

**DURABLE RESISTANCE TO *Puccinia triticina* BY
ACCUMULATION OF RESISTANCE GENES**

Jelena BOŠKOVIĆ¹ and Momčilo BOŠKOVIĆ²

¹Faculty of Biofarming, Bačka Topola, Megatrend University, Serbia

²Faculty of Agriculture, University of Novi Sad, Novi Sad

Bošković J., and M. Bošković (2009): *Durable resistance to Puccinia triticina by accumulation of resistance genes* - Genetika, Vol. 41, No. 3, 353 - 378.

The individual use of single race-specific resistance genes with major phenotypic effects has rarely provided lasting resistance. However, breeding and combining or pyramiding of resistance genes into individual cultivars has had considerable success, particularly in situations in which the pathogen does not reproduce sexually, as in the case of wheat leaf rust pathogen. In European-Mediterranean region performed international investigations of wheat leaf rust proved that breeding of new lines of wheat resistant to *Puccinia triticina* Eriks. for differentiation of pathogen population, as well as for sources of durable resistance is necessary. Breeding of such resistant lines has proved necessary due to the

Corresponding author: Prof. dr Jelena Bošković, Faculty of Biofarming, Megatrend University Belgrade Maršala Tita 39 24300 Bačka Topola, Phone: 381 24 718 580; Fax: 381 24 712 209; E-mail: jboskovic@biofarming.edu.rs

unsatisfactory survey results of these regions on standard isogenic Lr lines. It has become clear that these regions needed new, more efficient differential resistance genes, as well as sources of resistance. In the beginning, after extensive screening tests of several International Rust Nurseries, 18 donors of resistance had been selected as crosses with recurrent parents' varieties Princ and Starke. These hybrid lines had been comparatively tested with twenty six Lr single gene lines using twenty especially virulent cultures of *P. triticina* in order to check the presence of these known Lr genes in our hybrid lines. Considerable influence of recurrent parent to the number of resistant genes in used donors was demonstrated. On the other hand, considerable influence of the pathogen culture was established to the number of resistance genes in used donors. In order to enhance resistance and pyramiding genes in these hybrids, the most interesting selected eight lines have been crossed with only effective isogenic ones, containing the strong genes Lr9, Lr19 and Lr24. On the basis of different segregation ratios of all crossing combinations it was proved that no one of resistant donors contained the applied strong resistant genes. It means that our hybrid lines contained resistant genes from the donors, as well as three strong resistant genes Lr9, Lr19 and Lr24.

Key words: accumulation of resistance genes, hybrid lines, *Puccinia triticina*, resistant hybrid, wheat lines.

.INTRODUCTION

Leaf rust, caused by *Puccinia triticina* Eriks., is an important foliar disease of common wheat (*Triticum aestivum* L.) worldwide. Breeding wheat cultivars with resistance to leaf rust is the most effective, economical and environmentally friendly method of disease control and was used in numerous wheat breeding programs (KOLMER, 1996). Pyramiding several major rust resistance genes into one adapted cultivar is one strategy for obtaining more durable resistance. Variability of wheat rust pathogen has always represented the greatest problem of successful selection of resistant cultivars. Sixty years ago international studies of wheat rust pathogens and greater testing of resistant genotypes have stated. In 1966 (BOŠKOVIĆ, 1966) in our country the centre for national and international studies of leaf rust pathogens was founded. During selection for resistance toward leaf rust pathogen, studies have been carried out in immediate link with the results of *P. triticina* population analysis results, on national, as well as on international level. Since the very beginning of the forming of the international centre for what leaf rust population differentiation, pathotypes have been used for testing of seedlings of great number of wheat genotypes and generational material. Beside this, in the international nurseries for leaf rust numerous lines of generational material of the selection to resistance were grown and tested such as in the Central Nursery in Rimski Sancevi. In European – Mediterranean countries our resistant species were tested in the nursery ELRWN.

In the paper are presented results of our long term studies on durable resistance to wheat leaf rust pathogene *Puccinia triticina* Eriks.

Types of resistance toward wheat rust pathogens

In 1905, more intensive studies and use of resistance toward wheat rust begun when BIFFEN (1905) proved that resistance toward yellow rust pathogen has inheritable character. Van der Plank (1963) was the first one who differentiated two resistance types; specific and horizontal, common or non-specific. Specific type is the most frequently occurring in plants as hyper sensible reactions and it is efficient only toward some variants of virulent parasites. Hypersensible reactions *Gramineae* are causers of cell death after infection. This process has been carried out simultaneously with forming of haustoria in host cell (HEAT, 1976). Specific resistance is preconditioned by one or several genes, while interaction between host and parasite create gene-for-gene relationships. In praxis it has been proved that specific resistance has not always been durable. LOEGERING (1961) explained lost of resistance in wheat rust by selection pressure and other agents, and it occurs in shorter or longer time period. In such a manner cultivar Ceres in USA preserved its resistance toward stem rust pathogen for the period of over nine years, while cultivar Bowie lost the same as soon as its production increased.

Common or non-specific resistance is preconditioned by more minor genes that take effect to whole pathogen population in longer time interval. In VAN DER PLANK publication (1984) this resistance type was discussed in more details in epidemiological sense, but inadequate genetic categorization aroused high controversy in scientific circles. It has been proved that genetic categorization sometimes does not correspond to reality. It has been established that resistance is not of polygenetic character but it depends upon one or several specific genes. Good example for this is resistance of all maize lines and hybrids toward *Cochliobolis carbonum* U11. It has been assumed that resistance is of horizontal character, for it has been efficient for over thirty years. However, it has been experimentally proved that it is preconditioned by one mayor gene, which means that it is specific resistance (SHARP, 1983) Parasite non-specific resistance preconditioned by host's morphological or functional characteristics was found, and these act to whole parasitic population. Similar occurs with excluding leaf rust pathogen from mechanisms of the stoma apparatus (ROMING and COLDWELL, 1964).

Combinations of more specific major resistance genes can provide effects of durable horizontal resistance. In India in such a manner wheat cultivars with combinations Lr1, Lr10, Lr13 and Lr14 preserved resistance in longer time period, while those with single genes showed higher susceptibility (RAO *et al.*, 1972). Last years similar resistance effects toward leaf rust pathogen are obtained also by accumulation, i.e. 'pyramiding genes' in selection to resistance. Cytoplasm can also have impact to efficiency and longevity of single major genes transferred into different parents. WASHINGTON and MAN (1974) have proved that the same mayor resistance gene in wheat cultivar Chris, under influence of cytoplasm of relatives *Triticum timopheevi* and *Aegilops speltoides* has different longevity and efficiency. It means that in this case specific major genes can show features of horizontal resistance.

Example of durable gene effect for specific resistance is known in Australia for *Puccinia triticina*. Wheat cultivar Timgalen that contained specific genes kSr5, Sr6 and Sr36 showed resistance in field conditions in the period of over twenty years. In interaction of host and parasite horizontal resistance can be defined only in the case in which specific resistance could not be proved. In terms, in this case the most suitable expression is type of durable resistance that was suggested by JOHNSON (1983), and it can be of specific or horizontal character.

Durable resistance should remain efficient in longer time period under different environmental conditions (JOHNSON, 1983). Testing to durable resistance must include two elements, long time period and wide area. It can not be accepted if the cultivar has been grown in experiments, regardless to the repeated studies at more localities during several years.

In order to eliminate disadvantages of the specific resistance more strategies have been developed and systematized (MAC KAY, 1987). Breeding of resistant cultivars by synthesis has been stipulated first, i.e. by building in of several resistance genes into one cultivar. It can give efficient protection in longer time period for the appropriate spectrum of pathogen virulence. This approach would comprehend also combination of specific genes that in interaction with the parasite maintain significantly lower infection type in regard to genes used as single ones (SOMBORSKI and DYCK, 1982).

NELSON (1978) named synthesis of specific resistance genes "pyramiding genes". KOLMER *et al.* (1991) emphasized that the most significant strategy has been selection to durable resistance of pyramiding genes. Certain programs that have been directed to combination of genes proved very successful in increase of durable resistance (ROELFS *et al.*, 1992). With gene accumulation more durable resistance has been maintained, for there have been small possibilities of corresponding virulence gene mutations in pathogen population (MUNDT, 1990). Durable resistance that lasted in the period 1966 – 1980 (KOLMER *et al.*, 1991) toward leaf rust has been stipulated for cultivars Chris, Era (Lr13+Lr34) and Columbus (Lr13+Lr16). In our breeding program we have been oriented toward accumulation of the most efficient strong genes and their wild relatives Lr9, Lr19 and Lr24. Many wheat lines of high specific resistance that gave very good results in longer time period on wide European-Mediterranean region were made (BOŠKOVIĆ *et al.*, 1995; 1996; 1999; 2001).

The second variant of synthesis has been directed toward use of moderate, i.e. mean efficient specific resistance toward whole spectrum of virulence, by which selection pressure of host to parasitic population has been slowed-down. ZADOKS (1972) described similar type of resistance also in the system *Triticum: Puccinia striiformis* Westend.

The following approach refers to use of specific resistance genes with differentiation in time. Successive change of single genes enables stabilization of selective pressure to the pathogen population. In such cyclic changes identical genes can be used again after appropriate number of years (VAN DER PLANK, 1982).

