Interaction of a new leaf mutation *ins2* with *af*, *uni*^{tac} and *tl*^{w}

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In 1959 Lamprecht described a recessive mutation *insecatus* (*ins*), with apices of the first pair of leaflets incised. The midvein in the zone of incision was not only retained but also overdeveloped sometimes being transformed into a tendril. Unfortunately, this character had incomplete penetrance and highly variable expression, as well as showing reduced fertility (5). Thus genetic mapping of the relevant gene has been difficult.

In 1992 in the progeny of a *Twt* trisomic (see [1]) there was found a plant with a normal karyotype and a phenotype closely resembling that of *insecatus*, although the incisions of the leaflet apices were deeper and midveins frequently transformed into ramified tendrils. The first incisions appeared at the first pair of leaflets of the nodes 5-6, counting the first scale leaf as node 1. At higher nodes, incisions became deeper and appeared at the second pair of leaflets (Fig. 1a). Sometimes the first pinna pair position possessed three or four leaflets forming a butterfly-like structure (Fig. 1b). At the nodes 8-12, roughly at the first flowers, the expression of the mutant phenotype reached its maximum: the leaflets being replaced by compound tendrils and the phenotype may become indistinguishable from *afila* (Fig. 1c, d). On higher nodes the mutant phenotype gradually decreases. The mutant plant was selfed for 15 generations, producing an isogenic line AFD.

Pea lines WL021, WL025, WL1759, and WL1898 from Weibullsholm collection (Landskrona, Sweden) are designated as carrying the gene *ins*. However, we found poor visible expression of the mutant character in these lines and were unable to perform an analysis of allelism with the newly found mutation. We called the new mutation *ins*2. In the crosses of *ins*2 plants with wild type plants, the F_1 progenies had a normal phenotype, thus indicating the recessive mode of inheritance of *ins2*. It should be noted that a thorough analysis of the heterozygotes *ins2/Ins2* reveals a aberrant phenotype weakly expressed in some leaves, most frequently manifested in the pointing of the apical part of the leaflets.

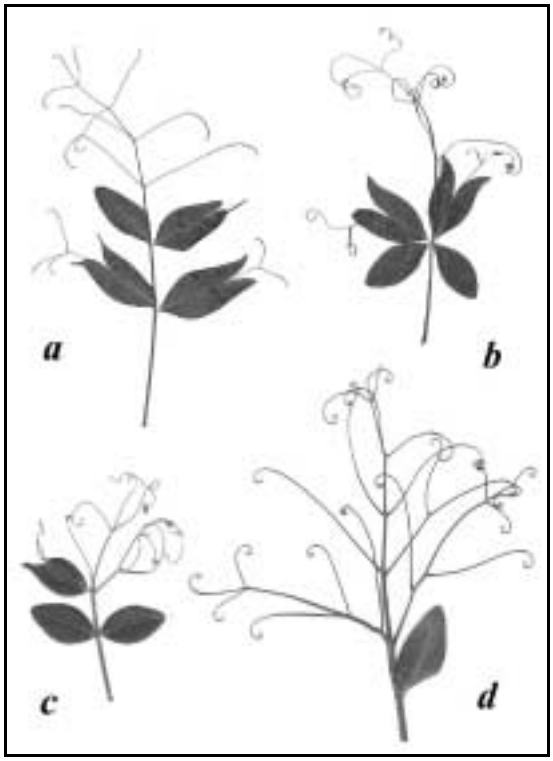

Fig. 1. The pea leaves of plants homozygous for ins2 of nodes 7-8 (a, b) and 10-12 (c, d).

The similarity of *ins2* and *afila* phenotypes may be evidence of allelism of the relevant genes. To test this possibility we crossed the line AFD (*Af, I, ins2*) with WL1746 (*af, i, Ins2*). F_1 plants displayed the *insecatus* phenotype, suggesting that *ins2* and *af* might be allelic. However, segregation data for these traits in the F_2 are in contradiction with this hypothesis (Table 1). One can see that all seven plants grown from *i/i* seeds possessed the *afila* phenotype, confirming a close linkage of *i* and *af*. We therefore used the color of the cotyledons in seeds produced on the plant to postulate the genotype (homozygous dominant or heterozygous) of the plant at the *Afila* locus. Of nine *I/I* plants (and, most probably, *Af/Af*) three displayed the *insecatus* phenotype, indicating non-allelism of the genes *af* and *ins2*. In addition, the data of Table 1 suggest the hypothesis that heterozygosity at *Af* causes the *ins2* allele to become dominant, manifesting its effect in the heterozygous state.

To study interaction of *ins2* with genes affecting development of a compound leaf we crossed the line AFD with an earlier synthesized stock GRT carrying the markers *i, af, tl*^{w} and *uni*^{tac} derived as F4 from a cross cv. Frontier x line A45 (kindly supplied by Prof. E. T. Gritton). As expected, F_1 plants had the phenotype *ins*. Phenotypes of the F_2 plants (144 in total) are given in Table 2. For convenience, this F_2 population will be referred to as VAR. All 35 plants of this population grown from *i* seeds possessed the *afila* phenotype and were excluded from the further analysis. Of 109 plants of VAR grown from *I* seeds 51 (47%) had leaflets incised to various extents. The greater than expected proportion of plants with the *ins* phenotype can be accounted for by an effect of heterozygosity for the gene *af*.

