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Analysis of wild *Helianthus annuus* and *H. petiolaris* populations for presence of Rf genes for PET-1 cytoplasm

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Abstract

Presence of genes for restoration of male sterile PET-1 cytoplasm and mode of their action have been determined in 8 populations of wild *Helianthus annuus* and one *H. petiolaris* ssp. *petiolaris* Nutt. population. One dominant gene was found to be present in three wild *H. annuus* populations (ANN-2159, ANN -221 3 and ANN-2226) and the populations of *Helianthus petiolaris* ssp. *petiolaris* Nutt. (PET-2167). The remaining 5 populations (ANN-2143, ANN-2172, ANN-2173, ANN-2199 and ANN-2229) had two complementary dominant genes.

Key words: Sunflower, cms, fertility restoration, dominant gene, complementary genes

Introduction

The sunflower (*Helianthus annuus* L.) is an important oil crop in many countries. Sunflower oil has a special place among edible oils on account of its quality and high biological and energy values. It had been recorded that American Indians used sunflower seeds for extracting oil and pigments which they used as body and hair paint. Large-scale use of sunflower seeds for production of edible oil, however, started in Russia in 1920s. Varieties developed through "farmer selection" had been used at the beginning, followed by varieties developed by qualified breeders. Presently, hybrids predominate in the commercial sunflower production.

Although research results have indicated that hybrids are higher yielding and more uniform in height, moisture content and maturation than varieties, their practical exploitation started fairly late. This was due to the bisexual nature of the flower in sunflowers, which necessitated previous discovery of suitable male sterile cytoplasm. Although Stube and Gundaev were first to report of having discovered cms [1], all breeding centers in the world use PET-1 cms which was discovered by Leclercq [2]. This cms is present in all currently grown commercial hybrids. Soon after the discovery of the latter cytoplasm, a gene for its restoration has been identified by several researchers [3-5].

To exploit any male sterile cytoplasm, of which more than 60 have been discovered so far [6], it is necessary to have a corresponding restorer gene. The objective of this paper was to analyze several previously disregarded populations of wild sunflower for gene(s) for restoration of male sterile PET-1 cytoplasm and to determine their modes of action.

Material and methods

Several scores of wild *H. annuus* and *H. oetiolans* ssp *petiolans* Nutt. populations collected in the central Great Plains of the United States [7] have been analyzed for presence of genes for restoration of male sterile PET-1 cytoplasm. Pollen collected from plants of the studied populations was applied to V-893-3-4 (L-1), a cytoplasmatic male sterile inbred line derived from the variety VNIIMK 8931 at Institute of Field and Vegetable Crops. Cms discovered by Leclercq [2] was subsequently backcrossed into L-1.

The plants of L-1 and the analyzed populations were isolated with paper bags before flowering. Pollen from 15-20 plants of each population was mixed and hand applied to sterile plants of L-1. The seeds obtained were sown the next year to obtain the F_1 plants which were inspected for the presence or absence of pollen. Nine crosses with all male fertile plants were selected for further work. Eight of these crosses included wild *H. annuus* as a parent, and one cross included *H. petiolaris*. All plants were again isolated with paper bags before flowering to produce the F_2 generation. Pollen collected from a large number of plants was mixed and hand applied to sterile L-1 plants to produce the BC_1 generation.

In the next year, the F_2 and BC_1 generations grown in the field were visually inspected for the presence or absence of pollen. In the Cytogenetics Laboratory, anthers were checked under the microscope for the presence of pollen and pollen viability was determined by the staining [8]. The segregation ratio of male sterile to male fertile F_2 plants was tested by the χ^2 analysis [9].

Results and discussion

The inspection of the plants from the F_1 generation showed that a large number of the studied populations possessed a gene for restoration of PET-1 cms. Some crosses had all fertile plants, some had both fertile and sterile plants and some had all sterile plants. Nine crosses with all fertile plants were identified for further work. These plants had a percentage of high fertile pollen (above 80%). Eight crosses included populations of wild *H. annuus*, and one included *H. petiolaris* ssp. *petiolaris*.

