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ORIGINAL PAPER

Sonali D. Gandhi · Adam F. Heesacker
Carrie A. Freeman · Jason Argyris · Kent Bradford
Steven J. Knapp

The self-incompatibility locus (*S*) and quantitative trait loci for self-pollination and seed dormancy in sunflower

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Abstract Wild populations of common sunflower (*Helianthus annuus* L.) are self-incompatible and have deep seed dormancy, whereas modern cultivars, inbreds, and hybrids are self-compatible and partially-to-strongly self-pollinated, and have shallow seed dormancy. Self-pollination (SP) and seed dormancy are genetically complex traits, the number of self-compatibility (*S*) loci has been disputed, and none of the putative *S* loci have been genetically mapped in sunflower. We genetically mapped quantitative trait loci (QTL) for self-incompatibility (SI), SP, and seed dormancy in a backcross population produced from a cross between an elite, self-pollinated, nondormant inbred line (NMS373) and a wild, self-incompatible, dormant population (ANN1811). A population consisting of 212 BC_1 progeny was subsequently produced by backcrossing a single hybrid individual to NMS373. BC_1 progeny produced 0–838 seeds per primary capitula when naturally selfed and 0–518 seeds per secondary capitula when manually selfed and segregated for a single *S* locus. The *S* locus mapped to linkage group 17 and was tightly linked to a cluster of previously identified QTL for several domestication and postdomestication traits. Two synergistically interacting QTL were identified for SP among self-compatible (*ss*) BC_1 progeny ($R^2 = 34.6\%$). NMS373 homozygotes produced 271.5 more seeds per secondary capitulum than heterozygotes. Germination percentages of seeds after-ripened for 4 weeks ranged from 0% to 100% among self-compatible BC_1S_1 families. Three QTL for seed dormancy were identified ($R^2 = 38.3\%$). QTL effects were in the predicted direction (wild alleles decreased self-pollination and seed germination). The present analysis differentiated between loci governing SI and SP and identified DNA markers for bypassing SI and seed dormancy in elite × wild crosses through marker-assisted selection.

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S. D. Gandhi · A. F. Heesacker · C. A. Freeman
Department of Crop and Soil Science, Oregon State University, Corvallis, OR 97331, USA

J. Argyris · K. Bradford
Department of Vegetable Crops, University of California, Davis, CA 95616, USA

S. J. Knapp (✉)
Center for Applied Genetic Technologies, The University of Georgia, 111 Riverbend Road, Athens, GA 30602, USA
E-mail: sjknapp@uga.edu
Tel.: +1-706-5424021

Introduction

Wild populations of common sunflower (*Helianthus annuus* L.) are self-incompatible (Heiser 1954; Heiser et al. 1969) and have deep seed dormancy (Heiser 1976; Seiler 1988, 1996, 1998), whereas modern cultivars, inbreds, and hybrids are self-compatible and partially-to-strongly self-pollinated (Luciano et al. 1965; Fick and Zimmer 1976; Fick and Redher 1977; Fick 1978), and have short-lived seed dormancy (Alissa et al. 1986; Corbineau et al. 1990). Self-incompatibility (SI) and seed dormancy complicate breeding in elite × wild hybrids (Seiler 1992), and the genetic mechanisms underlying SI, self-pollination (SP), and seed dormancy in sunflower are either unknown or superficially known.

The SI system in sunflower is sporophytic (Ivanov 1975; Fernandez-Martinez and Knowles 1978) and undoubtedly similar in complexity to the sporophytic SI systems found in other genera (Nasrallah 2002; Hiscock and McInnis 2003). The number of SI loci (*S* loci) in common sunflower has been disputed, and none of the putative *S* loci have been genetically mapped. One multiallelic *S* locus was identified by Fernandez-Martinez and Knowles (1978) from analyses of SI among several self-incompatible wild hybrids, whereas two multiallelic *S* loci were identified from analyses of SI

among self-compatible × self-incompatible hybrids (Habura 1957; Lofgren and Nelson 1977; Olivieri et al. 1988). SI was reported by Vranceanu et al. (1978) to be genetically complex (quantitative) and governed by an unknown number of loci.

Self-incompatibility enforces outcrossing in wild sunflower populations (Heiser 1954). Self-compatibility was apparently first discovered in elite germplasm (Russell 1952; Luciano et al. 1965) and was subsequently used to exploit genetic variability for SP in breeding programs. Selfing seems to be primarily affected by floret density, stigma orientation, and pollen agglutination (Segala et al. 1980; Miller and Fick 1997). Genetic, physiological, and morphological factors underlying selfing are complex; heritabilities for SP (seeds per capitulum produced by natural selfing) have been in the range of 0.1–0.5 (Luciano et al. 1965; Fick 1978; Leclerq 1980; Segala et al. 1980; Kovacik and Skaloud 1990). Despite the inherent complexity of the trait, selection for increased SP is straightfoward and routinely practiced in hybrid sunflower breeding programs and has pushed selfing percentages into the 80–100% range in modern inbred lines and hybrids (Fick and Zimmer 1976; Fick and Rehder 1977; Fick 1978).

The number and nature of loci controlling SP is unknown. Burke et al. (2002) identified two linked quantitative trait loci (QTL) for SP (seeds per capitulum produced by natural selfing) on linkage group (LG) 17 in an analysis of F_2 progeny from an elite × wild hybrid (HA89 × ANN1238). QTL alleles from the elite parent were recessive, had a large effect, and increased SP. Because the F_1 was self-compatible and capitula were not manually pollinated, Burke et al. (2002) concluded that the QTL affected autogamy (self-pollination) rather than SI.

