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A new method for hybrid seed production based on cytoplasmic male sterility combined with a lethal gene and a female sterile pollenizer in *Capsicum annuum* L.

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Summary. A new improved method for hybrid seed production was successfully tested. This method is based on using a cytoplasmic male sterile line possessing a lethal gene with action that can be easily inhibited and a female sterile pollenizer. The lethal gene ensures 100% purity of the F_1 crop. The female sterile pollenizer provides a permanent abundant flowering with excess of pollen grains that leads to increased hybrid seed production without additional labour expenses. The described scheme is applicable for other crops as well.

Key words: Cytoplasmic male sterility – Lethal genes – Female sterility – Hybrid seed production – *Capsicum annum* L.

Introduction

Cytoplasmic male sterility in pepper was described by Peterson (1958) but was not used widely in commercial hybrid seed production because of phenotype instability (Daskalov 1971/72; Novak et al. 1971; Shifriss and Frankel 1971; Shifriss and Guri 1977, 1979). For this reason, the production of hybrid seeds is based either on utilization of genic male sterility (Daskalov 1972, 1976; Breuils and Pochard 1975; Hirose and Fujime 1980) or on the classical method of emasculation and artificial hand pollination. The drawbacks of these methods are that sometimes a high percentage of non-hybrid seeds is produced and a considerable amount of manpower is required.

Daskalov and Mihailov (1983, 1986) described a method of hybrid seed production based on utilization of male sterility (CMS or GMS) combined with a lethal gene that can be easily inhibited. This method represents an improvement of the female parent so that 100% purity of the F_1 crop is ensured.

The aim of the present study was to further improve this method using a female sterile pollenizer.

Materials and methods

The investigations were carried out during 1982–1987 at the Institute of Genetics, Sofia. The following lines were used as initial material:

- *Zlaten medal (S) rfrf ll.* a cytoplasmic male sterile analogue of the CV Zlaten medal possessing a conditional lethal gene that can be easily inactivated with a specific treatment.
- *Borjana cfs.* a conditional female sterile line obtained by gamma irradiation of dry seeds.

The dynamics of the flowering and fruit setting as well as the seed setting of the female sterile line was investigated. This line was used as pollenizer and the yield of the hybrid seeds was compared with that of the normal pollen parent.

Results and discussion

In 1983 a female sterile mutant in a M_2 population of the cv Borjana was established. The genetical analysis revealed that the mutant phenotype is determined by a single recessive gene designated cfs (conditional female sterile).

The mutant plants are vigorous with compact habitus and are characterized by a permanent abundant flowering during the whole vegetation period (Table 1). They form hundreds of normally developed flowers with anthers containing fertile pollen grains. At the end of the vegetation period the mutant plants form fruits with a diverse number of seeds (Table 2). The mean yield of seeds per plant is approximately 1.5 g (15% of the con-

Table 1. Dynamics of flowering and fruit setting of the female sterile line Borjana cfs

Variants	May	June	July	August	September
Borjana (check)	Begin of flowering	Full flowering + Begin of fruit setting	Reduced flowering + Full fruit setting	End of flowering + Full fruit setting	No flowering
Borjana cfs	Begin of flowering	Full flowering	Full flowering	Full flowering + Beginn of fruit setting	Full flowering + Full fruit setting

Table 2. Seed setting of the female sterile line Borjana cfs

Variants	Year	Mean no. of seeds per fruit	Range	Mean yield of seeds per plant/g	Relative yield	Range	Calculated yield per ha/kg
Borjana (check)	1984	172	142–286	9.3	100.00	6.3–12.8	744
Borjana cfs	1984	41	2–187	1.4	15.05	0.4–2.7	112
Borjana (check)	1985	185	111–302	10.6	100.00	7.2–14.0	848
Borjana cfs	1985	44	1–216	1.6	15.09	0.8–3.1	128

Table 3. Yield of hybrid seeds on the basis of cytoplasmic male sterility combined with a lethal gene and female sterile pollenizer

Variants	Year	Removal of the young fruits to stimulate flowering	Mean yield per plants/g	Relative yield	Yield per ha/kg (50,000 male sterile plants/ha)
Zlaten medal (S) rfrf ll Cfs Cfs × Borjana (check)	1985	3 times	5.3	100.00	265
Zlaten medal (S) rfrf ll Cfs Cfs × Borjana cfs cfs (female sterile)	1985	0	6.1	115.09	305

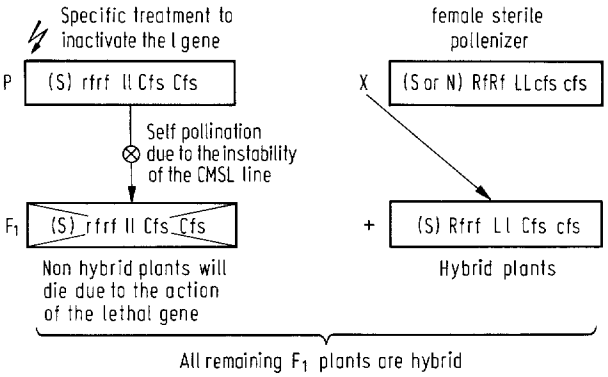

Fig. 1. Hybrid seed production based on cytoplasmic male sterility combined with a lethal gene and female sterile pollenizer with permanent flowering

trol), which is completely satisfactory for easy maintenance of the line.

Female sterility was reported for a number of crop plants, e.g. Casady et al. (1960) in sorghum, Tustus and Meyer (1963) in cotton, Honma and Pratak (1964) in tomato, Gotzov and Dzelepov (1974) in wheat, Hanna and Powel (1974) in pearl millet and Brown and Bingham (1984) in alfalfa, etc.

The possible use of female sterile pollenizer in hybrid seed production was pointed out by Gotzov and Dzelepov (1974), Hanna and Powel (1974), Brown and Bingham (1984) and Bingham and Hawkins-Pfeiffer (1984). There are, however, some problems of maintaining of the female sterile lines and for this reason such pollenizers are commercially still not widely used. A female sterile pollenizer in pepper hydrid seed production could be of great use, because to stimulate the flowering of the male parent it is necessary to remove the young fruits 2–3 times, which requires additional labour expenses. The mutant Borjana cfs represents certain interest in this connection.

In Fig. 1 a scheme for hybrid seed production using cytoplasmic male sterility combined with a lethal gene (CMSL) and a female sterile pollenizer is given. The CMSL line Zlaten medal (S)rfrf 11 can be maintained by self pollination during the winter months so that no maintainer line is required. A specific treatment must be

applied to inhibit the expression of the lethal gene in order to obtain normally growing female plants. At the hybrid seed production plot the female parent and the pollenizer must be planted in alternate rows. Pollination would be performed mainly by diverse insects – bees, trips, ants, etc.

If the CMSL line expresses phenotypic instability of sterility, all non-hybrid F_1 plants obtained by self-pollination will die soon after emergency due to the action of the lethal gene.

The female sterile pollenizer ensures permanent flowering and abundant pollen quantity that leads to an increased yield of hybrid seeds without additional labour for removing the fruits of the male parent (Table 3). This scheme is confined not only to sweet pepper but may be applied for other crops as well.

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