The last approach comprises in creation of spatial genetic diversity that can successfully counteract diversified virulence inside pathogen population. This is maintained by growing of multi-line, compound cultivars or by coordinated use of different resistance genes in certain geographic areas. Multi-line or compound cultivars attracted greater attention by way of creation and practical use. BORLAUG (1958) was the first one to initiate the idea of creation of these wheat cultivars in control of pathogen epidemics. Multi-line cultivar compiles of agronomic similar isogenic lines with differing resistance genes that should maintain parasite equilibrium. BORLAUG and GILBER (1953) used modified crossing method for creation of multi-line wheat cultivars against *P. graminis tritici*. BROWING and FREY (1969) distinguish that multi-line cultivars possess not only vertical but horizontal resistance as well, even tolerance. Many works in selection were directed to creation and growing of multi-line cultivars and their mixtures with the aim of obligatory parasite control. Multi-line cultivars have not been made especially for *P. triticina*.

Delayed sporulation of what rust pathogen is considered as very valuable characteristics of horizontal resistance type. ZADOKS (1972) analyzed components of horizontal type of resistance toward *P. triticina* in great number of wheat genotypes under identical conditions of inoculation. He found great differences between cultivars in pustule number and size, as well as in dynamic of pathogen penetration per unit of leaf area. Differences were also found between length of incubation period, sporulation dynamic, pustule development and infective period. Greater number of authors (PARLEVLIE, 1975, GUPTA and SINGH, 1982) differentiated components of delayed development of wheat and barley parasites; the frequency of infection, prolonged incubation period (latent period), size of a pustule and number of uredospores per pustule. Winter and summer wheat cultivars with delayed development of leaf rust significantly delay also epidemic in field. Delayed development of leaf rust parasite is more present in summer wheat cultivars. BJARKO and LINE (1986) established that this is horizontal type of resistance conditioned by three partially recessive genes. From the practical point of view significantly more valuable are cultivars with accumulation of resistance genes, for horizontal resistance does not offer complete protection (ROELFS *et al.*, 1992).

Partial resistance is the most important feature of horizontal resistance type, and it is expressed by significantly reduced diseases intensity, which results also with minor damages. In interaction of oat and *Puccinia hordei* Otth. basic component has partially extended period of incubation due to which number of rust uredo generations is reduced (PARLEVLIE, 1981). Studies have shown that this interaction causes mechanism of excluding parasite before forming haustoriae (NIKS, 1986). The author reported that partial resistance originates from association between reduction of forming haustoriae and reduction of infection without death of cells due to hyper sensible reaction. Presence of the susceptible infection type (PARLEVLIE and OMMEREN, 1875) results in great reduction in development of epidemics. Retarded fungi development after forming of haustoriae has been noticed, as well as smaller pustules at seedlings and adult plants (JACOBS, 1989). All listed components of the partial resistance are included in extended incubational period

(LP), and it is the most important in wheat infected by rust parasites (TANG *et al.*, 1977). In interaction of oat and *Puccinia hordei* inheritance of prolonged incubational period is preconditioned by several genes of minor effect, and majority of them express additive action (PARLEVLIET, 1981). For prolonged incubational period in system *Triticum aestivum*: *Puccinia triticina*, LEE and SHANER (1985) determined oligogenic inheritance two of four recessive genes with the identical effects. Two partially recessive resistance genes for LP have been reported for wheat genotype Suwon 85 as well (KUHN *et al.*, 1980).

Tolerance is the special type of resistance. Tolerant genotype is able to sustain corresponding infection level with significantly lower yield losses in contrast to other genotypes under identical conditions. For tolerance and horizontal resistance mutual feature is absence of specific selective pressure to the parasite population. Difference make occurrence of horizontal resistance from special natural obstacles of parasite development, while tolerance refers to adaptation of metabolism and energetic production of the host. Genetic bases of tolerance almost always have polygenic character, as it includes adaptation of whole host plant mechanism (SCHAFER, 1971). It has been defined (SCHAFER, 1971), but they have all accepted that tolerant cultivar suffers significantly lower losses in yield and quality. Selection to tolerance towards obligate and facultative parasites is very difficult, for it has not been able to select and evaluate material in generations of segregation. Selection to tolerance in some fungi and bacteria has been methodologically successful. SOTO *et al.* (1982) emphasizes that selection to tolerance can be successful in virus diseases that are transmitted by insects-vectors. Tolerance has been demonstrated by several authors in field trials, but there have not been special studies of physiology of mutual relationships of parasites and hosts. Due to this, selection in direction of tolerance arise special difficulties. BELL (1968) emphasizes that only knowledge of physiological causes of this phenomenon would enable direction of selection toward causing physiological processes related to tolerance. Tolerance toward *P. triticina* has been evaluated as in cases of other parasites by comparison of infection intensity and yield.

Finally, type of resistance that is based upon the mechanism of avoiding of the infection by host should be mentioned. This kind of resistance occurs when susceptible host stadium develops before occurrence of parasites and mass development of inoculums, as well as the most intensive attack. This resistance type is called pseudo-resistance. COLDWELL *et al.* (1957) have found that wheat cultivar Dual avoids harder infection of *P. triticina*, with only 4% of infected plants, whereas cultivar Trumbull had 90% infected ones.

Inheritance modes of wheat resistance to *Puccinia triticina*

Since the very beginning (MAINS *et al.*, 1926), studies of *P. triticina* resistance inheritance have provided basic scientific information for selection to resistance. MARTINEZ *et al.* (1953) performed comprehensive inheritance studies of resistance toward some parasite races in stadium of seedlings and adult plants, in combination of resistant wheat genotype NS No. II-39-2 (Premier x Bobin-Gaza-

Bobin) with susceptible cultivar Thatcher. Seedlings reactions depended upon two pair of resistance genes toward only some races, whereas field reactions were preconditioned with three pairs of genes.

Inheritance of resistance to several races of *P. triticina* was studied by FITZERALD *et al.* (1957) in combination of crossing of resistant wheat genotype Purdue Sel. 3369-61-1-10 with the cultivars AmBanner, Butler, Wabash, Seneca and Malakof. In some combinations of this crossings resistance depended upon two genes and it was inherited dominantly toward four races and recessively toward one. Other combinations indicated monogenetic inheritance. Depending upon races and parents pair's inheritance, resistance can depend upon one, two or three gene pairs (ALAN *et al.*, 1959). SCHAFER *et al.*, (1963) showed that incorporation of two or more resistance genes in one cultivar is much better, as parasite needs more virulence mutations to overcome this resistance.

For inheritance of resistance of the mature plant stadium for cultivars Rio Negro and Frondoza two complementary genes were established (RAO *et al.*, 1962). Studies of UPADHYAYA *et al.*, (1965) showed that in some crossings of three dominant resistance genes two proved to be complementary. Two complementary dominant and one recessive resistance gene were established in other crossing combinations. It was emphasized that modification genes are important for reaction toward *P. triticina* in the stadium of adult plants, but very susceptible to the influence of environmental factors (DYCK, 1987). Authors identified partially dominant resistance genes of the adult stadium Lr12 in the cultivar Exchange and Lr13 in the cultivar Frontana. In the same cultivar, beside gene Lr13 Frontana it was also found genes Lr1 and Lr15. It should be emphasized that this resistance of adult plants has been insufficiently studied regardless of the fact that for some of these resistance were established as monogenetic type and dependant upon single genes Lr12,13, 22a, 2b and 26 (ROELFS, 1984).

Inheritance of resistance in adult plants to cultivars, *P. triticina* was studied. Combinations of resistance genes of seedlings with genes resistance of adult plants are known. Lr1 and Lr10 stipulate resistance of seedlings, and Lr 22a is especially efficient in the stadium of third and fourth pair of leaves (BROWDER, 1980). The same, LrT2 with reaction of medium resistance of seedlings gives high resistance in the stadium of adult plants in the field (SAMBORSKI and DYCK, 1982). The same authors emphasize that LrT2 and Lr13 in interaction with other resistance genes influence to the significant increase of resistance level toward leaf rust pathogen. However, more resistance components of the adult plant stadium that are not sufficiently differentiable do not exist, especially when related to the influence of host development stadium, nutrition, quantity of inoculums, temperature and light (ROELFS, 1984). RUSSELL *et al.*, (1976) proved differences in expression of resistance of the adult plant stadium in wheat in interaction of host and pathogen.

In characterization of wheat resistance to leaf rust parasite MILUS and LINE (1980) identified seven resistance classes in the study of four components in the stadium of seedlings and adult plants. Phenotypically, these classes incorporated susceptibility, hyper sensibility and different levels of retarded growth of rust

included in resistance of adult plants. MODAWI *et al.* (1985) established that in susceptible cultivars Belinda and Thatcher in adult stadium of development significant reduction in number of uredopustals per cm² of the wheat flag leaf area.

According to MILUS and LINE (1980), retarded development of leaf rust as the basic component of resistance of adult plant stadium, can be easily identified in majority of wheat genotypes. According to some author, retarded development of leaf rust is inherited monogenetically or oligogenically (LEE and SHANER, 1985, JERKOVIĆ, 1992, PRIJIĆ and JERKOVIĆ, 2009), and it means that this form of resistance can be easily manipulated in breeding programs. Quantitative character of inheritance of retarded development of leaf rust was also reported. Inheritance of the prolonged incubational period (LP) in interaction of oat and leaf rust parasite *P. hordei* depends upon several genes with additive action. LEE and SHANER (1985) determined the oligogenetic inheritance of LP length in six wheat genotypes with retarded leaf rust development. Two to four partially recessive genes with identical effects for prolonged incubational period (LP) *P. triticina* were determined. KUHN *et al.*, (1980) found two partially dominant resistance genes with the identical effects in resistance inheritance of prolonged incubational period for the wheat cultivar Suwon 85. Similarly, BJARKO and LINE (1986) established two to three partially recessive genes for inheritance of this trait.

