TESTING OF WHEAT TO DURABLE RESISTANCE AND SURVEY APPROACH FOR *Puccinia recondita tritici*

Jelena BOŠKOVIĆ ¹, Momčilo BOŠKOVIĆ ² and Željana PRIJIĆ ¹

¹Faculty of biofarming, Sombor
²Faculty of Agriculture, Novi Sad


The main objective within new approach in international pathogenicity survey of *Puccinia recondita tritici* 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. recondita tritici* cultures useful in differentiating sources of resistance. Emphasis is placed on sources of resistance and their usefulness rather than on description of fungus populations. In this international survey new methods have been applied containing Central Field Nursery, Central Seedling Tests, Cooperative Seedling Tests and Regional Field Nurseries (ELRWN–European Leaf Rust of Wheat Nursery). The results have been reported from one year of investigations. ELRWN contained 20 winter wheat hybrid lines with pyramiding resistant genes including strong ones.
Lr9, Lr19 and Lr24. In addition, 16 spring wheat lines were included, as control lines were Lr9, Lr18, Lr19, Lr24 and Lr14. In that year ELRWN have been realized in 13 countries and cooperative seedling test in 8 countries using 22 pathotypes of \textit{P. recondita tritici}. The best results obtained by the winter wheat lines NS-66/5×Lr24, NS-77/2×Lr19, NS-37/2×Lr19 and spring wheat lines 647-CMA-14793 and 26TH-ESWYT-10. The results have shown losing almost complete resistance of Lr9 and Lr24, but much less Lr19.

\textit{Key words:} International survey, hybrid lines, leaf rust, resistant hybrid wheat lines.

**INTRODUCTION**

Leaf rust caused by \textit{Puccinia recondita} Rob. ex Desm. \textit{f.sp. tritici} Eriks. is the most widespread and regularly occurring of the three rusts found on wheat. It is found nearly wherever wheat is grown. Leaf rust fungus is adapted to a range of different climates, and the disease can be found in diverse wheat growing areas throughout the world.

Genetic resistance is the most economical and preferable method of reducing yields losses due to leaf rust. Wheat cultivars that are susceptible regularly suffer yield reductions of 5–15\% or greater, depending on the stage of crop development when the initial rust infections occur. To date, 51 leaf rust resistance genes have been designated and mapped in wheat. Resistance gene expression is dependent on the genetics of host-parasite interaction, temperature conditions, plant developmental stage, and interaction between resistance genes with suppressors or other resistance genes in the wheat genomes. The essential orientation for the international studies of the rust pathogens where their long distance dissemination was well established phenomenon. Wind is a great uncontrolled carrier of inoculums and urediospores of rust fungi are recognized as international travelers (ROELFS, 1985). 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 and BOŠKOVIĆ 1988, 2000, 2001; STUBBS et al. 1974).

Various wheat breeding programs throughout the world have had mixed results in producing cultivars with long-lasting, effective resistance to leaf rust. Spring and winter wheat breeding programs in the world have generally been very successful in producing cultivars that have had high levels of durable and effective resistance.

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 Cambridge, 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 *Puccinia recondita tritici* 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, 1980; Bošković and Bošković, 1988; Bošković et al., 2000). The most of these lines had not shown satisfactory efficiency for the surveys.

A comparison of pathogenicity of *Puccinia recondita* in Europe, The United States and Canada has shown big differences (Bošković and Browder, 1976). 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 extensive screening tests of several International rusts nurseries, for crossing with varieties Princ and Starke (Bošković and Bošković, 1993). 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 and Bošković, 1997, 2001; Bošković et al., 2006).

The main objective within new approach in international patogenicity survey of *Puccinia recondita tritici* 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. recondita tritici* 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. recondita tritici* 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 involve testing of
a uniform set of wheat lines to naturally occurring *P. recondita tritici* 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 differentiation 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 (BROWDER and
YOUNG, 1975). Reaction classes (R, I and S) comprised the following variation of
infections 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 other rusts the reactions are recorded by
severity (0-99) and response (VR-S). In the field are recorded disease severity, the
parentage of the surface of the plant tillers and leaves 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

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.
recondita tritici* from Regional Field Nurseries (ELRWN). Genetic differentiation in
resistance within these selected wheat hybrids or cultivars have been realized applying
computerized program with a Boolean algebraic approach to modeling the gene-for-
gene relationship in different environments (BROWDER, 1985).
Cooperative Seedling Tests in the second year included selected 36 winter and spring wheat entries in ELRWN. Seedling tests to particular pathotypes of *P. recondita tritici* 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. Average reactions to these pathotypes are summarized in 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. recondita tritici* and some other wheat pathogens: Germany (3 sites), Austria, Holland, Bulgaria, Israel, Sweden, Switzerland, Italy, Poland, Czechoslovakia, Spain, France and Chile. Summarized data of this ELRWN are presented in Table 1.

