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Genetic control of inflorescence development in pea

By

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Frances Sussmilch

Abstract

Angiosperm species exhibit incredible diversity in inflorescence architecture. Legumes comprise the third largest family of flowering plants, second only to the grasses in terms of agricultural importance. Several important crop legumes, including pea, lentil, common bean and chickpea, share a characteristic inflorescence form, the compound raceme, which has one extra level of inflorescence branching, the secondary inflorescence (I_2), relative to the simple raceme of *Arabidopsis*. Historically, pea has been a popular model legume for studies of flowering, often through the characterisation of flowering and inflorescence mutants. In this study, pea genes with an apparent or putative role in inflorescence development were investigated, in order to improve understanding of the genetic control of inflorescence development in pea.

Unlike *Arabidopsis*, where no single gene mutant has a non-flowering phenotype, mutations at any of three pea loci can prevent flowering: *GIGAS/FTa1*, *VEG1/FULc* and *VEG2*. In this study, the roles of *VEG2* during inflorescence development were investigated using two mutant alleles: the non-flowering *veg2-1* mutant, and the late-flowering *veg2-2* mutant. The results indicate that *VEG2* is important for the correct timing of the inflorescence transition, initial specification and maintenance of I_2 identity, and specification of floral meristems, under both LD and SD conditions.

Preliminary mapping results indicated a pea homolog of *FD* as a candidate for the *VEG2* locus. In this study, the legume *FD* gene family was characterised and the *VEG2* locus was shown to correspond to *FDa*. In the *veg2-1* mutant, the entire coding sequence was found to be deleted but putative flanking genes were unaffected. The *veg2-2* mutant was shown to contain a single nucleotide polymorphism (SNP), affecting a highly conserved amino acid within the DNA-binding, basic region of the bZIP domain.

The mechanisms of *FDa* action were further investigated through analysis of expression patterns of *FDa* and protein interactions with the pea FT and TFL1 homologs. *FDa* was found to be expressed in the wild-type apex throughout development. FDa protein was found to be capable of interacting with all five pea FT homologs, and DET (TFL1a), but not LF (TFL1c). Flowering genes regulated (either

directly or indirectly) by *FDa* were identified based on misregulation of expression in the *veg2* mutants. These included pea homologs of *FT*, *TFL1* and *LFY*, in addition to a range of MADS-box genes.

The *late5* mutant is a previously undescribed EMS-induced mutant that exhibits phenotypic similarity to *veg2-2*. To determine the role of *LATE5* during pea inflorescence development, the *late5* phenotype was characterised. The genetic interactions between *LATE5*, *DET* and *LF* were investigated through the phenotypes of double and triple mutants. The molecular roles of *LATE5* were also investigated by examining the effects of the *late5* mutation on expression of flowering genes. The map position of *LATE5* was refined to a region of less than 3.2cM towards the base of pea linkage group I, corresponding to a syntenic region of 0.6Mb containing 95 annotated genes in *Medicago*.

The legume family of *SVP*-like genes, which have important roles in flowering time, inflorescence branching and floral meristem identity in other species, was characterised and two new *SVP*-like genes (*SVPb* and *SVPc*) were isolated from pea. Investigation of expression patterns of pea *SVPa*, *SVPb* and *SVPc* genes, revealed developmental regulation of *SVPc* in wild-type pea, and misregulation of *SVPc* in the *veg2-2* mutant indicating regulation of *SVPc* (directly or indirectly) by *FDa/VEG2*.

Overall, the findings of this study make a significant contribution to knowledge of the genetic control of inflorescence development in pea.