Of 58 VAR plants without incisions 32 displayed the phenotype *uni*^{tac}, and 26 were of the normal phenotype *Uni*. At the same time the phenotypic class (*ins uni*^{tac}) was completely absent (no plant both possessed a leaflet in place of the terminal tendril and had the first pair of leaflets incised).

The impression is that the mutant genes *uni*^{tac} and *ins2* either are tightly linked or are antagonists in their effect on the leaf development. Since all the plants with the *uni*^{tac} phenotype lacked incisions in the leaflets despite variation of the dose of *ins* and *af* alleles, we could hardly expect appearance of the *ins* trait in their off-spring. For this reason the descendants of plants homozygous for the genes *uni*^{tac} or *af* were excluded from the further analysis. From VAR there were chosen 41 plants that produced no less than 12 seeds. Fifteen plants lacked incisions (*Ins*), eight had weak incisions (*ins*-w), seven had strong incisions (*ins*-s) and the remainder (eleven) had medium expression of the trait (*ins*-m). An

Table 1. Segregation for the *afila* and *insecatus-2* phenotypes among the F_2 progeny of the cross AFD (*Af, I, ins2*) x WL1746(*af, i, Ins2*).

Genotype with respect to *i*	Putative genotype with respect to *af*	Phenotypes of progeny		
			non-*afila*	
		afila	*ins2*	*Ins2*
i/i	***af/af***	**7**	**0**	**0**
i/+[1]	***af/+***	**0**	**13**	**3**
+/+	**+/+**	**0**	**3**	**6**

[1] **+ = dominant 'wild type' alleles.**

Table 2. Segregation for the *afila, uni* and *insecatus-2* phenotypes among the F_2 progeny (further designated as 'VAR') of the cross AFD (*Af, I, Tl, Uni, ins2*) x GRT (*af, i, tl*^{w}, *uni*^{tac} , *Ins2*).

Genotype with respect to *i*	Phenotypes of progeny				
		non-*afila*			
	afila	*ins2 Uni*	*Ins2 Uni*	*Ins2 uni*^{tac}	*ins2 uni*^{tac}
i/i	**35**	**0**	**0**	**0**	**0**
i/+[1]	**0**	**42**	**15**	**17**	**0**
+/+	**0**	**9**	**11**	**15**	**0**
Total	**35**	**51**	**26**	**32**	**0**

[1] **+ = dominant 'wild type' alleles**

Table 3. The number of plants with certain putative genotypes, derived from their progenies, among 41 plants of the VAR population which produced not less than 12 seeds and were not homozygous for *i, af,* or *uni*.

Phenotypes of VAR plants with respect to *ins*	Putative genotypes with respect to loci			Number of plants	Total
	af	*uni*	*ins2*		
Ins	+/+	***uni***^{tac}/+	***ins2***/+	**5**	
	+/+	+	+/+	**2**	
	+/+	+/+	+/	**3**	
	af/+	***uni***^{tac}/+	+/+	**3**	
	af/+[1]	+	+/+	**2**	**15**
		+/+			
		+/+			
***ins*-w**	***af***/+	+/+	***ins2***/+	**4**	
	+/+	***uni***^{tac}/+	***ins2***/+	**3**	
		+			**8**
	+/+	+/+	***ins2***/+	**1**	
***ins*-m**	***af***/+	+/+	***ins2***/+	**2**	
	af/+	***uni***^{tac}/+	***ins2***/+	**5**	
	af/+	+	***ins2/ins2***	**2**	
	+/+	***uni***^{tac}/+	***ins2/ins2***	**2**	**11**
		uni^{tac}/+			
***ins*-s**	***af***/+	***uni***^{tac}/+	***ins2/ins2***	**1**	
	af/+	+/+	***ins2/ins2***	**6**	**7**

[1] **+ = dominant 'wild type' alleles**

analysis of the progeny of these 41 plants permitted the determination of the genotype of these plants with respect to the loci *Af*, *Uni* and *Ins2* (Table 3).

Although classification of plants according to leaflet incision was made visually and should not be treated as completely accurate, several general conclusions can be made. All seven plants with deep incisions were found to be homozygous for *ins2* and heterozygous for *af*, six of these being homozygous *Uni/Uni*. Heterozygosity for both *ins* and *af* was typical for the phenotype *ins*-m. Plants with weak incisions were heterozygotes *ins2*/*Ins2* in the homozygous background *Uni/Uni* or *Af/Af*. The majority of plants without incisions were homozygous for the wild-type allele *Ins*, but in five cases they were heterozygotes *ins2*/*Ins2* and homozygous *Af/Af*. We can reject the hypothesis of the tight linkage of the genes *uni* and *ins2* because four of the plants analyzed from VAR were double homozygotes (*Uni/Uni Ins/Ins*). At the same time we failed to observe double homozygotes (uni^{tac}/uni^{tac} *ins2/ins2*). Probably, the missing class should be sought for among the plants of the uni^{tac} phenotype.

