# Construction of a first genetic map of distylous *Turnera* and a fine-scale map of the *S*-locus region

**J.D.J. Labonne, A. Vaisman, and J.S. Shore**

**Abstract:** As a prelude to discovery of genes involved in floral dimorphism and incompatibility, a genetic map of distylous *Turnera* was constructed along with a fine-scale map of the *S*-locus region. The genetic map consists of 79 PCR-based molecular markers (48 AFLP, 18 RAPD, 9 ISSR, 4 RAMP), 5 isozyme loci, one additional gene, and the *S*-locus, spanning a total distance of 683.3 cM. The 86 markers are distributed in 5 linkage groups, corresponding to the haploid chromosome number. Molecular markers tightly linked or co-segregating with the *S*-locus in an initial mapping population of 94 individuals were used to assay an additional 642 progeny to construct a map of the *S*-locus region. The fine-scale map consists of 2 markers (*IS864a* and *RP45E9*) flanking the *S*-locus at distances of 0.41 and 0.54 cM, respectively, and 3 additional markers (*OPK14c*, *RP45G18*, and *RP81E18*) co-segregating with the *S*-locus in the total mapping population of 736 individuals. The genetic map constructed will serve as a framework for localization of genes outside the *S*-locus affecting distyly, while molecular markers of the fine-scale map will be used to initiate chromosome walking to find the genes residing at the *S*-locus.

*Key words: Turnera*, distyly, heterostyly, genetic map, fine-scale map, *S*-locus.

**Résumé :** En vue de l'identification de gènes impliqués dans le dimorphisme floral et l'incompatibilité, une carte génétique du genre distylique *Turnera* a été produite en conjonction avec une cartographie fine de la région du locus *S*. La carte génétique s'étend sur une distance de 683,3 cM et compte 79 marqueurs PCR (48 AFLP, 18 RAPD, 9 ISSR, 4 RAMP), 5 locus isoenzymatiques, un gène additionnel et le locus *S*. Les 86 marqueurs sont distribués sur 5 groupes de liaison, soit le nombre haploïde de chromosomes. Des marqueurs moléculaires étroitement liés ou en co-ségrégation avec le locus *S* au sein d'une première population de 94 individus ont été analysés sur 642 descendants additionnels afin de produire une carte détaillée de la région du locus *S*. La carte détaillée comprend deux marqueurs (*IS864a* et *RP45E9*) qui sont respectivement à 0,41 et 0,54 cM de chaque côté du locus *S* ainsi que 3 marqueurs additionnels (*OPK14c*, *RP45G18* et *RP81E18*) qui affichent une co-ségrégation avec le locus *S* au sein de la population complète de 736 individus. La carte génétique servira de cadre pour positionner des gènes situés à l'extérieur du locus *S* mais contrôlant la distylie tandis que les marqueurs de la carte détaillée seront employés pour initier une marche chromosomique en vue d'identifier les gènes résidant au locus *S*.

*Mots-clés : Turnera*, distylie, hétérostylie, carte génétique, carte détaillée, locus *S*.

[Traduit par la Rédaction]

## Introduction

Distyly is a genetic polymorphism whereby two floral morphs (short- and long-styled) having reciprocal arrangements of styles and stamens occur within a population. Darwin (1877), who undertook the first comprehensive study of distylous plants, found that both short- and long-styled morphs possess an incompatibility system that allows maximum seeds to be set only in inter-morph crosses. It was later shown that both the floral polymorphism and the incompatibility are controlled by a single diallelic locus termed the *S*-locus (Bateson and Gregory 1905). Short-styled plants are heterozygous (*Ss*), while long-styled plants are homozygous recessive (*ss*). Subsequent studies have provided evidence that the *S*-locus in *Primula* spp. is actually a gene complex consisting of at least 3 tightly linked genes that control floral dimorphism and incompatibility (Ernst 1955; Dowrick 1956; Lewis and Jones 1992; Kurian and Richards 1997).

In the past decade, attempts have been made to find genes at the *S*-locus of distylous species. For *Fagopyrum* spp. (buckwheat), Yasui et al. (2004) constructed a map of the genome based on amplified fragment length polymorphism (AFLP), while Aii et al. (1998) used bulk segregant analysis and found 3 molecular markers linked to the *S*-locus, the closest at 0.6 cM. In *Primula vulgaris*, Manfield et al. (2005) identified an 8.8 kb genomic DNA sequence associ-

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ated with the *S*-locus region. Li et al. (2007) used fluorescent differential display and discovered a flower timing related gene flanking the *S*-locus of *P. vulgaris* and a gene encoding a putative membrane protein tightly associated with the *S*-locus. Differentially expressed genes that might be involved in downstream functions of distyly have also been identified in *P. vulgaris* (McCubbin et al. 2006). While all these efforts have contributed to initiating molecular characterization, the genes residing at the *S*-locus remain elusive.