Models of gene-for-gene relationship host: pathogen

Based upon FLOR'S (1956) hypothesis, PERSON (1959) was the first one to develop theoretical model in the system gene-for-gene including five corresponding gene pairs (CGP) and by this he provided new directions in data analysis of infection types (IT) of rust parasites. Basic rule in the model was that genes for resistance and genes for virulence result in the type of susceptibility. The author determined host genotypes based upon increasing number of genes for resistance, pathogen genes based upon increasing number of genes for virulence, and these relationships were mathematically processed in details.

In the model of ROBINSON (1980) was applied the identical Person's rule, by which resistance genes of hosts and genes of high pathogenicity of parasites result in susceptible infection types, but with differently classified genotypes. The author assorted all alleles as susceptible as well as resistant reactions at the identical locus. The other gene assortments at corresponding locuses that differ from Person's model were given. In any case, Robinson's arrangements of genotypes of host and parasite interaction provide higher basis for logical approach to analysis of phenotypic data (IT) in gene-for-gene system. However, his arrangements of interaction genotypes are not based upon bionomical widening proposed by PERSON (1959). ROBINSON (1980) suggested that there exist other mathematical relationships that can be applied during classification of genotypes based upon nature of alleles at each locus.

MCINTOSH and WATSON (1982) broadened gene-for-gene relationship model in another manner. In their models, only two corresponding gene pairs are treated, but genes of low pathogenicity and genes that lead to reaction characteristic for resistant plants that in interaction provide low infection type (IT) are assorted.

Boolean's model is based upon concept of agricornus. This model applies Boolean's algebra symbolization that is very useful, as there are no traditional mathematic or biological marks. It has its special genotype that is integration of parasite and host interaction, and agricornus phenotype qualifies its genotype. LOEGERING *et al.*, (1971) developed computerized method of grouping host lines toward their reaction to the corresponding parasite culture out of which conclusions upon host and parasite genotypes can be made. Agricornus possesses genotype which is interaction of host and parasite, and phenotype is preconditioned by agricornus genotype (LOEGERING, 1984). This leads to the conclusion that genetic of agricornus is needed as well as genetic of host and parasite, and that it specially categorizes genetic between organisms and genetic within them. In this system interaction of host and parasite genotype toward relationships gene-for-gene and with use of the above given symbols can be presented by Boolean's scheme. Symbols in the scheme clearly suggest that for definite phenotype of agricornus, definite genotype of parasite as well as of host are needed, and it is the essence of the gene-for-gene concept. In such a manner it can be determined if two or more host lines differ in pathogenicity genotype. It is assumed that two host lines or two parasite cultures have alleles for reaction, or alleles for pathogenicity. Definitive phenotype in wheat rust is usually connected with resistance, while in interaction of some other parasites and host exist exceptions.

Many studies have shown that the plant infected by rust parasites resistant at moderate temperatures become susceptible at significantly higher ones. Most of the authors related these changes in response to host resistance genes, which are called "temperature genes" (EYAL and PETERSON, 1967).

Wheat allele Lr13 for resistance to *Puccinia triticina* in adult plant stadium, can be found in stadium of seedlings when plants are inoculated with the corresponding culture of parasites at 25.5°C. Also, resistance gene of adult plant stadium of wheat cultivar Atlas 66 was found with some cultures of *Puccinia triticina* at 5°C, at it was not discovered with identical cultures at 19°C. Delayed parasite development of wheat rust parasites in cultivar Suwaon 85 can be even visually observed when infected seedlings are grown at 5 and 12°C, but not when grown at 19 and 26°C (BROWDER and EVERSMEYER, 1984).

LOEGERING (1984) has already emphasized in Boolean's model great influence of temperature to agricornus phenotype, but he has not incorporated this influence into logical symbols of the system. Other mathematical models for genetic interaction between plant population and their parasite have also been known.

Sources of resistance and Puccinia triticina pathotypes

In 1966. in Novi Sad the International European Centre for Wheat Rust was founded (BOŠKOVIĆ, 1966). Since then, extensive testing of the world germ-plasm, i.e. of International wheat rust nurseries as Yugoslav selection materials in the Central nursery for wheat rust in Rimski Sancevi have been carried out. In this nursery genotypes in adult plant stadiums under conditions of artificial inoculations have been tested. In a greenhouse the identical wheat genotypes in the stadium of

seedlings with the European pathotypes *Puccinia triticina* have been tested (BOŠKOVIĆ, 1980). Based upon obtained results of these testings, resistance sources have been chosen, as well as the other resistant wheat genotypes for European Leaf Rust Wheat Nursery (ELRWN).

After many years of testing in the central nursery in such a manner two groups of genetically different sources of resistance have been selected to wheat rust pathogen. The first group of eight sources of resistance exhibited greater genetic differentiations and it has been used for complex breeding. In the second group eight other sources of resistance have been included that participated only in the first phase of breeding. At the time wheat cultivar Sava from Novi Sad was resistant, and was used as a control genotype.

Majority Lr isogenic lines selected up to 1980 showed susceptibility toward European pathotypes, i.e. races of *Puccinia triticina* (BOŠKOVIĆ 1971, 1976, 1980, 1988; BOŠKOVIĆ and BROWDER, 1976).

Transfer of resistance genes toward *P. triticina* into identical recurrent parents

1980. in cooperation with Prof. Mac Kay from Sweden begun breeding program of creation of European resistant lines toward *Puccinia triticina*, by transfer of new, more efficient genes into identical recurrent parents. Hybrid combinations of the first back-crossing (F_1b_1) of the first group of eight resistance sources with cultivars Prince and Starke were tested in stadium of seedlings with three international cultures *P. triticina* (BOŠKOVIĆ *et al.*, 1998; 2001; 2006).

Cultures of the resistant type of reaction "1" were taken from strong genes of wide spectrum Lr9 and Lr19, as these are more virulent. Hybrid material from these crossings is grown and tested in the Central Nursery in Rimski Sancevi with use of the pedigree method of breeding of resistant offspring in conditions of artificial nursery inoculations.

Relationship of segregation with the culture Yu-13-19-1 with each of eight resistance sources (66,77,26,32,37,49, 95,146) have shown that line 66/2 with recurrent parent Princ segregated to two resistance genes, and hybrid 66/4 with the cultivar Starke showed activity of three resistance genes. This relation of segregation suggests that there exist complementary action of genes. Significance of recurrent parent has been noticed, as the identical source of resistance with the cultivar Princ showed two resistance genes, and with the cultivar Starke three genes. In contrast to these, two hybrids of the following hybrid combinations: 77/1 x Princ and 77/3 x Starke, as well as I 32/2 x Princ and 32/3 x Starke have shown presence of only one resistance gene in both recurrent parents. The identical combinations of resistance genes occurred in lines 26/1 x Princ and 26/2 x Princ x Starke in regard to hybrids 94/1 x Princ and 94/2 x Starke. Both sources (26 and 94) with Princ had one resistance gene, and identical sources with Starke had two resistance genes. Three resistance genes have been determined in relationships of segregation lines 37/2 x Princ and 37/3 x Starke, and two resistance genes in hybrids 146/3 x Princ and 146/1 x Starke. The influence of the recurrent parent in line 46/2 x Princ with two resistance genes toward line 46/1 x Starke with one gene has shown again.

The relationship of segregation with another culture H-13-9-1 revealed no differences between the parents in the recurrent parents in crossing combinations 77/1 x Princ and 77/3 x Starke, followed by 37/2 x Princ and 37/3 x Starke, as well as 146/3 x Princ and 146/1 x Starke. For these hybrid combinations the identical results were achieved with both of the used pathotypes. Namely, both recurrent parents with a source of resistance 77 showed the presence of one gene, and with the source 37 three resistance genes and the source 146 two resistance genes. The identical results for both cultures were obtained by remaining hybrids, except lines 32/2 x Princ and 32/2 x Starke. The first one segregated in the presence of one, and the second in the presence of three resistance genes with complementary effect.

Analysis of segregation toward the third culture CZ-13-Ar-3 to the number of present genes in some breeding combinations with one and other recurrent parent revealed the identical results as well as for the previous cultures. It is interesting to emphasize that difference again occurred only in hybrid 32/2 x Princ and 32/3 x Starke. With this culture both named lines had only one resistance gene, i.e., identically as with the first culture. From this it can be concluded that genetic of host and parasite interaction for these two hybrids in certain measure is more complex in regard to the remaining hybrid combinations.

In the presented results of segregation relationship of F_1b_1 with three used cultures it is obvious that presence of one, two or three resistance genes was shown. Inheritance of resistance was dominant as it was established in many papers. Even the first works of resistance inheritance toward leaf rust pathogen suggested that there exists possibility of a dominant, recessive and intermediary inheritance, depending on the combination of parents (BOŠKOVIĆ *et al.*, 2001; 2006; 2008a, 2008b).

The influence of recurrent parent has also appeared to be significant, such as for instance sources of resistance 26 and 94 with Prince that had one resistance gene, and the identical sources with the cultivar Starke had two genes. Similar influence of different recurrent parents was also found by other author (MOMČILOVIĆ, 1969).

The other authors found different races or pathogen cultures show different genetic diversities in virulence. It is also confirmed by these researches as the identical crossing combinations with one culture show one resistance gene, and with other three or one.