Short-term embryo-imposed dormancy in domesticated sunflower germplasm hampers the rapid cycling of seeds in breeding programs. Seed germination is often accelerated by harvesting physiologically immature achenes and culturing embryos. Long-term pericarp-imposed dormancy is ubiquitous in wild germplasm (Heiser 1951, 1976; Heiser et al. 1969), complicates breeding in elite × wild crosses (Chandler and Jan 1985; Seiler 1992), and hampers seed multiplication in germplasm preservation and wild species breeding programs. Genetic analyses of seed dormancy have not been reported in sunflower (Corbineau et al. 1990; Seiler 1992; Miller and Fick 1997). QTL mapping is a powerful tool for exploratory analyses of natural genetic variability for seed dormancy and other domestication traits, particularly in elite × wild crosses where phenotypic differences between the parents are extreme (Fennimore et al. 1999; Cai and Morishima 2000; Burke et al. 2002; Koornneef et al. 2002). By mapping QTL governing SI, SP, and seed dormancy in sunflower, strategies can be developed for identifying genes and genetic mechanisms underlying the QTL (Koornneef et al. 2002; Borevitz and Chory 2004), selecting against SI and seed dormancy genes segregating in elite × wild populations, and accelerating the introgression of genes from wild populations through marker-assisted selection (MAS). The goal of the investigation reported here was to gain a deeper understanding of the genetics of SI, SP, and seed dormancy in sunflower by mapping QTL in an elite line × wild hybrid (NMS373 × ANN1811) and identifying and genetically mapping the *S* locus or loci.

Materials and methods

Plant materials and phenotyping

We crossed an unpigmented (*tt*), male-sterile (ms_{10} ms_{10}) NMS373 individual to a pigmented (*TT*), male-fertile ($Ms_{10}Ms_{10}$) ANN1811 individual, and backcrossed a single hybrid plant (TMs_{10}/tms_{10}) to an unpigmented (*tt*), male-sterile (tms_{10}/tms_{10}) NMS373 individual. ANN1811 is a wild *Helianthus annuus* population collected from Skidmore, Texas, USA (PI 494567). NMS373 is a nuclear male-sterile line that is near-isogenic to the nuclear male-fertile, fertility restorer ® line RHA373 (Miller 1997). BC_1 seeds were planted in a pumice:peat moss:sandy loam growing media and grown for 4 weeks in a greenhouse; 212 four-week-old BC_1 seedlings were transplanted to a field site near Corvallis, Oregon in May 2002. BC_1 plants were planted 1 m apart in rows spaced 1 m apart.

SP was phenotyped by counting the number of seeds (achenes) produced per primary capitula by natural selfing, whereas SI was phenotyped by counting the number of seeds produced per secondary capitula by manual selfing. We bagged the primary and a single secondary capitula on each BC_1 plant before the onset of flowering. Once bagged, primary capitula were not handled or manually pollinated, whereas secondary capitula were manually selfed (pollen was transferred using paper towels). Flower development was checked every day throughout flowering. Secondary capitula were manually selfed twice or thrice between the onset and completion of flowering. Both capitula were harvested at physiological maturity and dried for 72 h at 42–44°C in a gas-heated, forced-air dryer. Seeds (achenes) were threshed, cleaned, and stored at room temperature. The number of seeds produced by primary and second capitula were counted. Nearly one-half of the BC_1 progeny (101/212) produced a sufficient number of BC_1S_1 seeds for seed dormancy phenotyping. BC_1S_1 seeds were stored at room temperature (approximately 24°C) for 4 weeks. Fifty seeds per BC_1S_1 family were then surface sterilized in 1 l water/2 g Benlate dissolved in ethanol, placed on moistened blotter paper, and germinated at 25°C under continuous light. Germination percentages were recorded 3 days and 7 days later.

DNA marker genotyping, genetic mapping, and QTL analyses

Leaf tissue was harvested from BC_1 plants, placed on ice, and frozen at −80°C. Genomic DNA was isolated as

described by Tang et al. (2002). We genotyped 132 simple sequence repeat (SSR), insertion-deletion polymorphism (INDEL), single-strand conformational polymorphism (SSCP), or single nucleotide polymorphism (SNP) markers on 212 NMS373 × ANN1811 BC_1 progeny. The DNA markers were selected from a framework of 350 sequence-tagged-site (STS) markers genotyped on a subset of 94 randomly selected BC_1 progeny (unpublished data). The 350 STS markers were chosen from a collection of previously described SSR and INDEL markers (identified by ORS and ZVG prefixes, respectively; Tang et al. 2002; Yu et al. 2002, 2003), new SSR, INDEL, SSCP, and SNP markers (unpublished data) developed from sunflower cDNA sequences (Kozik et al. 2002; identified by RGC and HT prefixes), and new INDEL and SNP markers developed for previously mapped restriction fragment length polymorphic (RFLP) marker loci (unpublished data) (identified by the ZVG prefix; Berry et al. 1997; Gedil et al. 2001). SSR and INDEL genotyping was performed as described by Tang et al. (2002), SSCP genotyping was performed as described by Hongtrakul et al. (1998), and SNP genotyping was performed as described by Kolkman et al. (2004). The primer sequences for previously unpublished public STS markers appearing on the 132-locus BC_1 map and on a 27-cM-long segment on LG 17 of the 350-locus BC_1 map, have been supplied as supplemental data (ESM). The genetic map was constructed using MAPMAKER (Lander et al. 1987), essentially as described by Tang et al. (2002).

NMS373 × ANN1811 was hypothesized to have segregated for a single SI locus (*S* locus). The *S* locus was genetically mapped using *S*-locus genotypes inferred from SI phenotypes (*Ss* for self-incompatible and *ss* for self-compatible BC_1 individuals). The goodness-of-fit of the observed to the expected segregation ratio for the *S* locus was tested using a χ^2-statistic (Sokal and Rohlf 1981). The wild parent (ANN1811) was presumed to be heterozygous for dominant or incompletely dominant SI alleles (*SS′*) and to have transmitted one of the alleles (*S*) to the hybrid (a single F_1 plant was backcrossed), while the elite parent (NMS373) was presumed to be homozygous for a recessive self-compatibility allele (*ss*). The expected segregation ratio in the BC_1 was 1 *Ss* (self-incompatible):1 *ss* (self-compatible).