Studies of a number of distylous species have shown that genes outside the *S*-locus can also affect distyly. Mather (1950) showed that two unlinked genes in *Primula sinensis*, *a* and *m*, can affect style and stamen length, respectively. Genetic analyses in buckwheat have shown the existence of modifier genes that affect style length and the degree of incompatibility (Matsui et al. 2004, 2007). In *Turnera subulata* and related species, a putative polygalacturonase (*TsPG*) and $\alpha$-dioxygenase have been detected only in the style transmitting tissue of short-styled plants. Although both of these morph-specific proteins may play a role in distyly, linkage studies have shown that the genes encoding them are not located at the *S*-locus (Athanasiou et al. 2003; Khosravi et al. 2004).

As a first step towards finding genes determining distyly, both at and outside of the *S*-locus, we have constructed a first genetic linkage map, exploiting an interspecific backcross of (*T. subulata* × *T. krapovickasii*) × *T. subulata* (Turneraceae). Furthermore, we have constructed a fine-scale map of the *S*-locus region. Discovery of genes determining distyly should increase our understanding of floral development and mechanisms of incompatibility (McCubbin et al. 2006) and might also have important applications in plant breeding, since it might allow discovery of genes determining style and stamen lengths, which can affect seed or fruit yield.

## Materials and methods

### Plant materials and mapping population

The female (segregating) parent (MhBry-9S) was obtained by backcrossing an interspecific $F_1$ hybrid plant of *Turnera subulata* × *T. krapovickasii* to *T. subulata*. Thus, this parent carries genes introgressed from *T. krapovickasii* into *T. subulata*. The male parent (S16L) was a self-incompatible long-styled plant of *T. subulata*. A cross was made between MhBry-9S and S16L to generate a mapping population (a second-generation backcross) consisting of short-styled and homo-styled plants (the latter are self-compatible plants with long stamens and long styles). The origin of the parental plants and further details of the cross are described in Tamari et al. (2005) and Labonne et al. (2007).

### Inter-simple sequence repeat (ISSR) analysis

A total of 100 simple sequence repeat (SSR) primers (UBC primer set No. 9, University of British Columbia, Vancouver) were used to screen for polymorphisms between the parental plants, S16L and MhBry-9S. Genomic DNA was extracted from parental plants and progeny following the protocol of Doyle and Doyle (1987) with a few modifications. Each PCR amplification was carried out in a total volume of 25 μL containing ~50 ng DNA, 5 pmol primer, and JumpStart Taq DNA Polymerase (Sigma-Aldrich, Oakville, Ontario). The PCR conditions described in Kojima et al. (1998) were followed and amplification products obtained were separated on 1.5% agarose gels containing ethidium bromide (1 μg/mL). Primers producing bright and reproducible bands unique to MhBry-9S were used to genotype the progeny.

### Random amplified microsatellite polymorphism (RAMP) analysis

Simple sequence repeat primers from UBC primer set No. 9 (see above) were used in combination with random amplified polymorphic DNA (RAPD) primers (Operon Technologies Inc., Alameda, California) for RAMP analysis to find bands specific to MhBry-9S but absent in S16L. The reaction mixture consisted of ~25 ng DNA, 5 pmol ISSR primer, 10 pmol RAPD primer, and JumpStart Taq DNA Polymerase in a total volume of 12.5 μL. The PCR conditions optimized by Becker and Heun (1995) were used and the amplification products obtained were separated on 7.5% polyacrylamide gels. After electrophoresis, the gels were silver-stained according to the protocol described in Labonne et al. (2007). Only clear and reproducible RAMP markers tightly linked to the *S*-locus were used in assaying the mapping population (below).