With each of eight sources of resistance in recurrent papers in the selection process more lines were maintained, and fifteen homozygous were chosen to be tested in European Leaf Rust Nursery (ELRWN) on wide epidemiological area (BOŠKOVIĆ *et al.*, 2008a, 2008b; BOŠKOVIĆ and BOŠKOVIĆ, 2007).

Boolean's algebraic approach to modelling of mutual gene-for-gene relationships in the system *P. triticina*: wheat resistant hybrids: external conditions.

Number of resistance genes in sources of resistance, i.e. hybrids was established by analysis of F_1B_1 generation from crossings of sources of resistance

with susceptible cultivars Princ and Starke, based upon relationship of segregation. Of eight sources of resistance, three genes were established only in: 37-Bowie/Quaderna, S 66 R 5803 66- Nadadores 63, Traper (2), Lancer, Ks 62 136, Co 701354

For the named sources with three resistance pairs, the genetic analysis was performed according to the Model I of Boolean's algebraic approach to mutual gene-for-gene relationships in identical or different environmental conditions (BOŠKOVIĆ *et al.*, 1996).

In conventional genetic analysis of F_1B_1 generation of used resistance sources based upon relationship of segregation it is possible to determine only number of present resistance genes. Application of Boolean's algebraic gene-for-gene approach relationship illustrates significant advantages in genetic analysis in regard to conventional methods. This provides possibility for determination of characters and quality of resistance source that are important for their practical use. Boolean's algebraic approach to the models gene-for-gene relationship with three pairs of corresponding resistance genes for two sources, 37 and 66 in recurrent parents Princ and Starke, and in their variants and different environmental conditions illustrated several different features of this model. Extensions of Model I, applied in this paper differ from PERSON'S (1959) in the following. He did not use the identical concept for definitive variants, but he used terms "genes for resistance", and "genes for virulence", that produce susceptible genotype (S). This phenotype occurs where there are neither resistance genes nor virulence genes. In applied model in this paper, $1p \times 1h \times 1e$ always provide definitive agricornus 1 ap. Parasite and host genotypes that he classified according to increasing number of genes for resistance and genes for virulence. In this paper the factor of multiplication system was used in arrangement of genotypes. Person used the binomial methods of the model expansion in calculation of the expected number of phenotypes in serials, and in this paper binary formulas were applied for these calculations.

There exists the assumption that in this model there are no complementary effects of two or more definitive agricornus genotypes, but papers on complementary effects in the frame of wheat rust system are known (LOEGERING, 1984). Even if there exist complementary effects, given model remains as valuable and usable. Occurrence of definitive phenotype can be changed, but relationship of definitive versus non-definitive phenotype remains the same. Complementary effects of definitive agricornus genotypes should be included, as the additional complexity of this model.

Literature data in the field of resistance toward parasites is in great part based upon VAN DER PLANK'S (1982) classification of vertical and horizontal resistance. According to this classification, vertical resistance is characterised simply by inherited differences in phenotype. It is considered that vertical resistance in majority cases is controlled by gene-for-gene relationships. Horizontal resistance, according to the same author, is characterized by small, but very important differences in development of parasites in regard to different host genotypes. These differences relate to prolonged incubational period, reduced reproduction of

pathogens and, finally, reduced infectiveness of host. Commonly, it is believed that these differences are complexly inherited and that they are not under influence of parasite genotype. Definitive genotypes of agricornus that produce small differences in one environmental condition can produce far bigger differences of phenotypes if they are exposed to long definitive environmental conditions. It means that this partial function of definitive agricornus genotypes in non-definitive environmental conditions in any case is bases of horizontal resistance, i.e. resistance of slow-rusting resistance.

Various studies and publications support explanation of horizontal resistance as specific character of relationship parasite: host: environmental conditions, controlled by relationships gene-for-gene in different environmental conditions.

Accumulation of genes toward *Puccinia triticina*

Results of long lasting studies in the frame of international and national projects were outlined under scheme in which international analysis of *P. triticina* population and testing of new lines of accumulation of efficient resistant genes toward leaf rust pathogen in nurseries on wide epidemiological territory were included. According to this approach classifications and nomenclatures of pathogens were excluded, and everything is directed to better and more comprehensive practical solution of selection to resistance toward leaf rust pathogen (BOŠKOVIĆ *et al.*, 2001; BOŠKOVIĆ and BOŠKOVIĆ, 2007).

Central field nursery of leaf rust

During two years lasting testing of International nurseries for wheat rusts, in the Central field nursery some nurseries were included (BOŠKOVIĆ *et al.*, 2006; BOŠKOVIĆ and BOŠKOVIĆ, 2007) in a manner given.

Based upon reaction of seedlings of 1118 numbers of the Central nursery, twenty cultures of *P. triticina* from various epidemiological area, as well as reactions of testing of adult stadium in the field, 410 numbers were previously chosen from International leaf rust nursery. Remaining 708 were tested for the first time in CIMMYT's nurseries of summer wheat lines. In reactions of winter wheat lines seedlings, of the total of 410 numbers, 48% was resistant to all cultures, 42% differentially, and only 10% was susceptible to all cultures. This is completely understandable as tested materials originated from International nursery were chosen as resistant in previous years. Very close proportion per reactions were obtained from total number of 708 summer genotypes of CIMMYT's nurseries. In total, there were 53% of resistant lines per cultures, differential or in segregation 38%, and susceptible to all cultures only 9%. It revealed that CIMMYT's summer nurseries have numerous resistant wheat genotypes toward *P. triticina*.

Reactions of seedlings from 1412 tested lines from the Central nursery is performed in regard to twenty other new cultures of *P. triticina* from European collection, as well as reaction of adult stadium in the field suggest that maintained infection was sufficient. Of the total of 704 lines of the first group (of winter lines) in

the field there were 57% of susceptible ones. Similar occurred with the other group (of spring lines) in which of the total of 708, susceptible reactions occurred in 51% of lines. Susceptibility was also more expressed in reactions of seedlings. Of the total number of spring lines (CIMMYT's nurseries), 57% were susceptible, and of the total number of the first group (of winter lines), lower number, i.e. 33% were susceptible lines. There were corresponding differences between these groups and in other reactions of seedlings. In category of resistant seedlings toward all cultures in the first group there was 24%, in the second 17%; in the category of differential resistance or segregation in the first group 43%, and in the second 26%. These differences showed that in regard to resistance toward *Puccinia triticina* lines from the first group are genetically more unstable in regard to the second group lines.

Testing of seedlings of lines with accumulation of resistance genes

In the first year, winter selection materials were tested toward twenty pathotypes of *P. triticina*. As these were hybrid offspring obtained by complex breeding for accumulation of resistant genes, more detailed analysis of reaction toward used cultures of *P. triticina* was required. Hybrid lines represented offspring of breeding of eight donors of resistance (66, 77, 26, 32, 37, 46, 94 and 146) with recurrent parents Prince and Starke; after this the best lines of these offspring were crossed with genes that were only efficient, Lr9, Lr19 and Lr24. Lacking are only combinations of crossings 37 x Lr19 and 146 x Lr24, as all offspring from these crossings were excluded due to inconvenient recombination features (BOŠKOVIĆ, *et al.*, 2008a).

The most interesting are crossing combinations with the highest number of lines and spectrums of reaction, VR – very resistant and R – resistant, and these are: 66 x Lr19; 66 x Lr24; 77 x Lr24; 32 x Lr24; 94 x Lr9 and 94 x Lr19. From all of this it can be concluded that in good combining capabilities Lr24 with donors 66, 77 and 32, as well as Lr19 with 66 and 94, and Lr 9 only with 94. The other categories of weaker combining capabilities is observed from numeric relationships, and negative evaluation had also combinations of crossings in which somewhat more lines with spectrum reactions in segregation (LD,S; MD,S; HD,S; R,S; and S,R) were found. This segregation confirms genetic heterogeneity of corresponding hybrid lines toward used cultures of *P. triticina*. In selection to resistance, the aim is certainly choice of lines that show genetic uniformity of resistance toward reactions. As inside each of crossing combination there are 33-39 lines, further testing and choice of genetically uniform lines are possible. Higher numerical participation of lines with spectrum of reactions in segregation was seen for crossing combinations 55 x Lr19; 26 x Lr24; 32 x Lr9 and 37 x Lr24. In such a manner combination 77 x Lr19 had 18 lines with spectrum LD,S, three lines MD,S and 6 lines R,S, which makes 27 of the total of 39 lines of this crossing combination. Only 12 lines with reaction spectrum VR and R could be evaluated with genetically homozygote resistance. Numerical relationships in other numbered crossing combinations were similar, and in the following period, due to this for these offspring significantly more detailed testing and choice of corresponding homozygote resistant line is needed.

Based upon results of testing of hybrid offspring of lines with accumulation of resistance genes toward *P. triticina* nine different spectrum of reaction was differentiated, of which five was in segregation. This sufficiently clear suggested that significant number of lines of some crossings was heterozygote for resistance.

The most interesting were combinations of crossing with greter number of lines in reaction spectrum VR – very resistant and R – resistant. This indicates good combining capabilities of donor 66 with Lr19 and Lr24; and with Lr9 and Lr19. From this it is obvious that Lr24 combines better with corresponding donors than Lr9 and Lr19.

