The *fa2* gene and molecular markers mapping in the *gp* segment of the *Pisum* linkage group V

M. Gawłowska și W. Święcicki

Received: 19 October 2015 / Revised: 21 December 2015 / Accepted: 23 December 2015 / Published online: 27 January 2016

© The Author(s) 2016. This article is published with open access at Springerlink.com

Abstract Review studies on the world *Pisum* genetic resources have shown that stem fasciation is controlled by three loci, i.e., *fa1* (LGIV; Wt 10006 - type line of the Polish Gene Bank), *fa2* (LGV, the line Wt 12185), and *fas* (LGIII, the line Shtambovii). Outstanding advantages of this character (e.g., pods gathered in upper part of a stem) resulted in breeding some cultivars. Preliminary investigations suggested linkages of the newly described *fa2* gene within the *gp*–*U* segment. Based on the further linkage test crosses, it was stated that the *fa2* is localized between the *gp* and *Pis_Gen_9_3_1* markers (in the LGV). Additionally, four molecular markers (AD175, AB146, AC58, and AD280) and the morphological marker *lk* were also localized in this segment. Moreover, *rms5*, *lum3*, and *cri* were found to map on the other side of *gp* with tight linkage observed between *lum3* and *cri*.

Keywords *Pisum* · LGV · Stem fasciation · Chromosome mapping · Gene markers

Introduction

Stem fasciation in peas appears to be a very interesting character from a theoretical as well as a practical point of view. This character not only changes the stem architecture but also the physiology of flowering and maturing (Fig. 1). Its advantage is that pods are gathered in upper part of a stem; but in consequence, pea plants lodge and are susceptible to drought during shortened flowering and pod formation periods. Outstanding advantages resulted in breeding some cultivars, for example, cvs. Buława (POL), Ornamenta, Rosacron, Golf (DEU) and Novella (USA). Pea fasciation was described for the first time in 1597 (Święcicki 2001, after Derbshire 1911), and since then different names have been used for its designation, such as the *Pisum umbellatum*, mummy pea, or crown pea. Furthermore, a taxon was separated in *Pisum* taxonomy, i.e., *P. sativum*, subsp. *sativum*, convar. *vulgare var. coronatum* (Lehmann and Blixt 1984).

Fasciation was one of the seven monohybrid characters studied by Mendel (Święcicki et al. 2000). Induced mutations resulted in a number of independent mutation cases with a similar phenotype in different genotypic backgrounds which are still maintained in world *Pisum* collections (e.g., USDA Pullman *Pisum* Genetic Stock Collection, John Innes Centre *Pisum* Collection, Wiatrowo *Pisum* Collection). Information on the character anatomy, morphology, and expression are available in several references such as Gottschalk and Wolf (1983), Marx and Hagedorn (1962) and Sinjushin and Gostimsky (2006), but different opinions are available on its mode of inheritance. It has been shown that this character is controlled by one to four independent genes or multiple alleles of a single locus (Marx and Hagedorn 1962; Blixt 1972; Lamprechtt 1974; Święcicki 2001). The most popular was the acceptance of two independent fasciata genes — *fa* in LG IV and *fas* in LG III (Lamprechtt 1974; Blixt 1977). Additionally, a similar mutation type, *dichotomous branching*, was selected and reported as a character governed by two polymeric genes *bif1* and *bif2* (Gottschalk and Wolf 1983); this alteration was associated with a fasciation of only a few upper nodes that results in a forked stem (Fig. 2). Results of subsequent complementation tests (locus identity test...
crosses) that explain the genetic basis of fasciata phenotype in pea lines from the Blixt’s, Gottschalk’s, Marx’s, Święcicki’s and Sinjushin’s collections were as follows (Święcicki 2001; Sinjushin et al. 2006):

- no typeline registered by Blixt (1977) exists in the main Pisum collections for the fas gene from LGIII; Sinjushin et al. (2006) and Sinjushin (2011) suggested that the two lines, JI2771 and mutant Shtambovii, have the fas gene,
- fasciation in most of the tested lines is controlled by the fa gene from LGIV,
- dichotomous branching appeared to be controlled by the allele in the fa locus (symbol fa1bif was suggested),
- an exception is the fasciation in the accession Wt 12185 as controlled by a gene different from the fa locus; for fa in LGIV, the symbol fa1 (and fa1bif) was suggested and fa2 for the new gene in the type line Wt 12185.