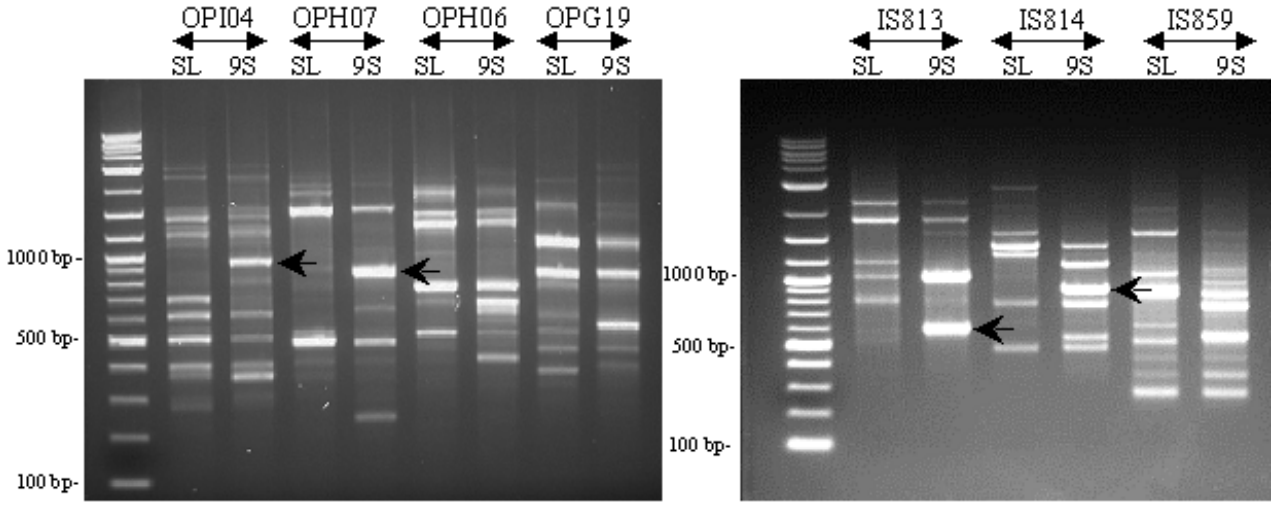
### AFLP analysis

AFLP analysis was performed using both the AFLP Analysis System I (Invitrogen, Burlington, Ontario), following the manufacturer's instructions, and an optimized protocol developed in our lab. Briefly, 250 ng of genomic DNA was digested with 2.5 units of both *Eco*RI and *Mse*I (New England Biolabs, Pickering, Ontario). Following restriction digestion, *Eco*RI and *Mse*I adapters were ligated to the ends of the fragments with T4 DNA ligase (QIAGEN, Mississauga, Ontario) and the mixture was incubated for 2 h at room temperature. After ligation, a first round of PCR was carried out with *Eco*RI and *Mse*I primers both having a degenerate nucleotide at the 3′ end (5′GACTGCGTACCAATTCN and 5′GATGAGTCCTGAGTAAN, respectively). A second round of PCR (selective PCR) was then performed using *Mse*I primers and fluorescently labeled *Eco*R1 primers (WellRED Dyes, Integrated DNA Technologies, Inc., San Diego, California). A total of 81 *Mse*I–*Eco*RI primer combinations were used to find polymorphisms between S16L and MhBry-9S. The PCR conditions described in the AFLP Analysis System I were followed in both rounds of PCR. Amplification products generated from the selective PCR were run on the CEQ 8000 Genetic Analysis System (Beckman Coulter Inc., California). All DNA fragments obtained were analyzed using the fragment analysis module and samples producing weak signals were repeated. Scoring of polymorphic bands was carried out using CEQ AFLP Dominant Scoring Software.

### Isozyme and RAPD analysis

Five isozyme loci, including aconitase (*Aco-1*), 6-phosphogluconate dehydrogenase (*Pgd-c*), leucine aminopeptidase (*LAP-1*), menadione reductase (*MNR-1*), and esterase

**Fig. 1.** Examples of polymorphisms identified using RAPD (left panel) and ISSR (right panel) analyses. Abbreviations SL and 9S correspond to parental plants S16L and MhBry-9S, respectively. Primers used are indicated above each pair of lanes. Some of the polymorphic bands mapped are indicated by arrows.




(*EST*), were assayed following Shore and Barrett (1987), while RAPD analysis followed Labonne et al. (2007).

### Single-strand conformation polymorphism (SSCP)

The style polygalacturonase gene, *TsPG* (Athanasiou et al. 2003), was amplified from genomic DNA with primers TPRSTY and NNSTY4 (5′GCCCCTGTTAGTTCCAAGATT and 5′CAGTACTTCCATAGAACCTCA, respectively). Each PCR product (4 μL) was mixed with 20 μL of SSCP buffer (95% formamide, 10 mmol/L NaOH, 0.25% bromophenol blue) in a 0.2 mL PCR tube and denatured at 95 °C for 10 min in a thermal cycler (Mastercycler Gradient, Eppendorf, Mississauga, Ontario). The tubes were then quickly chilled on ice and 5 μL was run on a 5% polyacrylamide gel for 45 min at 200 V. Following electrophoresis, the gels were silver-stained and scored as described in Labonne et al. (2007).

