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Linkages in *Pisum* L. VII. Locus for the sterile gene *calf* (*cabbage leaf*)

Wojciech K. ŚWIĘCICKI, Bogdan WOLKO, Katarzyna KRUSZKA

Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland

Abstract. Genetical analyses were conducted to find linkages and the locus of the gene *calf* on the *Pisum* chromosome map. The recessive, pleiotropic gene *calf* (enlarged and undulated leaflets, stipules, flowers and pods, plant sterile), artificially induced (the initial line – Large Podded G-20, the mutagene – DES and NMU) was described by Sharma in 1975. An identical mutant gene at the same locus was isolated in our research (the initial line – cv. Pegro, the mutagene – fast neutrons). Two lines were included in the *Pisum* gene bank – the type line for the gene *calf* - Wt 15873 and the representative line – Wt 16024. In linkage studies the representative line was crossed with tester lines bearing gene markers. Analyses of dihybrid segregation in F_2 generations revealed linkages of the gene *calf* with chromosome 2 markers. Two isozymic markers helped to reveal the *calf* locus on chromosome 2 with the following gene order: *Orp – Calf – K – Pgm-p – Fum*. This is in agreement with the current *Pisum* linkage map.

Key words: chromosome map, linkage, *Pisum*.

Introduction

The mutation type in pea named *cabbage leaf* has been described by SHARMA in 1975. Since its mode of segregation suggested that this mutation is of monohybrid, recessive nature, the gene symbol *calf* has been proposed. The mutation has been selected from the M_2 generation after dry seed treatment of cv. Large Podded G-20 with the diethyl sulfate and N-nitrosomethyl urea (SHARMA 1975). The characteristic features of the mutant are visible even in the seedling stage – stipules and leaves are markedly bigger and undulated

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Correspondence: W.K. ŚWIĘCICKI, Institute of Plant Genetics, Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland. E-mail: wswi@igr.poznan.pl.

in comparison with the initial genotype (Figure 1). This phenotype has justified the proposed name for the mutation type. Moreover, the stem is slightly thicker and more angular, plants are taller and flowers are larger. Pods are flat, curved and almost two times broader than in the initial line. Plants are fully sterile. Sometimes seeds develop in pods but without embryos. This mutation has been

