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ORIGINAL PAPER

High-density genetic maps for loci involved in nuclear male sterility (*NMS1*) and sporophytic self-incompatibility (*S*-locus) in chicory (*Cichorium intybus* L., Asteraceae)

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Abstract High-density genetic maps were constructed for loci involved in nuclear male sterility (*NMS1*-locus) and sporophytic self-incompatibility (*S*-locus) in chicory (*Cichorium intybus* L.). The mapping population consisted of 389 F1′ individuals derived from a cross between two plants, K28 (male-sterile) and K59 (pollen-fertile), both heterozygous at the *S*-locus. This F1′ mapping population segregated for both male sterility (MS) and strong self-incompatibility (SI) phenotypes. Phenotyping F1′ individuals for MS allowed us to map the *NMS1*-locus to linkage group (LG) 5, while controlled diallel and factorial crosses to identify compatible/incompatible phenotypes mapped the *S*-locus to LG2. To increase the density of markers around these loci, bulked segregant analysis was used. Bulks and parental plants K28 and K59 were screened using amplified fragment length polymorphism (AFLP) analysis, with a complete set of 256 primer combinations of *Eco*RI-ANN and *Mse*I-CNN. A total of 31,000 fragments were generated, of which 2,350 showed polymorphism between K59 and K28. Thirteen AFLP markers were identified close to the *NMS1*-locus and six in the vicinity of the *S*-locus. From these AFLP markers, eight were transformed into sequence-characterized amplified region (SCAR) markers and of these five showed co-dominant polymorphism. The chromosomal regions containing the *NMS1*-locus and the *S*-locus were each confined to a region of 0.8 cM. In addition, we mapped genes encoding proteins similar to S-receptor kinase, the female determinant of sporophytic SI in the Brasicaceae, and also markers in the vicinity of the putative *S*-locus of sunflower, but none of these genes or markers mapped close to the chicory *S*-locus.

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Introduction

In angiosperms, approximately 90 % of species are hermaphrodite, mostly in the form of co-sexuality where male and female organs are located within the same flower. Though this sexual strategy heightens the chance of producing offspring, it is frequently associated with mating systems that promote outcrossing and reduce self-fertilization and its deleterious consequence, inbreeding depression (Charlesworth 2002; Chuck 2010). Self-incompatibility (SI) is the widespread mating system promoting outcrossing and is present in over 50 % of species (Gaude and Cabrillac 2001; Takayama and Isogai 2005; Allen and Hiscock 2008). In most cases, SI is controlled by

a single multi-allelic locus, the *S*-locus, which contains closely linked genes encoding the male and female determinants of pollen–pistil recognition (Silva and Goring 2001; Franklin-Tong and Franklin 2003; Takayama and Isogai 2005). Two genetic forms of SI have been recognized (de Nettancourt 2001): in gametophytic SI (GSI), the incompatibility phenotype of the pollen is determined by its own haploid genotype, whereas in sporophytic SI (SSI), it is determined by the diploid genotype of the parent plant.

Molecular characterization of GSI has revealed at least three different molecular mechanisms (Allen and Hiscock 2008). In the Solanaceae, Rosaceae and Plantaginaceae, GSI is regulated by pistil-expressed S-RNases and pollen-expressed F-box proteins (Kubo et al. 2010), whereas in the Papaveraceae, GSI is controlled by an entirely different set of molecules, centered around a novel class of stigmatic S-protein that interacts with a pollen receptor to trigger a calcium-based signaling system in the pollen that leads to inhibition of pollen tube growth and eventual cell death (Wheeler et al. 2010). In the grasses, where GSI uses neither of these mechanisms, a third molecular mechanism of GSI occurs (Klaas et al. 2011). So far, pistil and pollen determinants involved in SSI have only been identified in the Brassicaceae as a serine threonine S-receptor kinase (SRK) and its cognate ligand (SCR/SP11, Iwano and Takayama 2012). In the Convolvulaceae the *S*-locus has been mapped (Rahman et al. 2007a) and several candidate genes for the male and female *S*-determinants identified, and while these remain to be validated, none are homologs of *SRK* or *SCR/SP11* (Rahman et al. 2007b; Kakeda et al. 2009).

Male sterility (MS) is another widespread mechanism promoting outcrossing in angiosperms. MS is defined as the failure of plants to produce functional anthers, pollen or male gametes, while female reproduction is normal (Kaul 1988; Horner and Palmer 1995). Numerous genes and pathways are involved in the developmental process leading to functional pollen production (Twell 2011), and, on the basis of inheritance patterns, male sterility has been divided into cytoplasmic male sterility (CMS) and nuclear or genic male sterility (NMS). In some cases, the MS phenotype is regulated by environmental factors such as temperature or photoperiod (Kempe and Gils 2011). In agronomically important species, the main breeding objective is to improve yield and the best way to achieve this in allogamous species is by encouraging heterosis. MS is frequently used by breeders to produce hybrid varieties to not only increase biomass production, but also resistance to biotic and abiotic stresses (Kempe and Gils 2011). The use of CMS is more widespread than NMS because of its modes of maintenance and restoration of fertility (Budar and Pelletier 2001; Wang et al. 2007). However, due to pleiotropic effects and alterations of the mitochondrial genome, CMS can be associated with undesirable effects or to unstable sterility, and to maintain CMS lines, it is necessary to identify fertility restorer nuclear genes (Wang et al. 2007; Kempe and Gils 2011). NMS presents the advantage of a stable and complete male sterility, numerous restorer lines and no effect on yields (Yi et al. 2006; Huang et al. 2007). NMS is generally a recessive trait, so half of the hybrids produced are fertile (Yi et al. 2006). This drawback may be overcome by using combinations of recessive genes and/or epistatic interactions, dominant alleles or environment-sensitive genes (Xing et al. 2003; Huang et al. 2007; Wang et al. 2007; Kempe and Gils 2011). Another efficient way is to use marker-assisted selection (MAS) so that MS genotypes can be identified early (Chen et al. 2006).

In the Asteraceae (daisy family), over 60 % of species are estimated to be self-incompatible (Ferrer and Good-Avila 2007) with sporophytic genetic determination (Hiscock 2000; Allen et al. 2011). To date, however, there is little information on the male and female determinants of the molecular mechanisms underlying SSI in this family. Using *Brassica SRK* sequences, a candidate gene approach was performed in *Senecio squalidus* (Oxford ragwort), but this analysis showed no link between SSI and *SRK*-like genes expressed in stigmas (Tabah et al. 2004). Further molecular analyses in *S. squalidus* identified an *S*-associated stigma-specific peroxidase gene (*SSP*), but again this proved not to be directly involved with SSI (McInnis et al. 2005). In sunflower, 1–4 QTL associated with self-fertility were identified (Burke et al. 2002; Gandhi et al. 2005; Wills and Burke 2007). It was reasoned that a QTL of major effect that mapped to LG17 could be the *S*-locus (Gandhi et al. 2005; Yue et al. 2009), but no further analysis of genes associated with this QTL has been reported. Other works on sunflower have focused on male sterility and have led to the identification of several NMS genes, and CMS and associated restorer genes, some of which have been mapped (Chen et al. 2006; Jan and Vick 2007; Yue et al. 2010).

Chicory (*Cichorium intybus* L., Asteraceae) is an important leafy vegetable crop in many European countries with a well-characterized system of sporophytic self-incompatibility (Kiers 2000; Lucchin et al. 2008; Kilian et al. 2009). Interestingly, this SSI system shows variability in its strength among cultivated varieties and in wild populations, with different individuals ranging from strongly self-incompatible to almost completely self-fertile—a feature of the SSI system that appears to be characteristic of the entire *Cichorium* genus (Stout 1917, 1918; Pécaut 1962; Cichan 1983; Coppens d'Eeckenbrugge et al. 1987; Castaño et al. 1997; Kiers et al. 2000). Chicory also possesses a spontaneous NMS mutation ('Edith'), which has been exploited by breeders for the production of

hybrids (Desprez et al. 1994; Doré and Varoquaux 2006). In contrast, no spontaneous CMS has been reported in chicory, but CMS has been introduced by breeders via hybrids obtained by protoplast fusions between chicory and CMS sunflower (Rambaud et al. 1993).

Identification of the genes regulating NMS and SSI in chicory is desirable to plant breeders, because it will allow the optimization of breeding programs by improving the selection of sexual phenotypes and compatible parents in crosses. The availability of molecular-genetic resources for chicory (Gonthier et al. 2010) may provide a way to implement MAS of NMS and SI, and thus to improve the production of hybrid lines. The aim of this study was to determine the number of genes underlying NMS and SI and to map them to provide a route to their positional cloning. From populations used to build the reference genetic map of chicory (Cadalen et al. 2010), we selected the cross K28 × K59 because it produced an F1′ progeny showing segregation for both strong SI phenotypes and MS ('Edith'). Molecular markers close to the chicory *NMS1*-locus and *S*-locus were identified and allowed us to construct high-density genetic maps around these loci. To explore the conservation of SSI genes in the Asteraceae and Brassicaceae, we also mapped candidate genes encoding proteins similar to the S-locus receptor kinase (SRK), the female determinant of SSI in species of Brassicaceae. In addition, we mapped molecular markers in the vicinity of a QTL proposed to contain the *S*-locus of sunflower (*Helianthus annuus*, Gandhi et al. 2005), another species of Asteraceae.

Materials and methods

Plant materials

Parental genotypes K28 (MS) and K59 (H) were obtained from seeds of a pre-breeding open pollinated population (Kospool) of *C. intybus* var. *sativum* provided by the Florimond-Desprez breeding company (Cappelle-en-Pévèle, France). K28 and K59 plants were first grown in vitro and cloned by somatic embryogenesis or organogenesis (Dubois et al. 1991; Decout et al. 1994) to increase the number of plants for crosses. Ten clones of each genotype were acclimated in a glasshouse at 20 °C, with a 16/8 h light/dark cycle and grown for about 3 months. To induce flowering, plants were vernalized at 5–7 °C for 12 weeks and then transferred to an insect-proof greenhouse cell under the same pre-vernalization conditions. At flowering, all K28 clones showed a male-sterile (MS) phenotype, whereas K59 plants produced capitula with fully fertile hermaphrodite flowers (H). Anthesis and stigma receptivity in the 15–25 flowers of each inflorescence (capitulum) was more or less synchronous and occured in the morning; the lifetime of each flower being just a few hours (MCQ and TH, personal observations). Crosses between K28 (female) and K59 (male) individuals were performed by gently rubbing detached capitula of K59 against capitula of K28 plants and seed from these crosses used to found the F1′ mapping population. The use of male-sterile K28 as female plant prevented emasculation and assured progeny not contaminated by self-fertilization. The same procedure was used later to perform BC1 crosses between F1′ plants and the parental genotype K28, and for crosses among F1′ individuals. Despite the highly self-incompatible phenotype of K59, some self-seeds were also obtained.

Cultivation and vernalization conditions for the different progenies were the same as those described for the parental genotypes. Progenies were cloned in vitro before being transferred to the glasshouse and vernalized to induce flowering. Selected genotypes were maintained in the glasshouse by vegetative propagation via cuttings.

Male sterility phenotyping

Presence/absence of pollen grains in 359 F1′ individuals was scored by several observations (>10) of flowers during the flowering period (4–6 weeks) and sexual phenotypes assigned as MS or H. Pollen presence and pollen viability in F1′ plants were verified by squashing dehiscent anthers in a drop of Alexander's stain (Alexander 1969) on glass slides and observation under an Olympus BH2 light microscope. Pollen grains of which the cytoplasm stained pink were supposed to be viable (fertile); in the absence of pink color, non-viable (sterile) pollen grains appeared green due to the coloration of the pollen wall.

Self-incompatibility phenotyping

For each cross and self-pollination performed, pollinated capitula were harvested 4–6 weeks after pollination and compatibility/incompatibility determined using seed set, meaning the ratio of fully formed colored achenes (i.e., seed-containing fruits) to the total number of ovaries present in each capitulum. In preliminary tests, 79 F1′ individuals (39 H and 40 MS) were crossed with K59 as pollen donor. The H plants were reciprocally crossed with K59 and used as pollen donors to perform crosses with K28. In addition, H plants, including K59, were selfed. Each cross combination was repeated at least five times. A sample of 39 F1′ individuals and the parental genotypes used for back-crosses were transferred outside the glasshouse to a nearby experimental area. Numerous insects (mainly bumble bees and hoverflies, TH personal observations) were observed visiting capitula on sunny days, and

ten capitula per plant were marked to determine seed set under open pollination conditions.

A subset of 33 F1′ plants (22 H and 11 MS) involved in back-crosses were propagated and used to perform diallel and factorial crosses under glasshouse conditions. The H plants were reciprocally crossed (diallel crosses) and used as male parent in crosses with MS plants (factorial crosses). For each combination, three to four crosses were performed.