All winter wheat hybrid lines with accumulation of resistant genes containing strong resistant genes Lr9, Lr19 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 loosened almost complete resistance as Lr9 and Lr24, but much less Lr19.

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 F₂ generations, that is presented in Table 2 (BOŠKOVIĆ et al. 1999, 2000, 2006). 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. That means, correlation of degree of resistance of cooperative seedling tests to particularly pathotypes of *P. recondita tritici*, as well as to degree of resistance in the field of the ELRWN in corresponding countries to the number of resistant genes in F₂ generations (Table 2) of each breeding combination.
Table 1. Seedling and field response in the second year ELRWN to P. recondita tritici

<table>
<thead>
<tr>
<th>Winter wheat lines</th>
<th>Cooperative seedling tests</th>
<th>Field response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reaction to 22 pathotypes of P. recondita tritici</td>
<td>Reactions in 13 ELRWN</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>Seg.</td>
</tr>
<tr>
<td>1 NS-66/5×Lr24</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>2 NS-66/2×Lr9</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>3 NS77/2×Lr19</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>4 NS-77/3×Lr24</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>5 NS-26/1×Lr9</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>6 NS-32/2×Lr19</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>7 NS-37/2×Lr9</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>8 NS-66/4×Lr19</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>9 NS-26/2×Lr19</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>10 NS-26/2×Lr24</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>11 NS-32/1×Lr9</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>12 NS-32/3×Lr24</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>13 NS-46/2×Lr9</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>14 NS-46/3×Lr19</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>15 NS-46/3×Lr24</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>16 NS-94/2×Lr9</td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td>17 NS-94/4×Lr19</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>18 NS-94/5×Lr24</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>19 NS-146/1×Lr9</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>20 NS-146/3×Lr19</td>
<td>18</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spring wheat lines</th>
<th>81-ND-582</th>
<th>417-ND-660</th>
<th>647-CMA-14793</th>
<th>11TH-ESWYT-20</th>
<th>11TH-ESWYT-25</th>
<th>11TH-ESWYT-30</th>
<th>26TH-ESWYT-3</th>
<th>26TH-ESWYT-10</th>
<th>26TH-ESWYT-36</th>
<th>26TH-ESWYT-49</th>
<th>26TH-ESWYT-50</th>
<th>Lr9</th>
<th>Lr18</th>
<th>Lr19</th>
<th>Lr24</th>
<th>Lr14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14</td>
<td>9</td>
<td>22</td>
<td>18</td>
<td>15</td>
<td>6</td>
<td>18</td>
<td>22</td>
<td>21</td>
<td>12</td>
<td>12</td>
<td>4</td>
<td>2</td>
<td>12</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>9</td>
<td>-</td>
<td>4</td>
<td>5</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>15</td>
<td>6</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>8</td>
<td>15</td>
<td>5</td>
<td>7</td>
<td>6</td>
<td>12</td>
<td>12</td>
<td>10</td>
<td>8</td>
<td>5</td>
<td>9</td>
<td>15</td>
<td>14</td>
<td>10</td>
<td>12</td>
</tr>
</tbody>
</table>

Legend: R – resistant; Seg. – segregations; S – susceptible.
Table 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 recondita tritici*.

