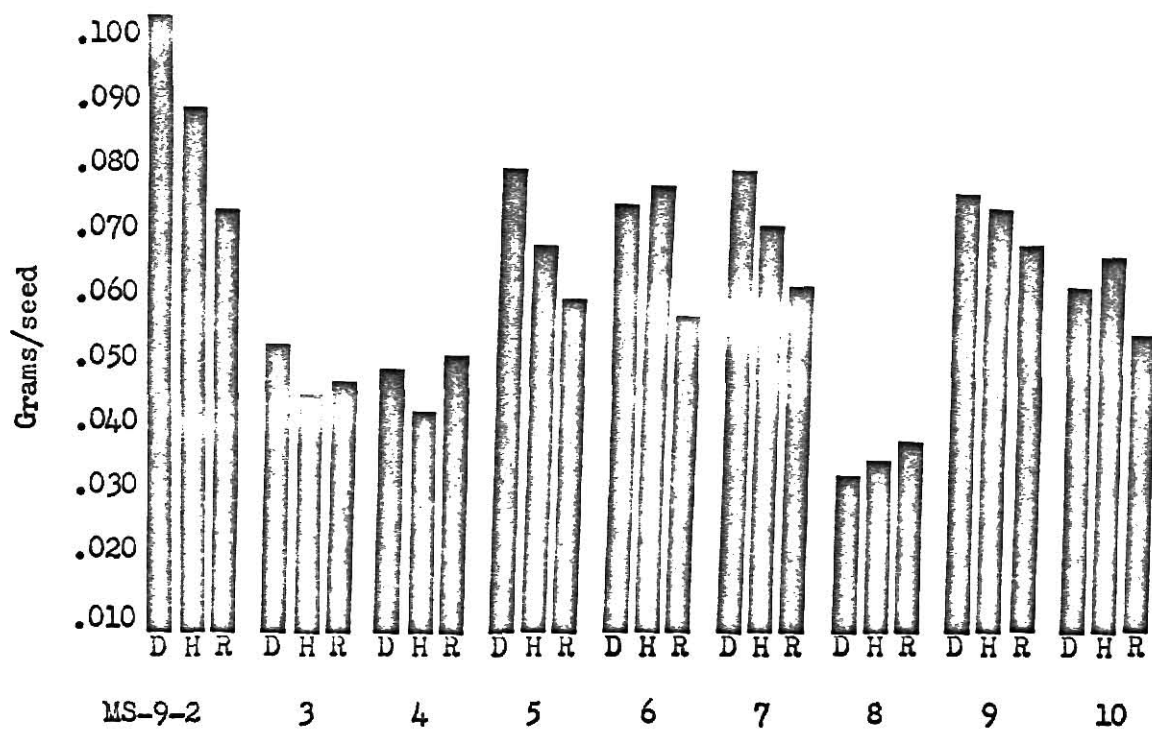


Fig. 5d. Comparison of seed weights.

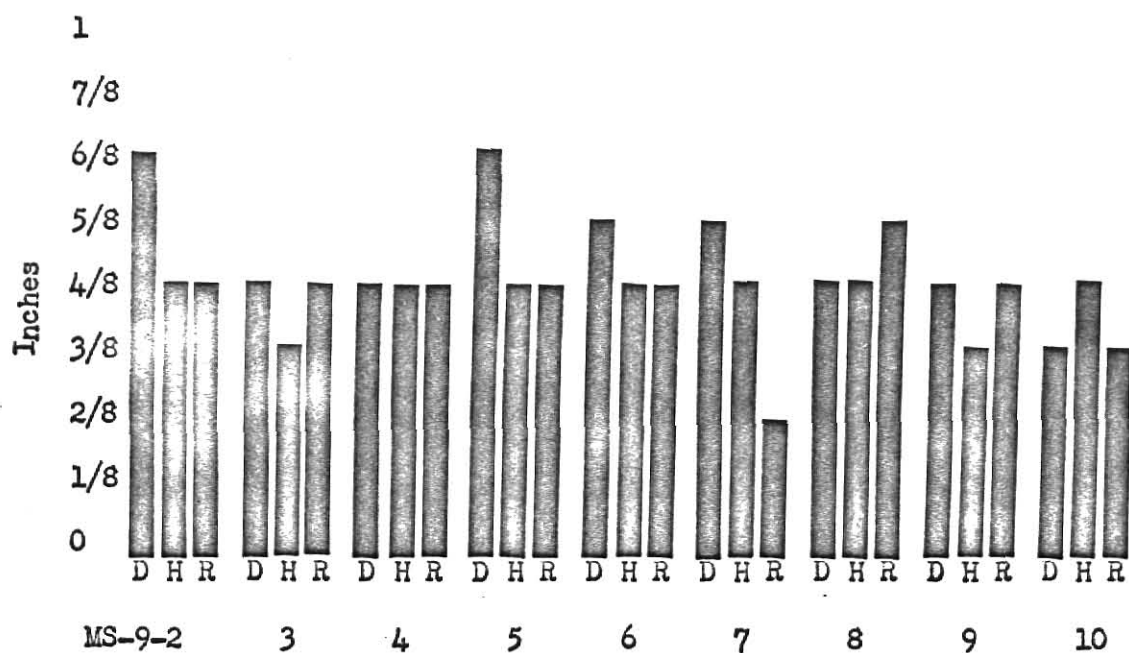


Fig. 5e. Comparison of rind thickness.

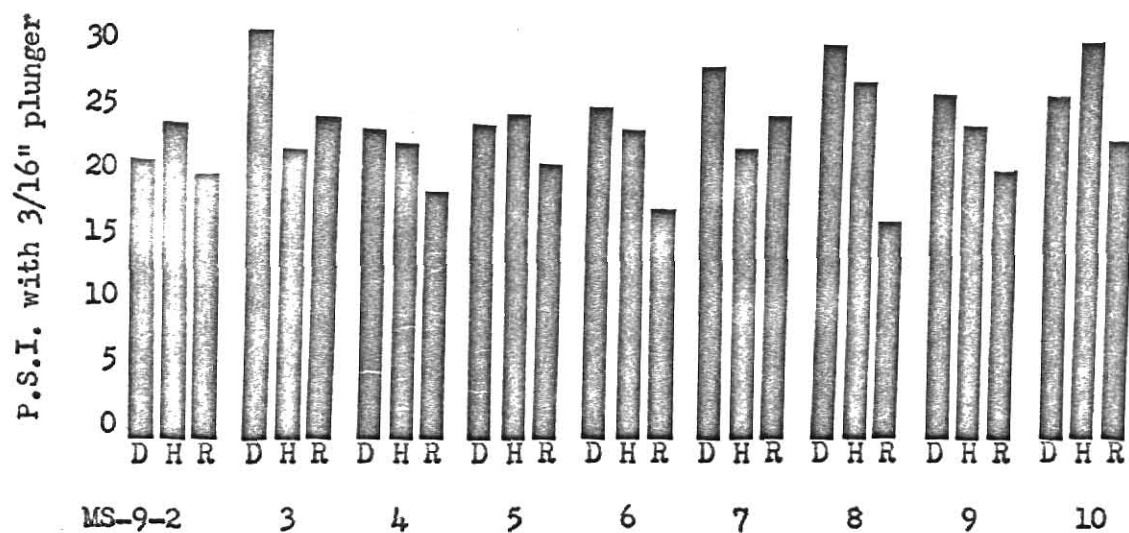


Fig. 5f. Comparison of rind toughness.

