Tl2, a new locus resembling *Tl* in its action

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Pea possesses the most complex compound leaves of all genetically studied plant species. Its unipinnate leaf is composed of a pair of basal stipules, one or more pairs of proximal leaflets, one or more pairs of distal tendrils, and a single terminal tendril. The gene *Tendril-less (tl)*, together with *Afila (af)* and *Unifoliata (uni)*, belongs to a group of genes able to drastically alter the leaf architecture. The mutation tl^w, appears to replace the tendrils of the distal region of the leaf by normal oval leaflets. The *Tl* gene displays incomplete dominance so that heterozygotes tl^w/+ in the distal region of the leaf possess pinnae morphologically intermediate between tendrils and leaflets, we call them 'Flat Tendrils' and designate the leaf phenotype of the heterozygotes tl^w/+ as *FT*. It should be noted that the wild-type allele *Tl* not only represses lamina formation in the pinnae of the distal part of the leaf but also contributes to branching potential of the rachis, this effect being especially evident in a *af/af* background.

Examination of the M_2 generation produced by treating the line SG (*Tl*, *R*) with γ-rays (7000R), revealed a plant with tendrils converted to very narrow leaflets (Fig. 1). Its phenotype resembled *FT*, exhibited by heterozygotes tl^w/+, suggesting that it was a new mutation at the *Tl* locus. We crossed this plant with the line WL1238 (tl^w *r*). Of seven F_1 plants examined, five had the phenotype FT and two had the phenotype *tendril-less (tl)*. The appearance of these phenotypes is consistent with the hypothesis that the mutant plant with flat tendrils was heterozygous *tl*/+. The F_1 plants with the phenotype *tl* produced 77 seeds, of which 29 were wrinkled and 48 round, indicating that segregation in the region around *R* was relatively normal. However, when we grew out the F_2, two unexpected phenotypic classes with respect to tendril shape were obtained. The F_2 contained three phenotypic classes: *tl* (42 plants), *FT* (26 plants) and *N* (9 plants with wild-type leaves). All of the 29 plants grown from the wrinkled seeds had the phenotype *tl.* This result was not surprising considering the tight linkage between the loci *R* and *Tl*. The segregation pattern indicated that the mutant in the line SG affected a hitherto unknown gene conditioning development of flat tendrils (*FT*) upon its inactivation. We designated this gene as *tendril-less2* (*tl2)*.

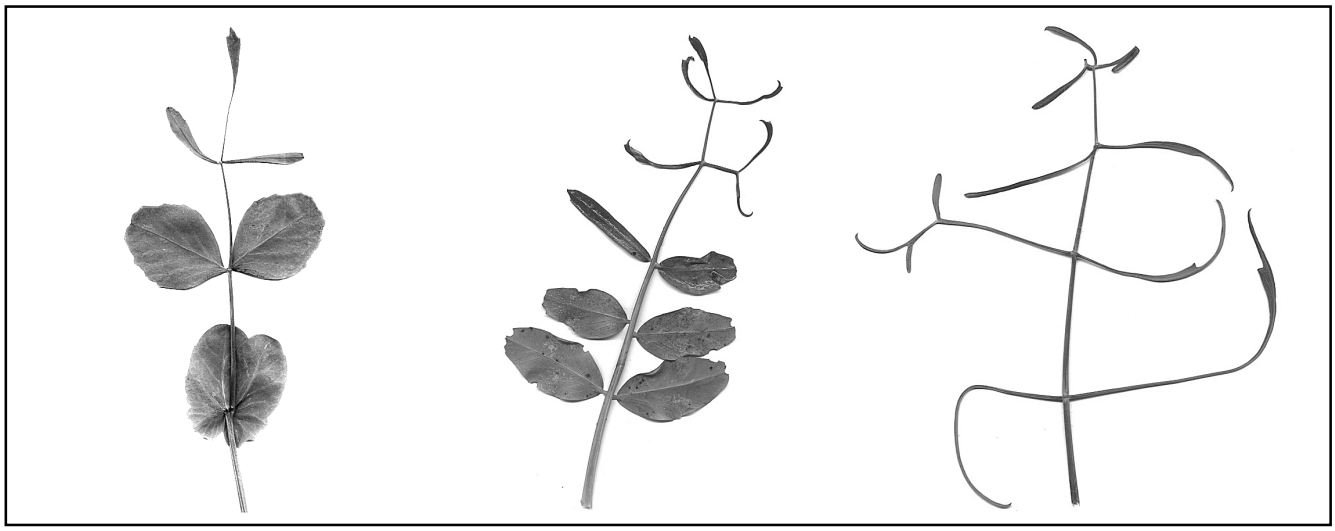

Fig. 1. Phenotypes of the tl2/Tl2 *heterozygotes of the line Flat-1: left, young leaf; center, mature leaf; right, distal portion of a compound leaf.*