For usage of a stem fasciation in breeding, the results of Gottschalk (1979) and Święcicki (2001) are important, indicating that lines Gott37B (fa1bif) and Wt12185 (fa2) are characterised by a full penetrance of mentioned genes and an increased seed production.

Preliminary results have shown that the new fasciata gene, fa2, is linked with gp in linkage group V (LGV) (Cr0 = 17.6 ± 7.6) (Święcicki and Gąwłowska 2004). The aim of this study was to map fa2 using more markers from the gp region, including molecular markers.

Material and methods

For the purpose, the type line for the gene fa2 (Wt 12185) from the Polish Pisum Gene Bank at Wiatrowo was selected. For the linkage test crosses, this line was crossed to tester lines with LGV morphological markers, particularly from the gp region: Wt 10498: lum3, cri (cross number: K. 3365); Wt 15294: gp, rms5 (K. 3548 and K. 3048); Wt 15860: creep, ce (K. 3528); Wt 10287: lk (K. 3364); and Wt 11238: gp, cp1 (K. 3338). Phenotypic expression of the markers is shortly given below:

- **Gp–gp** = green–yellow color of pods (luteo-legumina mutation type, Blixt 1977)
- **Rms5–rms5** = plant with one stem–with many basal branches (ramosus, Apsisitwanich et al. 1992)
- **Creep–creep** = perpendicular–creeping stem growth (creeping, Blixt 1977)
- **Ce–ce** = anthocyanin/cardinal–cherry flower color (cerise, Blixt 1977)
- **Lk–lk** = normal–dwarf plant plus other complex changes (erectoides, Święcicki 1989)
- **Lum3–lum3** = interveinal leaf areas green–yellowish (costata, Święcicki 1988)
- **Cri–cri** = leaf and stipules surface smooth–crinkled (crispa, Blixt 1977)
- **Cp1–cp1** = pods straight–concavely curved (concavum, Blixt 1977)

Additionally, a segregation of molecular markers was analysed: Pis_Gen_9_3_1 in K. 3548, Pis_Gen_9_3_1,
The Pis_Gen_9_3 marker (the abbreviation Pis_Gen_9_3 is used in the text) is a sequence tagged site marker obtained in a framework of the EU Grain Legumes Integrated Project (2004–2008). The primer information is available on the following website: http://bioweb.abc.hu/cgi-mt/pisprim/pisprim.pl. A polymorphism was identified after sequencing [A/G, SNP 312 bp] and visualized after AsuI digestion. PCR was conducted as follows: 1× buffer Go-Taq flexi (Promega, Madison, WI, USA), 1.5 mM MgCl2 (Promega), 1 mM dNTP (ThermoFisher Scientific), primer 1 (0.2 μM), primer 2 (0.2 μM), 0.6 U GoTaq polymerase (Promega), and 25 ng DNA. PCR temperature profile TD 60–40 °C was used (Hecker and Roux 1996).

SSR primers were designed by the Pea Microsatellite Consortium, Agrogene, France, and used in mapping by Loridon et al. (2005). Five molecular markers were used showing a polymorphism in investigated populations and linkages with the investigated LGV (Pis_Gen_9_3; Święcicki et al. 2012 and AB146, AC58, AD175, AD280; Loridon et al. 2005). PCR was conducted as follows: 1× buffer Go-Taq flexi (Promega), 1.5 mM MgCl2 (Promega), 0.6 mM dNTP (ThermoFisher Scientific), primer 1 (0.2 μM), primer 2 (0.2 μM), 0.6 U GoTaq polymerase (Promega), and 25 ng DNA. PCR profile: 95 °C (2 min); 35× [95 °C (30 s)], required TM [AB146 (64 °C), AD175 (58 °C), AD280 (64 °C), AC58 (58 °C) (60 s)], 72 °C (60 s), and 72 °C (5 min).