<table>
<thead>
<tr>
<th>Pathotype</th>
<th>B.g.s.1289</th>
<th>Is.w.889</th>
<th>Chl.w.1489</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross</td>
<td>Exp. Ratio</td>
<td>$\chi^2$</td>
<td>P</td>
</tr>
<tr>
<td>NS-66/5\texttimes Lr24</td>
<td>57:7</td>
<td>0.35</td>
<td>0.55</td>
</tr>
<tr>
<td>NS-66/2\texttimes Lr9</td>
<td>57:7</td>
<td>0.24</td>
<td>0.62</td>
</tr>
<tr>
<td>NS77/2\texttimes Lr19</td>
<td>57:7</td>
<td>0.67</td>
<td>0.40</td>
</tr>
<tr>
<td>NS-77/3\texttimes Lr24</td>
<td>15:1</td>
<td>0.36</td>
<td>0.53</td>
</tr>
<tr>
<td>NS-26/1\texttimes Lr9</td>
<td>9:7</td>
<td>3.20</td>
<td>0.08</td>
</tr>
<tr>
<td>NS-32/2\texttimes Lr19</td>
<td>45:19</td>
<td>2.65</td>
<td>0.12</td>
</tr>
<tr>
<td>NS-37/2\texttimes Lr9</td>
<td>54:10</td>
<td>0.17</td>
<td>0.65</td>
</tr>
<tr>
<td>NS-66/4\texttimes Lr19</td>
<td>13:3</td>
<td>0.35</td>
<td>0.55</td>
</tr>
<tr>
<td>NS-26/2\texttimes Lr19</td>
<td>9:7</td>
<td>0.02</td>
<td>0.90</td>
</tr>
<tr>
<td>NS-26/2\texttimes Lr24</td>
<td>13:3</td>
<td>0.14</td>
<td>0.70</td>
</tr>
<tr>
<td>NS-32/1\texttimes Lr9</td>
<td>3:1</td>
<td>0.95</td>
<td>0.75</td>
</tr>
<tr>
<td>NS-32/3\texttimes Lr24</td>
<td>15:1</td>
<td>1.60</td>
<td>0.24</td>
</tr>
<tr>
<td>NS-46/2\texttimes Lr9</td>
<td>9:7</td>
<td>0.02</td>
<td>0.90</td>
</tr>
<tr>
<td>NS-46/3\texttimes Lr19</td>
<td>15:1</td>
<td>0.09</td>
<td>0.75</td>
</tr>
<tr>
<td>NS-46/3\texttimes Lr24</td>
<td>15:1</td>
<td>0.18</td>
<td>0.65</td>
</tr>
<tr>
<td>NS-94/2\texttimes Lr9</td>
<td>9:7</td>
<td>0.07</td>
<td>0.80</td>
</tr>
<tr>
<td>NS-94/4\texttimes Lr19</td>
<td>9:7</td>
<td>1.47</td>
<td>0.30</td>
</tr>
<tr>
<td>NS-94/5\texttimes Lr24</td>
<td>15:1</td>
<td>0.65</td>
<td>0.42</td>
</tr>
<tr>
<td>NS-146/1\texttimes Lr9</td>
<td>15:1</td>
<td>3.15</td>
<td>0.08</td>
</tr>
<tr>
<td>NS-146/3\texttimes Lr19</td>
<td>15:1</td>
<td>0.18</td>
<td>0.94</td>
</tr>
</tbody>
</table>

**DISCUSSION**

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. recondita tritici*. Considerable arguments for durability of cultivars with pyramided race-specific resistance genes have been already reported (KOLMER *et al*. 1991; MUNDT, 1991).

Same differences in $F_2$ generation (Table 2) concerning the number of resistance genes related to particular pathotypes of *P. recondita tritici* was already reported by other authors stating that differences can depend from different donors and pathotypes used (SAMBORSKI and DYCK, 1982; SINGH and MCINTOSH, 1984).
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 (Bošković and Bošković, 1988, 1993, 1995; Bošković et al., 2000; Bošković et al., 2006; Paradies, 1980; Statler et al., 1985). Meanwhile, these lines loosened almost complete resistance, as Lr9 and Lr24 and much less Lr19 (Table 1). Recently has been reported that pathogenicity studies of European populations of *Puccinia recondita tritici* 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).

That demonstrated the complexity of genetic parasite: host interactions of these hybrids, which differ from the other hybrid combinations.

As we mentioned, further pyramiding resistant genes was realized by selection of the eight best hybrids with same resistant donors and crossed with only effective isogenic lines containing the strong genes Lr9, Lr19 and Lr24. Twenty progenies of F$_2$ generation with different crossing combinations have been tested with three different international pathotypes of *P. recondita tritici* (Bg.s.12/89, Is.w.8/89 and Chl.w.14/89). Differentiation of the pathotypes that used was from International survey of *P. recondita tritici* on differential Lr single gene lines with different virulence/avirulence formulae from distinct regions. The first culture was from Bulgaria, second from Israel and third was from Chile. Genetic analyses of twenty crossing combinations tested in the seedling stage with the first culture are presented in Table 2.

