SABRAO Journal
of Breeding and Genetics
42 (1) 46 – 50, 2010

INHERITANCE OF FERTILITY RESTORATION FOR DIFFERENT CMS SOURCES IN SUNFLOWER (Helianthus annuus L.)

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SUMMARY

Worldwide production of cultivated hybrid sunflower (Helianthus annuus L.) presently utilizes only the PET-1 source of cytoplasmic male sterility. The use of one sterile cytoplasm creates a high degree of genetic vulnerability. The present study was conducted to study the fertility restoration and inheritance pattern for three diverse cytoplasmic male sterile sources i.e., PET-1 (ARM 243A), CMS PEF (FMS 850A) and CMS I (IMS 850A). Four independent crosses were generated by crossing three diverse male sterile lines with four inbred lines (known restorers for the CMS sources). The inheritance studies in three crosses viz., ARM 243A x 95-C1, IMS 850A x GP-322-1 and FMS 850A x LTRR-5 revealed two dominant genes with complementary gene action were found to be responsible for restoration. In ARM 243A x 3376R, single dominant gene use for the fertility restoration. The study results indicated that the diverse CMS sources required different fertility restoring genes for their restoration.

Key words: Fertility restoration, *Helianthus spp*., inheritance, male sterility.

Sunflower (*Helianthus annuus* L.) is the third important oilseed crop in the world after soybean and groundnut for edible oil. The crop has gained importance after its introduction in 1972 and the area is expanding every year. The expansion is mainly due to development of hybrids, which have high yield potential, greater uniformity, increased self-fertility and resistance to diseases than the open pollinated varieties (Miller, 1987). Sunflower is now planted on over 23.7 million hectares worldwide (Damodaram and Hegde, 2007). Sunflower hybrids, produced by utilizing the cytoplasmic male sterility (CMS) and nuclear fertility restoration are grown on majority of sunflower production areas (Fick, 1989).

The first CMS source PET-1, was discovered by Leclercq (1969) and originated from a cross between *Helianthus petiolaris* Nutt. and cultivated *Helianthus annuus* L. The cytoplasm is commonly referred to as the French cytoplasm and designated as the PET 1 cytoplasm (Serieys, 1987). The identification of genes for fertility restoration is the beginning of commercial hybrid seed production in sunflower using the CMS source PET-1 (Kinman, 1970). Most of the present day sunflower hybrids utilize only PET-1 source of cytoplasm for

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hybrid production. Use of single source of cytoplasm may lead to high degree of genetic vulnerability to biotic stress. Hence, there is a need to diversify the cytoplasmic base. Sunflower breeding programs need to find new CMS sources to identify and use of new restorer lines to enhance the genetic diversity. CMS in plants derived from crosses of H. lenticularis Dougl. with cultivated sunflower. Subsequent crosses of CMS plants and the cultivar Armavirets produced a stable, male sterile line (Anashchenko *et al*., 1974). Serieys (1987) designated this cytoplasm as ANL1. Leclercq (1983) examined fertility of progenies from crosses of ANL1 and PET1 with 15 maintainer or restorer lines and concluded that ANL1 and PET1 were cytoplasmically different from one another. These results indicated that differential nucleo-cytoplasmic interactions in progenies from crosses involving CMS lines to maintainer and fertility restorer lines can be used to differentiate cytoplasmic sources. Miller (1996) crossed new cytoplasmic male sterile source, PEF1 derived from H. petiolaris sp. fallax Heiser with the restorer lines Zaira (PI 371935) and HA 60 (Ames 4035). Restoration by these two restorer lines is controlled by two complementary dominant genes with cumulative gene action. Availability of fertility restorers for the new CMS sources is very limited in the available germplasm. The inheritance of fertility restoration for PET-1 cytoplasm is controlled by a single dominant gene and in some cases two complementary dominant genes (Dominguez-Gimenez and Fick, 1975). Partial restoration of fertility has been observed in many sources indicating the presence of modifier genes. These modifier genes are influenced by the environment making their inheritance difficult to determine. The objective of the study was to determine the inheritance of fertility restoration in four crosses utilizing three diverse CMS sources.

MATERIALS AND METHODS

The experimental material comprising three diverse cytoplasmic male sterile sources *viz*., PET-1 (ARM 243A) from *H. petiolaris*, CMS PEF (FMS 850 A) from *H. petiolaris* sp. *fallax* and CMS I (IMS 850 A) from *H. lenticularis* were obtained from Directorate of Oilseeds Research, Rajendranagar, Hyderabad. The three CMS sources along with the four newly identified restorers were sown at Research Farm, Directorate of Oilseeds Research, Rajendranagar, Hyderabad during *rabi*, 2007-08. Four independent crosses were made between the CMS lines and the restorers to generate F_1 seed. During the *kharif*, 2008 the F_1 plants were studied for fertility reaction and the plants with complete male fertility (100%) based on visual observation were selfed to produce the F_2 seed. In *rabi*, 2008-09, the parents, F_1 and F_2 were planted, the parents and F_1s were raised in two rows, while the F_2 plants were grown in 40 rows of 5m length with a spacing of 60cm between the rows and 30 cm between plants within a row. Necessary package of practices and plant protection measures were taken to produce a healthy crop. The F_2 plants were classified as male fertile and male sterile based on anther development and amount of pollen production and the data was subjected to Chi-Square (χ^2) procedure. Pollen fertility was confirmed in the laboratory by using 1% acetocaramine staining (Chaudhary *et al*., 1981).

