Characteristics and inheritance of the leaf mutation *ins*

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Lamprecht was the first to describe *insecatus* leaves (gene symbol *ins*) (1). He studied a pea line with dissected tips of the first pair of leaflets. A central vein of the incised leaflet is overdeveloped into a small tendril (Fig.1, left).

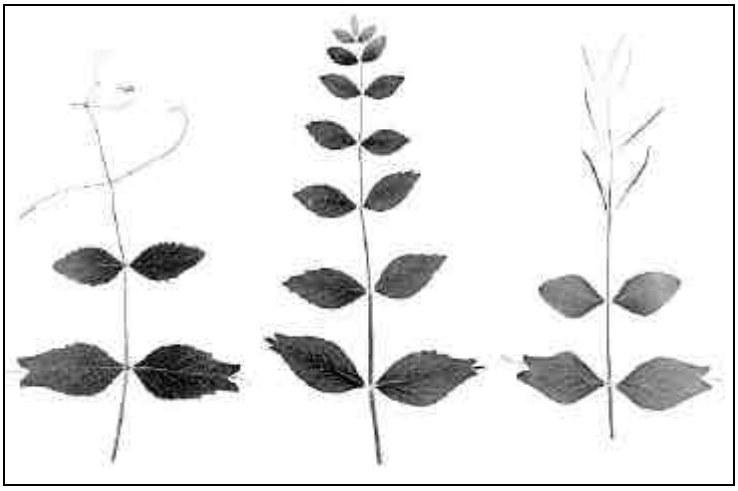

Fig.1. Insecatus *leaves from F_2 plants of the cross WL1238 x WIR2521 differed by Tl/Tl (left), tl^w/tl^w (middle) and Tl/tl^w (right) alleles*

In contrast to homeotic pea leaf development mutants *afila* and *tendril-less*(*tl*) the mutation of gene *ins* affects only a few compound pea leaves. We have observed the *insecatus* phenotype in several lines and determined the late flowering the *insecatus* phenotype appearance 2-3 nodes below the first flowering node. Following initial formation of *insecatus* leaves, they may also occur at the next 3-4 nodes. Thus, *insecatus* leaves are formed in the middle part of a plant whereas lower and upper nodes of such plants have normal leaves. Unknown developmental and/or physiological mechanisms led to this pattern of *insecatus* leave phenotype distribution. In our collection we have not observed the *insecatus* phenotype in early flowering pea lines. In order to analyze the influence of a flowering node on formation of *insecatus* character the F_2 hybrids differed in *lf* alleles were studied (Table 1). The lines OK7, OK14 (kindly provided by Dr. O.E. Kosterin) and WL1325 flower at node 6, counting the first scale leaf as node 1, and have *lf-a, Ins* genotype. The lines WL1751 (*Ins*), WIR2521 (*ins*) WIR319 (*ins*) belong to the late flowering class.

Table 1. Comparative analysis of the first flowering and *insecatus* nodes in F_2 *insecatus* plants from different crosses.

Cross	N	Flowering	First flowering node ±S.E.	First *insecatus* node ±S.E.
WL1325 x WIR2521	**16**	**Late**	**21.1 ± 0.6**	**19.5 ± 0.6**
WL1751 x WIR2521	**35**	**Late**	**19.8 ± 0.4**	**16.7 ± 0.5**
WIR319 x OK14	**30**	**Late**	**24.5 ± 0.8**	**19.5 ± 0.6**
WIR319 x OK7	**21**	**Late**	**24.9 ± 0.9**	**18.7 ± 0.6**
WIR319 x OK7	**3**	**Early**	**6.0 ± 0.0**	**15.7 ± 2.1**

Data for all late plants of five crosses presented in Table 1 were used to obtain a correlation between the first flowering node and the first *insecatus* node. The correlation coefficient is r=0.83. These results show a regular trend: the higher the first flowering node; the higher the first *insecatus* node.

The interaction of different leaf development mutants is of great interest because of the control of compound leaf development. We described the tl^w gene influence on exhibition of *insecatus* phenotype. Homozygous tl^w/tl^w, *ins/ins* plants had instead of overdeveloped central vein a small leaflet (Fig. 1, middle). In heterozygous Tl/tl^w plants the overdeveloped central vein looked like a flat tendril (Fig. 1, right). Thus, a form of central vein of *insecatus* plants is completely defined by gene *tl*. The gene *ins* itself is responsible for

extension of central vein beyond the edge of the blade and more or less symmetric leaflet dissection on both sides of this vein.

Lamprecht established a single recessive gene inheritance for *insecatus* leaves (1). We have analyzed five hybrid combinations differing for *ins* alleles. In our experiments all F_1 plants had wild type phenotype. F_2 plants with any degree of dissection were identified as a recessive homozygous class. With such an approach the number of plants in different phenotypic classes is in a good accordance with the theoretically expected 3:1 ratio for the monogenic recessive control of *insecatus* leaves.

Encouraged by the good 3:1 fit for *Ins – ins* segregation in F_2 we have studied segregation of *ins* with marker genes. The fact that ins was in repulsion phase relative to the most of marker genes made the interpretation of segregations data more complicated because of large standard errors for the determination of genetic distances. There was no linkage between *ins* and any of 29 marker genes listed here: *d, o, i, ar, M, b, gl, v, gp, te, Fs, U^{st}, Pl, r, tl^{w}, bt, le, Np, fas, k, wb, a, lf, blb*(2), *oh, st, SCA*(3), *PSP3, PSP7* (the two last code proteins separated by electrophoresis in acetic acid). Lamprecht also failed to locate *ins* (1). In his experiments the initial pea line has the gene *ins* with precise expressivity, while in the F_2 progeny the plants appeared with insignificant leaflet dissection. The continuous variability from strong to insignificant dissection created complexities at phenotypic classification.

Our experiments also show there are genes modifying the gene *ins* expression. Two lines WIR319 and WL6 each with the "normal" *insecatus* expressivity (5-6 incised leaflets per a plant) have been crossed. All F_2 offspring had dissected leaflets but the amount of *insecatus* leaflets varied very wide from plant to plant. As all F_2 plants were recessive homozygotes *ins/ins* and were growing in the same conditions of a greenhouse, it is obvious that a genetic background of a plant influences on an *insecatus* gene expression.

In some case the mutation *ins*, like *ins2* (4, 5), displays dominance. In the cross WL1238 x WIR2521 we have obtained 8 F_1 plants: 2 F_1 with WT and 6 F_1 with mutant *insecatus* phenotype. Seven F_1 plants were used to generate the test-cross WL1238 x (WL1238xWIR2521) (Table 2). Test-cross data reveal good 1:1 segregation. But varying dominance of *insecatus* phenotype in the same cross makes genetic mapping of *ins* gene difficult. For further genetic analysis, we will need to find *ins* alleles with better penetrance and stable expression.

Table 2. Segregation of WT and *insecatus* phenotypes in the test-cross WL1238 x (WL1238 x WIR2521).

No.	F_1 phenotype	Test cross	
		WT	mutant
1.	WT	3	5
2.	mut	-	-
3.	mut	11	4
4.	WT	6	4
5.	mut	11	10
6.	mut	4	4
7.	mut	4	7
8.	mut	8	8
	Total	46	41

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