Reduction in seed number is evident in the male sterile fruits. The effect of pollen sources on the number of seed produced was considered. The effect of sibmating and outcrossing is shown in Fig. 6. There were 74 fruits sibmated and 60 outcrossed. The range in the number of seed produced in the sibmated fruits was from none to 248 seed. The range in number of seed from outcrosses was from no seed in Crimson Sweet and glabrous Crimson Sweet mutant crosses to 354 seed also found in a glabrous Crimson Sweet mutant cross.

Effectiveness of the pollen sources is compared in Fig. 7. The sources of pollen used were breeding lines 69-1, 69-24, 69-29; varieties Crimson Sweet and Charleston Gray and a Crimson Sweet mutant. Interest in the mutant prompted close investigation of these seedlings. Out of 183 germinated seedlings, all were normal with no detectable reduction in pubescence. The estimated percentage pollen viability was 91.5% and fruits were selfed on these plants. The estimated percentage of pollen viability of the normal pubescent plants was 94.6%. The glabrous male sterile plants produced only a few flowers with any viable pollen to give a mean of 1.6% viability by the chemical test.

Morphological and Anatomical Studies

The morphological study of staminate bud development from initiation to meiosis revealed few differences in normal and male sterile buds. Plate IV shows young meristems with bud initiation and early development which progresses with differentiation of sepals, petals and stamens. Plate V shows male sterile and normal buds in later developmental stages.

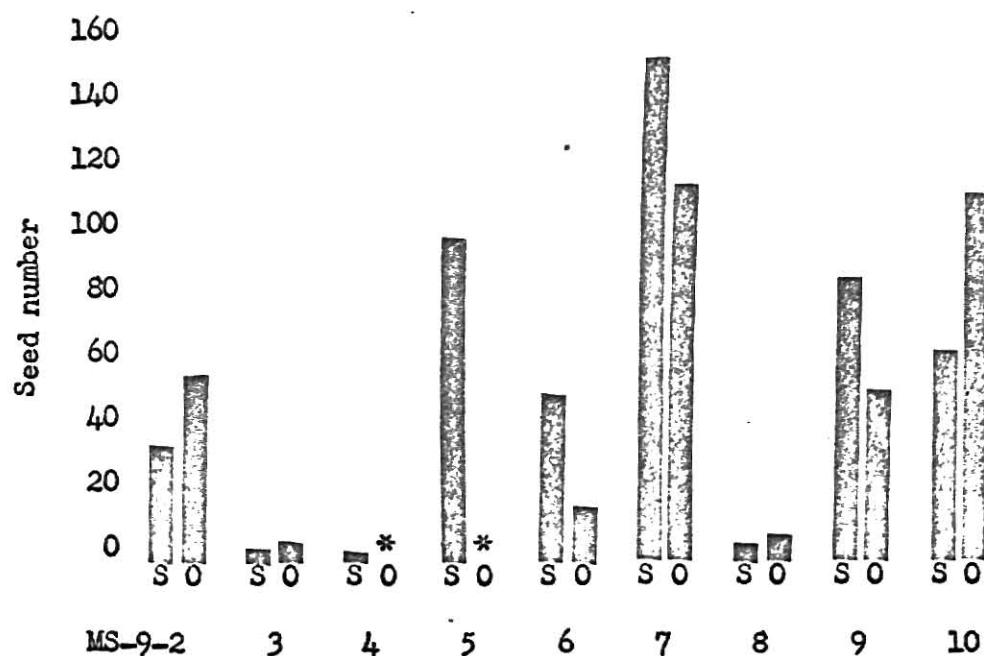


Fig. 6. Sibmating versus outcrossing effect on seed production.

* - No outcrossing attempted
 S - Sibmated
 O - Outcrossed

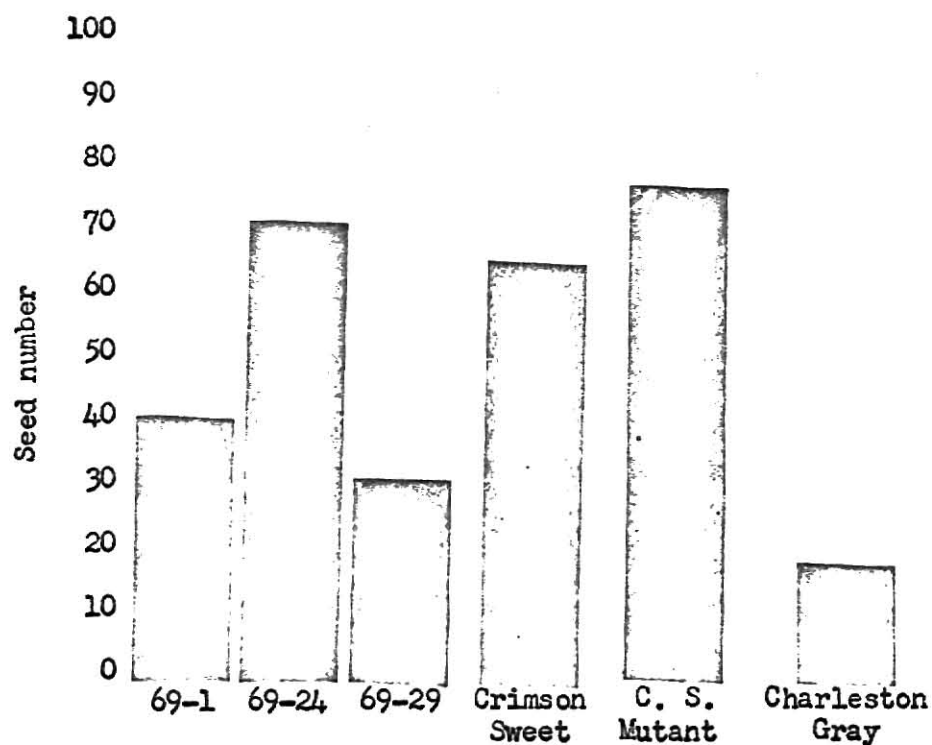


Fig. 7. Effect of pollen sources on seed production in male sterile fruits.

EXPLANATION OF PLATE IV

Watermelon apical meristematic tissue.

