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Full Length Research Paper

The level of resistance to late blight *Phytophthora infestans* (Mont.) de Bary in tomato breeding genotypes in Serbia

Jasmina Zdravkovic[1]*, Nenad Pavlovic[1], Mirjana Mijatovic[1], Milan Zdravkovic[1], Sladjan Adzic[1], Pavlovic Rados[2] and Ljiljana Boškovic-Rakocevic[2]

[1]Institute for Vegetable Crops, 71 Karadjordjeva St., 11420 Smederevska Palanka, Serbia.
[2]Faculty of Agronomy, Cara Dusana 34, Cacak, Serbia.

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Late blight (*Phytophthora infestans* (Mont.) de Bary) was identified as one of the major diseases causing significant economic losses in tomato production. Thus, the development of resistant cultivars and hybrids was included in tomato breeding programs at the Institute for Vegetable Crops, Smederevska Palanka. A line (S-31), Tomato late blight resistance gene *Ph-2* carrier, was diallely crossed with four susceptible lines possessing good production characteristics (S-49, S-31, hom-3 and hom-4) in order to obtain resistant high-yield and quality hybrids. Parental lines and five F1 hybrids, as well as three commercially grown susceptible cultivars (Narvik SPF, Adonis, SP-109, negative control) were tested for resistance toward this pathogen. Sensitivity was evaluated ten days after inoculation on the basis of leaf area necrotic spot, using I to VI level scale. The results imply dominant or partially dominant inheritance pattern. Three commercially grown cultivars were susceptible, as expected. Five of F1 hybrids including S-31 and hom-3 parents were resistant to late blight. The best hybrids were S-31 x hom-3 and S-31 x hom-4 when the morphological traits, yield and resistance were taken into account. Obviously, lines from group hom contributed to increased resistance to tomato late blight with respect to resistant parent.

Key words: Inheritance, *Ph-2,* resistance gene, *Solanum lycopersicum*.

INTRODUCTION

The first tomato cultivars resistant to some *Phytophthora infestans* isolates have been developed in 1950 and 1960. This resistance has been controlled by *Ph-1* gene (Foolad et al., 2008). However, during the strong epidemics of late blight, these tomato cultivars showed extreme sensitivity. On the other hand, cultivars with *Ph-2* gene showed significantly greater resistance to this pathogen (Danailov, 2002). The *Ph-2* gene introduced by Gallegy (1964) to West Virginia 63 cultivar has been used by Laterrot (1975), to breed cv. Pieraline and other, more stable cultivars (Gajc-Wolska and Michalska, 2002). The *Ph-2* gene resistance (Gallegy, 1960; Laterrot, 1994; cited by Fooland et al., 2008) has been incorporated in many tomato varieties both for industrial processing or fresh consumption and at the Institute for Vegetable Crops in Smederevska Palanka in the line S-31 (Markovic et al., 1992).

Tomato breeding program for resistance to late blight in Serbia started in 1980. Tomato genotypes containing the *Ph-2* gene for resistance and tomato genotypes with good yield and quality of fruits, but susceptible to this pathogen have been hybridised. Selected lines and tomato hybrids showed a higher level of resistance than the susceptible parents (Kamoun and Smart, 2005). Further selection resulted with new hybrids and cultivars of tomato, which have been tested for resistance in artificial conditions of infection. Resistance to different isolates of *P. infestans* is conferred by a greater number of genes such as *Ph-3* (Eshed and Zamir, 1995; Fooland et al., 2008), originating from *Solanum pimpinellifolium*. The latest researches indicate the existence of resistance

*Corresponding author. E-mail: jzdravkovic@institutpalanka.co.rs.

gene called *Ph-5* (Robert et al., 2001, Brouwer et al., 2004; cited by Foolland et al., 2008) originating from the wild forms of tomato *Solanum habrochaites*. New selections of tomatoes resistant to *P. infestans* are based on all the genes that contribute to the increase of resistance to the pathogen.

This study started from a familiar source of resistance to late blight S-31 gene *Ph-2*. However, new selected lines have higher level of tolerance to this pathogen, which proves the presence of some other gene of resistance to late blight. The aim of the research was to determine the level of tolerance of new lines and gene effects of these lines in progeny generations for trait: resistance to late blight.

MATERIALS AND METHODS

Plant material

Three tomato cultivars (Adonis, Narvik SPF and SP-109) and four parental lines (S-31, S-49, hom-3 and-4), six experimental hybrids (diallel without reciprocal crossing of four parental lines), and a line possessing the resistance gene to late blight have been the material for this research. Plants have been planted in pots of size 10 x 10 cm at the stage of two leaves. Plants have been grown in green house at 18 to 25°C.