Next year selection material, i.e. numerous lines of certain crossing combinations of eight resistance sources (66, 77, 26, 32, 37, 46, 94 and 146), with recurrent parents Prince and Starke, and each of these combinations crossed with Lr9, Lr19 and Lr24 were tested with twenty new cultures of *Puccinia triticina*. New cultures are considered the other cultures, i.e. virulence spectrum of parasite in regard to previous year. Results of reactions, without field reactions, as all lines were resistant excluding lower number with occurrence of traces or low intensity infections of resistant type suggest significant segregation of infection types per plants that resembled to those from previous year. Significant participation of segregation confirms that young hybrid material is heterogeneous in greater measure to the feature of resistance toward parasite (BOŠKOVIĆ *et al.*, 2008a, 2008b)

It should be noted that lines 37 x Lr19 and 146 x Lr24 were excluded due to bad recombination features.

From comparison of the total number of 759 lines in regard to 834 lines from previous year it can be concluded that 75 lines of some crossing combinations were excluded due to dominant susceptible reactions in field or in stadium of seedlings. Also, results of reactions of the chosen basic lines of resistance sources with the cultivar Princ and several lines of inter-crossing of source of resistance excluded four lines. In the first year 39 lines were presented, and in the second 35 of them. It is noticeable that more lines with spectrum of resistance R (resistant) and LD (low differential reactions) in the second year, and this result from selection to resistance in field conditions.

Selection of hybrids with accumulation of resistance genes toward *P. triticina*

Detailed crossings to gene resistance accumulation to *P. triticina* were performed. Lines from previous crossings with the highest degree of resistance in stadium of seedlings with different *P. triticina* cultures, as well as in field trial and European nurseries for leaf rust were approved. Those were wheat lines with resistance sources under numbers 66, 77, 26, 32, 37, 46, 94 and 146.

Each of these lines was crossed with efficient genes in isogenic lines Lr9, Lr19 and Lr24. In our country and in the world for these lines were known for they were the only one of the created Lr lines that possessed wider spectrum of resistance (BOŠKOVIĆ, 1985).

Similarly as in previous crossings, hybrid material was grown in field trials, with use of artificial inoculation and pedigree method of selection in choice of

resistant plants. With the aim of resistance monitoring, offspring from this crossings were tested in F₂ generation with three *P. triticina* cultures parallel with parental components and susceptible control. These cultures were chosen from previously named twenty cultures from European nurseries, and all three were taken from resistant wheat genotypes of infection type '1' and minimal fructification of parasites. (BOŠKOVIĆ *et al.*, 2001).

Comparison of the results with resistance gene accumulation, possessed by both parents, and in reactions with different cultures, i.e. virulence of *P. triticina*, lead to complex genetic interactions with different relationship of segregation. However, novel approaches in analysis of genetic of host: parasite and environmental conditions relationship, by use of mathematic models gene-for-gene relationship provide possibility for better choice of the most comprehensive resistance sources. In such a manner, presence of specific or horizontal resistance is possible to determine as slow rusting and partial resistance, and etc.

Diversity of genetic interactions of these crossings with great influence of different crossing components, as well as *P. triticina* cultures, including varying of environmental conditions directs to complexness these genetic interactions. It means that new approaches and models in analysis of gene-for-gene relationships that offer significant advantages in regard to conventional methods need to be applied (LOEGERING, 1984; BROWDER, 1985; BOŠKOVIĆ *et al.*, 1998; 1999; 2000).

According to the novel approach to international analysis of leaf rust pathogen population, testing of seedlings with international pathotypes and multilocational testing of adult plants in nurseries was performed. According to this program, appropriate series of resistant genotypes in European nursery of wheat leaf rust (ELRWN), and possible, some other potentially useful resistance sources is inoculated with several prevalent cultures in six to eight countries by collaborators in these researches, and countries were well distributed on European – Mediterranean territory.

Identification and marking of Lr resistance gene using of DNA markers and their marking

Last years, based on DNK markers great advantage has been made in facilitation and shorting time and financials in identification of resistance genes. Leaf rust resistance genes: Lr1, Lr9, Lr10, Lr13, Lr19, Lr23, Lr24, Lr25, Lr27, Lr28, Lr29, Lr31, Lr34, Lr35, Lr37 and Lr47 have been mapped on chromosomes, usually by RFLP molecular markers. Improved breeding programs demand a series of molecular markers that are connected to resistance genes (PURNHAUSER *et al.*, 2000) Up to now, 26 different molecular markers have been reported that had been tightly connected to 14 different Lr resistance genes. The widest applied marker system is based on RFLPs. However, RFLP markers have been very difficult for use in the practical program of breeding. Therefore, now the costly and technically complicated RFLPs marker system is abandoned and work is transferred to use of basic PCR markers. Results of the authors emphasize the importance of DNA markers in characterization of resistance genes. In this, the aim in wheat breeding is also

creation of long lasting resistance toward pathogen by assistance of appropriate markers.

In comprehension study of the same authors named markers were chosen from the following isogenic lines of wheat rust: in TcLr3, 3bg with ISSR prajmer (ACTG94) and TcLr20 with RAPD prajmer (OP/J-17), and for Lr29 gene with OP/Y-10. It is clear that each of polymorph PCR fragments was detected, but it had been developed as the result by exchange in genomes with back crossing for incorporation time Lr resistance gene by back crossing. All of this suggested to research of new binding target sites for prajmers RAPD, SSR and ISSR.

The first molecular STS marker was discovered by SCHACHERMAYER *et al.*, (1996) for Lr9 gene derived from *Aegilops umbellulata*. Resistance genes toward leaf rust resistance Lr1, Lr9, Lr24, Lr28, Lr29 and Lr37 were mapped on hromozomes with RAPD markers; Lr19, Lr26 and Lr46 were mapped on hromozomes with AFLP markers. However, resistance genes toward leaf rust Lr1, Lr9, Lr10, Lr24, Lr28, Lr35, Lr37 and Lr40 were mapped with hromozomes with STS markers and leaf rust resistance genes Lr13, Lr16, Lr22a, Lr22b, Lr39 and Lr50 were mapped with hromozomes with SSR markers. Leaf rust resistance gene Lr51 was mapped on hromozomes with CAPS marker, Lr37 with SCAR marker. RFLP markers are reliable but costly, demand intensive work and need high purity DNA, so they were not used for marker-assisted breeding. From the reasons of practical use, RFLP markers that are completely linked with given resistance genes transferred into specific PCR markers, STS or CAPS markers and RADP markers that can be converted into SCAR markers (SYBIL, 2007).

Enzymatic marker (Endopeptidase Ep-D1c) for Lr19 was also developed and used (WINZELER *et al.* 1995). Beside this, microsatellite markers (simple sequence repeats – SSR) were used for resistance genes Lr3bg and Lr18 (PURNHAUSER *et al.* 2000). Checking studies of the isogenic line group (NIL) that carry different resistance genes with the studied coding serin/treonin protein cinase revealed polymorph DNA fragment in line with Lr10 resistance gene. This fragment was mapped on Lr10 where infection oat resistance gene locus was performed. Molecular description of these genes in wheat provides unique biological system for research of molecular basis of specific relationship between wheat and pathogen. Accumulation of different resistance gene in the classic process of breeding for creation of lasting resistance will be made easier by molecular markers (BOŠKOVIĆ *et al.*, 2008a; 2008b; PALLOOIX *et al.*, 2009). Lasting resistance can be achieved with combination of several genes of coded partial resistance in one cultivar, rather than in single resistance gene (JERKOVIĆ and TODOROVA, 1999).

BARTOS *et al.* (2000) used STS marker for Lr10 gene to identify this resistance gene in Czech cultivars Alka and Siria. Presence of these genes was studied by SCHACHERMAYER *et al.* (1996) in 62 offspring of wheat lines from European program of breeding with different genetic origin. Lr10 marker was found specifically high for Lr10 resistance gene and it was derived in 12 offspring. Development of PCR-basic allele specific markers in kinds of polyploidy is more complex than in diploid species, because PCR can result in amplification of multiple

fragments of similar size from more than one genome (HELGUERA, *et al.*, 2000, VIDA *et al.*, 2009).

Presence of Lr9, Lr19 and Lr24 genes in off spring was confirmed through identification of seedling tests. The most efficient were lines with resistance genes Lr9, Lr19 and Lr24. Lr9 is introgressed from *Aegilops umbellutata*. Presence of Lr19 was confirmed by specific strengthening of single fragment 130bp in Thatcher NIL (Tc*Lr19) and INIA66//CMH81A575. Leaf resistance gene Lr24 was introgressed from *Agropyron elongatum*. Of 41 lines of differently crossed combinations tested to leaf rust pathogen, 38 lines showed presence of Lr19. Lr24 gene is known to bind to Sr24 gene for wheat stem rust that is combination efficient against all kinds of rusts (KNOTT, 1989).

These studies with molecular markers connected with Lr isogenic lines (NILs) are very significant also for future studies in our country, for better and more successful work on breeding to resistance toward leaf rust pathogen.

Mapping of leaf rust resistance genes in wheat

Location of chromosomes is basic step for identification and understanding of allele links of resistance genes (BOŠKOVIĆ *et al.*, 2001). Majority of known determined wheat rust resistance genes was identified through cytostatic analysis by use of aneuploidias. Monosomic analysis is more mutually used cytogenetical method in wheat (KNOTT, 1989) and adequate method for location of dominant genes. Until recently, chromosome locations of many mapped leaf rust resistance genes were determined by use of monosomic analysis and telocentric mapping. SEARS (1954) has developed set of 21 monosomics for each chromosome by use of bread wheat cultivar *Chinese spring*. Monosomic analysis includes crossing of 21 monosomics as female parent with resistant cultivar that possesses unknown resistance genes. F1 plants with 41 chromosome allowed self-pollination and F2 plants have evolved rust resistance. Monosomic that coincide with location of gene chromosome provides reverses segregation into F2 and the following segregation of generations (KNOTT, 1989). SEARS (1954) has also produced addition chromosome lines and other kinds of aneuploidia (multisomic, trisomic, tetrasomic) that have also been used in mapping. Monosomic analysis often have often been combined with telocentric mapping in which ditelosomics developed by SEARS (1966) used for discovering of chromosomes of location position (locus) and distance gene recombination from centromere. These cytogenetical stocks have also recently been used for determination of molecular marker's positions.

