Introgession of powdery mildew resistance into cultural pea from wild accession of *P. fulvum*

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Abstract. Wildlife pea specimen *P. fulvum* i-609881 of the UIP collection (Saint Petersburg, Russia) in a greenhouse box has demonstrated full resistance to powdery mildew. As a result of crossing with cultivated peas, interspecific hybrids of Stabil × i-609881 were obtained. In populations of interspecific hybrids, the BC₂F₃ Stabil × i-609881 line of the leafless morphotype (*af*), resistant to the powdery mildew pathogen, was isolated as a result of backcrossing. As a result of crossing plants of this line with susceptible plants of the leafy (*Af*) variety Temp, the following series of pea hybrids was obtained. Analysis of F₁ hybrids showed that all plants were characterized by resistance to the pathogen powdery mildew. In the population of F₂ hybrids, a splitting analysis was performed on the basis of resistance to powdery mildew. The analysis revealed that the hybrid population consisted of 66 resistant and 20 affected plants. The actual splitting corresponded to the expected one for monogenic inheritance with a phenotype ratio of 3:1 phenotype ratio (*χ₀²* test, *p*=0.7087438). Based on the analysis of populations of F₁ and F₂ hybrids, it is concluded that resistance to powdery mildew is the result of introgression of the genetic resistance factor from the genome of the *P. fulvum* i-609881 sample and is encoded by the dominant allele of a single gene.

1. Introduction

Mainly the causative agent of powdery mildew in peas is *Erysiphe pisi* [1]. However, other species that can infect pea plants in the field and greenhouses have now been identified, such as *E. baeumleri* in the Czech Republic and *E. trifolii* in the northwestern United States [1, 2, 3]. Powdery mildew is a dangerous disease that leads to a 25-50% decrease in yield and a noticeable deterioration in grain quality [4]. Powdery mildew is widespread all over the world, especially active in climatic conditions characterized by high temperatures during the day and low at night [5]. The use of pea varieties that are resistant to powdery mildew in agricultural production will significantly increase the yield and quality of agricultural products.

For the first time, sources resistant to powdery mildew were isolated from pea samples found in Peru [6]. Resistance to powdery mildew was determined by the recessive *er1* gene, which is widely used in pea breeding. If the *er1* gene provides almost complete resistance to the disease, the second recessive *er2* gene shows a protective effect only on leaves [7]. The use of the *er2* gene is problematic due to the fact that its protective effect is manifested in certain conditions of plant growth, and its expression depends on the temperature and age of the leaves [8]. Both genes are localized in different coupling groups, *er1* in VI [9], and *er2* in III [10].

The third dominant *Er3* gene, which provides resistance to the powdery mildew pathogen, was identified in a sample of the wildlife pea species *P. fulvum* and, as a result of introgression, became
available for use in the breeding process [5]. Studies revealed independent inheritance of the erl, er2, and Er3 genes [11]. Further studies have shown that the Er3 gene is localized in the IV linkage group [12]. Two RAPD markers were identified for the Er3 gene, localized in 2 alternative phases at a distance of 0 to 2.8 cm. They were converted to SCAR markers that allow marker selection with high reliability [13].

Resistance to powdery mildew in the erl gene is based on the barrier to penetration created as a result of loss of function in the susceptibility factor – the PsMLO protein encoded by this gene [14], and in the er2 and Er3 genes – on the effect of hypersensitivity after the pathogen entering cells [5].

To replenish the gene pool of cultivated peas with new genes of economically valuable traits, a series of interspecific hybrids of peas P. sativum × P. fulvum was obtained in our laboratory [15, 16]. The study of the formation process under the conditions of an infectious background of a greenhouse box in a backcrossed population of interspecific hybrids Stabili-609881 allowed identifying lines of BC2,F3 hybrids that are resistant to the powdery mildew pathogen, conducting crosses of a stable line with a susceptible Temp variety, and study hybrids of the first and second generation [17].

The aim of this study was to perform a genetic analysis of the introgression of the powdery mildew resistance factor obtained from the P. fulvum i-609881 sample from the UIP collection for use in basic research and pea breeding.

2. Material and methods

Interspecific pea hybrids for introgression of powdery mildew resistance were obtained in a combination of Stabil × i-609881. The parent variety Stabil was affected by powdery mildew, and the specimen P. fulvum i-609881 of the UIP collection (Saint Petersburg, Russia) was completely resistant to the pathogen. Interspecific hybrids were grown for several years in a greenhouse box, where they were re-crossed with the parent variety Stabil. The plants were grown in high temperatures in the summer and low temperatures in the autumn growing season. During the growing season, significant temperature changes were observed day and night, which created favorable conditions for the affection of plants with powdery mildew. Under these conditions, as a result of backcrossing and selection of resistant plants, a powdery mildew-resistant pea line BC2,F3 Stable × i-609881 was obtained with a 12.5% share of the P. fulvum genome.

Powdery mildew resistant plants of the specified pea line of leafless morphotype (af) were crossed with pathogen susceptible plants of the leafy (Af) Temp variety. Hybrids F1 and F2 were grown in a greenhouse box and analyzed for signs of resistance to powdery mildew and leaf morphology. The correspondence between the actual and expected splitting of F2 hybrids was evaluated by the $\chi^2$ criterion using the EXCEL 2010 computer program.