RESULTS AND DISCUSSION

The segregation pattern of male sterility and male fertility is presented in Table 1. All the four crosses showed complete fertile reaction in F_1 generation showing the dominant nature of fertility over sterility. The segregation analysis of F_2 data from the

crosses ARM 243A x 95-C_1, IMS 850A x GP-322-1 and FMS 850A x LTRR-5 segregated in the ratio of 9 fertile: 7 sterile indicating the presence of two complementary dominant genes were responsible for fertility restoration. Neither of the genes alone gives restoration. Instead, the presence of at least one dominant allele at each locus is required for fertility restoration. Similar results of two dominant genes control of fertility restoration for PET-1 source has been reported by Dominguez-Gimenez and Fick (1975) and Seilar and Jan (1994), whereas Miller (1996) and Rukmini Devi (2002) who reported two complementary dominant genes for fertility restoration of PEF cytoplasm. In the cross ARM 243A x 3376R (PET-1 source), a 3:1 segregation for fertility and sterility was observed indicating single dominant gene for fertility restoration. This is in line with the research findings of Leclercq (1971), Kural and Miller (1992), Seilar and Jan (1994), Horn and Friedt (1997), Vishnuvardhan Reddy *et al.* (2002) and Jan and Vick (2007). These results of the present investigations clearly indicated the existence of cytoplasmic diversity among the three different cytoplasmic male sterile sources used. The fertility in ARM 243A was reported by a single dominant gene contributed from 3376R and two complementary genes present in 95-C_1. These findings indicated existence of diverse mechanism for fertility restoration.

Table 1. Segregation pattern of male fertile (F) and male sterile (S) plants inP_1, P_2, F_1 and F_2 populations of three diverse cytoplasmic male sterile sources and restorer lines of four crosses in Sunflower

Generations	Total Plants	Observed frequencies		Expected frequencies		Ratio F: S	χ^2	Probability
		Fertile (F)	Sterile (S)	Fertile (F)	Sterile (S)			
ARM 243A x 95-C_1								
P_1	30	-	30	-	-	-	-	-
P_2	29	29	-	-	-	-	-	-
F_1	27	27	-	-	-	-	-	-
F_2	430	227	203	242	188	9:7	2.12	0.10-0.25
ARM 243A x 3376R								
P_1	28	-	28	-	-	-	-	-
P_2	26	26	-	-	-	-	-	-
F_1	30	30	-	-	-	-	-	-
F_2	514	368	146	385.50	128.50	3:1	3.18	0.05-0.10
IMS-850A x GP-322-1								
P_1	29	-	29	-	-	-	-	-
P_2	31	31	-	-	-	-	-	-
F_1	28	28	-	-	-	-	-	-
F_2	415	245	170	233.50	181.50	9:7	1.29	0.25-0.50
FMS-850A x LTRR-5								
P_1	27	-	27	-	-	-	-	-
P_2	30	30	-	-	-	-	-	-
F_1	28	28	-	-	-	-	-	-
F_2	392	208	184	220.50	171.50	9:7	1.61	0.10-0.25

Contradicting the present results, Horn and Friedt (1997) reported in the F_2 populations of the two crosses with PEF1 cytoplasm revealed that, in both cross combinations a single dominant restorer gene appears to be responsible for fertility restoration. Anashchenko and Kukosh (1985) reported fertility restoration controlled by

five *Rf* genes, two major genes *viz*., Rf_1 and Rf_2 with a common effect, two weaker genes with complementary action and the gene Rf_1 peculiar to CMS I cytoplasm. Kukosh (1984) and Horn and Friedt (1997) observed that fertility restoration of the CMS I cytoplasm was under the control of single dominant gene.

Vranceanu and Stoenescu (1978) reported that in three cases, fertility restoration was conditioned by three complementary genes and in one case by cumulative action of two non-allelic dominant genes. Dominguez-Gimenez and Fick (1975) and Whelan (1980) reported the cases in which fertility restoration was controlled by as many as four non-allelic genes. However, in the present study, no crosses revealed fertility restoration controlled by three or more genes are responsible for pollen fertility restoration. Thus, it can be concluded that the fertility restoration ability was controlled by single or two dominant genes with different interaction effects depending on the restorers involved and CMS lines used. Further studies are needed to know the precise mechanism of fertility restoration using different CMS sources and fertility restorers.

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