Finally, in total 325 F1′ individuals were used as pollen receptors and crossed with cloned H plants representing the two tester genotype groups identified in diallel and factorial crosses (see "Results"; Table 1). Each cross combination was repeated at least five times.

SSR and STS genotyping

Simple-sequence repeat (SSR) and sequence-tagged site (STS) marker genotype data (291 loci) were available for 174 F1′ individuals of the K28 × K59 cross (Cadalen et al. 2010) and were used for mapping the *NMS1*-locus and the *S*-locus on the chicory consensus map. For fine mapping, an additional set of 215 individuals were genotyped for 13 markers mapped to LG5 and for 15 markers mapped to LG2, surrounding the *NMS1*-locus and *S*-locus, respectively. The 389 genotyped plants included all plants phenotyped for NMS and SI.

Table 1 Compatibility groups, phenotypes and genotypes deduced from diallel and factorial crosses performed between K28 × K59 F1′ individuals, and back-crosses of F1′ individuals with K28 and K59 (see Online Resource 3)

		Pollen							
	Group	A (DxE)	B (K59)	B (F1')[a]	C (F1')	D (F1')	E (F1')[a]		
Stigma	A (K28)	-	+	+	+	-	-	[S3S4]	S_3S_4
	B (K59)	+	-	-	-	+	+	[S1]	S_1S_2
	B (F1')	+	-	-	-	+	+	[S1]	S_1S_3
	C (F1')	+	-	-	-	+	-	[S1S4]	S_1S_4
	D (F1')	-	+	+	+	-	+	[S3]	S_2S_3
	E (F1')	+	+	+	+	+	-	[S4]	S_2S_4
		[S3]	[S1]	[S1]	[S1]	[S3]	[S4]	Phenotype	
		S_3S_4	S_1S_2	S_1S_3	S_1S_4	S_2S_3	S_2S_4		Genotype

Pollen group A was determined from crosses between D × E progenies and genotypes representative of the different pistil compatibility groups. Gray squares correspond to compatible crosses, white squares to incompatible crosses, and striped squares indicate reciprocal crosses with different results

[a] Pollen tester groups B and E used for large-scale phenotyping of F1′ individuals

AFLP detection

Amplified fragment length polymorphism analysis was performed using primer sequences developed by Vos et al. (1995), adapted for capillary electrophoresis conditions (Myburg et al. 2001). Briefly, 500 ng of DNA was digested with *Eco*RI and *Mse*I enzymes and ligated to specific adapters. Pre-amplification was performed from a 1:30 dilution of the restriction-ligation mixture using *Eco*RI-A and *Mse*I-C primers. After restriction–ligation and pre-amplification steps, an aliquot of all samples was analyzed on a 1 % agarose gel to check for the presence of clear visible low-molecular-weight smears of DNA. Selective amplifications were carried out after dilution (1:10) of pre-amplification reactions, using primer combinations with two supplementary selective nucleotides (*Eco*RI-ANN/*Mse*I-CNN). The *Eco*RI-ANN primers were labeled with either 6-FAM or HEX fluorescent dyes for subsequent separation of selective amplification products on a 3100 Avant Genetic Analyzer capillary sequencer (Applied Biosystems). Sample preparation, denaturation and capillary electrophoresis fractionation were performed according to the User Bulletin ABI PRISM 3100 capillary array for high-throughput microsatellite and SNP genotyping (Applied Biosystems). Data were automatically collected and normalized using the Genescan 3.1 software, and the detection of polymorphic fragments was carried out with Genotyper 3.7 (Applied Biosystems). In addition, visual comparisons of chromatograms were systematically performed to ascertain the software-generated results.

Conversion of AFLP markers into SCAR markers

To generate low-complexity AFLP fingerprints and to clone AFLP fragments of interest, the mini-sequencing procedure of Brugmans et al. (2003) was applied to determine the fourth, fifth and sixth selective nucleotide following the *Mse*I-CNN primer 3′ end. The PCR products were separated in 8 % denaturing polyacrylamide gel using an LI-COR 4200 sequencer (Myburg et al. 2001). Fragments were extracted from the gel according to the protocol developed for chicory and *Arabidopsis halleri*, described in Paris et al. (2012). Gel-recovered fragments were cloned using the pGEM®-T Easy Vector system I (Promega), and clones sequenced from both ends (Genoscreen, France). For the SCAR markers, new primers were defined interior to the adapter positions. Polymorphisms were tested using size and single-strand conformational polymorphism (SSCP) fractionation or high-resolution melting (HRM) PCR assay (see below).

Identification of *SRK*-like genes

To determine whether protein(s) similar to the S-receptor kinase (SRK) female determinant of SSI in the Brassicaceae might be involved in chicory SSI, a search for *SRK*-like sequences was performed using the *Cichorium* sp. EST database accessible through the HeliaGene portal (http://www.heliagene.org/cgi/cichorium.cgi). This database contains 84,095 *C. intybus* and *C. endivia* ESTs obtained from NCBI dbEST (http://www.ncbi.nlm.nih.gov/dbEST; Legrand et al. 2007; Dauchot et al. 2009) and the Compositae Genome Project database (CGPdb, http://cgpdb.ucdavis.edu/), and assembled in 50,659 unisequences (i.e., contigs and singletons, prefix CIC). The collections of *Cichorium* EST were obtained from RNA extracted from whole plants (CGPdb: a mixture of different organs, including flowers), leaf tissue (Legrand et al. 2007; Dauchot et al. 2009) and roots (Dauchot et al. 2009). *Cichorium* sp. sequences were first selected by a TBLASTX search (Altschul et al. 1997) on the basis of their homology (*E*-value $<10^{-30}$) with a selection of ten *SRK* alleles identified in different Brassicaceae species. Because of a high number of positive hits (more than 100), selected sequences were then submitted to a BLASTX (Altschul et al. 1997) search (*E*-value $<10^{-30}$) against the *Arabidopsis lyrata* proteome (Hu et al. 2011) accessible through the PHYTOZOME portal (http://www.phytozome.net/). *Cichorium* sp. unisequences were selected for primer definition (Online Resource 1), and polymorphism testing if they were among the five best hits in a continuous alignment with the ortholog of the *Arabidopsis thaliana* ARK3 protein (the closest functional counterpart of SRK in *A. thaliana*) and among the 15 best hits in a continuous alignment with the AlSRK13 allele contained in the reference genome (Goubet et al. 2012).

Identification of candidate EST-derived markers from sunflower LG17

To determine the degree of co-linearity between LG2 of chicory and LG17 of sunflower (predicted to contain the *S*-locus, Gandhi et al. 2005), derived EST markers that mapped to an interval of approximately 60 cM encompassing the self-pollination/self-incompatibility (SP/SI) QTL (the putative *S*-locus QTL) were identified on the various sunflower maps of the CMap database (http://www.sunflower.uga.edu/cmap). Corresponding EST sequences were extracted from the CGP and the HeliaGene databases (http://cgpdb.ucdavis.edu/; http://www.heliagene.org/), using sequence information described in Lai et al. (2005) and Heesacker et al. (2008). Putative *Cichorium* sp. orthologs identified in *Helianthus* sp. were then subjected to a BLASTN search of sequences in the *Cichorium* sp. database (http://www.heliagene.org/cgi/cichorium.cgi) and those with an *E*-value $<10^{-30}$ selected for further analysis. These *Cichorium* sp. unisequences were submitted to reciprocal BLASTN searches against the *Helianthus* sp. database to select only those sequences with the best hit for the *Helianthus* sp. query used for the BLASTN search (minimum 75 % identity over 200 bp). Selected unisequences and primer sequences are given in Online Resource 2.

DNA isolation, PCR conditions and polymorphism detection

The total DNA was extracted from 100 mg of fresh leaves using the GenElute Plant Genomic DNA kit (Sigma-Aldrich). Yield and quality of the extracted DNA were determined using a biophotometer (Eppendorf).

PCR conditions for SSR and STS markers (developed previously for construction of the chicory reference map) and for SCAR markers (developed in this study) were the same as those described in Cadalen et al. (2010). Size and SSCP polymorphism detection were performed on an ABI 3100-Avant Genetic Analyzer sequencer (Applied Biosystems), again according to Cadalen et al. (2010).

For new markers, primer sequences were selected with the Primer3 v 0.4.0 software, with an optimum T_m of 60 °C (Rozen and Skaletsky 2000). Prior to designing primers for *SRK*-like genes and EST-derived markers, the presence and position of putative introns were established using the method of Cadalen et al. (2010). For genes containing introns, primers were designed in regions flanking the predicted intronic sequences. Polymorphism was tested using an HRM assay. PCR reactions were performed in 15 µL containing 1× HRM Master Mix (Roche), 2 mM $MgCl_2$ (Roche), 0.125 µM of each primer (Sigma-Aldrich) and 3 ng of template DNA. Amplifications and melting curves were generated on a Rotor-Gene thermocycler (Corbett) using the following conditions: initial denaturation at 94 °C for 5 min, then 55 PCR cycles of 30 s at 94 °C, 30 s at 60 °C and 30 s at 72 °C, followed by an extension step of 5 min at 72 °C, a denaturation step of 1 min at 95 °C and a cooling step of 1 min at 40 °C. Melting cycle was performed by increasing the temperature from 65 to 95 °C with 25 florescence acquisitions, each of 0.05 s. Melting curves were analyzed with the Rotor-Gene Q series software (v1.7, Corbett).

Data analysis

Seed-set data were arcsine-square root transformed prior to analyses. Analyses of variance were performed using the ANOVA procedure of SAS. Linkage analyses and map calculations were performed with JoinMap v3.0 (Van Ooijen and Voorrips 2001), using genotype nomenclature

adapted to cross-pollination data and setting parameters applied in Cadalen et al. (2010).

Results

Phenotypic analysis in F1′ progenies

Nuclear male sterility

Sexual phenotypes [i.e., male sterile (MS) and fertile hermaphrodite (H)] appeared stable throughout the flowering period for all individuals examined. H plants produced flowers with long anthers and abundant brilliantly white dehiscent pollen, deposited at anthesis on the outer side of the stigma branches and upper part of the style. In contrast, flowers of MS plants exhibited shorter anthers (about two-third of the length of fertile anthers) and visibly lacked pollen.

Alexander's stain of pollen from 40 H plants, including K59, showed a minimum of 85 % viability, based on the ratio of pink to green stained pollen grains, whereas pollen grains were absent in the squashes of anthers of the 41 MS plants examined, including K28 (data not shown). The sexual phenotypes of a total of 359 F1′ individuals were scored visually. A Chi-square test indicated that the segregation observed for sexual phenotype (176 H:183 MS) did not deviate from the 1:1 ratio expected for a nuclear recessive trait determined by a single gene, *NMS1* ($\chi^2 = 0.14$, $df = 1$, $P = 0.71$), indicating that K28 was homozygous (*nms1 nms1*) and K59 heterozygous (*NMS1 nms1*).

Preliminary test of the efficiency of seed set as a discriminator of compatible and incompatible crosses

A comparison of the mean seed-set distribution between H ($n = 39$) and MS ($n = 40$) F1′ individuals, obtained after back-crossing with parental genotypes, showed no significant differences (Mann–Whitney-test, $P = 0.52$), indicating that seed-set data from H and MS plants could be considered as a homogeneous sample. Seed-set values fell into two unambiguous classes (Fig. 1): one with a mean number of full achenes per capitulum of at least 7.6 (seed-set range 0.39–0.92), and a second with a mean number of full achenes per capitulum comprising between 0 and 2.0 (seed-set range 0–0.10). A similar seed-set range (0–0.12) was observed for the selfed H plants, which produced 0–2.2 achenes per capitulum (Fig. 1). Thus a distinction between compatible and incompatible crosses in the K28 × K59 progeny could be unambiguously made from seed-set values; mean seed-set values less than 0.15 were considered as resulting from incompatible crosses and values of at least 0.35 as resulting from compatibles crosses. Crosses producing intermediate numbers of achenes, referred to as pseudo self-compatibility (PSC, Stout 1917, 1918; Pécaut 1962; Eenink 1981; Cichan 1983; Coppens d'Eeckenbrugge and Evrard 1988; Varotto et al. 1995), were not observed for the plants analyzed.

The seed set of a sample of 39 F1′ plants (20 H and 19 MS) and the parental genotypes were determined under open pollination conditions. In contrast to controlled back-crosses, all the individuals tested produced achenes with a mean seed set (0.74 ± 0.14, range 0.44–0.95) (Fig. 1) not significantly different from that obtained for compatible crosses of the same genotypes pollinated with K59 (0.70 ± 0.13, range 0.39–0.92) (ANOVA F-test, $P = 0.07$). In addition, no significant differences between seed set of H and MS plants were observed after open pollination or after compatible back-crosses (ANOVA F-test, $P = 0.34$). In the same way, seed-set values observed for incompatible back-crosses were not significantly different for H and MS plants and were consistent with seed sets obtained for selfed plants (ANOVA F-test, $P = 0.33$). The absence of differences between seed sets of H and MS plants was confirmed by using the larger data set obtained from diallel and factorial crosses. These comparisons indicated that (1) the female fertility of the F1′ progeny depended on pollen compatibility and not on the pollination procedure (open pollination vs. manual pollination) and (2) the *nms1* mutation had no pleiotropic effect on female fertility as estimated by seed-set values.

