Mapping of two thiamin deficiency genes of pea

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Kumar and Sharma (1) isolated 20 stable auxotrophic mutations for thiamin biosynthesis in pea. These mutations were induced by 0.05% ethylmethane sulphonate (EMS) and 0.0057% nitrosomethyl urea (NMU). Based on complementation tests, the mutations were recessive alleles (*thiA, thiB, and thiC*) at three loci. The mutants are characterized by yellowing of the leaves about two weeks after germination, followed by death of the plants. The mutants were viable and indistinguishable from normal plants when thiamin was applied exogenously at the concentration of about 0.2 mg/ml.

ThiA has been mapped very close to *K* on linkage group II (10). *ThiB* was mapped near *Pl* in linkage group VI (11.3 crossover units), and *ThiC* was located 20 crossover units away from *St* in linkage group III (1). However, the precise positions of each locus in relation to other loci on the respective linkage groups have not been studied. The thiamin auxotrophic mutants can serve as good seedling markers in genetic studies.

Two of these three mutants, *i.e. thiB* (SK 25) and *thiC* (L 102), are maintained at the Division of Genetics, I.A.R.I., New Delhi. In order to determine the precise position of these two genes, the mutants were crossed with lines having several known markers of linkage groups III and VI. The data for *thiB* were obtained from the cross of SK 25 (*thiB*) with P 1746-2 (*Pl, Arg*) while the data for *ThiC* were obtained from crosses of line L 102 (*thiC*) with P 1297-1 (*apu, uni*), P 1759-2 (*wel*), and P 1563 (*st*). A cross between the lines P 841 (*st*) and P 1297-1 was also made to determine the distance between the loci *St* and *Apu.* These crosses also segregated for the genes *A, D, Af, Le, Gp, Tl, Con,* and *Wlo*, which belong to other linkage groups. The distance between *Apu* and *Wel* was estimated from a cross between P 1297-1 and P 1759-2. In all the crosses, the F_1 plants were fully fertile. The F_1 plants in the crosses involving the *thiB* and *thiC* mutants and normal green parents were normal, confirming the recessive nature of these two mutations. Details of the crosses, single locus and joint segregation with χ^2 values and recombination fractions (R.F) obtained by using a computer program, CROS, provided by Dr. S.M. Rozov (http://pisum.bionet.nsc.ru) are presented in Table 1. No mapping function was applied, and the map distances are expressed as recombination frequencies.

Table 1. Joint F_2 segregation of thiamin-deficient mutations (*thiB, thiC*) with other morphological markers.

			Phenotypes[2]					Chi-square			
Loci	**Phase [1]**	**Cross**	**DD**	**Dr**	**rD**	**rr**	**Total**	**Locus 1 (3:1)**	**Locus 2 (3:1)**	**Joint seg.**	**Recomb. Fract. ± Stan. Error**
thiB Arg	**C**	**SKxP 1746-2**	**197**	**26**	**20**	**43**	**286**	**2.343**	**0.116**	**85.95**[c]	**18.3 ± 2.6**
Pl ThiB	**C**	**SKxP 1746-2**	**199**	**18**	**24**	**45**	**286**	**0.116**	**1.347**	**88.76**[c]	**16.5 ± 2.4**
Arg Pl	**C**	**SKxP 1746-2**	**211**	**6**	**6**	**63**	**233**	**0.116**	**0.115**	**224.2** [c]	**4.34 ± 1.2**
ThiC Wel	**R**	**L 102xP 1759-2**	**132**	**59**	**53**	**7**	**251**	**0.16**	**0.224**	**8.80**[a]	**33.3 ± 5.5**
ThiC Apu	**R**	**L 102xP1297-1**	**140**	**71**	**66**	**2**	**279**	**0.05**	**0.202**	**25.49**[c]	**16.6 ± 5.7**
ThiC Uni	**R**	**L 102xP1297-1**	**148**	**63**	**66**	**2**	**279**	**0.05**	**0.430**	**19.45**[c]	**18.16 ± 5.7**
Uni Apu	**R**	**L 102xP1297-1**	**180**	**34**	**26**	**39**	**279**	**0.43**	**0.20**	**49.06**[c]	**24.67 ± 3.0**
Thi St	**R**	**L 102xP 1563**	**106**	**52**	**44**	**4**	**204**	**0.32**	**0.529**	**8.40**[a]	**27.5 ± 6.4**
Apu St	**R**	**P 1297-1xP 841**	**175**	**78**	**66**	**1**	**320**	**0.09**	**0.016**	**21.3** [c]	**12.94 ± 5.5**
Apu Wel	**R**	**P 1297-1xP 1759-2**	**92**	**46**	**58**	**2**	**198**	**0.08**	**0.060**	**22.89**[c]	**17.90 ± 6.8**

[a], [b], [c] Mean $P < 0.01$, $P < 0.001$, $P = 0.0001$, respectively.