### Linkage analysis

Segregation data from a total of 94 progeny were analyzed using MAPMAKER 3.0 (Lander et al. 1987; Lincoln et al. 1992). The "group" command was used at a LOD score of 3.0 and a maximum distance of 25 cM to establish the linkage groups. Up to 8 markers showing no distortion in segregation ratio ($\chi^2$ goodness-of-fit test, $P > 0.01$; expected ratio 1:1) were used to build a framework map for each linkage group using the "compare" and "map" commands. Additional markers were added to each linkage group using the "try" command and the order was verified using the "ripple" command. Map distances were calculated using the Haldane mapping function.

### Fine-scale mapping

Markers initially identified during RAMP analysis were used in a second RAMP-PCR on a total of 6 progeny (3 short-styled and 3 homostyles) of nonrecombinant genotypes to determine whether the RAMP markers are located on the chromosome bearing the *S*-locus. Primers producing markers on this chromosome were used to assay known recombinants (18 in total) on either side of the *S*-locus. This procedure allowed the identification of RAMP markers close to or co-segregating with the *S*-locus. Of all types of molecular markers identified, only the ones tightly linked or co-segregating with the *S*-locus in the initial mapping population of 94 progeny were used to genotype an additional 642 individuals.

## Results

### Molecular markers

RAPD and ISSR analyses were initially used to discover markers on the chromosome bearing the *S*-locus. Once polymorphisms using these markers were exhausted, we chose to use AFLP because it is known to produce high levels of polymorphism per primer (Vos et al. 1995; Lin et al. 1996). Being amenable to automation, AFLP would not only allow us to find additional markers on the chromosome bearing the *S*-locus but would also allow identification of markers on the remaining 4 chromosomes.

Both RAPD and ISSR analyses produced many bright, reproducible bands that could be easily scored (Fig. 1). Where polymorphic bands were of low intensity, for instance with RAPD primer OPH07 and ISSR primer IS859 (Fig. 1, band sizes of ~650 bp and ~900 bp, respectively), they were not considered for mapping. While RAPD analysis was found to generate more polymorphisms than ISSR analysis, it was AFLP analysis that produced the greatest polymorphism overall, averaging 5.1 bands per primer pair (Table 1). Not all polymorphic bands generated by the above three methods were used in assaying the mapping population of 94 progeny. The segregation patterns of two RAPD markers in 20 progeny are shown in Fig. 2.

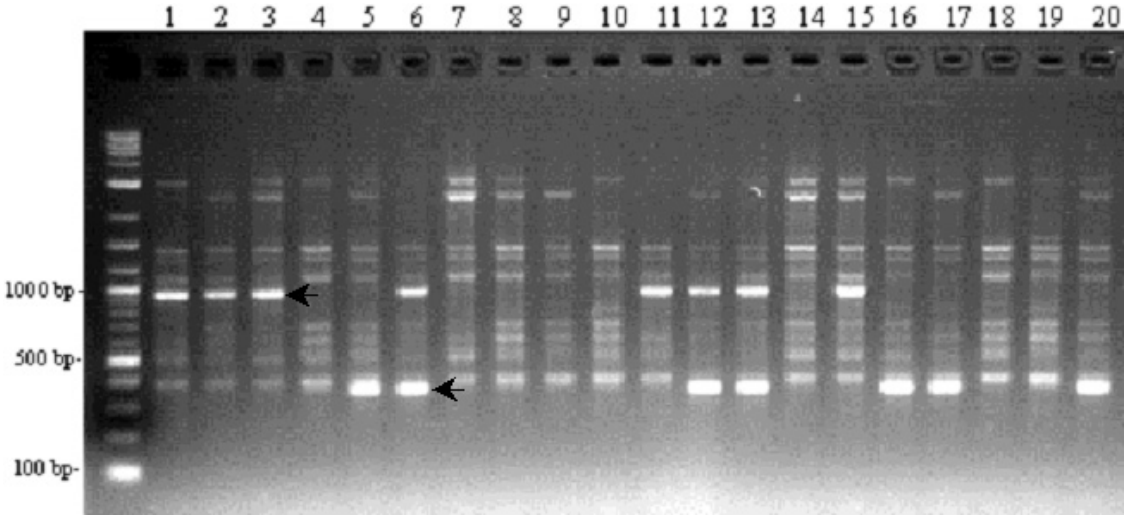
### Linkage map

Analysis of segregation data from the 94 progeny established 5 large linkage groups consisting of more than 12 markers each (Fig. 3) and 5 smaller ones having only 2–3 markers. A total of 23 markers remained unlinked. The 5 large linkage groups identified are consistent with the haploid number of 5 chromosomes in *T. subulata* and *T. krapo-*

