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The *ms1* mutation in soybean: involvement of gametes in crosses with tetraploid soybean *

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Summary. Previous studies indicated that *ms1ms1* male-sterile female-fertile soybean (*Glycine max* [L.] Merr.) plants can produce seeds with different ploidy levels. The codominant chlorophyll-deficient mutant *y11* was used in attempts to understand the embryo-endosperm relationship in seed production in *ms1ms1* plants and to determine the mechanism of gamete formation in the *ms1* mutation. Crosses were conducted between yellow-green male-sterile plants (*ms1ms1Y11y11*) and green fertile tetraploid cultivars (*Ms1Ms1Ms1Ms1Y11Y11Y11Y11*) in the greenhouse in the summers of 1987 and 1988. A total of 2,007 cross-pollinations were made. Thirty hybrid seeds were obtained, and plants were analyzed for chromosome number, fertility, and color. All the hybrid seedlings were tetraploid and fertile. No triploids were found. Among the 30 F_1 plants, 7 were green (*Y11Y11Y11Y11*), 17 were green-yellow (*Y11Y11Y11y11*), and 6 were yellow-green (*Y11Y11y11y11*). The segregation ratio was close to the expected 1 green:2 green-yellow:1 yellow-green ($X^2 = 0.38$; $0.90 > p > 0.75$). From the results of this experiment, we conclude that: (1) triploids were not produced by crossing diploid *ms1ms1* soybean plants with tetraploid plants; (2) tetraploid progeny can be produced from these crosses by the fusion of 2n *ms1* eggs, or fusion of other 2n gametophyte cells in the embryo sac with a 2x sperm from tetraploid plants; (3) the megaspore mother cell of male-sterile plants undergoes meiotic division without cytokinesis after telophase II and forms more than the normal number of gametes, which can fuse with each other to generate tetraploid gametophyte cells.

Key words: *Glycine max* – Male sterility – Embryo-endosperm relationship – Chlorophyll mutant

Introduction

Attempts to obtain triploid soybean plants (2n = 3x = 60 chromosomes) through natural cross-pollination and artificial cross-pollination between autotetraploids and diploids, and reciprocal crosses, were unsuccessful (Porter and Weiss 1948; Sadanaga and Grindeland 1981). In Porter and Weiss's report, 274 reciprocal crosses were made between diploid and tetraploid soybean plants. No hybrid seed were obtained. Natural cross-pollination was allowed between autotetraploid male-sterile and -fertile diploid soybean plants (Sadanaga and Grindeland 1981). Only 12 hybrid seeds were obtained. No triploid plants were found. Studies with other plant genera also indicated that producing triploids by reciprocal crossing between diploid and tetraploid plants is comparatively difficult. The success rate is far below expectation (Marks 1966a, b). This failure to produce triploids may be caused by unsuccessful endosperm development. Endosperm has played a significant role in the evolution of angiosperms because of its physiological and genetic relationship to the embryo. The success of the embryo depends on the normal development of the endosperm in almost all species (Brink and Copper 1947).

The embryo-endosperm relationship has been described by four major hypotheses developed by different investigators over the past 50 years. The first hypothesis is that endosperm development depends on a 3:2 ratio of

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endosperm to embryo genome (Watkin 1932). According to this hypothesis, triploids cannot be produced by reciprocal crosses between diploids and tetraploids because of a 4:3 or 5:3 ratio of endosperm to embryo genomes, respectively. The second hypothesis states that endosperm development depends on a 2:1 ratio of female to male genomes (Nishiyama and Inomata 1966; Lin 1975, 1984). Triploids also cannot be produced because of a 4:1 or 1:1 ratio of female to male genomes in reciprocal crosses between diploids and tetraploids, respectively. The third hypothesis indicates that each species has its own peculiar activating value. The ratio of the activating value of a male nucleus to the response value of two polar nuclei must be 1:2 to give rise to normal endosperm development (Nishiyama and Yabuno 1978). The last hypothesis concerns the Endosperm Balance Number (EBN). In any cross, the ratio of EBN of maternal to paternal parents must be 2:1 to give rise to viable progeny (Johnston et al. 1980; Johnston and Hanneman 1980; Arisumi 1982, Ehlenfeldt and Hanneman 1988). For instance, in the reciprocal interploidy crosses between diploid and tetraploid soybean, viable triploids are not expected to occur because the EBN ratio would be 4:3 or 1:1, respectively, assuming EBN equals ploidy.

