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ORIGINAL PAPER

Wild peas vary in their cross-compatibility with cultivated pea (*Pisum sativum* subsp. *sativum* L.) depending on alleles of a nuclear–cytoplasmic incompatibility locus

V. S. Bogdanova · O. E. Kosterin · A. K. Yadrikhinskiy

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Abstract

Key message **Divergent wild and endemic peas differ in hybrid sterility in reciprocal crosses with cultivated pea depending on alleles of a nuclear 'speciation gene' involved in nuclear–cytoplasmic compatibility.**

Background In hybrids between cultivated and wild peas, nuclear–cytoplasmic conflict frequently occurs. One of the nuclear genes involved, *Scs1*, was earlier mapped on Linkage Group III.

Results In reciprocal crosses of seven divergent pea accessions with cultivated *P. sativum*, some alleles of *Scs1* manifested incompatibility with an alien cytoplasm as a decrease in pollen fertility to about 50 % in the heterozygotes and lack of some genotypic classes among F2 segregants. Earlier, we defined monophyletic evolutionary lineages A, B, C and D of pea according to allelic state of three markers, from nuclear, plastid and mitochondrial genomes. All tested representatives of wild peas from the lineages A and C exhibited incompatibility due to *Scs1* deleterious effects in crosses with testerlines of *P. sativum* subsp. *sativum* (the common cultivated pea) at least in one direction. A wild pea from the lineage B and a cultivated pea from the lineage D were compatible with the testerline in both directions. The tested accession of cultivated *P. abyssinicum* (lineage A) was partially compatible in both directions. The *Scs1* alleles of some pea accessions even originating from the same geographic area were remarkably different in their compatibility with cultivated *Pisum sativum* cytoplasm.

Conclusion Variability of a gene involved in reproductive isolation is of important evolutionary role and nominate *Scs1* as a speciation gene.

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V. S. Bogdanova · O. E. Kosterin (✉) · A. K. Yadrikhinskiy
Institute of Cytology and Genetics of Siberian Division of Russian Academy of Sciences, Acad. Lavrentyev ave. 10, Novosibirsk 630090, Russia
e-mail: kosterin@bionet.nsc.ru

O. E. Kosterin
Novosibirsk State University, Pirogova str. 2, Novosibirsk 630090, Russia

Introduction

Wild relatives of cultivated plants are widely acknowledged as valuable resources for crop improvement (Tanksley and McCouch 1997). The development of molecular–genetic approaches to plant breeding aided in introgression of genes or gene blocks from wild relatives and landraces into cultivars (McCouch 2004; Hajjar and Hodgkin 2007). At present, the major breeding interest is focused on the yield management, resistance to pests and pathogens and adaptation of cultures to global climatic changes (Fernie et al. 2006). Grain legumes as an important group of food and fodder crops are involved in pre-breeding programmes for genetic enhancement (Sharma et al. 2013). There is a variety of wild pea forms with large potential as donors of agronomically important traits such as resistance to fungi (Wroth 1998; Fondevilla et al. 2007, 2011), or pea weevil (Clement et al. 2002; Byrne et al. 2008; Aryamanesh et al. 2014).

Although crop wild relatives are an attractive source of valuable traits, the first attempts of interspecies cross-hybridization faced substantial problems associated with

poor compatibility between cultivars and their wild relatives such as hybrid sterility, reduced recombination of chromosomes and genetic linkage of genes of potentially harmful effect with those of agronomic value (Fernie et al. 2006). Knowledge of the genetic basis of cross-compatibility of different wild and cultivated forms can substantially aid in overcoming reproductive barriers and utilizing wild genetic diversity to transfer beneficial traits to cultivars.

Some of the hybridization barriers between related species or subspecies were genetically analysed. In maize, systems of the *Tcb1*, *Ga1* and *Ga2* alleles governing interaction of pollen tubes with silk tissue were described that confer prezygotic barriers in crosses between the cultivated *Zea mays mays* and wild teosinte *Z. m. mexicana* (Lu et al. 2014). About 50 loci controlling postzygotic reproductive barriers between rice subspecies have been identified, and molecular products of some genes have been characterized [reviewed in Ouyang et al. (2010)]. Two loci involved in the sterility of interspecific hybrids were mapped in naturally occurring *Mimulus* species (Sweigart et al. 2006). In some plant species, crossing barriers are manifested as nuclear–cytoplasmic incompatibility, the best known example of which being probably cytoplasmic male sterility (CMS) arising because of a conflict between nuclear and mitochondrial genomes [reviewed in Chase (2007)]. Genomic conflict is supposed to play an eminent role in establishing reproductive isolation according to the widely accepted Bateson–Dobzhansky–Muller model (Johnson 2010). A conflict of plastid and nuclear genomes has been described in detail for a series of interspecies crosses in the genus *Oenothera* [reviewed in Greiner et al. (2011)]. Fertility of wide hybrids of wheat was shown to be related to nuclear–cytoplasmic compatibility as well (Aksyonova et al. 2005). The nuclear–cytoplasmic incompatibility in wheat has been subject to genetic analysis (Ohtsuka 1991) and shown to be governed by at least two unlinked nuclear genes, *Scs* and *Vi* (Anderson and Maan 1995). We described a conflict between nuclear and plastid genomes in crosses of wild and cultivated representatives of the garden pea *Pisum sativum* L. (Bogdanova and Kosterin 2006; Bogdanova 2007) and genetically mapped two nuclear genes, *Scs1* and *Scs2,* responsible for the conflict (Bogdanova et al. 2012).

