

Characterization of a New Male Sterile Mutant in Watermelon

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Watermelon, *Citrullus lanatus* (Thunb.) Matsum. and Nakai, is an important crop worldwide. Hybrid cultivars are replacing open pollinated cultivars because hybrids are thought to have higher yield and quality, and more uniform fruit size. However, the cost of hybrid seed is much higher than that of open-pollinated seed because of extra labor required for controlled pollination in producing hybrid seed. Male sterility has been found in many crop plants, and has been used to reduce the costs of hybrid seed production (Kaul, 1988).

The first male sterility in watermelon was reported by Watts (1962) who found a male sterile mutant in the X2 generation of 'Sugar Baby' irradiated with gamma rays. The mutant was described as a glabrous male sterile (*gms*) due to the associated lack of hairs on the plant foliage (Watts, 1962). Glabrousness and male sterility were inherited together as a single recessive nuclear gene, suggesting very close linkage or a pleiotropic effect of the locus involved (Watts, 1967). The *gms* gene not only disrupts the male reproductive function, but also reduces female reproduction (Watts, 1967). Therefore, there has been little commercial application of the *gms* gene (Zang et al., 1994).

A second male sterile mutant was reported by Zang et al. (1990). Two spontaneous male sterile mutants were found in a self-pollinated population of a commercial cultivar 'Nongmei 100' in China in 1983. These mutants were sib-mated and used to develop breeding line G17AB, which has desirable agronomic traits (Zang et al., 1990). In contrast to the *gms* trait, the Chinese male sterile line contains no gross morphological differences between sterile and fertile plants (Zang et al., 1990). Female fertility of the Chinese male sterile mutant is normal and there is stability for male-sterile and female-fertile characters despite varying environmental conditions (Zang et

al., 1994). The Chinese male sterile mutation was inherited as a single recessive nuclear gene (Zang et al., 1990), and assigned the gene symbol, *ms* (or *ms-1* if additional loci were identified). The cytological phenomenon associated with male sterility in the Chinese male sterile line is the lack of the tapetum, and tetrads that do not form (Zang et al., 1994).

In the study presented here, we investigate the characteristics of a new male sterile mutant found in Korea.

Methods. A third occurrence of male sterility was observed as a mutant in a breeding line, unrelated to the glabrous male sterility and Chinese male sterility in 1998 in Korea. This line originated from a cross between the commercial hybrid cultivar 'Fiesta' (Syngenta) and a breeding line with high quality, HL229 (Hungnong Seed Co.).

A male sterile mutant was found in the progeny during self-pollination to develop inbred lines. Five plants in a population of twenty failed to set fruit from self-pollinated flowers. The anthers of the five plants were smaller than those of their sibs, and they usually failed to dehisce. Microscopic examination disclosed that they contained no viable pollen.

This investigation was initiated to study the genetics of the the new male sterile mutant. Five male sterile plants set fruits readily when pollinated by fertile sibs. Five progenies were obtained from the five male sterile plants after cross-pollination with five of their fifteen fertile sibs. In addition, self-pollinated progenies were obtained from fifteen fertile sibs. A check watermelon line, heterozygous for the Chinese male sterile gene, *ms*, was kindly provided by Dr. G. E. Tolla, Seminis Vegetable Seeds / Asgrow Brand, USA.

Results. Progeny tests demonstrated that eight of the fifteen fertile sibs, which produced all-fertile progenies, were homozygous normal (Table 1). Seven, which produced progenies segregating approximately three male fertile to one male sterile, were heterozygous for a single, recessive factor for male sterility. The progenies from two male sterile plants pollinated by male fertile sibs segregated one male fertile to one male sterile, and the progenies from three other sterile plants produced all fertile progeny after sib mating. A total population of 210 plants from self-pollinated heterozygous parents, in seven generations, segregated 160 normal and 50 male sterile plants (chi-square 3:1, 0.16). Similarly, 80 plants in two progenies from male sterile plants pollinated by heterozygous sibs segregated 43 normal and 37 male sterile plants (chi-square 1:1, 0.45).

