Journal of Agricultural Science
http://www.ccsenet.org/jas
Vol. 2, No. 1
March 2010

Genetic and Morpho-Agronomic Evaluation of New Tomato Breeding Lines Resistant to Bacterial Speck (*Pseudomonas Syringae* pv. *Tomato)*

Elzbieta U. KOZIK (Corresponding author) & Marzena Nowakowska
Research Institute of Vegetable Crops
Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland
E-mail: ekozik@inwarz.skierniewice.pl

Abstract

A breeding program was initiated at the Research Institute of Vegetable Crops, Skierniewice, Poland to select new tomato lines resistant to bacterial speck (*Psedomonas syringae* pv. *tomato* (PST) and suitable for field growing. Two tomato cultivars with good agronomic characteristics Luban and Rumba were crossed with resistant to PST 'Ontario 7710'. Then backcross and pedigree breeding procedures were employed to obtain BC_5, BC_4S_1, BC_3S_2, BC_2S_3, BC_1S_4 populations. Screening test among those hybrid populations was performed in a greenhouse and exhibited a large amount of variation in response to PST infection. All plants within BC_1S_4 progeny of the 'Luban' x 'Ontario 7710' cross and two BC_1S_4, BC_3S_2 progenies of the 'Rumba' x 'Ontario 7710' cross had no symptoms of bacterial speck and could be assigned to the homozygous category. The remaining progenies were segregating for resistance and susceptibility and gave distribution of plants over all severity classes. That confirms heterozygosity for resistant gene *Pto* of their latter resistant single plant selections before backcrossing or/and selfing. The BC_5 populations had significantly higher disease severity rates (DSI = 2.4) than all other selfed populations (DSI = 1.2 – 1.6). After selection resistant genotypes of the populations were transplanted to the field for morphological traits evaluation. A noticeable progress in breeding regarding most of the plant and fruit characteristics of the recurrent parents was approached in all populations of the 'Luban' x 'Ontario 7710' cross. Although all progenies of the 'Rumba' x 'Ontario 7710' differed from their recurrent parent as to one feature at least, however the differences showed improved shifting toward the characteristics of susceptible parent.

Keywords: Lycopersicon, Bacterial speck, Morphological features, Yield quality

1. Introduction

Bacterial speck of tomato, caused by *Psedomonas syringae* pv. *tomato (*PST) occurs in all major tomato growing areas of the world. The disease attacks stems, buds, flowers, leaves and fruits, causing reduction of yield and sometimes leading to death of tomatoes plants (Louws *et al.*, 2001; Preston, 2000). In Poland, severe outbreaks of bacterial speck on tomatoes have been reported during past decade, causing lower fruit productivity and quality of cultivars (Macias, 1999). This is related to the susceptible tomato cultivars grown in Poland (Macias, 1996) and to the lack of effective chemical control of the disease (Alexander *et al.*, 1999; Jones and Jones, 1989; Ramos *et al.,* 1989; Silva and Lopes, 1995). Therefore breeding of tomatoes for genetic resistance against bacterial speck seems to be very important and promising perspective. It was found that resistance of *Lycopersicon esculentum* 'Ontario 7710' to bacterial speck is determined by a single dominant gene (Pitblado and MacNeill, 1983). Also, the evidence that this resistance is governed by semi-dominant gene was found (Kozik, 2002). However, the horticultural traits of 'Ontario 7710' are not very desirable for agronomic conditions and preferences of Polish farmers. The project was initiated to incorporate the resistance to bacterial speck into Polish cultivars with good horticultural characteristics. Newly developed breeding material has to be at least as good as, if not better than the standard cultivars in regards to their high and good quality of productivity improved by the addition of resistance to bacterial speck.

This paper includes progress information on the development of tomato breeding lines derived from crosses of susceptible cultivars Rumba and Luban with resistant to bacterial speck 'Ontario 7710'. The results obtained in 2007-2008 from evaluation of the most advanced in breeding progenies of those crosses with regard to resistance level and horticultural characteristics are also discussed.