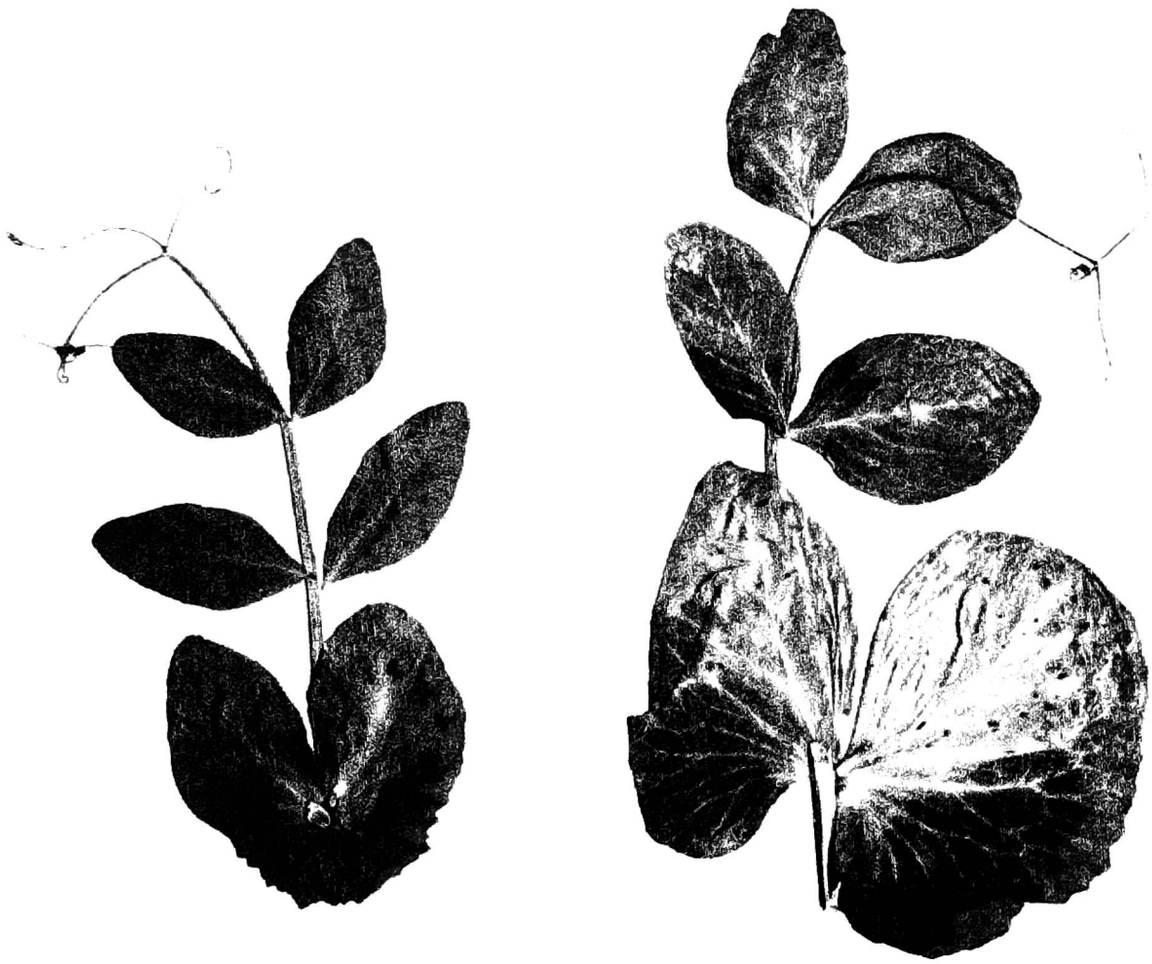

Figure 1. Expression of the gene *calf*: normal (left), mutant leaf (right)

classified by BLIXT (1977) as complex sterile and has been included in the *Pisum* Gene Bank in the heterozygous stage (the catalogue number in the gene bank at Weibullsholm Plant Breeding Institute (Sweden) – Wl 5873, at the Plant Breeding Station Wiatrowo (Poland) – Wt 15873).

The gene *calf* has not been localized hitherto, most probably because of difficulties in studies on a sterile gene or no suitable markers available. There are still some more complex sterile mutations in the *Pisum* gene bank that have not been mapped yet. Some of them cause very strong changes in plant morphology, which makes observations of many standard markers impossible. But it seems that for evaluation of the *Pisum* chromosome map also linkage studies for such genes are important thanks to much more suitable markers available at present. For example 25 years after the discovery of the gene *def*

its locus was found using isozymic markers *Aldo* and *Gal 2* (WOLKO, ŚWIĘCICKI 1991). The aim of the present study has been to find linkages and the locus of the gene *calf*.

Material and methods

During investigations on mutation induction in pea at Wiatrowo (Poznań Plant Breeders) dry seeds of cv. Pegro (the catalogue number – Wt 6048) were irradiated by fast neutrons. In the M_2 generation a mutant was selected with a phenotype identical to that described by SHARMA (1975). The mutant line (in the heterozygous stage) was included in the *Pisum* Gene Bank at Wiatrowo after multiplication, with the catalogue number Wt 16024. The locus identity test cross showed that both mutants are controlled by a gene from the same locus. Thus, additionally to Wt 15873 – the type line for the gene *calf*, Wt 16024 is considered as a representative line.

For linkage studies on the gene *calf* the representative line Wt 16024 was used because its initial line Wt 6048 was available for comparisons. The following testerlines with standard markers for different *Pisum* chromosomes and linkage groups were crossed with the line Wt 16024 (WEEDEN et al. 1996):

Wt 11238 – *A* – linkage group IA, *d*, *i* – linkage group IB; *s*, *wb*, *k* – linkage group II; *b* – linkage group III; *r*, *tl*, *cp*, *gp*, *te*, U^{st} – linkage group V (the code number in a data base for the crossing to Wt 16024 = K. 1209; marker gene segregation was observed on F_2 generation plants in the field in 1993),

Wt 11288 – *M*, *st*, *b* – linkage group III (K. 1210; in the field in 1993),

Wt 11143 – *n*, *fa*, *v* – linkage group IVA and IVB (K. 1208; in the field in 1993),

Wt 15860 – *creep*, *ce*, *Fs* – linkage group V (K. 1207; in the field in 1993),

Wt 11745 – *r*, *tl*, *coch* – linkage group V (K. 1212; in the field in 1993).