Back-crosses of 79 F1′ individuals with K59 used as pollen donor resulted in 40 incompatible combinations (22 H, 18 MS) and 39 compatible combinations (17 H, 22 MS). This suggested a single locus segregation for compatibility with K59 pollen and genetic independence between the loci

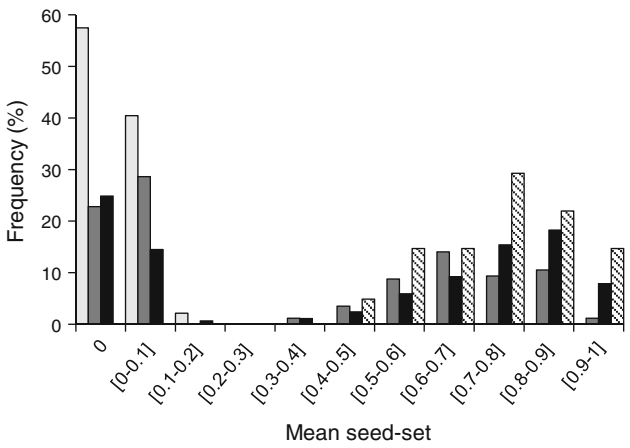


Fig. 1 Distribution of mean seed-set frequencies for open pollinations (*striped bars*, $n = 41$), controlled selfings (*light gray bars*, $n = 47$), back-crosses (*dark gray bars*, $n = 171$) and controlled factorial and diallel crosses (*black bars*, $n = 663$)

involved in male fertility and self-incompatibility ($\chi^2 = 1.03$, $df = 1$, $P = 0.31$). Among the 31 H individuals back-crossed in both directions with K59, and used as pollen donor to pollinate K28, 15 were compatible with K59, whatever the direction of the crosses, and incompatible with K28, the remaining 16 individuals being incompatible with K59 and compatible with K28 (Fig. 2). These back-crosses results indicated that *S*-haplotypes in K59 exhibited the same dominance/co-dominance relationships in pollen and pistil, and that K28 possessed a different allelic combination.

Diallel and factorial crossing analyses

A total of 33 F1′ plants (22 H, 11 MS), used previously for back-crosses, were crossed using diallel or factorial designs, depending on their sexual phenotype. A total of 663 crosses were performed and a complete data set was obtained for 29 F1′ individuals (Table 1; Online Resource 3).

The distribution of seed-set values showed, as for back-crosses, a clear distinction between compatible and incompatible crosses (Fig. 1). The results revealed that F1′ plants and parental genotypes could be classified into five incompatibility groups (Online Resource 3): K28 being the unique member of group A and K59 having the same pollen and pistil phenotype as group B individuals. F1′ plants were classified among the four pistil incompatibility groups independently of their sexual phenotype. Reciprocal crosses between H individuals of groups C and E showed different results, indicating different *S*-haplotype dominance relationships in pollen and pistil for individuals belonging to these groups.

To account for the best genetic model explaining the compatibility groups observed in the F1′ progeny and the parents, all possible models involving a single *S*-locus with two to four haplotypes, and including all possible dominance relationships between alleles in pollen and pistil, were tested. The results could be explained by a sporophytic self-incompatibility system involving four *S*-haplotypes (S_1, S_2, S_3 and S_4) with both parents being heterozygous (K59: S_1S_2; K28: S_3S_4). The four compatibility groups found in the F1′ progeny thus corresponded to a 1:1:1:1 segregation of the four genotypes S_1S_3, S_1S_4, S_2S_3 and S_2S_4 (Table 1).

Pollen behavior of the K59 and F1′ progeny could be explained by dominance of the S_1 allele over S_2, S_3 and S_4, and with S_2 being the most recessive allele (Fig. 3). Because K28 was MS, the dominance relationship between the S_3 and S_4 alleles in pollen was determined with additional crosses performed between seven S_3S_4 plants (pollen donor) obtained from progenies between the D and E groups, and seven individuals representing the 5 pistil compatibility groups. The results are summarized in Table 1, and revealed dominance of allele S_3 over S_4 in pollen. In pistil, the most recessive allele was S_2, and S_1 was dominant over S_2 and S_3, as in pollen (Fig. 3). The differences between pollen and pistil behavior in reciprocal crosses between individuals belonging to groups C and E and groups A and E could be explained by the co-dominance in the pistil of S_1 and S_4, and S_3 and S_4, respectively (Fig. 3; Table 1).

Large-scale phenotyping of SI

As half the F1′ progenies were male sterile, the most efficient strategy to determine the *S*-locus genotype for a maximum number of plants would be to test their pistil behavior by pollinating them with tester genotypes. Examination of the results of the diallel and factorial crosses (Table 1) indicated that pollinating F1′ progenies with H genotypes of groups B and E would be sufficient to identify the four pistil groups segregating in the F1′ progeny. In total, the compatibility patterns of 321 individuals crossed with both tester groups were unambiguously determined. The four genotypes S_1S_3, S_1S_4, S_2S_3 and S_2S_4, segregated as 97:70:83:71 with no significant difference from the 1:1:1:1 ratio expected for the genetic model

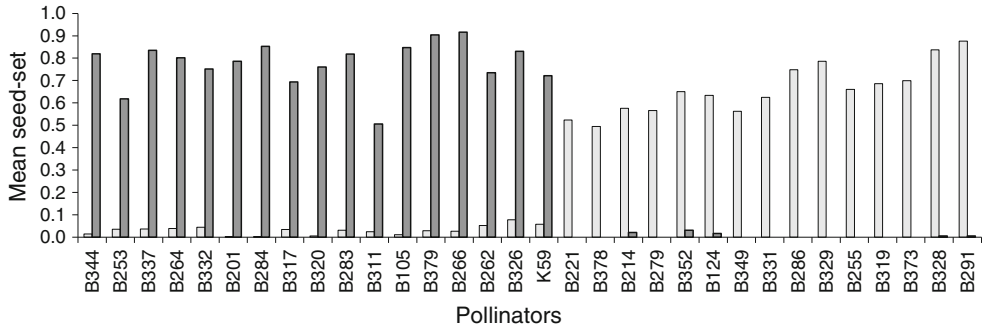


Fig. 2 Mean seed-set values obtained from back-crosses of 31 F1′ individuals (B105–B373) used as pollinators of K59 (*light gray bars*) and K28 (*dark gray bars*)

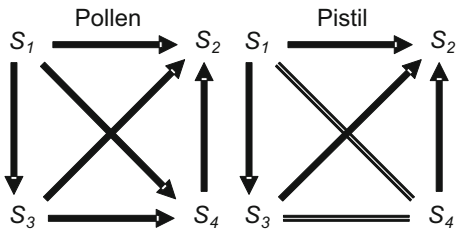


Fig. 3 Dominance relationships in pollen and pistil between the four *S*-haplotypes segregating in the K28 × K59 progeny. *Arrows* indicate dominance and *double lines* indicate co-dominance

deduced from diallel and factorial crosses ($\chi^2 = 5.97$, $df = 3$, $P = 0.1$). Upon selfing, all 158 H plants produced on average fewer than 1.5 achenes per capitulum, thus confirming the self-incompatibility of the F1′ progeny. Furthermore, the segregation data obtained for *NMS1* and *S* (320 individuals) confirmed that both loci were independent ($\chi^2 = 0.381$, $df = 3$, $P = 0.9$).

Mapping of the *NMS1*-locus and *S*-locus on the reference map

To map the *NMS1*-locus and *S*-locus, the K28 × K59 map described in Cadalen et al. (2010) was used. Using this map (174 genotyped individuals), *NMS1*-locus and the *S*-locus were mapped to distal regions of LG5 and LG2, respectively (Fig. 4a). Subsequently, 215 additional F1′ individuals were genotyped for markers flanking both loci. On LG5, the *NMS1*-locus was mapped to a 1.54 cM region between the markers PeroxL1R2 and EU14H12. On LG2, the *S*-locus was mapped to a 1.80 cM interval between the SSR markers EU08B02 and EU12G02 (Fig. 4b).

High-density map of the *NMS1*-locus and *S*-locus

Bulked segregant analysis (BSA) strategies

To increase the density of molecular markers around the *NMS1*-locus, a standard BSA strategy, based on plant phenotypes, was used (Michelmore et al. 1991). DNA of MS and H plants were pooled in four bulks of 14 samples each, bulks B1 and B2 corresponding to MS plants, and bulks B3 and B4 corresponding to H plants.

As 4 *S*-haplotypes with various dominance relationships segregated in the K28 × K59 progeny, a marker-assisted BSA strategy was used to target only markers in the vicinity to the *S*-locus. Eight DNA samples and bulks were generated to find markers specific for the S_1 or S_2 alleles (Fig. 5) and located within the 3.21 cM interval between the EU08B02 and EU03D06 markers (Fig. 4b). Bulks $P1_a$, $P1_b$ and $P2_a$, $P2_b$ were constructed from homozygous individuals S_1S_1 and S_2S_2, respectively. DNA was obtained from rare progenies of the K59 selfings and genotyped for EU08B02 and EU03D06 markers. As the homozygous region is not restricted to this interval, markers related to S_1 or S_2 identified from the bulks P1 and P2 could be localized outside this range. Thus, to target specific markers of S_1 or S_2 in the EU08B02–EU03D06 interval, four additional bulks were constructed from the 389 genotyped F1′ individuals. Bulks P3 and P4 resulted in DNA pools of non-recombinant genotypes in the EU08B02–sw2H09 interval. Bulks P5 and P6 included DNA of individuals presenting one recombination event on the K59 chromosome in the EU03D06–sw2H09 interval (Fig. 5). Consequently, markers specifically linked to the S_1 haplotype should be detected only in bulks P1, P3 and P5, and in K59, and markers specifically linked to the S_2 haplotype should be present only in bulks P2, P4 and P6, and in K59.

AFLP screening of the bulks

DNA bulks B1 to B4, P1 to P6, plus the K28 and K59 parents were screened using the complete set of 256 primer combinations *Eco*RI-ANN and *Mse*I-CNN. A total of 31,000 fragments were generated, and of these 2,350 revealed a polymorphism between K59 and K28.

A total of 18 AFLP markers were found present in B3 and B4 bulks (coupling phase with the wild-type allele) and absent in B1 and B2 bulks (MS). One marker could not be mapped because of bad amplification from F1′ DNA, and four did not map on LG5. The 13 remaining markers were mapped in an interval of 3.6 cM surrounding the *NMS1*-locus (Fig. 4c). Five of them were mapped in an interval of 0.8 cM encompassing the *NMS1*-locus. In addition, for all 359 F1′ plants phenotyped, marker cgat97 showed an absolute co-segregation with the *NMS1*-locus.

The marker-assisted BSA strategy resulted in 14 AFLP markers potentially linked to the *S*-locus, with six and eight markers detected in coupling phase with the S_1 and the S_2 haplotypes, respectively. Two markers could not be mapped because of bad amplifications from F1′ DNA, and six did not map in the region of the *S*-locus. Finally, six markers were mapped on LG2, at an interval of 1 cM encompassing the *S*-locus (Fig. 4c), reducing to about 0.8 cM the region containing the *S*-locus. Two markers (tacg193 and aaca83) were associated in coupling phase with the S_1 haplotype, the remaining four markers (ggat125, tcaa300, ttaa440 and aacc134) being associated with the S_2 haplotype. Marker tcaa300 did show an absolute co-segregation with the *S*-locus ($n = 321$). Markers tacg193 and ggat125 mapped 0.13 cM above the SRR marker EU08B02, which was found as the most distal marker of this end of LG2 in the reference map (Cadalen et al. 2010).

