

## A new xantha-type mutation *golden (gld)* on the short arm of chromosome 5

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Structural heterozygotes for translocations are of interest for gene mapping in several respects. First, the translocation breakpoints (T-points) behave as genetic markers manifested by pollen semisterility in heterozygotes versus pollen fertility in both types of structural homozygotes. Second, two intact and two interchange chromosomes are involved in the analysis. Third, translocations suppress recombination in the vicinity of T-points, facilitating recognition of linkage with distant markers. Finally, tertiary trisomics can be found among the progeny of translocation heterozygotes from selfing, permitting trisomy analysis.

We have performed a large experiment with the goal of adding markers to the genetic map in the regions adjacent to the centromeres of linkage groups II and V. For the sake of clarity in this manuscript we will use chromosome numbers paralleling the linkage group numbers as published in the most recent version of the pea genetic map (4). It is well known (1-3) that the centromeric regions are in the vicinity of T-points of Hammarlund translocation, involving chromosomes 2 and 5. We decided to treat the heterozygotes for this translocation by EMS and then identify new mutations linked to T-points and to the markers situated in the centromeric regions of chromosomes 2 and 5 of the normal karyotype. The plant material used was hybrid stock designated 'Tau-2.' This hybrid was generated from the line WL1476 [translocated karyotype (K)] with genotype *Cri*, *Gp*, *A*, *His(2-6)*<sup>2121</sup> (1), and a partially inbred line with normal karyotype (N) and genotype *cri*, *gp*, *a*, *His(2-6)*<sup>1123</sup>. The latter stock had a complicated pedigree, being in general derived from the lines WL1238, WL1018, our line SG, and a mutation *cri* induced in our laboratory with EMS in line SG.

In the hybrid Tau-2 (karyotype N/K) a cross structure is resolved in meiosis I, shown on Fig. 1. The hyperploid gametes carry the additional minor interchange chromosome 5<sup>2</sup>, composed of the short arms of the chromosomes 2 and 5. After combining with euploid gametes, viable and fertile trisomics are formed (1-3).

Selfing Tau-2 results in five main classes of progeny: structural homozygotes N/N for the normal and interchange (K/K) karyotype, structural heterozygotes (N/K), and tertiary trisomics with karyotypes N/N+5<sup>2</sup> and N/K + 5<sup>2</sup>. The recessive alleles *cri*, *gp*, and *a* are located near the T-point of chromosomes of normal karyotype, while the dominant alleles reside on the interchange

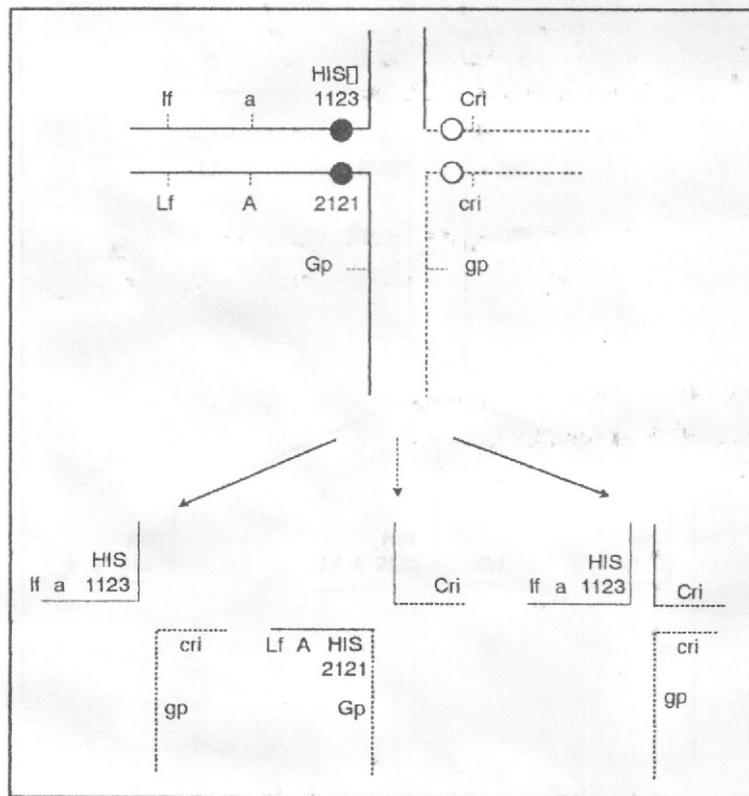


Fig. 1. A scheme of a translocation cross forming in meiosis in the hybrid Tau-2 and the types of gametes produced. It should be noted that only one class (N+2<sup>5</sup>) of hyperploid gametes is produced by the structural heterozygote (3).

chromosomes. Therefore we can easily distinguish structural homozygotes N/N (with the phenotype *cri gp a*) and trisomics N/N+5<sup>2</sup> (phenotype *Cri gp a*). Trisomics (*Cri gp a*) can be confused with the very rare cross-overs between the breakpoint and *cri*, but they can be easily recognized by slightly reduced pollen fertility, large bracts (3), and by examination of their progeny. Class N/K (with the phenotype *Cri Gp A*) can be distinguished from K/K by pollen semisterility and codominant expression of histone H1 subtypes (1123+2121) encoded by the gene cluster *His(2-6)*. This same phenotype can be exhibited by another class of trisomics N/K+5<sup>2</sup>. However, this last genotype can be recognized by a weak growth habit and large bracts.