P. infestans isolate

For testing of tomato resistance to *P. infestans* isolate PI -55 Pilatovac of this pathogen was used. The isolate was extracted from potato sampled in the region of Guca, Serbia. The isolate belong to A1 mating type according to previous research of Ivanović et al. (2004).

Inoculation

Inoculation has been performed on tomato leaves and whole plants. Six leaves of each genotype have been inoculated while two leaves of each genotype have been used as control. These leaves have been placed face up on moistened filter paper. They have been inoculated with approximately 10^4 sporangia/ml suspension. The suspension has been prepared on rye agar and spent 3 h at 5°C before inoculation (Ivanović et al., 2004). Inoculated leaves have been incubated for 10 days at 15°C. Plants have been inoculated with suspensions with concentration of approximately 10^5 sporangia/ml. The inoculum has been prepared from *P. infestans* culture 15 days old, grown on rye agar and on 18°C. Fifteen plants of each genotype have been inoculated while five have been control.

After inoculation plants spent 48 h at 18°C in high humidity (relative humidity, RH - 100%) and after that, they have been moved to green house where it was 20°C and 80% RH (Dorrance and Inglis, 1997). The level of infection and sensitivity has been tested twelve days after inoculation, respectively. The spread of symptoms of disease on leaf surface has been expressed at the scale from 0 to 6 where 0 = no symptoms; 1 = 1 to 10% leaf area affected (LAA); 2 = 11 to 20% LAA; 3 = 21 to 40% LAA; 4 = 41 to 70% LAA, 5 = 71 to 90% LAA; and 6 = 91 to 100% LAA or plant dead. Regarding the level of infection, plants have been divided into five categories; immune (disease index of 0%), resistant (1 to 20%), moderately susceptible (21 to 40%), susceptible (41 to 70%), highly susceptible (71 to 100%).

Statistical analysis

Trial has been set by a randomised complete block design with four repetitions. The level of infection of the leaves and plants has been estimated in percentages. Percentage values have been interpreted into real by using the table for the transformation sqrt (arcsin)% (Snedecor, 1956). Components of genetic variance in diallel crossing and regression coefficients have been determined by using the methods of Mather and Jinks (1971). General and specific combining abilities of parental lines have been determinate by using method 2, mathematic model 1 by Griffing (1956).

RESULTS

Experimental hybrids S-49 × hom-4, S-49 × hom-3, S-31 × hom-4, S-31 × hom-3, and hom-3 × hom-4, were resistant to this pathogen. Resistance of the parental line S-31 was 2 on our scale. Breeding lines hom-3 and hom-4 with unknown level of resistance showed 2 on scale, which proves high level of tolerance to late blight same as S-31 for familiar carrier of gene *Ph-2*. Tomato cultivars Adonis, Narvik and the SP-109 have been used as negative control. Narvik and Adonis showed high sensitivity (on a scale of 4) as well as variety SP-109 (on a scale of 3). The percentage of infection for all these genotypes has been from 9.6 to 18.3% for treated leaves and from 8.2 to 14.6% or whole plants (marks on the tolerance scale 2). Cultivars S-49 and SP-109 and experimental hybrid have been less susceptible. Cultivars Adonis and Narvik SPF were the most susceptible. This line and other resistant genotypes have been created by crossing cultivars (Wva'63, Wva'700, Pieraline) with the resistance gene *Ph-2* and lines with good combining abilities for yield and quality traits (Table 1). A similar level of tolerance of breeding line S-31 and its progeny (hybrids S-31 × hom-4 i S-31 × hom-3) was expected. Sice breeding line S-49 was sensitive to late blight on scale 3 and its progeny (hybrids S-49 × hom-4 i S-49 × hom-3) tolerant on scale 2, carriers of tolerance are lines hom 3 and hom 4. Which gene is responsible for this heredity can not be determined, but its presence can be proved. This can be proved by mark 2 on scale for hybrid 3 x hom 4 (Table 1).

