Utilizing Male Sterility from *Beta maritima* in Sugarbeet Breeding

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ABSTRACT

CMS plants originating from natural populations on stony beaches of the Brittany Peninsula in France were crossed with sugar beet O-type plants in order to incorporate the sterile cytoplasm from wild subspecies to sugar beet. F_1, B_1, B_2, and B_3 generations have been obtained. Pollen sterility from these hybrids has been found to be induced by both cytoplasmic and genetic factors. Studies of meiosis have shown that meiotic divisions proceeded normally with only small deviations until the tetrad stage, followed by the degeneration of the pollen grain. In the F_1 and B_1 generations, all plants were self-sterile with white stamens lacking viable pollen, suggesting dominance of the wild morphological features. A great variation in degree of pollen sterility and morphological features were found in the B_2 generations. Full sterility was found in some of the analyzed progenies. It can be assumed that the variation is due to the degree of recombination with nuclear genes from wild subspecies, and that the maintainer nuclear genes from sugar beet O-type are similar but not the same as in *Beta maritima*.

Additional Key Words: *Beta vulgaris,* pollen, CMS

Cytoplasmic male sterility (CMS) is used in the production of commercial hybrid seed of maize, sugarbeet, rice, sunflower, *Brassica,* and sorghum. In sugarbeet *(Beta vulgaris* L.), the CMS source identified by Owen (1945), called S type, is still the only cytoplasm used worldwide for the production of hybrid seed. This single source has led to a very narrow and vulnerable cytoplasmic base in the sugarbeet crop. Therefore, it is important to broaden the cytoplasmic variation by identifying or creating additional sources of male sterility. There are reports of CMS types different from the Owen type (Coe and Stewart, 1977; Mikami, et al., 1985). Most of these cytotypes originated from *B. maritima.* Seeds from a *B. maritima* CMS plant found on the Brittany peninsula of France have been added to our beet collection. Research to determine the potential of this CMS cytoplasm was initiated in 1988. The male sterile cytoplasm was transferred to sugarbeet nuclear backgrounds by backcrossing.

MATERIAL AND METHODS

The CMS plants originated from seeds collected from the stony shores of the Brittany peninsula (Port Blanc - Port de Golf). This material was multiplied at the Institute in Bydgoszcz. The offspring of this population (about 50 plants) were biennial, male sterile with white anthers, and uniform in morphological characters. Only one pollen fertile plant was found.

A few male sterile plants were crossed with O-type plants from our breeding program. In this way, F_1 and backcross generations B_1, B_2, and B_3 have been obtained. Seeds were harvested from individual plants and sown for vernalization. Beginning with the B_2 generation, part of the seeds has been sown in the field together with O-type plants for further backcrossing.

A phenotypical and cytological evaluation of pollen was made. Pollen sterility was determined in Belling's solution. Anthers from flowers on the main stalk of wild CMS plants and hybrids have been analyzed for meiosis. The material was fixed in Carnoy's fixative (3:1) and stained with 2% orcein. Meiosis was observed on squashed preparations. In all hybrid generations, variation in morphological features was observed. Roots from the B_2 generation were evaluated for sugar content.

RESULTS

All F_1 plants (46) were male sterile with white anthers and resembled the wild parent morphologically. The B_1 generation was obtained by backcrossing with sugarbeet O-type plants. All of the 158 plants in this generation were male sterile with white anthers.

Backcrossings were continued to obtain the B_2 generation. 595 plants from 24 progenies of the B_2 generation have been studied. Contrary to the previous observations, variation in pollen sterility was observed.

Phenotypical and microscope pollen evaluation revealed three groups of plants: (1) white anthers with sterile pollen, (2) yellow anthers with sterile pollen, and (3) yellow anthers with partially sterile pollen. The differentiated pollen sterility is demonstrated in Figure 1 (photos 8-12).