The *ms1* soybean mutation can cause male sterility due to failure of cytokinesis after telophase II, which results in quadrinucleate structures termed coenocytic microspores (Albertsen and Palmer 1979) and the production of polyembryony, haploidy, and polyploidy (Beversdorf and Bingham 1977; Chen et al. 1985). Reduced female fertility is the result of the failure of cytokinesis after telophase II, which produces abnormal megagametophytes that contain supernumerary nuclei (Cutter 1975; Cutter and Bingham 1977; Kennell and Horner 1985). Kennell and Horner (1985) also reported that abnormal megagametophyte development may cause the nuclei in the megaspores to fuse with each other before fertilization and produce polyembryony and polyploidy in *ms1* progeny. According to their report, 2n gametes may be formed by the fusion of egg cells or other gametophyte cells in *ms1ms1* ovaries. When crossing an *ms1ms1* plant that produces a 2n gamete with a tetraploid plant, it might be possible to select for 2n egg cells. If successful, the ratio of the genome of the endosperm to that of embryo and the ratio of female to male genomes of endosperm or EBN will be 3:2 and 2:1, respectively, which fits the hypotheses of endosperm development. Therefore, tetraploid progeny would be expected to occur in these crosses.

The objectives of this experiment were: (1) to determine if tetraploid progeny can be produced by crossing diploid *ms1ms1* soybean planats with tetraploid cultivars; (2) to investigate the involvement of diploid female gametes in *ms1ms1* mutant; and (3) to study the endosperm-embryo relationship in these crosses.

Materials and methods

The diploid *ms1ms1* plants were from crosses between the Urbana male-sterile line (T266H) and a white-flowered chlorophyll-deficient line derived from a cross between T219H and trisomic C. The chlorophyll-deficient gene *y11* is inherited codominantly as 1 green (*Y11Y11*):2 yellow-green (*Y11y11*):1 yellow lethal (*y11y11*) plants. The flower color mutant gene *w1* is inherited recessively and segregates 3 purple (*W1-*):1 white (*w1w1*). The diploid genotype, therefore, was *ms1ms1Y11y11w1w1*.

The male plants used in this experiment were tetraploid cultivars 'Lincoln', 'Clark', 'Dunn', and 'Dunfield'. They were all fertile green plants (*Ms1Ms1Ms1Ms1Y11Y11Y11Y11*). Lincoln, Clark, and Dunn had purple flowers (*W1W1W1W1*). Dunfield had white flowers (*w1w1w1w1*).

Seeds of the female parent and tetraploid cultivars were germinated in pots and grown in isolation in the agronomy greenhouse at Iowa State University during the summers of 1987 and 1988. About 15 days after planting, all the green plants were discarded. Yellow plants died at the young seedling stage.

Flower buds (1–3 days before flowering) were collected in 70% ethanol every morning. Anthers were squashed under the dissecting microscope and stained with iodine-potassium iodide (I_2KI). Pollen from fertile plants (*Ms1-*) should be plump and round with a golden brown color. Coenocytic pollen from male-sterile plants (*ms1ms1*) stains dark brown or black and is large with an irregular shape. Male-fertile plants were discarded.

When plants started flowering (about 6 weeks after planting), all the purple-flowered plants were discarded. The remaining plants were the desired genotype (*ms1ms1Y11y11w1w1*).

Crosses were made between the male-sterile, yellow-green, white-flowered mutant (*ms1ms1Y11y11w1w1*) as the female parent and tetraploid cultivars as the male parent. Hybrid seeds were harvested at maturity. All the F_1 seeds were germinated in paper towels, and root tips were obtained to check the chromosome number (Palmer and Heer 1973). F_1 plants were transferred into pots and plant color, flower color, and plant fertility were checked. When tetraploid progeny was produced in these crosses, it was very difficult to distinguish phenotypically a green-yellow tetraploid plant (*Y11Y11Y11y11*) from a green plant (*Y11Y11Y11Y11*). Therefore, we collected F_2 seeds from these plants, germinated them in a sand bench, and observed whether or not plant color segregated in the F_2 generation. If the F_2 seeds were from green F_1 plants, no segregation would occur, and only green plants would be found. If the F_2 seeds were from green-yellow F_1 plants, segregation should occur and yellow-green plants would be found in the F_2 generation.