Aneupliods have not been so well tolerated in comparison tetraploids with hexaploids (JOPPA, 1987). Monosomics in tetraploid wheat's often exhibit slight advantage and instability. JOPPA (1987) have produced a set of 14 different disomic-replaceable lines with 13 pairs of hard wheat (*cv. Langdon*) chromosome and a pair from D-genome of chromosome by replacing homologous chromosome pair from A i B genomes.

Recent studies have shown that aneuploidias have been studied and obtained by may efficiently be used for discovering of positioning of chromosome features in

wheat (HUSSIEN *et al.*, 2005; SINGH *et al.*, 2004). Two novel genes have temporarily been determined; Lrac104 and Lrac124 have been located on chromosomes 6B and 4A, separately for each, have used trisomics for determination of chromosome loci of recessive genes at chromosome 2B in wheat HD 4502.

Position of chromosomes of several leaf rust resistance genes created from hard *Emmer* wheat has been determined. One of the alleles, Lr14, known as Lr14a, had been created from *Emmer* wheat of the cultivar *Yaroslav* and it has been transformed into *T. aestivum* cultivars *Hope* and *H-44* that had been used in mapping. (MCINTOSH *et al.*, 1967; MCINTOSH *et al.*, 1995). Lr23 had been transformed in hexaploid of wheat from *T. turgidum ssp. durum cv. Gaza* and located in chromosome 2BS. Determined location of gene that was transferred into wheat from *T. dicoccoides* into chromosome 6BS. DYCK and SYKES (1994) transferred two wheat rust resistance genes from *T. turgidum ssp. dicoccoides* into hexaploid wheat, the one that was identical with Lr33.

In last decades molecular markers were used for mapping gene chromosomes that determine simple inheritance of traits, or for finding of new quantitative features of loci (QTL) that are often connected with more complex features (ZHOU *et al.*, 2003). The most numerous mutually applied molecular techniques for the start up leaf rust resistance genes were RFLP (polymorphism of restriction fragments length). However, later on, markers that are closely related to gene of interest and PCR- basic, easy to use markers (STS, SSRs, ISSRs) were identified and developed for some of these genes.

P. triticina is wheat parasite that often and in very short period can cause serious yield losses up to 40%. In order to reduce such losses, with their researches, breeders reached ecological and the most efficient method and it is use of molecular markers. Molecular markers have found wide application in breeding of plants. They do not depend upon environmental conditions, can be found in each tissues and phases of plant growth and owe high polymorphism. Breeders have isolated 51 resistance gene toward wheat leaf rust pathogen by molecular markers. Of all resistance genes, Lr9, Lr19 and Lr24 are considered as the strong ones. And long-lasting resistance is achieved with combination of two or more Lr genes. It is considered that improved strategy of breeding relates to integration of molecular markers into conventional breeding programs, for this leads to breeding efficiency that could not be reached earlier; this shortened the process, and financial costs have been reduced.

ACKNOWLEDGEMENTS

The authors appreciate financial support by international and national projects and collaborators in projects. This paper has been dedicated to Academition Slavko Borojevic for his contribution in development of this field of science, as well as for his constant support to the authors of the paper.

Received September 7th, 2009.

Accepted December 11th, 2009.

REFERENCES

- ALAN, E.R., E.G. HEYNE, E.T. JONES, and C.O. JOHNSTON, (1959): Genetic Analysis of Ten Sources of Hessian Fly Resistance, Their Relationship and Association with Leaf Rust Reaction in Wheat. Technical Bulletin 104.
- BARTOS, P., A.HANZALOVA, and V.BLASKOVA (2000): Leaf rust resistance of winter wheat cultivars registered in the Czech Republic. Acta Phytopathol. Entomol. Hung. 35(1-4): 149-151.
- BELL, G.D.H. (1968): Plant Breeding for crop improvement in Britain: methods, achievements and objectives, Proc. Roy Soc. B. 171,145-173.
- BIFFEN, R.H. (1905): Mendel's laws of inheritance and wheat breeding. J.Agr.Sci. 1,4-48.
- BJARKO, M.E. and R.F. LINE (1986): Inheritance of latent period, infection type and area under disease progress curve in two slow rusting spring wheat cultivars. (Abstract). Phytopathology 73,841-842.
- BORLAUG, N.E. (1958): The use of multiline or composite varieties to control airborne epidemic disease of self-pollinated crops. 1st International Wheat Genet. Symp.Proc., of Univ. of Manitoba, Winnipeg, Canada, p. 12-27. Public Press, Ltd. Winnipeg, 1958.
- BORLAUG, N.E. and J.W. GIBLER (1953): The use of flexible composite wheat varieties to control the constantly changing stem rust pathogen. Agron. Abstr., 81.
- BOŠKOVIĆ, J., M. BOŠKOVIĆ, and G. PRODANOVIĆ (1995): Enhancement of resistance to *Puccinia recondita tritici* of wheat by incorporation of the strong genes in sources of resistance. European Journal of Plant Pathology XIII International Plant Protection Congress, The Hague, The Netherlands, 2-7 July 1995, Paper No. 1002.
- BOŠKOVIĆ, M. BOŠKOVIĆ, and Z.JERKOVIĆ (1996): Combinations of unknown and strong resistance genes of wheat to *Puccinia recondita* f. sp. *tritici*. Cereal Rust and Powdery Mildews Bulletin, Volume 24, Supplement: 248.
- BOŠKOVIĆ, J., M. BOŠKOVIĆ, V. PEŠIĆ, G. PRODANOVIĆ, and V. PEŠIĆ (1998): Durable resistance to wheat leaf rust pathogen by breeding genotypes with pyramiding resistant gene. Abstracts, XVIIIth International Congress of Genetics, August 10-15, 1998, Beijing, China, 8P268.
- BOŠKOVIĆ, J., M. BOŠKOVIĆ, Z. JERKOVIĆ, G., PRODANOVIĆ, and V. PEŠIĆ (1999): Durable resistance to wheat leaf rust pathogen combination of resistance genes. XIV International Plant Protection Congress, Tel Aviv Israel. Abstract. p. 76.
- BOŠKOVIĆ, J., M. BOŠKOVIĆ, M. BABOVIĆ, Z. JERKOVIĆ, and V. PEŠIĆ (2000): Pyramiding strategy for durable resistance to wheat leaf rust pathogen. Wheat in a Global Environment. 6th International Wheat Conference, 5-9 June, 2000 Budapest, Hungary, p. 55.
- BOŠKOVIĆ, J., M. BOŠKOVIĆ, M. BABOVIĆ, Z. JERKOVIĆ, and V. PEŠIĆ (2001): Pyramiding strategy for durable resistance to wheat leaf rust pathogen. Wheat in a Global Environment. Developments in Plant Breeding, Volume 9, Eds. Z. Bedo and L. Lang. Kluwer Academic Publishers, p.804. Hardcover, May 31, 2001, 1st edition.
- BOŠKOVIĆ, J., M. BOŠKOVIĆ, and Ž. PRIJIĆ (2006): Strategija akumulacije gena za dugotrajnu otpornost prema patogenu lisne rđe pšenice. Treći simpozijum sekcije za oplemenjivanje organizama. Zlatibor, 16-20 maja 2006. Zbornik apstarkta, str.15, I-Usm-10.
- BOŠKOVIĆ, J., M. BOŠKOVIĆ, and Ž. PRIJIĆ (2008a): Accumulations of genes for durable resistance to wheat leaf rust pathogen. Journal of Agriculture Science. Vol. 53, No 3. 2008. p. 163-172.