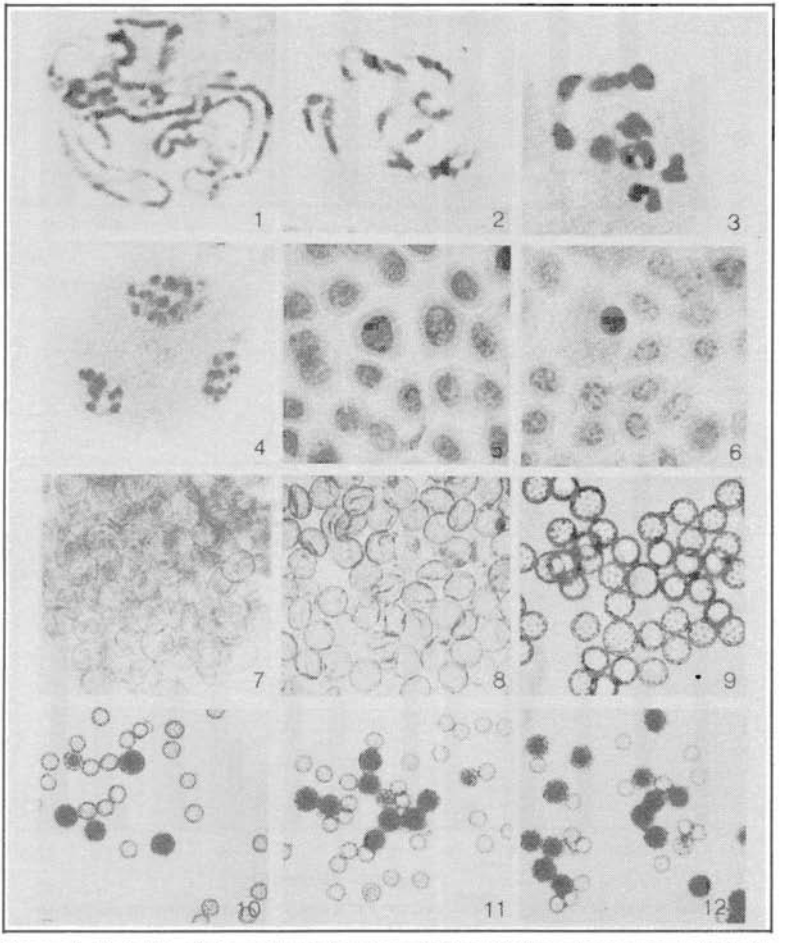

Figure 1. Hybrids of the male sterile *B. maritima* x O-line of sugar beet. Meiosis and pollen sterility. 1-3 = chromosomes conjugation; 4 = A II chromosomes fusion at the neighboring poles; 5 = dyad; 6 = monad; 7 = sterile pollen of *Beta maritima;* 8 = sterile pollen of hybrid plants from white anthers; 9 = sterile pollen of hybrid plants from yellow anthers; 10-12 = partially sterile pollen of hybrid plants.

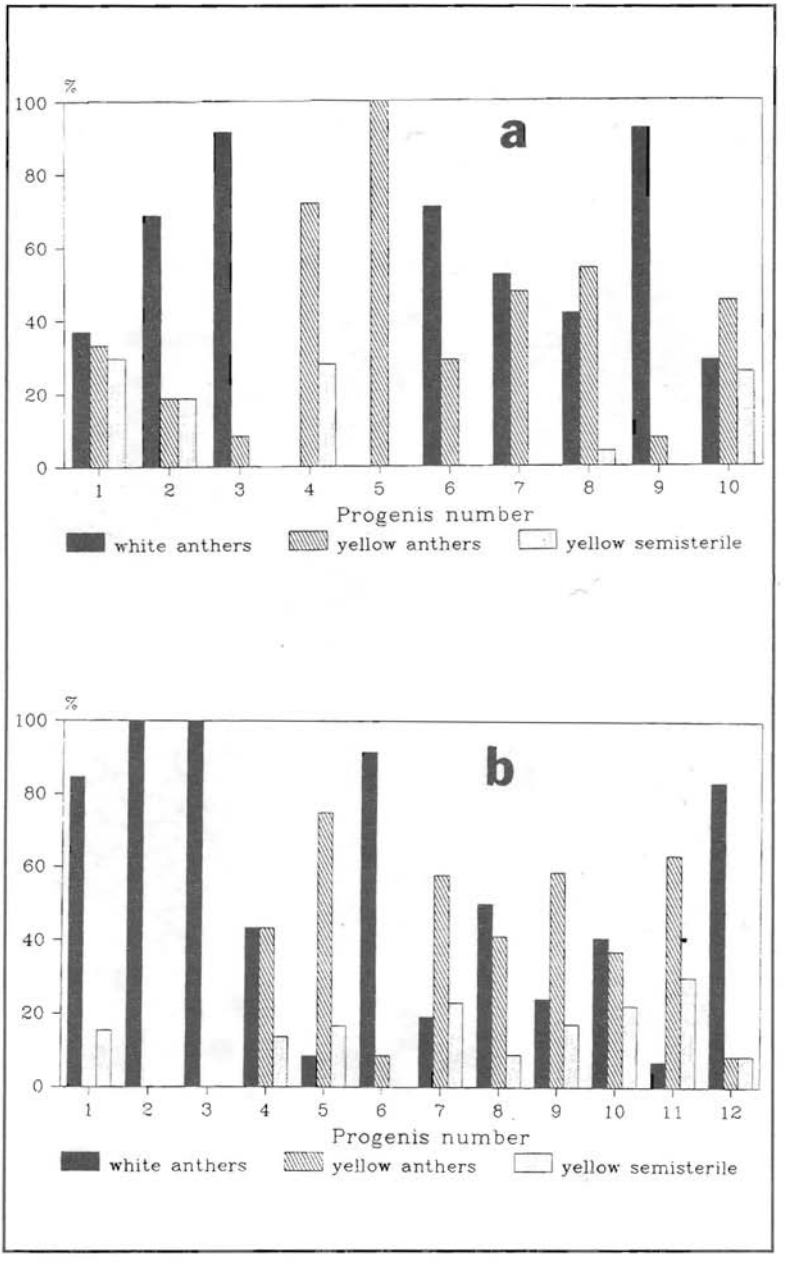

Figure 2. Pollen sterility of hybrids: (a) B_2 generation; (b) B_3 generation.

