Additional *Rf* genes for CMS GIG2 and Their Molecular Mapping

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Abstract

A new alloplasmic cytoplasmic male-sterile (CMS) line with *Helianthus giganteus* cytoplasm and HA 89 nucleus was developed, named CMS GIG2. The male-fertility restoration (*Rf*) genes were discovered from four interspecific amphiploids, and introgressed into a cultivated background. A half-diallel test among four homozygous restoration lines suggested that the *Rf* genes derived from *H. maximiliani*, *H. grosseserratus* and *H. atrorubens* were closely localized, whereas the one derived from *H. angustifolius* was different. This study reports the mapping of the *Rf* genes from *H. grosseserratus* and *H. angustifolius*, designated as Rf_{10}, and Rf_{11}. Bulked segregant analysis with SSR and EST-SSR primers from LGs 3, 7, and 13 of the sunflower genetic map identified polymorphic markers on LG 3 for Rf_{10}, and LG 13 for Rf_{11}. Further analysis mapped Rf_{10} between two co-dominant markers, ORS13 and ORS822, at a genetic distance of 0.7 cM and 3.1 cM, respectively, with nine markers covering a genetic distance of 49.5 cM. The mapping of Rf_{11} is currently underway. The closely linked markers for *Rf* genes will facilitate marker-assisted selection (MAS), and provide a basis for studying the interaction between nuclear and cytoplasmic genes, and cloning of the two genes.

Introduction

The combination of CMS and the corresponding *Rf* genes is a critical tool in large-scale hybrid seed production of many crops, including cultivated sunflower (*Helianthus annuus* L.). Nine *Rf* genes have been mapped, including Rf_1, Msc_1, Rf_3-RHA 340, Rf_3-RHA 280, and Rf_5 for CMS PET-1, Rf_4 for CMS GIG2, *Rf*-PEF1 for CMS PEF1, Rf_6 for CMS 514A, and Rf_9 for CMS ANN3 (Gentzbittel et al. 1995; Horn et al. 2003; Abratti et al. 2008; Feng and Jan 2008; Schnabel et al. 2008; Liu et al. 2012, 2013, 2015; Qi et al. 2012). Alternative CMS/*Rf* gene systems will expand the diversity of sunflower germplasm for breeding and elucidate the mechanism of the interaction between nuclear and cytoplasmic genes. A new alloplasmic CMS line with *H. giganteus* cytoplasm and HA 89 nucleus was recently developed, named CMS GIG2. A single dominant *Rf* gene was identified from four interspecific amphiploids. The *Rf* genes have been introgressed into a cultivated background using backcrossing. The objectives of this study were to (1) study the allelic relationship among the *Rf* genes derived from four sources; and (2) molecularly map the *Rf* genes from *H. grosseserratus* and *H. angustifolius* on a sunflower genetic map.

Materials and Methods

1. **Half-diallel test:** Six testcross progeny populations of four homozygous *Rf* lines derived from four interspecific amphiploids were tested for male fertility in 2014, including *H. maximiliani*, *H. angustifolius, H. grosseserratus,* and *H. atrorubens*.
2. **Mapping population:** An F_2 population was used for the mapping of Rf_{10}, with 152 individuals derived from G12/438a x HA 234 [Pedigree: ((CMS GIG2 x Gro Amp) $HA89^2$, F_4) × HA 234]. Another F_2 population was used for the mapping of Rf_{11}, with 162 individuals derived from G12/1228 x HA 821 [Pedigree: ((CMS GIG2 x Ang Amp) $HA89^2$, F_5) × HA 821].
3. **Male fertility analysis and F_2 genotype determination:** The male fertility of the F_2 progenies was determined visually. The F_3 progenies of the F_2 population were visually scored to determine the genotype of each F_2 plant in the field at Fargo, ND in 2014, using 25-50 progenies from each F_2 individual.
4. **Polymorphism screening:** Bulked segregant analyses were conducted using SSR and EST-SSR primers reported to be closely linked to sunflower LGs 3, 7, and 13. Polymorphism screening between parents: SSR and EST-SSR markers mapped to LGs 3 and 13, respectively.
5. **Statistical analysis and linkage map construction:** Chi-square test and the MAPMAKER/Exp version 3.0b program (Lander et al. 1987).

Results and Discussion

1. **Allelism test for the *Rf* genes in four homozygous *Rf* lines derived from four interspecific amphiploids suggested that the *Rf* genes derived from *H. maximiliani*, *H. grosseserratus* and *H. atrorubens* were closely localized, whereas the one derived from *H. angustifolius* was different (Table 1).**

Table 1. Testcross progeny test of CMS GIG2 with six F_1s from half-diallel crosses of four Rf lines for male fertility (MF/MS).

♀ \ ♂	Rf Max	Rf Ang	Rf Atr
Rf Ang	198:26		
Rf Atr	126:0	169:45	
Rf Gro	201:1	188:17	220:3

2. **Fertility segregation in the mapping population confirmed a single dominant gene controlling the fertility restoration for CMS GIG2, derived from amphiploids *H. grosseserratus* x P 21 and *H. angustifolius* x P 21, respectively (Table 2).**

Table 2. Segregation of the Rf_{10} locus, SSR (ORS) markers, and EST-SSR (HT) markers linked to Rf_{10} in the F_2 population of the cross G12/438a x HA 234.