In the genus *Pisum*, a clear-cut wild species *Pisum fulvum* Sibth. et Smith. is recognized (Maxted and Ambrose 2001), while other main taxa variably receive status of species, subspecies or variations within *P. sativum* L. The cultivated taxa are the well-known garden pea *P. sativum* subsp. *sativum* L. and *P. abyssinicum* A. Br. from South Arabia and Ethiopia. Presently, the vast diversity of wild peas beyond *P. fulvum* has been proposed to consider as a single subspecies *P. sativum* subsp. *elatius* (Bieb.) Schmahl. (Maxted and Ambrose 2001) that is a useful compromise although making this subspecies, in broad sense, loose and paraphyletic (Kosterin and Bogdanova 2008). Independently of a taxonomic status assigned, wild peas comprise a broad continuum of forms (Jing et al. 2012) with a variable degree of reproductive isolation among representatives of wild and cultivated peas (Ben-Ze'ev and Zohary 1973; Bogdanova and Berdnikov 2001; Bogdanova and Kosterin 2006; Yadrikhinskiy and Bogdanova 2011). Since taxonomy of the genus is still unsettled (Maxted and Ambrose 2001), it is of biologic interest to isolate natural groups among the diversity of wild peas. Earlier, we put forward an approach to operational (not taxonomic) classification of pea forms based on three genetic markers referring to three cellular genomes—nuclear, plastid and mitochondrial (Kosterin and Bogdanova 2008). Based on allelic combinations of these markers, we isolated pea evolutionary lineages A, C, D and B, here enumerated in the order of their evolutionary origin one from another (Kosterin et al. 2010). An analysis of their geographic distribution led to a conclusion that the cultivated pea ascends to representatives of *P. sativum* subsp. *elatius*, belonging to the lineage B, currently inhabiting the north-eastern Mediterranean (Ibid.). An analysis of nuclear–cytoplasmic incompatibility in the crosses involving representatives of the mentioned evolutionary lineages was undertaken to clarify their evolutionary relationships.

In the present work, we performed reciprocal crosses of a number of wild and cultivated forms referring to the lineages A, B, C and D with the common cultivated pea *P. sativum sativum* and analysed phenotypic manifestation of alleles of the gene conferring nuclear–plastid incompatibility, *Scs1* (Bogdanova et al. 2009, 2012). This gave us a basis to classify the accessions studied into groups compatible or incompatible with the cytoplasm of *P. sativum sativum*. Such an approach was employed in identification of the hybrid sterility gene in rice (*Oryza sativa*) subspecies where three alleles of the *S5* locus were found to be characteristic of the groups of varieties compatible with *O. sativa indica*, compatible with *O. sativa japonica* and those of wide compatibility (Chen et al. 2008). We anticipate that the analysis undertaken will aid in future identification and characterization of the nuclear–cytoplasmic compatibility gene *Scs1* in pea.

Materials and methods

Pea taxonomy

We adopt here the taxonomy suggested by Maxted and Ambrose (2001) and consider the accessions studied in this work as belonging to two species, the cultivated *P. abyssinicum* and *P. sativum,* which in turn consist of two subspecies, *P. sativum sativum,* including cultivated forms, and *P. sativum elatius,* embracing all the diversity of wild peas beyond *P. fulvum*.

Plant material

The tester lines WL1238, WL1072, WL851 were received from the Weibullsholm collection (Landscrona, Sweden). These lines belong to *P. sativum* subsp. *sativum* and are fully compatible with other germplasm of this subspecies. WL1238 is marked by the alleles *le, b, k, gp*, *tl-w*; WL1072 is marked by the alleles *st*, *gp*; WL851 is marked by the alleles *le, k, st*, *tl-w*. The description of markers may be found at http://data.jic.bbsrc.ac.uk/cgi-bin/pgene.

Other germplasm used was received from Vavilov Research Institute of Plant Industry (St. Petersburg, Russia), John Innes Centre (Norwich, UK), and Neil O. Polans. The accessions are listed below with their attribution to evolutionary lineages A, B, C, D (Kosterin et al. 2010):

Lineage A: VIR320 (Palestine), *P. s.* subsp. *elatius.*
721 (Israel), *P. sativum* subsp. *elatius* [of the lines used by Ben-Ze'ev and Zohary (1973)].
VIR2759 (Yemen). *P. abyssinucum*
Lineage C: JI1096 (Greece), *P. sativum* subsp. *elatius*
Lineage D: VIR3439 (Egypt), *P. sativum* subsp. *sativum* ("*Pisum jomardi*" auct.)
Lineage B: JI1794 (Israel), *P. sativum* subsp. *elatius* [= 716 by Ben-Ze'ev and Zohary (1973)]

The marker combinations characteristic of the lineages are given in Online Resource 1

Plant growing

Seeds were sown in a greenhouse in hydroponic beds filled with claydite/vermiculite mixture and watered thrice a day with a standard Knop nutrient solution (0.8 g/l calcium nitrate; 0.2 g/l magnesium sulphate; 0.2 g/l acid potassium phosphate; 0.2 g/l potassium nitrate and traces of ferric phosphate). Plants were illuminated by 8 h daylight/16 h incandescent light of 10,000–12,000 lux intensity.