Two QTL analyses were performed for SI, SP, and seed dormancy. First, QTL were identified and statistics estimated using composite interval mapping (CIM) (Zeng 1993, 1994), as implemented in QTL CARTOGRAPHER (Basten et al. 2002). Second, DNA marker loci tightly linked to QTL identified by CIM were used as independent variables in mixed model analyses, where intralocus and interlocus QTL effects were estimated (described below). CIM analyses were performed using a 2-cM window and one to five cofactors; LOD scores were compared to an empirical genome-wide significance threshold calculated from 1,000 permutations for $P = 0.05$ (Doerge and Churchill 1996). QTL effects (backcross genotype mean differences) and coefficients of determination (R^2) were estimated by CIM for each QTL. One-LOD support intervals were calculated as described by Conneally et al. (1985) and Lynch and Walsh (1997).

The intralocus and interlocus effects of QTL for SI and SP were estimated by using the *S* locus and DNA marker loci (ORS292 and ORS349) as independent variables in a 2^3 factorial mixed linear model (*S*, ORS292, and ORS349 were tightly linked to QTL identified by CIM). The 2^3 effects of genotypes were fixed, whereas the effects of BC_1 progeny nested in genotypes were random. The intralocus and interlocus effects among loci were estimated using linear contrasts among least square means (Littel et al. 1996): $y_{AA} - y_{Aa}$ for single-locus effects (*S*, ORS292, and ORS349), $(y_{AABB} - y_{AaBB} - y_{AABb} + y_{AaBb})/2$ for two-locus interaction effects (*S* × ORS292, *S* × ORS349, and ORS292 × ORS349), and $(y_{AABBCC} - y_{AaBBCC} - y_{AABbCC} + y_{AaBbCC} - y_{AABBCc} + y_{AaBBCc} + y_{AABbCc} - y_{AaBbCc})/4$ for the three-locus interaction effect (*S* × ORS292 × ORS349), where A, *B*, and *C* index loci in the three-locus model, y_{AA} and y_{Aa} are least square means for *AA* (NMS373 homozygotes) and *Aa* genotypes, respectively, y_{AABB}, y_{AaBB}, y_{AABb}, and y_{AaBb} are least square means for *AABB*, *AaBB*, *AABb*, and *AaBb* genotypes, respectively, and y_{AABBCC}, y_{AaBBCC}, y_{AABbCC}, y_{AaBbCC}, y_{AABBCc}, y_{AaBBCc}, y_{AABbCc}, and y_{AaBbCc} are least square means for the *AABBCC*, *AaBBCC*, *AABbCC*, *AaBbCC*, *AABBCc*, *AaBBCc*, *AABbCc*, and *AaBbCc* genotypes, respectively. Type-III sums of squares, Type-III *F*-statistics, and least square means for genotypes were estimated using SAS PROC MIXED (http://www.sas.org), where $F = MS_G/MS_{P:G}$ is an *F*-statistic, MS_G is the mean square for the three intralocus or four interlocus effects, and $MS_{P:G}$ is the mean square for BC_1 progeny nested in genotypes (the residual). *S*, ORS349, ORS292, *S* × ORS349, *S* × ORS292, ORS349 × ORS292, and *S* × ORS349 × ORS292 effects were estimated using the complete BC_1 mapping population ($n = 212$). ORS349, ORS292, and ORS349 × ORS292 effects were separately estimated among self-compatible (*ss*) BC_1 progeny ($n = 91$). The intralocus and interlocus effects of QTL for seed dormancy were estimated by using three DNA marker loci (ZVG3, ZVG9, and ORS1114) as independent variables in a 2^3 factorial mixed linear model (ZVG3, ZVG9, and ORS1114 were tightly linked to QTL identified by CIM).

Results

Genetic mapping in NMS373 × ANN1811

We genotyped 132 STS markers on 212 NMS373 × [NMS373 × ANN1811] BC_1 progeny. The loci assembled into 18 linkage groups (the complete map has been supplied as ESM). Two of the linkage groups were found to be upper and lower fragments of LG 10 (Tang et al. 2002; Yu et al. 2003). The other 16 linkage groups

matched previously identified linkage groups. The two LG 10 fragments were oriented, and the gap between fragments was identified by using previously mapped SSR and INDEL marker loci and found to span the ORS910-ORS595A interval. The 132 DNA marker loci were chosen from a collection of 350 DNA marker loci mapped on a subset of 94 BC_1 progeny (unpublished data) so as to maximize genome coverage and pull linkage groups together and included the 34 endmost STS markers from each linkage group. Groups and locus orders were identical for common STS marker loci on the 132- and 350-locus NMS373 × ANN1811 and previously described maps (Tang et al. 2002; Yu et al. 2003). The 132-locus BC_1 map was 1,450 cM long and had a mean density of 11 cM per locus (ESM). The BC_1 did not segregate for male-sterility as expected. We suspect that the original hybrid (NMS373/ANN1811) was homozygous male-fertile ($Ms_{10}Ms_{10}$), not heterozygous male-fertile ($Ms_{10}ms_{10}$).

Genetic mapping of the SI locus (*S*)

NMS373, ANN1811, and the BC_1 progeny were branched and produced multiple capitula per plant. The SI and SP phenotypes of the parents (ANN1811 and NMS373) were ascertained by naturally and manually selfing primary capitula on separate plants; male-fertile ($Ms_{10}ms_{10}$) NMS373 plants were phenotyped. The self-incompatible parent (ANN1811) produced no seeds when naturally or manually selfed, whereas the self-compatible parent (NMS373) produced significantly more seeds per capitulum by manual than natural selfing (375.4 and 250.2 seeds per capitulum, respectively) ($P < 0.0001$) (Fig. 1). NMS373 was not fully self-pollinated. One-third of the disk flowers within NMS373 capitula failed to produce seed by natural selfing (250.2/375.4 × 00 = 67%).

