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Genetic diversity of *Puccinia triticina* populations from Romania analysed by randomly amplified polymorphic DNA technique

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Abstract

The objective of the study was to analyse 10 populations of Puccinia triticina collected between the years 2011 and 2014 from different Romanian fields (Fundulea, Livada, Albota-Pitesti), using randomly amplified polymorphic DNA (RAPD) technique with 8 UBC primers. In the local populations were detected 19 RAPD phenotypes of P. triticina and the molecular phenotypes distinguished by each primer generally varied by 1 - 3 bands. There are currently 3 major groups of P. triticina in Romania as determinate by the UPGMA clustering analysis. This clustering arrangement has been influenced by the host selection and by the geographical area. A high degree of genetic diversity was found among leaf rust fungus populations isolated in the same year (2012) and field (Fundulea) from wheat and Triticales, with a similarity of 25%. The molecular variation observed within the P. triticina population isolated from wheat grown on Fundulea field suggested that a new phenotype arise in the year 2014. To our knowledge, this is the first report on the genetic diversity in the local populations of cereal rust pathogen, isolated from Romania.

Keywords: local population of *Puccinia triticina*, RAPD technique, genetic diversity

1. Introduction

Leaf rust is an important disease of wheat produced by *Puccinia triticina* Ericks, which annually causes significant yield losses in Europe and worldwide. To control the disease virulence surveys has been conducted in some European countries as well as the breeding of wheat cultivars with leaf rust resistance and regularly applied of fungicides (KOLMER & al. [5]; MANTOVANI & al. [6]). However, little is known about the virulence and genetic diversity of the *P. triticina* populations from southeast part of Europe, especially from Romania. The isolation and characterization of local fungal populations offer important information to understand the genetic mechanisms underlying variations in leaf rust fungus and to development effective strategies to control the disease. In the last years molecular changes in populations of rust fungus *Puccinia triticina* have been detected using several methods: amplified fragment length polymorphism – AFLP (KOLMER & al. [4]), random amplified polymorphic DNA – RAPD (KOLMER & al. [2]; KOLMER & al.[3]) and simple sequence repeat SSR markers (MANTOVANI & al. [6]; SZABO & al. [7]; VISSER & al. [8]). Using different molecular methods studies have showed that *P. triticina* isolates collected from durum and common wheat have a highly divergent selection driven by host genotype and by geographic location. Thus, isolates of *Puccinia triticina* from durum wheat collected from Ethiopia were distinct for SSR genotype compared with isolates obtained from

Europe, South America, and Mexico (KOLMER & al.[3]; MANTOVANI & al. [6]). Another study on the virulence and molecular polymorphism of the wheat leaf rust fungus isolates obtained from international collections distinguished 9 groups of isolates based on RAPD marker differences (KOLMER & al.[3]). Moreover, the research have proved there are a general relationship between virulence on wheat lines with resistance genes and RAPD phenotype (KOLMER & al.[3]). The objective of our work was to detect genetic variability in local populations of *P. triticina* collected during the last four years from different Romanian fields using RAPD technique with 8 UBC primers. To our knowledge, this is the first report on the genetic diversity in the local populations of cereal rust pathogen, isolated from Romania.

2. Materials and Methods

***P. triticina* isolates:** The populations of *P. triticina* were collected between 2011 - 2014 from wheat (W) and *Triticales* (T) from different Romanian fields: Fundulea (FUN), Livada (LIV) and Albota-Pitesti (ALB) (*Table 1*).

Table 1. *Puccinia triticina* populations used in RAPD analysis

No.	Population	DuRes no.	Abbreviation
1	I	42	FUN2011Wc42I
2	II	43	FUN2011Tc43II
3	III	19	FUN2012Wc19III
4	IV/V	5	ALB2012W5V
5	VI	14	FUN2012Wp14VI
6	VII	23	LIV2013W23VII
7	IX	17/18/25/26/27	FUN2013W19IX
8	XI	44	FUN2012T44XI
9	XIV	48	FUN2014Wc48XIV
10	XVII	51	FUN2014W51XVII

DNA isolation from *P. triticina* spores: It was performed using the commercial kit *OmniPrep for Fungus* (*Geno Tech*, USA) according to the manufacturer's instructions, with some modifications: the spores were incubated with 2 µl lyticase (*Sigma*) solution (200 U final concentration), at 37°C, for 45 minutes and mechanical disintegrated using Minibead beater equipment (*Biospec Products*, UK) for 90 seconds (DINU & al. [1]). DNA concentration was determined using using NanoDrop ND-1000 Spectrophotometer (*ThermoScientific*, USA).