A plant with the phenotype *FT*, homozygous for *R* and supposedly heterozygous for *tl2,* was crossed with the line Sparkle (*r, a, Tl*) and for three generations a single most vigorous plant with the phenotype *FT* was selected. Self-pollination of these plants never produced plants with the phenotype *tl*, while wild-type and *FT*

phenotypes were present in approximately equal proportions, the latter slightly predominating. Such segregation pattern suggests that in the case of the *tl2* gene, the *FT* phenotype reflects heterozygosity at *Tl2*. The absence of the *tl* phenotype suggests lethality of the homozygotes *tl2/tl2*. The explanation for the observed FT:N ratio of close to 1:1 instead of 2:1 may reside in reduced transmission of the lethal through the germ line. Two F_4 plants with the *FT* phenotype became progenitors of the lines Flat-1 (*a, tl2/+, R*) and Flat-2 (*A, tl2/+, R*). We made an extensive cross between the line Flat-1 (♀) and the line DSP (*a, Tl*) (♂) derived from cv. Dark Skinned Princess. The success of the cross was confirmed by electrophoretic analysis of the cotyledon protein SCA. The F_1 produced 112 seeds and 97 F_2 plants, of which 48 had the phenotype *FT* and 49 the wild-type phenotype. The striking equality in the numbers of both phenotypic classes showed that transmission of *tl2* through female germ line did not differ from the wild-type allele *Tl2*. We conclude that the deviation of the phenotype ratio in the progeny produced by self pollination is due to a decreased transfer of *tl2* through the male germ line. It should be noted that all *FT* plants had fully fertile pollen implying that no translocation was involved.

Lethality and the difference in gamete competitive ability precludes standard analysis of recombination relationships in the F_2. However, if *tl2* is tightly linked to some marker, the proportion of plants with this marker should be strongly biased in the classes *FT* and *N*. An example of such an analysis is given in Table 1. If the loci *Tl2* and *R* were tightly linked, the plants grown from the wrinkled seeds would mostly have the wild-type phenotype; however, in the experiment we did not observe a noticeable deviation in the ratio *FT:N* in classes *r* and *R*. We tested the loci *Curl*, *Wlo*, *Uni. I, Af*, and *Cri* in the same manner and found no evidence of linkage with *Tl2*.

Table 1. Phenotypes of the progeny of self-pollinated F_1 plants of the *FT* phenotype, resulting from the cross Flat-2 (*R/R, tl2/Tl2*) X DRC (*r/r, Tl2/Tl2*). N stands for the wild-type phenotype of tendrils.

Phenotype of seeds	Phenotype of tendrils		Total
	FT	N	
R	**88**	**80**	**168**
r	**22**	**24**	**46**
Total	**110**	**104**	**214**

Joint segregation χ^2 = 0.30 (P~0.6), calculated with the expected numbers derived from the observed segregation for each gene and a null hypothesis of the absence of linkage.

To check more loci, we crossed the line Flat-1 with our line HT1 (*A, Tl2, gp*) homozygous for the Hammarlund translocation (Table 2). (This line was described in ref. 4). Analysis of 119 F_2 plants revealed an obvious linkage of *Tl2* with the translocation breakpoint and the locus *Gp* residing near the breakpoint in chromosome V (here we use the linkage group numbers for desig-nation of the corresponding chromosomes). The majority (54 of 65) of the structural heterozygotes, registered by pollen semisterility had the phenotype *FT*, that is, were heterozygotes *tl2/+*. The majority of fully fertile plants (44 of 54) had wild-type tendrils. One can conclude that *Tl2* resides near the translocation breakpoint. In the line HT1 the longer interchange chromosome bears the allele *gp* near the T-point. Thirty-two of 35 F_2 plants with the phenotype *gp* had normal tendrils and only three had flat tendrils. These data provide evidence in favor of *tl2* being located on a non-translocated chromosome, either V or II.

Table 2. Tendril phenotypes of F_2 plants resulting from the cross Flat-1 (*a, tl2/+, Gp*) x HT-1 (*A, Tl2, gp,* Hammarlund translocation). *FT* refers to flat tendrils, *N* - to wild-type phenotype.

Phenotypes in respect of *gp* and *a*		fertile pollen		semisterile pollen	
		FT	N	FT	N
Gp	***A***	**0**	**8**	**54**	**11**
	a	**7**	**4**	**0**	**0**
gp	***A***	**3**	**32**	**0**	**0**
	a	**0**	**0**	**0**	**0**

Joint segregation χ^2 for *tl2* and the breakpoint is 49.4 (P<0.001), for *tl2* and *gp* is 40.8 (P<0.001). For χ^2 calculation, expected values were derived from the observed segregation for each factor and null hypothesis of the absence of linkage. Joint segregation of *tl2* and *a* was not evaluated since some of the *a* plants were trisomics.

