A gene for stem fasciation is localized on linkage group III

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Fasciation is one of the most widespread abnormalities of higher plant development. An understanding of the inheritance of the trait is very important, not only for theoretical purposes dealing with genetic control of meristem activity but also for practical use. Stem and fruit fasciation is used as an agriculturally valuable trait in selection of many species including pea (*Pisum sativum* L). The peculiarities of genetic control of fasciation in pea are still being discussed. There are few genes responsible for fasciation development; these genes form the *fasciata* family although little is known about their structure, protein products and even localization on the genetic map. The gene *Fa* (or *Fa1* as was proposed by Święcicki and Gawłowska (8)) is localized in linkage group IV (4), *Fa2* is in LG V (8) and *Fas* is supposed to be associated with LG III (1).

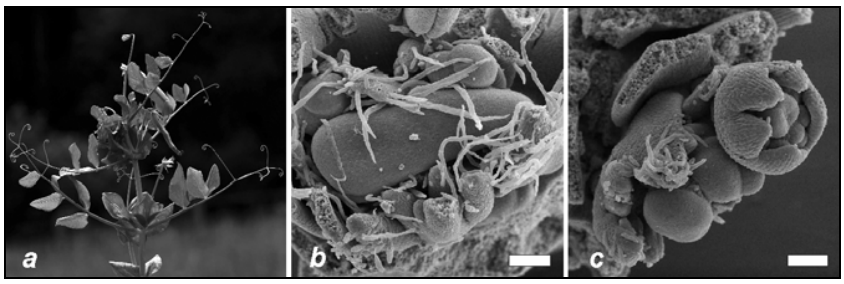

Fig. 1. Fasciated plant of "Shtambovy" mutant line (a) and scanning electronic microphotographs depicting stem apical meristems of "Shtambovy" mutant (b) and wild type plant (Nemchinovsky cultivar, c). Scale bar = 100 μ.

Table 1. Analysis of segregation at single loci in an F_2 population. A – homozygote as the first parental line ('Shtambovy'), B – homozygote as the second parental line (WL1238), H – heterozygote, N – total number of plants analyzed.

Loci	A	H	B	N	χ^2 (P>0.05)
Egl1	27	58	29	114	0.11
PK4	25	32	25	82	3.95
Pepcn	18	35	23	76	1.13
Le	91		27	118	0.28
Fas	90		30	120	0.00

The fasciated mutant 'Shtambovy' was produced by induced chemical mutagenesis (ethylmethane sulfonate) from the cultivar 'Nemchinovsky' (6). This mutant exhibits strong features of fasciation such as stem flattening, phyllotaxis abnormalities, clustering of axillary racemes on top of the stem, etc. (Fig. 1a). Such phenotype is connected with stem apical meristem enlargement which can be seen with usage of scanning electron microscopy. The apex of mutants becomes ridge-like (Fig. 1b) instead of hemispheric in wild-type plants (Fig. 1c) thus producing ribbon-like stem with multiple bundles and a striated surface. The morphology, anatomy and growth characteristics of fasciated plants compared with normal ones have been previously described (7).

The fasciation in a new mutant line is caused by a recessive mutation in a single gene (see Table 1). Allelism tests revealed that the gene responsible for fasciation in 'Shtambovy' is not allelic to gene *Fa* from JI 5 ('Mummy Pea'): all F_1 plants from cross 'Shtambovy' x JI 5 were non-fasciated.

In order to determine the possible relationship between 'Shtambovy' mutation and genes *Fas* and *Fa2*, an effort was made to localize the new *fasciata* locus on the pea linkage map. The F_1 and F_2 progeny of a cross 'Shtambovy' x WL

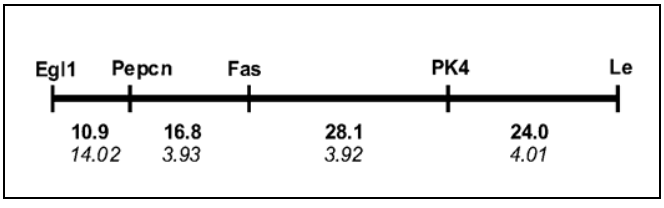

Fig. 2. Region of LG III containing gene Fas. Top numbers are genetic distances (cM), bottom numbers (in italics) are meanings of LOD score.

1238 were planted in the field. All F_1 hybrids were monomorphic and exhibited a non-fasciated phenotype. In the second filial generation the genetic analysis was performed involving the trait of interest and morphological markers carried by parental lines. According to some previous data (not shown) the gene of interest appeared to be associated with linkage group III. In order to check this hypothesis PCR-based CAPS markers (Cleaved Amplified Polymorphic Sequences) distributed across LG III were tested for linkage with the gene of interest. Primer sequences and reaction conditions were as described earlier (2, 3). The polymorphism was revealed by digestion of PCR products with restriction endonucleases *Tru*9I (for *PK4*), *Rsa*I (for *Pepcn*) and *Alu*I (for *Egl1*). F_2 segregation data was processed using the program Mapmaker/EXP 3.0 (5). The logarithm of odds (LOD) threshold for the linkage estimation was set at 3.0; the recombination frequencies were converted to map distances in cM using the Kosambi mapping function. The chi-square values for all marker pairs are presented in Table 2.

Table 2. Segregations and joint chi-square values for the selected loci in F_2. A – homozygote as the first parental line ('Shtambovy'), B – homozygote as the second parental line (WL1238), H – heterozygote, C – dominant phenotype like in the second parental line, N – total number of plants analyzed.

Loci	Classes in segregation						N	Joint χ^2
	CH	CA	CB	AH	AA	AB		
Fas-Le	70		19	21		7	117	0.71
***Fas-Egl*1**	44	10	24	9	14	0	101	67.14
Fas-Pepcn	28	5	17	5	8	0	63	38.36
***Fas-PK*4**	23	8	17	7	16	3	74	122.81

We found significant linkage between the gene responsible for *fasciata* phenotype in 'Shtambovy' and CAPS markers from the bottom part of linkage group III. According to results the map of region containing this gene was constructed with morphological marker *Le* included (although the latter shows no linkage with *fasciata* gene in this cross).

As *Fas* is the only known *fasciata* gene associated with LG III, we propose that the gene causing fasciation in the 'Shtambovy' mutant is identical to *Fas*. More investigations on this point are needed including additional allelism tests. Regardless of the outcome of these tests, the new mutation can be used as an additional morphological marker in LG III and may provide new information concerning genetic control of stem development in pea.

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