Inheritance of Fertility Restoration for Two Cytoplasmic Male Sterility Sources of Helianthus pauciflorus (rigidus) Nutt.

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ABSTRACT

New sources of cytoplasmic male sterility (cms) and fertility restoration genes would reduce the genetic vulnerability of commercial sunflower (Helianthus annuus L.) hybrids because of the current use of a single male sterile H. petiolaris Nutt. cytoplasm and a few fertility restoration genes. The objectives of this study were to evaluate the inheritance of fertility restoration, to compare the cytoplasmic similarity between the two cms sources, and to confirm the vigor reducing effect of combining perennial species cytoplasms with cultivated nuclei. Cms-RMX plants, maintained by backcrossing with inbred line HA89, were crossed with 21 prospective restoration lines. Male-fertile F1 progeny were observed in crosses with ‘Luch’ and ‘RCMG1’. Segregation of male sterility in F2, and testcrosses with HA89 indicated fertility restoration was controlled by two complementary dominant genes. Identical segregation ratios of male fertile to male sterile in both F2 and testcross F1 were obtained with cms-RIG1 using the fertility restoration genes identified for cms-RIGX. These results suggest a single origin of the two cms sources. In a field test, cms-RIGX plants produced no seeds after self-pollination, and 99% seed set from open-pollination, indicating complete male sterility and female fertility. The new cms-RIG sources and corresponding fertility restoration genes will provide cytoplasmic diversity for sunflower hybrid production.

SINCE THE BEGINNING of the commercial hybrid sunflower era, genetic vulnerability due to the use of a single H. petiolaris (Leclercq, 1969) male-sterile cytoplasm (PET1) for worldwide production has been recognized. At least 62 cms sources have been identified in sunflower (Serieux, 1999). Eight cms sources were derived from wild perennial and 54 from wild annual species, and fertility restoration genes were reported for 30 cms sources. However, inheritance studies were reported for only 17 of the cms sources. The only reports on the inheritance of fertility restoration for perennial species cytoplasm are for cms-MAX2 and cms-RIGX (Jan and Zhang, 1994; Jan et al., 1994). The cms-RIG1 identified by Vulpe (1972) remained nonrestorable until we crossed it with restoration sources that restore fertility in cms-RIGX (Jan et al., 2000), which was obtained from the former Soviet Union in 1987.

The lack of identified cms sources from perennial Helianthus species could be largely due to the difficulties of cross incompatibility and the sterility of F1 and BC1F1 progeny (Jan, 1997). However, perennial H. tuberosus L. contributed substantially to sunflower improvement in the early 1900s in the former Soviet Union. As a result, most of today’s sunflower cultivars are thought to have H. tuberosus cytoplasm. This was not generally recognized until the discovery of the vigor restoration genes in many cultivated lines obtained by crossing them with vigor-reduced HA89 in perennial Helianthus species cytoplasm (Jan and Ruso, 2000). Plants with pale-green leaves and retarded growth were obtained when backcrossing inbred line HA89 into the cytoplasms of five perennials species: H. mollis Lam, H. maximiliani Schrader, H. grosseserratus Martens, H. divaricatus L., and H. angustifolius L. (Jan, 1992). Dominant vigor restoration genes identified in 11 of the 14 lines were at the same locus, suggesting a common perennial species donor, H. tuberosus (Jan and Ruso, 2000). Cms-RIGX produced typical male-sterile (MS) plants, without extruding anthers or pollen production and with normal plant height and vigor. However, segregation of normal and reduced vigor plants similar to those observed previously (Jan, 1992) was observed after several backcrosses with HA89.

The objectives of this study were to identify fertility restoration genes for cms-RIGX and cms-RIG1, to evaluate the inheritance of fertility restoration, to compare the cytoplasmic similarity between the two cms sources, and to confirm the vigor reducing effect of combining perennial species cytoplasms with cultivated nuclei.