SI and SP segregated in NMS373 × ANN1811 (Figs. 1, 2). The SI distribution was approximately exponential and was inferred to be a mixture of an exponential and a normal distribution (Fig. 2). The left-hand distribution was leptokurtic, right-skewed, and approximately exponential with a range of 0 to approximately 50 seeds per capitulum, whereas the right-hand distribution was platykurtic and approximately normal, with a range of approximately 50–518 seeds per capitulum; one-half of the BC_1 individuals produced fewer than 47 seeds per capitulum (Fig. 2).

CIM identified a single QTL for SI on LG 17 (*si17.1*) (Figs. 3, 4; Table 1). The QTL was flanked by ZVG80 and ORS735 (Fig. 3). Because this QTL had a large effect (LOD = 52.1; $R^2 = 66.2$), no other QTL were identified, and one-half of the progeny were tightly clustered in the lower end of the SI distribution. The BC_1 was inferred to have segregated for a single SI locus (*S*), where the *S* allele was transmitted by ANN1811, the *s* allele was transmitted by NMS373, *S* was completely or incompletely dominant to *s*, and BC_1 progeny were

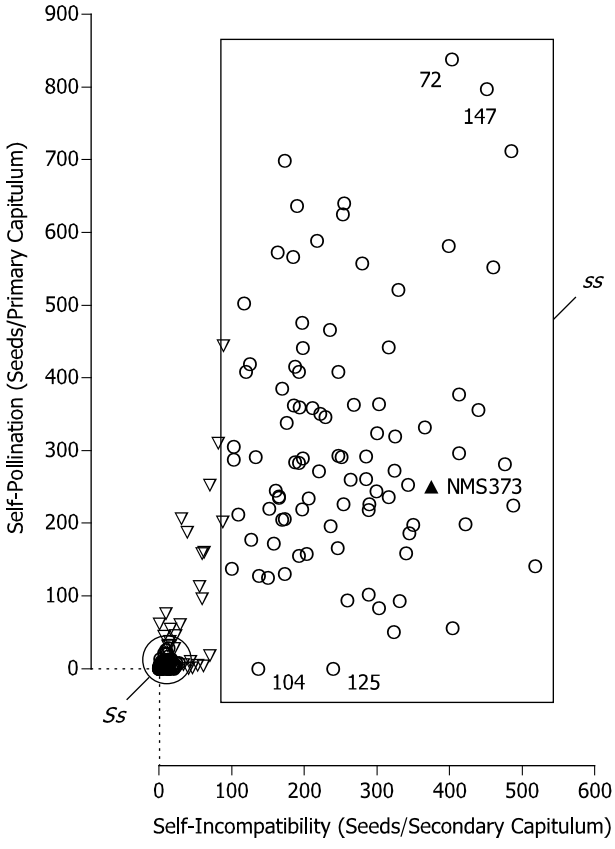

Fig. 1 Seeds per primary capitulum produced by natural selfing (SP, self-pollination) and seeds per secondary capitulum produced by manual selfing (SI) among 212 NMS373 × (NMS373 × ANN1811) BC_1 progeny and male-fertile NMS373 plants (*solid black triangle*). SP and self-incompatibility (SI) phenotypes are not shown for ANN1811 (0.0 seeds per capitulum for both). BC_1 progeny classified as heterozygous and self-incompatible (*Ss*) are identified by *open circles* (*encircled* and labeled *Ss*). BC_1 progeny classified as self-compatible (*ss*) are identified by *open circles* (*boxed* and labeled *ss*). Unclassified BC_1 progeny are identified by *inverted triangles*

either heterozygous and partially to fully self-incompatible (*Ss*) or homozygous for the NMS373 allele and self-compatible (*ss*) (Fig. 1).

The *S* locus was genetically mapped using genotypes inferred from SI phenotypes (Figs. 1, 2). We used conservative rules to assign BC_1 individuals to genotypic classes (*Ss* and *ss*) to minimize misascertainment errors and maximize mapping accuracy. *S*-locus genotypes were inferred for 179 BC_1 individuals with unambiguous phenotypes; the phenotypes for 26 BC_1 individuals were ambiguous (identified by inverted triangles in Fig. 1). Of the latter, 18 produced 29–89 seeds per capitulum when manually selfed, and these spanned the overlapping upper tail of the self-incompatible (*Ss*) and lower tail of the self-compatible (*ss*) classes; eight produced 1–23 seeds per capitulum when manually selfed, but 31–75 seeds per capitulum when naturally selfed. BC_1 progeny producing fewer than 27 seeds per capitulum by manual *and* natural selfing (87) were classified as heterozygotes (*Ss*), whereas BC_1 progeny producing more than 100

Fig. 2 Seeds per secondary capitulum produced by manual selfing (SI) among 212 NMS373 × (NMS373 × ANN1811) BC_1 progeny

seeds per capitulum by manual selfing (92) were classified as *ss* homozygotes (Fig. 1). The observed segregation ratio for *S* (87 *Ss*:92 *ss*) was not significantly different from 1:1 ($\chi^2 = 0.089$; $P = 0.76$). The *S* locus mapped to LG 17, was flanked by ZVG80 and ORS735, and was centered directly under the maximum LOD for the SI QTL identified by CIM (Fig. 3).

We aligned a 19.3-cM interval spanning ORS1245-*S*-ZVG152 on LG 17 of the NMS × ANN1811 map with a 27-cM interval spanning ORS561-ORS1245-*P*-ZVG152 on LG 17 of an updated rendition of the RHA280 × RHA801 map (unpublished data) to identify additional STS markers linked to *S* and to assess the proximity of *S* to the QTL identified by Burke et al. (2002) (Fig. 5). Forty-four STS marker loci have been mapped to the ORS1245-ZVG152 interval spanning *S*; however, none seem to be closer than ORS592, ORS625, or ORS735, a cluster of three SSR marker loci 2.4 cM downstream of *S* (primer sequences for previously unpublished STS markers in the ORS561-ZVG152 interval have been supplied as ESM).