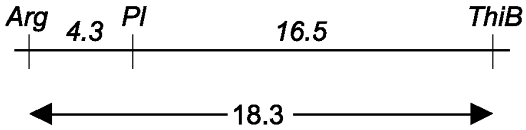
[1] D = dominant allele and r = recessive allele

[2] C = coupling and R = repulsion phase linkage

The F_2 segregation analysis showed that *ThiB* and *ThiC* as well as all other marker loci segregated in the 3:1 ratio (Table 1). In the cross SK 25 x P 1746-2, *thiB* was estimated to be located at about 18.3 map units away from *Arg*, and 16.5 map units from *Pl.* The linkages *ThiB–Arg* ($P < 0.0001$) and *Pl–ThiB* ($P < 0.0001$) were highly significant. The *Pl–ThiB* map distance of 16.5 is reasonably close to 11.3 reported by Kumar and

Sharma (1). The map distance between *Pl* and *Arg* on the same chromosome was 4.3 (Table 1). This distance was reported as 3 map units by Marx (2), 2 map units by Weller and Murget (10) and 2.3 map units by Święcicki and Kruszka (9).

Based on the segregation analysis of *ThiB, Arg* and *Pl,* these loci can be arranged in the following order:



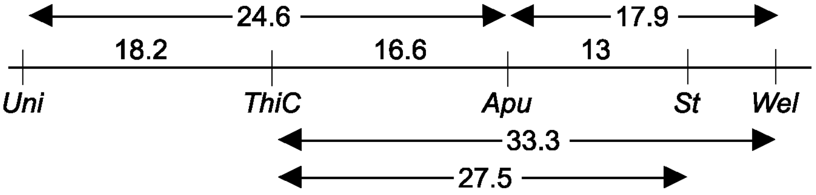
From the cross L 102 x P 1759-2, *ThiC* has been located at the distance of 16.6 map units from *Apu*, 27.5 map units from *St*, and 33.3 map units from *Wel.* Marx (3) located *St* about 8.8 map units away from *Wel.* Subsequently this distance was reported as 12.5 map units (5). Sharma and Kumar (8) did not find any linkage between *St* and *Uni*, but this linkage was estimated to be 36.4 map unitsd by Marx (5). However, Rozov et al. (6) reported 57 Kosambi units as the distance between *St* and *Uni.*

The results of the cross L 102 x P 1297-1 showed that *ThiC* is located about 18.2 map units away from *Uni.* The distance between *Uni* and *Apu* being 24.6 shows that *ThiC* is placed between these two genes. The linkages *ThiC-Apu* and *ThiC-Uni* were highly significant (P < 0.0001).

Marx (4) reported 23 map units distance between *Apu* and *Uni*, 10 map units between *St* and *Apu*, and 30 map units between *St* and *Uni.* Marx (5) located *Apu* at the distance of 33.9 map units from *Uni*, while Sarala and Sharma (7) reported 20 map units distance between these two loci.

From the cross P1297-1 x P841, *St* has been mapped 13 map units away from *Apu.* This distance is reasonably close to 15 map units reported by Sarala and Sharma (7). Finally, from the cross P1297-1 x P1259-2, the distance between *Wel* and *Apu* was estimated about 17.9 map units which is very close to the distance (19.8) reported by Marx (5).

On the basis of joint segregation of *thiC, wel, apu, uni* and *st*, the following arrangement is proposed:



Thus, our results confirm that the two thiamin auxotrophic mutations, *thiB* and *thiC,* are recessive. The locus *ThiB* is located in the lower part of linkage group VI and *ThiC* belongs to linkage group III between *Apu* and *Uni* . The exact position of *ThiC* with respect to the isozyme markers (*Adh*-1 and *Acp*-3) is under investigation. The two thiaminless mutations can be used for further studies on the biochemical pathway of thiamin biosynthesis as well as in mapping programs.

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