Pollen counts

To estimate pollen fertility, acetocarmine staining was used (Singh 2003). 100–300 pollen grains per flower and 3–4 flowers per plant were counted under light microscope: pollen grains with unstained cytoplasm were regarded as not viable; pollen fertility was estimated as the proportion of the viable pollen grains among the pollen grains counted.

Genomic DNA extraction, PCR analysis and endonuclease digestion

Genomic DNA extraction, PCR analysis (including primer sequences) and endonuclease digestion were carried out as described in Bogdanova et al. (2012). The description of the procedures can be found in Online Resource 1.

Crossing scheme and experimental design

A series of reciprocal crosses were performed using pea accessions and testerlines (Online resource 1, Table 1S). In all crosses mentioned, the maternal parent is indicated first. To denote the alleles inherited from the specific parent, for example, WL1238 or JI1794, we use marker name followed by underscore and parent designation, for example, *PhlC_1238* and *PhlC_1794*. The accessions 721, JI1794, VIR3439 were crossed in a reciprocal manner with WL1238. Plants of the JI1096 accession were crossed as pollen parents with WL1238. The reciprocal cross JI1096 × WL1238 produced no progeny since hybrid seeds did not set because of some prezygotic barrier. To test phenotypic manifestation of the *Scs1_1238* allele in the background of the JI1096 cytoplasm, we pollinated JI1096 plants with pollen of F3 segregants of the cross (WL1238 × JI1096) carrying molecular markers of LGIII linked to *Scs1* inherited from WL1238. VIR2759 (*P. abyssinicum*) was crossed to WL1072 as pollen parent. To obtain the reciprocal combination of nuclear *Scs1_1072* and cytoplasm of VIR2759, which cannot be obtained by a direct cross due to prezygotic barrier, F2 plants of the cross (WL1072 × VIR2759) with appropriate markers of LGIII were used to pollinate VIR2759. WL1072 was used as a counterpart in the crosses since it carried the visible marker *st* (reduced stipules) close to *Scs1* on LGIII. Also, reciprocal crosses were made of VIR320 and JI1794.

Phenotypic manifestation of the *Scs1* alleles from different pea germplasm in different cytoplasmic backgrounds was estimated in two aspects: (1) an effect on pollen fertility, and (2) gametophyte/sporophyte lethality as revealed by an absence of some genotypic classes in the F2 segregation, the effects earlier observed in crosses of VIR320 and L100 with WL1238 (Bogdanova et al. 2012). In the F2 of each cross, genomic DNA was extracted and pollen counts were made from individual plants. Since biparental plastid inheritance is possible and the situation of the nuclear–plastid conflict is favourable for propagation of paternal plastids (Bogdanova and Kosterin 2006), all plants were tested for the origin of cytoplasm by CAPS-analysis. In most cases, the amplified portion of *RbcL* was tested for the presence of the recognition site for the *Asp*LEI endonuclease (Kosterin and Bogdanova 2008), CAPS-analysis of *psbA-trnH* intergenic spacer was used to distinguish between plastid DNA of WL1238 and JI1794 (Zaytseva et al. 2012) since digestion pattern of their *RbcL* alleles with *Asp*LEI did not differ. In the F2 populations studied, up to ten plants were observed to contain paternal plastid DNA. Only plants with maternally inherited plastids were further analysed. In the

crosses with VIR3439 (cultivated *P. sativum* of the lineage D), origin of the plastids was not tested since we had no appropriate markers.

In the crosses where the cultivated *P. sativum sativum* participated, all plants were genotyped for *PhlC* which is tightly linked to the incompatibility-conferring locus *Scs1* (Bogdanova et al. 2009, 2012). In some crosses, a molecular marker *AJ832139,* tightly linked to *PhlC* and *Scs1* (Bogdanova et al. 2012) was also genotyped. In the crosses between VIR320 and JI1794, *PhlC* and *AJ832139* alleles could not be distinguished by CAPS-analysis, therefore, we genotyped the F2 plants for *Gsn* which is tightly linked to *PhlC* and *Scs1* (Bogdanova et al. 2009). Mean pollen fertility was estimated in the genotypic classes of homozygotes for the maternal allele of *PhlC*, heterozygotes, and homozygotes for the paternal allele of *PhlC*, where available. Statistically significant differences in pollen fertility, if observed, between these genotypic classes were attributed to the effect of the *Scs1* allele. Presence or absence of lethal effect was estimated as statistically significant deviation of segregation ratio in the *PhlC* locus from the Mendelian 1:2:1.

Results

Phenotypes of the carriers of *Scs1* alleles from cultivated *P. sativum sativum* in alien cytoplasm

We studied effects on pollen fertility of the allele of *Scs1* from *P. sativum sativum* in the background of the cytoplasm of accessions from different evolutionary lineages in F2 sergregants of crosses involving WL1238 or WL1072 testerlines as pollen parents and the accessions tested as seed parents. To follow the *Scs1* allelic state, we monitored the tightly linked molecular marker *PhlC*. The effects were manifested as decreased pollen fertility in the heterozygotes for *PhlC*, as compared with the homozygotes for its maternal allele. Pollen fertility in different genotypic classes for *PhlC* in the studied F2 populations is given in Table 1. Pollen fertility in phenotypic classes for other markers scored are given in Table 3S of Online Resource 1.

