Genetic variability analysis of Romanian local populations of *Puccinia triticina* using SSR-PCR technique

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The aim of the study was to highlight differences at the molecular level between populations and monosporales of *Puccinia tritticina*. 19 populations and 14 monosporales from fields from Albota, Livada and Fundulea were used. Molecular tests have involved the use of 15 SSR primers and the electrophoretic separation of the amplificons.

The analyzes revealed a series of differences at molecular level between the populations as well as the monosporales. Based on analyzes conducted on populations / monosporales from 2015, compared to the previous years, it can be said that the degree of polymorphism at the molecular level is relatively low, too. Also, the results do not allow a clear correlation of the differences at the molecular level with the harvesting location and year. Instead, it seems that it can be made a grouping of *P.triticina* populations, by host plant of the pathogen: the analyzed populations were grouped into two main clusters (isolates from Durum wheat and common wheat).

Materials and methods

Table 1. Populations and monosporales of *Puccinia triticina*

No.	Population DuRes #	City/Origin	Year	Monosporales
1	IV/5	Albota/wheat, field	2012	MP5.28
2	VI/14	Fundulea/ wheat, plant	2012	MP14.1/14.6/14.7/MP14.26
3	IX/17	Fundulea/ wheat, field	2013	MP17.1/17.2/17.4/17.5
4	IX/18	Fundulea/ wheat, field	2013	
5	VII/23	Livada/ wheat, plant	2013	MP23-2
6	X/30	Fundulea/ durum wheat, field	2013	
7	I/42*	Fundulea/ wheat, field	2011	
8	XI/44	Fundulea/triticale, field	2012	
9	XIV/48	Fundulea/ wheat, field	2014	
10	XVI/50	Fundulea/ durum wheat, field	2014	
11	XVI/50bis	Fundulea/ durum wheat, field	2014	
12	XVII/51	Fundulea/ wheat, field	2014	
13	XXII/53	Albota/ wheat	2014	
14	XVII/54	Fundulea/ wheat, field	2015	Analyzed monosporales MP54.1/54-2
15	XIX/55	Livada/ wheat	2015	Analyzed monosporales MP55-2/55-4
16	XX/56	Albota/ wheat	2015	In progress
17	XXVI/57	Fundulea/ wheat, field	2015	In progress
18	XXVII/58	Albota/ wheat	2015	In progress
19	XXVIII/59	Livada/ wheat	2015	In progress

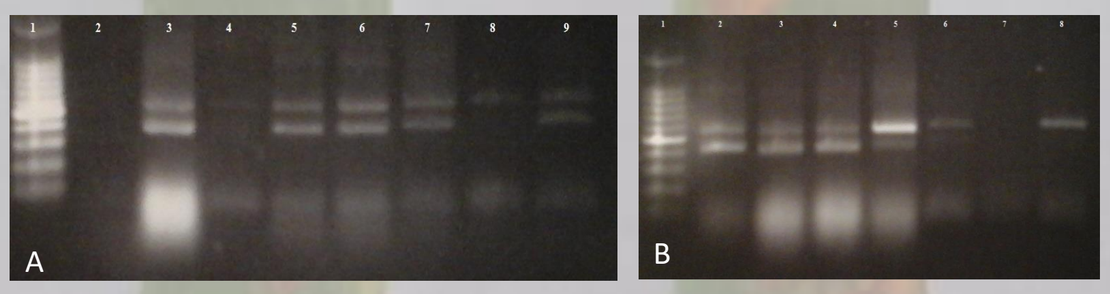
Isolation of genomic DNA from the spores of *Puccinia triticina* was performed using the method OmniPrep.

For PCR-SSR analysis, primers recommended by Szabo et al. (2007) and Duan et al. (2003) were used. The polymorphism in the resulting amplicons was obtained with primers PtSSR76/92/151a/154/173/184/186. The alleles have been emphasized by electrophoresis on 3% Metaphor agarose gel (Lonza) and on 6% polyacrylamide gel.