In crosses 2, 6, 7 and 9, the segregation to fertile and sterile plants in the BC_1 generation was close to the 1:1 ratio (Table 1). After it was found that the segregation ratio in the F_2 generation was approximately 3:1 in favour of fertile plants, it was concluded that the populations ANN-2159, ANN-2213, ANN-2226 and PET-2167 possessed one dominant gene (Rf1) for restoration of PET-1 cms. Control of fertility restoration of PET-1 cytoplasm by a single dominant gene was reported earlier [3, 4, 10].

In the remaining five crosses, which included the populations ANN-2143, ANN-2172, ANN-2173, ANN-2199 and ANN-2229, there were two complementary dominant genes (Rf1 and Rf2) as indicated by the segregation ratio of approximately 9:7 in the F_2 generation and the approximate ratio 1:3 in the BC_1 generation. The segregation of fertile and sterile F_2 plants in the 9:7 ratio is the result of the heterozygous state of the hybrids which formed four kinds of gametes, Rf1 Rf2, Rf1 rf2, rf1 Rf2 and rf1 rf2.

Although literature sources claim so, we could not find a case of control of restoration of PET-1 cms by more than two genes. Earlier studies [11-13] reported cases involving three or four dominant genes. Vran ceanu and Stoenescu [14] reported three recessive genes with complementary action.

For breeding purposes, a genotype with a single dominant gene for fertility restoration is typically used. A polygenic system, especially if it contains several genes, is difficult to use because this system is sensitive to various environmental influences and also it requires elaborate methods of breeding and seed processing. According to Vranceanu and Stoenescu [15] the restoration of pollen fertility controlled by polygenic

Table 1. Number of male-fertile (MF) and male-sterile (MS) plants in the F_1, F_2 and BC_1 generations and Chi-square analysis

No.	Cross	Generation	No. of plants		Expected ratio	χ^2	P
			MF	MS			
1.	L1 × ANN-2143	F_1	30	0			
		F_2	80	55	9:7	0.49	0.50-0.60
		BC_1	25	80	1:3	0.08	0.75-0.99
2.	L1 × ANN-2159	F_1	32	0			
		F_2	66	23	3:1	0.03	0.60-0.75
		BC_1	30	29	1:1	0.16	0.60-0.75
3.	L1 × ANN-2172	F_1	22	0			
		F_2	73	56	9:7	0.04	0.80-0.99
		BC_1	20	75	1:3	0.79	0.40-0.50
4.	L1 × ANN-2173	F_1	15	0			
		F_2	80	60	9:7	0.04	0.80-0.90
		BC_1	68	53	1:3	0.33	0.60-0.75
5.	L1 × ANN-2199	F_1	19	0			
		F_2	70	50	9:7	0.21	0.60-0.75
		BC_1	28	68	1:3	0.89	0.40-0.50
6.	L1 × ANN-2213	F_1	28	0			
		F_2	82	30	3.1	0.10	0.75-0.99
		BC_1	45	40	11	0.30	0.60-0.75
7.	L1 × ANN-2226	F_1	20	0			
		F_2	65	17	3:1	0.80	0.40-0.50
		BC_1	23	25	1:1	0.08	0.75-0.99
8.	L1 × ANN-2229	F_1	26	0			
		F_2	68	47	9:7	0.39	0.60-0.75
		BC_1	33	88	1:3	0.33	0.65-0.75
9.	L1 × PET-2167	F_1	28	0			
		F_2	84	31	3:1	0.24	0.65-0.75
		BC_1	45	50	1:1	0.26	0.65-0.75

complexes also produces a series of surprises in the process of creation of cms inbred lines. When such a polygenic complex occurs in the genotype of the fertile analogous line B, the analogous line A is completely sterile in the first generations of backcross, but as soon as its genotype becomes saturated with these polygenes the phenomenon of pollen fertility restoration appears, in the most cases as a partial fertility. One should avoid these lines in the programme of sunflower hybrid develpoment.

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