Application of RGAP technique for genotype screening of introgressive wheat lines resistant to powdery mildew

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Introduction

The interspecific hybridization is a widely used and effective way for the development of genetically resistant crops. Nevertheless, such hybridization leads to genomic stress in the newly formed hybrids that complicates investigation of the resistance origination. In purpose to detect a present variety of resistance genes in the plant genotypes the Resistance Gene Analog Polymorphism technique [1], which is based on genomes screening with primers to conserved regions of resistance genes, is frequently used. This technique displays a wide range of components in the electrophoretic spectrum that represent different parts of R genes and, therefore, could be associated with a resistance trait.

Plant materials & methods

In this work the RGAP technique was used in order to investigate resistance trait control in introgressive lines with different goat-grass origin.

These introgressive wheat lines are derived from crosses between Aurora variety of common winter wheat (AABBDD) and its amphidiploids with substituted D genome by genomes from diploid *Aegilops* species. There were 10 lines originated from Aurotica (Aurora's amphidiploid with TT genome from *Amblyopyrum mutica*), 18 lines derived from Aurodes (amphidiploid with SS from *Ae. speltoides*), and 13 lines from Aurolata (DD genome substituted by UU from *Ae. umbellulata*). These introgressive lines demonstrate durable resistance to powdery mildew, unlike the susceptible Aurora variety.

Eight RGAP primers specific to the conservative motifs of five R genes were used. Primers to the conservative NBS (Kinase 1) and LRRs regions of the *Cre3* [2, 3], *Rps2*, and *Xa21* genes [1] were selected from literature data. Also, three RGAP primers to the MHD and LRRs regions were developed to corresponding conservative sequences between *Pm8* and *Pm3b* genes (their characteristics are shown in **Table 1**). All lines were screened by 6 combinations of RGAP primers. Electrophoresis was provided in 1,9% agarose gel.

Primer	Tm (°C)	GC %	Sequence
Pm8-LRR-L	59.07	45.00	GCATGCCTTGAAGCTTTGTA
Pm8-LRR-R	60.02	50.00	CTGTCGCAAACAGAAGTCCA
Pm8-MHD-L	55.87	38.10	CGATCTTATGCATGATATTGC

Table 1. Characteristics of RGAP primers designed to conservative motifs of wheat *Pm8* and *Pm3b* genes.

Results

Analysis of different combinations of RGAP primers revealed two types of variation in introgressive lines' genomes spectra: presence/absence variation (PAV) and the presence of new components differed from parental spectra. Results of lines polymorphism are summarized in **Table 2** (for Aurolata and Aurodes derivatives) and in **Table 3** (for Aurotica derivatives).

Probably, PAV can occur due to the introgressions events in the plants' genomes whereas the presence of new components indicates genomic rearrangements that are initiated in the resident genome by the process of distant hybridization. In **Fig.1** and **Fig.2** are represented few RGAP electrophoretic spectra of Aurolata-, Aurodes- and Aurotica-derivative wheat lines with marked variability.

RGAP primers combination		Results of Aurolata derivatives: Number of unique components	Results of Aurolata derivatives: Presence/Absence variation	Results of Aurodes derivatives: Number of unique components	Results of Aurodes derivatives: Presence/Absence variation
Pm8 LRR-L	XLRR rev	5	6	8	6
Pm8 LRR-L	Pm8 LRR-R	2	5	2	3
Pm8 MHD-L	RLRR rev	4	7	6	7
Cre3 Lr-L	Pm8 LRR-R	4	9	3	13
Cre3 P-loop	XLRR rev	8	2	2	8
Cre3 P-loop	RLRR for	4	5	7	7

Table 2. Characteristics of variability in RGAP electrophoretic spectra of Aurolata-derivative introgressive lines and Aurodes-derivative introgressive lines

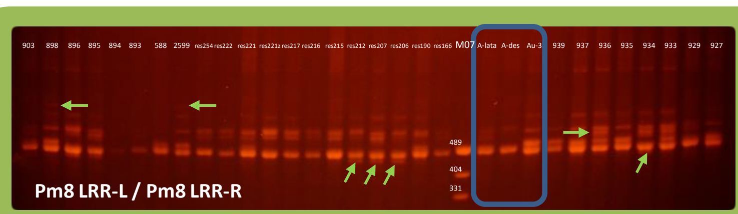

Fig.1 RGAP electrophoretic spectra of introgressive wheat lines that derived from Aurolata and Aurodes. Au-3 – Aurora cultivar; A-lata – amphidiploid Aurolata; Au-des – amphidiploid Aurodes; res166-res254, 588-903, 2599 – Aurolata-derived introgressive lines; 927-939 – Aurodes-derived introgressive lines.

Green arrows indicate new components in lines spectra in comparison with parental spectra.

RGAP primers combination		Number of unique components	Presence/Absence variation
Pm8 LRR-L	XLRR rev	2	3
Pm8 LRR-L	Pm8 LRR-R	4	2
Pm8 LRR-R	Cre3 Lr-L	2	2
Cre3 P-loop	Pm8 MHD-L	2	5
Cre3 P-loop	XLRR rev	4	3
Cre3 P-loop	RLRR for	3	4

Table 3. Characteristics of variability in RGAP electrophoretic spectra of Aurotica-derivative introgressive lines

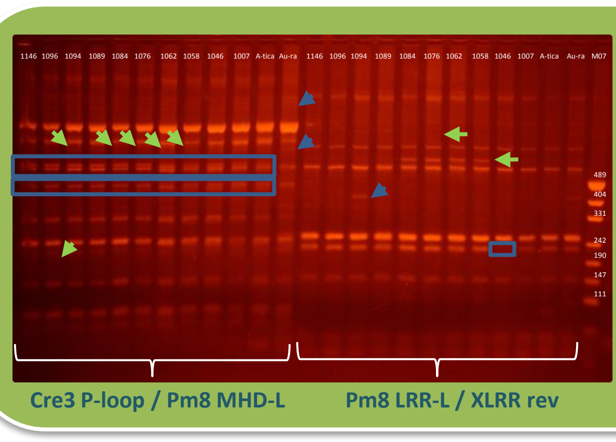

Fig.2 RGAP electrophoretic spectra of Aurotica-derived wheat introgressive lines.

A-tica – amphidiploid Aurotica, Au-ra – Aurora cultivar, 1007-1146 – introgressive lines.

Green arrows indicate new components in lines spectra in comparison with parental spectra. Blue arrows and rectangles indicate presence/absence variation.

Conclusions

According to the resistant phenotypes of introgressive lines and susceptible one of Aurora, all variations observed within RGAP spectra compared to the same in Aurora spectrum potentially can be associated with the powdery mildew resistance. Nonetheless, considering to the RGAP technique specificity (low annealing temperature), possible amplification of pseudogenes and LRR-containing receptor kinases regions should be taken into account. Consequently, determination of components associated with the resistance trait requires the further screening of F_2 segregating populations.

References

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