The results of mono- and dihybrid segregations were calculated using a computer program based on the product-ratio method for linkage estimation (Święcicki et al. 1998). For graphic presentation of the loci order, the MapChart program was used (Voorrips 2002).

## Results and discussion

Mono- and dihybrid segregations in the F2 generation of seven populations were analysed by the fa2 gene and eight table 1

| Cross combination | Gene | Total chi square (3:1) |
|-------------------|------|-----------------------|
|                   |      | Dominant  | Recessive |
| K. 3365 Lum3      | 86   | 23        | 109       | 0.88   |
| K. 3365 Cri       | 86   | 23        | 109       | 0.88   |
| K. 3548 Fa2       | 152  | 42        | 194       | 1.16   |
| K. 3048           | 261  | 65        | 326       | 4.45   |
| K. 3528           | 194  | 49        | 243       | 3.00   |
| K. 3364           | 204  | 65        | 269       | 0.10   |
| K. 3365           | 84   | 22        | 106       | 1.02   |
| K. 3548 Gp        | 148  | 47        | 195       | 0.08   |
| K. 3365           | 243  | 82        | 325       | 0.01   |
| K. 3319           | 80   | 24        | 104       | 0.20   |
| K. 3338           | 102  | 40        | 142       | 0.76   |
| K. 3548 Rms5      | 148  | 47        | 195       | 0.08   |
| K. 3048           | 263  | 86        | 349       | 0.02   |
| K. 3528 Creep     | 199  | 54        | 253       | 1.80   |
| K. 3364 Lk        | 223  | 63        | 286       | 1.35   |
| K. 3338 Cp-1      | 85   | 32        | 117       | 0.34   |
| K. 3548 Pis_Gen_9_3| 49   | 19        | 68        | 0.31   |
| K. 3319           | 69   | 22        | 91        | 0.03   |
| K. 3338           | 86   | 36        | 122       | 1.32   |
| K. 3319 AD 175    | 63   | 23        | 86        | 0.14   |
| K. 3338           | 94   | 22        | 116       | 2.25   |
| K. 3319 AB 146    | 59   | 11        | 70        | 3.22   |
| K. 3338 AD 280    | 73   | 24        | 97        | 0.00   |

Linkage test crosses: K. 3548 = Wt 12185 × Wt 15294, K. 3048 = Wt 15294 × Wt 12 185, K. 3528 = Wt 12185 × Wt15860, K. 3364 = Wt 12 185 × Wt 10287, K. 3319 = Wt 3527 × Wt 11238, K. 3365 = Wt 12185 × Wt 10498, K. 3338 = Wt 15989 × Wt 11238
morphological and five molecular markers (Table 1). Preliminary results suggest that most of the selected markers originate from the \( Gp- Fa2 - U \) region (Świecińska and Gawłowska 2004). Markers \( lum3, cri, \) and \( cp-1 \) localized on the opposite side of \( gp \) (Blixt 1977; Świecińska 1988; Weeden et al. 1998) additionally should confirm a selection of appropriate chromosome region for the \( fa2 \) localization. And it appeared (Table 2, K. 3365) that for gene pairs \( Fa2- Cri \) and \( Fa2- Lum3 \), there were no deviations from correct dihybrid segregation and no linkages. A valuable result, hitherto not known, is the strong linkage revealed for \( Lum3- Cri \) (Cr-0 = 1.9).

