IDENTITY AND LINKAGE OF THE COSTATA MUTANT IN PISUM*

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Abstract

Six induced mutants of the *costata* type are described. It was found that three mutants were allelic. Linkage analysis confirmed the localization of the *costata* gene hum in chromosome 4.

Introduction

Among higher plants the Pea holds a special place when it comes to genetic investigations including gene mapping. It is also now one of the genetically best known of the higher plants with at present more than 3500 lines available in the Nordic Gene Bank of which about 1000 are mutant lines. Information on more than 350 genes and 40 characters are available on this material. Certain lines particularly suited for linkage studies, so called tester lines (Blixt, 1976) are also available from this collection.

Work to increase the knowledge about the gene map of *Pisum* and to investigate the genetic background of different characters is continuously in progress. This work includes also crosses and linkage analysis. The present paper deals with how to identify and localize genes determining the costata mutant type in peas through origin test crosses, identity test crosses and linkage studies.

Material and Method

The identification and mapping of mutant genes was carried out in three steps, which involved three types of crosses, the origin test cross (ORX), identity test crosses (IDX) and linkage test crosses (LIX). The origin cross involved the cross of a mutant with its variety of origin in order to check the general genetic behaviour of the mutant (Blixt, 1972). The identity test cross, involved crosses between morphologically identical to similar mutants, as it has been established that morphological similarity does not necessarily mean genotypic similarity. Particularly in the case of induced mutants morphologically similar mutants often turned out to be mutations at different loca (Gottschalk, 1964). The third step involved one to several linkage test crosses of a mutant to suitable tester lines and the analysis of the F₂ generations. Tester lines carrying marker genes were obtained from the Nordic Gene Bank and also through the Pisum Genetic Association (PGA).

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The analysis of F₂ generations of LIX-crosses involved carrying out monohybrid as well as dihybrid combination and the calculations of chi-square and CrO values. All possible dihybrid combinations were calculated and evaluated. Those combinations giving significant indications of linkage was then used for mapping the investigated mutant gene.

The following mutant lines were used for ORX and IDX crosses.

- Line 5160, a costata mutant obtained by Blixt (1948) through EI treatment of the cultivar Weitor.
- Line 5161, costata, obtained by Blixt (1958) after EI-treatment of the cultivar Weitor.
- Line 5366, costata, obtained by Blixt (1958) after EI-treatment of the cultivar Weitor.
- Line 5937, obtained by Blixt (1972/76) after EMS treatment of the cultivar Parvus.
- 5. Line 6011, obtained by Prof. Luigi L. Monti after DES-treatment of the variety Parvus at CNEN, Rome 1975/76. This line is the type line for the gene lum (Monti, 1970).
- 6. Line 5958, obtained by Blixt (1976) after EMS treatment of the cultivar Parvus.

The cultivars Parvus and Weitor, line 1107 and 1263, respectively, were used for the origin test crosses.

Results and Discussion

Results from ORX-crosses are presented in Table 1. Lines 5366, 5937, 5958 and 6011 were all observed to have F_1 plants phenotypically normal, which would indicate recessivity of the mutant. In the case of line 5937, 5958 and 6011 the F_2 segregation observed fits a 3:1 segregation with the mutant as recessive and thus the costata mutant of these three lines can be considered as determined by a single recessive gene. Line 5366 shows a significant deficit of mutant plants which, however, is found for many induced mutations. The mutant in line 5366, therefore, may also be assumed to be determined as a monogenic recessive.

The results of the IDX-crosses (Table 2) indicate that the mutants of lines 5160, 5161, 5366, 5937 and 5958 are not mutants at the lum-locus. Further, that the lines 5160, 5161 and 5366 are mutated at the same locus and that this locus is a different

Table 1. Origin test crosses (ORX)	Table	M.	Origin	test	crosses	(ORX)
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Cross Pare	ents phenotype	F _i fertility	F ₂ -s normal	egregation mutant	Chisqr 3:1
5591 1263 x 53 5638 1107 x 59 5645 1107 x 59 5657 1107 x 60	Normal Normal Normal	89.5 75.5 98.3 88.8	1023 135 10 351	265 41 5	13.45 0.27 0.56 0.18

one from the loca mutated in lined 5937 and 5958. Further, in the lines 5937 and 5958 the mutation seems to have occurred at different loca.