Among 800 plants produced from selfing the structural heterozygote, Tau-2, we obtained 44 trisomics (N/N+5<sup>2</sup>). In addition we identified seven cross-over events between the T-point and *gp*, three between the T-point and *a*, none between the T-point and *cri* or between T-point and *His(2-6)*.

We imbibed 2165 F<sub>4</sub> seeds from Tau-2 in 0.15% solution of EMS overnight and grew the resulting plants hydroponically in the greenhouse. Among the M<sub>1</sub> plants only structural heterozygotes were selected and their progeny germinated. Similarly, among M<sub>2</sub> plants only structural heterozygotes (one M<sub>2</sub> plant of each family) were allowed to produce seeds. By this procedure we eliminated about half of the mutations not linked to the T-points. The analysis of new mutations was carried out on M<sub>3</sub> families grown in the field. We paid particular attention to families in which visible alterations of phenotype co-segregated or were negatively correlated with markers *cri* and *a*. Several mutations in the vicinity of the T-points were obtained. Here we report the results for one of them, a chlorophyll mutant of the xantha type.

Among the M<sub>3</sub> families we found one in which 9 of 10 plants with phenotype *cri* had no chlorophyll but, judging by their yellow color, normal level of carotinoids. Another 34 plants of this family were normal green (Table 1). This new chlorophyll mutation was called *golden (gld)*. All the nine *gld* plants died after producing 5 to 6 nodes but no flowers; however, absence of an anthocyanin ring at the base of the stipules indicated the phenotype *a*. In addition, all the mutants had a *His(2-6)* haplotype 1123. Thus, all mutants clearly had a normal karyotype (N/N). The plants with phenotype *Cri Gld* exhibited three phenotypic classes with respect to other markers (Table 1). Three, presumably trisomics, had white flowers with large bracts and the haplotype 1123. Two other classes had colored flowers with vexillary or no bracts. The heterozygotes (1123/2121) had a semisterile pollen (karyotype N/K), while the homozygotes (2121/2121) had normal pollen.

One can make two inferences from these results. The mutation is tightly linked to the markers on chromosomes 2 and 5 of the normal karyotype, and it resides in the short arm of either of the chromosomes 2 or 5, because addition of the small interchange chromosome reconstitutes the normal phenotype.

Table 1. Segregation for phenotype classes in the original M<sub>3</sub> family (resulted from selfing of a structural heterozygote) in which the mutation *gld* was found.

	1123, a <sup>1</sup>	1123+2121, A	2121, A
<i>cri, gld</i>	9	0	0
<i>cri, Gld</i>	1	0	0
<i>Cri, Gld</i>	3 <sup>2</sup>	17	13

<sup>1</sup> phenotype *a* was determined by absence of the anthocyanin ring.

<sup>2</sup> This is a trisomic class. While calculating recombination fractions it was not taken into account.

Recombination fractions (% of cross-over, calculated with the method of maximal likelihood with a program CROS): *cri* - [*His(2-6)*, *a*] = 0; *gld* - [*His(2-6)*, *a*] = 2.8 +/- 2.6 (JSCS-joint segregation chi-square = 34.8); *cri* - *gld*: 2.6 +/- 2.5 (JSCS = 34.8). All linkages are significant at the 0.1% level.

If the karyotype of the plants with phenotype *Cri Gld a 1123* was indeed  $N/N+5^2$  then their genotype with respect to the locus *Golden* should be  $gld/gld/Gld$  and their progeny should comprise diploids  $gld/gld$  and trisomics like the parents. Two presumed trisomic plants differed from their diploid counterparts by less vigor and fertility. Only one of them gave 20 seeds, that were sowed into a greenhouse bed and gave rise to 19 seedlings, 15 of which had the phenotype *cri gld* and four had the parental phenotype *Cri Gld* and flowers with large bracts. Cytological analysis of PMC of the four plants revealed a small additional chromosome.

Unfortunately, the *golden* mutation appeared in a family in which the allele *Gp* was fixed in a homozygous state most probably due to a cross-over event between the T-point and the locus *Gp* happened in the  $M_2$  stage. For restoration of the marker lost we crossed one trisomic plant with our original line HT1, a structural homozygote for the Hammarlund translocation with the long interchange chromosome marked with A, *His(2-6)<sup>2121</sup>*, *lf<sup>a</sup>*, and *gp*. This line was derived from the hybrid Tau-2 by fixation of two cross-over events between T-points and the markers mentioned. An  $F_1$  plant obtained had a phenotype *lf A 2121+1123 Gp Cri*.