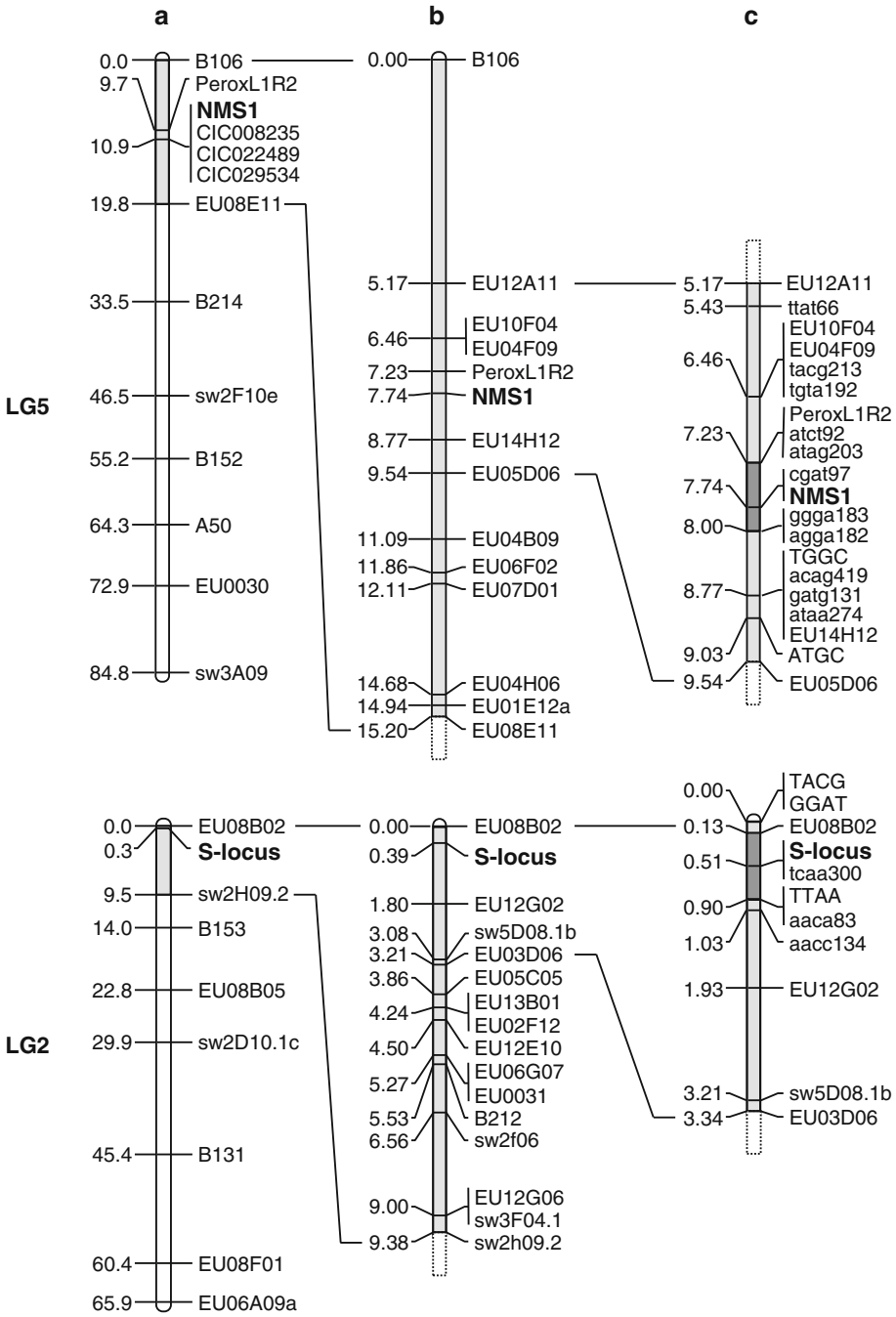
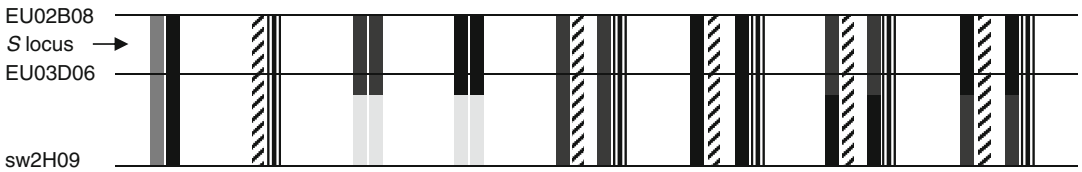


Fig. 4 Genetic mapping of *NMS1*-locus and *S*-locus on LG5 and LG2, respectively, of the K28 × K59 map. **a** Selection of SSR and STS markers covering the complete LG of the reference map ($n = 174$) (Cadalen et al. 2010). The position of the three *SRK*-like derived markers (CIC008235, CIC022489 and CIC029534) mapped on LG5 is indicated. **b** SSR and STS makers flanking the *NMS1* and *S*-loci ($n = 389$). **c** Chromosomal segments including AFLP (*lower-case*) and SCAR derived markers (*capital*) identified in this study ($n = 389$). Cumulative map distances (cM) are indicated on the *left* of the linkage groups

Transformation of AFLP markers into SCAR markers

A mini-sequencing procedure (Brugmans et al. 2003; Paris et al. 2012) was undertaken to reduce the complexity of AFLP profiles for four fragments linked to the *NMS1*-locus (atag203, agga182, tggc219 and atgc311) and five fragments linked to the *S*-locus (tacg293, ggat125, tcaa300, ttaa440 and aacc134). Highly linked fragments cgat97 (*NMS1*-locus) and aaca83 (*S*-locus) were not considered in view of their small sizes. The mini-sequencing procedure was successful for eight AFLP fragments (all but atag203) and allowed the isolation and sequencing of fragments of the expected size. In addition, the AFLP profile simplification for markers ggat125 (S_2-haplotype) and tacg293 (S_1-haplotype) revealed the presence of a second fragment associated with the S_1, S_3 and S_4 haplotypes (ggat128) or the S_2, S_3 and S_4 haplotypes (tacg294). These fragments were also excised from acrylamide gels, cloned and sequenced. Fragments ggat125 and ggat128 differed only by an insertion of 3 bp, whereas fragments tacg293 and tacg294 showed a 1 bp InDel polymorphism and nine SNPs, allowing the distinction of the four segregating



Bulks and parents	K59	K28	$P1_a$ $P1_b$	$P2_a$ $P2_b$	P3	P4	P5	P6
Number of DNA samples/bulks			n = 8	n = 15	n = 13	n = 9	n = 9	n = 13
S-locus genotype	S_1S_2	S_3S_4	S_1S_1	S_2S_2	S_1S_3+S_1S_4	S_2S_3+S_2S_4	S_1S_3+S_1S_4	S_2S_3+S_2S_4

Fig. 5 Marker-assisted BSA strategy for the identification of *S*-locus linked AFLP markers. Each parental chromosome corresponding to LG2 is represented by a singular graphical genotype (*dark gray* and *black* for K59, *oblique* and *vertical stripes* for K28). Corresponding genotypes in bulks are represented by the same graphical features. In P1 and P2 bulks, *light gray* segments corresponded to indeterminate genotypes. $P1_a$ and $P1_b$ as well as $P2_a$ and $P2_b$ correspond to duplicated bulks of S_1S_1 and S_2S_2 genotypes, respectively. In P5 and P6 bulks, one recombination event between markers EU03D06 and sw2H09 in the chromosome of K59 is indicated

S-haplotypes. The sequences of the AFLP fragments obtained during this study were deposited at NCBI as accession numbers GF112132 to GF112141 (Table 2).

For the eight AFLP fragments, primer pairs were designed and size, SSCP or HRM polymorphisms were screened for in the F1′ progeny (Table 2). SCAR markers were renamed AGGA, TGGC and ATGC (*NMS1* locus), and TACG, TCAA, TTAA, AACC and GGAT (*S*-locus). For the *NMS1*-locus, the AGGA marker showed no polymorphism. SCAR markers TGGC and ATGC revealed a size and HRM polymorphism, respectively, and co-segregated with AFLP fragments tggc219 and atgc311 (Table 2; Fig. 4c).

For the *S*-locus, the AACC marker turned out to be monomorphic. A multiple band profile was obtained for the TCAA marker, but no polymorphism associated with *S*-haplotypes was detected. SSCP polymorphism was detected for the TTAA marker with four alleles associated with the four *S*-haplotypes. Similarly, HRM analysis revealed four groups for the TACG marker that were associated with the four *S*-haplotype combinations present in the K28 × K59 progeny (Fig. 4c). The size polymorphism previously observed for the AFLP markers ggat125/128 was preserved with two corresponding alleles for the GGAT marker (Table 2).

Mapping of *SRK*-like sequences and EST-derived makers from LG17 of sunflower

From the *Cichorium* sp. EST database, ten unisequences were selected on the basis of their highest homology and continuous alignment with the *S*-domain containing genes *ARK3* and *AlSRK13* of the self-incompatible species *A. lyrata* (Online Resource 1). The highest homologies with both genes were found at the protein level for the unisequence CIC008235 (>75 % similarity), followed by CIC029534 and CIC023810 (>70 % similarity). Similarity ranges of 55–70 % were found for the seven remaining sequences (CIC006729, CIC022489, CIC029017, CIC030577, CIC019748, CIC013468 and CIC024179). PCR products were obtained for the ten markers in the K28 × K59 progeny, and the amplification products of six of them showed HRM polymorphisms. Gene markers were mapped on LG1 (CIC013468), LG3 (CIC030577), LG4 (CIC006729) (Online Resource 4) and LG5 (CIC008235, CIC022489 and CIC029534; Fig. 4a). Thus, none of these gene markers mapped on LG2. On LG5, the three markers CIC008235, CIC022489 and CIC029534 showed absolute co-segregation among the 174 individuals genotyped. As no overlap was found between the three sequences, it was not possible to determine whether they corresponded to different regions of the same gene or that they represented up to three tandemly repeated genes. Surprisingly, these three markers also co-segregated with *NMS1*, representing the closest gene markers found for this locus (Fig. 4a).

The degree of co-linearity between LG2 of chicory and LG17 of sunflower was assayed by developing chicory EST-derived markers from sunflower map data (designed as HT markers). Five HT markers of the NMS373 × ANN1811 BC1 LG17 map were selected. They were mapped in an interval of about 60 cM (markers ORS561-bottom end of LG) encompassing the major QTLs found for SP/SI (CMap database: http://www.sunflower.uga.edu/cmap; Gandhi et al. 2005). An additional set of 20 HT markers were selected from the sunflower RHA280 × RHA801 RIL map of the CMap database, on the basis of their location on the same chromosomal segment, representing about 40 cM (Fig. 6; Online Resource 2). Significant homologies with *Cichorium* sp. unisequences were found for 21 *Helianthus* sp. sequences. Sixteen of them were considered as putative orthologs, on the basis of

Table 2 AFLP and derived SCAR markers linked to the *NMS1*-locus and *S*-locus

GeneBank Id	AFLP	Coupling phase	SCAR	Polymorphism	No. of alleles	Allele specificity	Size (bp)	Forward primer 5′ → 3′	Reverse primer 5′ → 3′
GF112141	ggat125	S_2	GGAT	InDel	2	$S_2/S_1\ S_3\ S_4$	93	ATTCAGGTGCAGGAGGACAT	CAAGTCAGCCTCCCAAACAT
GF112137	ggat128	$S_1/S_3/S_4$					96		
GF112134	tacg293	S_2	TACG	HRM	4	$S_1/S_2\ S_3\ S_4$	191	TCCCTTCAATGAGTCGATGT	TGGAATAAATTCAGCCATCCT
GF112133	tacg294	$S_1/S_3/S_4$					191		
GF112140	tcaa300	S_2	TCAA	No	Multiband profile		272	AATTCATCGCAAGGAGGTCA	GGGGTGTTGTTGGTAGCATT
GF112136	ttaa440	S_2	TTAA	SSCP	4	$S_1/S_2/S_3/S_4$	378	CAATGCGTGCCTTTTGTATG	CAACCAAATCATCTCTTCTCTCTC
GF112135	aacc134	S_2	AACC	No	1		109	ACCCCCAAATTTCAGGTTTC	CAAAATAGTTCAGGTGACTTACGC
GF112132	agga182	*NMS1*	AGGA	No	1		150	AACAATGAAAATAGATGGCGAGT	CGAGACGAGCCGTTTATCA
GF112138	tggc219	*NMS1*	TGGC	InDel	2	*NMS1*/*nms*1	118	CCTTGGAATAATGGTTTTCACA	CCCCTTCTACAAGCAATCT
GF112139	atgc311	*NMS1*	ATGC	SSCP	2	*NMS1*/*nms*1	139	TTGTTCTTATTCTTTCTTGCTTGC	GAAATACATGTTAGACATATGAAAAA

AFLP marker names were defined using the second and third selective bases of *Eco*RI and *Mse*I primers, followed by the fragment size. Only selective bases in capital letters were conserved to name SCAR markers

reciprocal best-hit tests and showed 77–86 % identity between *Cichorium* sp. and *Helianthus* sp. *Cichorium* sp. sequences (cHT markers) were used for PCR primer design and polymorphism test (Online Resource 2).

