ADDITIONAL MARKERS FOR CHROMOSOME 6

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Although chromosome 6 is one of the largest of the pea chromosomes, relatively few morphological mutants and only one isozyme marker have been assigned to the corresponding linkage group (1, 3, 5). Here we present data from seven F_2 populations that permit us to place two additional isozyme loci, Acp-4 and Gpi-c, and one morphological marker on this chromosome.

The parental lines used in the crosses were obtained from the Northeast Regional Plant Introduction Station, Geneva, NY (PIs 169607, 220189, 227258, 343980, 344001, and 429839), from the John Innes Institute, Norwich, England (JI12 and JI1794), and from the Department of Horticultural Sciences, NYSAES, Geneva, NY (A73-91, A1078-239, and Slow). The genotypes of the lines, carrying Gpi-c or Acp-4 rare alleles are as follows:-

PI344001: Wlo, Pl, Gty, Acp-4^c, Gpi-c^a, Prx-3^b
PI227258: Wlo, pl, gty, Acp-4^a, Gpi-c^a, Prx-3^b
JI1794: Wlo, Pl, Gty, Acp-4^c, Gpi-c^b, Prx-3^b
PI343980: Wlo, pl, gty, Acp-4^b, Gpi-c^b, Prx-3^c
PI220189: Wlo, pl, gty, Acp-4^a, Gpi-c^b, Prx-3^b

Extracts were made for electrophoresis from imbibed seeds by slicing off a 5-10 mg piece from one cotyledon and mashing this tissue in extraction buffer. Extraction buffers and conditions for electrophoresis were as described in (4) with glucosephosphate isomerase (GPI, EC 5.3.1.9) being assayed on the lithium borate/tris citrate gel and beta acid phosphatase (β-ACP, EC 3.1.3.-) on the morpholine/citrate gel. The assay for GPI contained 0.1 M tris HCl (pH 8.0), 2 mM $MgCl_2$, 10 mM fructose 6-phosphate, 0.5 mM NADP, 1 unit/ml glucose 6-phosphate dehydrogenase (Sigma G8S78), 0.4 mM MTT and 0.1 mg/ml meldola blue. The 3-ACP assay was as described in (4) except that β-naphthyl acid phosphate was substituted for α-naphthyl acid phosphate.

The GPI phenotype typical of most pea lines contains a plastld and a cytosolic isozyme. Several wild accessions (possessing dehiscent pods) displayed mobility variants for the cytosolic form. F_1 hybrids between lines fixed for alternate allozymes showed the 3-banded phenotype typical of a GPI heterozygote. Three phenotypes were observed in the F_2: the two parental and that of the hybrid.

The β-ACP assay visualized two zones of activity. The more anodal zone matched the ACP-3 phenotype seen in an α-acid phosphatase assay. The other zone was found near the origin (both anodal and cathodal) and showed considerable polymorphism among the lines. This zone was designated ACP-4. Hybrids generated from parents with different ACP-4 phenotypes exhibited the additive phenotype, although for certain allozyme combinations the separate bands were not well resolved.

Single locus segregation data for loci known to be on linkage group 6 (Pl, Wlo, and Prx-3), as well as the allozyme polymorphisms of interest, are presented in Table 1. Segregation at Gty, a locus previously assigned to chromosome 3 (2), also is included for crosses in which it segregated. Nearly all polymorphisms displayed segregation ratios close to those expected for single gene traits, indicating that the variation observed in the GPI-2 and ACP-4 phenotypes is generated by different alleles at the structural loci. We designate the loci responsible for the polymorphisms Gpi-c and Acp-4, respectively.

Table 1. Single locus segregation of loci mapping to chromosome 6.