Molecular analysis by RAPD: RAPD-polymerase chain reaction conditions, running and scoring of gels were previously described, as well as the 10-base primers UBC 402, 450, 489, 517, 519, 521, 538, 556 (*Table 2*). Briefly, 10 – 100 ng/µl DNA was amplified in 25 µl reaction volume using AmpliTaq mixture (*Applied Biosystems*, USA) and 2.5 µl primer (10 µM). The thermocycler was programmed at 94°C for 3 min; followed by 40 cycles of 1 min at 94°C, 2 min at 36°C and 2 min at 72°C; then 10 min at 72°C (KOLMER & al. [2]; KOLMER & al.[3]). The PCR products have been visualized by gel electrophoresis in agarose 0.8%. Sizes were estimated by comparison with DNA size markers, Benchtop 100 bp DNA Ladder (*Promega*, USA).

Table 2. The RAPD primer sequences (Kolmer et al., 1995; Kolmer et al., 2000)

Primer	Sequence(5'-3')
UBC517	GGTCGCAGCT
UBC519	ACCGGACACT
UBC521	CCGCCCCACT
UBC538	TGACCTCTCC
UBC556	ATGGATGACG
UBC450	CGGAGAGCCC
UBC489	CGCACGCACA
UBC402	CCCGCCGTTG

Data analysis: Primers UBC 519 and UBC 556 generated four and three polymorphic phenotypes, respectively, that were scored independently. All other primers generated only 2 polymorphic phenotypes. A 10-digit binary number based on the RAPD banding pattern for each of the 8 UBC primers was generated for each *P. triticina* population. The RAPD data were used to construct dendrograms with *DendroUPGMA clustering program* (Dice coefficient to compare between set of variables). The program calculates a similarity matrix, transforms similarity coefficients into distances, calculates cophenetic correlation coefficient (CP) and makes a clustering using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm.

3. Results and Conclusions

The genetic diversity in Romanian populations of *P. triticina* isolated between the years 2011 and 2014 from wheat and *Triticales* from different fields (Fundulea FUN, Livada LIV, Albota-Pitesti ALB) was detected using RAPD technique. Eight primers were selected based on the ability to amplify major DNA bands there were polymorphic between the *Puccinia triticina* isolates from worldwide collections (KOLMER & al. [3]).

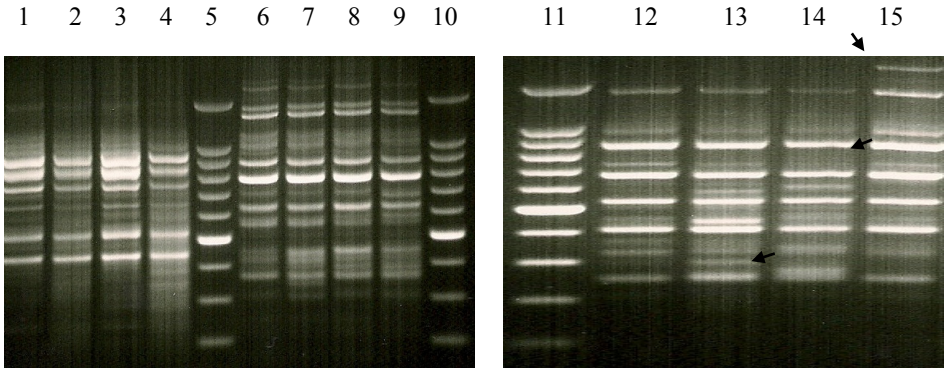

Figure 1. The RAPD analysis with UBC 489 and UBC 521 primers (left) and UBC 519 (right) for *Puccinia triticina* populations FUN2012Wp14VI(lanes 1, 6, 12), FUN2013W19IX (lanes 2, 7, 13) şi RB2012 (lanes 3, 8, 14) and single uredinial isolate MP26 (lanes 4, 9, 15). Benchop 100bp DNA Ladder (*Promega*) (lanes 5, 10, 11)

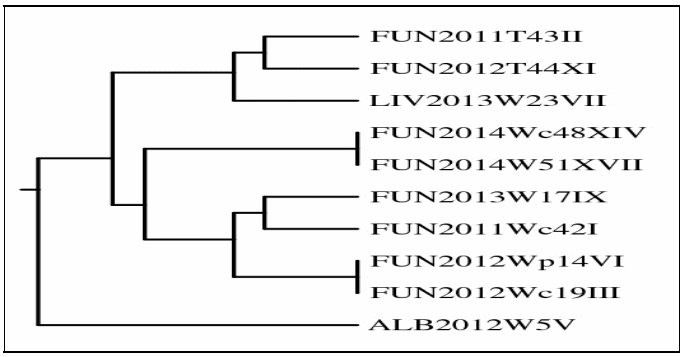

Figure 2. Dendrogram of *Puccinia triticina* populations based on DNA banding patterns of RAPD with 8 random decameric primers. The populations were isolated between 2011 – 2014 from different fields (FUN – Fundulea, LIV – Livada, ALB – Albota-Pitesti), from wheat (W) and *Triticales* (T)