Results and discussion

A total of 2,007 crosses were made in the agronomy greenhouse during the summers of 1987 and 1988, and 30 F_1 seed were obtained (Table 1). All F_1 plants had 80 chromosomes ($2n=4x=80$). The hybrid plants from crosses between diploid *ms1ms1* plants and tetraploid 'Lincoln', 'Clark', or 'Dunn' plants had purple flowers, and hybrid plants from crosses between *ms1ms1* plants and tetraploid 'Dunfield' plants had white flowers. All the F_1 plants were fertile and were green or yellow-green (Table 2). Progeny of the green F_1 plants allowed us to separate the green phenotypic F_1 class into two genotypic classes (Table 3).

Table 1. Results of crosses between *ms1ms1Y11y11* plants and tetraploid soybean cultivars

Year	No. of sterile plants	No. of crossed flowers	No. of seeds
1987	67	336	5
1988	363	1,671	25
Total	430	2,007	30

Table 2. Plant color of F_1 soybean plants from crosses between *ms1ms1* diploid plants and tetraploid cultivars

Year	Crosses	F_1 plant color and no. of seeds		
		Green[a] *(Y11Y11Y11Y11 or Y11Y11Y11y11)*	Yellow-green *(Y11Y11y11y11)*	Total
1987	*ms1ms1* × 'Lincoln'	1	0	1
	ms1ms1 × 'Clark'	2	1	3
	ms1ms1 × 'Dunn'	1	0	1
	Subtotal	4	1	5
1988	*ms1ms1* × 'Lincoln'	0	1	1
	ms1ms1 × 'Clark'	12	4	16
	ms1ms1 × 'Dunn'	5	0	5
	ms1ms1 × 'Dunfield'	3	0	3
	Subtotal	20	5	25
	Total	24	6	30

[a] This group may contain both green (*Y11Y11Y11Y11*) and green-yellow (*Y11Y11Y11y11*) plants. Green-yellow plants could be differentiated from green plants in the F_2 population by segregation for plant color, The F_2 segregation data for differentiating green and green-yellow F_1 plants are shown in Table 3

No triploids were obtained from crosses between diploid and autotetraploid soybean plants. These results confirmed the previous reports that triploids could not be obtained through cross-pollination between diploid and autotetraploid soybean (Porter and Weiss 1948; Sadanaga and Grindeland 1981). Sadanaga and Grindeland (1981) noted that failure to obtain triploids from crosses between diploids and induced tetraploids could be explained either by the hypothesis that endosperm development depends on a 2:1 ratio of female to male

Table 3. Summary of F_2 plant color segregation of 30 tetraploid F_1 plants from crosses between *ms1ms1Y11y11* diploid plants and *Ms1Ms1Ms1Ms1Y11Y11Y11Y11* tetraploid soybean cultivars

Summary	F_1 plant color and genotype		
	Green *(Y11Y11Y11Y11)*	Green-yellow *(Y11Y11Y11y11)*	Yellow-green *(Y11Y11y11y11)*
No. of F_1 plants	7	17	6
χ^2 (1:2:1)	0.3	0.27	0.09
Pooled χ^2		0.38	
P (df=2)		0.90–0.75	

genomes (Nishiyama and Inomata 1966) or by the Endosperm Balance Number (EBN) hypothesis (Johnston et al. 1980). Under either hypothesis, crosses between diploid and autotetraploid soybean failed to produce triploids because the maternal:paternal genome or EBN ratio is 4:1 when the autotetraploid is the female, and the ratio is 1:1 when the diploid is the female parent.

According to those hypotheses, if 2n gametophytes could be produced by a female parent, tetraploid progeny can be produced by crossing this female parent with autotetraploid cultivars. In our experiment, tetraploids were produced from crosses between diploid *ms1ms1* plants and autotetraploid cultivars. The *ms1* gene causes male sterility due to failure of cytokinesis following telophase II (Albertsen and Palmer 1979) and results in polyembryony, haploidy, and polyploidy (Beversdorf and Bingham 1977; Chen et al. 1985). The genetic test for F_1 plant color segregation supports the hypothesis that the abnormal development of the megagametophyte resulted in gamete fusion and generated 2n gametophytes in *ms1* embryo sacs before fertilization. The fusion between the 2n gametes from *ms1* embryo sacs and 2n sperms from tetraploid cultivars gave rise to the viable tetraploid progeny. This experiment confirmed the conclusion of the previous studies that triploids cannot be produced by crossing diploid and autotetraploid soybean plants, and indirectly supported those hypotheses of endosperm development. Our data also suggested that polar nuclei underwent simultaneous fusion (duplication) with egg cells to give normal endosperm development (according to the hypotheses of endosperm-embryo relationship); tetraploid progeny could not be produced without successful endosperm development.

