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LINKAGE BETWEEN GENES FOR HAIRY FIRST LEAF AND CHLOROPHYLL DEFICIENCY IN *BRASSICA OLERACEA*

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ABSTRACT

The fourth linkage group of *B. oleracea* L. has two genes: *Hr-1*, (hairy first leaf), a dominant seedling marker from "Dwarf Green" curly kale, and *pg-2*, (pale green seedling), a recessive chlorophyll mutant from green sprouting broccoli. Recombination between *Hr-1* and *pg-2* ranged from 7.4 to 20.1% in the six progenies studied, with a mean of 13.15 ± 0.68%. *Hr-1* segregated independently of the three other linkage groups (two genes of each were tested) and of two unlocated genes for male sterility.

INTRODUCTION

Tests among nine genes of sprouting broccoli (*B. oleracea* L. var. *italica* PLENCK) demonstrated three linkage groups (SAMPSON, 1966). Broccoli was used as the standard genotype for these linkage analyses because of its annual habit. Genes from the biennial varieties of *B. oleracea* are now being backcrossed into broccoli to facilitate genetic analysis. This paper gives the results of linkage tests between a gene for hairy first leaf margin from curly kale (*B. oleracea* L. var. *sabellica* L.) and the nine previously analysed genes of broccoli.

MATERIALS

The broccoli genes white petal *(Wh)*, cream petal *(cr)*, persistent sepals *(ps)*, glossy foliage *(gl-1)*, pale green foliage *(pg-1* and *pg-2)* and male sterility *(ms-1* and *ms-4)*, together with a gene for purple stems and ovaries *(A^{ck})* from curly kale, were described earlier (SAMPSON, 1966). The gene for hairy first leaf margin was first reported from marrow-stem kale (THOMPSON, 1956). It is here designated *Hr-1* to distinguish it from *Hr-2* which gives hairy leaves and petioles (THOMPSON, unpublished). *Hr-1* is an excellent seedling marker with normal viability. I obtained the gene from plant 59-10-01 of "Dwarf Green" curly kale. Hairs were present on the margins of new leaves throughout the life of this plant. However, in most of the segregating progenies reported here the marginal hairs were restricted to the first two or three true leaves, as in marrow-stem kale.

The identity of the *Hr-1* gene from curly kale with the gene for hairy first leaves in marrow-stem kale was demonstrated by an F_2 of the cross curly kale plant 59-10-01 × marrow-stem kale homozygous for hairy first leaf. The marrow-stem kale parent was

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supplied by Dr. K. F. THOMPSON in 1964. This F_2 contained 242 plants, all with hairy first leaves.

Hairs on the leaf margin is a rare trait in *B. oleracea*, apart from the kales. I have examined 27 varieties (10 cabbage, 8 broccoli, 6 cauliflower, 2 Brussel sprouts, 1 kohl rabi) and found only two with marginal hairs: Savoy cabbage "Perfection Drumhead" had 12 plants with hairs and 45 with none, "Early Purple Head" broccoli had nine plants with hairs and fifteen without.

METHODS

Controlled pollinations were made during winter in a screened greenhouse. The broccoli × kale hybrids were backcrossed to broccoli for linkage tests between *Hr-1* and floral characters. The progenies for *Wh* and *ms-1* tests were first backcrosses; those for *cr* and *ps* were second backcrosses to broccoli. Almost all plants in these progenies had the annual habit of broccoli.

Seedlings were germinated in the greenhouse where they were scored for *Hr-1*, *gl-1*, *pg-1* and *pg-2*. If the progenies were to be scored for *A*^{ck} *Wh*, *cr*, *ps*, *ms-1* or *ms-4*, the seedlings were transplanted to the field in May. Usually some plants died or failed to flower so that seedling data may represent more plants than adult data for the same progeny. Each plant was individually labeled and classified at least twice for each gene.

Because the original kale parent was *A*^{ck} *A*^{ck} *c c* for the complementary anthocyanin genes, whereas the broccoli parents were *a a C C*, most of the backcrosses studied gave 1:1 segregations for *A*^{ck} : *a*.

Segregation data were analysed for independence between gene pairs by means of 2 × 2 contingency tests. A joint estimate of linkage was obtained by the method of maximum likelihood. If the contingency tests showed independent segregation, data from several progenies were combined (Table 1), provided that the heterogeneity tests for segregation at each of the two loci and for contingency permitted.