Correct, monohybrid segregation for the \( fa2 \) and selected markers (Table 1) allowed us to analyse a dihybrid segregation to look for linkages and the \( fa2 \) locus (Table 2). In K. 3548 and K. 3048 populations

| Cross combination | Pair of genes | Phase | Phenotype | Total | Joint chi square | Cr-0 value ± SE (per cent) |
|-------------------|---------------|-------|-----------|-------|------------------|----------------------------|
| K.3365            | \( Fa2-Lum3 \) | R     | 60 20 19 3 106 1.3 | 40.4 ± 8.0 |
| \( Fa2-Cri \)     | R             | 60 20 19 3 106 1.3 | 40.4 ± 8.0 |
| \( Lum3-Cri \)    | C             | 86 0 0 23 109 105.6 | 1.9 ± 1.3 |
| K. 3548           | \( Fa2-Gp \)  | R     | 105 47 42 0 194 16.7 | 15.8 ± 6.9 |
| \( Fa2-Rms5 \)    | R             | 106 46 42 0 194 16.8 | 16.0 ± 7.0 |
| \( Fa2-Pis\_Gen\_9\_3 \) | C         | 48 9 1 10 68 26.7 | 10.8 ± 4.0 |
| \( Rms5-Gp \)     | C             | 144 4 4 43 195 159.1 | 4.2 ± 1.5 |
| \( Rms5-Pis\_Gen\_9\_3 \) | R         | 28 16 21 3 68 5.0 | 31.2 ± 10.8 |
| \( Gp-Pis\_Gen\_9\_3 \) | R         | 26 16 23 3 68 5.0 | 29.2 ± 10.9 |
| K. 3048           | \( Fa2-Gp \)  | R     | 179 81 64 1 325 23.2 | 12.9 ± 5.4 |
| \( Fa2-Rms5 \)    | R             | 183 78 63 2 326 20.3 | 18.5 ± 5.3 |
| \( Rms5-Gp \)     | C             | 234 11 9 71 325 229.6 | 6.3 ± 1.4 |
| K. 3528           | Creep- Fa2    | R     | 141 49 53 0 243 17.9 | 15.9 ± 6.2 |
| Creep- Ce         | C             | 117 27 17 20 181 17.3 | 29.1 ± 4.1 |
| \( Fa2-Ce \)      | R             | 96 43 37 4 180 7.9 | 30.7 ± 6.6 |
| K. 3364           | \( Fa2- Lk \) | R     | 147 57 65 0 269 23.6 | 13.8 ± 6.0 |
| K. 3319           | \( Gp-Pis\_9\_3 \) | C     | 62 6 5 16 89 41.2 | 13.4 ± 3.9 |
| \( Gp-AD175 \)    | C             | 59 4 2 19 84 53.0 | 6.9 ± 2.9 |
| \( Gp-ACS8 \)     | C             | 55 4 6 13 78 33.6 | 14.0 ± 4.3 |
| \( Gp-AB146 \)    | C             | 55 3 2 8 68 27.2 | 9.3 ± 3.7 |
| \( Pis\_Gen\_9\_3-AD175 \) | C    | 59 3 2 19 83 53.7 | 6.0 ± 2.7 |
| \( Pis\_Gen\_9\_3-AC58 \) | C    | 54 4 7 12 77 27.0 | 15.7 ± 4.6 |
| \( Pis\_Gen\_9\_3-AB146 \) | C     | 56 0 1 10 67 48.4 | 3.6 ± 2.3 |
| \( AD175-ACS8 \)  | C             | 54 2 7 14 77 41.5 | 10.7 ± 3.8 |
| \( AD175-AB146 \) | C             | 58 1 0 10 69 48.4 | 3.5 ± 2.2 |
| \( AC58-AB146 \)  | C             | 50 3 2 6 61 32.2 | 11.1 ± 4.3 |
| K. 3338           | \( Cpl-Gp \)  | C     | 74 10 8 24 116 47.6 | 15.9 ± 3.8 |
| \( Cpl-Pis\_9\_3 \) | C     | 63 10 10 21 104 31.9 | 19.9 ± 4.5 |
| \( Cpl-AD280 \)   | R             | 44 16 24 2 86 3.1 | 30.1 ± 9.7 |
| \( Cpl-ACS8 \)    | C             | 56 13 22 6 97 0.3 | 47.7 ± 7.4 |
| \( Gp-Pis\_Gen\_9\_3 \) | C    | 81 6 5 30 122 77.9 | 8.9 ± 2.7 |
| \( Gp-AD280 \)    | R             | 47 21 26 3 97 4.3 | 31.6 ± 9.0 |
| \( Gp-ACS8 \)     | R             | 70 15 24 7 116 0.3 | 45.7 ± 6.6 |
| \( Pis\_Gen\_9\_3-AD280 \) | R    | 44 22 27 2 95 6.7 | 25.1 ± 9.5 |
| \( Pis\_Gen\_9\_3-AC58 \) | C     | 71 9 21 12 113 10.3 | 30.4 ± 5.4 |
| \( AC58-AD280 \)  | R             | 52 20 15 1 98 3.7 | 26.8 ± 9.8 |

