The position of *Age* and *Wsp* on linkage group IV

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The locus *Age* (*Ageotropic*) has been determined to be on linkage group (LG) IV approximately 14 cM distal to the STS marker P393a (3). Last year the senior author reported that the locus *Wsp* is linked to *Age* (2); however, the distance between *Age* and *Wsp* was only roughly approximated, and the relative order of the loci on the linkage group was not determined. Both *age* and *wsp* are easily scored in seedlings, and the region of LG IV distal to P393a has relatively few convenient markers. Hence, both loci have great potential as standard anchor markers for general mapping studies, and it is important to clearly establish their relative positions. We present here the order of the loci on LG IV and a more precise estimation of the distance between them. In addition, we present evidence that the number of RAPDs in this region is relatively low, further emphasizing the importance of these morphological mutants for mapping studies.

Three experiments were performed to more precisely determine the linkage relationships among markers around *Age*. One involved a classical linkage analysis with the alleles *age* and *wsp* in coupling phase. The second attempted to place *Wsp* directly on the consensus map, and the third was an attempt to use bulked segregant analysis (1) to identify additional DNA markers near *Wsp*. Only the first experiment was successful, but the lack of success of the other two may reveal important information about the DNA sequences in this region of the genome.

A plant homozygous for both *age* and *wsp* was selected from the F_2 mapping population described in (2). This plant was crossed to two *Age Wsp* lines of unrelated parentage (B285-500-5 and cv. Sparkle). The two F_2 populations derived from these crosses were grown in a 1:1 peat:silt soil mix in Conetainers in the greenhouse. Seed were examined after five days to insure *age* plants were oriented so that the primary root was directed downward and the epicotyl had a clear route to the surface. After two weeks, seedlings were scored for wax deposition and root orientation as follows: plants lacking wax on stem and upper surface of stipule were scored *wsp*; plants with normal wax on stem and upper surface of stipule were scored *Wsp*; plants with secondary roots protruding above soil surface were scored *age*; plants without roots on surface as *Age*. Most plants were then transferred to pots, enabling the root orientation to be further examined. In all cases the appearance of roots at the soil surface had correctly revealed the homozygous *age* genotype.

Segregation ratios indicated that in both populations each gene segregated normally. Joint segregation analysis gave distances of 16 and 23 cM for the single population recombination estimates (Table 1). Heterogeneity tests indicated the two results could be combined, and the combined linkage value was 19 $\pm$ 11 cM. This distance is in reasonable agreement with the previous estimate of <15 cM (2), which was based on a small population with alleles in repulsion phase. The distance is also similar to that between *Age* and P393a (3). Hence, *Wsp* must either be very near P393a or at the distal end of the known map for LG IV.

Table 1. Joint segregation analysis of the loci *Age* and *Wsp* in two F_2 populations

		No. F_2 plants with designated phenotype				χ^2	Recomb.	Stand.
Cross	N	+/+	+/-	-/+	-/-	3:1	Fract.	Error
C00-29	36	21	4	2	8	13.0	16	16
C00-5	39	28	4	3	4	7.0	23	15
Combined	75	49	8	5	12	21.2	19	11

In order to determine the relative order of P393a, *Age* and *Wsp*, a cross was made between a recombinant inbred line from a mapping population derived from the cross MN313 x JI1794 (J. Przyborowski and N.F. Weeden, unpublished) and a line homozygous for *wsp*. An F_2 population was generated from this cross and joint segregation analysis performed on P393a, *wsp* and several RAPD markers known to be on LG IV. Only very weak (non-significant) linkage was observed between P393a (or RAPD markers closely flanking this STS) and *wsp* (data not presented). Although *age* was not segregating in this population, the combination of a lack of significant linkage between P393a and *wsp* and the 14 cM linkage between *Age* and P393a reported in (3), suggest that *Wsp* is distal to *Age* and that this region of LGIV can be represented as follows:

P393a——14 cM————*Age*——19 cM————*Wsp*.

Although a number of RAPD markers were identified distal to P393a on the consensus map (4), none of these segregated in the F_2 used in the second experiment. Hence, we could not locate *Wsp* very precisely on the consensus map. The approximately 33 cM between P393a and *Wsp* is similar to the distance (34 cM) between P393a and the most distal marker on the consensus map. Thus, it would appear that *Wsp* is an excellent marker for the most distal portion of this arm of LG IV (chromosome 7).

Our third experiment attempted to identify RAPD markers linked to *Wsp* by bulked segregant analysis. We used F_3 plants from a cross made by Dr. G.A. Marx (A686-353 [*wsp U*] x A486-115 [*det*]). Ten F_3 plants were grown from each of 30 F_2 plants. F_2 plants homozygous *wsp* or homozygous *Wsp* were identified by examination of the F_3. DNA was extracted from five homozygous *wsp* F_3 plants (each derived from a different F_2) and from five homozygous *Wsp* F_3 plants (again derived from different F_2 plants). The DNA was combined into a *wsp* bulk and a *Wsp* bulk and the bulks compared using 281 primers obtained from Operon Technologies, Inc. or the University of British Columbia biotechnology laboratory. We failed to identify a RAPD marker linked to *Wsp* despite the low stringency of the screen (markers within 20 cM should have been easily recognized in this survey because we included only homozygous lines and only five individuals in each bulk). The few differences that were observed between bulks proved to be 'false positives' unlinked to *Wsp* when tested on the whole population.

In other bulked segregant analysis studies in pea involving this number of primers, we have usually been able to identify several RAPD markers linked to the locus of interest. Hence, we conclude that this region of the pea genome contains a relatively low number of RAPD-generating sequences. Whether this low level of RAPDs is due to a lack of amplifiable sequences in this region or a low level of sequence polymorphism could not be determined from our data.

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