Preliminary results suggested that linkages should be looked for in the linkage group II. Therefore, the line with the gene *calf* was crossed additionally with the following tester lines:

Wt 10659 – *orp*, *k*, *Pgm-p*, *Fum* – K. 1676; in the field in 1996,

Wt 11685 – *Pgm-p*, *Fum* – K. 1677; in the field and in a greenhouse in 1996.

Expression of most of the gene markers was presented in earlier papers (ŚWIĘCICKI 1984, 1986, WOLKO, ŚWIĘCICKI 1991). A short description of the expression of the marker not presented hitherto, is given below:

Coch – *coch* = normal stipules – reduced, spatula-like stipules.

Segregation of two isozymic markers *Fum* and *Pgm-p* was observed. Isozyme phenotypes were determined using horizontal starch gel electrophoresis as described by WOLKO and WEEDEN (1989). Two isozyme systems: fumarase (FUM) and phosphoglucomutase (PGM) were assayed on extracts from young leaves. The discontinous tris-citrate lithium borate buffer mixture was used for electrophoresis.

The line with the sterile gene *calf* was sown in the heterozygous stage (6 families, 16 seeds each). Crossings were done using pollen from plants with the normal phenotype (probable heterozygotes) from families segregating for *calf*. Observations for the investigated gene and gene markers were carried out in the F_2 generation in families segregating for *calf*.

Results of observations of mono- and dihybrid segregation were calculated using a computer program based on the product-ratio method for linkage estimation (ŚWIĘCICKI 1984, 1989). To determine the gene order the Mapmaker v. 3.0 program from the Whitehead Institute/USA was used (LINCOLN et al. 1990).

Results and discussion

Genetical analyses for genes controlling complex sterile characters are more difficult because of: (1) sterility which requires a large number of crossings with heterozygotes and abundant plant material for selection of segregating families; (2) marked changes in phenotype (eg. the gene *fil* or *rui*) making observations of many standard markers difficult or even imposible. These difficulties maybe less important when using molecular markers (WOLKO 1989).

SHARMA (1975) is the author of the investigated character and the gene symbol. His conclusion on the monohybrid nature of the mutation was based on segregating mutant families. Our own investigations on the *calf* segregation in F_2 populations confirmed Sharma's conclusion (Table 1).

Despite the pleiotropic effect of the gene *calf* causing sterility, morphological changes still make it possible to observe most of the standard gene markers (WEEDEN et al. 1993), excluding genes for generative organs, of course. Therefore, for the linkage test the line Wt 16024, representative of *calf*, was crossed with most of the standard tester lines. In the F_1 generation of these cross combinations the karyotype was tested. In all combinations about 100% fertility was observed. Thus, a model *Pisum* karyotype was involved in the investigations (the tester line Wt 11238 has the same karyotype as Wl 110) (BLIXT 1977).

Dihybrid segregation for the gene *calf* and 22 gene markers was analysed in an F_2 generation in 1993. Chi-square testing confirmed undisturbed dihybrid segregation for most gene pairs with the *calf*. The exception was the gene pair

Table 1. Monohybrid segregation for the investigated gene *calf* and gene markers in chromosome 2 observed in the F_2 population of the following linkage test crosses: K. 1209 = Wt 16024 × Wt 11238; K. 1676 = Wt 16024 × Wt 10659; K. 1677 = Wt 16024 × Wt 11685

Cross combination	Gene	Allele		Total	Chi-square* (3:1)
		dominant	recessive		
K. 1209	*Calf*	46	23	69	2.56
K. 1676		43	13	56	0.10
K. 1677		166	74	240	4.36
K. 1209	*Wb*	51	18	69	0.04
K. 1676	*Orp*	37	15	52	0.41
K. 1676	*K*	31	17	48	2.78
K. 1676	*Fum*	35	21	56	4.67
K. 1677		167	72	239	3.35
K. 1676	*Pgm-p*	38	18	56	1.52
K. 1677		164	76	240	5.69