**Table 1.** Number of polymorphic bands identified with four molecular methods.

| Molecular method | No. of primers or primer pairs | No. of polymorphic bands identified | No. of polymorphic bands per primer | No. of markers mapped |
|---|---|---|---|---|
| RAPD | 111 | 43 | 0.39 | 18 |
| ISSR | 100 | 29 | 0.29 | 9 |
| AFLP | 81 | 410 | 5.06 | 48 |
| RAMP | 292 | 323 | 1.11 | 4 |

**Fig. 2.** Segregation of two polymorphic RAPD markers in 20 progeny from the initial mapping population. Both markers (~900 bp and ~375 bp) were produced using primer OPI04. For the ~900 bp marker, 8 progeny show presence of the marker and 12 progeny lack the marker, while for the ~375 bp marker, 7 progeny have the marker and 13 progeny do not.




*vickasii* (Solís Neffa and Fernández 2000). The linkage group bearing the *S*-locus carried 24 markers, while the remainder possessed 13 to 18 markers. The length of the linkage groups varied from 82.6 to 204.4 cM (Table 2). Although markers were mapped irrespective of which parental species they were derived from, most of the dominant molecular markers were found to come from *T. krapovickasii* and only 9 originated from *T. subulata*. Four molecular markers, *IS864a*, *OPK14c*, *RP45G18*, and *RP81E18*, were found to co-segregate with the *S*-locus in the initial mapping population. The flanking markers next closest to the *S*-locus were at distances of 1.1 cM.

The 6 genes mapped — *Aco-1*, *Pgd-c*, *LAP-1*, *MNR-1*, *EST*, and *TsPG* — were all codominant markers. The style polygalacturonase gene (*TsPG*), previously shown to be loosely linked (4.6 cM) to the *S*-locus in the reciprocal cross (Athanasiou et al. 2003), was mapped to the very end of linkage group 1. The total map length of all linkage groups was 683.3 cM (Table 2).

## Segregation distortion

A total of 35 markers were distorted when assessed at a type I error rate of 5% ($P < 0.05$) per locus, and this represents 40.2% of all markers mapped. For a type I error rate of 1% ($P < 0.01$), the percentage of markers exhibiting distortion decreased to 24.4% (Table 2). Linkage group 5 contained the largest number of distorted markers (13 at $P < 0.01$), while markers in the linkage group bearing the *S*-locus did not show any distortion at $P < 0.01$. Distorted markers were usually positioned next to each other, especially in linkage groups 2–4 (Fig. 3). All *T. krapovickasii*–derived markers that showed segregation distortion at $P < 0.01$ were underrepresented in the progeny. The one *T. subulata* dominant marker that showed distortion at $P < 0.01$ was present in excess (Table 2).

## Fine-scale mapping

RAMP analysis provided an inexpensive and reliable method to find more markers on the chromosome bearing the *S*-locus. Not only does RAMP analysis provide greater levels of polymorphism than RAPD or ISSR analyses (Table 1), but the number of primer combinations that can be used is almost unlimited. For these reasons, RAMP analysis was used in targeting the *S*-locus to find tightly linked or co-segregating markers. After confirmation that the RAMP markers were linked (see Materials and methods), we used known recombinants for *Aco-1* and *Pgd-c* from the initial mapping population to screen for markers located within ~4 cM of the *S*-locus, on either side. Once a marker of interest was identified using RAMP analysis, all 94 progeny were subsequently genotyped.

To construct a fine-scale map, we assayed an additional 642 progeny with the RAPD and ISSR markers that co-segregated with the *S*-locus in the initial mapping population. The ISSR marker *IS864a* was mapped to one side of the *S*-locus at a distance of 0.41 ± 0.23 cM, while the RAPD marker *OPK14c* continued to co-segregate with the *S*-locus after analysis of the additional 642 progeny (Fig. 4). Marker *RP45E9* (a RAMP marker), which produced only one recombinant with the *S*-locus in the segre-

**Fig. 3.** Linkage map of distylous *Turnera.* RAPD and ISSR markers start with the letters OP and IS, respectively. For AFLP, the last three nucleotides at the 3′ end of the *Eco*RI and *Mse*I primers were used to name each marker. Markers showing segregation distortion at $P < 0.01$ are marked with an asterisk.

