Two waxless mutants of somaclonal origin in pea

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Two monogenic recessive waxless mutants were obtained from long-term callus cultures of pea cv. Ranny Zeleny (1). They were named as *waxy1* and *waxy2*. This paper reports on the distribution and ultrastructure of their wax cover and the results of linkage tests for the *waxy1* and *waxy2* mutations.

Materials and Methods

The ultra-structure of epicuticular wax on both sides of untreated leaflets was studied by scanning electron microscopy using a Hitachi S-405A microscope. Mutant lines *waxy1* and *waxy2* and the initial cultivar, Ranny Zeleny, carried the genes *i, a, Af, lf, Le* and *R*. To make crosses, the following marker lines were used: R1 (*Lf*; R1 is a regenerant line obtained from a callus culture of Ranny Zeleny), "moustached" line (*I, A, af, le*), L1072 (*wb*) and Hobart line L63 (*I, A, le, r*). The joint segregation χ^2 was obtained using a 2 x 2 contingency table and the recombination fraction was calculated using the product ratio method.

Results

Surface examinations were made on plants grown in the quite warm and dry 1991 summer field conditions. These conditions promoted fully the production of wax. It was found that *waxy1* plants had a normal wax cover on the upper surface of the leaflets but lacked wax on the under surface of the leaflets and both sides of the stipules; the stem carried a reduced quantity of wax. The *waxy2* plants were covered with a normal quantity of wax only on the upper surface of the leaflets while other parts were without wax. Thus the *waxy1* and *waxy2* mutants look very much like the known mutants *was* and *wsp* as described in Pisum Newsletter 10:81 (1978).

A study using scanning electron microscopy revealed that on the adaxial side of leaflets of the normal, glaucus line R9, wax was present as a multitude of platelets, 1-2 μm in length, forming a dense cover over the surface (Fig. 1). The same structures, both in shape and number, were also present on the adaxial surface of *waxy1* and *waxy2* (Fig. 3) leaflets. Crenated ribbons of wax, 4-5 μm in length, were present on the abaxial surface of the leaflets of the normal line R9 (Fig. 2). On the abaxial side of *waxy1* leaflets, wax occurred as shorter 2-4 μm ribbons, but mainly as rods and granules (Figs 5 and 6). The greatest reduction in wax occurred with the *waxy2* mutant where only very small rods of less than 0.6 μm length and granules were found on the abaxial surface of leaflets (Fig. 4).

The substantial reduction of crystalline wax on *waxy2* plants resembles the observation by Holloway et al (2) for the phenotypically identical *wsp* mutant. So *waxy2* and *wsp* may be identical mutations and the minor differences in details of wax structure may be due to differences to environmental conditions and genetic background.

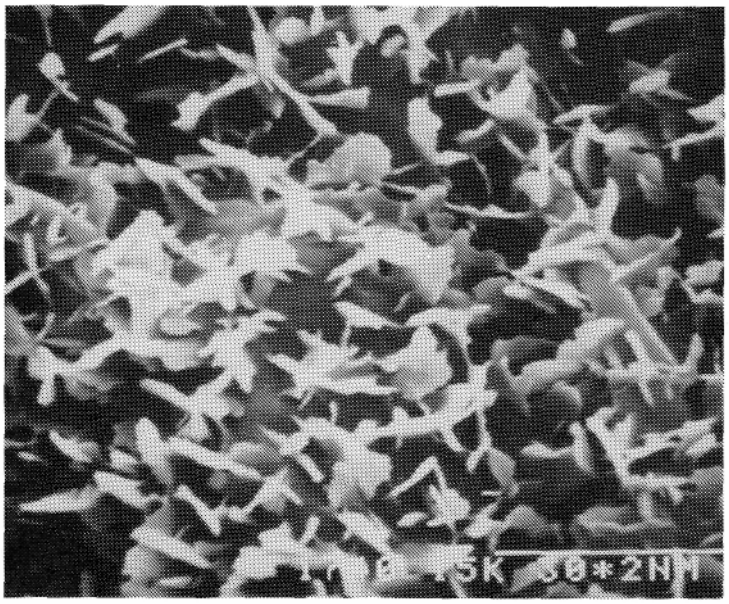

Fig. 1. Line R9: adaxial side of the leaf; x 10000; the bar above 30* 2 nm = 3 μm.

