Relationship between different fasciated lines of pea

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Introduction

Although data on genetic control of fasciation in pea (*Pisum sativum* L.) appeared together with genetics itself (5), some aspects of it remain unclear. Until recently, even the number of genes involved in the development of this trait was under discussion: the hypothesis of monogenic control (5) was in controversy with one proposing the existence of two polymeric genes (2). At present, the former seems more probable, being supported by numerous experiments (4). The *Fa* locus proposed to be identical to that studied by Mendel, is localized on linkage group (LG) IV (2). Nevertheless, a few additional genes are known to cause fasciation: *Fas* (LG III, 1), *Fa2* (LG V, 12), *Nod4* (LGV, 8), and *Sym*28 (no linkage data, 7).The two latter also take part in the nodulation process.

Currently, a wide range of fasciated mutants, lines, recombinants and cultivars of pea are known. Their origin in some cases is unclear and samples from different germplasm collection may have different designations, obscuring the genetic relationship between them. For this reason, the relation between different *fasciata* lines needs to be investigated to finally determine the number of genes influencing this character, and to avoid synonyms. In addition, further study of fasciation in pea is needed, not only for practical purposes (some highly productive fasciated pea cultivars exist, reviewed in (11)) but also to solve the fundamental problem of genetic control of stem apical meristem (SAM) in higher plants.

Materials and Methods

The following fasciated pea lines from the collection of Genetics Department of Moscow State University (MSU) served as the plant material for the current study: 'Shtambovy' mutant originating from 'Nemchinovsky' cultivar via ethylmethane sulfonate treatment (6), 'Rosacrone' cultivar (provided by Vavilov Institute of Plant Industry, St. Petersburg, Russian Federation), 'Lupinoid' line from All-Russia Research Institute of Legumes and Groats Crops (Orel, Russian Federation), lines from John Innes Collection (Norwich, UK) (JI 5, JI 2671, JI 2771), and P 64 (*sym*28) from the collection of All-Russia Research Institute of Agricultural Microbiology (Pushkin, Russian Federation). The lines and F_1 hybrids were planted in open field conditions of Skadovskii Biological Station of MSU (Zvenigorod, Moscow District). Quantitative trait measurements were taken on growing plants and then processed with usage of Statistica 6 software package (Statsoft, Inc., Tulsa, OK, U.S.A.). The following characteristics were compared: number of the first node with clustered leaves (the phyllotaxis abnormalities reflect stem apical meristem size, 9) and width of internode preceding the node of flower initiation (NFI).

PCR-based microsatellite markers were used to confirm the hybrid origin of some F_1 plants (when no morphologic markers' segregation confirmed it). The primer sequences and conditions of PCR were chosen according to those described in Lorindon et al. (3). The restriction products were separated via electro-phoresis in agarose gel (Amresco) and then stained with ethidium bromide and visualized under UV-light.

Results and Discussion

Table 1 represents the results of allelism tests based on the phenotype of F_1 hybrids of clearly confirmed origin (Fig. 1). It is evident that three genes are responsible for fasciation inheritance in the lines. Studied lines 'Shtambovy' and JI 2771 are mutants at a gene on LG III (10) which is the only known *fasciata* gene on LG III and thus needs to be designated *Fas*, as has been discussed in work cited previously. Currently, however, line JI 2771 is designated as *fas-2* (Catalogue of *Pisum* Genetic Stock in John Innes Centre, http://www.jic.ac.uk/germplas/pisum/pgs2.txt), which seems improper.

Table 1. Results of allelism test-crosses between the lines examined.

	Shtambovy	Rosacrone	Lupinoid	JI 5	JI 2671	JI 2771	P 64
Shtambovy							
Rosacrone	n/a						
Lupinoid	n/a	a					
JI 5	–	a	–				
JI 2671	–	a	–	–			
JI 2771	a	n/a	–	–	n/a		
P 64	n/a	n/a	–	–	–	–	

Key: a, allelic; n/a, non-allelic; dash, cross not made or data absent.