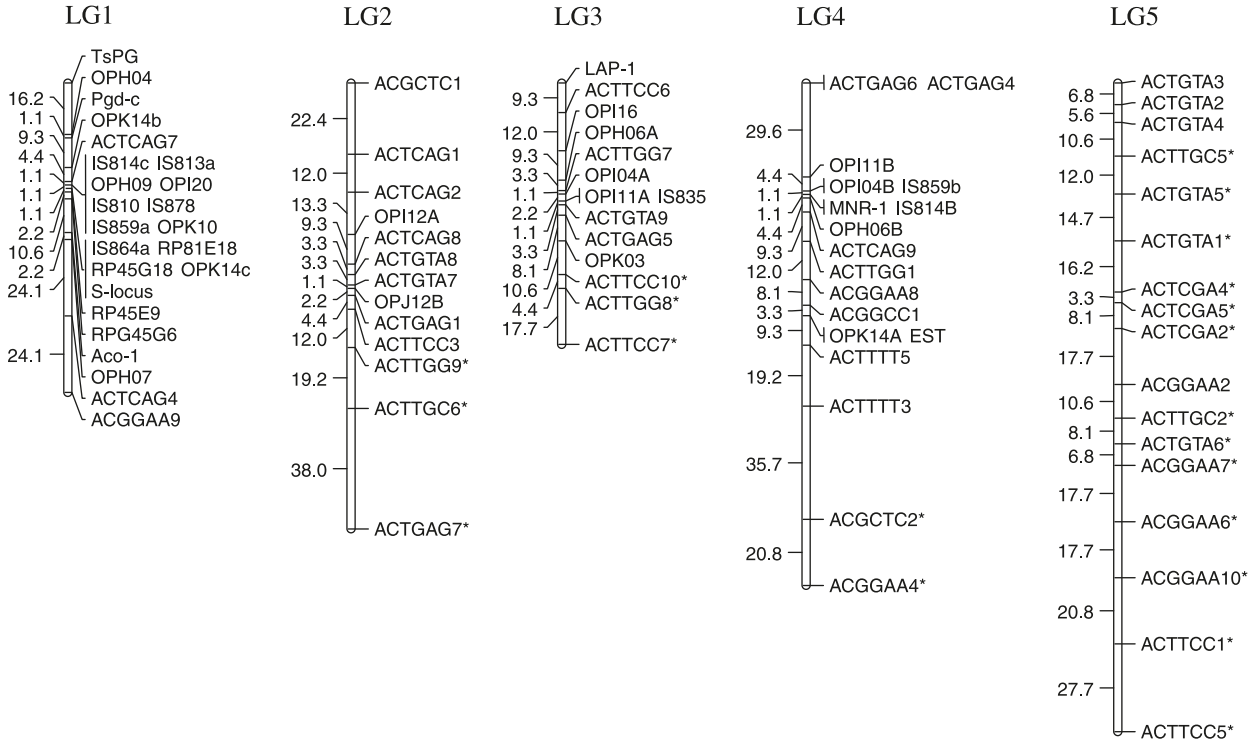



**Table 2.** Characteristics of linkage groups.

| Linkage group | Map length (cM) | No. of markers | | No. of distorted markers | |
|---|---|---|---|---|---|
| | | *T. krapovickasii* | *T. subulata* | $P < 0.01$ | $P < 0.05$ |
| LG1 | 97.5 | 18 | 6 | 0 | 2 $K_e$ |
| LG2 | 140.5 | 10 | 3 | 2 $K_d$, 1 $S_e$ | 3 $K_d$, 1 $S_e$ |
| LG3 | 82.6 | 14 | 0 | 3 $K_d$ | 13 $K_d$ |
| LG4 | 158.3 | 18 | 0 | 2 $K_d$ | 2 $K_d$ |
| LG5 | 204.4 | 17 | 0 | 13 $K_d$ | 14 $K_d$ |
| Total | 683.3 | 77 | 9 | 21 | 35 |

**Note:** Codominant markers were arbitrarily considered to be derived from *T. krapovickasii* and none showed distortion. $K_e$ indicates a marker derived from *T. krapovickasii* is in excess, $K_d$ indicates a marker derived from *T. krapovickasii* is in deficiency, and $S_e$ indicates a marker derived from *T. subulata* is in excess.

