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INTERNATIONAL SURVEY APPROACH FOR *PUCCINIA TRITICINA* OF WHEAT

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ABSTRACT – International Survey Approach for *Puccinina triticina* of Wheat
The main objective within new approach in international pathogenicity survey of *Puccinia triticina* was to provide genetically diverse sources of resistance (wheat lines with pyramiding resistant genes) to be used in a survey of wheat leaf rust pathogen in European-Mediterranean regions and to search for and document pathogenicity of *P. triticina* cultures useful in differentiating sources of resistance. Emphasis is placed on sources of resistance and their usefulness rather than on description of fungus populations.

Keywords: Puccinia triticina, wheat, International survey, hybrid lines.

INTRODUCTION

Leaf or brown rust caused by *Puccinia triticina* (*Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* Eriks. & E. Henn.) is probably the most important disease on the worldwide basis and yield losses may reach 40% in susceptible cultivars. Strategy for durability of leaf rust resistance in cultivars after the are released in agriculture is perhaps more important than achieving resistance in the first instance. The objective of cultivar management, regardless of epidemic probability, is to maximize the potential durability of deployed resistance. The global leaf rust population varies in virulence and this variation may result from one or more factors. The essential orientation for the international studies of the rust pathogens where their long distance dissemination as well established phenomenon. Wind is a great uncontrolled carrier of inoculum and urediospores of rust fungi are recognized as international travelers (ROELFS, 1985; BOŠKOVIĆ AND BOŠKOVIĆ JELENA, 2007). This was the mean reason why the best method of rust pathogen control was a network of international cooperative studies which would cover large epidemiological areas (BOŠKOVIĆ JELENA ET. AL., 2001; 2008A; MESTERHÁZY ET AL, 2000; GOYEAU ET AL, 2006; LI ET AL., 2010).

The importance and necessity of cooperative international investigations of the wheat rusts was especially emphasized by the European and Mediterranean Cereal Rusts Foundation. That was included first time in resolutions of Cereal Rust Conferences in Cambrige, 1964 and later on the others. Cooperative research of yellow rust of wheat for Europe had been organized in Netherlands, for stem rust in Portugal and Italy, and for leaf rust in Yugoslavia. The European Project of Wheat Leaf Rust Research had been started in Novi Sad dealing primarily with pathogenicity surveys of P. triticina in European-Mediterranean regions and breeding for resistance (BOŠKOVIĆ, 1966). From that time in International surveys for European-Mediterranean regions different sets of Lr lines have been used (BOŠKOVIĆ JELENA ET. AL., 2001). The same Lr lines used hadn't any value for European-Mediterranean regions. It was clear, even years ago, that these regions needed

new more efficient resistance genes and large testing and crossing program started in that time. At the beginning 18 donors of resistance had been selected after an extensive screening tests of several International rusts nurseries, for crossing with varieties Princ and Starke. Later on, eight of these hybrid lines with the most interesting donor, 66, 77, 26, 32, 46, 94 and 146, have been crossed with only effective genes Lr9, Lr19 and Lr24 (BOŠKOVIĆ JELENA ET AL., 2008B).

The main objective within new approach in international patogenicity survey of Puccinia triticina was to provide genetically diverse sources of resistance (wheat lines with pyramiding resistant genes) to be used in a survey of wheat leaf rust pathogen in European-Mediterranean regions and to search for and document pathogenicity of P. triticina cultures useful in differentiating sources of resistance. Emphasis is placed on sources of resistance and their usefulness rather than on description of fungus populations.

MATERIALS AND METHODS

The methods are applied according to the following approaches and procedure:
Central Field Nursery Each year in this field nursery numerous field materials from International rust nurseries as well as numerous breeding wheat lines from our program have been tested in the condition of artificial inoculations.
Central Seeding Test *P. triticina* collections from regional nurseries (ELRWN) have been sent to Novi Sad where has been cultured and there virulence to the source lines confirmed. When virulence to a given line is found and confirmed by greenhouse tests, that line should be removed from the field nursery and replaced by another line with potential value. This procedure is based on the concept of maximizing the number of sources of resistance to be studied. It is assumed that once virulent cultures are available, these cultures can be used to separate that line from other sources of resistance. Analysis of infection-type data has been done to distinguish between sources of resistance and to evaluate the usefulness of different sources of resistance in various places of the European-Mediterranean regions.