2. Materials and methods

2.1 Plant material

Two tomato cultivars Luban and Rumba with good agronomic characteristics but susceptible to bacterial speck (PST), and 'Ontario 7710' resistant to PST and ten BC_5, BC_4S_1, BC_3S_2, BC_2S_3, BC_1S_4 populations were used in both greenhouse and field experiments.

Crosses were made between 'Rumba' and 'Luban' and the bacterial speck resistant 'Ontario 7710'. Resistant F_1 plants from each of these crosses were used in procedures of backcross and inbreeding based on a one-way program up to the Bc_5. Also after each backcross plants were selfed in consecutive generations. Each population was screened for seedling PST-resistance and only resistant seedlings were transplanted into the field for horticultural traits evaluation (such as plant vigor, fruit size, fruit weight, number of locules) or were transplanted into 10-liter plastic pots under greenhouse conditions for backcrossing or selfing. All crosses were made by hand pollination.

2.2 Screening for bacterial speck resistance (greenhouse experiments)

The bacterial strain Pst3, race 0 *Psedomonas syringae* pv. *tomato*, from the Collection of Plant Pathogens, Institute of Plant Protection, Poznan, Poland was used in all tests. This strain was found to be the least variable and the most virulent among other tested in our previous studies (Kozik and Sobiczewski, 2000). Stock cultures of bacteria were maintained on nutrient agar with 1.6% glycerol under paraffin oil at 5°C. For the preparation of inoculum, bacteria were incubated at 24°C on nutrient agar medium supplemented with 1% glucose. The inoculum was prepared by washing off 24h-old cultures of bacteria with sterile distilled water. Concentration of bacteria in the suspension was adjusted spectrophotometrically to 10^8 cfu/ml and confirmed by a serial dilution plating method. Before inoculation of tomato plants, the ability of Pst3 strain to induce hypersensitivity reaction (HR) on tobacco plants cv. Samsun was checked according to the method of Klement (1963).

Ten days after sowing the tomato seedlings were transplanted to 10 cm diameter plastic pots containing sphagnum peat substrate (Kronen Mix) and kept on benches in a greenhouse. When the plants reached the four leaf stage they were sprayed with inoculum. After inoculation the plants were kept under a plastic cover for 4 – 5 days to obtain a relative humidity of 100%. Afterwards the covers were taken out and the humidity fluctuated between 50 and 70%. The temperature set points were 27°C by day and 21ºC by night. Disease was assessed 14 days after inoculation. The lesions of bacterial speck on leaves per plant were counted and plants were classified using the scale of Chambers and Merriman (1975) as follows: 0 = no lesions; 1 = 1-10 lesions per plant; 2 = 11-20 lesions; 3 = 21-40 lesions; and 4 = more than 40 lesions per plant. Data were collected as means over all leaves on the plants within each line. Disease intensity was evaluated on the basis of disease severity index (DSI). Low DSI per genotype implies a high level of resistance and low susceptibility, while a high DSI means that resistance level is low and thus susceptibility is high (Eenink, 1981). Additionally, considering the number of spots, plants from class 0 were regarded as resistant (R), those from 1 and 2 were tolerant (T) because of the very low number of spots and lack of expansion, and those from classes 3 and 4 were considered susceptible (S), as they were typical for 'Rumba' and 'Luban'.

2.3 Morphological evaluation (field experiments)

In a second decade of May 2007 and 2008, after the selection for bacterial speck resistance, plants were transplanted to the experimental field of the Research Institute of Vegetable Crops in Skierniewice. The experiment was set up according to an experimental design with four randomized blocks with two rows of twenty plants per genotype per block. A plot size was 10 m^2 with 2.4 plants·m^{-2}. Fertilization and all other cultural practices, including irrigation in the rainfall deficiency periods, were conducted according to standard recommendations. Standard fertilizer practices were used.