The mean pollen fertility in the classes of homozygotes for the maternal and paternal allele of *PhlC* did not differ significantly. Significant effects were associated with reduced pollen fertility in heterozygotes in the cytoplasms of 721, which is wild *P. sativum elatius*, and VIR2759, which is cultivated *P. abyssinicum*, both belonging to evolutionary lineage A, and the cytoplasm of JI1096, which is wild *P. sativum elatius* from evolutionary lineage C. Lethal effects, manifested as absence of some genotypic class were not observed. Although the homozygotes for *PhlC_1794* were somewhat underrepresented, this was not

Table 1 Mean pollen fertility (%) with standard errors, of plants in genotypic classes for *PhlC* in F2 populations of the crosses involving *P. sativum sativum* testerlines as the pollen parent and diverse pea accessions as seed parents

Cross	Source of cytoplasm with indication of evolutionary lineage	Maternal homozygotes for *PhlC* (M)	Heterozygotes for *PhlC* (H)	Paternal homozygotes for *PhlC* (P)	Tst (M–H)	Tst (P–H)	Tst (M–P)	Chi-square 1:2:1
721 × WL1238	721 (A)	$85.28 \pm 4.49\ \sigma = 14.89$ $n = 11$	$69.69 \pm 2.66\ \sigma = 14.08$ $n = 28$	$82.35 \pm 4.13\ \sigma = 19.39$ $n = 22$	3.06**	2.67*	0.44	4.38
VIR2759 × F2(WL1072 × VIR2759)	VIR2759 (A)	$70.10 \pm 3.05\ \sigma = 16.69$ $n = 30$	$64.42 \pm 2.34\ \sigma = 15.33$ $n = 43$	$73.21 \pm 3.44\ \sigma = 17.20$ $n = 25$	1.50	2.18*	−0.68	1.98
JI1096 × F3(WL1238 × JI1096)	JI1096 (C)	$79.26 \pm 3.26\ \sigma = 19.28$ $n = 35$	$68.00 \pm 2.32\ \sigma = 20.33$ $n = 77$	$73.63 \pm 3.28\ \sigma = 18.55$ $n = 32$	2.76**	1.35	1.22	0.82
VIR3439 × WL1238	VIR3439 (D)	$96.53 \pm 0.52\ \sigma = 2.20$ $n = 18$	$95.33 \pm 0.48\ \sigma = 2.13$ $n = 20$	$95.19 \pm 0.46\ \sigma = 1.71$ $n = 14$	1.71	−0.20	1.88	3.38
JI1794 × WL1238	JI1794 (B)	$88.14 \pm 5.45\ \sigma = 16.34$ $n = 9$	$87.83 \pm 1.79\ \sigma = 11.75$ $n = 43$	$91.13 \pm 1.24\ \sigma = 5.96$ $n = 23$	0.07	1.26	−0.77	6.84*

σ standard deviation, n number of plants. *Tst* Student's criterium

* $0.01 < P < 0.05$

** $0.001 < P < 0.01$

related to nuclear–cytoplasmic interaction since these are homozygotes for the maternal allele in the background of the maternal cytoplasm.

Phenotypes of the carriers of *Scs1* alleles from different pea germplasm in *P. sativum sativum* cytoplasm

Pollen fertility in different genotypic classes of the F2 hybrids in the crosses of the reciprocal direction, involving *P. sativum sativum* as donor of cytoplasm and the accessions tested as pollen parents is given in Table 2. Effects of different *Scs1* alleles on pollen fertility in the background of the alien cytoplasm of *P. sativum sativum* were observed in the crosses WL1238 × 721 and WL1072 × VIR2759, both of the pollen parents belonging to the lineage A. Note that allelic states of the *Scs1* also had an effect on pollen fertility in the hybrids of the reciprocal nuclear–cytoplasmic combination of both these cases (Table 1). At the same time, in the crosses where the cytoplasm came from the cultivated *P. sativum sativum*, the mean pollen fertility of heterozygotes for *Scs1* was about 50 % which was lower than that observed in the reciprocal nuclear–cytoplasmic combination.

Statistically significant deficit of homozygotes for the paternal allele of *Scs1* was observed in the crosses WL1238 × 721, WL1238 × JI1096 and WL1238 × JI1794. In the latter case, the shortage of homozygotes for *PhlC_1794* was very similar to that of the reciprocal cross (Table 1) where the same class of homozygotes was underrepresented, therefore, we attribute it to some kind of nuclear–nuclear interaction independent of the cytoplasm origin. The drastic deficit of one class of homozygotes for *PhlC* in the crosses WL1238 x 721 and WL1238 × JI1096 appeared to be quite different. Unlike in the reciprocal nuclear–cytoplasmic combination, it affects the homozygotes for the paternal allele and hints a possible lethal effect of the closely linked *Scs1*. If *Scs1* is in fact a recessive sporophytic/gametophytic lethal, the plants homozygous for the closely linked *PhlC* should have been heterozygous for *Scs1*. We tested the presence of the *Scs1_1238* allele in two of the three homozygotes for *PhlC_721* which appeared in the F2 population of the cross WL1238 × 721 (Table 2). As we have seen, in the background of the WL1238 cytoplasm, homozygotes for *PhlC_1238*, most of which must also be homozygotes for *Scs1_1238*, had more or less fertile pollen, about 70 % on average (Table 2). Therefore, to confirm the presence of *Scs1_1238* allele in the genome of the plants in question, we could perform a cross with WL1238 and see if they produce progenies with fertile pollen (Fig. 1).