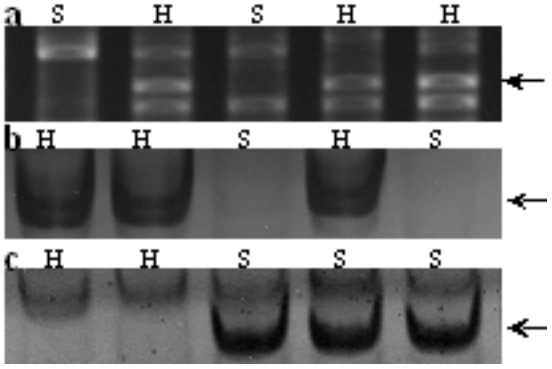
gating population of 94 individuals, was mapped at a distance of 0.54 ± 0.27 cM on the other side of the *S*-locus relative to *IS864a*. Two additional RAMP markers, *RP45G18* and *RP81E18*, were found to co-segregate with the *S*-locus in the total mapping population of 736 individuals (Fig. 4). Assuming that the next individual to be assayed for these markers is a recombinant, the distance of the three co-segregating markers would be 0.14 ± 0.14 cM on one side or the other of the *S*-locus.

While markers *OPK14c* and *RP45G18* were assayed for all 736 progeny, RAMP marker *RP81E18*, once found to be linked to the *S*-locus, was mapped by assaying only the recombinant progeny identified with markers flanking the *S*-locus (*RP45E9* and *IS864a*). The approach is reasonable, as it is highly improbable that a double cross-over would occur in the interval between the two closely adjacent flanking markers. In the absence of cross-over interference, the probability of a double cross-over is given by the product of the recombination frequencies between each locus and the *S*-locus, or $0.0054 \times 0.0041$, which is about $2.2 \times 10^{-5}$. It is therefore highly unlikely that any recombinants for this co-segregating marker would have been missed using this strategy. Segregation data obtained from the 5 markers mentioned above were used to construct a fine-scale map of the *S*-locus region (Fig. 5).

## Discussion

The present study reports a first genetic linkage map of distylous *Turnera* along with a fine-scale map of the *S*-locus

**Fig. 4.** Markers co-segregating with the *S*-locus for five different progeny assayed for each. (*a*) RAPD marker *OPK14c*, (*b*) RAMP marker *RP45G18*, which runs as a doublet, and (*c*) RAMP marker *RP81E18*. S, short-styled plants; H, homostyled plants.

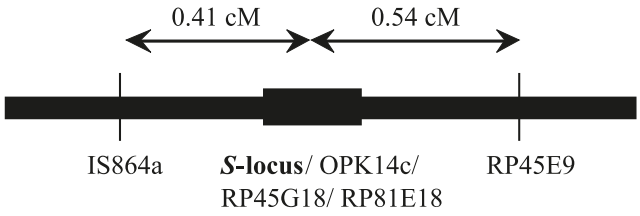



region. A cross was designed to enhance heterozygosity in regions around the *S*-locus, but we also exploited this opportunity to obtain the first map of the genome. The genetic map consists of 86 markers distributed in 5 linkage groups spanning a total distance of 683.3 cM, with an average spacing of one marker every 10.7 cM.

Clustering of markers occurred in most linkage groups where numerous markers were mapped within a few centimorgans. These clusters were not biased towards any marker type; i.e., clusters were heterogeneous with respect to the markers they contained. Clustering of markers of one type, for instance, RAPD, AFLP, or RFLP, has been reported in sugar beet, soybean, and barley (Nilsson et al. 1997; Young et al. 1999; Becker et al. 1995). In the case of AFLP, clustering has sometimes been associated with the type of restriction enzymes used and their sensitivity to DNA methylation (Young et al. 1999). Chromosomal regions experiencing lower levels of recombination are expected to accumulate markers in clusters, as opposed to other regions where there is no suppression of recombination (Ma et al. 2004). It is known that recombination rate is reduced in regions around the centromeres (Mather 1939; Tanksley et al. 1992). The clustering of markers observed in our study occurred near the center of the linkage groups, suggesting that the clusters may represent centromeric regions. This inference is supported by the observation that the karyotype of *Turnera subulata* and *T. krapovickasii* comprises 4 approximately equal-sized metacentric chromosomes and one submetacentric chromosome (Solís Neffa and Fernández 2000).