Nineteen genotypes of *P. triticina* were distinguished by RAPD in the local analysed populations and the genotypes distinguished by each primer generally varied by 1 - 3 bands. Primers UBC 519 and UBC 556 generated four and, respectivelly, three polymorphic genotypes, while the others primers only two (*Figure 1*). In a study that analysed 64 single-uredinial isolates of *Puccinia recondita* f. sp. *tritici* from Canada using RAPD technique with 10 UBC primers showed that primers UBC 556 detected three genotypes, primer UBC 519 detected four genotypes, while primer UBC 531 detected five (KOLMER & al. [2]). The first cluster included the leaf rust fungus populations isolated from *Triticales* (Fundulea field) and from wheat (Livada area) with a similarity of 66.7 – 80%. Cluster 2 consisted of six populations isolated in the last 4 years from wheat grown on Fundulea field. First, the data showed that there is no difference between the population isolated from field (FUN2012Wc19III) and the same population obtained by germination of urediniospores in artificial laboratory conditions (temperature, humidity) (FUN2012Wp14VI). The similarity between population isolated in the year 2011 (FUN2011Wc42I) and populations collected in the years 2012 and 2013 (FUN2012W/FUN2013W) was 80%, while compared to population from year 2014 (FUN2014W) was 66.7%. This molecular variation within the *P. triticina* populations isolated between years 2011 and 2014 from Fundulea field wheat opens the possibility that a new phenotype arise in the year 2014. However, more fungus populations from year 2014 and further isolates need to be examined to address this issue.The *Puccinia triticina* population collected in the year 2012 from Albota-Pitesti field was found to have a highly different RAPD pattern compared to others populations and generated the 3rd cluster.

The present day arrangement of the three major clusters has been influenced by the host selection (*Triticales versus* wheat) and by the geographical area (Fundulea, Livada, Albota-Pitesti) of the *Puccinia triticina*. A high degree of genetic diversity was found among populations of leaf rust fungusisolated in the same year (2012) and field (Fundulea) from wheat and *Triticales,* with a similarity of 25%. Moreover, molecular analysis has revealed that fungal populations isolated from wheat from Fundulea, Livada, Albota-Pitesti fields were grouped in different clusters. The similarity coefficientbetween *P. triticina* population isolated in the same year (2012) from Albota area (ALB2012W5V) and Fundulea area (FUN2012Wp14VI) was 44.4%.

Table 3. Similarity matrix computed with Dice coefficient

	FUN 2011T 43II	FUN 2012T 44XI	ALB 2012W 5V	LIV 2013W 23VII	FUN 2014Wc 48XIV	FUN 2014W 51XVII	FUN 2013W 17IX	FUN 2012Wp 14VI	FUN 2012Wc 19III	FUN 2011Wc 42I
FUN2011T 43II	1	0.800	0.33	**0.667**	0.800	0.80	0.667	0.444	0.444	0.571
FUN2012T 44XI		1	**0.00**	**0.800**	0.500	0.50	0.500	**0.250**	0.250	0.333
ALB2012W 5V			1	0.333	0.400	0.40	**0.222**	**0.444**	0.444	0.286
LIV2013W 23VII				1	0.400	0.40	0.444	0.444	0.444	0.286
FUN2014W c48XIV					1	1.00	0.500	0.500	0.500	0.667
FUN2014W 51XVII						1	0.500	0.500	0.500	**0.667**
FUN2013W 17IX							1	0.667	0.667	**0.800**
FUN2012Wp14VI								1	1.000	**0.800**
FUN2012Wc 19III									1	0.800
FUN2011Wc 42I										1

3. Conclusions

The genetic diversity of ten leaf rust fungus populations collected from different Romanian areas was analyzed by RAPD technique with eight UBC primers distinguished nineteen phenotypes. The UPGMA cluster analysis showed that there are currently three major clusters of *Puccinia triticina*, arrangement that has been influenced by the host selection (*Triticales versus* wheat) and by the geographical area (Fundulea, Livada, Albota-Pitesti). This genetic variation within the *P. triticina* populations collected between years 2011 and 2014 from wheat grown on the Fundulea field suggested the possibility that a new phenotype arise in the year 2014.

4. Acknowledgements

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