Among the  $F_2$  plants one can distinguish all karyotypes by their phenotypes: *cri Gp 1123 a* - for  $N/N$ , *Cri gp 2121 A* - for  $K/K$ , *Cri Gp 2121+1123 A* - for  $N/K$ , and *Cri Gp 1123 a* - for trisomics  $NN+5^2$ . Pollen fertility was an additional indication of karyotype: in structural homozygotes it was 100% fertile, in structural heterozygotes - 50% fertile, in trisomics - 80-90% fertile. The segregation of these phenotypes in  $F_2$  is shown in Table 2. We can see that all *gld* plants displayed markers of the normal karyotype. Pollen mother cells were analyzed from 10 of the 12 presumed trisomic plants and the presence of a small additional chromosome was confirmed (Fig. 2). The  $F_3$  progenies of the trisomics contained *Cri Gld* plants in an overall fraction of 0.26 (Table 3).

To determine on which chromosome of the standard karyotype *Gld* resides, we crossed a structural heterozygote from the above described  $F_2$  with the testerline WL1255 possessing a genotype  $A/A 1221/1221$ . A heterozygote  $1221/1123$  with fully fertile pollen, i.e. a structural homozygote for the normal karyotype without the additional chromosome, was chosen from the  $F_1$ . It

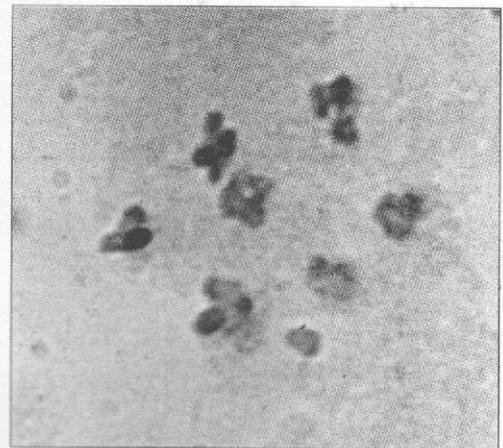


Fig. 2. Metaphase I in PMC of trisomic plants among  $F_2$  of the cross (trisomic *Gld/gld/gld* x HPI)

Table 2. Segregation for phenotype classes in the  $F_2$  progeny of the cross of the trisomic *Gld/gld/gld* with the interchange line HT1.

	2121 A	2121+1123 A	1123a
<i>cri gld Gp</i>	0	0	26
<i>cri Gld Gp</i>	0	0	1
<i>Cri Gld Gp</i>	0	83	12 <sup>1</sup>
<i>Cri Gld gp</i>	35	0	0

<sup>1</sup> This is a trisomic class. While calculating recombination fractions, it was added to the disomic class *cri gld Gp*, the allelic state of the diploid chromosome set being tested by their progenies.

Recombination fractions: *cri* - [*His(2-6)*, *a*] = 0; *gld* - [*His(2-6)*, *a*] = 0.6 +/- 0.6 (JSCS-joint segregation chi-square = 151.7); *cri* - *gld*: 0.6 +/- 0.6 (JSCS = 151.7).

All linkages are significant at the 0.1% level.

Table 3. Segregation of phenotypes in the progeny of trisomics from F<sub>2</sub> explained in Table 2

No of trisomic	<i>cri gld</i>	<i>cri Gld</i>	<i>Cri Gld</i>
1	22	0	3
2	23	0	6
3	36	0	16
4	11	1	2
5	31	1	18
6	45	1	18
7	14	0	5
8	25	0	9
9	19	0	5
10	17	0	5
11	18	1	4
Total	261 (73.3%)	4 (1.1%)	91 (25.6%)

gave rise to 211 F<sub>2</sub> plants, of which only four had phenotype *gld*, all being *cri* (Table 4). The *gld* mutation assorted independently of the locus *His(2-6)* but showed linkage to *cri*, although the distance of 0.6 % cross-over obtained is not reliable due to a strongly biased monogenic segregation for both *cri* and *gld*.

The evidence indicates that *Gld* is located on linkage group V distal to *cri* and possibly very close to it.

Table 4. Segregation of phenotypes in progeny of the plant with normal karyotype (1123/1221) taken from the cross of WL1255 (1221, *Cri, Gld*) with the structural heterozygote (2121/1123, *Cri/cri, Gld/gld*)

	1221	1221+1123	1123
<i>Cri Gld</i>	51	102	46
<i>cri gld</i>	1	2	1
<i>cri Gld</i>	3	3	4

Recombination fractions: *cri - His(2-6)* = 49.0 +/- 0.4, JSCS = 1.3; *gld - His(2-6)* = 47.5 +/- 4.2, JSCS—joint segregation chi-square = 0.003; *cri - gld* = 7.0 +/- 1.8, JSCS = 57.9, P < 0.001.

All linkages are significant at the 0.1% level

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