Cooperative Seeding Tests Uniform sets in European Leaf Rust Wheat Nursery (ELRWN) and possibly some other potentially useful sources of resistance, should be inoculated with several prevalent cultures by 6-8 cooperators in several countries well-disposed on European-Mediterranean territory.

Regional Field Nurseries (ELRWN) This approach should involvee testing of a uniform set of wheat lines to naturally occuring *P. triticina* populations at 20-30 sites in Europe and Mediterranean regions. The materials included should emphasize only wheat lines previously tested and shown to be highly resistant, and for which there is indication of diverse resistance genotype. Observations of leaf rust severity should be made by cooperators and sent to Novi Sad for assembling and summarization. The materials in these nurseries will also provide a basis for collecting uredial cultures which are virulent to some or all of the wheat lines. These cultures are used in further greenhouse and laboratory studies for differentation sources of resistance. The seedlings in the greenhouse where scored for infection type according to a scale 0-9 and variations were classified for easier computerization. Reaction classes (R, I and S) comprized the following variation of inffections types »R« - 1, 2, 3, 4, (0, 0; 1, 2) »I« – 5, 6, (X^-, X^+) and »S« - 7, 8, and 9 (3^-, 3^+, 4). Since the segregation was very frequent in the seedlings and in the field, that was designated by »,« For leaf rust and ather rusts the reactions are recorded by severity (0-99) and response (VR-S). In the field are recorded desease severity, the parentage of the

surface of the plant tillers and leavs affected, using the modified Cobb scale (PETERSON ET AL, 1984). Host response, the type of infections observed (R - resistant, I - all intermediate types and S – susceptible).

Severity is reduced to a single digit as follows: 0=0; 10=1; 11-25=2; 26-35=3; 36-45=4; 46-55=5; 56-65=6; 66-75=7; 76-85=8; 86-100=9. Host response is changed from R, I and S to 0-9 scale to computerization and deriving coefficient of infection. R= 0-3 or 2; I=4-6 or 5; S=7-9 or 8.

As a material have been used our hybrid lines with pyramiding resistant genes and other highly resistant wheat genotypes in ELRWN selected according to above explained procedures. In Central Field Nursery are included complete International Rust Nurseries and numerous of our breeding lines.

RESULTS AND DISCUSSION

In Central Field Nursery have been tested in the field eight International Rust Nurseries with total of 410 entries and seven spring wheat – CIMMYT Nurseries with 708 entries. In addition to Central Nursery have been tested hybrid progenies from the breeding program of accumulation, or pyramiding resistant genes. In breeding material were included 834 hybrid lines. Some selected of all these material have been tested in the greenhouse (seedling stage) to twenty-two international cultures of *P. triticina* from Regional Field Nurseries (ELRWN). Cooperative Seedling Tests in the second year included selected 36 winter and spring wheat entries in ELRWN. Seedling tests to particular pathotypes of *P. triticina* have been realized in the following countries: Germany (one pathotype), Czechoslovakia (two pathotypes), Sweden (one path.), China (three path.), France (four path.), Italy (two path.), Bulgaria (four path.) and Israel (five path.) – in total 22 pathotypes (Table 1).

A Regional Field Nursery (ELRWN) comprised in second year twenty of winter wheat hybrid lines with pyramiding resistant genes from our breeding program and sixteen highly resistant spring wheat lines, again selected from tested and analyzed International Wheat Rust Nurseries. Field ELRWN nurseries with 36 entries have been realized in 13 countries and evaluated to *P. triticina* and some other wheat pathogens: Germany (3 sites), Austria, Holland, Bulgaria, Israel, Sweden, Switzerland, Italy, Poland, Czechoslovakia, Spain, France and Chile.

