Development of Genic Male-sterile Watermelon Lines with Juvenile Albino Seedling Marker

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Additional index words. breeding, *Citrullus lanatus*, chlorophyll deficiency, hybrid, seed production, virescent mutant

Abstract. This research was conducted to develop genic male-sterile lines of watermelon (*Citrullus lanatus* Matsum & Nakai) homozygous for the juvenile albino (*ja*) seedling marker. Male-sterile plants (*msms*) of the genic male-sterile line G17AB were crossed with a Dixielee plant that was heterozygous for the *ja* locus. Male-fertile, juvenile albino recombinants of the F₂ progeny were self-pollinated, resulting in F₃ progeny. The male-sterile normal green recombinants of the F₂ progeny were crossed with an F₁ hybrid plant with genotype *MsmsJaja*, and three populations (93JMSB-1, -2, and -3) were obtained from these crosses. Juvenile albino recombinants were confined to 93JMSB-1. Of the juvenile albino plants of 93JMSB-1, male-sterile plants were sib-crossed with male-fertile plants, resulting in 93JMSB-1-1. Progeny of 93JMSB-1-1 was homozygous for *ja* and segregated for *ms* in a 127 male-sterile : 128 male-fertile ratio, fitting a 1:1 ratio. The male-sterile juvenile albino plants of F₃ were crossed with male-fertile juvenile albino plants of 93JMSB-1, resulting in 93JMSF3-1 and -2. Plants 93JMSF3-1 and -2 were homozygous for *ja* but segregated for *ms* at 10 male-sterile : 13 male-fertile and 15 male-sterile : 19 male-fertile for 93JMSF3-1 and 93JMSF3-2, respectively, fitting the 1:1 ratio. These three genic male-sterile lines with the *ja* seedling marker provide valuable germplasm for introducing *ms* and *ja* genes into diverse genetic backgrounds and for studying cross-pollination and gene flow in watermelon populations.

The first genic male-sterile mutation in watermelon was found in an X₂ population of 'Sugar Baby' irradiated with gamma rays and described as a glabrous male sterile (*ms_g*) (Watts, 1962). Glabrous leaves and male-sterility characters have never been separated and are inherited as a single recessive nuclear gene, suggesting close linkage or a pleiotropic effect of the locus involved. The possibility of rapid identification of male-sterile plants at the seedling stage via the glabrous phenotype generated considerable interest in introducing the *ms_g* gene into diploids and even tetraploids for hybrid seed production (Love et al., 1986). However, there has been little commercial application because the *ms_g* gene not only

disrupts the male reproductive function but also considerably reduces female reproduction.

A spontaneous, genic, male-sterile watermelon mutant (*ms*) was found in China (Xia et al., 1988; Zhang and Wang, 1990). Extensive evaluations of this mutant have shown that the *ms* mutant is stable for male sterility and female fertility in a wide range of genetic backgrounds and environmental conditions (Zhang et al., 1994). This mutant has great value in watermelon breeding and hybrid seed production.

The primary disadvantage to using a genic male-sterile mutant in hybrid seed production in any crop is that pure stands of male-sterile plants are difficult to obtain. Homozygous recessive (*msms*) male-sterile plants must be maintained by sib-crossing to heterozygous (*Msms*) male-fertile plants, yielding a 1 male-sterile : 1 male-fertile plant population. In hybrid seed production, the male-fertile segregants are removed as soon as they can be recognized. Efforts have been made to simplify this system by identifying seedling markers that are tightly linked to male-sterile genes in several crop species (Driscoll, 1986; Jorgensen, 1987; Rao et al., 1990).

Three chlorophyll-deficient seedling marker mutants have been described in watermelon (Rhodes, 1986; Zhang and Rhodes, 1994; Zhang et al., 1996a, 1996b). Spotted (*Sp*) is a dominant mutant that produces yellow spots on the cotyledon, leaf, and fruit. Delayed green (*dg*) is a recessive mutant that produces yellow cotyledons and pale-green, new, true leaves that turn green as development progresses. Juvenile albino (*ja*) is a recessive mutant in which chlorophyll deficiency is expressed under short-day conditions. No linkage relationships were found between *ms* and *Sp* (Murdock and Ferguson, 1992) or between *ms* and *dg* (Zhang et al., 1996a). Similarly, evidence of linkage between *ms* and *ja* was lacking in a breeding study population (unpublished).