In order to determine the nature of the genetic relationship between the Chinese male sterile and the new male sterile, a plant homozygous for the new male sterile factor was crossed with a plant heterozygous for the Chinese male sterile gene. The reciprocal cross was made in the spring of 1999. All of the 367 F1 plants grown in the greenhouse in the autumn of 1999 revealed no segregation for sterility in the genotypes, indicating that there were two loci involved, with fertile x sterile sibs having genotypes $ms-1/ms-1 Ms-2/Ms-2$ x $Ms-1/Ms-1 Ms-2/ms-2$ or $Ms-1/Ms-1 ms-2/ms-2$ x $Ms-1/ms-1 Ms-2/Ms-2$ (Table 2). The data indicated that the Chinese male sterile gene and the new male sterile were non-allelic. Murdock et al. (1990) reported that the Chinese male sterile gene, *ms*, and the glabrous male sterile gene, *gms*, were non-allelic. We propose the symbol *ms-2* for the new Korean male sterile, making it the second in the series of male sterile genes, after the Chinese male sterile, *ms* (or *ms-1* for clarity).

The cross of genotypes $ms-1/ms-1 Ms-2/Ms-2$ x $Ms-1/Ms-1 Ms-2/ms-2$ produced all fertile F1 progeny. Thirteen of these F1 plants were self-pollinated in the autumn of 1999, and 50 F2 progeny from each were evaluated for the presence of *ms-1* and *ms-2* in spring of 2000. Half of the F2 progenies made by crossing with F1 hybrids heterozygous for only *ms-1* would be expected to segregate normal and male sterile plants at a 3:1 ratio. Seven of the F1 plants (termed P3, P4, P8, P9, P10, P11, and P13) segregated in this manner (Table 3). The 331 plants in the population included 251 normal and 80 male sterile plants. The observed numbers fit a 3:1 ratio (chi-square 0.12) and differed

almost significantly from a 9:7 ratio (chi-square 51.6). Therefore, the progenies were determined to have the $Ms-1/ms-1 Ms-2/Ms-2$ genotype. Half of the F2 progenies, from F1 hybrids heterozygous for both *ms-1* and *ms-2* would be expected to segregate normal and male sterile plants in a ratio differing from the 3:1 ratio. If *ms-1* and *ms-2* were independently inherited, the F1 progenies would be expected to segregate normal and male sterile plants in a 9:7 ratio. Six of the F1 plants (termed P1, P2, P5, P6, P7, and P12) segregated in this manner (Table 3). The 307 plants in the population included 179 normal and 128 male sterile plants. The observed numbers fit a 9:7 ratio (chi-square 0.53) and differed almost significantly from a 3:1 ratio (chi-square 45.6). Therefore, the progenies were determined to have the $Ms-1/ms-1 Ms-2/ms-2$ genotype.

The new male sterile mutant was first observed in 5 of 20 plants in watermelon progeny 1255. That F7 progeny was derived from the cross between cultivar 'Fiesta' and breeding line HL229. Progeny tests demonstrated that the gene, *ms-2*, occurred in the 330-10 parent of 1255 (Fig. 1). The mutation probably occurred in one of the two germ cells from plant 1121-4 that produced 330-10 or as a somatic mutation in 330-10 itself. The first alternative could result from unequal crossing-over or another type of minute chromatin loss, gain, or qualitative change at meiosis. The second alternative could result from failure of minute chromatin duplication, loss of a minute chromatin fragment, or a qualitative change during meiosis.

Development of staminate flowers was affected by the new male sterile mutant. The size of the sterile staminate flower bud was same as that of fertile staminate flower until 3 to 4 days before anthesis. Thereafter, the size of the fertile staminate flower increased greatly in one day before anthesis, whereas the size of sterile staminate flower remained relatively small during its development (Fig. 2 C, D). The same phenomenon was reported in the Chinese male sterile line (Zang et al., 1994). The size of sterile staminate flower in the newly found male sterile mutant is bigger than that of the sterile staminate flower in the Chinese male sterile line one day before anthesis (Fig. 2 B, D). The development of anthers showed great difference between the fertile and sterile flowers (Fig. 3). The size of the fertile anther increased greatly in the day before anthesis, whereas the size of the sterile male anther remained

Table 1. Progeny test of male sterile plants and their male fertile sibs.

Progeny	Total	Number of plants		Ratio Obs:Exp	Chi- square	Probability
		mf	ms			
ms(3) x mf(2)	40	22	18	1 : 1	0.4000	0.750>P>0.500
ms(10) x mf(12)	40	21	19	1 : 1	0.1000	P=0.750
ms(11) x mf(13)	40	40	0			
ms(15) x mf(16)	40	40	0			
ms(20) x mf(19)	40	40	0			
mf(2) selfed	30	23	7	3 : 1	0.0400	0.900>P>0.750
mf(5) selfed	30	24	6	3 : 1	0.4000	0.750>P>0.500
mf(8) selfed	30	23	7	3 : 1	0.0400	0.900>P>0.750
mf(9) selfed	30	22	8	3 : 1	0.0400	0.900>P>0.750
mf(12) selfed	30	21	9	3 : 1	0.4000	0.750>P>0.500
mf(17) selfed	30	24	6	3 : 1	0.4000	0.750>P>0.500
mf(18) selfed	30	23	7	3 : 1	0.0400	0.900>P>0.750
mf(1) selfed	30	30	0			
mf(4) selfed	30	30	0			
mf(6) selfed	30	30	0			
mf(7) selfed	30	30	0			
mf(13) selfed	30	30	0			
mf(14) selfed	30	30	0			
mf(16) selfed	30	30	0			
mf(19) selfed	30	30	0			