- BOŠKOVIĆ, J., M. BOŠKOVIĆ, and Ž. PRIJIĆ (2008B): Testing of wheat to durable resistance and survey approach for *Puccinia recondita tritici*. Genetika, vol. 40, N^o. 2, 95-107.
- BOŠKOVIĆ, M.M. (1966): The European Project of Wheat Leaf Rust Research. Contemporary Agriculture. Vol. 14, N^o. 11-12, 607-611.
- BOŠKOVIĆ, M.M. (1971): Uticaj lisne rde i pepelnice na prinos nekih sorti pšenice u Jugoslaviji. savremena poljoprivreda, XIX 4:81-88.
- BOŠKOVIĆ, M.M. (1976): International Pathogenetic Survey of *Puccinia f. sp. recondita tritici*. proc. of the 4th Europ. and Mediter. Cereal Rust Conf., Interlaken, 75-78.
- BOŠKOVIĆ, M.M. (1980): The use of some new differentials in international pathogenetic surveys of *Puccinia f. sp. recondita tritici*. proc. of the fifth Europ. and Mediter. Cereal Rust Conf. Bari, Italy, 185-190.
- BOŠKOVIĆ, M.M. (1985): Ispitivanje virulentnosti *Puccinia recondita f. sp. tritici* i testiranje materijala pšenice na specifičnu otpornost. Zaštita bilja, Vol. 36 (4), br. 174. 345-356.
- BOŠKOVIĆ, M.M. (1988): International Pathogenetic Survey of wheat Leaf Rust Pathogen and Sources of resistance. Arab Journal of Plant Protection 4:128-135.
- BOŠKOVIĆ, M.M. and L.E. BROWDER (1976): A comparison of pathogenicity of *Puccinia recondita tritici* in Europe, The United States and Canada. Plant Dis. Repr. 60:278-280.
- BOŠKOVIĆ, M. and J. BOŠKOVIĆ (2007): Sistem nomenklature u analizi populacije *Puccinia recondita tritici*. Str. 287-321. Poglavlje. Naučna publikacija. NAUKA OSNOVA ODRŽIVOG RAZVOJA. Izdavač Društvo genetičara Srbije..Str. 339.
- BOŠKOVIĆ, M., L. BROWDER, J. BOŠKOVIĆ, and Z. JERKOVIĆ (2000): International survey approach for *Puccinia recondita tritici* of wheat using multilocation testing. Acta Phytopathologica et Entomologica Hungarica 35 (1-4), 351-358.
- BROWDER, L. E. (1980): A compendium of information about named genes for low reaction to *Puccinia recondita tritici* in wheat. Crop Sci. 20, 775-779.
- BROWDER, L. E. (1985): Parasite:host :environment specificity in the cereal rusts. Annu. Rev. Phytopathology. 23, 201-222.
- BROWDER, L. E. and M. G. EVERSIMEYER (1984): The influence of temperature on "slow-leaf-rusting" in *Triticum aestivum* Suwon 85. Phytopathology, 74,877. (Abstr.)
- BROWNING, J.A. and K.J. FREY (1969): Multiline cultivars as a means of disease control Ann.Rev. Phytopathology, 7, 355-382.
- CALDWELL, R.M., J. F. SCHAFER, L.E. COMPTON, and F. L. PATTERSON (1957): A new plant type of wheat leaf rust resistance of composite origin. Phytopathology, 47, 691-692.
- DYCK, P.L. (1987): The association of a gene for leaf rust resistance with the chromosome 7D suppressor of stem rust resistance in common wheat. Genome 29, 467-469.
- DYCK, P.L., and E.E. SYKES (1994): Genetics of leaf-rust resistance in three spelt wheats. Canadian journal of plant science 74, :231-233.
- EYAL, Z. and J.L. PETERSON (1967): Uredo-spore production of five races of *Puccinia recondita* Rob. ex desm. as affected by light and temperature. can. j. Bot. 45, 537-540.
- FITZGERALD, J.P., M. R. CALDWELL, and E. O. JR. NELSON (1957): Inheritance of resistance to certain races of leaf rust of wheat. Agron. J. 49, 539-543,
- FLOR, H.H. (1956): The Complementary genetic systems in flax and flax rust. adv. Genet. 8:29-54.
- GUPTA, R.P. and A.SINGH (1982): Slow rusting in wheat infected with brown rust. Indian J. Genet. 42,176-182.

- HEATH, M.C.(1976): Hypersensitivity, the cause or consequence of rust resistance? *Phytopathology* 66:935-936.
- HELGUERA, M., I. A. KHAN, and J. DUBCOVSKY (2000): Development of PCR markers for the wheat leaf rust resistance gene *Lr 47*. *theor. appl. genet.* 100, 1137-1143
- HUSSEIN, S., J.J. SPIES, Z.A. PRETORIUS, and M.T. LABUSCHAGNE (2005): Chromosome locations of leaf rust resistance genes in selected tetraploid wheat through substitution lines. *Euphytica* 141, 209-216.
- JACOBS, T. (1989): The occurrence of cell wall appositions in flag leaves of spring wheats susceptible and partially resistant to wheat leaf rust. Submitted to the *Journal of Phytopathology*.
- JERKOVIĆ, Z. (1992): Identifying genes for *Puccinia f.sp. tritici* resistance in wheat lines. *J. Genet. Breed.* 46, 163-166.
- JERKOVIC, Z., M TODOROVA (1999): Pseudo resistance, nonhost resistance, field resistance to *Puccinia spp.* *Agricultural science, Sophia*, XXXVI, 4, 29-32.
- JOHNSON, R. (1983): Genetic background of durable resistance. In *Durable Resistance in Corps*, ed. F. Lamberti, J.M. Waller, N.A. Van der Graaff, p. 5-24. New York: Plenum.
- JOPPA, L.R. (1987): Section 5F: Aneuploid analysis in tetraploid wheat. In: Heyne (ed.). *Wheat and wheat improvement*. American Society of Agronomy, Inc. USA.. 255-267.
- KOLMER, J.A., P.L. DYCK, and A.P. ROELFS (1991): An appraisal of stem rust and leaf rust resistance and the probability of multiple mutations to virulence in populations of cereal rust fungi. *Phytopathology* 81.
- KOLMER, J.A. (1996): Genetics of resistance to wheat leaf rust. *Annual Review of Phytopathology*, September 1996, vol.34, 435-455.
- KNOTT, D.R. (1989): The wheat rust – breeding for resistance. In: *Monographs on theoretical and applied genetics*. No. 12. Springer-Verlag, Berlin.
- KUHN, R.C., H.W. OHM, and G. SHANER (1980): Inheritance of leaf-rusting resistance in Suwon 85 wheat. *Corp, Science* 20, 655-659.
- LEE, T.S. and G. SHANER (1985): Oligogenic inheritance of length of latent period in six slow leaf –rusting wheat cultivars. *Phytopathology* 75, 636-643.
- LOEGERING, W.Q. (1961): Physiologic specialization in the rust fungi and its relation to changes in varietal reaction. "Recent Advances in Botany", 568-571, Toronto Press.
- LOEGERING, W.Q. (1984): Genetics of the pathogen-host association, pages 165-192 in *The Cereal Rust, Vol.I. Origins, specificity, structure and Physiology*. W. R. Bushnell and A.P. Roelfs, eds. Academic Press, New York. 546 pp.
- LOEGERING, W.Q., R.A.MCINTOSH, and C.H. BURTON (1971): Computer analysis of disease data to derive hypothetical genotypes for reaction of host varieties to pathogens. *Can. J. Genet. Cytol.* 13, 742-748.
- MAC KEY, J. (1978): Genetic of race-specific phytoparasitism on plants. 14th Inter.Congr. Genet., Moscow, USSR, August p.21-30.
- MAINS, E.B., C.E. LEIGHTY, and C.O. JONSTON (1926): Inheritance of resistance to Leaf Rust, *Puccinia Triticina Erikss*, in Crosses of Common Wheat, *Triticum vulgare*. *Journal of Agriculture Research*, Vol. XXXII, N^o , 931-972.
- MARTINEZ, L.M., E.R. AUSEMUS, and C.R. BURNHAM (1953): Inheritance of Reaction to Leaf Rust, *Puccinia Rubigo-Vera Tritici* (Erikss.) Carleton, and of Certain other Characteristics in Wheat Cross. *technical Bullten* 205.

- MCINTOSH, R.A., N.H. LUIG, and E.P. BAKER (1967): Genetic and cytogenetic studies of stem rust, leaf rust, and powdery mildew resistances in Hope and related wheat cultivars. *Australian journal of biological sciences* 20, 181-1192.
- MCINTOSH, R. A. and I.A. WATSON (1982): Genetics of host-phytopathogen interaction in rusts. In *The Rust Fungi*, ed. K.J. Scott, A.K. Chakravorty, pp. 121-149. New York : Academic Press, 288.
- MILUS, E.A. and R.F. LINE (1980): Characterization of resistance to leaf rust in Pacific Northwest Wheat. *Phytopathology* 70, 167-172.
- MODAWI, R.S., L.E. BROWDER, and E.G. HAYNE (1985): Reduced receptivity of infection associated with wheat gene Lr2c for low reaction to *Puccinia recondita*. *Phytopathology* 75, 573-576.
- MOMČILOVIĆ, V. (1969): Nasleđivanje otpornosti prema *Puccinia recondita* f.sp. *tritici* Rob. ex Desm. u cilju prenošenja u nove sorte pšenice. "Savremena poljoprivreda", N^o 1.p. 27-46.
- MUNDT, C.C. (1990): Probability of mutation to multiple virulence and durability of resistance gene pyramids. *Phytopathology* 80, 221-223.
- NELSON, R.R. (1978): Genetics of horizontal resistance to plant diseases. *Annu. Rev. Phytopathol.* 16, 359-378.
- NIKS, R.E. (1986): Failure of haustorial development as a factor in slow growth and development of *Puccinia hordei* in partially resistant barley seedlings. *Physiological and Molecular Plant Pathology* 28, 309-322.
- PALLOIX, A.V., V. AYME, and B. MOURY (2009): Durability of plant major resistance genes to pathogens depends on the genetic background, experimental evidence and consequences for breeding strategies. *New Phytologist*. Volume 183 Issue 1. p. 190-199.
- PARLEVIET, J.E. (1975): Partial resistance of barley to leaf rust, *Puccinia hordei*. II. effect of cultivar and development stage on latent period. *Euphytica* 24, 21-27.
- PARLEVIET, J.E. (1981): Race non-specific disease resistance. P.45-54. In *Strategies for the Control of Cereal Disease*, eds. J.F. Jenkyn and R.T. Blackwell Sci. Publ., London.
- PARLEVIET, J.E. and A.VAN OMMEREN (1975): Partial resistance of barley to leaf rust *Puccinia hordei*. II. Relationship between field trials, microplot tests and latent period. *Euphytica* 24:, 93-303.
- PERSON, C. (1959): Gene-for-gene relationship in host: parasite system. *Can. J. Bot.* 37, 1101-1130.
- PRIJIĆ, Ž. and Z. JERKOVIĆ (2009): Common to *Puccinia triticina* durable resistance genes combinations in Serbia. *Book of abstracts IV congress of Serbian genetic society*, 226
- PRUNHAUSER, L., G. GYULAI, M. CSOSZ, L. HESZKY, and A. MESTERHAZY (2000): Identification of leaf rust resistance genes in common wheat by molecular markers. *Acta Phytopathologica et Entomologica Hungarica* 35, (1-4) , 31-36.
- RAO, M.V., A.N. PAHUJA, and K. BLAKOTINA (1972): Field reaction to Indian races of leaf rusts of wheat lines and varieties known genes for resistance. *Indian Journal of Genetics and Plant Breeding* 32, 102.
- RAO, M.V., R.L. SOMAYAJULU, and S.G. AHIRL (1962): Inheritance of field Resistance of Some Bred Wheat Varieties to Indian Races of Brown Rust. *Ind. Jour. Gen. and Pl. Br.* Vol, 22, No. 2.
- ROBINSON, R.A. (1980): New concepts in breeding for disease resistance, *Ann. Rev. Phytopathology* 18, 89-210.
- ROELFS, A. P. (1984): Race specificity and methods of study. Pp. 131-164 in A. Roelfs and W. R. Bushnell, eds. *The Cereal Rusts Vol. I; Origins, Specificity, Structure and Physiology*, Academic Press, Orlando.