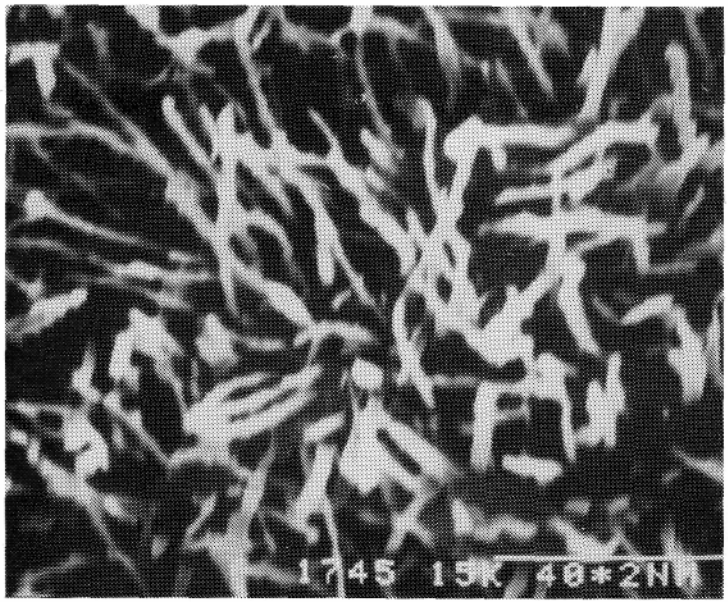

Fig. 2. Line R9: abaxial side of the leaf; x7500.

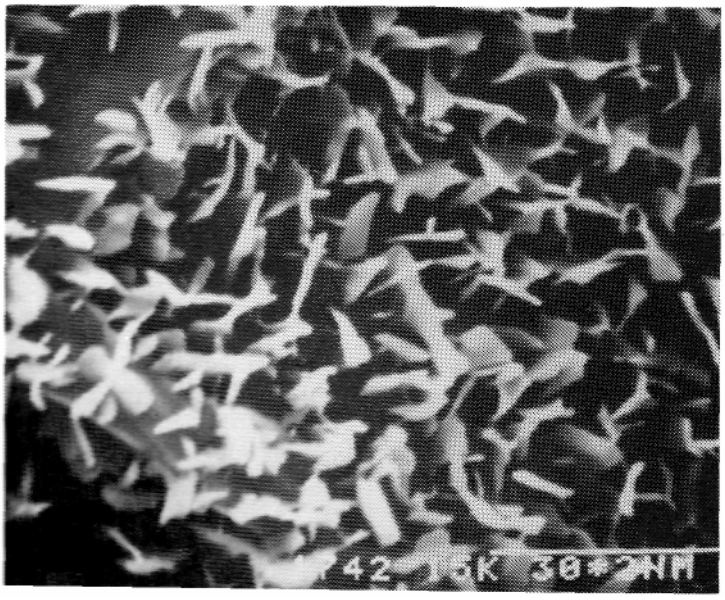

Fig 3. Line *waxy2*: adaxial side of the leaf; x 10000.

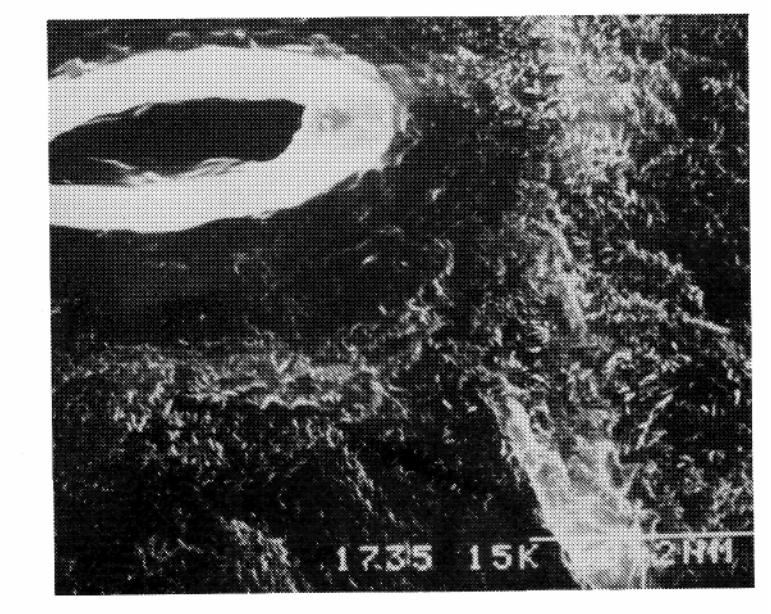

Fig. 4. Line *waxy2:* abaxial side of the leaf; x 5000.