Cross	Locus	N	Phenotype[1]			Ratio	χ^2
			aa	ab	bb		
344001 x 169607	Acp-4	31	7	18	6	1:2:1	0.87
	Gpi-c	31	7	16	8	1:2:1	0.10
	Gty	26	18	--	8	3:1	0.46
	Pl	26	20	--	6	3:1	0.05
	Wlo	26	22	--	4	3:1	1.28
344001 x A1078-239	Gpi-c	17	2	9	6	1:2:1	1.94
	Gty	17	10	--	7	3:1	2.37
	Wlo	17	12	--	5	3:1	0.18
JI12 x 227258	Acp-4	44	9	21	14	1:2:1	1.23
	Gpi-c	44	16	19	9	1:2:1	3.05
	Prx-3	44	13	26	5	1:2:1	4.36
	Wlo	44	32	--	12	3:1	0.12
Slow x 227258	Acp-4	30	9	13	8	1:2:1	0.60
	Gpi-c	30	9	11	10	1:2:1	2.20
	Prx-3	24	10	6	8	1:2:1	6.33*
A73-91 x 227258	Acp-4	32	13	13	6	1:2:1	4.19
	Gpi-c	32	6	15	11	1:2:1	1.69
	Prx-3	27	10	10	7	1:2:1	2.48
429839 x 227258	Acp-4	114	35	56	23	1:2:1	2.56
	Gpi-c	114	24	56	34	1:2:1	1.79
	Prx-3	90	28	33	29	1:2:1	6.42*
Slow x JI1794	Acp-4	41	11	23	7	1:2:1	1.39
	Prx-3	45	15	22	8	1:2:1	2.20
	Pl	45	33	--	12	3:1	0.07
	Gty	46	36	--	10	3:1	0.26

[1]aa = dominant or homozygous fast, ab = heterozygous, bb = recessive or homozygous slow.

*Indicates deviation from expected ratios was significant at $P<0.05$.

Table 2. Joint segregation analysis of loci on chromosome 6

Cross / Loci	Number of progeny with designated phenotype[1] a/a	a/h	a/b	h/a	h/h	h/b	b/a	b/h	b/b	χ^2	Rec. frac.	SE
344001 x 169604												
Pl:Gty	17[P]	--	3	--	--	--	1	--	5[P]	10.1**	16	19
Pl:Gpi-c	4[P]	13	3	--	--	--	1	1	4[P]	7.4*	25	11
Gty:Acp-4	1	3	4[P]	--	--	--	5[P]	3	1	9.7**	15	8
Gty:Gpi-c	5[P]	12	1	--	--	--	0	2	6[P]	12.7**	11	8
Gty:Wlo	18[P]	--	0	--	--	--	4	--	4[P]	10.6**	17	19
Wlo:Acp-4	2	16	4[P]	--	--	--	4[P]	0	0	15.8***	8	6
Wlo:Gpi-c	5[P]	14	3	--	--	--	0	0	4[P]	11.9**	13	8
Acp-4:Gpl-c	0	0	7[P]	2	15	1	5[P]	1	0	40.3***	7	3
344001 x A1078-239												
Wlo:Gty	10[P]	--	2	—	--	—	0	--	5[P]	10.1**	11	24
Gty:Gpi-c	2[P]	8	0	—	—	—	0	1	6[P]	13.3**	5	5
Wlo:Gpi-c	2[P]	8	2	—	—	—	0	1	4[P]	7.3*	16	12
JI12 x 227258												
Wlo:Acp-4	4	19	9[P]	--	—	—	10[P]	2	0	18.6***	13	6
Wlo:Prx-3	5[P]	22	5	—	—	—	0	4	8[P]	13.5**	20	8
Wlo:Gpi-c	9[P]	17	6	—	—	—	0	2	10[P]	19.4***	17	8
Acp-4:Prx-3	1	5	3[P]	2	17	2	10[P]	4	0	21.6***	19	5
Acp-4:Gpi-c	0	2	7[P]	2	17	2	14[P]	0	0	55.5***	7	3
Prx-3:Gpi-c	10[P]	2	1	6	16	4	0	1	4[P]	24.0***	19	5
Slow x 227258												
Acp-4:Gpi-c	0	0	9[P]	1	11	1	8[P]	0	0	49.5***	3	2
Gpi-c:Prx-3	5[P]	3	0	5	1	2	0	2	6[P]	13.0**	25	8
Acp-4:Prx-3	0	2	6[P]	5	1	2	5[P]	3	0	13.0**	25	8
A73-91 x 227258												
Acp-4:Gpi-c	0	2	11[P]	0	13	0	6[P]	0	0	55.5***	3	2
Gpi-c:Prx-3	5[P]	0	1	5	8	1	0	2	5[P]	17.6**	20	6
Acp-4:Prx-3	0	4	5[P]	5	6	1	5[P]	0	1	14.4**	24	7
429839 x 227258												
Acp-4:Gpi-c	0	3	32[P]	2	52	2	22[P]	1	0	183***	4	1
Gpi-c:Prx-3	14[P]	4	0	11	22	10	3	7	19[P]	38.6***	23	4
Acp-4:Prx-3	5	7	18[P]	10	22	11	13[P]	4	0	32.1***	26	4
220189 x 343980												
Acp-4:Prx-3	6	5	16[P]	5	50	6	16[P]	8	4	59.6***	22	3
Slow x JI1794												
Pl:Gty	15[P]	--	4	--	--	--	3	--	3[P]	1.9	none	
Pl:Prx-3	0	11	4[P]	--	--	--	3[P]	3	0	18.0***	36	13
Pl:Acp-4	5	12	2[P]	--	--	--	3[P]	3	0	1.6	none	
Gty:Prx-3	5[P]	10	1	--	--	--	0	3	3[P]	6.6*	19	9
Acp-4:Prx-3	0	3	3[P]	4	9	0	1[P]	2	1	8.2	none	
Gty:Acp-4	3	13	5[P]	--	--	--	5[P]	0	1	10.5**	20	9
Combined												
Pl:Gty	32[P]	--	7	--	--	--	4		8[P]	10.5**	24	13
Acp-4:Prx-3	7	13	22[P]	11	76	8	27[P]	14	6	78.9***	23	2
Gpi-c:Prx-3	29[P]	10	1	18	46	12	4	13	28[P]	75.4***	22	2
Gty:Acp-4	4	26	9[P]	--	--	--	10[P]	3	1	18.4***	17	6
Gty:Gpi-c	7[P]	20	1	--	--	--	0	3	12[P]	24.7***	8	5
Wlo:Gty	28[P]	--	6	--	--	--	0	—	9[P]	21.2***	14	14
Acp-4:Gpi-c	0	7	66[P]	7	108	6	55[P]	2	0	383***	4	1
Wlo:Acp-4	6	35	13[P]	--	--	--	14[P]	2	0	31.8***	11	4
Wlo:Gpi-c	16[P]	39	11	--	--	--	0	3	18[P]	37.5***	16	5