Top left. Glabrous meristem at the second true leaf stage. (35X)

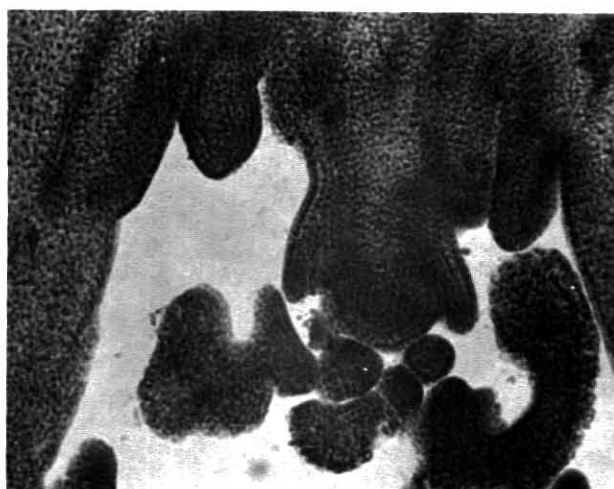
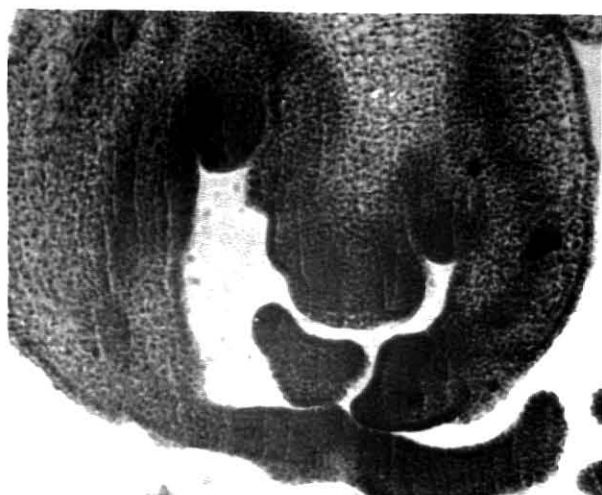
Top right. Glabrous meristem at the third true leaf stage. (35X)

Center left. Glabrous meristem showing bud development. (35X)

Bottom left. Normal meristem at the third true leaf stage. (35X)

Bottom right. Normal meristem showing development of staminate buds. (35X)

PLATE IV



EXPLANATION OF PLATE V

Watermelon staminate buds.

Top left. Glabrous staminate bud. (35X)

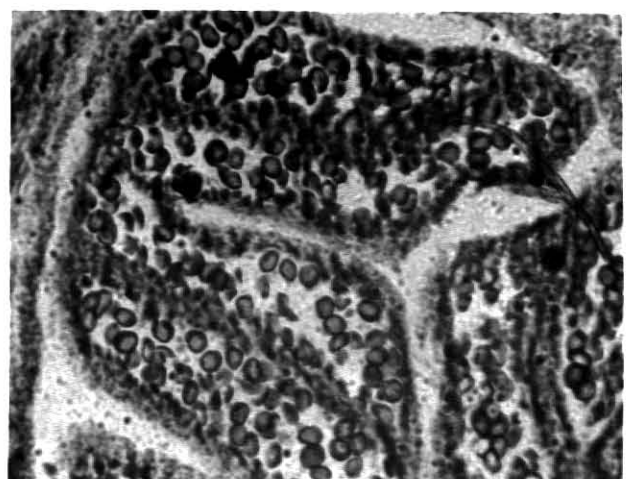
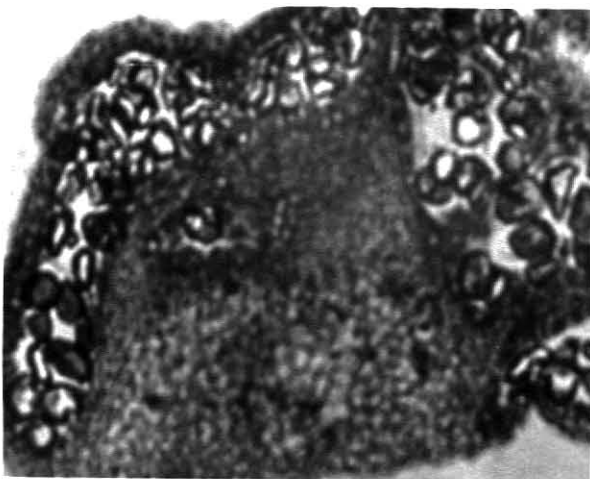
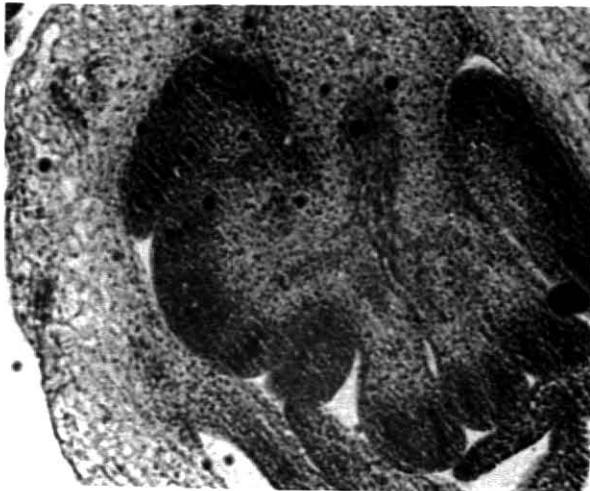
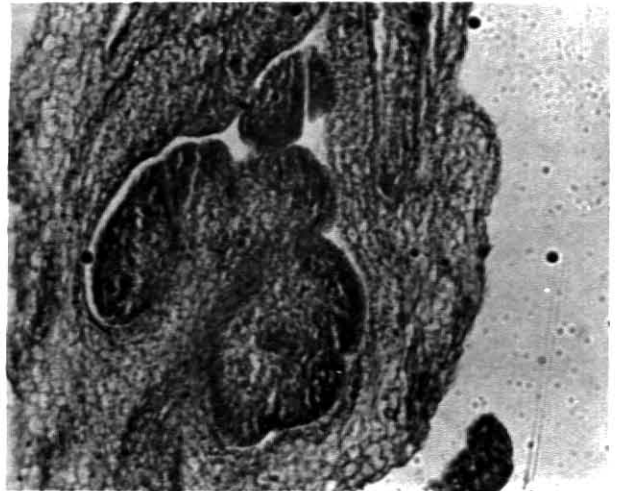
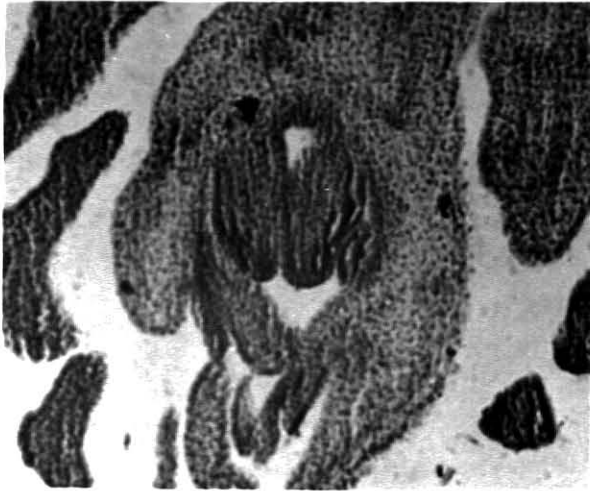
Top right. Glabrous staminate bud with
stamen lobes developing. (35X)

Center left. Glabrous staminate bud fully
developed. (35X)

Bottom left. Anthers with pollen of male
sterile plant. (35X)

Bottom right. Normal anther with pollen. (35X)

PLATE V



Stamen lobes become more evident as microsporogenesis approaches. The anthers become pollen sac-like structures when pollen matures. Stamens of the sterile buds cease to increase in size as the pollen matures so that at anthesis the stamens of the normal buds are much larger and dehiscent while sterile buds have small shrivelled anthers and are non-dehiscent.