RESULTS AND DISCUSSION

Linkage. – In 1961 a chlorophyll deficient *(pg-2 pg-2)* broccoli was pollinated by plant 59-10-01 of curly kale. The F_1 plants had hairy leaves and normal green color. Two F_1 plants were selfed at the bud stage and both F_2 progenies showed linkage between *Hr-1* and *pg-2*. Progeny 62-34, from F_1 plant 61-35-01, gave 16.7 percent recombination as opposed to 7.4 percent in progeny 62-35 from F_1 plant 61-35-02 (Table 1). Also the two progenies were heterogeneous for *Hr-1* segregation and for independence.

Backcross progenies 63-23 and 63-24 confirmed the linkage of *Hr-1* and *pg-2* (Table 1). The two were homogeneous with respect to single gene segregations and independence chi-squares, although progeny 63-23 had a statistically significant deficiency of both *hr-1* and *pg-2* plants. As with the F_2's, progeny 63-23 showed almost twice as much recombination from F_1 plant 61-35-01 as progeny 63-24 showed from its F_1 parent, 61-35-02 (Table 1).

Two backcrosses, 64-10 and 64-11, with *Hr-1* and *Pg-2* in repulsion were raised in 1964. Progeny 64-10 had a great deficiency of *pg-2* plants and the two progenies were heterogeneous for *pg-2* segregation. Progenies 64-10 and 64-11 showed 20 and 13

TABLE 1. SEGREGATION DATA FROM *Brassica oleracea* THAT SHOW LINKAGE BETWEEN THE GENES *Hr-1* AND *pg-2*, AND INDEPENDENCE OF *Hr-1* FROM EIGHT OTHER GENES. THE CHI-SQUARES FOR SINGLE GENE SEGREGATIONS, INDEPENDENCE TESTS (χ^2_I) AND HETEROGENEITY TESTS FOR SIMILAR PROGENIES AND (χ^2_R) FOR JOINT ESTIMATES OF THE RECOMBINATION FRACTIONS ARE LISTED

Mating, phase and pedigree number	Test gene (T)	Observed frequency				χ^2*Hr-1*	χ^2*T*	χ^2I	Recombination	
		Hr-1 T	*Hr-1 t*	*hr-1 T*	*hr-1 t*				Percent	χ^2R
Linkage:									13.15 ± 0.68	
F_2, C[1] 62-34	*pg-2*	120	15	16	40	1.9	1.5	70.2***	16.7 ± 3.0	1.1
62-35	*pg-2*	75	5	2	16	2.3	.7	59.6***	7.4 ± 2.8	3.0
heterogeneity						4.1*	1.9	4.7*		
BC, C 63-23	*pg-2*	289	35	44	212	8.0**	12.8***	303.3***	13.6 ± 1.4	.1
63-24	*pg-2*	348	24	35	317	.6	2.4	507.4***	8.1 ± 1.0	23.7***
heterogeneity						2.6	2.6	2.4		
BC, R[1] 64-10	*pg-2*	72	146	224	21	1.6	35.9***	170.6***	20.1 ± 1.9	13.6***
64-11	*pg-2*	19	209	211	45	1.2	1.6	265.4***	13.2 ± 1.5	< .1
Heterogeneity						< .1	26.8***	14.2***		
Independent segregation:										
BC, R 62-36	*Wh*	121	106	116	89	1.1	4.1*	.5	48.6 ± 2.4	
BC, C 64-16	*gl-1*	137	131	251	244	67.5***	.2	< .1	50.1 ± 1.8	
BC, C 64-21	*ps*	125	80	107	96	< .1	7.7**	2.8	45.8 ± 2.5	
BC, C 62-36, 64-08, 64-09, 64-11, 64-18 and 64-20	A^{ck}	557	581	553	586	< .1	1.4	< .1	49.8 ± 1.0	
heterogeneity						5.4	4.4	2.7		
64-19	A^{ck}	51	57	45	35	4.1*	< .1	1.5	54.3 ± 3.6	
64-21	A^{ck}	122	83	111	92	< .1	8.2**	1.0	47.5 ± 2.5	
BC, C 64-18	*cr*	95	102	120	97	1.0	.6	2.1	53.6 ± 2.5	
BC, C 64-08 and 64-09	*pg-1*	121	130	130	94	1.5	1.5	4.6*	54.7 ± 2.3	
heterogeneity						.5	.1	< .1		
BC, C 64-19	*ms-1*	49	51	33	40	4.2*	.5	.2	48.6 ± 3.8	
64-20	*ms-1*	88	122	81	129	0.0	16.0***	.5	48.3 ± 2.4	
F_2, C 65-64	*ms-4*	225	55	84	23	1.4	4.8*	.2	48.4 ± 3.7	