Ideally, seedling markers that are useful for hybrid seed production should be controlled by a single recessive gene and incorporated into the seed parent because the abnormal phenotype(s) conferred by the recessive gene will not be expressed in the hybrid. Furthermore, off-types in the seed-parent, resulting from outcrosses during reproduction and mixes during seed handling, can be recognized before pollination.

Because a linkage between *ms* and seedling marker has not been identified, our approach to improving watermelon breeding and hybrid seed production has been to develop genic male-sterile lines with a recessive seedling marker. The seedling marker is not used for distinguishing male-sterile and male-fertile plants. The seedling marker is used to recognize inbreds in the hybrid population and to detect off-types in the parent line (Zhang et al., 1996a). Our report describes the introduction of the *ja* gene into genic male-sterile watermelon lines. Breeding line 93JMSB-1-1, a genic male-sterile line with the *ja* seedling marker developed during our study, has been used to test the new hybrid seed production system (unpublished).

Materials and Methods

Two genetic stocks, G17AB and Dixielee, were used in this study. G17AB is a genic male-sterile line (Xia et al., 1988; Zhang and Wang, 1990; Zhang et al., 1994) that contributes the *ms* gene to the crosses involved. Male-sterile (*msms*) G17AB plants were used. Dixielee is a high-quality, disease-resistant, open-pollinated cultivar developed by Crall and Elmstrom (1979) and inbred in our program for several generations. The mutation of *ja* occurred in a Dixielee plant grown in the greenhouse in Spring 1991 when the cross G17AB x Dixielee was initiated. This mutation was confirmed by the appearance of the *ja* phenotype in the F₂ progeny grown in the field in 1992 (Zhang and Rhodes, 1994). Therefore, Dixielee contributed the *ja* gene to the crosses involved. Dixielee initially was chosen as a parent to improve fruit quality [i.e., flesh firmness, soluble solids concentration (SSC), and rind toughness] and disease resistance [i.e., fusarium wilt [*Fusarium oxysporum* f. sp. *niveum* (E.F. Smith) Snyder and Hansen] and

Received for publication 27 Nov. 1995. Accepted for publication 7 Mar. 1996. This article is technical contribution no. 4136 of the South Carolina Agricultural Expt. Station, Clemson Univ., Clemson, S.C. This work was supported in part by the U.S. Dept. of Agriculture, Office of International Cooperation and Development. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

anthracnose [*Colletotrichum lagenarium* (Pass.) Ell. and Halst]} of the genic male-sterile line G17AB. However, efforts to develop genic male-sterile lines that include the *ja* seedling marker were initiated after observing *ja* recombinants in a breeding population that was involved in the male-sterility breeding study.

The cross G17AB (*msms*) x Dixielee (*Jaja*) was made in Spring 1991 (Fig. 1). Four F₁ plants were self-pollinated in the greenhouse on the Clemson Univ. campus in Fall 1991. Plants of an F₂ and the F₁ populations were grown in the field at Edisto Research and Education Center (EREC) of Clemson Univ., Blackville, S.C., from Spring to Summer 1992. For the F₂ family that was segregating for the *ja* trait, male-fertile plants with the *ja* phenotype were self-pollinated and resulted in progeny 93JMSF3. Normal, green, male-sterile, F₂ plants were crossed with F₁ hybrid plants and resulted in progenies 93JMSB-1, -2, and -3. Progenies 93JMSB-2 and -3 were discarded after learning that they did not carry the *ja* gene and are not included in Fig. 1. In 1993, these progenies were grown in the field at EREC. From the segregating progeny 93JMSB-1, juvenile albino male-sterile plants were sib-crossed with juvenile albino male-fertile plants, and the resulting progeny was designated 93JMSB-1-1. This line was tested for male fertility in the field at EREC in 1994. The juvenile albino male-sterile 93JMSF3 plants were crossed with the juvenile albino male-fertile 93JMSB-1 plants, and the resulting progenies were designated 93JMSF3-1 and -2. These two sister lines were tested for male fertility in 1995 in plots at Clemson Univ. Musser Fruit Research Farm (MFRF), Seneca, S.C. A seed increase and further selection of 93JMSB-1-1 was conducted at MFRF in 1995.