A total of 30 tetraploid seeds has been obtained by crossing diploid *ms1ms1* plants and tetraploid cultivars. Among the progeny, there were 7 green plants (*Y11Y11Y11Y11*), 17 green-yellow plants (*Y11Y11Y11y11*), and 6 yellow-green plants (*Y11Y11y11y11*) based upon F_2 segregation data

(Table 3). Sen and Vidyabhusan (1960) studied tetraploid soybeans and found that the frequency of abnormal progeny of tetraploid soybean was low, being about 1%–3% of the population. From their studies, it is clear that meiosis in tetraploid soybeans is fairly normal and that 2n gametes are formed that can participate in fertilization. In our experiment, all the tetraploid parents were green plants that produced *Y11Y11* 2n gametes. To generate tetraploid progeny, diploid eggs must be formed in the diploid *ms1ms1* parent to fuse with the 2x sperms produced by tetraploid parents through artificial crossing. The *ms1ms1* plants were yellow-green (*Y11y11*) in plant color.

If the diploid eggs came from megaspore mother cells (*Y11y11*) or some other diploid cells (*Y11y11*) directly, all the tetraploid progenies should be green-yellow (*Y11Y11Y11y11*) plants only. No green (*Y11Y11Y11Y11*) or yellow-green (*Y11Y11y11y11*) plants should be found. If the diploid eggs came from duplication of haploid eggs (*Y11* or *y11*) or some other gametophyte cells (*Y11* or *y11*) themselves, i.e., *Y11* became *Y11Y11* and *y11* became *y11y11*, the tetraploid progenies should be either green (*Y11Y11Y11Y11*) or yellow-green (*Y11Y11y11y11*) plants. No green-yellow (*Y11Y11Y11y11*) plants should be found. If the diploid eggs derived from random fusion between any two of the gametophyte cells (*Y11* or *y11*), all three phenotypes (genotypes) of plant color, green (*Y11Y11Y11Y11*), green-yellow (*Y11Y11Y11y11*), and yellow-green (*Y11Y11y11y11*), should be found in the tetraploid progenies in a 1 green:2 green-yellow:1 yellow-green ratio. Our experiment showed that 7 green, 17 green-yellow, and 6 yellow-green tetraploid plants have been obtained.

The results indicated that the duplicated egg cells in diploid *ms1ms1* plants were produced from fusion between haploid gametes that came from meiotic division of the megaspore mother cell (MMC). In the normal meiotic divisions, MMCs form four haploid cells, of which three degenerate and one undergoes three mitotic divisions to form an embryo sac with eight nuclei. In the *ms1ms1* mutation, MMCs may undergo meiotic division without cytokinesis after telophase II, and all four haploid nuclei may develop, forming more than the normal number of nuclei (8–32) in the embryo sac (Kennell and Horner 1985). Those nuclei may fuse with one another to form higher ploidy cells. In our experiment, MMCs could produce *Y11* or *y11* haploid cells in equal probability through meiosis and mitosis. Those haploid cells may fuse with one another to form diploid eggs in a 1 *Y11Y11*:2 *Y11y11*:1 *y11y11* ratio. When the diploid sperm (*Y11Y11*) from autotetraploid cultivars fertilized these eggs, tetraploid embryo was generated. Tetraploid progenies were produced in a ratio of 1 green (*Y11Y11Y11Y11*):2 green-yellow (*Y11Y11Y11y11*):1 yellow-green (*Y11Y11y11y11*) in plant color. Although the F_1 population was very small, it fits the expected 1:2:1 ratio ($X^2 = 0.38$; $0.90 > p > 0.75$, Table 3) very well.

Formation of 2n gametes may be associated with fused or parallel spindles (First Division Restitution, FDR) or with incomplete second meiotic division (Second Division Restitution, SDR) in potato (Hermsen 1984). In *ms1ms1* soybean plants, both first and second meiotic divisions are complete in both male and female sporogenesis. Failure of cytokinesis causes the male sterility and the reduction in female fertility (Albertsen and Palmer 1979; Kennell and Horner 1985).

This experiment also confirmed the study on *ms1ms1* plant megasporogenesis done by Kennell and Horner (1985). They reported that failure of cytokinesis after telophase II occurred within developing *ms1ms1* megaspores, and that development of all four nuclei may lead to four times the normal number of nuclei (8–32), resulting in up to four egg cells, as observed in some ovules.

Because *ms1* megagametophyte cells can fuse with one another, as suggested by our data, diploid, triploid, or tetraploid egg cells may occur in *ms1ms1* embryo sacs.

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