Plate VI shows a cross-section of staminate flowers from male sterile and normal buds. The anthers and surrounding tissue are similar in both types of buds. Cross-sections of stem tissue show multicellular, hair-like projections on pubescent stems which are not present in glabrous tissue.

Cytological Investigations

Meiotic divisions were studied cytologically in sterile and normal pollen mother cells. Divisions were found between 4 and 5 A.M. Central Daylight Saving Time. Plate VII shows chromosomes in the first and second divisions, mostly in metaphase plates. Aberrant division figures found in the sterile sporocytes are shown in Plate VIII. Tetrads are irregular with many scattered bodies of chromatin material. Pollen from the sterile anthers is abnormal compared with functional pollen.

EXPLANATION OF PLATE VII

Watermelon cytological investigations.

Top left. Chromosomes of sterile pollen mother cell showing univalents. (450X with B. & L. 1.25 N.A. objective)

Top center. Sterile sporocytes with polar and side views of metaphase I. (450X)

Top right. Sterile sporocytes at metaphase II with plates at right angles. (450X)

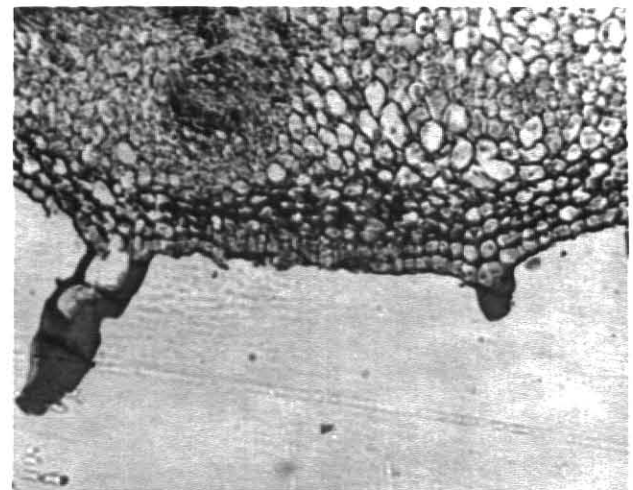
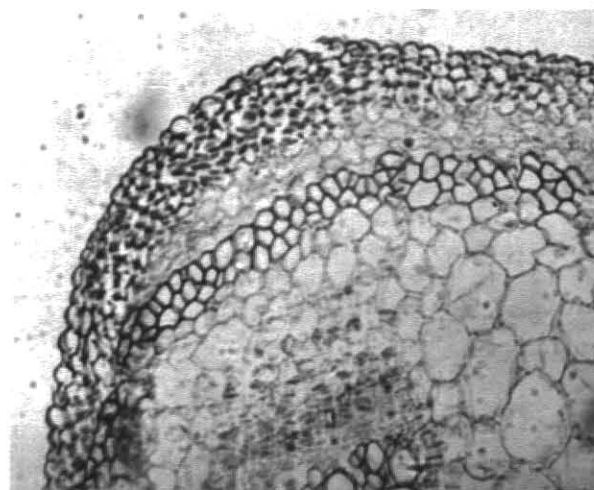
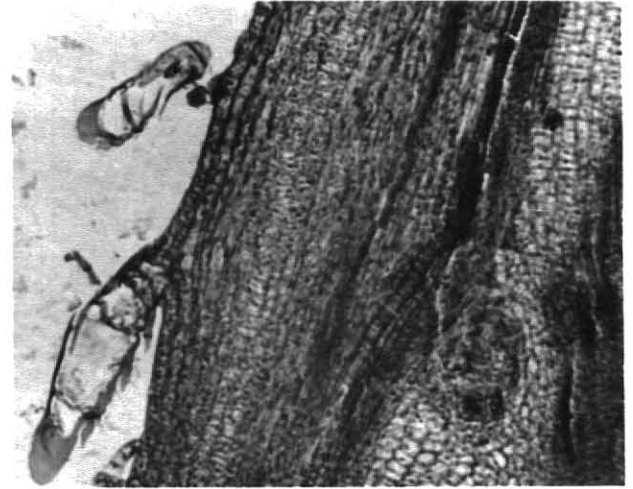
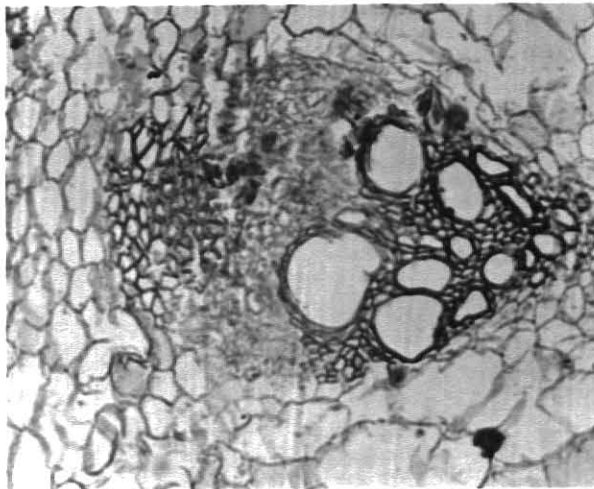
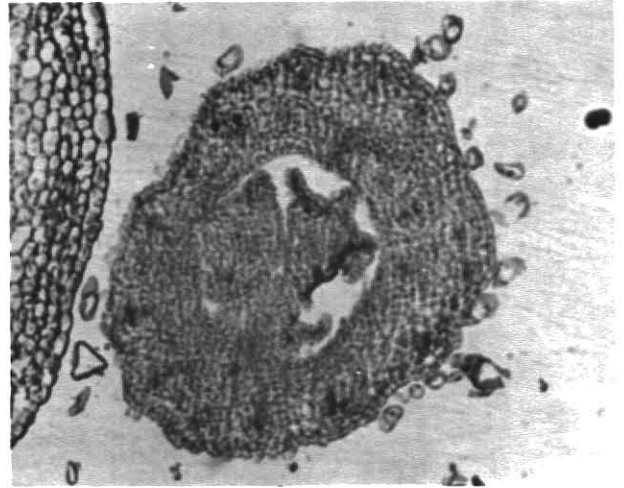
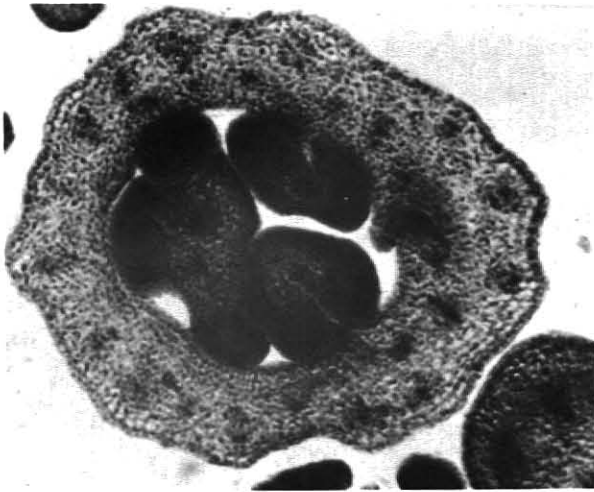
Center left. Chromosomes of normal sporocyte. (450X)

Center right. Normal sporocytes with 11 bivalents. (450X)

Bottom left. Bivalents in a normal sporocyte. (1000X)

Bottom right. Sporocytes showing normal orientation of chromosomes after first division. (1000X)

PLATE VI



EXPLANATION OF PLATE VI

Anatomy of watermelon bud and stem tissue.