*, **, *** = significant at .05, .01 and .001 levels of probability respectively.
[1]C = Coupling phase, R = Repulsion phase.

percent recombinations but, curiously, they were both descended (via three backcrosses to broccoli) from F_1 plant 61-35-01 that gave 7 and 8 percent recombination in previous tetsts.

A joint estimate of 13.15 ± 0.68 recombination between *Hr-1* and *pg-2* was obtained for the six progenies by the maximum likelihood method. Two progenies did not fit this estimate, one being too high, the other too low (Table 1).

Independent segregation. – Previously *pg-2* was shown to segregate independently from eight other broccoli genes (SAMPSON, 1966). Now, one F_2 and nine backcross progenies showed independent segregation between *Hr-1* and the eight other genes, i.e., *Wh* and *gl-1* of linkage group 1, *ps* and A^{ck} of group 2, *cr* and *pg-1* of group 3 and two unlinked male sterility genes (Table 1). Aside from the previously discussed *Hr-1*:*pg-2* segregation of progeny 64-11, these progenies had 26 single gene segregations, seven of which had statistically significant deviations from the expected 1:1 or 3:1 ratios. The deficiencies of both *ps* plants and *a* plants in progeny 64-21 probably arose from the same cause because the two genes were on the same chromosome. Except for the deficiency of *Hr-1* plants in progeny 64-16 nothing can be said to explain the observed deviations.

The serious deficiency of hairy plants in backcross 64-16 (observed 268 *Hr-1* and 495 *hr-1*; expected, 1:1) may have resulted from the failure of the character to be ex-

pressed in many *Hr-1* plants. However, proof for this is inconclusive. Progeny 64-16 (= hybrid plant 64-62-05 × broccoli plant 59-18-01) was first sown on Nov. 10, 1964, during the poorest season for growing *Brassica* seedlings in the greenhouse. This sowing gave 141 *Hr-1*:335 *hr-1* plants (χ^2 for 1:1 = 79.0). The residual seed of 64-16, sown on Dec. 18, 1964, gave better results (127 *Hr-1*:160 *hr-1*; $\chi^2 = 3.8$). The two sowings were heterogeneous for the *Hr-1* segregation but both sowings gave nearly perfect *Gl-1*:*gl-1* segregations. These *Gl-1* results provide strong evidence that neither pollen contamination nor admixture of foreign seed caused the *Hr-1* deficiency.

Was low light intensity and short day length responsible for the deficiency of *Hr-1* plants in the first sowing of 64-16? Progeny 64-10, which gave 218 *Hr-1*:245 *hr-1* (Table 1), was grown at the same time but was not significantly deficient for *Hr-1* plants. Was another gene from plants 62-62-05 or 59-18-01 suppressing hair development in progeny 64-16? Plant 62-62-05, itself from the cross 62-35-01 × 59-18-01, was selfed and the seed sown on December 2, 1965. Again there was a large deficiency of *Hr-1* plants (observed 318 *Hr-1*:173 *hr-1*; expected 368:123; $\chi^2 = 39.4$; $.001 > P$) and an excellent *Gl-1*:*gl-1* segregation.

To determine whether broccoli plant 59-18-01 carried genes that affected hair development in *Hr-1 hr-1* plants, the cross 59-10-01 (*Hr-1 Hr-1* curly kale) × 59-18-01 *(hr-1 hr-1)* was made and sown on Dec. 2, 1965. Most of the plants had many hairs on the leaf margins but careful searching revealed only a single hair on some plants, and none was found on three of the 246 plants. Thus no simple genetic hypothesis can explain the deficiency of *Hr-1* plants in progeny 64-16. Nevertheless it is clear that *Hr-1* expression is subject to modification.

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References

1. Sampson, D. R., Genetic analysis of *Brassica oleracea* using nine genes from sprouting broccoli. Can. Jour. Genet. Cytol. **8** (1966) 404–413.
2. Thompson, K. F., Production of haploid plants of marrow-stem kale. Nature **178** (1956): 748.