The *S* allele transmitted by ANN1811 was deduced to be incompletely dominant because very few BC_1 progeny (5/212) were completely self-incompatible (produced no seeds when manually selfed). ANN1811 individuals produced seeds when intercrossed but not when selfed. Moreover, two of three elite × wild (self-compatible × self-incompatible) hybrids screened for SI (CMS372 × PI 435593, CMS372 × PI 468575, and CMS372 × PI 531022) were self-compatible; hence, incompletely dominant or weak *S* alleles seem to be present, but are not necessarily common, in wild sunflower populations.

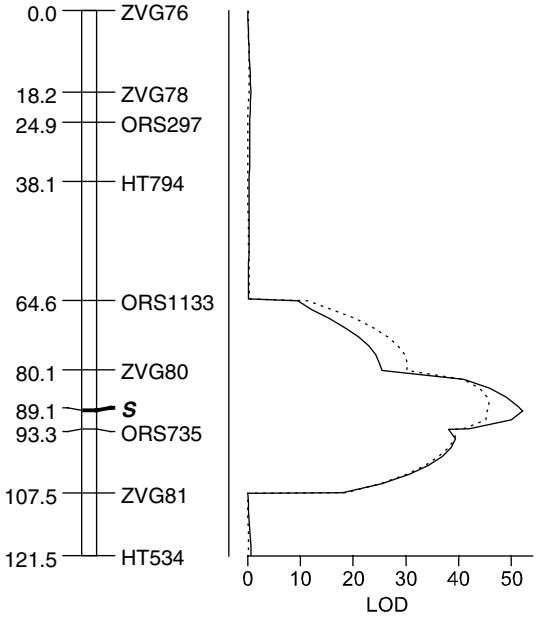

Fig. 3 Likelihood odds (LODs) for SP (*dashed line*) and SI (*solid line*) QTL identified by composite interval mapping on LG 17 in NMS373 × ANN1811

Genetic mapping of QTL for self-pollination

The phenotypic correlation between SI and SP was 0.71 ($P < 0.0001$) among BC_1 progeny (Fig. 1). The mean and maximum number of seeds per capitulum among BC_1 progeny was significantly greater for primary capitula (phenotyped for SP) than secondary capitula (phenotyped for SI) because the number of disk flowers was generally greater in the former than the latter. Because phenotypic differences for SP per se cannot be ascertained between self-incompatible and self-compatible parents, the segregation of SP self-pollination QTL in NMS373 × ANN1811 could not be predicted a priori but was observed among self-compatible NMS373 × ANN1811 BC_1 progeny. Several BC_1 progeny were self-compatible but not strongly self-pollinated, e.g., two self-compatible individuals ($BC_{1\text{-}104}$ and $BC_{1\text{-}125}$) produced 137 and 240 seeds per capitulum, respectively, when manually selfed and no seeds when naturally selfed (Fig. 1). Conversely, several BC_1 progeny were self-compatible and strongly self-pollinated; for example, $BC_{1\text{-}72}$ and $BC_{1\text{-}147}$ produced 451 and 403 seeds per capitulum, respectively, when manually selfed

and 797 and 838 seeds per capitulum when naturally selfed. Nearly one-half of the progeny (97/200) produced fewer seeds by natural than manual selfing.

Three QTL for SP were identified by CIM (Figs. 3, 4; Table 1). One mapped to LG 17 (*sp17.1*), had a very large effect, was coincident with *si17.1* and the *S* locus, and was inferred to have been caused by the *S* locus. Mixed model QTL analyses, performed using genotypes for the *S* locus and two DNA marker loci (ORS292 and ORS349) as independent variables, shed light on effects of and interactions among SP QTL (Tables 2, 3). ORS349 and ORS292 were tightly linked to *sp6.1* and *sp15.1*, respectively (Fig. 4). The seven intralocus and interlocus effects of *S*, ORS292, and ORS349 were significant for SP, whereas only *S* was significant for SI (Table 2). The R^2 for the three-locus (3^2 factorial) model for SP was 68.0%, the self-compatible, self-pollinated parent (NMS373) transmitted alleles for increasing SP, and the four epistatic interactions were synergistic (increased SP). The $S \times$ ORS349, $S \times$ ORS292, and $S \times$ ORS349 $\times$ ORS292 interaction (epistatic) effects were caused in part by the absence of QTL effects among *Ss* individuals and by the presence of QTL effects among *ss* individuals (ORS292- and ORS349-linked SP QTL had no effect in *Ss* individuals).

We performed a separate analysis among self-compatible BC_1 progeny to estimate the effects of SP QTL in the absence of the masking effect of the *S* locus (Table 3). The effects of the two SP QTL (*sp6.1* and *sp15.1*) were found to be several-fold greater when analyses were performed on self-compatible (*ss*) BC_1 progeny using only ORS349 and ORS292 as independent variables (91 out of 178 BC_1 progeny were *ss*). ORS292- and ORS349-linked QTL effects were significant and large ($y_{AA} - y_{Aa} = 135.8$ seeds per capitulum for both), and the ORS292 $\times$ ORS349 epistatic effect was significant and synergistic [$(y_{AABB} - y_{AaBB} - y_{AABb} + y_{AaBb})/2 = 73.6$ seeds per capitulum]; BC_1 progeny homozygous for NMS373 alleles at both loci (*AABB*) produced significantly more seeds per capitulum (478.1) than progeny heterozygous for one of the two loci (*AaBB* or *AABb*) (268.7 seeds per capitulum for both). Double heterozygotes (*AaBb*) produced only slightly fewer seeds per capitulum (206.6) than single heterozygotes; hence, QTL alleles transmitted by the elite parent synergistically interacted to increase SP. Two-thirds of the phenotypic variability among *ss* BC_1 progeny for SP, however, was not explained by the two QTL; the R^2 for the two-locus (2^2 factorial) model was 34.6%.