Joint segregation of gene pairs in chromosome 5 (K. 3365 = Wt 12185 × Wt 10498), K. 3548 = 12185 × Wt 15294, K. 3048 = Wt 15294 × Wt 12185, K. 3528 = Wt 12185 × Wt 15860, K. 3364 = Wt 12185 × Wt 10287, K. 3319 = Wt 3527 × Wt 11238, K. 3338 = Wt 15989 × Wt 11238
substantial deviations from a dihybrid segregation for most of the gene pairs were stated. Exceptions were as follows: rms5—Pis_Gen_9_3 and gp—Pis_Gen_9_3 with Cr-0 values of about 30. Taking into account Cr-0 values obtained from the K. 3548 population, the following loci order can be accepted: Gp/Rms5–Fa2–Pis_Gen_9_3. The supplemental analysis of the K. 3048 population allows us to accept the gene order presented in Fig. 3. Substantial deviations from correct dihybrid segregation and linkages for Fa2–Creep and Creep–Ce and no deviations for Fa2–Ce (K. 3528 population, Table 2) additionally confirm the fa2 localization and the presented loci order (see also consensus Pismum map, Weeden et al. 1998). A valuable supplement for this region is the linkage Fa2–Lk=13.8, revealed in the population K. 3364. Together with the earlier result for Gp–Lk=12.3 (Święcicki 1989), it is emphasized that the Lk gene is also localized in the investigated Gp–U region.

Supplemental linkage data in the above-mentioned region supply an analysis of K. 3319 and K. 3338 populations covering the Gp locus and five molecular markers: Pis_Gen_9_3, AD175, AB146, AC58, and AD280. Obtained results suggest the loci order given in Fig. 3.

Conducted analyses localized the new fa2 gene in the Gp–U segment of the LGV between Gp and Pis_Gen_9_3 markers. Four additional molecular markers (AD175, AB146, AC58, AD280) and morphological lk were also localized in this segment. Moreover, the locus rms5 and a strong linkage between lum3 and cri were found from the other side of the Gp locus.

Compliance with ethical standards

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.
Święcicki WK (2001) Supplemental data on fasciata genes in Pisum resources. Pisum Genet 33:19–20
Święcicki WK, Gawłowska M (2004) Linkages for a new fasciata gene. Pisum Genet 36:22–23
Święcicki WK, Wolko B, Kruszka K (1998) Linkages in Pisum. VII. Locus for the sterile gene calf (cabbage leaf). J Appl Genet 39:163–169
Święcicki WK, Wolko B, Weeden NF (2000) Mendel’s genetics, the Pisum genome and pea breeding. Vortrage Pflanzenzüchtg 48:65–76
Święcicki W, Gawłowska M, Bednarowicz M, Knopkiewicz M (2012) Localization of the common markers on the pea maps Wt10245xWt11238, CarnevalsMP1401 and P665xMessire (Pisum sativum L.). Sci Med 3(3):229–234
Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93(1):77–78
Weeden NF, Ellis THN, Timmerman-Vaughan GM, Swiecicki WK, Rozov SM, Berdnikov VA (1998) A consensus linkage map for Pisum sativum. Pisum Genet 30:1–4