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Abbreviations

ABA	Abscisic acid
AF	*AFILA*
AG	*AGAMOUS*
AGL24	*AGAMOUS-LIKE24*
AGL79	*AGAMOUS-LIKE79*
ALOG	*Arabidopsis* LIGHT-SENSITIVE HYPOCOTYL 1 *Oryza* GI
AN	*ANANTHA*
AP1	*APETALA1*
AP2	*APETALA2*
AP3	*APETALA3*
APO1	*ABERRANT PANICLE ORGANIZATION1*
AREB3	ABA-Responsive Element Binding protein 3
ATC	*ARABIDOPSIS THALIANA CENTRORADIALIS HOMOLOG*
BiFC	Bimolecular fluorescence complementation
bp	Nucleotide base pairs
BRC1	*BRANCHED1*
bZIP	Basic region leucine zipper
CAL	*CAULIFLOWER*
CAPS	Cleaved amplified polymorphic sequence
cDNA	Complementary DNA
CDS	Coding sequence
CEN	*CENTRORADIALIS*
CETS	*CENTRORADIALIS/TERMINAL FLOWER 1/SELF-PRUNING*
CO	*CONSTANS*
cv.	Cultivar
DAM	Dormancy-associated MADS-box
dbEST	GenBank Database of Expressed Sequence Tags
dCAPS	Derived cleaved amplified polymorphic sequence
DET	*DETERMINATE*
DFCI TGI	Dana Farber Cancer Institute Gene Indices
DJC23	*DNA J PROTEIN C23*
DJC24	*DNA J PROTEIN C24*
DLF1	*DELAYED FLOWERING1*
DNA	Deoxyribonucleic acid
DNE	*DIE NEUTRALIS*
DPBF4	*Dc3 promoter-binding factor 4*
DTF	Plant age at first open flower
EMS	Ethyl methanesulfonate
FA	*FALSIFLORA*
FDP	*FD PARALOG*
FLC	*FLOWERING LOCUS C*

FT	*FLOWERING LOCUS T*
FUL	*FRUITFUL*
gDNA	Genomic DNA
gi	*gigas*
Hd3a	*Heading date 3a*
I_1	Primary inflorescence
I_2	Secondary inflorescence
INCO	*INCOMPOSITA*
J	*JOINTLESS*
KAL	Kaliski
kb	1000 nucleotide base pairs
LARP1C	*LA RELATED PROTEIN 1C*
LATE1	*LATE BLOOMER 1*
LATE5	*LATE BLOOMER 5*
LB	Lurio-Bertoni media
LD	Long day photoperiod
LF	*LATE FLOWERING*
LFY	*LEAFY*
LG	Linkage group
LSH3	*LIGHT-DEPENDENT SHORT HYPOCOTYLS 3*
LSH4	*LIGHT-DEPENDENT SHORT HYPOCOTYLS 4*
LUX	*LUX ARRHYTHMO*
Mb	1,000,000 nucleotide base pairs
MC	*MACROCALYX*
miR156	microRNA156
NFI	Node of flower initiation
PCR	Polymerase chain reaction
phyA	*phytochrome A*
PI	*PISTILLATA*
qRT-PCR	Quantitative reverse transcription PCR
QTL	Quantitative trait loci
RACE	Rapid amplification of cDNA ends
RAPD	Random amplified polymorphic DNA
RN	Reproductive node
RNA	Ribonucleic acid
RNAi	RNA interference
SAM	Shoot apical meristem
SD	Short day photoperiod
SDW	Autoclaved Milli-Q water
SE	Standard error
SEM	Scanning electron microscopy
SEP1	*SEPALATA1*
SEP2	*SEPALATA2*
SEP3	*SEPALATA3*
SEP4	*SEPALATA4*

SFT	*SINGLE FLOWER TRUSS*
SN	*STERILE NODES*
SNP	Single nucleotide polymorphism
SOC1	*SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*
SP	*SELF PRUNING*
SPGB	*SELF-PRUNING G-BOX*
SPL	*SQUAMOSA PROMOTER BINDING PROTEIN-LIKE*
SQUA	*SQUAMOSA*
SVP	*SHORT VEGETATIVE PHASE*
TAE	Tris acetate ethylenediamine tetra-acetic acid
TAIR	The Arabidopsis Information Resource
TER	Térèse
TFL1	*TERMINAL FLOWER1*
T_m	Optimal annealing temperature
TN	Total nodes
TSA	Transcriptome Shotgun Assembly
UFO	*UNUSUAL FLORAL ORGANS*
UNI	*UNIFOLIATA*
UTR	Untranslated region
VEG1	*VEGETATIVE 1*
VEG2	*VEGETATIVE 2*
V/I_1 transition	Transition of the SAM from vegetative to I_1 meristem identity
VRN1	*VERNALIZATION1*
WT	Wild-type
WUS	*WUSCHEL*
YFC	C-terminal half of yellow fluorescent protein
YFN	N-terminal half of yellow fluorescent protein
YFP	Yellow fluorescent protein
ZCN8	*Zea mays CENTRORADIALIS8*
ZFL1	*Zea mays FLO/LFY 1*