The variation in pollen sterility for 10 representative progenies of the B_2 generation are presented in Figure 2a. Progenies Number 3 and 9 were completely male sterile. This type of male sterility has been found in five progenies of the 24 tested.

The B_3 backcross was made in all progenies except those with only yellow anthers. Seed were harvested from 66 backcrosses. Twelve backcrosses with different ratios of male sterile types were vernalized. The remaining were multiplied in a two year regeneration cycle. 348 plants from the B_3 generation were evaluated for pollen sterility (Figure 2b).

A great amount of variation in pollen sterility also occurred in the B_3 generation. Segregation for the three groups of sterile pollen was found in all but two backcrosses. In comparison with the B_2 generation, changes have been observed in the group of plants with partially sterile pollen. The pollen fertility decreased and ranged from 1.5 - 98%. Plants which had above 30% fertile pollen shed pollen even though the anthers were small and shriveled. Two progenies (numbers 3 and 9) from the B_2 generation were found with complete male sterility. However, they produced segregating offspring.

Meiosis in the male sterile plants from *B. maritima* was regular. In the F_1, B_1, B_2, and B_3 generations, diakinesis and MI chromosomes usually formed 9 bivalents. Sometimes 8 bivalents were found and in two progenies, univalents were observed. At AI and AII single bridges and lagging chromosomes occurred infrequently. Tetrads were regular with four nuclei of the same size. Some plants of all generations sporadically showed dyads and monades (2% of pollen mother cells). They were formed as a result of the chromosomes grouped at the neighboring poles. Degeneration of monospores occurred after release from the tetrads (Figure 1, photos 1-6).

Male sterile plants of non-segregating offspring are being micropropagated in in vitro culture to obtain genetically identical plants for further studies.

Variation has been observed also in morphological traits. The B_1 generation plants produced large rosettes before shooting. The inflorescence were very leaved. In some plants the shoots had red vertical stripes. Considerable variation has been found in root shape. The most frequent root shape types in the B_2 generation are pictured in Figure 3.

Sugar content was determined in roots of the B_2 generation. The results (mean of 10 roots) are as follows: male sterile *B. maritima* = 4.6%; sugarbeet-like roots = 15.3%; B_2 *"maritima"*-like roots = 12.6%; sugarbeet CMS line number 9 = 16.8%.

In the first generation, plants were mainly bigerm, similar to the

maternal parent *(B. maritima).* In the B_2 generation, 16% of the plants were monogerm and the remaining monogerm-bigerm type. In the B_3 generation, the number of monogerm plants increased and varied among progenies from 8.3 to 23%. The most common type in the B_3 generation was mono-bigerm. Most of the bigerm seeds were located on the upper part of the main stem.