Top left. Cross-section of glabrous staminate
bud. (35X)

Top right. Cross-section of normal staminate
bud. (35X)

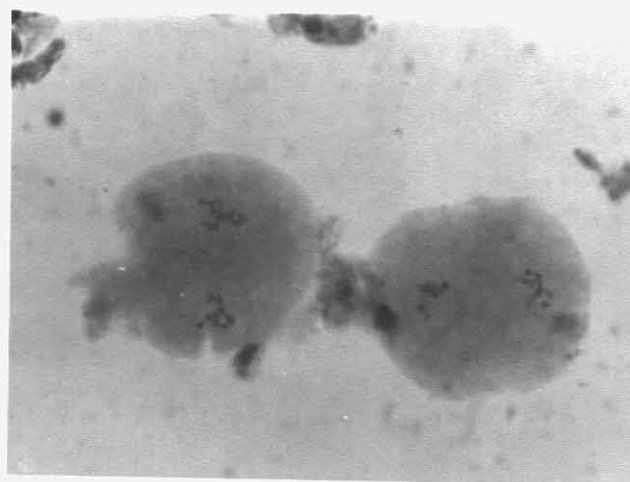
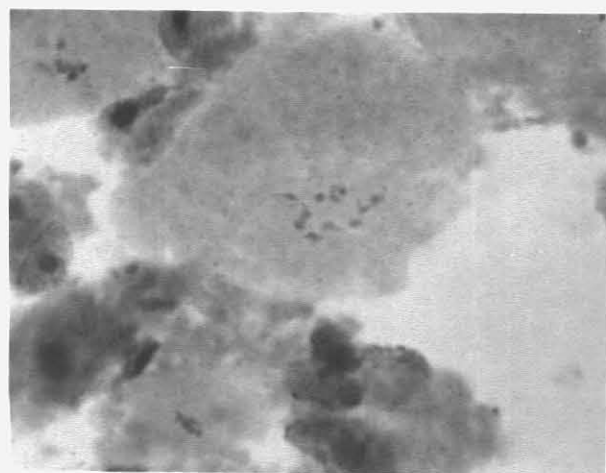
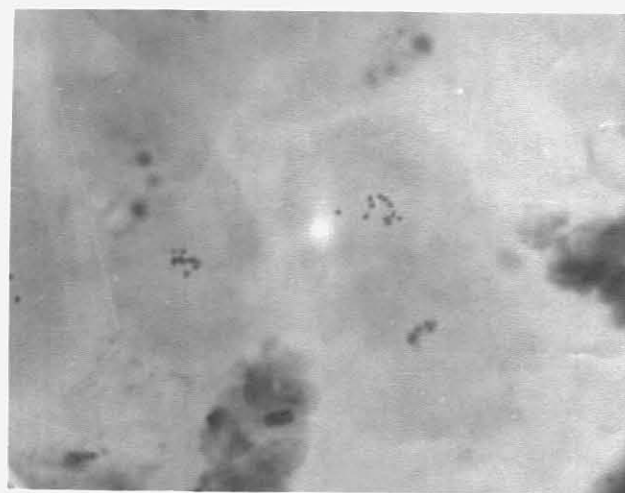
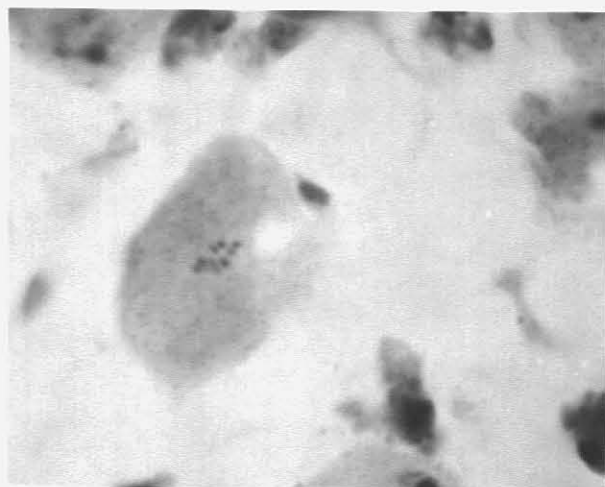
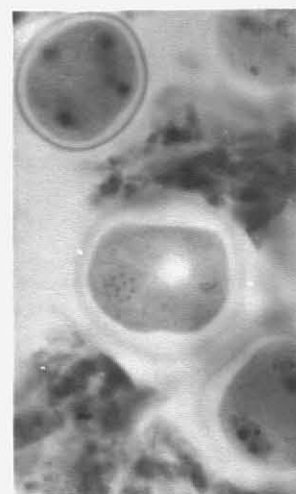
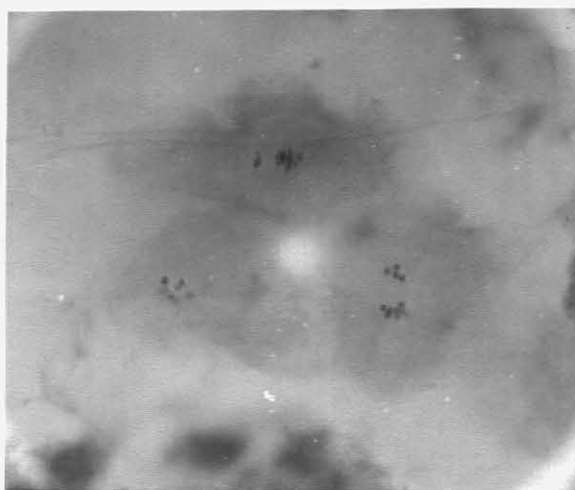
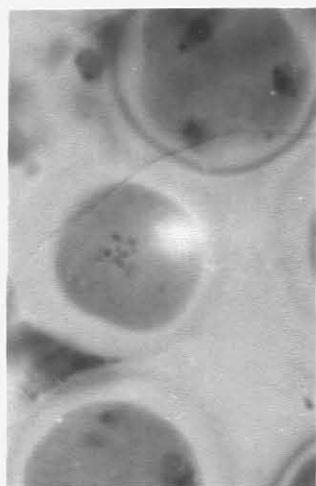
Center left. Cross-section of glabrous stem
showing vascular bundles. (35X)

Center right. Longitudinal section of normal
stem. (35X)

Bottom left. Cross-section of older glabrous
stem with secondary supporting fibers. (35X)

Bottom right. Cross-section of normal stem
showing epidermal layer with pubescence. (35X)

PLATE VII



EXPLANATION OF PLATE VIII

Watermelon cytological investigations.