Different segregation ratios of resistant and susceptible plants in all crossing combinations proved that no one of the hybrids used did not posses strong genes Lr9, Lr19 and Lr24. Trigenic control of resistance was present in the hybrids: NS-66/2 × Lr9, NS-66/5 × Lr24 and NS-77/2 × Lr19. In crossing combinations NS-66/4 × Lr19 and NS-26/2 × Lr24 segregation’s ratio 13R : 3S showed epistatic dominant gene on one locus and epistatic recessive gene on the other. In F$_1$ × Lr (Bošković and Bošković, 2007; Bošković et al., 2008) of the same donors have had two or three resistance genes depending of the pathotype applied. The mentioned hybrid with strong genes proved the accumulation of these genes by progenies segregation to resistance susceptible plants. Incorporations of the strong Lr genes with segregation ratio 9R : 7S and two dominant complementary genes have shown the hybrids: NS-77/1 × Lr9, NS-26/1 × Lr9, NS-26/2 × Lr19, NS-46/2 × Lr9, NS-94/2 × Lr9 and NS-94/4 × Lr19, since the majority of these hybrids in F$_1$ × Lr have had only one resistant gene. Two isoepistatic resistant genes according to ratios 15R : 1S were present in the hybrids: NS-77/3 × Lr24, NS-32/3 × Lr24, NS-37/3 × Lr24, NS-46/3 × Lr19, NS-46/3 × Lr24, NS-94/5 × Lr24, NS-146/1 × Lr9 and NS-146/3 × Lr19. It is typical that these ratios were mostly with Lr24 and again the majority in F$_1$ × Lr has had only one resistant gene, which confirms accumulation of the strong resistant gene.

The same hybrid combinations tested with second culture of *P. recondita tritici* in Table 2. According to segregation ratios have proved successful incorporation of Lr9, Lr19 and Lr24. Comparing segregation ratios with the first
pathotype, it is expressed considerable influence of the culture used, which is related to the segregation ratio and number of resistant genes. Thus, the segregation ratios and number of the genes in all crossing combinations were different, except in the hybrids NS-94/2 × Lr9, NS-94/4 × Lr19, NS-94/5 × Lr24, NS-66/4 × Lr19, NS-77/1 × Lr9, and NS-32/3 × Lr24 where digenic resistant genes were found with both pathotypes and tregenic combination only in NS-66/5 × Lr24.

Segregation ratios with the third culture (Chl.w.14/89) of \textit{P. recondita tritici} in Table 2. have confirmed transferring of the strong resistance genes, with corresponding differences related to the pathotype applies, as with the previous two cultures.

As it is presented (BOŠKOVIĆ and BOŠKOVIĆ J., 2007; BOŠKOVIĆ et al. 2008) segregation ratios in F$_1$b$_1$ of eight resistant donors in the same recurrent parents Princ and Starke tested with three International cultures of \textit{P. recondita tritici} have shown presence of one, two and three resistance genes. Inheritance of resistance was mostly dominant, as it was reported by many authors (ROELFS \textit{et al.} 1992; JERKOVIĆ, 1992). Yet, the first investigations found that inheritance of resistance to \textit{P. recondita tritici} could be dominant, recessive and intermediate, depending of parents combinations (JERKOVIĆ, 1992; SCHACHERMAYR \textit{et al.} 1996).

Complementary acting resistance genes have been found in the hybrids NS-66/4 × Starke$^2$ and NS-32/2 × Starke$^2$ containing three resistance genes, similar with investigations of (SINGH and HUERTA-ESPINO, 2003). They reported in some crosses three resistance genes and two of them complementary acting. In identification of resistance genes Lr33 and Lr34 (DYCK, 1987; DYCK \textit{et al.} 1987; SINGH and HUERTA-ESPINO, 2003) it was found complementary effect of Lr34 with Lr33 or LrT3. It was expressed the influence of recurrent parent to the number resistance genes. Resistant donors 26 and 94 with Princ have had one resistant gene, but with Starke two genes. Similar influence of different recurrent parents was already reported (BOŠKOVIĆ, \textit{et al.} 2006).