Comparing the observed segregation ratio for male sterility and juvenile albino and the expected ratio from progenies 93JMSB-1, 93JMSB-1-1, 93JMSF3-1, and 93JMSF3-2 was accomplished using a χ^2 analysis.

Results and Discussion

Our effort to combine *ms* and *ja* into the same line began in 1992, after observing *ja* plants in the F₂ progeny of G17AB x Dixielee. Eleven juvenile albino recombinants appeared among 45 F₂ individuals at the seedling stage. The occurrence of albino plants in the F₂ population suggested that the F₁ plant, from which the F₂ progeny was derived, was heterozygous at the *ja* and *ms* loci and had a genotype *MsmsJaJa*. Growth of the albino plants was severely retarded in April and May in the field. Because of the dry and then wet weather, many albino recombinants did not set fruit. Some albino plants were covered by adjacent normal plants and were too small to even determine male fertility before the breeding plot was terminated in late July. Only two self-pollinated fruit were recovered from two juvenile albino male-fertile plants.

Because juvenile albino is controlled by the single recessive gene *ja* (Zhang et al., 1996b), all the F₃ plants resulting from self-

pollinated juvenile albino F₂ plants should be albino. When 93JMSF3 was tested in 1993, all the plants had the typical phenotype of a *ja* mutant. Four male-sterile plants were obtained from 19 juvenile albino 93JMSF3 plants. These data suggested that the parental F₂ plant had a genotype *MsmsJaJa*.

Three normal, green, male-fertile, F₂ plants were crossed with normal, green, male-fertile, F₁ plants. When the three resulting families (93JMSB-1, -2, and -3) were tested, one of them (93JMSB-1) segregated for *ja* in a ratio of 128 normal green : 42 juvenile albino, fitting a 3:1 ratio ($P = 0.90$ to 0.95). This segregation of the *ja* trait suggested that 93JMSB-1 was from a cross between an F₂ plant with *msmsJaJa* genotype and an F₁ plant with *MsmsJaJa* genotype. Of 24 *ja* plants of 93JMSB-1 examined, nine plants were male-sterile. This ratio (9:15) fits the expected 1 male-sterile : 1 male-fertile ratio ($P = 0.10$ to 0.25) for independent inheritance of *ja* and *ms* loci. Determining the genetic relationship between *ms* and *ja* was not one of our objectives, and confirmation of these preliminary results requires a larger population.

Since 93JMSB-1 was from a cross between a male-sterile F₂ individual (*msmsJaJa*) and a male-fertile F₁ plant (*MsmsJaJa*), the juvenile, albino male-fertile 93JMSB-1 plants should have a genotype *MsmsJaJa*. Sib-crossing juvenile albino male-sterile with juvenile albino male-fertile plants within 93JMSB-1 family

should result in progenies that are homozygous for juvenile albino but are segregating for male fertility in a 1:1 ratio. When 93JMSB-1-1 (a line from sib-crossed male-sterile and male-fertile juvenile albino plants of 93JMSB-1) was tested in 1994 in the field at EREC, it was homozygous for *ja* and segregated for *ms* in a ratio of 127 male-sterile : 128 male-fertile. This ratio fits the expected 1:1 ratio ($P = 0.95$ to 0.98). Agronomic trait evaluation of 93JMSB-1-1 was not completed because of the extremely dry weather and animal damage of fruit. In 1995, 93JMSB-1-1 produced red-fleshed fruit weighing 3.1 ± 0.8 kg with an SSC of $9.4\% \pm 1.3\%$. In this line, variation in fruit size, SSC, and rind toughness still remains such that improvements can be made through selection.