Lines 'Rosacrone', 'Lupinoid', JI 5 ('Mummy Pea'), and JI 2671 are all allelic. The fasciation in them is caused by gene *Fa* localized on LG IV, as JI 5 ('Mummy pea', syn. WL 6) is regarded as type line for *fa* (11) identical to one described in Mendel's work (2, 5). Line JI 2671 also needs to be designated as *fa* instead of *fas*.

Line P 64 shows no allelism with any of the other mutants, but rather is homozygous at gene *sym*28 as stated in (7).

The study of quantitative traits in fasciated lines provides additional data confirming the relationship between fasciated lines (Fig. 2). The two *fas* forms are characterized with strongly expressed fasciation resulting in development of widely flattened main stem and phyllotaxis distortions, which can be clearly seen even in seedlings. Usually two or three leaves form in the third node, i.e. true (not scalar cataphylls) leaves exhibit abnormalities in their arrangement. In contrast, *fa* lines are weakly fasciated and features of stem flattening and clustering of leaves can be seen only at late stages of development. The line 'Lupinoid' has unusual leaf arrangement: the formation of leaf whorls is usually observed on the first nodes and then on 10-11th (and more). Such enhancement of fasciation expression can be explained by existence of modifying genes altering manifestation of *fa* in different recombinants. The stem and leaf arrangement in the P64 line (*sym*28) are also weakly affected. Such differences were seen even during observations in the very dry summer of 2007 when all features of fasciation were expressed weaker then usual due to drought stress.

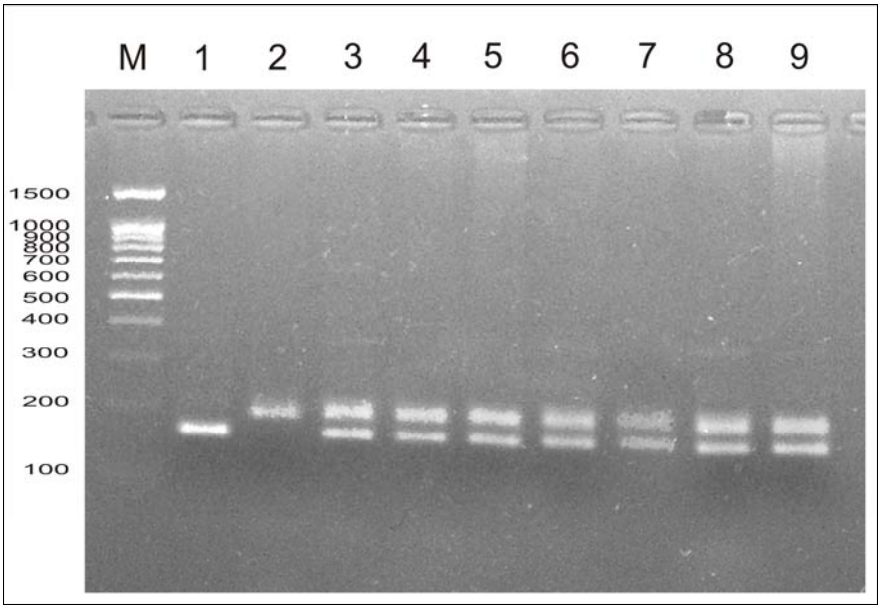

Fig. 1. Confirmation of hybrid origin of F_1 plants from cross between 'Shtambovy' and JI 2771 via amplification of AA122 microsatellite marker. Key: M, marker of molecular weight (100 bp + 1.5 Kb, Sibenzyme); 1, 'Shtambovy'; 2, JI 2771; 3-9, spectra of individual F_1 hybrids.

In conclusion, fasciation for the lines studied is produced by three independent genes, and its manifestation in different genotypes is phenotypically distinguishable. Certain changes in the designation of type lines are recommended. Further investigations on gene interactions including analysis of F_2 and double mutants are needed to get more information on genetic control of SAM development in pea and higher plants in general.