Approximately 24% of loci mapped showed departures from the expected Mendelian ratio of 1:1 at a level of $P < 0.01$. For most linkage groups, loci showing segregation distortion tended to be positioned next to each other, with linkage group 5 containing most of the distorted loci (Fig. 3). Segregation distortion is a common occurrence in genetic mapping, particularly in crosses between strains, cultivars, and species. Various factors are thought to be the cause of the non-Mendelian ratios, including differential viability or lethality of pollen (Xu et al. 1997), differential survival of zygotes (Fishman et al. 2001), differences in genome size (Jenczewski et al. 1997), and linkage to distorting factors (Zamir and Tadmor 1986; Xu et al. 1997; Fishman and Willis 2005). A majority of the loci exhibiting aberrant ratios showed a deficiency of *T. krapovickasii* markers, suggesting

**Fig. 5.** Fine-scale map of the *S*-locus region consisting of the two closest flanking and three co-segregating molecular markers.




that gametes or zygotes carrying chromosomal segments from *T. krapovickasii* introgressed into the *T. subulata* background tend to be less viable. It is also possible that gametes containing mostly the *T. subulata* genome tend to outcompete gametes of other genotypes, although for this cross competition among ovules would be required. In an attempt to find the cause of the observed deficiency of homostyled progeny for the cross used in the present study (and its reciprocal), Tamari et al. (2005) identified pollen tube competition as the most likely cause of segregation distortion, as there is considerably greater distortion when the male parent is segregating.

We explored segregation data for 5 markers on linkage group 5 in the reciprocal cross (male parent was segregating) and found extremely marked distortion with very limited evidence for transmission of any *T. krapovickasii* markers (J.D.J. Labonne and J.S. Shore, unpublished data). This is in contrast to the female cross reported here, which was distorted but far less so. While several factors could be responsible for the aberrant ratios, our data point to the hybrid nature of the cross and the reduced viability of progeny carrying increasing amounts of the *T. krapovickasii* genome as plausible causes of the segregation distortion.

There is evidence from distylous species that genes outside the *S*-locus affect incompatibility and floral morphology (Mather 1950; Matsui et al. 2004, 2007). A map of the genome may be of considerable value in the genetic localization and ultimate cloning and identification of such genes. In a recent study, McCubbin et al. (2006) identified 11 classes of cDNA that were differentially expressed between short-styled and long-styled morphs of *Primula vulgaris*. Linkage analysis showed that none of the genes appear to be located on the chromosome bearing the *S*-locus, implying that they might be involved in downstream functions of distyly (McCubbin et al. 2006). An unusual self-compatible short-styled plant of *T. subulata* appears to be the result of a gene(s) not linked to the *S*-locus, although the inheritance of the "mutation" abolishing the incompatibility response of the style was not fully resolved (Shore and Barrett 1986). The genetic map obtained here will aid in resolving the linkage relationships of the gene(s) involved. Recently, we used X-ray mutagenesis to obtain mutations influencing distyly in *T. subulata* and *T. scabra*. We have generated a self-compatible short-styled plant, one long homostyle, and three long-styled X-ray mutants (J.D.J. Labonne and J.S. Shore, unpublished data). A series of controlled crosses are being made to investigate the inheritance of the mutations. The genetic map and markers identified here should be useful in localizing the gene(s) responsible for the mutant phenotypes.

Estimates of the genome size of *T. subulata* or *T. krapo-*

*vickasii* are not available. Using flow cytometry, however, the "1C" value of hexaploid ($2n = 30$) *T. ulmifolia* was estimated to be 1.51 pg (K.H. Keeler, personal communication 1993). *Turnera subulata* and *T. krapovickasii* are diploid. We roughly estimate the genome size of these species to be one-third the size of the *T. ulmifolia* genome, giving a 1C estimate of ~0.5 pg or approximately 500 Mb. The sum of the map length of all linkage groups in the present study is 683.3 cM and it is very likely an underestimation of total map length. Using method 4 in Chakravarti et al. (1991), following the approach of Hall and Willis (2005), we estimate the total map length to be ~772.5 cM. We can infer that 1 cM is approximately 650 kb in *T. subulata* and *T. krapovickasii*. The kb/cM ratio is very useful in the planning of positional cloning experiments (Durrett et al. 2002).

We have now constructed a bacterial artificial chromosome (BAC) library of *T. subulata*, and tightly linked or cosegregating markers identified in the present study are being used to initiate chromosome walking to find the gene(s) residing at the *S*-locus. Comparable approaches are underway in buckwheat (Nagano et al. 2005) and *P. vulgaris* (Li et al. 2007). The data obtained will likely be of considerable interest, since each of these species has had an independent origin of distyly, and will help in identification of genes involved in self-incompatibility and floral development.

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