MATERIALS AND METHODS

Seed of a H. pauciflorus (rigidus) cms source in a cultivated background was obtained through a scientific exchange with former Soviet Union scientists in 1987. The cms source was temporarily designated cms-RIGX and was increased by crossing with inbred line HA89 and bulk pollen of several wild H. annuus (WA) accessions. Male-sterile plants of cms-RIGX/HA89//WA/3/HA89 and cms-RIGX/2*HA89/3/cms-RIGX/HA89//WA were pollinated with 20 restoration tester lines in 1988 (Table 1). The F1 progenies were grown in the field in 1989. Male-fertile F1 plants having normal anther extrusion and pollen production were self-pollinated.

Testcrosses were made in 1992 with remnant F1 seed of crosses segregating for fertility in 1989 onto male-sterile plants of cms-RIGX/2*HA89/3/cms-RIGX/HA89//WA/4/2*HA89. Testcross progenies were grown in the greenhouse and visually scored for segregation of male-fertile and male-sterile plants. Typical male-sterile cms-RIGX plants without anther extrusion and pollen production were classified as male sterile, all other plants were classified as male fertile. Male-fertile testcross progenies were crossed to cms plants, selfed, and sib-mated. Pollen stainability was used to indicate the degree of fertility restoration in the progeny (Alexander, 1969). Seed of self-pollinated or sib-pollinated male-fertile testcross progenies were grown in the greenhouse in 1993 and 2000 to obtain F2 male-fertile to male-sterile segregation ratios. Pollen stainability of male-fertile F1 plants grown in the greenhouse was scored. Cms-RIGX was repeatedly backcrossed to HA89, and the progenies observed for male sterility and for reduced vigor associated with perennial species cytoplasms.
The cms-RIG1 germplasm with an RHA274 nuclear component was obtained from Dr. H. Series in 1996. This cms source was pollinated with pollen from male-fertile RIGX testcross progenies (Table 2), heterozygous for the fertility restoration genes from Luch and RCMG1 (Miller and Wolf, 1991) in 1997. Progeny were tested in 1998 and 2000 in the greenhouse. Male-fertile progenies were examined for pollen stainability and self-pollinated. Self-pollinated F₁ progenies with Luch and RCMG1 restoration genes in cms-RIG1 were grown in the field in 1999 and observed for segregation of male-fertile and male-sterile plants.

RESULTS AND DISCUSSION

The cms-RIGX has the characteristic cytoplasmic male sterility property of producing no visible anthers during flowering, similar to the cms-PET1 used in all commercial hybrids. Six cms-RIGX × HA89 hybrids were male sterile. Cms-RIGX × wild H. annuus (WA) produced 12 male-sterile progeny and a single male-fertile head among other male-sterile heads on one plant. This male-fertile head was designated 34F. Crosses of cms-RIGX with 34F produced all male-sterile progeny, indicating an absence of fertility restoration genes. F₁ progeny of 34F × HA89 produced one male-fertile and 28 male-sterile plants. This male-fertile plant was designated 741-1F, and it continued to produce male-fertile progenies through self-pollination. Because of the obvious lack of fertility restoration genes, 741-1F was considered to be the result of a rare cytoplasmic reversion. Cms-RIGX and 741-1F have identical cytoplasmic genes except for the one(s) determining male-fertile and male-sterile characteristics. Thus, 741-1F and cms-RIGX provide useful material for molecular studies of cytoplasmic male sterility.

All the USDA RHA lines have the Rf₁ restoration gene which did not restore fertility to cms-RIGX (Table 1). Only crosses with the open-pollinated cultivar Luch and restoration line RCMG1 produced male-fertile F₁ progenies at a low frequency. RCMG1 was previously identified as a restorer line for cms-PET2 (Miller and Wolf, 1991). Testcross segregation ratios of one male-fertile to three male-sterile plants were obtained for both Luch and RCMG1, suggesting fertility restoration was conditioned by two complementary dominant genes (Table 2). F₂ progenies of heterozygous self-pollinated fertile plants for both restoration sources fit the expected nine male-fertile to seven male-sterile segregation ratio, further confirmation of a two complementary dominant gene control mechanism.