Crossing the juvenile albino male-sterile plants (*msmsJaJa*) of the F₃ progeny with the juvenile albino male-fertile plants (*MsmsJaJa*) of the 93JMSB-1 progeny (Fig. 1) resulted in a 1 male-sterile : 1 male-fertile segregation ratio in the subsequent progenies. The same objective may be achieved by sib-crossing juvenile albino male-sterile plants (*msmsJaJa*) with juvenile albino male-fertile plants (*MsJaJa*) within the F₃ progeny. However, one-third of the time, crosses between *msmsJaJa* and *MsmsJaJa* plants may occur, resulting in 100% male-fertile progeny. In the 1995 test, 93JMSF3-1 was homozygous for *ja* (Fig. 2) but segregated for male fertility in a 10 male-

Fig. 1. Pedigree of 93JMSF3-1, 93JMSF3-2, and 93JMSB-1-1 genic male-sterile lines with juvenile albino seedling marker. The desirable genotypes used for making crosses during breeding are underlined.

sterile : 13 male-fertile ratio, fitting the expected 1:1 ratio ($P = 0.50$ to 0.75). Similarly, 93JMSF3-2 was homozygous for *ja*, but segregated for male-fertility in a 15 male : 19 male-fertile ratio, fitting the expected 1:1 ratio ($P = 0.25$ to 0.50). Under the same conditions, 93JMSF3-1 and 93JMSF3-2 produced round fruit that were larger and higher in SSC than 93JMSB-1-1. Fruit weight and SSC were 6.1 ± 1.1 kg and $11.1\% \pm 0.6\%$ for 93JMSF3-1, and 3.8 ± 0.7 kg and $11.2\% \pm 0.7\%$ for 93JMSF3-2, respectively. Flesh color of both lines was bright red. A portion of the striped rind (Fig. 3) was an uneven yellow. Rind toughness, as determined by a penetrometer, still varies in both lines. Improvement of the existing lines for the agronomic traits noted can be achieved by selection.

Blossoming of the sterile male flowers of the three juvenile albino genic male-sterile lines (93JMSB-1-1 and 93JMSF3-1 and -2)

was usually delayed until growth of the plants improved in June in South Carolina with >14-h daylength. Fully expanded petals of the sterile male flowers were about half the size of the fertile male flowers. Anthers of the sterile male flowers always were much smaller than those of fertile male flowers and lacked pollen grains. The female flowers from male-sterile and male-fertile plants were indistinguishable. The fertility of female flowers on the male-sterile plants was normal, as we have observed in other genetic stocks (Zhang et al., 1994).

Conclusion

We have successfully developed three genic male-sterile lines with a *ja* seedling marker. These three lines are useful to watermelon breeders and geneticists in several ways. First, these lines provide valuable genetic stocks for

introducing *ms* and *ja* genes into various genetic backgrounds using backcrossing without labor-intensive, manual, cross- and self-pollination (Carter et al., 1983; Zhang et al., 1996a). Second, desirable, inbred, genic male-sterile lines with the *ja* seedling marker can be expected from these lines, especially 93JMSF3-1 and 93JMSF3-2, by sib-crossing and selecting for several more generations. This expectation is based on the fact that the parental line G17AB has good fruit set under a wide range of environmental conditions (Xia et al., 1988; Zhang et al., 1994). The wild-type parental line Dixielee has outstanding fruit quality and good resistance to diseases (Crall and Elmstrom, 1979). However, more efficient selection may be achieved under the growing conditions with long days, under which the *ja* plants grow almost normally (Zhang et al., 1996b). Third, these lines also will provide unique genetic stocks for geneticists studying cross-pollination events and gene flow in watermelon populations. One of the lines, 93JMSB-1-1, has been used to test a new hybrid seed production system that uses male sterility and the *ja* seedling marker (unpublished). Preliminary results showed that the male sterility effectively increased the cross-pollination between seed parent and pollen parent, and the *ja* seedling marker was useful for recognizing the hybrids and inbreds. The new system can greatly reduce the costs of hybrid watermelon seed production because roguing male-fertile plants is much more efficient than hand-pollinating individual flowers. Genetic purity test of the hybrid seeds can be accomplished in 7 to 10 days in the greenhouse or laboratory with the assistance of seedling marker. This system also allows commercial watermelon production to use pure hybrids because any inbred can be removed at seedling stage based on seedling marker.

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Fig. 2. Plant of the juvenile albino genic male-sterile line 93JMSF3-1 grown in the field in early June.

Fig. 3. Fruit of the juvenile albino genic male-sterile line 93JMSF3-1.

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