High-resolution melting polymorphism was detected for 13 cHT markers in the K28 × K59 progeny. Mapping data allowed their assignment to four linkage groups (LG3, LG6, LG7 and LG8), with one to five markers mapping on each linkage group (Fig. 6; Online Resource 4). These results indicated that none of the markers mapped to LG2. In addition, a shared macrosynteny between a large fragment of sunflower LG17 and a unique chicory linkage group seems not to be the case. However, on a smaller scale, segments of chicory LG3, LG6 and LG7 could be related to the sunflower LG17 targeted region. The three cHT markers mapped to chicory LG3 were spread over a segment of about 19 cM in the central region of this linkage group; cHT758 and cHT122 were separated by 0.7 cM. A similar configuration was found in sunflower maps, as these markers were located in an interval of 10–25 cM, depending on the map, and with HT758 and HT122 separated by less than 1 cM in the RHA280 × RHA801 RIL map. On LG6, the five cHT markers mapped were located on one end, with cHT289, cHT719 and cHT538 showing absolute co-segregation at position 0.3 cM and cHT230 and cHT109 at position 11.5 cM. Markers HT230 and HT109 also co-segregated in sunflower. The corresponding interval in sunflower maps represented 4–29 cM and corresponded, as in chicory, to one end of the linkage group. From the four cHT markers mapped on LG7, two showed co-segregation (cHT272 and cHT254) as they did in sunflower. In addition, these markers defined one of the ends of LG7, mapping distal to marker EU05D03 in the reference chicory map. Markers cHT78 and the pair cHT272 and cHT254 mapped 13.8 cM apart. The corresponding segment in sunflower LG17 represented 10–25 cM and was located in the central part of LG17.

Discussion

As a first step to positional cloning of genes regulating NMS and SSI in chicory, high-density genetic maps were constructed for the *NMS1*-locus and the *S*-locus, respectively. This allowed us to map the *NMS1*-locus to a 0.8 cM region on LG5 and the *S*-locus to a 0.8 cM region on LG2. Candidate genes encoding proteins similar to SRK (the female determinant of SSI in the Brassicaceae) were also mapped, but none of them mapped close to the chicory *S*-locus. Similarly, the mapping of molecular markers corresponding to markers in the vicinity of the predicted *S*-locus on LG17 of sunflower showed no linkage with the *S*-locus in chicory.

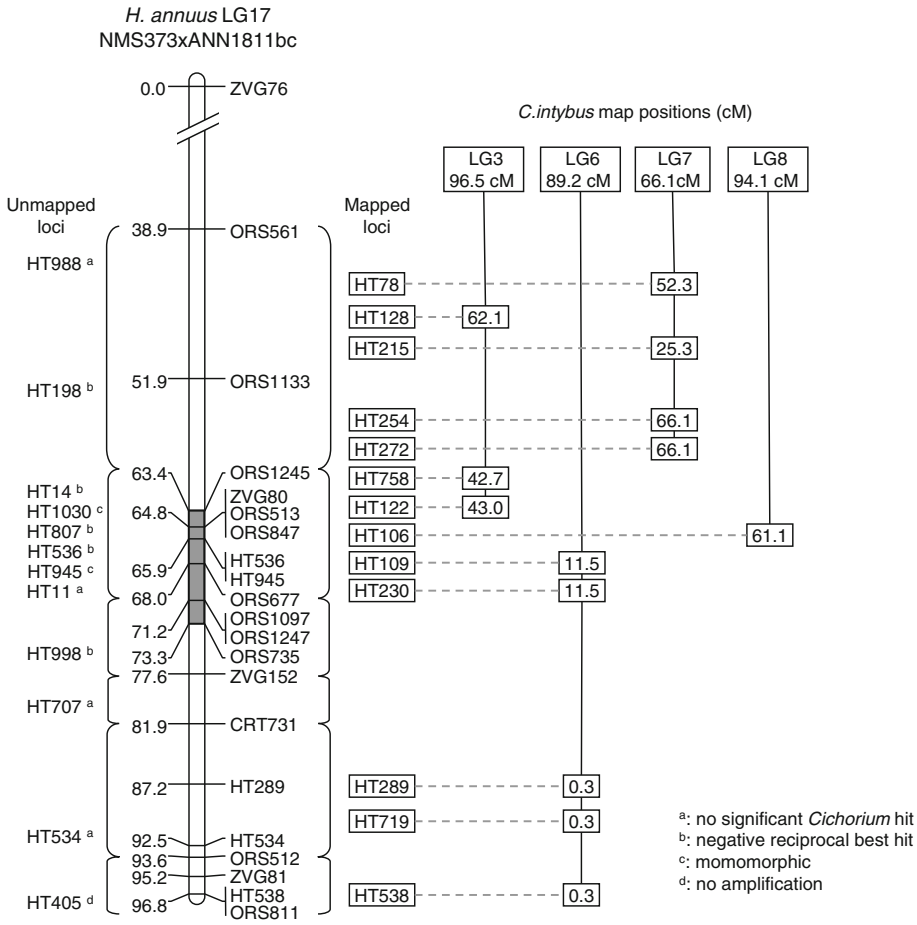


Fig. 6 Mapping of derived EST markers (HT markers) of sunflower LG17 on chicory linkage groups. HT markers were positioned on both sides of LG17 according to their relative position to common markers of NMS373 × ANN1811 BC1 and RHA280 × RHA801 RIL maps (http://www.sunflower.uga.edu/cmap). HT markers with the same map position are listed on the *same line*. HT markers positioned on the *left* side of LG17 correspond to unmapped loci in chicory; those positioned on the *right* side of LG17 were mapped in chicory and their position in chicory linkage groups is indicated in *squared boxes* linked to marker names. Total map distance of each chicory linkage group is indicated below the LG number. The *gray box* on sunflower LG17 indicates the interval encompassing the SP/SI QTL in the NMS373 × ANN1811 BC1 map (Gandhi et al. 2005)

The K28 × K59 progeny as material of choice for genetic studies on SI

The K28 × K59 progeny used in this study allowed a clear distinction to be made between incompatible and compatible crosses because their seed-set scores (the ratio between the number of seeded achenes produced and the number of ovules per capitulum available) showed a bimodal distribution (Fig. 1). In incompatible crosses seed set was less than 0.15, whereas in compatible crosses seed set exceeded 0.35. With a mean of 18 ovules per capitulum (18 ± 1, $n > 1{,}000$), these results were consistent with previous observations in witloof chicory (Eenink 1981) and Italian red chicory (Varotto et al. 1995) where the authors considered crosses as incompatible when they resulted in less than three achenes per capitulum and as compatible when at least six achenes per capitulum were produced. However, in contrast to these and other studies in chicory (e.g., Stout 1917, 1918; Pécaut 1962; Cichan 1983; Coppens d'Eeckenbrugge and Evrard 1988), as well as in other Asteraceae species (Cheptou et al. 2000; Brennan et al. 2005; Ferrer and Good-Avila 2007; Crawford et al. 2010), no PSC was observed among the K59 × K28 progeny. This plant material can thus be considered as highly self-incompatible, making it well suited to study the genetics of SI in chicory. Moreover, the segregation of NMS in the K28 × K59 progeny was very valuable, since it eliminated the possibility of selfing in the K28 maternal plant, precluding any need for difficult and laborious emasculation procedures.

Genetic determination of NMS and SI

The Mendelian segregation of compatibility phenotypes in the K28 × K59 progeny allowed us to assign genetic determination of SI to a single *S*-locus and identify four segregating *S*-haplotypes and their dominance relationships. This further confirms the sporophytic inheritance of SI in chicory previously shown by Eenink (1981) and Varotto et al. (1995). Resolving the pollen and pistil dominance relationships also allowed the identification of a small number of pollen testers that could be used for large-scale phenotyping of MS and H individuals.

Similarly, phenotype segregations of MS versus H revealed a single locus with a recessive allele underlying NMS, confirming the previous report on male-sterile

'Edith' (Desprez et al. 1994). More importantly, NMS segregated independently of the *S*-locus, and showed no pleitropic effect on female functions or on incompatibility phenotypes. The latter suggests that NMS can also safely be used as a tool for studies of the genetic basis of PSC in chicory.

Fine mapping of the *NMS1*-locus and *S*-locus as a first step to positional cloning of the underlying genes

Initial large-scale phenotyping allowed mapping of the *NMS1*-locus to a 1.54 cM region on LG5 and the *S*-locus in a 1.80 cM region on LG2. Considering a genome coverage of 800–1,100 cM (Cadalen et al. 2010) and the physical size of the *C. intybus* genome being 1,400–1,500 Mb (P. Devaux, personal communication, Koopman 2002), a 1.3–1.9 Mb/cM relationship can be estimated to exist at the whole genome level. Assuming this estimate holds true for the regions containing the *NMS1*-locus and *S*-locus, they would represent physical lengths exceeding 2 Mb. To facilitate fine mapping and positional cloning of these loci via screening two available BAC libraries for chicory (Gonthier et al. 2010), a higher density of markers around the *S*-locus and *NMS1*-locus was desirable.

The standard BSA strategy (Michelmore et al. 1991) coupled with AFLP adopted for the *NMS1*-locus has been widely used in plants and animals to find markers linked to a gene or QTL of interest (Xiao et al. 2007; Wei et al. 2009; Zhang et al. 2009; de Brito et al. 2010). The marker-assisted BSA strategy adopted for the *S*-locus is less common (Meksem et al. 1995; Cnops et al. 1996; Lahaye et al. 1998; Jordan et al. 2006), but is more efficient when more than two alleles are involved in the phenotype under analysis. Obviously, markers flanking the locus of interest and large segregating progenies with individuals representing recombination events in a region encompassing only a few centimorgan are needed to construct the bulks. When these conditions are fulfilled, however, it allows a quick identification of interesting markers among the large number of polymorphic markers detected by the AFLP technique.

Both these strategies, phenotypic BSA and marker-assisted BSA, resulted in an increased density of markers and reduced the marker intervals around both the *NMS1*-locus and the *S*-locus to 0.8 cM, and thus to a physical distance of 1–1.5 Mb, if the whole genome Mb/cM ratio applied to these regions. This means that map-based cloning of the *NMS1*-locus and *S*-locus will be feasible, provided that they are not in regions in which recombination is suppressed, for instance in centromeric regions. Karyotype analyses indicate that chromosomes in chicory are meta- to sub-metacentric (Haque and Godward 1984; Ma et al. 1984; Rambaud et al. 1992; Doré et al. 1996; Ge et al. 2002). As both *NMS1* and *S* mapped to distal LG positions, and the reference genetic map is close to saturation (Cadalen et al. 2010), it is unlikely that they are located in centromeric regions.

Suppression of recombination is expected for the *S*-locus region, because any recombination between the genes encoding male and female determinants would lead to the breakdown of SI (Castric and Vekemans 2004; Uyenoyama 2005) and evidence from studies of other *S*-loci shows that there is indeed extensive repression of recombination (Kawabe et al. 2006; Goubet et al. 2012). The *S*-locus of *Petunia inflata*, and possibly also in *Papaver rhoeas*, has a sub-centromeric location (Wang et al. 2003; Wheeler et al. 2003), whereas in *Antirrhinum hispanicum*, *Ipomoea trifida* or *Brassica oleracea* the *S*-locus mapped to a telomeric region (Casselman et al. 2000; Suzuki et al. 2004; Yang et al. 2007). In *Petunia inflata* and *Ipomoea trifida*, the relative Mb/cM ratios were reported to be between 10 and 20 times higher in the *S*-locus region than for the entire genome, i.e., 17.6 Mb/cM vs 0.9 Mb/cM and 2.3 Mb/cM vs 0.2 Mb/cM, respectively (Wang et al. 2003; Suzuki et al. 2004). In contrast, in *Brassica oleracea* there was no difference in recombination frequencies between the *S*-locus region and the whole genome (0.4 Mb/cM), although at the *S*-locus itself suppression of recombination was observed (Casselman et al. 2000). The variability of Mb/cM ratios reported for *S*-locus regions makes it difficult to estimate the time and number of steps required to physically map (by chromosome walking) the region between the markers defining the 0.8 cM region containing the *S*-locus.

Mapping of *SRK*-like sequences

SSI has so far been described in Asteraceae, Betulaceae, Caryophyllaceae, Polemoniaceae, Convolvulaceae and Brassicaceae (Allen and Hiscock 2008), but only in the latter family have the *S*-locus genes encoding the male and female determinants (*SCR/SP11* and *SRK*, respectively) been identified (Stein et al. 1991; Schopfer et al. 1999; Suzuki et al. 1999; see for review Iwano and Takayama 2012). As a potential route to identifying the female determinant of SSI in Convolvulaceae and Asteraceae, Kowyama et al. (1996) and Tabah et al. (2004) used a candidate gene approach to screen stigma RNAs of *Ipomoea trifida* (Convolvulaceae) and *S. squalidus* (Asteraceae), respectively, for expression of *SRK*-like gene sequences. In both cases, it was concluded (from an absence of stigma-specific expression and specific *S*-allele association) that the *SRK*-like genes identified were unlikely to be involved in SSI (Kowyama et al. 1996; Tabah et al. 2004). This was later confirmed for *Ipomoea* when the *S*-locus was mapped and no *SRK*-like

(or *SCR/SP11*-like) sequences were found at the *S*-locus (Rahman et al. 2007b). In *S. squalidus*, a full-length cDNA (*SSRLK1*) and two partial cDNAs (*SSRLK2* and *SSRLK3*) encoding members of the *S*-domain receptor kinase subfamily were obtained, but none were polymorphic or associated with specific *S*-alleles (Tabah et al. 2004).

