A deletion covering the *Tl* locus in *Pisum sativum*

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Over 1,500 hybrid seeds were produced from Sprint-1 (*R Tl* $His1^f$) x L-1018 (*r* tl^w $His1^s$) crosses. These seeds were subjected to gamma radiation (7,000 R) and planted in the field. From the 1,208 plants that developed we found 15 chimeric individuals possessing branches with the *tl* phenotype (tendrils were transformed into the leaflets). Normal plants and normal branches had the flattened tendrils characteristic of heterozygotes (Tl/tl^w), suggesting that the maternal allele *Tl* was disabled in mutant branches of chimeric plants. In most cases these branches proved to be sterile, but one chimeric plant (tl-7) produced 29 seeds (11 wrinkled and 18 round) on its large '*tl*' branch. The histone H1 phenotype for the leaves of the mutant branch displayed both alleles of the *His1* locus. Therefore, both alleles of loci flanking gene *Tl* (*R* and $His1^f$) were conserved in the maternal chromosome. We suggest that gamma rays induced a new mutation at the *Tl* locus which we called tl^x.

Four round seeds were planted, and each produced a plant with a phenotype indistinguishable from classical *tl*. These plants were all heterozygous for *His1* but, unfortunately, only one plant set seed - five wrinkled and six round. The M3 plants grown from the wrinkled seeds were homozygous for histone locus *His1*. All six plants from round seeds proved to be heterozygous for *His1* and for locus *R*. In total, 275 wrinkled and 259 round seed were obtained from the six plants. We planted 200 of the wrinkled seed, and of the 192 plants examined for histone H1 phenotype, only 20 were heterozygous, the remaining being $His1^s/His1^s$ homozygotes. Recombination frequency was estimated to be 5.2%. This result reflected a decreased level of crossing over in the vicinity of locus *Tl* (5.2% in comparison to the usual 11% [3]), suggesting a possible chromosome aberration involving the *Tl*-region.

To follow tl^x more directly, a round-seeded F6 descendant of the chimeric plant, tl-7, was crossed with a mutant line having genotype *r/r, det/det, TllTl.* This line originated from a single mutant plant we found in 1988 after gamma-ray treatment of line SG (*R Det Tl*), The mutant plant had wrinkled seeds (*r*), normal tendrils (*Tl*), and, unexpectedly, determinate growth behaviour (*det*). One round F_1 seed developed into a plant heterozygous for both *Tl* and *His1.* This plant produced five seeds: two round and three wrinkled. The wrinkled seeds produced plants with normal tendrils and determinate growth (genotype *Tl/Tl*, *det/det*), whereas the two plants grown from round seeds had flattened tendrils and normal growth (*Tl/tl, Det/-*). The stronger of the *Tl/tl*, *Det/-* plants produced 49 seeds (18 round and 31 wrinkled). The F_3 generation produced 893 seeds (325 round and 568 wrinkled). Of the 450 wrinkled seeds planted in greenhouse, 431 germinated and all were *Tl/Tl*, *det/det.* This result indicated that we had no crossovers in the region covering loci *R, Tl,* and *Det* Analysis of H1 histone revealed 25 crossovers that corresponded to about 3% of recombination between genes *Tl* and *His1*.

Of the 286 F_4 plants grown in the field from round seeds, all had flattened tendrils (Tl/tl^x) and normal (*Det/-*) growth behaviour. Moreover, all 256 plants that matured produced both kinds of seeds, i.e. all these plants were heterozygous for locus *R.* We concluded from these data that progeny of plants heterozygous for mutation tl^x lacked mutant homozygotes tl^x/tl^x. This conclusion was indirectly confirmed by the absence of homozygotes of the *R/R*, *Tl/Tl* type in our experiments.

All previously described recessive alleles of locus *Tl* do not affect viability; therefore, we suggest that tl^x is caused by a deletion covering gene *Tl* and some essential locus nearby. The absence of crossing over between loci *R* and *Tl* accompanied by the considerable decrease of recombination between genes *Tl* and *His1* is consistent with this hypothesis. Thus we can postulate the existence of a recessive lethal *lth* on chromosome 5 in the vicinity of locus *Tl*. In contrast to the usual pattern for recessive lethals, the ratio of homozygotes (*r Tl Lth / r Tl Lth*) to heterozygotes (*r Tl Lth / R* tl^x *lth)* strongly deviates from a classical 1:2.

The mutation *r* involved in the above experiments was always accompanied by the recessive trait *det.* The absolute absence of *Det* plants grown from wrinkled seeds suggests that either we have a case of simultaneous appearance of two different mutations (probably caused by a small deletion) or the new *r* mutation has a pleiotropic effect on the growth habit. It was reported that the *det* mutant obtained by Popova [2] cosegregated with *r* [1]. Thus our *r det* mutant may be produced by a deficiency covering two tightly linked genes. If such is the case, the reduced level of recombination observed between *R* and *His1* may, at least in part, be attributed to this deletion.

In order to determine if mutation tl^x affects gametophytic viability, we crossed heterozygous F_5 plants (*Det R* tl^x */ det r Tl*) with tester line 5-11 (*Det r* tl^w */ Det r* tl^w). The proportion of wrinkled seeds (Table 1) reflects the viability of pollen grains (cross #2) and ovules (cross #1) carrying chromosome *det r Tl* with the wild type allele *Lth* in comparison with gametophytes with the lethal *lth* (*DetR* tl^x).

Table 1. Amount of wrinkled and round seeds in crosses:
#1. F_5 (*R* tl^x */ r Tl*)* x Line 5-11 (*r* tl^w */ r* tl^w)
#2. Line 5-11 (*r* tl^w */ r* tl^w)* x F_5 (*R* tl^x */r Tl*)
#3. F_5 (*R* tl^x */ r Tl*) x F_5 (*R* tl^x */ r Tl*)

Cross	Number of round seeds	Number of wrinkled seeds	Proportion of wrinkled seeds
#1	80	134	0.63
#2	16	70	0.81
#3	171	210	0.55

*Maternal parent

We can see that both kinds of gametes carrying the mutation tl^x can survive but have inferior chances to take part in fertilization. Male gametophytes appear to be particularly sensitive to the presence of tl^x. It follows from data in Table 1 that the proportion of wrinkled seeds arising from selling should be equal to 0.51 (= 0.63 x 0.81) if all sporophytic genotypes are equally viable. If the *R* tl^x/*R* tl^x genotype is lethal, however, the 7% of the seeds expected to possess this genotype may not be produced. If so, the share of the wrinkled seed would be 0.51/0.93 or 0.55, precisely matching our experimental data. We conclude that locus *lth,* affected by the mutation tl^x can be considered to be a sporophytic lethal.

This deficiency covering the region including codominant morphological marker *tl* and the recessive sporophytic lethal should be a convenient tool for constructing a balancer system that might be used for many genetical applications.

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