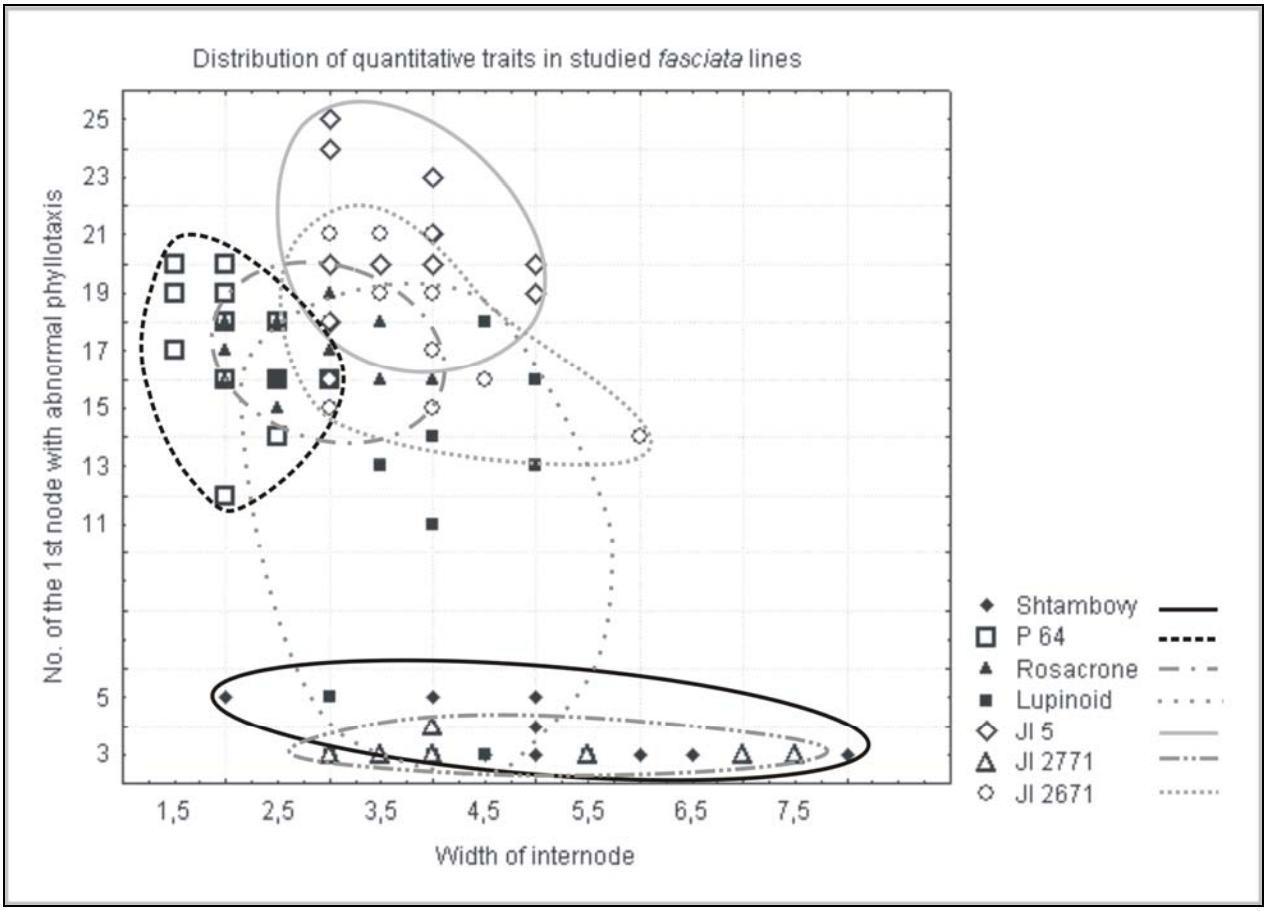

Fig. 2. Scatterplot distribution of quantitative traits in fasciated lines (explanation in text).

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