To test the conclusion of Tabah et al. (2004) that *SRK*-like genes were not involved in SSI in the Asteraceae, we decided to increase the number of *SRK*-like candidate genes by screening the large collection of *Cichorium* sp. ESTs available (see "Materials and methods") and locate their corresponding genes on the K28 × K59 map. The *SRK*-like *Cichorium* sp. unisequences were selected on the basis of their homology with *ARK3* and *SRK* of the self-incompatible species *A. lyrata*. Of the ten *SRK*-like sequences selected, six could be mapped to four linkage groups of the K28 × K59 map (Online Resource 4), but none of them mapped to LG2 (the location of the *S*-locus). PCR products for the four remaining sequences showed no polymorphisms, despite the segregation of four *S*-haplotypes in the K28 × K59 descendants. As genes determining SI are expected to be highly polymorphic, we concluded that these genes were also unlikely to encode the female determinant of SSI. This means that despite the enlarged number of *SRK*-like genes analyzed, our study supported the conclusion of Tabah et al. (2004) that an *SRK*-like gene was not the female determinant of SSI in the Asteraceae and that this family, like Convolvulaceae, had a different molecular mechanism of SSI from that of Brassicaceae.

Interestingly, three of the *SRK*-like gene markers were found to co-segregate with the *NMS1*-locus on LG5. Since the targeted sequences did not overlap, it was not possible to determine whether they corresponded to the same gene or represented different genes. To our knowledge, there are no examples of *S*-domain *RLK* genes involved in male sterility. However, in *Arabidopsis* and rice, *RLK* genes belonging to the *LRR*- (Leucine-rich repeat) and *lectin-RLK* gene subfamilies have been shown to be required for normal anther development and their impaired expression alone, or in combination, resulted in male sterility (Canales et al. 2002; Zhao et al. 2002; Nonomura et al. 2003; Albrecht et al. 2005; Colcombet et al. 2005; Hord et al. 2006, 2008; Mizuno et al. 2007; Wan et al. 2008). A combination of genetic analyses in large segregating populations and expression analyses is required to determine whether or not a mutation in an *S-domain RLK* gene underlies *nms1* male sterility in chicory.

Mapping of the sunflower self-incompatibility/self-pollination QTL

In sunflower, two research groups claim to have mapped the putative *S*-locus (Gandhi et al. 2005; Yue et al. 2009). Based on seed-set numbers obtained by manual selfings among BC1 progeny of the cross NMS373 × ANN1811, Gandhi et al. (2005) inferred two unambiguous genotypic classes corresponding to heterozygous self-incompatible genotypes (*Ss*) and homozygous self-fertile genotypes (*ss*). The observed Mendelian (1:1) segregation was attributed to segregation of *S*-haplotypes, considering a loss-of-function mutation at the *S*-locus in the self-fertile cultivated genotype NMS373, and the presence of a functional *S*-haplotype in the wild *H. annuus* individual (ANN1811) used as progenitor. Similarly, Yue et al. (2009) found a Mendelian segregation (1:3) for seed set upon self-pollination in the progeny of a cross between an oilseed sunflower inbred maintainer line, HA89, and a confection sunflower genotype (LSS) introduced from China, and mapped the corresponding locus close to the putative *S*-locus of Gandhi et al. (2005).

Assuming a unique determinism of SSI in Asteraceae, as documented for Brassicaceae (Sherman-Broyles and Nasrallah 2008), the sunflower claims prompted us to test the possible correspondence of the proposed sunflower LG17 *S*-locus and the *S*-locus in chicory, by trying to localize the sunflower LG17 chromosomal region containing the putative *S*-locus on the chicory map. Molecular HT markers in the region harboring this locus in sunflower were converted into markers specific for chicory (cHT), but none of the selected 13 cHT markers mapped to chicory LG2. The location and order of these markers on LG17 was confirmed in the recently published sunflower high-density genetic map (Bowers et al. 2012).

Three possible explanations may account for the lack of a correspondence between the sunflower chromosomal region containing the proposed *S*-locus and the chicory *S*-locus. A first possibility is that the markers we mapped do not represent orthologs of the sunflower markers, even though the procedure for their selection was stringent, including a reciprocal best-hit test. The power of our approach could be limited by the fact that *Helianthus* sp. and *Cichorium* sp. EST databases are not exhaustive and that the distinction between orthologs and paralogs is complicated by the polyploid ancestry of the Asteraceae family (Funk et al. 2005; Barker et al. 2008), as well as by an extra genome duplication in members of the Heliantheae subfamily after its separation from other Asteraceae subfamilies, among which is the Cichorioideae (Barker et al. 2008).

A second explanation is that the number of markers that we mapped was too low to detect common chromosomal segments between chicory LG2 and sunflower LG17. Extensive chromosomal rearrangements have been described in annual sunflower species (Burke et al. 2004; Lai et al. 2005) and it may be that only a small chromosomal fragment containing the *S*-locus has been conserved.

Indeed, it was not possible to establish shared synteny between a large fragment of sunflower LG17 and a unique chicory linkage group. On a smaller scale, however, distal segments of LG6 and LG7 and a central region of LG3 in chicory ($n = 9$) may represent traces of reshuffling of the targeted LG17 region in sunflower ($n = 17$).

A third explanation is that the sunflower QTL on LG17 does not define the *S*-locus. Neither of the mapping studies performed on sunflower (Burke et al. 2002; Gandhi et al. 2005; Wills and Burke 2007; Yue et al. 2009) was designed explicitly to characterize and map the *S*-locus, as exemplified by the lack of any diallel crossing analyses that would have allowed characterization of *S*-allele segregation and pistil and pollen dominance relationships. In fact, the trait analyzed addressed only the genotype's ability to produce seed upon self-pollination, and mechanisms that result in the appearance of complete or partial self-fertile genotypes in a self-incompatible species are variable and complex and could be the result of mutations not linked to the *S*-locus (e.g., Murase et al. 2004; Vilanova et al. 2006; Isokawa et al. 2010; Cachi and Wünsch 2011; Zuriaga et al. 2012). For instance, mutations leading to self-fertility can occur in non *S*-locus genes involved in the signaling cascade that leads to the rejection of pollen, in genes regulating/modifying the expression of *S*-genes or affecting the turnover of their products (see for review Good-Avila et al. 2008).

In our opinion, the first explanation is the least probable, as it is unlikely that none of the 13 markers we mapped represented an ortholog of the sunflower markers linked to the proposed sunflower *S*-locus. An answer to the two other explanations, however, has to await an unambiguous identification of the sunflower *S*-locus (diallel analysis) and genomic comparison of the chromosomal region controlling the SSI determinism in both species. This can be envisaged once the sequencing of the sunflower genome is completed (Kane et al. 2011) and we have realized the positional cloning of the chicory *S*-locus.

Conclusion

Taken together, the results obtained in this study represent an important step forward toward the positional cloning of genes underlying NMS and SSI in chicory. Our results offer already the possibility of applying MAS to these important traits in chicory breeding programs. In addition to providing a valuable resource for future analyses of genes underlying these important reproductive traits, our new data for the chicory *S*-locus add significantly to the assertion that the Asteraceae possess a novel system of SSI that is different from the well-characterized system of the Brassicaceae (Hiscock and Tabah 2003; Allen et al. 2011). These results therefore also indicate that, in the absence of obvious candidates, positional cloning of the *S*-locus is probably the most appropriate strategy to identify its genes encoding the male and female determinants.

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References

Albrecht C, Russinova E, Hecht V, Baaijens E, de Vries S (2005) The *Arabidopsis thaliana* SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASES1 and 2 control male sporogenesis. Plant Cell 17:3337–3349. doi:10.1105/tpc.105.036814

Alexander MP (1969) Differential staining of aborted and nonaborted pollen. Biotech Histochem 44(3):117–122. doi:10.3109/10520296909063335

Allen AM, Hiscock SJ (2008) Evolution and phylogeny of self-incompatibility systems in angiosperms. In: Franklin-Tong VE (ed) Self-incompatibility in flowering plants—evolution, diversity, and mechanisms. Springer, Berlin, pp 73–101. doi:10.1007/978-3-540-68486-2

Allen AM, Thorogood CJ, Hegarty MJ, Lexer C, Hiscock SJ (2011) Pollen–pistil interactions and self-incompatibility in the Asteraceae: new insights from studies of *Senecio squalidus* (Oxford ragwort). Ann Bot 108(4):687–698. doi:10.1093/aob/mcr147

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25(17):3389–3402. pii: gka562

Barker MS, Kane NC, Matvienko M, Kozik A, Michelmore RW, Knapp SJ, Rieseberg LH (2008) Multiple paleopolyploidizations during the evolution of the Compositae reveal parallel patterns of duplicate gene retention after millions of years. Mol Biol Evol 25(11):2445–2455. doi:10.1093/molbev/msn187

Bowers JE, Bachlava E, Brunick RL, Rieseberg LH, Knapp SJ, Burke JM (2012) Development of a 10,000 locus genetic map of the sunflower genome based on multiple crosses. G3 2(7):721–729. doi:10.1007/s11103-012-9908-z

Brennan AC, Harris SA, Hiscock SJ (2005) Modes and rates of selfing and associated inbreeding depression in the self-incompatible plant *Senecio squalidus* (Asteraceae): a successful colonizing species in the British Isles. New Phytol 168(2):475–486. doi:10.1111/j.1469-8137.2005.01517.x

Brugmans B, van der Hulst RGM, Visser RGF, Lindhout P, van Eck HJ (2003) A new and versatile method for the successful conversion of AFLP markers into simple single locus markers. Nucleic Acids Res 31(10):e55. doi:10.1093/nar/gng055

Budar F, Pelletier G (2001) Male sterility in plants: occurrence, determinism, significance and use. C R Acad Sci III 324(6): 543–550. doi:10.1016/s0764-4469(01)01324-5

Burke JM, Tang S, Knapp SJ, Rieseberg LH (2002) Genetic analysis of sunflower domestication. Genetics 161(3):1257–1267

Burke JM, Lai Z, Salmaso M, Nakazato T, Tang S, Heesacker A, Knapp SJ, Rieseberg LH (2004) Comparative mapping and rapid karyotypic evolution in the genus *Helianthus*. Genetics 167(1):449–457. doi:10.1534/genetics.167.1.449

Cachi AM, Wünsch A (2011) Characterization and mapping of non-*S* gametophytic self-compatibility in sweet cherry (*Prunus avium* L.). J Exp Bot 62(6):1847–1856. doi:10.1093/jxb/erq374

Cadalen T, Morchen M, Blassiau C, Clabaut A, Scheer I, Hilbert JL, Hendriks T, Quillet MC (2010) Development of SSR markers and construction of a consensus genetic map for chicory (*Cichorium intybus* L.). Mol Breed 25(4):699–722. doi:10.1007/s11032-009-9369-5

Canales C, Bhatt AM, Scott R, Dickinson H (2002) EXS, a putative LRR receptor kinase, regulates male germline cell number and tapetal identity and promotes seed development in *Arabidopsis*. Curr Biol 12(20):1718–1727. doi:10.1016/s0960-9822(02)01151-x

Casselman AL, Vrebalov J, Conner JA, Singhal A, Giovannoni J, Nasrallah ME, Nasrallah JB (2000) Determining the physical limits of the *Brassica S* locus by recombinational analysis. Plant Cell 12(1):23–33. doi:10.1105/tpc.12.1.23

Castaño CI, Demeulemeester MAC, De Proft MP (1997) Incompatibility reactions and genotypic identity status of five commercial chicory (*Cichorium intybus* L.) hybrids. Sci Hortic 72(1):1–9. doi:10.1016/S0304-4238(97)00111-8

Castric V, Vekemans X (2004) Plant self-incompatibility in natural populations: a critical assessment of recent theoretical and empirical advances. Mol Ecol 13(10):2873–2889. doi:10.1111/j.1365-294X.2004.02267.x

Charlesworth D (2002) Plant sex determination and sex chromosomes. Heredity 88:94–101. doi:10.1038/sj/hdy/6800016

Chen JF, Hu JG, Vick BA, Jan CC (2006) Molecular mapping of a nuclear male-sterility gene in sunflower (*Helianthus annuus* L.) using TRAP and SSR markers. Theor Appl Genet 113(1): 122–127. doi:10.1007/s00122-006-0278-2

Cheptou PO, Imbert E, Lepart J, Escarre J (2000) Effects of competition on lifetime estimates of inbreeding depression in the outcrossing plant *Crepis sancta* (Asteraceae). J Evol Biol 13(3):522–531

Chuck G (2010) Molecular mechanisms of sex determination in monoecious and dioecious plants. In: Kader J-C, Delseny M (eds) Advances in botanical research, vol 54. Academic Press, London, pp 53–83. doi:10.1016/S0065-2296(10)54002-3