Top left. Aberrant orientations of chromosomes of sterile sporocyte at second division. (1000X with B. & L. 1.25 N.A. oil objective)

Top center. Chromosomes in sterile sporocytes. (1000X)

Top right. Scattered chromosomes of sterile sporocyte at second division. (1000X)

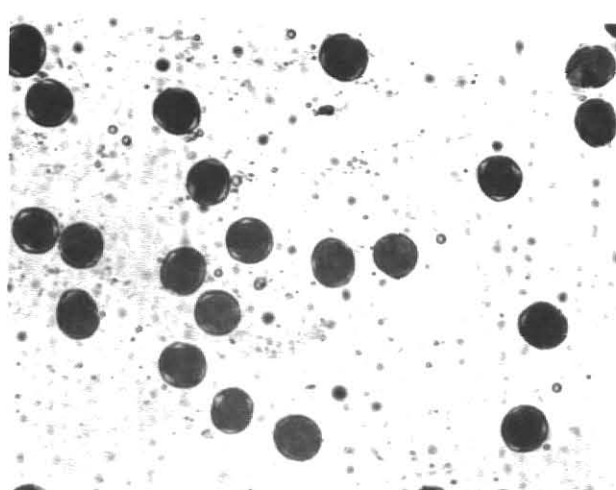
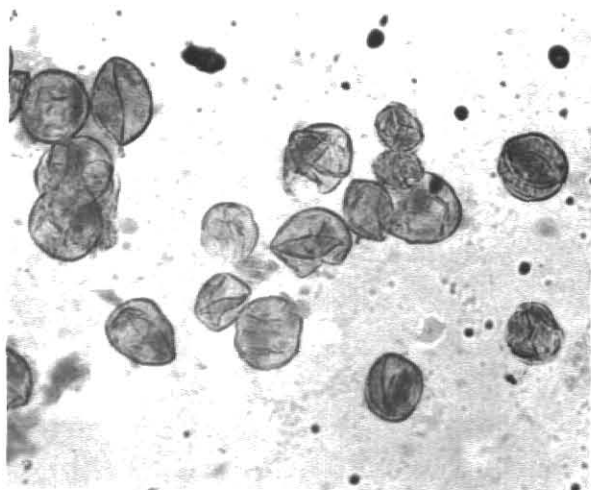
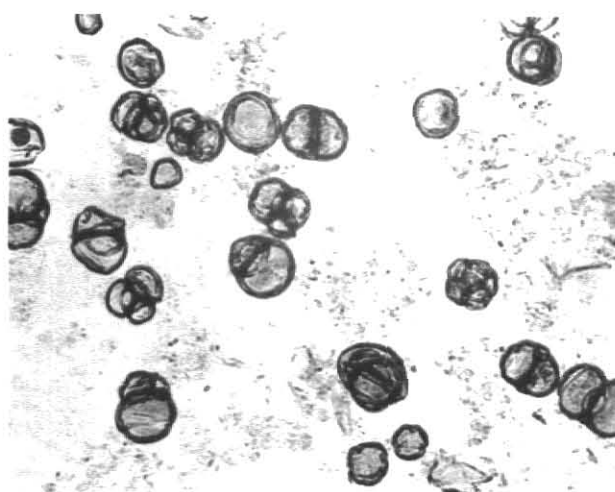
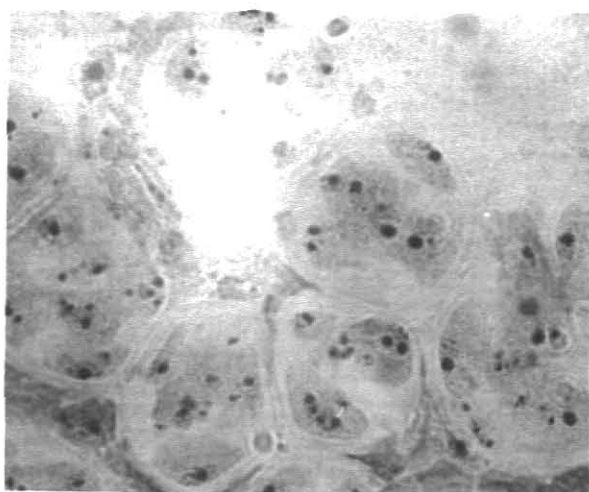
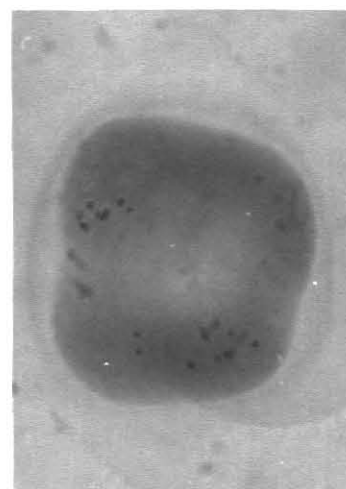
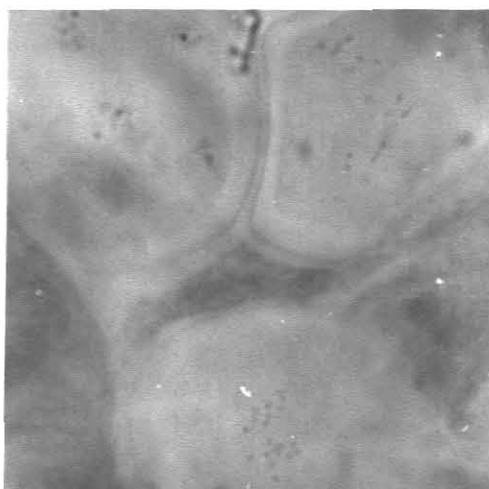
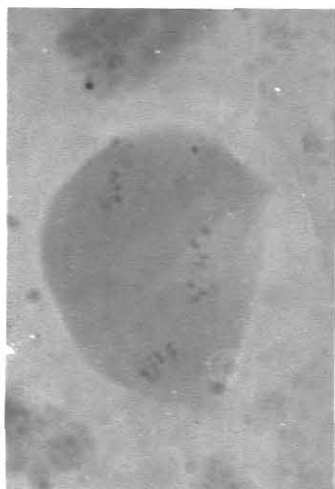
Center left. Tetrads with aberrant orientations and many micronuclei-like bodies. (140X)

Center right. Sterile pollen showing irregular quartets. (70X)

Bottom left. Sterile pollen showing abnormal shapes. (70X)

Bottom right. Normal pollen. (70X)

PLATE VIII



DISCUSSION

Quality Associated with Male Sterility

The male sterility mechanism controlled by the " ms_g " gene seems to be a stable, dependable method to prohibit pollen production. Its association with the glabrous character increases its usefulness by ease of identification of male sterile plants, even at the seedling stage. In all plants examined in this study, no separation in these two characters was found. The characters are expressed as if it were a pleiotropic effect but it would seem likely that the characters are closely linked, perhaps linked in a way preventing recombination.

The male sterile plants are slightly later in development and less vigorous but not to the extent that their usefulness would be limited. Disease resistance and productivity have been improved through breeding. The lines now carried are uniformly disease resistant and vigorous. In vine characteristics the plants could compete favorably with commercial varieties.