3. Results

All F1 pea hybrids obtained as a result of hybridization of resistant plants of the BC2,F3 Stabil × i-609881 line of the leafless morphotype (af) with plants of the leafy (Af) susceptible variety were characterized by resistance to powdery mildew, which indicated the dominant character of the resistance trait.

To determine the number of genes encoding resistance to the powdery mildew pathogen, a study of splitting on the basis of resistance in the F2 hybrid population was performed. 86 F2 hybrids were analyzed in the experiment. Firstly, we performed a test analysis of the quality of the splitting population on the basis of “leaf type” with known monogenic inheritance (af) (table 1).

**Table 1.** Test analysis of pea hybrid F2 segregation on type of leaf with known monogenic inheritance.

| Morphotype of plant | Leafy ($\sum$ AfAf, Afaf) | Semi-leafless (afaf) | Total | $\chi^2$ test, p |
|---------------------|---------------------------|---------------------|-------|-----------------|
| Number of plants (fact) | 62                        | 24                  | 86    | 0.5335653 |
| Number of plants (expected as 3 : 1) | 64.5                      | 21.5                |       |                 |
The actual splitting for the control trait “leaf type” corresponded to the expected 3 : 1 ($\chi^2$ test, $p=0.5335653$), which indicated a high quality of the splitting population.

As a result of the study of splitting on the basis of resistance to the powdery mildew pathogen, 66 resistant and 20 affected plants were identified from 86 plants (table 2), which corresponded to the 3 : 1 splitting expected for monogenic inheritance ($\chi^2$ test, $p=0.7087438$).

**Table 2. Segregation of pea hybrids $F_2$ on resistance to powdery mildew.**

| Type of plant | Resistant | Susceptible | Total | $\chi^2$ test, $p$ |
|---------------|-----------|-------------|-------|-------------------|
| Number of plants (fact) | 66        | 20          | 86    |                   |
| Number of plants (expected as 3 : 1) | 64.5      | 21.5        | 86    | 0.7087438         |

4. Discussion

In the greenhouse box, cultivated and wild pea plants were grown for 10 years. Every year, pea plants were affected by powdery mildew, creating a local infectious background. Powdery mildew affected both cultivated and wild peas, including *P. fulvum* specimens. However, a sample of i-609881 with complete resistance to the powdery mildew pathogen was found among wild pea samples. To obtain interspecific hybrids, plants of the sample i-609881 were crossed with plants of the pea variety Stabil.

The formation process was studied in backcrossed populations of interspecific pea hybrids under conditions of local infectious background of the greenhouse box. Selection of plants of interspecific pea hybrids on an infectious background over several generations allowed identifying the line of the $BC_2F_3$ Stabil × i-609881 leafless morphotype ($af$), whose plants were resistant to powdery mildew. This line, but of late generation $BC_2F_3$ Stabil × i-609881, showed resistance to powdery mildew in the field conditions (figure).

**Figure 1.** Pea introgression lines $BC_2F_3$ Stabil × i-609881 susceptible (left) and resistant (right) to powdery mildew.

Analysis of populations of $F_1$ and $F_2$ hybrids showed that resistance to powdery mildew is the result of introgression of the dominant allele of a single gene from the genome of the *P. fulvum* i-609881 sample of the UIP collection. To determine whether resistance is determined by the previously described *Er3* gene [3, 10] or by a new gene (allele), an allelism test should be performed. At least samples of wildlife pea species may have different alleles of the *Er3* gene, providing different degrees of resistance to the powdery mildew pathogen.

In contrast to the *er1* gene, which is the result of a mutation that led to the synthesis of an aberrant susceptibility factor protein PsMLO, which creates a barrier to pathogen penetration [14], the dominant nature of the new gene opens up new prospects for studying alternative molecular mechanisms of plant resistance to diseases.
Long-term use of the er1 gene to prevent powdery mildew from infecting pea plants may lead to overcoming resistance because of pathogen evolution. Identification of new resistance genes increases the competitive advantages of peas over powdery mildew pathogen in the process of permanent genome coevolution. In the future, the pyramiding of various genes will increase the resistance of pea plants to the powdery mildew pathogen and buy time for the pathogen evolution necessary to overcome resistance. It is known that for the previously described powdery mildew resistance genes, genetic markers, RAPD and SCAR for the er1, Er3 and AFLP for the er2 gene have been developed, which make it easy to combine these genes in one genotype using marker selection [13].

5. Summary
Currently, three identified powdery mildew resistance genes in peas are known: er1, er2, and Er3. The Er3 gene is the result of introgression from a sample of the wild pea species *P. fulvum*. It is known that for the previously described powdery mildew resistance genes, genetic markers have been developed, RAPD and SCAR for the er1, Er3 and AFLP for er2 genes, which make it easy to combine these genes in one genotype using marker selection. Our research shows that resistance to powdery mildew is the result of introgression of the dominant allele of a single gene from the genome of the *P. fulvum* i-609881 sample of UIP collection. Further research is needed to determine whether resistance is determined by the previously described *Er3* gene or whether it is a new gene (allele). At least different samples of wild pea species may have different alleles of the *Er3* gene. Allelic variants of the gene are important for use in breeding practice, providing different degrees of protection against the pathogen powdery mildew.

6. References
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