Genetic mapping of QTL for seed dormancy

Seed dormancy was phenotyped by germinating field-produced ANN1811, NMS373, and BC_1S_1 seeds har-

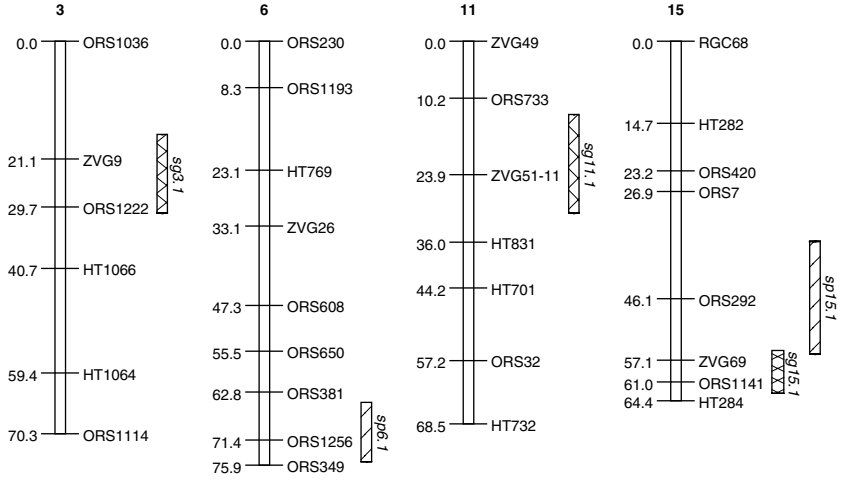

Fig. 4 One-LOD support intervals for SP (*sp*) and seed dormancy (*sg*) QTL identified on LGs 3, 6, 11, and 15 in NMS373 $\times$ ANN1811

Table 1 Statistics for self-pollination (SP), self-incompatibility (SI), and seed dormancy QTL identified by composite interval mapping among 212 NMS373 $\times$ (NMS373 $\times$ ANN1811) BC_1 progeny

Trait	QTL	Linkage group	Position (cM)	Closest DNA Marker	LOD	Effect	R^2
Self-incompatibility	*si17.1*	17	89.1	ORS735	52.1	228.1	66.2
Self-pollination	*sf6.1*	6	75.4	ORS349	3.2	61.3	2.6
	sf15.1	15	46.1	ORS292	4.7	74.6	3.8
	sf17.1	17	88.1	ORS735	45.9	290.1	57.5
Seed dormancy	*sd3.1*	3	25.1	ORS1222	3.5	17.2	9.7
	sd11.1	11	20.2	ZVG51	5.9	22.7	16.5
	sd15.1	15	63.0	HT284	4.5	19.3	12.1

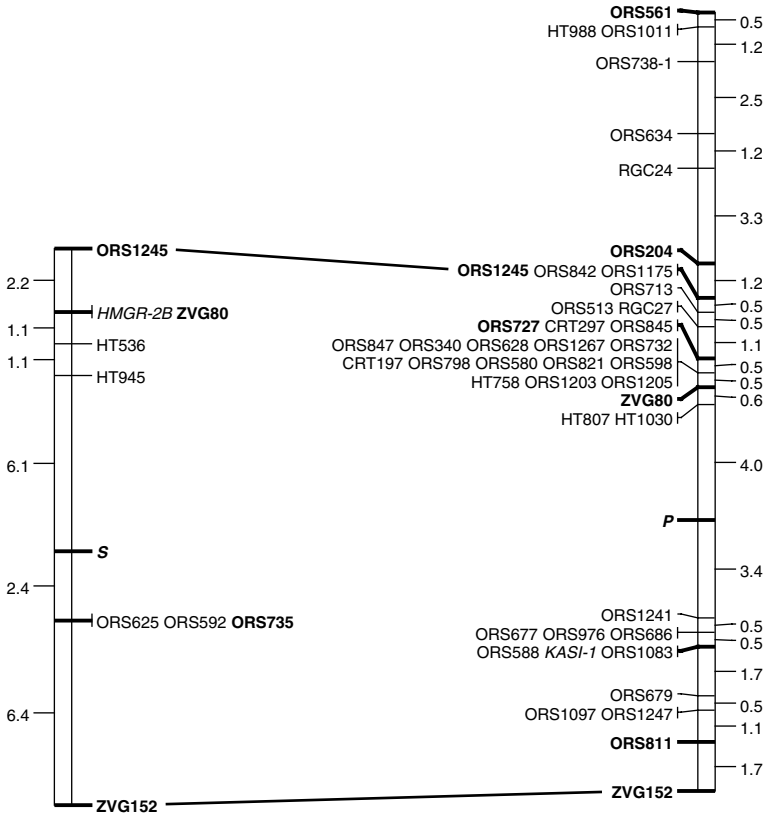

Fig. 5 Chromosomal segments on LG 17 flanking *S* and *P* and mapped by genotyping STS markers on 94 NMS373 × (NMS373 × ANN1811) BC_1 progeny (*left*) and 94 RHA280 × RHA801 recombinant inbred lines (*right*)

Table 2 Type-III *F*-test probabilities ($Pr > F$) and coefficients of determination (R^2) for intralocus and interlocus effects (seeds per capitulum) of SI and SP QTL segregating among 212 NMS373 × (NMS373 × ANN1811) BC_1 progeny