Fruits were harvested once a week. Quantity of a total and marketable yield was subjected with regard to fruit fractions based on fruit diameter: marketable (>6.0 cm, 4.5-6.0 cm, 3.5-4.5 cm), nonmarketable (small = <3.5 cm, irregular, cracked, spoiled). The yield quality was recorded on the bases of the participation of marketable yield and subsequent fruit fractions in total yield in percent. Twenty fruits of each population were evaluated for morphological traits: fruit weight, number of locules and fruit shape (shape coefficient).

The share of marketable yield and other fruit fractions in total yield for parental cultivars Rumba, Luban and Ontario 7710 and their segregating crosses were similar in 2007 and 2008 and indicated that the data could be pooled. Therefore, data for each generation were pooled for the two crosses and statistical analysis were processed with STATISTICA 5.0 software. Significant differences among means were verified using Newman-Keuls' range test at alfa = 0.05.

3. Results

3.1 Variation in resistance to bacterial speck

The screening test indicated that severity of bacterial speck on all plants of the recurrent parents 'Luban' and 'Rumba' was high. Both cultivars, affected in classes 3 and 4, were assigned to the susceptible category (DSI = 3.2 and 3.5, respectively) (Table 1). It shows that applied inoculation procedure gave infection of 100% susceptible parents plants.

The donor parent 'Ontario 7710', one progeny of the Luban x Ontario 7710 cross (BC_1S_4) and two progenies of the Rumba x Ontario 7710 cross (BC_1S_4, BC_3S_2) were highly resistant with all symptomless plants within each population (DSI = 0.0). Intermediate disease severity was found in all other progenies of the Rumba and Luban x Ontario 7710 crosses. Both BC_4S_1, BC_2S_3, BC_5 and one BC_3S_2 populations showed large variation range and gave distribution of plants over all severity classes. In both BC_5 progenies a segregation ratio of 1 tolerant (classes 0, 1, 2)*:* 1 susceptible (classes 3, 4) was obtained and they had significantly higher disease severity rates (DSI = 2.4) than all other selfed populations (DSI = 1.2 – 1.6).

3.2 Variation in morphological traits

A significant progress in breeding for bacterial speck resistance in populations of cv. Luban was noticed in total yield components and fruit characteristics for most of the breeding methods used (Table 2, 3). Percentage of marketable yield, small, irregular and cracked fractions of fruits was similar to those of 'Luban'. All populations expressed lower number of diseased fruits (2.0-3.6%) than the susceptible parent (6.9%). However, there was a large variation in fruit fractions in total yield observed among populations of cv. Rumba except marketable fruits that showed similar or higher values (83.8-91.1%) to those of the susceptible parent (85.1%). Population Bc_2S_3 with respect to the most of fruit fractions in total yield were approaching to those of 'Rumba'. The best results were recorded for the most advanced in backcrossing populations BC_4S_1 and BC_5 where percentage of irregular and diseased fruits was lower than in their susceptible parent 'Rumba' (Table 2).

Data on morphological traits of fruits revealed no significant differences between 'Luban' and its consecutive populations (Table 3). Fruit weight, shape coefficient, number of locules and fruit hardness for all tested progeny of the 'Luban' x 'Ontario 7710' cross were similar to those of susceptible parent. In contrast to the populations of cv. Luban, progenies of the 'Rumba' x 'Ontario 7710' cross constituted a more diversified group. The most advanced in backcrossing populations BC_5 and BC_4S_1 had the most lighter fruit (91.9 and 94.8g, respectively), typical for 'Rumba'. In remaining populations, more heavy than for susceptible parent fruit persisted. All tested populations of the 'Rumba' x 'Ontario 7710' cross were characterized by higher fruit hardness than their recurrent parent (Table 3).

