Sym28, a gene controlling stem architecture and nodule number, is localized on linkage group V

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Introduction

Garden pea *(Pisum sativum* L.) represents one of the most important models for studying plant developmental genetics. This species serves as a model object for most investigations on the genetic control of formation of compound inflorescence, compound leaf and of symbiotic interaction with nitrogen-fixing bacteria (nodulation). The latter phenomenon is typical for most legumes and is of significant theoretical and practical interest. By now, multiple genes involved in the genetic control of nodulation have been identified (for review see (2)).

One of the key processes in plant development is the regulation of stem apical meristem (SAM) activity. Studies on the model plant *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) demonstrated that equilibrium between SAM proliferation and keeping its volume stable is reached via the *CLAVATA-WUSCHEL* regulatory feedback loop. Expression of the gene *WUSCHEL* (WUS) maintains the meristematic condition of cells and activates expression of the *CLAVATA (CLV1, CLV2* and *CLV3)* gene family. CLV proteins negatively regulate expression of *WUS* thus limiting its expression pool (14). Mutations in *CLV* genes lead to abnormal enlargement of the *WUS*-expressing zone resulting in flower and stem fasciation. Genes of the *FASCIATA (FAS1* and *FAS2)* family also serve as negative regulators of *WUS* (5).

Although no functionally confirmed homologs of CLV, *FAS* or *WUS* genes were identified in pea previously, some data exist suggesting that the same genes are involved in both nodulation processes and control of SAM activity in legumes (10). In pea, there are two gene mutations which are known to cause stem fasciation and hypernodulation: *Nod4* (11) and *Sym28* (9). *Nod4* was localized earlier in linkage group (LG) V (11). There is also some evidence that *Sym28* also belongs to LGV (J. Weller, pers. comm.). Further dissection of the activity, interactions and expression of these genes may provide greater understanding of the link between SAM activity and symbiotic nodule formation. Major macrosynteny observed between the chromosomes of pea and alfalfa *(Medicago sativa* L.) (4) together with the availability of a nearly complete genome sequence of *Medicago truncatula* Gaertn. is expected to be helpful in identifying the candidate genes and their map-based cloning in pea.

Our current study was aimed at the genetic mapping of *Sym28* along with the putative pea homologs from *A. thaliana* for *CLV2* and *FAS1,* which can be considered as candidate loci responsible for the fasciation trait. A brief characteristic of these fragments is presented in Table 1. The allelism test between *nod4* and *sym28* mutants was also performed in order to clarify the relationships between these genes.

Table 1. Putative pea homologs of CLV2 and FAS1 used as markers.

Fragment	Length, b.p.	Nucleotide sequence homology with *Arabidopsis*	Translated sequence homology*
PsCLV2	835	92% ($2 * 10^{-140}$)	87%
PsFASI	933	67% ($3 * 10^{-9}$)	71%

, synonymic replacements left out of account. E- value for homology to Arabidopsis nucleotide sequences is presented in parentheses.

Materials and Methods

Plant material and growth conditions

The following lines were used as material for this study: K301 *(nod4)* from the Institute of Cytology and Genetics (Novosibirsk, Russia) collection; P64 *(sym28)* from the All-Russia Research Institute of Agricultural Microbiology (Pushkin, Russia) collection and marker line WL1238 from the Genetics Dept. of Moscow State University (MSU) collection. Lines and filial progeny were planted in an experimental field on a territory of S.N. Skadovskii Biological Station of MSU (Moscow District, Russia) in 2007-2008 and also in greenhouse conditions. In total, 158 F_2 plants from the cross P64/WL1238 were examined for morphological traits and 87 were sampled for further DNA analysis. This sample included all fasciated recombinants together with a group of non-fasciated plants.

DNA extraction, PCR conditions and genetic mapping

DNA was extracted from freshly collected leaflets of parental lines and individual F_2 progeny from the cross P64/WL1238 using a modified CTAB procedure (12). PCR was carried out in a MC2+ thermal cycler (DNA Technology, Russia) according to the protocol described earlier (6). Both morphological *(gp,* te, r, tl) and molecular (see Table 2) markers of LGV were used to localize gene *Sym28* more precisely. Two of the CAPS markers used in our study, *PsCLV2* and *PsFAS1,* represent putative pea homologs of the genes *CLV2* and *FAS1* of *A. thaliana* respectively (unpublished data) thus being candidate genes for the fasciated phenotype in P64. The CAPS markers have been tested for polymorphism between parental lines using 12 restriction endonucleases: *Tru*9I, *Taq*I, *Rsa*I, *Hae*III, *Alu*I, *Bst*FNI, *Hpa*II, *Hinf*I, *Psp*N4I, *Bst*DEI, Bst4CI, and AspLEI (SibEnzyme, Russia). *PsCLV2* turned out to be monomorphic in the cross P64/WL1238 (data not shown). Distances between markers were calculated using Mapmaker/EXP 3.0 software package using the Kosambi mapping function and LOD score threshold set at 3.0 (7).