Parameter	Self-incompatibility			Self-pollination		
	Effect	$Pr > F$	R^2	Effect	$Pr > F$	R^2
ORS292	−5.9	0.6	0.0	68.9	< 0.0001	2.9
ORS349	10.2	0.4	0.1	70.4	< 0.0001	3.1
S	245.9	< 0.0001	71.3	299.5	< 0.0001	55.0
ORS292 × ORS349	−4.9	0.7	0.0	37.2	0.02	0.9
ORS292 × *S*	−7.5	0.5	0.1	66.8	< 0.0001	2.7
ORS349 × *S*	7.2	0.5	0.1	65.4	< 0.0001	2.6
ORS292 × ORS349 × *S*	−3.0	0.8	0.0	36.4	0.02	0.8

Table 3 Type-III *F* test probabilities ($Pr > F$) and coefficients of determination (R^2) for intralocus and interlocus effects (seeds per capitulum) of SP QTL segregating among 91 self-compatible (*ss*) NMS373 × (NMS373 × ANN1811) BC_1 progeny

Parameter	Effect	$Pr > F$	R^2
ORS292	135.7	< 0.0001	15.1
ORS349	135.8	< 0.0001	15.1
ORS292 × ORS349	73.6	0.02	4.4

vested from primary and secondary capitula and afterripened for 1 month, the period typically required to break dormancy in physiologically mature seeds of domesticate (elite) genotypes. Sufficient BC_1S_1 seeds were produced on 101 BC_1 progeny for seed dormancy phenotyping (the other progeny were either SI or weakly self-pollinated and produced insufficient seed for germination tests) (Fig. 1). Seven-day germination percentages for ANN1811 and NMS373 were 0% and 100%, respectively, and ranged from 0% to 100% among BC_1S_1 families (Fig. 6). The speed of germination was assessed by comparing 3-day and 7-day germination percentages and varied among BC_1S_1 families. The phenotypic correlation between 3-day and 7-day germination percentage was 0.80 ($P < 0.001$). QTL were mapped using 7-day germination percentages only. The phenotypic distribution for 7-day germination percentage was approximately normal and slightly right-skewed (towards the elite parent). CIM identified three seed dormancy QTL (*sg3.1*,*sg11.1*, and *sg15.1*) (Fig. 4). The intralocus effects were positive for each QTL

(17.2%, 22.7%, and 19.3% for *sg3.1*,*sg11.1*, and *sg15.1*, respectively); hence, alleles transmitted by the elite parent increased seed germination. Slightly more than one-half of the phenotypic variability was not explained by QTL (the R^2 for the three-QTL model was 43.4%). No significant epistatic interactions were found when a mixed model QTL analysis of the 2^3 factorial was performed on DNA marker loci closest to *sg3.1*,*sg11.1*, and *sg15.1* (ZVG9, ZVG51, and ORS1141).

Discussion

SI is governed by a single, genetically complex, multiallelic *S* locus in several genera of flowering plants (de Nettancourt 1977; Nasrallah 2002; Hiscock and McInnis 2003). By performing a genome-wide search and distinguishing between QTL for autogamy (natural SP) and SI, we identified a single SI locus (*S*) in sunflower (Figs. 1, 2), as was hypothesized by Fernandez-Martinez and Knowles (1978), but contrary to the findings of Habura (1957), Lofgren and Nelson (1977), Olivieri et al. (1988), and Vranceanu et al. (1988). Earlier genetic analyses of SI in sunflower were performed before the development of DNA markers and STS maps (Tang et al. 2002, 2003; Yu et al. 2003), thereby precluding comparative mapping and genome-wide scans for loci controlling SI and SP. *S* loci could be duplicated and hemizygous in interspecific hybrids carrying rearranged chromosomal segments (Rieseberg et al. 1993, 1995; Rieseberg 1998; Burke et al. 2004); for example, the two independent *S* loci reportedly segregated in *H. annuus* × *H. bolanderi* Gray and *H. annuus* × *H. exilis* Gary hybrids (Olivieri et al. 1988) and could have been produced by a translocation or inversion of the chromosomal segment harboring *S* on LG 17 in *H. annuus* (Figs. 3, 5). Searches for unlinked *S* loci can be rapidly performed using STS marker loci linked to *S* (Fig. 5) and systematically screening STS marker loci strategically positioned throughout the sunflower genome (Tang et al. 2002, 2003; Yu et al. 2003).

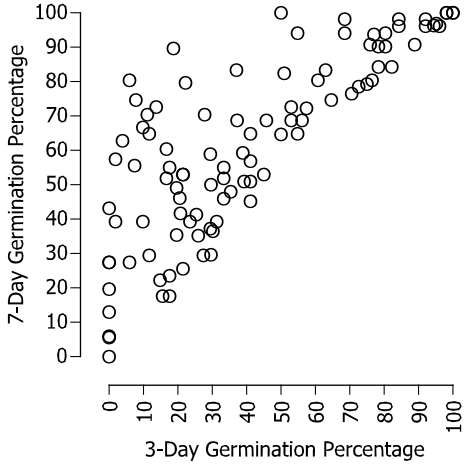

Fig. 6 Three- and seven-day germination percentages among seeds of 101 NMS373 × (NMS373 × ANN1811) BC_1S_1 families afterripened for 4 weeks

Self-compatible *S* alleles arise through loss-of-function mutations (Nasrallah 2002; Hiscock and McInnis 2003) and have undoubtedly arisen more than once in sunflower. Mutations in either of the physically linked *S* genes in *Brassica* disrupt the receptor-ligand protein interaction, thereby causing the loss of SI (Nasrallah 2002). The original sources of self-compatibility (loss-of-function mutations in *S*) used in sunflower breeding are not known. Self-compatibility was known in the pre-hybrid era in sunflower (Russell 1952; Luciano et al. 1965). Selection for self-compatibility and SP became increasingly important in sunflower breeding once cytoplasmic-genic male-sterility was discovered and single-cross hybrids were introduced (Fick and Rehder 1977; Fick 1978; Miller and Fick 1997). SI was needed for inbred line development, and SP was needed to reduce insect pollinator requirements, maximize seed yields, and produce oilseed sunflower on a commodity scale (Fick and Zimmer 1976; Fick and Rehder 1977; Miller and Fick 1997). Hybrids mask the effects of deleterious recessive alleles exposed by inbreeding in inbred line development and, consequently, are the ideal vehicle for utilizing self-pollination in a heterotic, insect-pollinated, allogamous species.