Seeds from two F2 plants homozygous for *PhlC_721* were grown into two F3 plants which were crossed as pollen parents with WL1238 and WL851, marked with

Table 2 Mean pollen fertility (%), with standard errors, of plants in genotypic classes for *PhlC* in F2 populations of the crosses involving *P. sativum sativum* testerlines as the seed parent and the tested pea accessions as pollen parents

Cross	Pollen parent with indication of evolutionary lineage	Maternal homozygotes for *PhlC* (M)	Heterozygotes for *PhlC* (H)	Paternal homozygotes for *PhlC* (P)	Tst (M–H)	Tst (P–H)	Tst (M–P)	Chi-square 1:2:1
WL1238 × 721	721 (A)	$68.18 \pm 3.77\ \sigma = 19.21$ $n = 26$	$52.91 \pm 3.28\ \sigma = 17.35$ $n = 28$	$45.2 \pm 6.47\ \sigma = 11.21$ $n = 3$	3.07**	−0.75	2.01	18.58***
WL1072 × VIR2759	VIR2759 (A)	$89.89 \pm 3.09\ \sigma = 9.15$ $n = 12$	$72.95 \pm 2.97\ \sigma = 11.87$ $n = 16$	$74.30 \pm 2.74\ \sigma = 5.47$ $n = 4$	3.89***	0.22	2.75*	4.00
WL1238 × JI1096	JI1096 (C)	$58.98 \pm 3.65\ \sigma = 17.88$ $n = 24$	$52.83 \pm 2.96\ \sigma = 13.9$ $n = 22$	$51.07 \pm 14.66\ \sigma = 25.4$ $n = 3$	1.29	−0.19	0.69	18.51***
WL1238 × VIR3439	VIR3439 (D)	$94.85 \pm 0.52\ \sigma = 1.65$ $n = 10$	$94.63 \pm 0.92\ \sigma = 4.87$ $n = 28$	$95.53 \pm 0.65\ \sigma = 1.85$ $n = 8$	0.14	0.51	−0.82	2.35
WL1238 × JI1794	JI1794 (B)	$83.78 \pm 3.94\ \sigma = 15.75$ $n = 16$	$88.36 \pm 1.28\ \sigma = 8.28$ $n = 42$	$91.18 \pm 2.60\ \sigma = 7.36$ $n = 8$	−1.44	0.90	−1.25	6.85*

σ standard deviation, n number of plants. *Tst* Student's criterium

* $0.01 < P < 0.05$

** $0.001 < P < 0.01$

*** $P < 0.001$

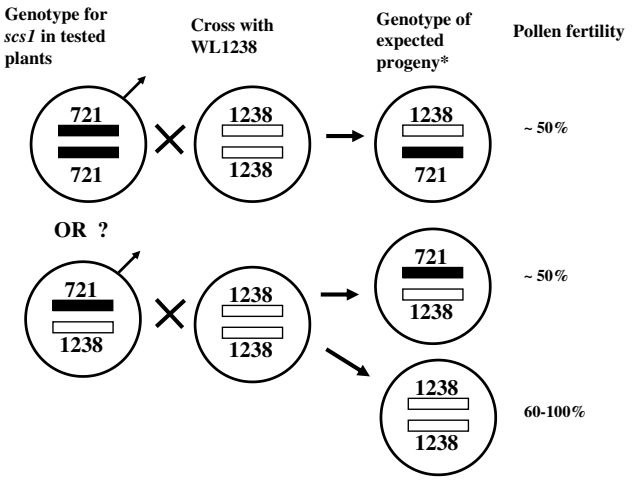

Fig. 1 A scheme for genotype testing in respect of the *Scs1* locus in homozygotes for *PhlC_721* in F2 of the cross WL1238 x 721 in the background of the WL1238 cytoplasm. *Asterisk* indicates genotypes of the progeny expected if *Scs1_721* is not lethal for male gametophytes. Otherwise only the lowest class with fertile pollen is expected

recessive alleles to ensure cross-fertilization. Seven progenies were obtained with pollen fertility ranging from 0.64 to 0.97. Therefore, we concluded that the plants homozygous for *PhlC_721* did carry the allele *Scs1_1238*, and homozygotes for the allele *Scs1_721* do not exist in the background of the cytoplasm of WL1238. The same we assume for the allele *Scs1_1096*, although F2 plants from the cross WL1238 × JI1096 homozygous for *PhlC_1096* were not tested.