Table 2. Allelism test for male sterility genes between the Chinese male sterile line and the new male sterile line.^z

Female parent	Male parent	Total	Number of plants	
			mf	ms
Chinese ms plant (<i>ms-1/ms-1 Ms-2/Ms-2</i>)	New mf plant (<i>Ms-1/Ms-1 Ms-2/ms-2</i>)	178	178	0
New ms plant (<i>Ms-1/Ms-1 ms-2/ms-2</i>)	Chinese mf plant (<i>Ms-1/ms-1 Ms-2/Ms-2</i>)	189	189	0

^z Proposed gene symbols for watermelon male sterile, designating *ms-1* for the Chinese allele and *ms-2* for the new Korean male sterile.

Table 3. Segregation of F2 progeny from sib mated F1 plants (*ms-1/ms-1 Ms-2/Ms-2 x Ms-1/Ms-1 Ms-2/ms-2*).

F2 progeny from F1 plants	Total	Number of plants		Ratio Obs:Exp	Chi- square	Probability
		mf	ms			
P3	53	42	11	3:1	0.5094	0.500>P>0.250
P4	53	39	14	3:1	0.0566	0.900>P>0.750
P8	53	44	9	3:1	1.8176	0.250>P>0.100
P9	43	30	13	3:1	0.6279	0.500>P>0.250
P10	42	34	8	3:1	0.7937	0.500>P>0.250
P11	42	31	11	3:1	0.0317	0.900>P>0.750
P13	45	31	14	3:1	0.8963	0.500>P>0.250
Pooled	331	251	80	3:1	0.1219	0.750>P>0.500
P1	53	33	20	9:7	0.7790	0.500>P>0.250
P2	53	32	21	9:7	0.3669	0.750>P>0.500
P5	53	30	23	9:7	0.0027	P>0.950
P6	53	29	24	9:7	0.0506	0.900>P>0.750
P7	53	28	25	9:7	0.2519	0.750>P>0.500
P12	42	27	15	9:7	1.1020	0.500>P>0.250
Pooled	307	179	128	9:7	0.5274	0.500>P>0.250

Year	Season	Progeny generation	Diploid -plant	Genotype (ms)
1995	spring	F1	343-6	+/+
			sibs	+/+
1996	spring	F2	328-7	+/+
			sibs	+/+
1997	spring	F3	329-6	+/+
			sibs	+/+
1997	fall	F4	1121-4	+/+
			sibs	+/+
1998	spring	F5	330-10	+/ <i>ms-2</i>
			sibs	+/+
1998	fall	F6	1255-1	+/+
			-2	+/ <i>ms-2</i>
			-3	<i>ms-2/ms-2</i>
			-4	+/+
			-5	+/ <i>ms-2</i>
			-6	+/+
			-7	+/+
			-8	+/ <i>ms-2</i>
			-9	+/ <i>ms-2</i>
			-10	<i>ms-2/ms-2</i>
			-11	<i>ms-2/ms-2</i>
			-12	+/ <i>ms-2</i>
			-13	+/+
			-14	+/+
			-15	<i>ms-2/ms-2</i>
			-16	+/+
			-17	+/ <i>ms-2</i>
			-18	+/ <i>ms-2</i>
			-19	+/+
			-20	<i>ms-2/ms-2</i>

Figure 1. Probable origin of the recessive allele (*ms-2*) responsible for the new male sterility of watermelon found in 1998 in Korea.



Figure 2. Development of sterile and fertile staminate flower bud (from 1 to 7, staminate flower bud from the next node of bloomed staminate flower to the seventh node): A, Fertile of the Chinese male sterile line. B, Sterile of the Chinese male sterile line. C, Fertile of the new male sterile mutant. D, male sterile of the new male sterile mutant.