Results and discussions

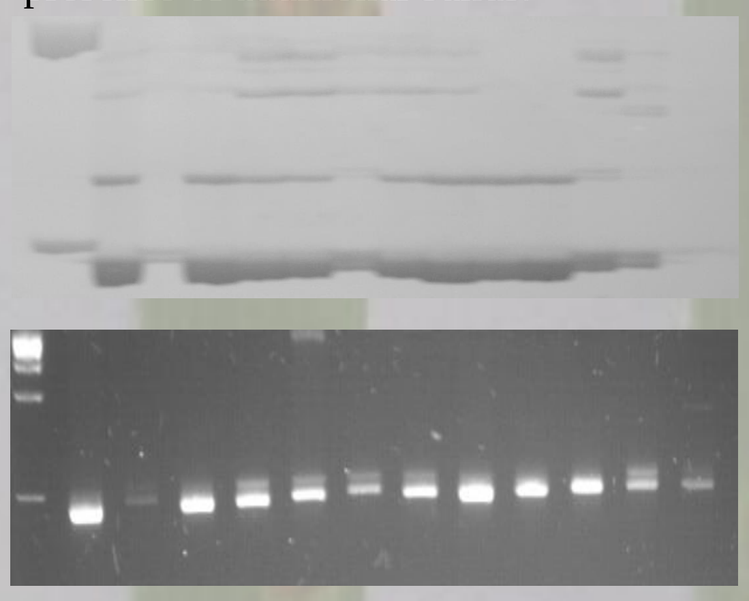
In the performed experiments were obtained clear two amplification products of ~590bp, ~430 bp respectively.

Comparative analysis of the amplicons obtained with the primer PtSSR184, using DNA from different populations of *P.triticina* (2012-2015) as well as from monosporales, has shown that, in general, the profile of the resulting amplicons is similar, irrespective of the year or location of isolation.



A. Line 3-9 DuRes 51/50BIS/53/54/MP54-1/MP54-2/56 2-8 DuRes
B. Line 57/58/59/17/MP55-2 /-/MP55-4.
Line 1 – 100 bp DNA ladder (Promega).

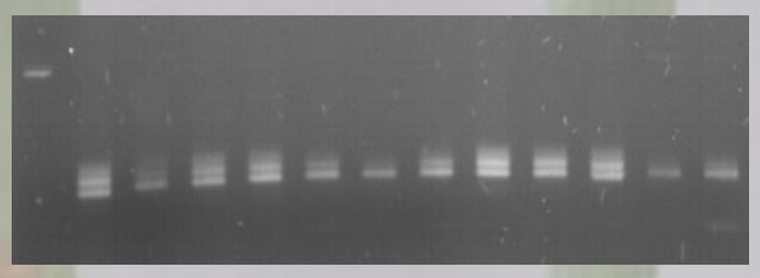
Analysis of the amplicons on 6% polyacrylamide gel, obtained by amplification of DNA from different populations (2014 and 2015) and monosporales of *Puccinia triticina*, allowed to emphasized some differences in the distribution of bands in the 470-495 bp domain, and the presence of additional bands.



Line 2-13 DuRes 51/50bis/53/54/MP54-1/MP54-2/56/57/58/59/MP55-2/MP55-4. Line 1-1Kbp ladder.

Line 2-13 DuRes 51/50bis/53/54/MP54-1/MP54-2/56/57/58/59/MP55.2/MP55.4. Line 1 – 1 Kbp DNA-ladder (Roth)

Separation of amplicons in 3% Metaphor agarose gel confirmed that the majority of the population and monosporales have at least two alleles with very similar dimensions (~ 500bp).



Line 2-13 DuRes 51/50bis/53/54/MP54-1/MP54-2/56/57/58/59/MP55-2/MP55-4. Line 1-1Kbp DNA ladder

In SSR-PCR analysis with primer PTR 92 alleles were identified in the range of 237 – 255 bp ± 5 bp, most populations analyzed revealed the presence of three distinct alleles, while in the monosporales were detected only 1-2 amplicons.

Line 2-13 DuRes 51/50bis/53/54/MP54-1/MP54-2/56/57/58/59/MP55-2/MP55-4. Line 1-1Kbp DNA ladder

ACKNOWLEDGEMENTS

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Conclusions

- When analyzing the pattern of DNA amplicons obtained from PCR-amplification with primers SSR 184/151a/154 there is a differentiation between certain populations harvested from durum wheat and common wheat.
- Differences between populations analyzed at the molecular level are relatively low and can not be correlated with the harvest year or location.
- Among those primers who allowed the detection of a polymorphism intra- and interpopulaţional were PtSSR 151a, PtSSR154 and PtSSR184 and they will be used in subsequent experiments.