Summarizing, the results of Table 2, as presented in Figure 1, indicate that the costata mutation type may be caused by at least genes:

The lum-locus in line 6011;

another locus represented in lines 5160, 5161 and 5366;

- a third locus in line 5937;
- a fourth locus in line 5958.

Table 2. Identity test crosses (IDX)

Cross number	Parents	$\begin{matrix} F_l \\ phenotype \end{matrix}$	No of F_1 plants
2976	5160 x 5161	costata	3
2977	5160 x 5366	costata	1
4576	5160 x 6011	Normal	6
4577	5161 x 6011	Normal	5
4583	5958 x 6011	Normal	11
6253	5366 x 6011	Normal	10
6254	5937 x 6011	Normal	3
6259	5160 x 5937	Normal	5
6263	5161 x 5366	Normal	9
6264	5161 x 5937	Normal	14
6270	5366 x 5958	Normal	10
6277	5937 x 5958	Normal	3

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	5	5	5	5	5	6	1	1
	1	1	3	9	9	0	1	2
	6	6	6	3	5	1	0	6
	0	1	6	7	8	1	7	3
5160		m	m	N		N		
5161			\mathbb{N}	N		N		
5366					N	N		N
5937					N	N	N	
5958						N	N	
6011							N	

Fig. 1. ORX and IDX corsses made. (N indicates normal, m mutant Fl phenotype).

To study the linkage of one of the costata mutant the cross 4364 was carried out between line 6011, type line for lum, and line 577, tester line. As far as genes here studied are concerned this line has the genotype: Lum, td, le. The corresponding genotype for line 6011 is: lum, Td, Le.

The monohybrid segregations in the crosses were as follows:

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436 Lum : 54 lum; expected 3:1 = 367.5 : 122.5; Chisqr 3:1 = 51.07***
387 Td : 103 td; expected 3:1 = 367.5 : 122.5; Chisqr 3:1 = 4.14*
366 Le : 124 le; expected 3:1 = 367.5 : 122.5; Chisqr 3:1 = 0.02NS
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A significant deficit of mutants was found, which, in the case of induced mutants may be considered almost normal.

The dihybrid segregations were as follows:

1) rDLum - Td DDDr Sum rr 7 Found 340 96 47 490 Expected 9:3:3:1 275 91 91 30 490 15.03 0.19 21.92 18.23 55.37 Chisgr 2) 344 92 43 11 490 Expected corr. 0.05 0.17 0.37 1.45 2.04 Chisqr corr. 3) CrO-R 41.07 Stand. err. 3.71

Lum — le									
Found	314	122	52	2	490				
Expected 9:3:3:1	175	91	91	30	490				
Chisqr	5.34	9.88	17.31	26.76	59.29				
Expected corr.	326	110	40	14	490				
Chisqr corr.	0.44	1.31	3.60	10.29	15.64				
CrO-R 18.53 Stand. err. 8.82									
Td – Le									
Found	309	78	57	46	490				
Expected 9:3:3:1	275	91	91	30	490				
Chisqr	4.04	2.10	13.24	7.72	27.10				
Expected corr.	289	98	77	26	490				
Chisqr corr.	1.38	4.08	5.19	15.38	26.03				

CrO-K 34.49 Stand. err. 2.75

These results show the linkage of the costata-mutant determined by the gene lum with the gene le in chromosome 4, as previously found by Monti (1970). The linkage relations appearing from cross 4364 are presented in Fig. 2. Further work on the costata mutation type is in progress.

lum 21 le 35 td.

Fig. 2. Map of the genes lum-le-td in chromosome 4.

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¹⁾ DD denotes both genes dominent, Dr. first gene dominant and second recessive, rD the reverse, rr both genes recessive.

²⁾ The corrected expected segregation was calculated according to STERN (1933).

³⁾ R denotes repulsion and K coupling phase.