Traits or markers	Number of F_2 plants	Observed number[a] A	H	B	C	Ratio tested	χ^2	*P*
Rf_{10}	152	36	84	32		1:2:1	1.89	0.388
ORS1080	151	38			113	3:1	0.00	0.963
ORS949	152	33	87	32		1:2:1	3.20	0.202
HT1029	152	37	83	32		1:2:1	1.62	0.445
HT441	152	34	85	33		1:2:1	2.14	0.342
HT734	152	39	80	33		1:2:1	0.89	0.639
ORS488	152	34	85	33		1:2:1	2.14	0.342
ORS1114	152	35	84	33		1:2:1	1.74	0.420
ORS13	152	36	83	33		1:2:1	1.41	0.495
ORS822	151	38	80	33		1:2:1	0.87	0.648

[a] Symbols: A, homozygous MS (*rfrf*); H, heterozygous MF (*Rfrf*); B, homozygous MF (*RfRf*); C, *RfRf* or *Rfrf*.

3. **Molecular mapping localized the Rf_{10} gene on LG 3 of the sunflower SSR map (Figure 1).**

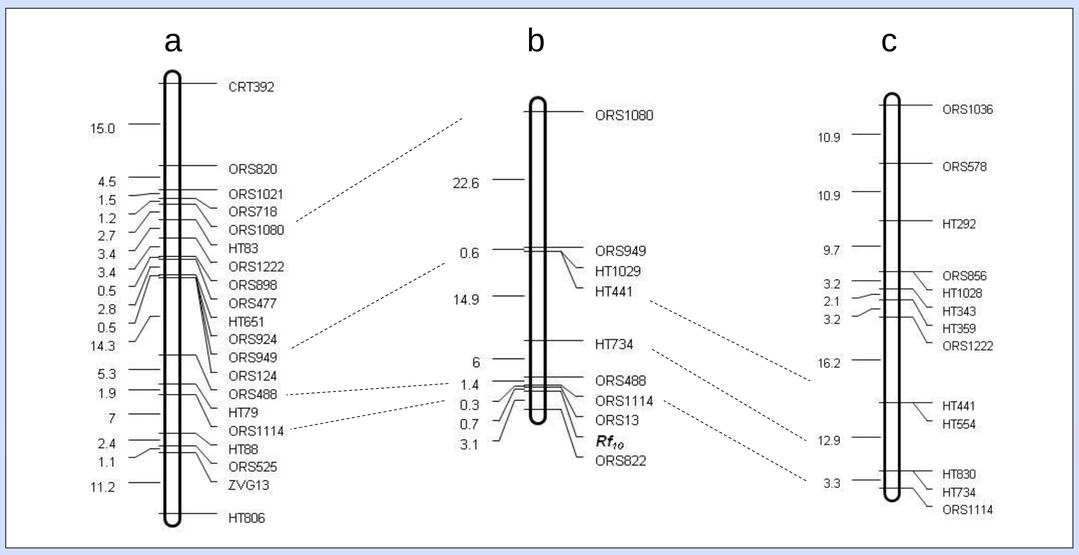

Figure 1. a) Corresponding region of LG 3 of the map RHA 280 x RHA 801_RIL (in press) in the Sunflower CMap Database. b) Mapping result of the Rf_{10} locus on LG 3, including six SSR (ORS), three EST-SSR (HT) linked markers, based on the analysis of 152 F_2 plants derived from the cross of G12/438a x HA 234. c) Corresponding region of LG 3 of the map NMS373 x ANN1811_BC (in press) in the Sunflower CMap Database (http://sunflower.uga.edu/cgi-bin/cmap/map_search). The distances are given in centimorgans (cM). The corresponding markers are aligned by dashed lines between the maps.

4. **Identification of two co-dominant SSR markers ORS511 and ORS799 that are closely linked to Rf_{11} on LG 13.**

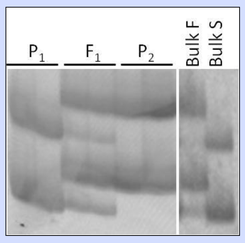

Figure 2. Representative gel image of the two parents, F_1s, Bulk F and Bulk S for the F_2 population derived from the cross of G12/1228 x HA 821 with a co-dominant marker ORS511 on a non-denaturing gel. P_1: HA 821; P_2: G12/1228.

Future work

1. Molecular mapping of Rf_{10} provides a closely linked marker ORS13 at a genetic distance of 0.7 cM on LG 3, which will be useful for MAS. More markers, such as SNPs, resistance gene candidate (RGC) markers, and newly designed SSR markers are required to fine map Rf_{10}.
2. The Rf_{11} gene from *H. angustifolius* was assigned to LG 13. Further analysis with the whole mapping population will be needed to map this gene.
3. Molecular mapping of Rf_{10} and Rf_{11} will help better understand the interaction of the nuclear gene and mitochondrial genome in sunflower.

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