In this study, the genotypes with negative GCA values have been separated, because they reduce the level of infection on leaf surface and fruit. This means that ranked genotypes determine the degree to which the holders of the resistance of parental lines are the bearers of resistance in progeny. Tests on leaves and plants showed the same range of genotypes. Line S-31 established the highest GCA. Line S-49 is the worst combiner, and also the most susceptible. Specific combining ability implies that some good combinations have a high degree of tolerance to *P. infestans* (Table 2). Line S-31 has been resistant to *P. infestans*, because it has in its pedigree a *Ph-2* gene for resistance (Markovic

Table 1. Reaction of tomato genotypes to cause of *Phytophtora infestans.*

Genotypes	Level of infection (%)		Resistance categories
	Leaf	Plant	
Adonis	94.6	89.4	5
Narvik	82.3	78.4	5
S-31	9.6	8.2	2
S-49	35.7	29.3	3
SP-109	34.8	31.6	3
S-49 x hom-4	12.4	8.6	2
S-49 x hom -3	10.6	9.3	2
S-31 x hom-4	17.8	12.4	2
S-31 x hom-3	10.6	9.4	2
S-31 x S-49	23.8	22.4	3
hom 3 x hom 4	14.2	16.5	2
hom 3	18.3	14.6	2
hom 4	17.4	12.8	2

1 – Immune, 2 – resistant, 3 - low sensitivity, 4 – susceptible, and 5 - very susceptible.

Table 2. Values of general (GCA) and special (SCG) combining abilities for leaf and plant for parents and F1 generation.

Parents	Leaf				Plant			
	GCA	SCG			GCA	SCG		
		S-49	hom -4	hom -3		S-49	hom -4	hom -3
S-31	-2.981	3.819	-2.320	-0.148	-1.883	3.691	-1.164	-0.620
S-49	4.492		-2.626	-7.353	4.272		-3.753	-7.076
hom 3	-0.369			2.608	-0.972			1.069
hom 4	-1.142				-1.417			
$LSD_{0.05}$	*1.2*		*2.43*		*1.571*		*3.032*	
$LSD_{0.01}$	*1.61*		*3.23*		*2.016*		*4.032*	

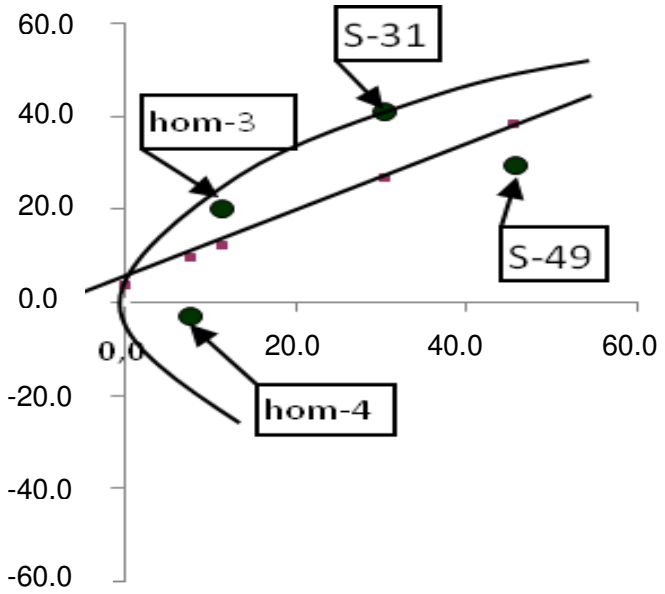

Figure 1. Graph of spreading spots around the limit parabole.

et al., 1992). Cultivars that are used as a negative control have the outstanding sensitivity to the parasites (Table 1). Leaves and the plants have reacted similarly to the infection with *P. infestans*, although the genotypes reacted differently among themselves. Genetic variability is almost identical both on the leaf and the entire tomato plant. Variance component (H1) belonging to the dominant activity has been higher than the variance belonging to the additive action of genes.

Dominant genes influenced more the inheritance properties of resistance to tomato *P. infestans*, which has been proved by positive interaction of additive and recessive alleles (F). The values of the average degree of dominance, analyzed both for leaf and fruit have been close to one, which indicates full dominance in the inheritance of resistance to Late blight. Points closer to coordinate system (hom-3 and hom-4) have been considered to be parents with the largest number of dominant genes (Figures 1 and 2). The parent who has been on a cross-section of the parabola and the regression line has not been identified in our research, which