All winter wheat hybrid lines with accumulation of resistant genes containing strong resistant genes Lr9, Lr 19 and Lr24 have shown very good results. But, there is a very slight difference between them in degree of resistance. The best were the lines NS-66/5×Lr24, NS-77/2×Lr19, NS-37/2×Lr19, then NS-66/2×Lr19, NS-77/3×Lr24, NS-66/4×Lr19, NS-26/2×Lr19, and NS-26/1×Lr9, NS-32/2×Lr19, NS-94/4×Lr19. These hybrid lines have had a little better combining ability from the genes of the donors and strong resistant genes Lr9, Lr19 and Lr24, which resulted, with some higher degree of resistance. Within spring wheat lines in ELRWN, the best results obtained were the lines 647-CMA-14793 and 26TH-ESWYT-10. Less resistance have had 26TH-ESWYT-36, 11TH-ESWYT-20 and 26TH-ESWYT-3. For these spring lines it can be supposed that they contain several resistant genes. Other spring lines have had insufficient resistance or quite susceptible reactions. The most typical were the lines Lr9, Lr19 and Lr24 which had been used in our breeding program for accumulation of resistant genes. It is clear that these lines loosed almost complete resistance as Lr9 and Lr24, but much less Lr19 (BOŠKOVIĆ JELENA AND BOŠKOVIĆ, 2009). It is important to compare these results of twenty wheat lines containing accumulated resistant genes with the same lines where have been reported

the segregation ratios of F_2 generations (BOŠKOVIĆ JELENA ET AL, 2001; 2008B). The number of resistant genes of these twenty lines in the table is very good correlated with results obtained in the seedlings and adult plants in ELRWN nurseries in Table 1.

Tab.1 – Seedling and field response in the second year ELRWN to *Puccinia triticina*

Winter wheat lines		Cooperative seedling tests			Field response	
		Reaction to 22 pathotypes of *P. triticina*			Reactions in 13 ELRWN	
		R	Seg.	S	R	S
1	NS-66/5×Lr24	22	-	-	13	-
2	NS-66/2×Lr9	21	1		13	-
3	NS77/2×Lr19	22	-	-	13	-
4	NS-77/3×Lr24	21	1	-	13	-
5	NS-26/1×Lr9	20	2	-	12	1
6	NS-32/2×Lr19	20	2	-	13	-
7	NS-37/2×Lr9	22	-	-	13	-
8	NS-66/4×Lr19	21	1	-	13	-
9	NS-26/2×Lr19	21	1	-	13	-
10	NS-26/2×Lr24	19	3	-	11	2
11	NS-32/1×Lr9	18	2	2	12	1
12	NS-32/3×Lr24	19	3	-	12	1
13	NS-46/2×Lr9	16	4	2	11	2
14	NS-46/3×Lr19	17	3	2	10	3
15	NS-46/3×Lr24	18	2	2	11	2
16	NS-94/2×Lr9	18	4	-	12	1
17	NS-94/4×Lr19	20	2	-	12	1
18	NS-94/5×Lr24	19	2	1	13	-
19	NS-146/1×Lr9	16	4	2	11	2
20	NS-146/3×Lr19	18	4	-	12	1
Spring wheat lines						
1	81-ND-582	14	1	7	10	3
2	417-ND-660	9	4	9	8	5
3	647-CMA-14793	22	-	-	12	1
4	11TH-ESWYT-20	18	4	-	8	5
5	11TH-ESWYT-25	15	5	2	10	3
6	11TH-ESWYT-30	6	7	9	7	6
7	26TH-ESWYT-3	18	2	2	10	3
8	26TH-ESWYT-10	22	-	-	12	1
9	26TH-ESWYT-36	21	1	-	10	3
10	26TH-ESWYT-49	12	6	4	8	5
11	26TH-ESWYT-50	12	3	7	9	4
12	Lr9	4	9	9	6	7
13	Lr18	2	5	15	5	8
14	Lr19	12	6	4	11	2
15	Lr24	6	2	14	8	5
16	Lr14	2	8	12	3	10

That means, correlation of degree of resistance of cooperative seedling tests to particularly pathotypes of *P. triticina*, as well as to degree of resistance in the field of the ELRWN in corresponding countries to the number of resistant genes in F_2 generations of each breeding combination (Table 2). Recently has been reported that pathogenicity studies of European populations of *Puccinia triticina* using pathogenicity and molecular markers resulted in 35 pathotypes identified from 68 isolates examined, all of which were avirulent for the genes

Lr9, Lr19 and Lr24, as well as to the other Lr's - Lr21, Lr25 an Lr29 (PARK ET AL, 1996; HYSING, 2007; ORDOÑEZ, AND KOLMER 2009).