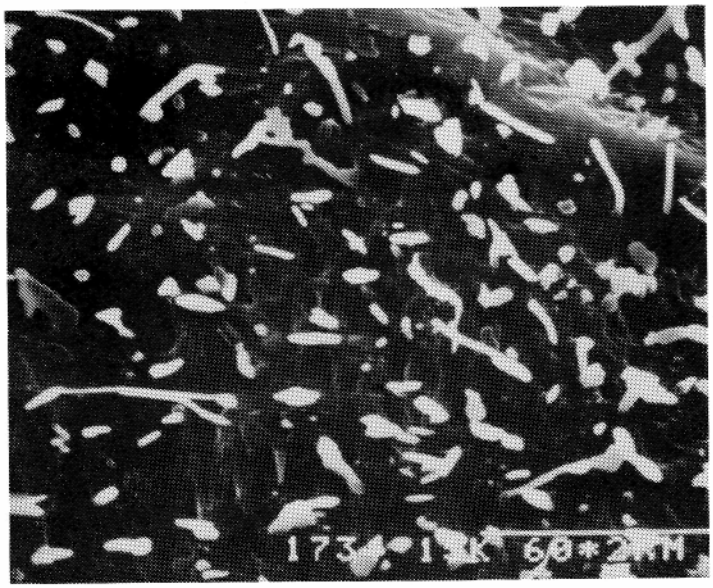

Fig. 5. Line *waxy1*: abaxial side of the leaf; x5000.

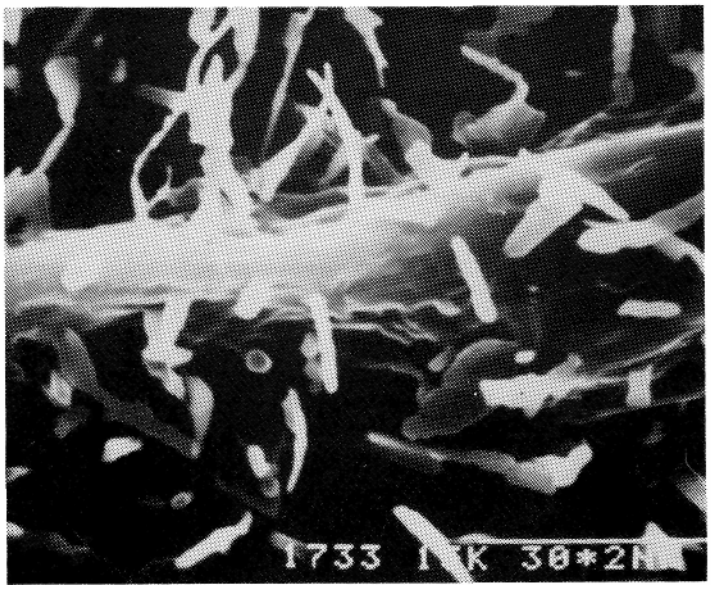

Fig. 6. Line *waxy1*: abaxial side of the leaf; x 10000.

Table 1. F_2 segregation data for crosses between *waxy1* and marker lines for chromosome 1 loci.

Marker gene (X)	Phase	Marker lines	Phenotype				Chi-square				Recomb fract	SE
			Waxy1 X	*Waxy1 x*	*waxy1 X*	*waxy1 x*	Total	*Waxy1*	*X*	Joint		
A-a	Coup.	Moustached; L63	530	125	125	98	878	0.07	0.07	54.27***	34.0	2.1
Lf-lf	Coup.	R1	252	54	46	51	403	0.19	0.24	46.64***	28.9	2.8
Af-af	Rep.	Moustached	369	106	112	30	617	1.30	2.88	0.09	49.0	3.1
I-i	Coup.	Moustached; L63	496	159	178	47	880	0.15	1.19	1.07	52.7	2.6

***P< 0.001

Crosses of *waxy1* and *waxy2* plants with each other and with the *wb* mutant produced only normal plants in F_1. Hence the three genes are not allelic. Unfortunately, we had no other known waxless mutants in our collection so a complete set of allelism tests was not possible. Crosses of our mutants with marker lines gave no evidence of linkage between *waxy2* and *a, lf, af, i, le* or *r*. However, there was strong evidence ($P < 0.001$) of linkage between *waxy1* and chromosome 1 markers *a* (34 cM) and *lf* (29 cM) (Table 1). The linkage data with *Lf* were obtained by crossing with regefterant line R1 which differed from the *waxy1* line only at the *Waxy1* and *Lf* loci. So other flowering genes which might hamper an interpretation of cosegregation data were absent in the cross. Our result is in accord with the known value of the *A-Lf* linkage [about 3 cM by our own studies and about 10 cM from published data (3)]. Therefore we propose the gene sequence *A-Lf-Waxy1* in Chromosome 1. *Waxy1* did not show linkage with markers *af* and *i* in the lower section of chromosome 1 (Table 1). None of the previously described wax loci is on chromosome 1. Hence *waxy1* may be a new locus. However, a complete set of allelism tests is necessary to fully resolve the identity of the *waxy1* and *waxy2* mutants.

1. Ezhova, T.A., Bagrova, A.M., Hartina, G.A. and Gostimski, S.A. 1989. Genetica (Russ.) 25(5):875-885.
2. Holloway, P.J., Hunt, G.M., Baker, E.A. and Macey, M.J.K. 1977. Chem. Phys. Lipids 20:141-155.
3. Murfet, I.C. 1971. Heredity 27:93-110.