Similarity between *Scs1* alleles of *P. sativum sativum* and a wild pea of the evolutionary lineage B

As seen from Tables 1, 2, JI1794 is the only wild pea which has *Scs1* with no adverse effects in the background of the cytoplasm of WL1238 and vice versa, *Scs1_1238* is compatible with the cytoplasm of JI1794 belonging to the lineage B. To test if *Scs1_1794* is equivalent to *Scs1_1238* in respect of compatibility to alien cytoplasm, we analysed pollen fertility in the F2 population of the crosses JI1794 × VIR320 and VIR320 × JI1794, where VIR320 belongs to the wild subspecies *P. sativum* subsp. *elatius* and evolutionary lineage A (Table 3). In these crosses, we used *Gsn* as molecular marker closely linked to *Scs1* since the *PhlC* alleles of the accessions involved could not be distinguished in our CAPS-assay. As seen from Table 3, in the background of JI1794 cytoplasm, neither deviation from the expected genotype segregation nor substantial drop in pollen fertility in any genotype class was observed. The reciprocal cross, in which the cytoplasm was inherited from VIR320, produced no homozygotes for paternal *Gsn_1794* while the heterozygotes had about 50 % fertile pollen.

Discussion

Compatible and incompatible nucleus–cytoplasm combinations

The effects of *Scs1* alleles in alien cytoplasm, including results of our earlier studies (Yadrikhinskiy and Bogdanova 2011; Bogdanova et al. 2012), are summarized in Tables 4, 5. The *Scs1* alleles of the accessions studied, being combined with an alien cytoplasm, exerted different effects manifested as a decrease of pollen fertility to various extent and/or absence of some genotypic classes among sporophytes. Only one wild accession, JI1794, did not demonstrate detrimental effects associated with the nuclear–cytoplasmic conflict. JI1794 belongs to *P. sativum* subsp. *elatius* in broad sense (Maxted and Ambrose 2001) (together with VIR320, L100, 721 and JI1096) but shares evolutionary lineage B with the common cultivated *P. sativum* subsp. *sativum.* Therefore, the allele *Scs1_1794* was supposed to be equivalent to *Scs1_1238* in respect of compatibility with alien cytoplasm. This was confirmed in the crosses of JI1794, in both directions, with the accession VIR320. The pattern of interaction between the *Scs1* alleles and the cytoplasms of wild peas VIR320 and JI1794, belonging to different evolutionary lineages A and B, respectively, is very

Table 3 Mean pollen fertility (%), with standard errors, of plants in genotypic classes for *Gsn* in F2 populations of the crosses JI1794 × VIR 320 and VIR320 × JI1794

Cross	Maternal homozygotes for *Gsn* (M)	Heterozygotes for *Gsn* (H)	Paternal homozygotes for *Gsn* (P)	Tst (M–H)	Tst (P–H)	Tst (M–P)	Chi-square 1:2:1
JI1794 × VIR320	86.72 ± 1.34 $\sigma = 5.51$ $n = 17$	82.99 ± 2.10 $\sigma = 12.76$ $n = 37$	84.43 ± 3.94 $\sigma = 12.45$ $n = 10$	1.15	0.32	0.66	3.09
VIR320 × JI1794	71.04 ± 3.63 $\sigma = 20.53$ $n = 32$	54.01 ± 2.69 $\sigma = 16.36$ $n = 37$	$n = 0$	3.83***	n/a	n/a	30.04***

n/a not available, σ standard deviation, *n* number of plants. *Tst* Student's criterium

*** $P < 0.001$

Table 4 Effect of the *Scs1* alleles from peas representing different evolutionary lineages in the background of the cytoplasm of the cultivated *P. sativum sativum*

Origin of *Scs1*	Evolutionary lineage	Effect on pollen fertility	Segregation distortion
VIR320	A	No	No
L100	A	Not studied	Yes
721	A	Decreases to about 50 % in heterozygotes	Yes
VIR2759	A	Decreases to about 70 % in heterozygotes and paternal homozygotes	No
JI1096	C	No	Yes
VIR3439	D	No	No
JI1794	B	No	No

Data for VIR320 are taken from Bogdanova et al. (2012), for L100 from Yadrikhinskiy and Bogdanova (2011)

Table 5 Effect of the *Scs1* alleles from *P. sativum sativum* in the background of the cytoplasm of peas representing different evolutionary lineages

Origin of cytoplasm	Evolutionary lineage	Effect on pollen fertility	Segregation distortion
VIR320	A	Decreases to about 50 % in heterozygotes	Yes
L100	A	Decreases to about 50 % in heterozygotes	Yes
721	A	Decreases to about 70 % in heterozygotes	No
VIR2759	A	Decreases to about 70 % in heterozygotes	No
JI1096	C	Decreases to about 70 % in heterozygotes	No
VIR3439	D	No	No
JI1794	B	No	No

Data for VIR320 are taken from Bogdanova et al. (2012), for L100 from Yadrikhinskiy and Bogdanova (2011)

similar to that observed for the *Scs1* alleles and cytoplasms of VIR320 and WL1238, namely, lethality of paternal homozygotes for *Scs1* and pollen semisterility in the cytoplasm of VIR320, and full compatibility in the reciprocal crosses.