A linkage relationship is indicated between the glabrous character and a dark green rind color. The Sugar Baby cultivar (originally irradiated) has a dark green rind. All the homozygous recessive fruits had dark green rinds, some with a faint striping. Homozygous ($Ms_g Ms_g$) dominant fruits had light green backgrounds because genes for light green color were introduced into the breeding lines along with genes for splashed and striped rind patterns. Some fruits on MS-9-6 were dark green because the line still carries many genes from

the Sugar Baby cultivar. Heterozygous fruits had dark green background rind color with the exception of two plants which were carrying the "ms_g" gene but had light green backgrounds. These are the result of recombination, so breaks in the linkage appear possible. The normal plant rind colors segregated very nearly 2:1 which follows the expected ratios if linked to the "ms_g" gene.

The "ms_g" gene is hypothesized to give a simple recessive expression of the glabrous character and male sterility. Plants used in this study were tested for conformity to standard Mendelian ratios. Percentages of glabrous plants were only slightly less than expected in most parental lines. In progeny tests the heterozygotes produced seedlings close to the three dominant to one recessive expected. The chi-square values show very good fit on most lines and all within the 1% α level. In progeny of homozygous recessive crossed to heterozygous, the actual number of seedlings in each group was near the expected. The "ms_g" gene expressed itself in a Mendelian ratio as a recessive factor in this study. The slightly lower number than expected of glabrous plants could have been caused by reduced vigor, disease resistance, germination potential and other factors that resulted from the radiation treatment. It would seem logical to have fewer of the aberrant forms survive than the normal. The deviation from expected ratios still fall within the range of possible values of the hypothesized recessive expression for a single genetic factor.

Comparing quality factors of the fruits after identifying their genetic constitution for the "ms_g" gene gave interesting results. The glabrous, male sterile fruits were consistently lower than normal fruits

in fruit weight, total soluble solids and extremely reduced in seed production. These characteristics have carried through from the original mutant plant discovered by Dr. Watts. It lacked vigor and produced small fruits which were nearly seedless. The quality was severely reduced in flesh and rind characters. Through breeding work, quality of the glabrous plants has been greatly improved but they still fall short of the quality of normal plants not carrying the "ms_g" gene. Heterozygous plants seem to be intermediate in quality for each of the factors considered. The possibility of other linkage relationships is indicated. Some of the genes influencing quality factors such as fruit weight, total soluble solids and seed production may be linked to the "ms_g" gene. An intensive backcrossing program could be used to try to break these linkage groups and put the "ms_g" gene in a new genetic environment. The expression of male sterility could be affected but its stability through the course of this study indicates that such a program would be possible.

Variability among the lines reveals a wealth of genetic diversity for further breeding work. Combinations of desired traits could be selected from the breeding lines. Seed production was extremely low in male sterile fruits in MS-9-3, 4 and 8. These lines also had smaller seed than the other lines. However, MS-9-3 produced the largest number of seed for both homozygous dominant and heterozygous fruits. This indicates that some degree of female sterility may also be associated with the male sterility. Rind thickness and toughness were fairly uniform among the lines.

Comparisons of the effect of pollen sources were made because of the extreme reduction in seed production on the male sterile plants.

Difficulties had been encountered in earlier generations with outcrossing to unrelated materials resulting in completely seedless fruits. Breeding for improved seed production was attempted. Although much improvement has been made, reduction in seed numbers still exists. Male sterile plants were sibmated to normal plants of the same line and outcrossed to other breeding lines and varieties. The outcross pollen was nearly as effective as sibling pollen and, in MS-9-2 and 10, more effective in producing seed. The number of seed seemed to be more dependent on the ability of the line to produce seed than on the source of pollen. This is encouraging for the prospect of F_1 hybrid watermelons using the male sterile plants as seed parents. If a line such as MS-9-7 was used as the female parent, then an unrelated pollen parent could be used for the hybrid and sufficient seed production could be anticipated. Male sterile plants capable of producing fruits with 200 to 300 or more seeds (as were found in this study) could be useful as seed parents for hybrids.

There was a degree of variability in the effect of different pollen parents in the outcrosses. Fewer seeds were produced from crosses using Charleston Gray pollen than other sources. Some fruits had no mature seeds in the outcrosses but this also occurred in certain sib-pollinated fruits. The Crimson Sweet mutant had the highest average seed number but its slightly higher average could have been due to particular care in making the crosses because of the very limited pollen source. This mutant was of particular interest because of the expression of the glabrous characteristic without male sterility. The pollen used in crosses produced viable seeds. Self-pollinated fruits set on this

plant but no seed developed as the plant was apparently female sterile. The glabrous condition of the plant resembled the glabrous male sterile plants but the leaves were more distorted and curled under at the margins. The flower sepals and petals were also distorted by a crinkled, curled condition and green in color. A somatic mutation is indicated because the crosses to glabrous male sterile plants yielded all pubescent plants in the F_1 generation. These plants had reduced pollen fertility but selfed fruits were obtained. The reduced pollen viability could have been due to other detrimental mutations from the radiation treatment.

Morphological and Anatomical Relationships

The initiation of staminate flowers begins very early in plant development when only one to two true leaves have expanded. This is only two to three weeks after germination. Plate IV shows young meristematic regions undergoing initiation. Older meristems show buds developing first sepal, then petal and stamen lobes. Normal and male sterile plants seem to follow the same pattern of development, as can be compared in the photomicrographs.

Plate V follows later staminate bud development. The anthers increase in size and expand into a series of convoluted lobes. After microsporogenesis the lobes become large pollen chambers with functional pollen, but sterile anthers at anthesis are reduced, non-dehiscent structures.

Plate VI shows cross-sections of sterile and normal buds. The structure and organization of the buds seem to be similar. Cross-sections

of stem tissue show the structure of multicellular hairs forming the pubescence of the normal plants. This is completely absent in the glabrous tissue. The epidermal layer of cells of the glabrous plants may not be as highly organized and uniform in size and shape as the normal epidermis. The cells are more spherical than the rectangular normal cells and the cuticle layer seems to be thinner on the glabrous tissue. Older stems develop layers of fiber cells. The vascular systems appear to have the same general organization. The morphological development and anatomical structure of the glabrous plants do not seem to depart appreciably from normal except for the absence of epidermal pubescence.

Cytological Observations

Cytological investigations of meiotic divisions help to explain the resultant sterility of the glabrous plants. The sporocytes undergo divisions for only a brief period at pre-dawn. The sterile sporocytes seemed to take longer to pass through the second division than the normal sporocytes. The chromosomes were very small so oil immersion objectives were used and still little could be ascertained of the nature of the individual chromosomes. The normal plants go through meiotic divisions in a regular manner producing a high percentage of functional pollen. Failure of complete synapsis was observed with some chromosome arms not paired as shown in photomicrographs in Plate VII. However, in many counts of 11 bivalents at diakinesis and metaphase I, regular pairing was observed.