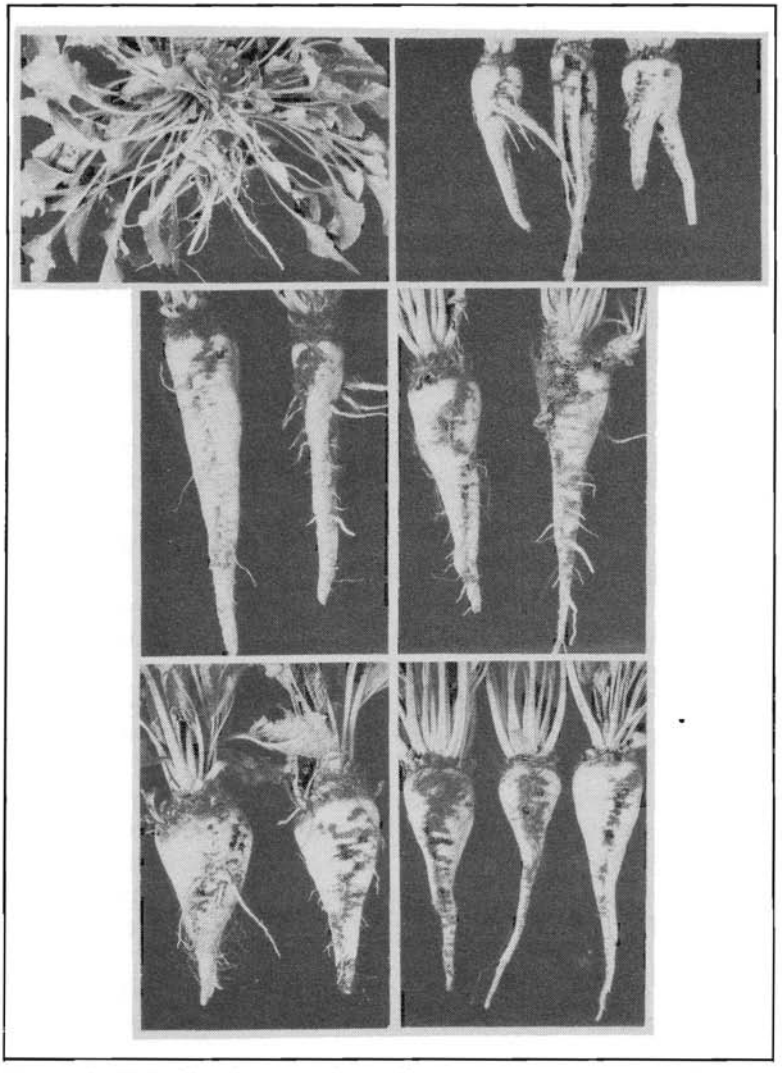

Figure 3. Hybrids of the male sterile *B. maritima* x O-line of sugar beet. Root shape variation in the B_2 generation.

DISCUSSION

The objective of these studies was to combine the male sterile cytoplasm of *B. maritima* (originated from Brittany) with the sugarbeet nuclear background. The O-type line with two pairs of recessive genes in the homozygous state was used as the pollen parent; which according to Owen's classical work (1942, 1945) permits the maintenance of male sterility. The results indicated that in this wild population of *B. maritima* maintainer nuclear genes are different from those presently utilized in sugarbeet breeding. Thus, sugarbeet O-type lines cannot fully maintain male sterility in the hybrid progenies.

Coe and Stewart (1977) reported incomplete transmission of male sterility into hybrids between sugarbeet and male sterile plants of *B. maritima* from England. Hallden, et al. (1988) and Mann, et al. (1989) mentioned that male sterile cytotypes of *B. maritima* differed from Owen's S type in mitochondrial DNA and required maintainers different from that of O-types. Such maintainers, however, have not been found.

It is our opinion that the complete male sterility in the F_1 and B_1 generations and the great variation in further backcross generations is due to the recombination taking place during meiosis. Male sterility was complete and stable when 25% of the nuclear background was from the *B. maritima* donor. However, in the B_2 and B_3 generations when only 12.5 and 6.2%, respectively, came from *B. maritima* segregation occurs for the different sterility groups. The degree of male sterility was influenced by recombination between chromosomes of the wild ssp. and sugarbeet O-type. It seems that the complete sterile progenies found in the B_2 and B_3 generations have incorporated genes from *B. maritima* during crossing-over. The desirable recombination are not stable, resulting in variation between progenies and individual plants. This result should be taken into consideration when new CMS from *B. maritima* will be transferred to sugarbeet by asymmetric fusion.

LITERATURE CITED

Boutin, V., G. Pannenbecker, W. Ecke, G. Schewe, P. Saumitou-Laprade, R. Jean, Ph. Vernet, and Michaelis. 1987. Cytoplasmic male sterility and nuclear restorer genes in a natural population of *Beta maritima:* Genetical and molecular aspects. Theor. Appl. Genet. 73:625-629.

Coe, G. E. and D. Stewart. 1977. Cytoplasmic male sterility, self-fertility and monogermness in *Beta maritima* L. J. Amer. Soc. Sugar Beet Technol. 19:257-261.

Hallden, C., T. Bryngelsson and N. O. Bosemark. 1988. Two new types of cytoplasmic male sterility found in wild *Beta* beets. Theor. Appl. Genet. 75:561-568.

Mann, V., L. McIntosk, C. Theurer, J. Hirschberg. 1989. A new cytoplasmic male sterile genotype in the sugar beet *Beta vulgaris* L.: a molecular analysis. Theor. Appl. Genet. 78:293-297.

Mikami,T., Y. Kishima, M. Sugiura, and T. Kinoshita. 1985. Organelle genome diversity in sugar beet with normal and different sources of male-sterile cytoplasm. Theor. Appl. Genet. 71:166-177.

Owen, F. V. 1942. Male sterility in sugar beets produced by complementary effects of cytoplasmic and mendelian inheritance. Am. J. Bot. (abstract) 29:692.

Owen, F. V. 1945. Cytoplasmatically inherited male sterility in sugar beets. J. Agric. Res. 71:423-440.