Our aim was to classify pea accessions as compatible or incompatible with the commonly cultivated *P. sativum* subsp. *sativum* with respect to the nuclear–cytoplasmic interaction. We consider an accession to be incompatible, if a lethal effect, that is, lack of some genotypic class in the F2 segregation has been registered; otherwise, we call it compatible or partially compatible. If some homozygous class is absent, the lethality may be either sporophytic or gametophytic affecting male or female gametophytes. Earlier, we have shown that in the background of the VIR320 cytoplasm, *Scs1_1238* is both a male gametophytic and sporophytic lethal, but can be transferred via female gametes (Bogdanova et al. 2012). In this study, we have not tested what kind of lethality is associated with *Scs1* alleles, either gametophytic or sporophytic, or both. The segregation ratio of homozygotes for the maternal allele of a LGIII marker and heterozygotes was close to 1:1 in F2 of the crosses WL1238 × 721, WL1238 × JI1096 (Table 2), and VIR320 × JI1794 (Table 3), resembling segregation in a testcross where one of the parents produces only one class of gametes. Thus, the observed segregation ratios imply male gametophyte lethality of the *Scs1* alleles in the indicated crosses. In all but one case where the lethal effect was registered, pollen fertility of heterozygotes was significantly lower than that of homozygotes for the maternal allele. The only exception was the cross WL1238 × JI1096 (Table 2) where we did not register significant difference of pollen fertility of heterozygotes and maternal homozygotes. This might be ascribed to the death of male gametophytes at some stages of their development later than the stage analysed, e.g. failure to form a pollen tube.

The following accessions were found to be incompatible with *P. sativum sativum* at least in one direction of the cross: VIR320 (lineage A), L100 (lineage A), 721 (lineage A), JI1096 (lineage C), that is, all studied *P. sativum elatius* from the evolutionary lineages A and C.

The accessions VIR2759 (lineage A), VIR3439 (lineage D) and JI1794 (lineage B) were compatible with cultivated *P. sativum sativum* in both directions. Of these, VIR2759 is a cultivated but independently domesticated pea species *P. abyssinicum* and its *Scs1* allele in heterozygote with *Scs1* of *P. sativum sativum* decreases pollen fertility in cytoplasms of either parent. For this reason, we consider

it as partially compatible. VIR3439 is a cultivated pea representing a dubious Egyptian taxon "*Pisum jomardii* Schrank". Formally, it belongs to the evolutionary lineage D, supposed to be the ancestor of the lineage B (Kosterin et al. 2010), and hence considered as a subspecies *P. sativum* subsp. *jomardii* (Schrank) Kosterin in Kosterin and Bogdanova (2008). However, most probably, this pea does not represent the actual line D but resulted from introgression of some genes from wild peas to commonly cultivated pea subspecies *P. sativum sativum* (Kosterin et al. 2010) and should be considered within the latter. The only wild accession compatible with *P. sativum* subsp. *sativum* is JI1794 belonging to the evolutionary lineage B considered to be a progenitor of the latter subspecies (Kosterin et al. 2010). The results of this study on the nuclear–cytoplasmic compatibility of different pea forms are concordant with those of Zaytseva et al. (2012) suggesting the existence of two evolutionary branches embracing the lineages A + C and B + D. It should be noted that we have tested compatibility of a cultivated and dubious representative of the lineage D (see above) with *P. sativum sativum*. As for a wild representative of the lineage D, we failed to obtain viable hybrids due to severe prezygotic barrier: as a result of several dozens of cross-pollinations between PI344537 (Sicily, lineage D) and WL1238 in both directions, we obtained one seed from the cross WL1238 x PI344537 which did not germinate.

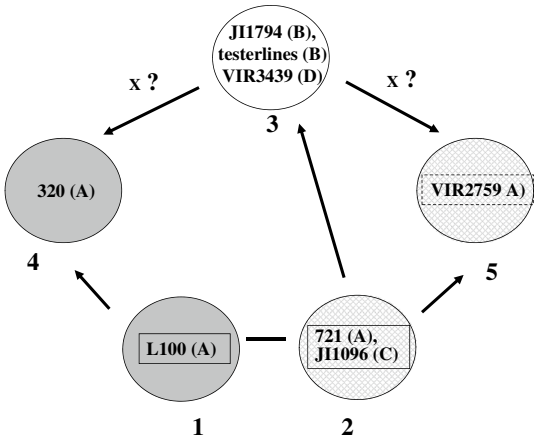

Fig. 2 A hypothetical scheme of evolutionary relationships among the studied pea accessions [with evolutionary lineages sensu Kosterin et al. (2010) indicated] grouped into states 1–5 with respect to nuclear–cytoplasmic compatibility with the common cultivated pea *P. sativum* subsp. *sativum*. A combination of a certain nucleus with a certain cytoplasm is considered compatible if *Scs1* exerts neither lethal effects nor decrease in pollen fertility. If lethality has been observed, a combination is considered incompatible, if only decrease in pollen fertility but no lethality observed, a combination is considered partially compatible. The cytoplasms compatible with *Scs1* of cultivated *P. sativum sativum* are given in *white*, incompatible in *grey*, partially compatible in *chequered*. Nuclei with *Scs1* incompatible with *P. sativum sativum* cytoplasm are *boxed*, partially compatible placed in *dashed box*. *Arrows* indicate the supposed evolutionary pathways; simple line connects the two states of which we cannot recognize the ancestral one; "X ?" indicates the supposed cases of introgression/hybridisation