Tab.2 – The segregation ratios in the F_2 generation of crosses between eight sources of resistance and Lr lines Lr9, Lr19 and Lr24 using three pathotypes of *Puccinia triticina.*

Cross	Pathotype								
	B.g.s.1289			Is.w.889			Chl.w.1489		
	Exp. Ratio	χ^2	P	Exp. Ratio	χ^2	P	Exp. Ratio	χ^2	P
NS-66/5×Lr24	57:7	0.35	0.55	57:7	0.53	0.48	54:7	0.16	0.82
NS-66/2×Lr9	57:7	0.24	0.62	9:7	0.15	0.70	45:19	0.77	0.37
NS77/2×Lr19	57:7	0.67	0.40	13:3	0.01	0.92	54:10	0.01	0.92
NS-77/3×Lr24	15:1	0.36	0.53	45:19	2.65	0.12	57:7	0.35	0.55
NS-26/1×Lr9	9:7	3.20	0.08	54:10	0.01	0.99	57:7	0.01	0.92
NS-32/2×Lr19	45:19	2.65	0.12	15:1	0.59	0.44	54:10	0.17	0.65
NS-37/2×Lr9	54:10	0.17	0.65	3:1	0.08	0.75	54:10	0.17	0.65
NS-66/4×Lr19	13:3	0.35	0.55	15:1	0.60	0.53	54:10	0.16	0.70
NS-26/2×Lr19	9:7	0.02	0.90	57:7	0.04	0.82	15:1	1.44	0.24
NS-26/2×Lr24	13:3	0.14	0.70	3:1	1.16	0.32	45:19	0.33	0.58
NS-32/1×Lr9	3:1	0.95	0.75	57:7	0.05	0.82	9:7	0.02	0.90
NS-32/3×Lr24	15:1	1.60	0.24	15:1	1.07	0.32	57:7	0.01	0.92
NS-46/2×Lr9	9:7	0.02	0.90	57:7	0.27	0.58	15:1	1.44	0.24
NS-46/3×Lr19	15:1	0.09	0.75	51:13	0.01	0.92	13:3	0.01	0.92
NS-46/3×Lr24	15:1	0.18	0.65	57:7	0.27	0.58	9:7	0.02	0.02
NS-94/2×Lr9	9:7	0.07	0.80	15:1	1.12	0.32	54:10	1.87	0.16
NS-94/4×Lr19	9:7	1.47	0.30	15:1	0.32	0.58	45:19	2.65	0.12
NS-94/5×Lr24	15:1	0.65	0.42	9:7	1.02	0.32	54:10	0.15	0.70
NS-146/1×Lr9	15:1	3.15	0.08	54:10	0.19	0.65	15:1	0.82	0.27
NS-146/3×Lr19	15:1	0.18	0.94	45:19	0.01	0.92	15:1	1.60	0.24

CONCLUSIONS

It is well known in the last time that combining or pyramiding of resistance genes into individual cultivars has had considerable success in reducing the rate of evolution of pathogens particularly in the situations where the pathogen does not reproduce sexually, as in the case of *P. triticina.* Considerable arguments for durability of cultivars with pyramided race-specific resistance genes have been already reported Samedifferences in F2 generation concerning the number of resistance genes related to particular pathotypes of *P. triticina* was already reported by other authors stating that differences can depend from different donors and pathotypes used.

In the time when we used the lines with strong genes Lr9, Lr19 and Lr24 in our breeding program that lines have had very high resistance on the large epidemiological territory, meanwhile, these lines loosed almost complete resistance, as Lr9 and Lr24 and much less Lr19.

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