Cichan MA (1983) Self fertility in wild populations of *Cichorium intybus* L. Bull Torrey Bot Club 110(3):316–323

Cnops G, denBoer B, Gerats A, VanMontagu M, VanLijsebettens M (1996) Chromosome landing at the *Arabidopsis TORNADO1* locus using an AFLP-based strategy. Mol Gen Genet 253(1–2):32–41. doi:10.1007/s004380050293

Colcombet J, Boisson-Dernier A, Ros-Palau R, Vera CE, Schroeder JI (2005) *Arabidopsis* SOMATIC EMBRYOGENESIS RECEPTOR KINASES1 and 2 are essential for tapetum development and microspore maturation. Plant Cell 17(12):3350–3361. doi:10.1105/tpc.105.036731

Coppens d'Eeckenbrugge G, Evrard B (1988) Aspects quantitatifs de l'auto-incompatibilité chez la chicorée witloof (*Cichorium intybus* L.). II. Distribution des akènes entre capitules autopollinisés d'une même plante. Rev Agric 41(1):59–62

Coppens d'Eeckenbrugge G, Evrard B, Louant B-P (1987) Aspects quantitatifs de l'auto-incompatibilité chez la chicorée witloof (*Cichorium intybus* L.). I. Relation entre fertilité et autofertilité. Rev Agric 40(1):35–40

Crawford DJ, Archibald JK, Kelly JK, Mort ME, Santos-Guerra A (2010) Mixed mating in the 'obligately outcrossing' *Tolpis* (Asteraceae) of the Canary Islands. Plant Species Biol 25(2):114–119. doi:10.1111/j.1442-1984.2010.00275.x

Dauchot N, Mingeot D, Purnelle B, Muys C, Watillon B, Boutry M, Van Cutsem P (2009) Construction of 12 EST libraries and characterization of a 12,226 EST dataset for chicory (*Cichorium intybus*) root, leaves and nodules in the context of carbohydrate metabolism investigation. BMC Plant Biol 9:14. doi:10.1186/1471-2229-9-14

de Brito GG, Caixeta ET, Gallina AP, Zambolim EM, Zambolim L, Diola V, Loureiro ME (2010) Inheritance of coffee leaf rust resistance and identification of AFLP markers linked to the resistance gene. Euphytica 173(2):255–264. doi:10.1007/s10681-010-0119-x

de Nettancourt D (2001) Incompatibility and incongruity in wild and cultivated plants, 2nd edn. Springer, Berlin

Decout E, Dubois T, Guedira M, Dubois J, Audran JC, Vasseur J (1994) Role of temperature as a triggering signal for organogenesis or somatic embryogenesis in wounded leaves of chicory cultured in vitro. J Exp Bot 45(281):1859–1865. doi:10.1093/jxb/45.12.1859

Desprez BF, Delesalle L, Dhellemmes C, Desprez MF (1994) Génétique et amélioration de la chicorée industrielle. C R Acad Agric 80(7):47–62

Doré C, Varoquaux F (2006) La chicorée endive ou witloof. In: Doré C, Varoquaux F (eds) Histoire et amélioration de cinquante plantes cultivées. Quae, pp 207–217

Doré C, Prigent J, Desprez B (1996) In situ gynogenetic haploid plants of chicory (*Cichorium intybus* L.) after intergeneric hybridization with *Cicerbita alpina* Walbr. Plant Cell Rep 15(10):758–761. doi:10.1007/BF00232223

Dubois T, Guedira M, Dubois J, Vasseur J (1991) Direct somatic embryogenesis in leaves of *Cichorium*—a histological and SEM study of early stages. Protoplasma 162(2–3):120–127. doi:10.1007/BF02562555

Eenink AH (1981) Compatibility and incompatibility in witloof-chicory (*Cichorium intybus* L.). 2. The incompatibility system. Euphytica 30(1):77–85. doi:10.1007/BF00033662

Ferrer MM, Good-Avila SV (2007) Macrophylogenetic analyses of the gain and loss of self-incompatibility in the Asteraceae. New Phytol 173(2):401–414. doi:10.1111/j.1469-8137.2006.01905.x

Franklin-Tong N, Franklin FCH (2003) Gametophytic self-incompatibility inhibits pollen tube growth using different mechanisms. Trends Plant Sci 8(12):598–605. doi:10.1016/j.tplants.2003.10.008

Funk VA, Bayer RJ, Keeley S, Chan R, Watson L, Gemeinholzer B, Schilling E, Panero JL, Baldwin BG, Garcia-Jacas N, Susanna A, Jansen RK (2005) Everywhere but Antarctica: using a supertree to understand the diversity and distribution of the Compositae. Biologiske Skrifter 55:343–374

Gandhi SD, Heesacker AF, Freeman CA, Argyris J, Bradford K, Knapp SJ (2005) The self-incompatibility locus (*S*) and quantitative trait loci for self-pollination and seed dormancy in sunflower. Theor Appl Genet 111(4):619–629. doi:10.1007/s00122-005-1934-7

Gaude T, Cabrillac D (2001) Self-incompatibility in flowering plants: the *Brassica* model. C R Acad Sci III 324(6):537–542

Ge R-C, Zhao M-L, Gao H-W, Zhao B-C, Li G-L (2002) Study on C-banding and karyotype analysis of Puna Chicory. Acta Agrestia Sin 10(3):190–193

Gonthier L, Bellec A, Blassiau C, Prat E, Helmstetter N, Rambaud C, Huss B, Hendriks T, Berges H, Quillet MC (2010) Construction

and characterization of two BAC libraries representing a deep-coverage of the genome of chicory (*Cichorium intybus* L., Asteraceae). BMC Res Notes 3(1):225. doi:10.1186/1756-0500-3-225

Good-Avila SV, Mena-Alí JI, Stephenson AG (2008) Genetic and environmental causes and evolutionary consequences of variations in self-fertility in self incompatible species. In: Franklin-Tong VE (ed) Self-incompatibility in flowering plants—evolution, diversity, and mechanisms. Springer, Berlin, pp 33–51. doi:10.1007/978-3-540-68486-2

Goubet PM, Berges H, Bellec A, Prat E, Helmstetter N, Mangenot S, Gallina S, Holl AC, Fobis-Loisy I, Vekemans X, Castric V (2012) Contrasted patterns of molecular evolution in dominant and recessive self-incompatibility haplotypes in *Arabidopsis*. PLoS Genet 8(3):13. doi:10.1371/journal.pgen.1002495

Haque MZ, Godward MBE (1984) Effects of seed-irradiation on meiosis in Ml plants of *Lactuca* and related plants. Genetica 65:179–186. doi:10.1007/BF00122903

Heesacker A, Kishore VK, Gao WX, Tang SX, Kolkman JM, Gingle A, Matvienko M, Kozik A, Michelmore RM, Lai Z, Rieseberg LH, Knapp SJ (2008) SSRs and INDELs mined from the sunflower EST database: abundance, polymorphisms, and cross-taxa utility. Theor Appl Genet 117(7):1021–1029. doi:10.1007/s00122-008-0841-0

Hiscock SJ (2000) Self-incompatibility in *Senecio squalidus* L. (Asteraceae). Ann Bot 85:181–190

Hiscock SJ, Tabah DA (2003) The different mechanisms of sporophytic self-incompatibility. Philos Trans R Soc Lond Ser B Biol Sci 358(1434):1037–1045

Hord CLH, Chen CB, DeYoung BJ, Clark SE, Ma H (2006) The BAM1/BAM2 receptor-like kinases are important regulators of *Arabidopsis* early anther development. Plant Cell 18(7):1667–1680. doi:10.1105/tpc.105.036871

Hord CLH, Suna YJ, Pillitteri LJ, Torii KU, Wang HC, Zhang SQ, Ma H (2008) Regulation of *Arabidopsis* early anther development by the mitogen-activated protein kinases, MPK3 and MPK6, and the ERECTA and related receptor-like kinases. Mol Plant 1(4):645–658. doi:10.1093/mp/ssn029

Horner HT, Palmer RG (1995) Mechanisms of genic male sterility. Crop Sci 35:1527–1535. doi:10.2135/cropsci1995.0011183X003500060002x

Hu TT, Pattyn P, Bakker EG, Cao J, Cheng JF, Clark RM, Fahlgren N, Fawcett JA, Grimwood J, Gundlach H, Haberer G, Hollister JD, Ossowski S, Ottilar RP, Salamov AA, Schneeberger K, Spannagl M, Wang X, Yang L, Nasrallah ME, Bergelson J, Carrington JC, Gaut BS, Schmutz J, Mayer KFX et al (2011) The *Arabidopsis lyrata* genome sequence and the basis of rapid genome size change. Nat Genet 43(5):476–481. doi:10.1038/ng.807

Huang Z, Chen YF, Yi B, Xiao L, Ma CZ, Tu JX, Fu TD (2007) Fine mapping of the recessive genic male sterility gene (*Bnms3*) in *Brassica napus* L. Theor Appl Genet 115(1):113–118. doi:10.1007/s00122-007-0547-8

Isokawa S, Osaka M, Shirasawa A, Kikuta R, Komatsu S, Horisaki A, Niikura S, Takada Y, Shiba H, Isogai A, Takayama S, Suzuki G, Suwabe K, Watanabe M (2010) Novel self-compatible lines of *Brassica rapa* L. isolated from the Japanese bulk-populations. Genes Genet Syst 85(2):87–96. doi:10.1266/ggs.85.87

Iwano M, Takayama S (2012) Self/non-self discrimination in angiosperm self-incompatibility. Curr Opin Plant Biol 15(1):78–83. doi:10.1016/j.pbi.2011.09.003

Jan CC, Vick BA (2007) Inheritance and allelic relationships of fertility restoration genes for seven new sources of male-sterile cytoplasm in sunflower. Plant Breed 126(2):213–217. doi:10.1111/j.1439-0523.2007.01350.x

Jordan T, Romer P, Meyer A, Szczesny R, Pierre M, Piffanelli P, Bendahmane A, Bonas U, Lahaye T (2006) Physical delimitation of the pepper *Bs3* resistance gene specifying recognition of the AvrBs3 protein from *Xanthomonas campestris* pv. *vesicatoria*. Theor Appl Genet 113(5):895–905. doi:10.1007/s00122-006-0349-4

Kakeda K, Urabayashi T, Ohashi T, Oguro T, Kowyama Y (2009) Agrobacterium-mediated transformation of *Ipomoea trifida*, a diploid relative of sweet potato. Breed Sci 59(1):95–98. doi:10.1270/jsbbs.59.95

Kane NC, Gill N, King MG, Bowers JE, Berges H, Gouzy J, Bachlava E, Langlade NB, Lai Z, Stewart M, Burke JM, Vincourt P, Knapp SJ, Rieseberg LH (2011) Progress towards a reference genome for sunflower. Bot 89(7):429–437. doi:10.1139/B11-032

Kaul MLH (ed) (1988) Male sterility in higher plants. Monographs on Theoretical and Applied Genetics, vol 10. Springer, Berlin

Kawabe A, Hansson B, Forrest A, Hagenblad J, Charlesworth D (2006) Comparative gene mapping in *Arabidopsis lyrata* chromosomes 6 and 7 and *A. thaliana* chromosome IV: evolutionary history, rearrangements and local recombination rates. Genet Res 88(1):45–56

Kempe K, Gils M (2011) Pollination control technologies for hybrid breeding. Mol Breed 27(4):417–437. doi:10.1007/s11032-011-9555-0

Kiers AM (2000) Endive, chicory and their wild relatives. A systematic and phylogenetic study of *Cichorium* (Asteraceae). PhD Thesis, Leiden University, The Netherlands

Kiers AM, Mes THM, van der Meijden R, Bachmann K (2000) A search for diagnostic AFLP markers in *Cichorium* species with emphasis on endive and chicory cultivar groups. Genome 43(3):470–476

Kilian N, Gemeinholzer B, Lack HW (2009) Cichorieae. In: Funk VA, Susanna A, Stuessy TE, Bayer RJ (eds) Systematics, evolution, and biogeography of Compositae. International Association for Plant Taxonomy, Vienna, pp 343–383

Klaas M, Yang BC, Bosch M, Thorogood D, Manzanares C, Armstead IP, Franklin FCH, Barth S (2011) Progress towards elucidating the mechanisms of self-incompatibility in the grasses: further insights from studies in *Lolium*. Ann Bot 108(4):677–685. doi:10.1093/aob/mcr186

Koopman WJM (2002) Zooming in on the lettuce genome: species relationships in *Lactuca* s.l., inferred from chromosomal and molecular characters, PhD Thesis. Wageningen University, The Netherlands

Kowyama Y, Kakeda K, Kondo K, Imada T, Hattori T (1996) A putative receptor protein kinase gene in *Ipomoea trifida*. Plant Cell Physiol 37(5):681–685