Haplotypes of the highly polymorphic *S* locus in *Brassica* are typically incompletely or completely dominant (Nasrallah 2002). The *S* allele segregating in NMS373 × ANN1811 was inferred to be incompletely dominant because fully self-incompatible BC_1 individuals were rare, the wild parent (ANN1811) produced no seeds when manually selfed, the phenotypes produced by *SS′* and *Ss* genotypes differed, and 94.3% of heterozygote (*Ss*) BC_1 progeny produced 1–30 seeds per capitulum (Fig. 1). Seeds may have been produced in *Ss* individuals by bypassing SI through the transfer of pollen from mature anthers on outer whorls of disk flowers to immature stigmas on inner whorls of disk flowers, so-called 'bud-pollination' (de Nettancourt 1977) or by weak or leaky SI phenotypes, such as those produced by class-II *S* haplotypes in *Brassica* (Nasrallah and Nasrallah 1993; Cabrillac et al. 1999). We discounted bud-pollination because seed could not be produced by manually selfing the wild parent.

LOD peaks for two large-effect self-pollination QTL identified by Burke et al. (2002) on the lower end of LG 17 in HA89 × ANN1811 were separated by a distance of 16.3 cM. We suspect that one of the QTL was an artifact of a locus ordering error and that a single QTL for self-pollination was present on LG 17 in HA89 × ANN1238. When we aligned the HA89 × ANN1238 map (Burke et al. 2002) with three other maps (Tang et al. 2002; Yu et al. 2003), a locus ordering error was discovered on the lower end of LG 17 in HA89 × ANN1238. ORS204 mapped to the bottom of LG 17 in HA89 × ANN1238, below the second QTL for self-pollination (Burke et al. 2002), but mapped near the middle of LG 17 in

RHA280 × RHA801 and PHA × PHB (Yu et al. 2003), above ORS727, ORS811, and the second QTL identified by Burke et al. (2002) and below ORS297 and ORS561 (Fig. 5). ORS204 was deduced to be several centiMorgans upstream of *S* and the SI and SP QTL we identified on LG 17 in NMS373 × ANN1811 (Figs. 2, 4). Shifting the position of ORS204 to the ORS561-ORS727 interval on the HA89 × ANN1238 map eliminates the erroneous ORS804 (EG825)-ORS204 interval and the lower QTL for SP identified by Burke et al. (2002). The upper QTL for self-pollination on LG 17 in HA89 × ANN1238 was centered on ORS811 (Burke et al. 2002), an SSR marker a few centiMorgans upstream of ORS735 on LG 17 (Tang et al. 2002, 2003; Yu et al. 2003). ORS735 is tightly linked to *S* (Fig. 3); hence, the upper QTL for self-pollination identified by Burke et al. (2002) was undoubtedly caused by the segregation of the *S* locus. The *S* locus had the largest effect on self-pollination in NMS373 × ANN1811 and produced an R^2 estimate similar in magnitude to the R^2 estimate for the SP QTL identified in HA89 × ANN1238 (Table 1; Fig. 3).

S genes have not yet been identified in sunflower. The sunflower *S* locus is undoubtedly genetically complex, as in the crucifers where two molecularly complex, physically linked genes (*SRK* and *SCR*) confer SI (Nasrallah 2002; Hiscock and McInnis 2003). The products of *SRK* and *SCR* function as receptors and ligands in self-recognition. *S* loci in the crucifers are highly polymorphic, and 50–60 haplotypes have been identified in *Brassica* (Boyes and Nasrallah 1993; Nou et al. 1993; Boyes et al. 1997; Ockendon 2000; Ruffio-Chable and Gaude 2001). The number of *S*-locus variants is not known in sunflower. The *S* locus is under strong selection and should be highly polymorphic in SI wild populations. It resides in an apparently gene-rich region on LG 17 that harbors a cluster of 20 QTL for domestication, morphological, and agronomic traits (Burke et al. 2002; Tang et al. 2005). Genetic variability for loci linked to *S* should be perpetuated by heterozygote selection and hitchhiking. Until *S* genes are cloned in sunflower, the *S* locus can be tracked and SI alleles can be selected in elite × wild crosses using flanking DNA markers identified in the present study. The closest STS markers (HT945 and ORS735) flank *S* by 2.4 cM and 6.1 cM (Fig. 5), so double-crossovers should be rare in the HT945-ORS735 interval.

Significantly larger seed samples and additional after-ripening and germination treatments are needed to validate the seed dormancy QTL identified in NMS373 × ANN1811. The seed supply was limited in the present study, and backcrossing to the elite parent was necessary because the wild parent was self-incompatible, as are most dormant genotypes in sunflower (Heiser 1951, 1976; Heiser et al. 1969; Seiler 1988, 1992, 1996, 1997, 1998); however, certain elite × wild hybrids carry incompletely dominant or leaky *S* alleles and can be selfed to produce inbred progenies and recombinant inbred lines (RILs) for developmental and QTL analyses; for example, self-compatibility was observed in two of the hybrids we tested (CMS372 × PI 468575 and CMS372 × PI 531022) and in HA89 × ANN1238 by Burke et al. (2002).

RILs facilitate the analysis of large, homogenous seed populations (Koornneef et al. 2002; Clerkx et al. 2004) that are free of the confounding effects of genotypic differences between the pericarp (maternal parent) and embryo (offspring). Such effects are critical in sunflower where embryo- and pericarp-imposed seed dormancy mechanisms seem to be operating (Corbineau et al. 1990; Connor and Hall 1997). QTL analyses in additional genetic backgrounds, on a larger scale, and at a higher resolution are needed to further unravel the genetics of seed dormancy in sunflower.

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