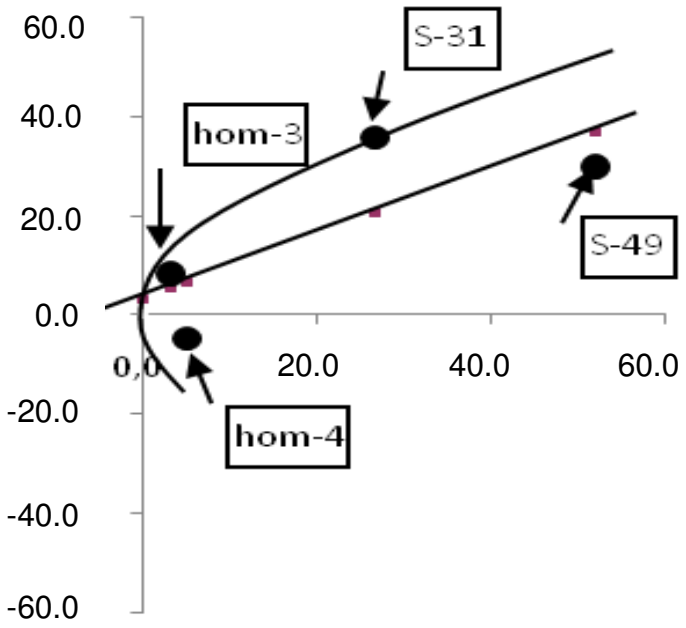

Figure 2. Graph of spreading spots around the limit parabole.

Table 3. Components of genetic variance of resistance to tomato late blight on leaf.

Components	Values
D	59.138
H1	67.6
H2	61.59
F	32.38
E	1.103
H2/4H1	0.228
U	0.649
V	0.35
sqrt(H1/D)	1.07
Kd/Kr	1.69
h^2	0.96648

D – Variance component belonging to aditive gene reaction; H1 i H2 – Variance component belonging to dominant gene reaction; F – interaction aditive x dominant effect; E – non-heritable variance counted bz analysing variance in random block system; sqrt(H1/D) – the average level of dominance; u – frequency of dominant genes; v – frequency of recesive genes; Kd/Kr – total number of dominant and recesive alelles; and h^2 heritability in broader sense.

proves that none of the parents had all dominant or recessive genes for the resistance to late blight. Heritability in the wider sense, which has been the ratio between the total genetic and phenotypic variance, has been high 0.9664 to 0.9423, leaf- fruit (Tables 3 and 4). There has been no significant difference in the intensity of infection on the entire plant or tomato leaf, where the infection first appears.

DISCUSSION

Late blight of tomato is an important economic disease. The first tomato lines resistant to late blight obtained in Serbia have been the lines with gene *Ph-2*, originating from Wva 63 (Markovic et al., 1992). Resistant hybrids have been obtained by crossing varieties and lines (originating from Wva'63, Wva'700, Pieraline) with the

Table 4. Components of genetic variance of resistance to tomato late blight on fruits.

Components	Values
D	49.551
H1	68.4
H2	58.92
F	32.14
E	1.723
H2/4H1	0.215
U	0.686
V	0.31
sqrt(H1/D)	1.17
Kd/Kr	1.76
h^2	0.94235

D – Variance component belonging to aditive gene reaction; H1 i H2 – variance component belonging to dominant gene reaction; F – interaction aditive x dominant effect; E – non-heritable variance counted bz analysing variance in random block system; sqrt(H1/D) – the average level of dominance; u – frequency of dominant genes; v – frequency of recesive genes; Kd/Kr – total number of dominant and recesive alelles; and h^2 heritability in broader sense.

gene *Ph-2*, on one hand and lines and varieties with good combining ability for yield and quality characteristics of fruit but not resistant to the parasites, on the other. The achieved resistance in this line has been incorporated in the hybrid combinations (Zdravkovic et al., 2001, 2004) and the nature of late blight isolates in Serbia (Mijatovic et al., 2004) has been examined. The gene (*Ph-2*) has been incorporated by many researchers (Gajc-Wolska and Michalska, 2002). Races of *P. infestans* isolates have been changing and to the beginning of the 21st century, 14 different races specific to tomato have been determined (Cohen, 2002). The mechanism of inheritance of resistance to tomato parasites from the aspect of this polymorphism is very difficult and complex. Inheritance in progeny generations is based on polygenic resistance to the pathogens (Foolad et al., 2008).

Our research proved that a large number of dominant genes that contribute to greater resistance, which is in accordance with the research of Kim et al. (2005), who found epistatic gene effects in the inheritance of these traits. In the determination of resistance in the F1 generation, the combining ability of parental lines indicate the existence of genes for resistance (in the parents lines hom-3 and hom-4) besides the line S-31, which is known as the donor of resistance genes to *P. infestans*. These results are important in the process of selection to resistance, both lines and F1 hybrids. Heritability in the wider sense, which is high, also confirmed that the F1 progeny will have larger number of genes of resistance to the parasites. Line selection could produce lines that have a higher degree of resistance than line S-31. Future selection work should be based on the study and incorporation of sources of resistance *Ph-2*, *Ph-3* and *Ph-5* (Foolad et al., 2008).

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