Pollen stainability of male-fertile testcross progenies averaged 88 and 55% for the Luch and RCMG1 sources, respectively. On the average, the Luch restoration genes had stronger dominant effects than RCMG1 restoration genes, as shown in both the testcross and the F₂ progenies. It is not clear if this effect is caused by genes at different loci, different alleles, or modifier genes. However, the variation in pollen stainability (16–98%) in progenies having the RCMG1 restoration genes suggests selection for genes that enhance fertility would be possible. Seed set of cms-RIGX plants crossed with male-fertile testcross progenies heterozygous for restoration genes was over 95%, indicating normal female fertility. In the 1990 field test, average seed set was 99% for open-pollination and 0% for self-pollination for male-sterile plants with the pedigree of cms-RIGX/3*HA89/4/cms-RIGX/HA89//WA/3/HA89/5/HA89. This provides strong evidence for full female fertility and complete male sterility of cms-RIGX.

Crosses of cms-RIG1 with plants heterozygous for the RIGX restoration genes produced 14 male-fertile and 36 male-sterile plants for Luch and RCMG1 restoration sources. This corresponds to a theoretical one male-fertile to seven male-sterile segregation ratio, further confirmation of a two complementary dominant gene control mechanism.
fertile to three male-sterile segregation ratio characteristic of two complementary dominant genes (Table 3). Subsequently, the F2 plants were grown in the field in 1999, and produced 56 male fertile and 51 male sterile for the Luch sources, and 90 male-fertile and 71 male-sterile plants for the RCMG1 sources. These ratios were not significantly different from nine male-fertile to seven male-sterile segregation ratios indicating two complementary independent genes controlling fertility restoration. Respective pollen stainability of male-fertile testcross progenies with Luch and RCMG1 restoration genes was 82 and 62%, similar to the results obtained with the cms-RIGX. These results indicated an identical reaction pattern of cms-RIG1 to the restoration genes identified specifically for cms-RIGX. It is likely that cms-RIGX and cms-RIG1 are the same, and cms-RIGX was not properly identified when distributed to the former Soviet Union by Vulpe. Molecular studies are needed to elucidate the origin of cms-RIGX and cms-RIG1.

Some reduced vigor plants were observed in progeny of cms-RIGX backcrossed with HA89 in greenhouse plantings. Backcross progeny of normal cms-RIGX having the pedigree of cms-RIGX/2*HA89/3/cms-RIGX/HA89/HA89/4/2*HA89 with HA89 produced 30 normal and 25 reduced vigor progeny plants. This ratio is consistent with the single dominant gene control of vigor restoration previously reported (Jan, 1992).

To utilize the cms-RIG germplasm for hybrid production, a breeder needs to select for both vigor restoration and fertility restoration genes. However, our recent study of cytoplasmic diversity and vigor restoration genes among cultivated lines indicated an abundance of vigor restoration genes in cultivated lines. This is hypothesized to be the result of the widespread use of H. tuberosus accesses for improving resistance to diseases and broomrape (Orobanche cumana Wall.) in the former Soviet Union in the early 1900s. The high frequency of vigor restoration genes in cultivated lines are believed to be the result of linkage to important agronomic traits. Cultivated lines in the cytoplasm of H. annuus do not require a vigor restoration gene to be normal and therefore are lacking those genes. This discovery provides important information for breeders and will make the cms-RIG or other cms from perennial Helianthus species suitable for hybrid development.

ACKNOWLEDGMENTS

The authors wish to thank L.G. Campbell and G.J. Seiler for thoughtful discussion and critical review of the manuscript. Technical assistance from L.A. Brown and J.A. Ruso is also greatly appreciated.

REFERENCES