Analyses of F$_2$ progenies in "pyramiding genes" and transferring of the strong Lr genes (Lr9, Lr19 and Lr24) tested with three different cultures of \textit{P. recondita tritici}, it was proved successful incorporation of these genes (Table 2). That was on the base of essential genetic rule that segregation ratios of all crossing combinations were to the resistant and susceptible plants in the progenies. If all progenies of the corresponding crossing combinations would expressed highly resistant reactions, as in the lines Lr9, Lr19 and Lr24, that would means the identity of resistant genes of the both parents used (BARTOŠ \textit{et al.} 1969). Inheritance of resistance in F$_2$ progenies was similar as in F$_1$b$_1$. The dominance of resistance prevailed, as in the papers (BOŠKOVIĆ, \textit{et al.} 1999) and complementary effects (JERKOVIĆ, 1992; BOŠKOVIĆ \textit{et al.} 2001a, 2001b, 2006). Considerable influence of the pathotype used to different segregation ratios and number of resistant genes in F$_2$ (Table 2), was reported by some other authors (GUPTA \textit{et al.} 1995).

Our further investigations in Serbia need to use reliable MAS which may help in resistance breeding of wheat and resistance gene pyramiding, providing durable resistance. However, the resistance gene that is actually present in the
material may be inactive or inefficient. Available markers should be verified with a possibly wide selection of wheat lines and, if possible, combined with studies of field resistance to rust fungi. The use of DNA markers helps to identify desirable genes more precisely and will facilitate transfer of R genes into wheat and even their pyramiding to derive cultivars with durable resistance to diseases. However, DNA gene marker reliability should be verified in diverse genetic backgrounds. It is particularly important in wheat accessions, considering the complex structure of their hexaploid genome. Gene bank accessions and cultivars of wheat often have additional numerous translocations from wild species. The development of new gene transfer techniques in the last decade allows direct biolistic or Agrobacterium-mediated wheat transformation. New biotechnological methods complement the conventional wheat breeding have opened new ways of transferring resistance genes into wheat cultivars and these new methods enrich conventional breeding methods, but cannot replace them (LUKASZ et al. 2003; BOŠKOVIĆ and BOSKOVIĆ J., 2007; BOŠKOVIĆ J. et al. 2008).

ACKNOWLEDGEMENTS
The authors are grateful for financial support by international and national projects and cooperators in projects.

Received May 17th, 2008
Accepted July 10th, 2008

REFERENCES


Glavni cilj u okviru novog pristupa međunarodne analize populacije patogeniteta *Puccinia recondita tritici* satojao se u uključenju genetički različiti izvora otpornosti (linije pšenice sa akumulacijom gena otpornosti) koji su upotrebljeni u analizi populacije patogenina lisne rde pšenice Evropsko-Mediterskog područja kao i analiza dobijenih podataka patogeniteta kulture *P. recondita tritici* korisnih u diferencijaciji izvora otpornosti. Istaknut je značaj izvora otpornosti i njihova korisna primena pre nego opisivanje populacije. U ovim međunarodnim analizama primenjene su nove metode u koje se uključuju testiranje centralnog poljskog rasadnika pšenice, centralno testiranje sejanaca, kooperativna testiranja sejanaca i regionalni poljski rasadnici (ELRWN - Evropski Rasadnik za Patogenu Lisne Rde Pšenice). Rezultati su saopšteni za jednogodišnja istarživanja. ELRWN je sadržavao 20 ozimih hibrida linija pšenice sa akumulacijom gena otpornosti uključujući jake gene Lr9, Lr19 i Lr24. Dodatno je uključeno 16 jarih linija pšenice kao i kontrolne linije Lr9, Lr18, Lr19, Lr24 i Lr14. U toj godini rasadnik ELRWN je bio realizovan u 13 zemalja, a kooperativno testiranje sejanaca u 8 zemalja upotrebom 22 patotipa *P. recondita tritici*. Dobijeni su najbolji rezultati sa ozimim linijama pšenice NS-66/5×Lr24, NS-77/2×Lr19, NS-37/2×Lr19 i jarim linijama pšenice 647-CMA-14793 i 26TH-ESWYT-10. Ovi rezultati su pokazali gubitak skore potpune otpornosti linija Lr9 i Lr24, ali mnogo manje linije Lr19.

Primljeno 17. V. 2008.