Figure 3. Development of male sterile and fertile anther (from 1 to 7, anther from the next node of bloomed staminate flower to the seventh node). A: male fertile of the Chinese male sterile line. B: male sterile of the Chinese male sterile line. C: male fertile of the new male sterile mutant. D: male sterile of the new male sterile mutant.



Figure 4. Comparative morphology of staminate flower between the newly found male sterile mutants and the Chinese male sterile line. Top left: sterile staminate flower of the Chinese male sterile line. Bottom left: fertile staminate flower of the Chinese male sterile line. Top right: sterile staminate flower of the newly found male sterile mutant. Bottom right: fertile staminate flower of the newly found male sterile mutant.

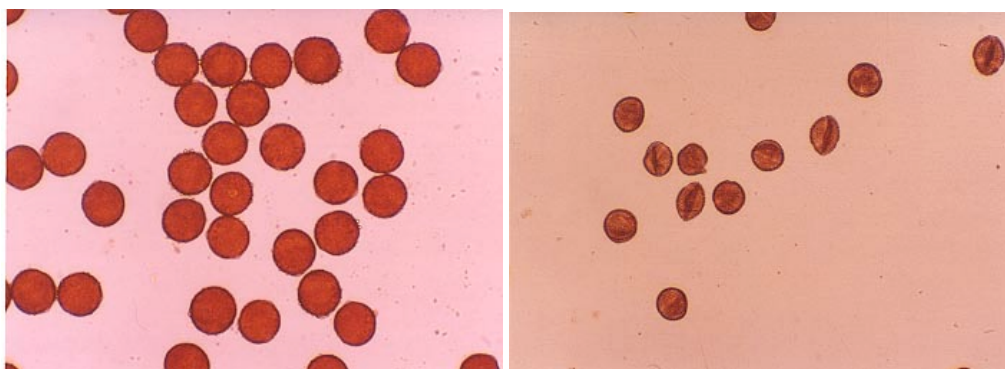


Figure 5. Specimens from the anthers of mature watermelon staminate flowers. Left: normal pollen from a male fertile sib (x100). Right: sterile pollen from male sterile plant of the new male sterile mutant (x100).

relatively small during its development (Fig. 3 C, D). The size of sterile anther in the new male sterile mutant is larger than that of sterile staminate flower in the Chinese male sterile line in the day before anthesis (Fig. 3 B, D). The buds from the male sterile plants became yellowish and almost fail to dehisce. The staminate flowers from the male sterile plants were open sometimes, but the size was smaller than that of the normal flower and no viable pollen was produced (Fig. 4 and 5).

Literature Cited

1. Bohn, G.W. and T.W. Whitaker. 1949. A gene for male sterility in muskmelon (*Cucumis melo* L.). Proc. Amer. Soc. Hort. Sci. 53: 309-314.
2. Cucurbit Genetics Cooperative, Cucurbit Gene List Committee. 1979. New genes for the Cucurbitaceae. Cucurbit Genetics Coop. Rpt. 2: 49-53.
3. Cucurbit Genetics Cooperative, Cucurbit Gene List Committee. 1982. Update of cucurbit gene list and nomenclature rules. Cucurbit Genetics Coop. Rpt. 5: 62-66.
4. Kaul, M.L.H. 1988. Male sterility in higher plants. Berlin/Heidelberg: Springer - Verlag.
5. Murdock, B.A., N.H. Ferguson, and B.B. Rhodes. 1990. Male-sterile (*ms*) from China is apparently non-allelic to glabrous-male-sterile (*gms*) watermelon. Cucurbit Genetics Coop. Rpt. 13: 46.
6. Rhodes, B.B., B.A. Murdock, and J.W. Adelberg. 1989. A second look at the glabrous male-sterile (*gms*) character in watermelon. Cucurbit Genetics Coop. Rpt. 12: 58.
7. Rhodes, B.B. and F. Dane. 1999. Gene list for watermelon. Cucurbit Genetics Coop. Rpt. 22: 61-74.
8. Watts, V.M. 1962. A marked male-sterile mutant in watermelon. Proc. Amer. Soc. Hort. Sci. 81: 498-505.
9. Watts, V.M. 1967. Development of disease resistance and seed production in watermelon stocks carrying *ms_g* gene. J. Amer. Soc. Hort. Sci. 91: 579-580.
10. Zhang, X.P., H.T. Skorupska, and B.B. Rhodes. 1994. Cytological expression in the male-sterile *ms* mutant in watermelon. J. Hered. 85: 279-285.
11. Zhang, X.P. and M. Wang. 1990. A genetic male-sterile (*ms*) watermelon from China. Cucurbit Genetics Coop. Rpt. 13: 45-46.