We mentioned above that no linkage was observed between *Tl2* and *Cri*, the latter being located on chromosome V immediately adjacent to the breakpoint. To explore the relationship between *Tl2* and *Cri* further we examined F_2 plants from the cross of our lines Cricytar (*cri*, *a*, *r*, *Tl2*) X Flat-1 (*Cri*, *a*, *R*, *tl2/+*), the F_1 plants having a FT phenotype. Of 34 *Cri* plants in the F_2, 18 had the wild-type phenotype and 16 had the *FT* phenotype. This ratio did not differ significantly from the ratio of these phenotypes among the *cri* F_2 plants: 7 *Tl* : 5 *FT*. (χ^2= 0.105, P~0.75, calculated for a model implying equal proportion of *FT* plants in both classes *Cri* and *cri*). Because *Tl2* and *Cri* do not appear to be linked and *Cri* is closer to the breakpoint than *Gp* (1, 4), *Tl2* most probably resides on chromosome II. This hypothesis is supported by a decreased proportion of plants with the phenotype *a* (11 of 119, instead of 30 expected). Moreover, as discussed below, some of these 11 *a* plants were in fact trisomics. None of the *a* plants was semisterile or homozygous for the allele *gp*. These observations indicate that the *a* plants are structural homozygotes for the chromosomes of the standard karyotype.

Self pollination of lines heterozygous for the Hammarlund translocation is known to produce tertiary trisomics which have the karyotype represented by two standard sets of chromosomes with an additional small interchange chromosome (1, 4, 5). If the sporophytic lethal *tl2* resides on the short arm of chromosome II above the breakpoint (Fig. 2), structural homozygotes for chromosome II will survive as trisomics if the extra chromosome carries the wild-type allele *Tl2*. Such a trisomic would have the phenotype *Gp a FT* and fertile pollen. Of seven plants with this phenotype four exhibited distinct external characteristics of trisomics. An analysis of the karyotype in pollen mother cells revealed the presence of seven bivalents plus a small univalent chromosome. The four plants with the phenotype *Gp a,* normal tendrils and fertile pollen could result from two cross-over events between the loci *A* and *Tl2*. Thus, most probably, *Tl2* resides in the short arm of chromosome II, although its location could be determined more reliably in crosses involving plants with the normal karyotype.

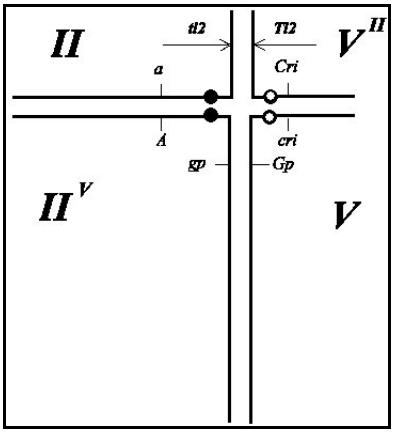

Fig. 2. A scheme of a translocation cross formed in meiosis of the hybrid Flat-1 x HT-1. Arrows indicate putative position of the

The phenotype of heterozygote *tl2/+* is very similar to that of tl^w/+, although in *tl2/+* the flat tendrils are somewhat wider and often are denticulate (Fig. 1). Moreover, the double heterozygote *tl2/+*, tl^w/+ has a phenotype indistinguishable from that of the homozygote tl^w/tl^w. The only important difference is lethality of *tl2* homozygotes. However, considering that the *tl2* mutant was generated by γ-radiation, the mutation might represent a small deletion that removes, in additon to *tl2,* an adjacent essential gene. According to its action, *tl2* resembles a lethal mutation tl^x in the locus *tl* described previously (2, 3).

Most legume species lack tendrils. It is therefore reasonable to suppose that conversion of the distal leaflets to tendrils appeared quite late in evolution, namely in the ancestors of the tribe Vicieae. This hypothesis is supported indirectly by viability of the null-mutation in the gene *tl*. However, loss-of-function mutation *tl2*, the effect of which in heterozygous state is almost indistinguishable from tl^w/+, seems to arrest development in early stages of embryogenesis. We propose that the locus *Tl* appeared via duplication of the locus *Tl2*, which had some function important for viability. This hypothesis is supported by the observation that in both genomic regions the two *Tl*-type genes are linked to legumin genes and histone H1 genes (6). Sequencing of the genes *Tl* and *Tl2* would provide an excellent test of our hypothesis.

Acknowledgement: This work was partly supported by the Russian State Program 'Russian Fund for Fundamental Research', grant No 99-04-49970.

1. Berdnikov, V.A., Gorel, F.L. and Temnykh. S.V. 1993. Pisum Genetics 25: 18-20.
2. Berdnikov, V.A., Gorel, F.L., Bogdanova, V.S., Kosterin, O.E., Trusov, Y.A. and Rozov. S.M. 1999. Genet. Res. 73: 93-109.

3. Gorel, F.L., Berdnikov, V.A. and Temnykh. S.V. 1994. Pisum Genetics 26: 16-17.
4. Gorel, F.L., Kosterin, O.E. and Berdnikov. V.A. 1999. Pisum Genetics 31:5-8.
5. Pellew, C. 1940. J. Genet. 39: 363-390.
6. Weeden, N.F., Ellis, T.H.N., Timmerman-Vaughan, G.M., Swiecicki, W.K., Rozov, S.M. and Berdnikov. V.A. 1998. Pisum Genetics 30: 1-4.