* $\chi^2_{1;0.05} = 3.83$, $\chi^2_{1;0.01} = 6.63$

Calf-Wb in the cross combination K. 1209 (Wt 16024 × Wt 11238). The joint chi-square value suggested a linkage and as a consequence a locus for the *calf* gene on the linkage group II (Table 2). Because of this markers from the *wb*-segment were used for further analyses. In this segment standard markers are *k* and *orp* which, as controlling characters of generative organs, are difficult or even imposible to observe in the *calf* genotype. More suitable are isozymic markers *Pgm-p* and *Fum* (WEEDEN et al. 1996). Allozyme analyses revealed the fast allele for *Pgm-p* and the slow for *Fum* in the line Wt 16024. Two lines with opposite alleles were selected from the *Pisum* Gene Bank at Wiatrowo for crossings: Wt 10659 (*Calf*, *Pgm-p*/slow, *Fum*/Fast and also *orp*, *k*) and Wt 11685 (*Calf*, *Pgm-p*/slow, *Fum*/Fast). An analysis of dihybrid segregation in their F_2 generations supplemented first suggestions from Table 2 (for K. 1209) – the calf locus in the linkage group II. Substantial deviations in dihybrid segregation for most of the gene pairs were observed. The cross combination

Table 2. Distribution of phenotypes in F_2 populations and the linkage tests for the *calf* gene (Wt 16024 – representative line × tester lines). Joint segregation of gene pairs in chromosome 2 (K. 1209 = Wt 16024 × Wt 11238; K. 1676 = Wt 16024 × Wt 10659; K. 1677 = Wt 16024 × Wt 11685)

Cross combination	Pair of genes	Phase	Phenotype				Total	Joint chi-square	Cr-O value (per cent)
			DD	Dr	rD	rr			
K. 1209	*Calf–Wb*	R	29	17	22	1	69	8.46	18.8±11.5
K. 1676	*Calf–Orp*	R	25	15	12	1	53	6.32	24.4±12.9
K. 1676	*Calf–K*	R	25	17	6	1	49	3.76	30.9±12.9
K. 1676	*Orp–K*	C	26	5	3	12	46	15.07	16.4±6.1
K. 1676	*Orp–Pgm–p*	R	21	16	15	1	53	10.73	19.9±13.2
K. 1676	*Orp–Fum*	R	19	18	13	2	52	6.28	26.1±12.8
K. 1676	*K–Pgm–p*	R	20	11	17	1	49	8.18	21.8±13.6
K. 1676	*K–Fum*	R	17	14	17	1	49	11.00	18.1±13.9
K. 1676	*Calf–Fum*	C	33	10	2	11	56	15.28	17.4±5.7
K. 1677		C	146	19	21	53	239	87.68	16.9±2.7
K. 1676	*Calf–Pgm–p*	C	38	5	1	13	57	37.83	8.12±3.8
K. 1677		C	162	4	2	72	240	212.97	2.2±1.0
K. 1676	*Pgm–p–Fum*	C	30	8	5	13	56	12.49	22.5±6.5
K. 1677		C	147	16	20	56	239	100.40	14.9±2.5

K. 1676 (Wt 16024 × Wt 10659) gives linkage data for more gene pairs than in K. 1677 (Wt 16024 × Wt 11685) but the size of the analysed plant population is markedly smaller (56 and 240 plants, respectively). Moreover, the *calf* gene (changed and sterile flowers) disturbed segregations for genes *orp* (*orange pod*) and *k* (wings reduced). As a consequence Cr-O values for *Calf – Orp* and *Calf – K* did not agree with those expected on the basis of results for other gene pairs and influenced a multipoint analysis. Nevertheless, loci for the selected markers are the same as on the current *Pisum* linkage map (WEEDEN et al. 1996).

Because of the above comments, data from both cross combinations (K. 1676 and K. 1677) were calculated for gene order estimation using the Mapmaker (LINCOLN et al. 1990). For K. 1677 the following gene order was identified: *Calf – Pgm-p – Fum*. This suggested that the *calf* locus is below *Pgm-p*, in *K* segment. Calculations for K. 1676 confirmed the above, localizing additionally the *calf* gene between *k* and *orp* loci (*Orp – Calf – K – Pgm-p – Fum*).

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