Further interesting information was gained from analysis of a particular plant (#99) (phenotype *ins*-m, *Uni*, *Af*) from VAR. None of its 17 descendants had the *afila* phenotype, 11 of them with the phenotype *Uni* had incisions, while the remaining six lacked incisions and were uni^{tac}. Thus, the genotype of the plant #99 most probably was (*Af/Af* uni^{tac}*/Uni ins2*/*ins2*). If such were the case, the progeny with the uni^{tac} phenotype should be double homozygotes (uni^{tac}/uni^{tac} *ins2*/*ins2*). However, the small size of progeny of the plant #99 may have prevented the observation of the *Ins*2 phenotype and hence been a source of an error in genotype determination. For this reason we extended our analysis by examining the progeny of the eight most productive descendants of plant #99 with the *insecatus* phenotype. All the progeny of four of them had the phenotype (*Uni ins*), while segregation for uni^{tac} was observed in the progeny of each of the other four families. All 12 plants with uni^{tac} phenotype lacked incisions, while the remaining 35 plants had incised leaflets. This result confirms that the genotype of plant #99 was (uni^{tac}/*Uni ins2*/*ins2*). A thorough examination of the plants of the uni^{tac} phenotype revealed a number of peculiarities: the mutant trait was clearly expressed only at the first three nodes, at a higher level leaves did not differ from normal ones, and only at some flowering nodes was the terminal tendril replaced by a leaflet. Thus, in the double homozygote (*ins2*/*ins2* uni^{tac}/uni^{tac}) the uni^{tac} trait is not expressed in all leaves.

In the present work we have shown that the gene *Ins2* acts as a synergist of the gene *Af* and as an antagonist of the gene *Uni*. Antagonistic interaction of the genes *Af* and *Uni* has been demonstrated by several authors (2, 6, 7) and recently confirmed by Hofer and Ellis (4). The striking phenotype *pleiofila* of the double homozygote (*af/af tl/tl*) is well known. Taking into account synergism of *Af* and *Ins2*, we might expect a similar phenotype to be displayed by the double homozygote (*ins2*/*ins2 tl/tl*).

In VAR we found several double homozygotes (*ins2*/*ins2 tl/tl*). These plants had in the leaflet incisions instead of an overdeveloped midvein a tiny leaflet at a tiny petiolule. As the size of incision increased, the midvein was replaced by a small compound leaf, its size correlating with the size of an incised leaflet that produced it (Fig. 2a, b). At higher nodes the incisions became deeper and, finally, the first pair of leaflets disappeared and in place of the second and third pairs there appeared compound leaves of the second order (Fig. 2c, d) closely resembling the leaves of plants with (*af/af tl/tl* uni^{tac}/uni^{tac}) genotype (3). The impression is that the effect of the homozygote *ins2*/*ins2* is equivalent to that of the double homozygote (*af/af* uni^{tac}/uni^{tac}).

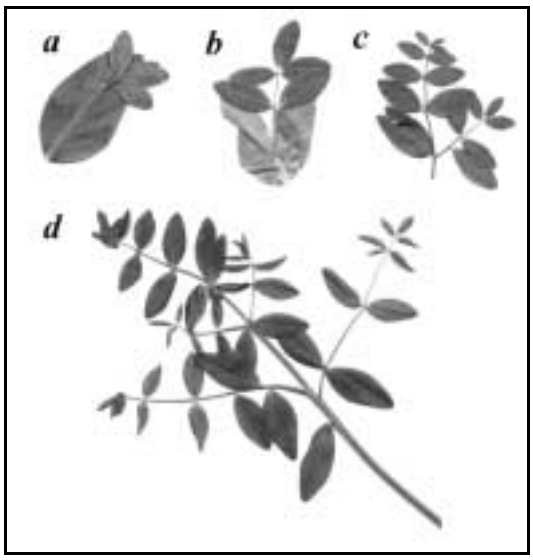

Fig. 2. Phenotype of plants homozygous for ins2 and tl^{w}*. a, b, compound leaves formed in leaflet incisions; c, d, bipinnate leaves at higher nodes.*

In the Fabaceae, bipinnate leaves are not found in the subfamily Papilionoideae, but they are very common in two related subfamilies - Mimosoideae and Caesalpinoideae. A function of the gene *Ins2*, as well as that of

the gene *Af* seems to consist in decreasing the rank of branching of the leaf axis. Weakening of this function together with inactivation of the *Tl* gene makes the structure of the compound pea leaf similar to what is perhaps a more archaic bipinnate form.

A thorough examination of leaves of different pea samples often reveals incisions of the *insecatus* type. It appears to be an evidence of existence of a number of genes that can produce the *insecatus* phenotype. Recently we have found another mutation with expression similar to *ins2*. At present it is being investigated.

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