[1]a = dominant or homozygous fast, h = heterozygous, b = recessive or homozygous slow.
P designates parental genotype.
* P<0.05, ** P<0.01, *** P<0.001.

Joint segregation analysis indicated that the two isozyme loci, as well as Gty, were linked to markers on chromosome 6 (Table 2). The data should be interpreted with some caution because all of the crosses with lines containing the rare GPI-2 variant (lines PI344001 and PI277258) showed sterility in some F_2 plants. This sterility may indicate that there exist chromosomal rearrangements between these lines (originally collected as P. sativum ssp. elatius) and the alternate parents used. However, tight linkage was found between Gpi-c and Acp-4 in each of these crosses and the location of Acp-4 on chromosome 6 was confirmed in the Slow x JI1794 progeny. We believe that any chromosomal rearrangements in the elatius parents did not seriously disrupt the linkage relationships on chromosome 6.

In a previous publication (3) the second author suggessted that Gpi-c might be located on chromosome 3 because of absolute linkage between it and Lap-1 in a progeny from a cross between a wild accession and a cultivated type. This result is now believed to be due to the parents differing for a reciprocal translocation involving chromosomes 3 and 6. Marx (2) presented data indicating that Gty was on chromosome 3. The data consisted of weak linkage (>25 recombination units) to Wel, B, and St, and strong linkage to Er (2). Our results consistently place Gty between Wlo and Pl on chromosome 6, although there is considerable between-progeny variation for the recombination frequencies. Furthermore, Gty did not show linkage with any of the 12 chromosome 3 markers segregating in the cross Slow x JI1794 (data not presented). If Gty is on chromosome 6, it is possible that Er also is on this chromosome.

For several crosses relatively few plants were available for analysis. In order to obtain a more robust estimate of the linkage values between marker loci we combined data from several crosses when homogeneity tests indicated such action was possible. Only two joint segregation data sets, those involving the: Prx-3 locus in the Slow x 227258 progeny, did not pass the homogeneity test and had to be excluded from the combined analysis. The combined data are presented in the last section of Table 2. Our best concept of the linkage relationships among the loci studied is given below.

Wlo--- 11 ---Acp-4-- 4 --Gpi-c--- 15 ---Gty--- 15 ---Prx-3---- 20 ----Pl

1. Blixt, S. 1974. In Handbook of Genetics, Vol. 2, Ed. R.C. King, Plenum Press, New York, pp. 181-221.
2. Marx, G.A. 1971. PNL 3:18-19.
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4. Weeden, N.F. and G.A. Marx. 1984. J. Hered. 75:365-370.
5. Wolko, B. 1989. PNL 21:80-81.