Some chromosome aberrations could be expected in irradiated material such as chromosome breaks for translocations, inversions or deficiencies. The chromosomes appear to have regular reduction divisions to produce functional pollen in heterozygous material. No condition of semi-sterility was found in the heterozygotes so a translocation or inversion must not be restricting recombinations unless disjunction is altered so that nearly all chromosomes undergo alternate instead of adjacent distribution.

The sterile sporocytes also contained 11 bivalents in several metaphase plates. However, in some cells not all the bivalents were oriented on the plate. Several figures were found with univalents having 2, 4 or 6 chromosomes failing to synapse or to remain paired. Most of the chromosomes went into second division still in a fairly normal condition. At second metaphase some of the plates were oriented very precisely with the full complement of chromosomes on each plate. The metaphase II figure in Plate VII has the plates oriented at right angles. Some figures were not normally oriented. Plate VIII shows aberrant configurations for second division. One figure seems to have three metaphase plates. Others have scattered chromosomes some of which will probably be lost from the nuclei of the microspores. These widely dispersed, incongruous orientations of the chromosomes were frequently found in second division figures of the sterile pollen mother cells.

Tetrad nuclei contained several bodies of darkly stained, chromatin-like material of various sizes resembling micronuclei. The tetrads were not oriented in a precise tetrahedral configuration as occurs normally. Some sporocytes did not have all four tetrads. Others had three clustered together on one side of the cell while the other

occupied a position toward the opposite side. This orientation resulted in an oblong, egg-shaped sporocyte. This aberrant orientation suggests possible interference with spindle activities. The polarity of cells could be disturbed, spindle fiber development or attachment could be altered or centromere activity could be affected. This would help to explain the abnormal movements and locations of chromosomes in the second division sporocytes.

Microspores remained in the sporocyte cell for one or 2 days before mature pollen grains developed. Pollen grains remain associated as quartets until dehiscence. Functional pollen grains were spherical, single and stained deeply with aniline blue. Sterile pollen grains were misshapen and often remained associated in quartets or in pairs of unequal sizes. The pollen did not stain with aniline blue but remained transparent.

SUMMARY AND CONCLUSIONS

This study was undertaken to investigate the practicality of the "ms_g" gene for genetic emasculation of seed producing lines for F₁ hybrid watermelons. The genetic mutation was induced by irradiation and discovered by Dr. V. M. Watts, former Head, Department of Horticulture, University of Arkansas. Breeding work has been done toward improving the quality of lines carrying the "ms_g" gene. With improvements that have been made it seems feasible to continue development of lines for hybrid seed production.

Lines carrying the "ms_g" gene are vigorous, productive and disease resistant. Fruit weight, rind thickness and toughness, and seed sizes

vary with the lines but are acceptable. Some improvement still needs to be made in total soluble solids content and in seed production of the male sterile plants, but fruits evaluated in this study are nearing that goal. The association between male sterility and the glabrous characteristics remained unchanged throughout the study.

Linkage is indicated between the " ms_g " gene and a dark green rind color but fruits were found with this linkage broken. The " ms_g " gene is expressed as a single recessive factor following Mendelian ratios. The homozygous dominant fruits determined by progeny tests were usually of higher quality for all factors than the heterozygotes which, in turn were superior to the homozygous recessive glabrous fruits. Some association of detrimental characteristics with the " ms_g " gene is indicated, particularly in seed production. Comparison of pollen source effect on seed production showed that outcrosses could be made without severe reduction in seed production below numbers produced from sibmatings.

Morphological study of staminate bud initiation and development showed similarity in normal and sterile buds. Cross-sections of stem tissue showed differences in the epidermal layer between glabrous and normal stems. The shape and size of the epidermal cells differed with a thinner cuticle layer on the glabrous stem. The most obvious difference was the absence in glabrous tissue of the multicellular hair-like projections forming the normal pubescence.

Cytological investigations of the pollen mother cell meiotic divisions revealed irregularities in the sterile cells. The chromosomes do not always align on the metaphase plate or move to their

tetrahedral configurations at anaphase. Some cells had chromosomes randomly scattered in the cell so that tetrads consisted of many small micronuclei-like bodies. Many tetrads had irregular shapes due to the orientation of the nuclei. These abnormalities resulted in pollen sterility. The normal sporocytes had nearly regular chromosome configurations which resulted in a high percentage of pollen fertility.

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SOME MORPHOLOGICAL, CYTOLOGICAL AND QUALITY FACTORS
ASSOCIATED WITH GENETIC MALE STERILITY
IN WATERMELON CITRULLUS LANATUS (THUNBERG) MATSUMURA

by

GALE L. FULLER

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Department of Horticulture

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Inbred varieties of Citrullus lanatus (Thunb.) Mats. have been available for many years but now there is increasing interest in F_1 hybrid watermelons. The difficulty in hand cross-pollination to produce hybrids has resulted in costly seed which has not been widely accepted. Use of genetic male sterility could eliminate hand emasculation procedures. A gene for male sterility induced by radiation was discovered by Dr. V. M. Watts, former Head, Department of Horticulture, University of Arkansas. The gene, notated " ms_g " is recessively expressed by a completely glabrous condition of stems and leaves combined with male sterility.

Breeding lines carrying the " ms_g " gene were developed at Kansas State University. This study was undertaken to determine the practicality of using this gene for developing lines for hybrid seed production. The " ms_g " gene expression was analyzed and found consistently following Mendelian ratios for a single recessive genetic factor. No separation of the glabrous phenotype and male sterility was found. Fruits carrying the " ms_g " gene were evaluated for weight, total soluble solids, seed number and weight, rind color, thickness and toughness. Quality of fruits from male sterile plants was substantially reduced from fruits in the same line not carrying the gene. Heterozygotes were intermediate in quality. Rind background colors of male sterile recessive and heterozygous fruits were dark green while fruits not carrying the gene had light backgrounds indicating a linkage between rind color and male sterility.

Seed production was severely reduced in male sterile fruits whether sibmated or outcrossed. However, some fruits produced over 300 seeds. Outcross pollen was nearly as effective as sibling pollen for seed production. A mutant plant with a glabrous expression but male fertile was evaluated but the mutation proved to be somatic with all pubescent progeny.

A morphological study was made of staminate bud development from initiation to anthesis. The sterile staminate flower development was similar to normal. Anatomical study of the epidermal layer of stem tissue from glabrous plants revealed slight variations in cell structure with no multicellular hair-like pubescence.

Cytological investigations of the meiotic divisions of pollen mother cells were made using an acetocarmine smear technique. Metaphase I plates appeared nearly normal in the sterile sporocytes except for occasional univalents or bivalents lying off the plate. Second division sporocytes had many aberrant configurations with chromosomes scattered sporadically losing orientation. The tetrads of the sterile sporocytes often were not oriented tetrahedrally and contained bodies of chromatin material resembling micronuclei. An aniline blue staining technique for pollen viability indicated about 98.4% or more sterile pollen in the male sterile flowers. The normal plants had 94.6% functional pollen.

The results of this study indicate that the "ms_g" gene will affect genetic emasculation with reliability. Further breeding work to improve seed production and quality is recommended. Results to date attest to the feasibility of using lines carrying the "ms_g" gene for hybrid seed production.