Kubo K, Entani T, Takara A, Wang N, Fields AM, Hua ZH, Toyoda M, Kawashima S, Ando T, Isogai A, Kao T, Takayama S (2010) Collaborative non-self recognition system in S-RNase-based self-incompatibility. Science 330(6005):796–799. doi:10.1126/science.1195243

Lahaye T, Hartmann S, Topsch S, Freialdenhoven A, Yano M, Schulze-Lefert P (1998) High-resolution genetic and physical mapping of the *Rar1* locus in barley. Theor Appl Genet 97(4):526–534. doi:10.1007/s001220050927

Lai Z, Nakazato T, Salmaso M, Burke JM, Tang S, Knapp SJ, Rieseberg LH (2005) Extensive chromosomal repatterning and the evolution of sterility barriers in hybrid sunflower species. Genetics 171(1):291–303. doi:10.1534/genetics.105.042242

Legrand S, Hendriks T, Hilbert JL, Quillet MC (2007) Characterization of expressed sequence tags obtained by SSH during somatic embryogenesis in *Cichorium intybus* L. BMC Plant Biol 7:27. doi:10.1186/1471-2229-7-27

Lucchin M, Varotto S, Barcaccia G, Parrini P (2008) Chicory and endive. In: Prohens J, Nuez F, Carena MJ (eds) Vegetables I, vol

1. Handbook of plant breeding. Springer, New York, pp 3–48. doi:10.1007/978-0-387-30443-4_1
Ma X-H, Qin R-L, Xing W-B (1984) Chromosome observation of some medical plants in Xinjiang. Acta Phytotaxono Sin 22(3): 243–249
McInnis SM, Costa LM, Gutierrez-Marcos JF, Henderson CA, Hiscock SJ (2005) Isolation and characterization of a polymorphic stigma-specific class III peroxidase gene from *Senecio squalidus* L. (Asteraceae). Plant Mol Biol 57(5):659–677
Meksem K, Leister D, Peleman J, Zabeau M, Salamini F, Gebhardt C (1995) A high-resolution map of the vicinity of the *R1* locus on chromosome V of potato based on RFLP and AFLP markers. Mol Gen Genet 249(1):74–81. doi:10.1007/BF00290238
Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis—a rapid method to detect markers in specific genomic regions by using segregating populations. PNAS 88(21):9828–9832. doi:10.1073/pnas.88.21.9828
Mizuno S, Osakabe Y, Maruyama K, Ito T, Osakabe K, Sato T, Shinozaki K, Yamaguchi-Shinozaki K (2007) Receptor-like protein kinase 2 (RPK 2) is a novel factor controlling anther development in *Arabidopsis thaliana*. Plant J 50(5):751–766. doi:10.1111/j.1365-313X.2007.03083.x
Murase K, Shiba H, Iwano M, Che FS, Watanabe M, Isogai A, Takayama S (2004) A membrane-anchored protein kinase involved in *Brassica* self-incompatibility signaling. Science 303(5663):1516–1519. doi:10.1126/science.1093586
Myburg AA, Remington DL, O'Malley DM, Sederoff RR, Whetten RW (2001) High-throughput AFLP analysis using infrared dye-labeled primers and an automated DNA sequencer. BioTechniques 30(2):348
Nonomura KI, Miyoshi K, Eiguchi M, Suzuki T, Miyao A, Hirochika H, Kurata N (2003) The *MSP1* gene is necessary to restrict the number of cells entering into male and female sporogenesis and to initiate anther wall formation in rice. Plant Cell 15(8): 1728–1739. doi:10.1105/tpc.012401
Paris M, Meyer C-L, Blassiau C, Coissac E, Taberlet P, Després L (2012) Two methods to easily obtain nucleotide sequences from AFLP loci of interest. In: Pompanon F, Bonin A (eds) Data production and analysis in population genomics, vol 888., Methods in Molecular Biology SeriesHumana Press, New Jersey, pp 91–108
Pécaut P (1962) Etude sur le système de reproduction de l'endive (*Cichorium intybus* L.). Ann Amélior Plantes 12:265–296
Rahman MH, Tsuchiya T, Suwabe K, Kohori J, Tomita RN, Kagaya Y, Kobayashi I, Kakeda K, Kowyama Y (2007a) Physical size of the *S* locus region defined by genetic recombination and genome sequencing in *Ipomoea trifida*, Convolvulaceae. Sex Plant Reprod 20(2):63–72
Rahman MH, Uchiyama M, Kuno M, Hirashima N, Suwabe K, Tsuchiya T, Kagaya Y, Kobayashi I, Kakeda K, Kowyama Y (2007b) Expression of stigma- and anther-specific genes located in the *S* locus region of *Ipomoea trifida*. Sex Plant Reprod 20(2):73–85. doi:10.1007/s00497-007-0045-9
Rambaud C, Dubois J, Vasseur J (1992) The induction of tetraploïdy in chicory (*Cichorium intybus* L. var Magdebourg) by protoplast fusion. Euphytica 62(1):63–67
Rambaud C, Dubois J, Vasseur J (1993) Male-sterile chicory cybrids obtained by intergeneric protoplast fusion. Theor Appl Genet 87(3):347–352. doi:10.1007/BF01184922
Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, pp 365–386
Schopfer CR, Nasrallah ME, Nasrallah JB (1999) The male determinant of self-incompatibility in *Brassica*. Science 286(5445):1697–1700. doi:10.1126/science.286.5445.1697
Sherman-Broyles S, Nasrallah JB (2008) Self-incompatibility and evolution of mating systems in the Brassicaceae. In: Franklin-Tong VE (ed) Self-incompatibility in flowering plants—evolution, diversity, and mechanisms. Springer, Berlin, pp 123–147. doi:10.1007/978-3-540-68486-2
Silva NF, Goring DR (2001) Mechanisms of self-incompatibility in flowering plants. Cell Mol Life Sci 58(14):1988–2007. doi: 10.1007/PL00000832
Stein JC, Howlett B, Boyes DC, Nasrallah ME, Nasrallah JB (1991) Molecular-cloning of a putative receptor protein-kinase gene encoded at the self-incompatibility locus of *Brassica oleracea*. PNAS 88(19):8816–8820
Stout AB (1917) Fertility in *Cichorium intybus*: the sporadic occurence of self-fertile plants among the progeny of self-sterile plants. Am J Bot 4(7):375–395
Stout AB (1918) Fertility in *Cichorium intybus*. Self-compatibility and self-incompatibility among the offspring of self-fertile lines of descent. J Genet 9:71–103. doi:10.1007/BF02984312
Suzuki G, Kai N, Hirose T, Fukui K, Nishio T, Takayama S, Isogai A, Watanabe M, Hinata K (1999) Genomic organization of the *S* locus: identification and characterization of genes in *SLG*/*SRK* region of S^9 haplotype of *Brassica campestris* (syn. *rapa*). Genetics 153(1):391–400
Suzuki G, Tanaka S, Yamamoto M, Tomita RN, Kowyama Y, Mukai Y (2004) Visualization of the *S*-locus region in *Ipomoea trifida*: toward positional cloning of self-incompatibility genes. Chromosom Res 12(5):475–481. doi:10.1023/B:CHRO.0000034749.58877.b7
Tabah DA, McInnis SM, Hiscock SJ (2004) Members of the *S*-receptor kinase multigene family in *Senecio squalidus* L. (Asteraceae), a species with sporophytic self-incompatibility. Sex Plant Reprod 17(3):131–140. doi:10.1007/s00497-004-0222-z
Takayama S, Isogai A (2005) Self-incompatibility in plants. Annu Rev Plant Biol 56:467–489. doi:10.1146/annurev.arplant.56.032604.144249
Twell D (2011) Male gametogenesis and germline specification in flowering plants. Sex Plant Reprod 24(2):149–160. doi: 10.1007/s00497-010-0157-5
Uyenoyama MK (2005) Evolution under tight linkage to mating type. New Phytol 165:63–70
Van Ooijen JW, Voorrips RE (2001) JoinMap® version 3.0: software for the calculation of genetic linkage maps. Plant Research International, Wageningen
Varotto S, Pizzoli L, Lucchin M, Parrini P (1995) The incompatibility system in Italian red chicory (*Cichorium intybus* L.). Plant Breed 114(6):535–538. doi:10.1111/j.1439-0523.1995.tb00851.x
Vilanova S, Badenes ML, Burgos L, Martinez-Calvo J, Llacer G, Romero C (2006) Self-compatibility of two apricot selections is associated with two pollen-part mutations of different nature. Plant Physiol 142(2):629–641. doi:10.1104/pp.106.083865
Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23(21):4407–4414
Wan JR, Patel A, Mathieu M, Kim SY, Xu D, Stacey G (2008) A lectin receptor-like kinase is required for pollen development in *Arabidopsis*. Plant Mol Biol 67(5):469–482. doi:10.1007/s11103-008-9332-6
Wang Y, Wang X, McCubbin AG, T-h Kao (2003) Genetic mapping and molecular characterization of the self-incompatibility (*S*) locus in *Petunia inflata*. Plant Mol Biol 53(4):565–580. doi: 10.1023/B:PLAN.0000019068.00034.09
Wang DJ, Guo AG, Li DR, Tian JH, Huang F, Sun GL (2007) Cytological and molecular characterization of a novel

monogenic dominant GMS in *Brassica napus* L. Plant Cell Rep 26(5):571–579. doi:10.1007/s00299-006-0276-5

Wei P, Feng H, Piao ZY, Li CY, Liu ZY, Wang YG, Ji RQ, Zou T, Ji SJ (2009) Identification of AFLP markers linked to *Ms*, a genic multiple allele inherited male-sterile gene in Chinese cabbage. Breed Sci 59(4):333–339. doi:10.1270/jsbbs.59.333

Wheeler MJ, Armstrong SA, Franklin-Tong VE, Franklin FCH (2003) Genomic organization of the *Papaver rhoeas* self-incompatibility S_1 locus. J Exp Bot 54(380):131–139. doi:10.1093/jsb/erg006

Wheeler MJ, Vatovec S, Franklin-Tong VE (2010) The pollen S-determinant in *Papaver*: comparisons with known plant receptors and protein ligand partners. J Exp Bot 61(7):2015–2025. doi:10.1093/jxb/erp383

Wills DM, Burke JM (2007) Quantitative trait locus analysis of the early domestication of sunflower. Genetics 176(4):2589–2599. doi:10.1534/genetics.107.075333

Xiao QJ, Wibowo TA, Wu XL, Michal JJ, Reeves JJ, Busboom JR, Thorgaard GH, Jiang ZH (2007) A simplified QTL mapping approach for screening and mapping of novel AFLP markers associated with beef marbling. J Biotechnol 127(2):177–187. doi:10.1016/j.jbiotec.2006.06.021

Xing QH, Ru ZG, Zhou CJ, Xue X, Liang CY, Yang DE, Jin DM, Wang B (2003) Genetic analysis, molecular tagging and mapping of the thermo-sensitive genic male-sterile gene (*wtms1*) in wheat. Theor Appl Genet 107(8):1500–1504. doi:10.1007/s00122-003-1385-y

Yang QY, Zhang DF, Li Q, Cheng ZK, Xue YB (2007) Heterochromatic and genetic features are consistent with recombination suppression of the self-incompatibility locus in *Antirrhinum*. Plant J 51(1):140–151

Yi B, Chen YN, Lei SL, Tu JX, Fu TD (2006) Fine mapping of the recessive genic male-sterile gene (*Bnms1*) in *Brassica napus* L. Theor Appl Genet 113(4):643–650. doi:10.1007/s00122-006-0328-9

Yue B, Cai X, Yuan W, Vick B, Hu J (2009) Mapping quantitative trait loci (QTL) controlling seed morphology and disk diameter in sunflower (*Helianthus annuus* L.). HELIA 32(50):17–36. doi:10.2298/HEL0950017Y

Yue B, Vick BA, Cai X, Hu J (2010) Genetic mapping for the *Rf1* (fertility restoration) gene in sunflower (*Helianthus annuus* L.) by SSR and TRAP markers. Plant Breed 129(1):24–28. doi:10.1111/j.1439-0523.2009.01661.x

Zhang ZZ, Guo ML, Zhang JD (2009) Identification of AFLP fragments linked to hydroxysafflor yellow A in Flos Carthami and conversion to a SCAR marker for rapid selection. Mol Breed 23(2):229–237. doi:10.1007/s11032-008-9228-9

Zhao DZ, Wang GF, Speal B, Ma H (2002) The *EXCESS MICROSPOROCYTES1* gene encodes a putative leucine-rich repeat receptor protein kinase that controls somatic and reproductive cell fates in the *Arabidopsis* anther. Genes Dev 16(15):2021–2031. doi:10.1101/gad.997902

Zuriaga E, Molina L, Badenes ML, Romero C (2012) Physical mapping of a pollen modifier locus controlling self-incompatibility in apricot and synteny analysis within the Rosaceae. Plant Mol Biol 79(3):229–242. doi:10.1007/s11103-012-9908-z