Nuclear–nuclear incompatibilities

Along with the nuclear–cytoplasmic incompatibility involving the nuclear *Scs1* gene, evidences for some nuclear–nuclear incompatibilities were observed. One such case was shortage of homozygotes for *Gsn_1794* in the F2 populations of the reciprocal crosses WL1238 × JI1794 and JI1794 × WL1238. Whether it is due to *Scs1* or some other gene(s) is not known. After all, JI1794 is a wild pea rather distant from cultivated forms (Ben-Ze'ev and Zohary 1973). Similar cases of underrepresentation of genotypic/phenotypic classes including visible markers were observed in the F2 of the crosses WL1238 × 721 and 721 × WL1238 and WL1238 × JI1096 (Online Resource 1, Tables 4S–9S). Statistically significant shortage of homozygotes for *AJ832139_1238* in combination with the dominant allele *K* (not reduced flower wings, linkage group II) was registered in both WL1238 × 721 and 721 × WL1238 crosses (Online Resource 1, Tables 4S, 6S). Shortage of homozygotes for *AJ832139_1238* in combination with the dominant allele *B* (purple, not pink flower pigmentation) on the same linkage group was registered in the crosses WL1238 × 721 and WL1238 × JI1096 (Online Resource 1, Tables 5S, 9S). It is possible that the product of some gene linked to the allele *AJ832139_1238* (may be *Scs1*) does not form a fully functional complex with products of some genes linked to *B_721*, *B_1096* and *K_721*.

Evolutionary relationships among nucleus–cytoplasm combinations

Compatibility of nuclei and cytoplasms in the groups of the accessions studied is schematically represented in Fig. 2. A question arises how specific combinations of co-adapted nuclear and organellar genomes evolve from one to another. Such combinations are further referred to as 'states' and are numbered 1–5 in Fig. 2. Evolution from a wild progenitor to the cultivated pea of the state 3 probably started from the plesiomorphic state which may be either 1 or 2. These both states are represented by doubtless wild forms belonging to the evolutionary lineages A and C which form the basic clade of *P. sativum* (Zaytseva et al. 2012). The state 2 includes a representative of the lineage C thought to be transitory between A and B (Kosterin et al. 2010), hence it may be transitory to the state 3 proper of the lineage B (and perhaps the entire common cultivated pea, *P. sativum* subsp. *sativum*). In such a scenario, the state 4, so far represented solely by VIR320, cannot be an evolutionary intermediate

between the states 1 and 3. Then, we suppose that VIR320 resulted from hybridisation between some peas of the states 1 and 3. Indeed, the original accession VIR320 had no exact provenance (it was received by N.I. Vavilov from Sutton, France, in 1922) and was very heterogeneous (Makasheva 1979; Kosterin and Bogdanova 2008), VIR320 actually used in this study was a highly homozygous subline isolated from the original accession. The state 5 of *P. abyssinicum* could hardly be an evolutionary intermediate between states 2 and 3, because it lacks wild representatives and has a restricted and marginal geographical range. Also, the allelic states of markers typical of the lineage A hardly could evolve into those typical of the lineage B with no obvious intermediates between *P. abyssinucum* and common garden pea of lineage B. We rather support the view of Govorov (1937) of the hybrid nature of *P. abyssinicum*.

Scs1 as a speciation gene

We examined a number of wild *P. sativum elatius* for their nuclear–cytoplasmic compatibility with cultivated *P. sativum sativum* and found that phenotypic manifestation of *Scs1* alleles from different accessions is rather different as to the pollen fertility and lethality among F2 segregants. Three wild pea accessions belonging to the same evolutionary lineage A and originating from the same country of Israel, namely, VIR320, L100 and 721, all display nuclear–cytoplasmic incompatibility with WL1238 as a representative of *P. sativum sativum,* associated with the alleles of the *Scs1* locus. However, the patterns of the incompatibility are rather different. While VIR320 is incompatible with WL1238 as donor of cytoplasm (Table 5), 721 is incompatible as pollen parent and L100 is incompatible in both directions. JI1794 originates from the same area as VIR320, L100 and 721 (Israel), but has the *Scs1* allele even more different from these accessions but similar to that of *P. sativum sativum* being fully compatible with the latter in both directions. This is not surprising since JI1794 belongs to the evolutionary lineage B, like *P. sativum sativum*.

Genes contributing to the splitting of lineages by cessation of gene flow may be defined as speciation genes (Rieseberg and Blackman 2010). In plants, the genes underlying reproductive barriers frequently contribute to hybrid sterility and may exhibit intraspecific polymorphism (Ibid.). For example, in *Mimulus*, one of the loci involved in hybrid sterility was found to be polymorphic over populations and to have uneven geographic distribution (Martin and Willis 2010). We observed among pea accessions a remarkable variability of the gene related to hybrid fertility and viability and suppose that it plays an important evolutionary role in creating postzygotic reproductive barriers that allow us to nominate this gene as a candidate speciation gene.

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Ethical statement The experiments comply with the current laws of the country in which they were performed.

Conflict of interest The authors declare that they have no conflict of interest.

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