Table 2. Characteristics of molecular markers used for mapping.

Marker / Endonuclease*	Genbank entry	Primer sequences	Marker type	Source
PsCLV2 / -	DQ478949	F: 5'- CCTGAGAGTTTRCTKTATTTGAAGTC- 3' R: 5'- GAGAAAGATACYTGAGGTTKGWCC- 3'	CAPS	This study
PsFAS1 / TaqI	DQ480717	F: 5'- CGTTGTCKAAGCTTGTWGATG- 3' R: 5'- AGCTTCWCWTCTATTTYTMTCC- 3'	CAPS	This study
Fbpp / Hpal 1	L34806	F: 5'- CCTTACTCTCCTTCACGTCT- 3' R: 5'- CTTTTCAACCTTCTCCACCT- 3'	CAPS	(3)
AD79	-	F: 5'- ACAAGACTTCCAGAAATTTTGCAT- 3' R: 5'- AGGACTGATGACGGAGACAAAG- 3'	SSR	(8)
AD175	-	F: 5'- CTTGTGCAGAAGCATTTGATTA- 3' R: 5'- AGAGACAATGGATGCTCATAGT- 3'	SSR	(8)

, for CAPS markers only. Note: in degenerate primer sequences R stands for A or G, K for G or T, W for A or T, Y for C or T.

Results and Discussion

Allelism tests

Sym28 has been shown to have significant linkage with markers of LGV (see below; J. Weller, pers. comm.); therefore, it was proposed that *sym28* may be allelic to another mutation causing both stem fasciation and hypernodulation, *nod4,* since *nod4* is known to be on LGV (11). To test this, the cross was made between lines P64 and K301. These lines possess somewhat different manifestation of fasciation. Phyllotaxis abnormalities are observed beginning from node 16.65 ± 4.61 in P64 and from 3.82 ± 0.88 in K301 (mean ± standard deviation). Axillary flower-bearing axes terminate with an abnormal flower in K301 (resembling ectopic flowers described in (1), while no such phenomena were observed in P64. This evidence supports the idea that fasciation is caused by different mutations in these lines and this hypothesis was confirmed by allelism tests: all F_1 progeny were non-fasciated thus evidencing for different genetic mechanisms underlying the described abnormalities.

Mapping of Sym28

Significant linkage was found between *Sym28* and LGV markers. Based on the linkage data, a map fragment surrounding *Sym28* was constructed (Fig. 1b). The arrangement of sequence-tagged markers (CAPS) in the resulting map was compared with the positions of putative homologous loci (according to BLAST search results) on the physical map of *Medicago truncatula* Gaertn. chromosome 7 (Fig. 1c) which was reported to be syntenic with LGV of pea (4). The obtained map was also compared with the one containing *Nod4* and morphological markers, as reported in (11). Map position of genes *Sym28* and *Nod4* was similar. Obviously, more linkage analyses are needed to reveal a more certain position of *Nod4* in relation with *Sym28* and possible correspondence between the genes studied in this report and known genes controlling meristem identity in *Arabidopsis.*

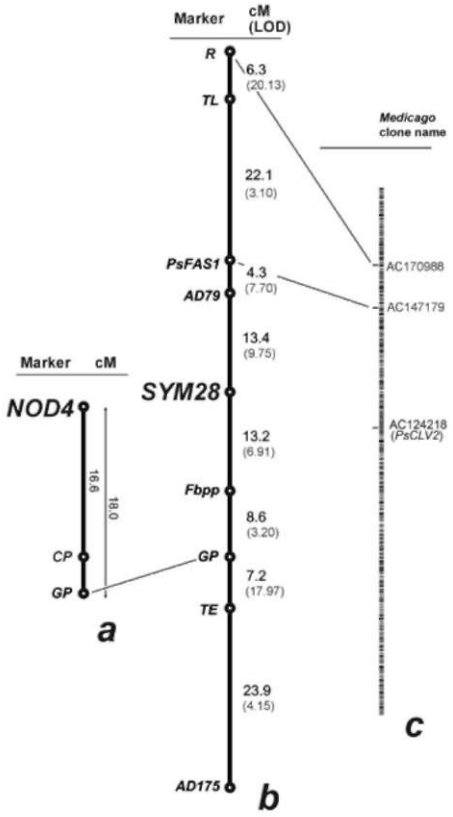

Figure 1. Correspondence between regions of pea LGV containing genes nod4 (a, after (11)), sym28 (b) and a physical map of chromosome 7 of Medicago truncatula (c) generated by Medicago CviT- BLAST tool (http://www.medicago.org/genome/cvit_blast.php). Chromosome orientation was established according to (13). Numbers between markers designate distances, cM; numbers in parentheses stand for LOD score.

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