- ROELFS, A.P., R.P. SINGH, and E.E. SAARI (1992): Rust diseases of wheat: Concepts and methods of disease management. Mexico, D. F. : CIMMYT, 81 pages
- ROMING, R.W. and R.W. CALDWELL (1964): Stomatal exclusion of *Puccinia recondita* wheat peduncles and sheaths, *Phytopathology*, 54, 214-218
- RUSSEL, G.E., C.R. ANDREWS, and C.D. BISHOP (1976): Development of powdery mildew on leaves of several barley varieties at different growth stages. *Annals of Applied Biology* 82, 467-476.
- SAMBORSKI, D.J. and P. L. DYCK (1982): Encancement of resistance to *puccinia recondita* by interactions of resistance genes wheat. *Canadian Journal of plant Pathology* 4, 152-156,
- SCHAFFER, J.F. (1971): Tolerance to plant disease. *Ann. Rev. Phytopathol.* 9:235-252.
- SCHAFFER, J.F., R.M. CALDWELL, F.L. PATTERSON, and L.E. COMPTON (1963): Wheat Leaf Rust Resistance Combination. *Phytopathology*, 53, 569-573.
- SCHACHERMAYR, G., C. FEUILLET, M. MESSMER, M. WINZELER and B.KELLER (1996): Development of molecular markers linked to the Lr1, Lr9 and Lr24 leaf rust in wheat. Proc. of the 9th European and Mediterranean cereal & powderz Mildews Conference, Lunteren, The Netherlands: 188-191.
- SEARS, E.R. (1954). The aneuploids of common wheat. *Missouri agricultural experiment station research bulletin* 572. 58 pp.
- SEARS, E.R. 1966. chromosome mapping with the aid of telocentrics. In: Mac Key, J. (ed.). *Proceedings 2nd international wheat genetic symposium, Lund. Hereditas Suppl.* 2, 370- 381.
- SHARP, E.L.(1983): Experience og using durable resistance in the U.S.A. In *Durable Resistance in Corp,ed F Lamberti, J.M. Waller,N.A. Van der Graaff*, p. 385-395.
- SINGH, R., D. DATTA, S. SING, and R. TIWARI (2004): Marker-assisted selection for leaf rust resirance genes Lr 19 and Lr24 in weath. Regional station, directorate of wheat research, Flowerdale, Shimla, India. *J. Appl. Genet.* 45 (4) pages: 399-403.
- SMITH, J.S., SMITH, O.S. (1992): Fingerprinting crop varieties. *Advances in agronomy*, V.47, 85-140
- SOTO, P.E., I.W. BUDDENHAGEN, and V.I. AUSUANI (1982): development of streak virus resistant maize population through improved challenge abd selection metods. *ANN. Biol.*,100:539-546.
- SYBIL, A. (2007): Enhancing the genetic diversity and durability of leaf rust resistance in durum and bread wheat. Dept. of Forest Mycology and Pathology, Swedish university of agricultural sciences, Uppsala, Sweden. Doctoral thesis.
- TANG, P.S., M.J. BLACKIE, and R.C. CLOSE, (1977): A simulation analysis of corp yield loss due to rust disease. *Agriculture Systems* 2, 189-198.
- UPADYAYA, Y.M.M., S.G. PAKRIVAL, and P.N. NARULA (1965): Inheritance of Brown rust resistance of Wheat. *Ind. Jour. of Gen and Pl.* No.2.
- VAN DER PLANK, J. E. (1982): Host-pathogen interactions in plant disease. Academic Press. 207 p.
- VAN DER PLANK, J. E. (1984): Disease Resistance in Plants, Academic Press, INC. New York.
- VIDA, G., M. MARIANN GÁL, A. ANDREA UHRIN, O.OTTÓ VEISZ, N. H. NAEEM HASAN SYED, J. A. FLAVELL, Z.WANG, and Z.BEDÓ (2009): Molecular markers for the identification of resistance genes and marker-assisted selection in breeding wheat for leaf rust resistance. *Euphytica*. (Online).
- WASHINGTON, W.J. and S.S. MAAN (1974): Disease reaction of wheat allein cytoplasm. *Corp Science* 0 14, 903.
- WINZELER, M., H. WINZELER, B. KELLER, PH. STRECKEISEN, S. HITZ, M. MESSMER, G.SCHACHERMAYR, and C. FEUILLET (1995): Strategien der Züchtung auf Braunrostresistenz bei Weizen, Bericht 46.

- Arbeitstagung der Arbeitsgemeinschaft der Saatzuchtleiter, Vereinigung österreichischer Pflanzzüchter, BAL Gumpenstein, 83-88.
- ZADOKS, J.C. (1972): Modern concepts of disease resistance in cereals. In Proceedings of Sixth Congress of Eucarpia, Cambridge, 1971, p. 89. Ed by F.G.H. Lupton, G. Jenkins and R. Johanson. EUCAPRIA, Cambridge.
- ZHOU, W.C., F.L. KOLB, G.B. BAI, L.L. DOMIER, L.K. BOZE, and N.J. SMITH (2003): Validation of a major QTL for scar resistance with SSR markers and use of marker-assisted selection in wheat. *Plant breeding* 122, 40-46.

DUGOTRAJNA OTPORNOST PREMA *Puccinia triticina* AKUMULACIJOM GENA OTPORNOSTI

Jelena BOŠKOVIĆ¹ i Momčilo BOŠKOVIĆ²

¹Fakultet za biofarming, Bačka Topola, Megatrend univerzitet Beograd

²Poljoprivredni fakultet, Univerzitet u Novom Sadu, Novi Sad

I z v o d

Pojedinačna upotreba specifičnih gena otpornosti prema *Puccinia triticina* Eriks. retko je osiguravala dugotrajniju otpornosti. Međutim, selekcija i kombiniranje, odnosno akumulacija gena otpornosti u isti genotip pšenice je imalo mnogo većeg uspeha, posebno u situaciji kada se patogen ne reprodukuje seksualno, kao što je slučaj kod prouzrokovala lisne rđe pšenice. U međunarodnim ispitivanjima lisne rđe pšenice bilo je potrebno stvoriti selekcijom nove otporne linije prema *P. triticina* za diferencijaciju populacije patogena, kao i za izvore otpornosti u Evropsko-Mediterranskom području. To je bilo značajno, pošto analize populacije patogena u ovim područjima upotrebom standardnih izogenih Lr linija nisu pokazale zadovoljavajuće rezultate. Bilo je sasvim jasno da su za ta područja potrebni novi, znatno efikasniji diferencijalni geni otpornosti i izvori otpornosti. U početku je bilo odabrano 18 donora otpornosti posle intenzivnog testiranja nakon nekoliko godina internacionalnih rasadnika za rđe žitarica. Donori su ukršteni sa rekurentnim roditeljima Princ i Starke. Ove hibridne linije i 26 Lr linija su komparativno testirane sa 20 posebno virulentnih kultura *P. triticina*, da bi utvrdili prisustvo ovih poznatih Lr gena u našim hibridnim linijama. Odnosi cepanja u F₁b₁ su pokazali prisustvo jednog, dva ili tri gena otpornosti. U daljoj akumulaciji gena otpornosti ovih hibridnih linija, odabrano je osam najinteresantnijih linija sa visokim stepenom otpornosti i ukršteno sa jedino efikasnim izogenim linijama koje su sadržavale jake gene otpornosti Lr9, Lr19 i Lr24. Na osnovu različitih odnosa cepanja svih kombinacija ukrštanja dokazano je da ni jedan od otpornih donora nije posedovao upotrebne jake gene otpornosti. To znači da su naše hibridne linije posedovale akumulirane gene otpornosti od upotrebljenih donora i u dodatku tri jaka gena otpornosti Lr9, Lr19 i Lr24.

Primljeno 07. IX. 2009.

Odobreno 11. XII. 2009.