4. Discussion

Previous studies have shown that resistance to bacterial speck (*Psedomonas syringae* pv. *tomato*) (PST) in 'Ontario 7710' is controlled by a single dominant gene (Pilovsky and Zutra, 1982) or semi-dominant gene (Carland and Staskawicz, 1993; Kozik, 2002). Backcross pedigree breeding program and selection for bacterial speck resistance was required to maintain resistance and to obtain breeding lines with uniform resistance and good plant and fruit characteristics typical for both susceptible recurrent parent "Rumba' and Luban'. Data in this study revealed that plants that carry resistant gene *Pto* to bacterial speck can be found in all of the tested populations, but genetic backgrounds of the families were different and depended on the homo/heterozygous status of resistant gene *Pto*. All plants within BC_1S_4 progeny of the 'Luban' x 'Ontario 7710' cross and two BC_1S_4, BC_3S_2 progenies of the 'Rumba' x 'Ontario 7710' cross had no symptoms of PST. These three progenies were uniformly resistant and could be assigned to the homozygous category. That also suggests that the previous single plant selection, before selfing, was homozygous for resistant gene *Pto*. The remaining progenies were segregating for resistance and susceptibility that confirms heterozygosity for resistant gene *Pto* of their latter resistant single plant selections before backcrossing or/and selfing. Presumably it may take one or two selfings to obtain other breeding lines with uniform, stable resistance to bacterial speck in case of more advanced in breeding populations and three or more selfings in case of two backcross populations BC_5.

Generally, if backcross breeding is to be successful, the genotype of the recurrent parent must be recovered in its essential plant and fruit features. This is primarily a function of the number of backcrosses, although selection for the type of the recurrent parent in the early backcross generations is effective in shifting the population towards the characteristics of that parent. It is believed that six backcrosses coupled with rigid selection in early generations produce plants that resemble the recurrent parent effectively (Allard, 1960). Processing tomato lines resistant to bacterial speck, developed in a backcross program employing tomato cv. UC 82 as the recurrent parent and the accession Ontario 7710 as the donor of the *Pto* resistance gene were reported earlier (Candilo and Calzolari, 1992). Backcross lines exhibited a resistance similar to the donor parent and significantly higher than the recurrent parent. Several lines achieved marketable yield similar or superior to those of cultivar UC 82. In our study a noticeable progress in breeding regarding most of the plant and fruit characteristics of the recurrent parents was approached in all populations of the 'Luban' x 'Ontario 7710' cross. Although all progenies of the 'Rumba' x 'Ontario 7710' differed from their recurrent parent as to one feature at least, however the differences showed improved shifting toward the characteristics of susceptible parent. The results also revealed that backcross pedigree programs coupled with a particularly high intensity of selection for bacterial speck resistance and the type of recurrent parent made variation among methods insignificant.

5. Conclusions

Three of 10 developed progenies of 'Luban' x 'Ontario 7710' cross (BC_1S_4) and 'Rumba' x 'Ontario 7710' cross (BC_1S_4, BC_3S_2) were uniformly resistant to bacterial speck and could be assigned to the homozygous category.

Resistant plants with good horticultural characteristics were obtained in all seven remaining generations that segregated in resistance responses to bacterial speck.

Several additional selfs will be required to develop other bacterial speck resistant breeding lines with uniform resistance and good horticultural characteristics.

A noticeable progress in breeding regarding most of the plant and fruit characteristics of the recurrent parents was approached in all populations of the 'Luban' x 'Ontario 7710' cross.

Although all progenies of the 'Rumba' x 'Ontario 7710' differed from their recurrent parent as to one feature at least, however the differences showed improved shifting toward the characteristics of susceptible parent.

References

Alexander, S. A., Kim, S. H., & Waldenmaier, C. M. (1999). First report of copper-tolerant *Pseudomonas syringae* pv. *tomato* in Virginia. *Plant Disease*, 83, 964.

Allard, R. W. (1960). *Principles of Plant Breeding*. New York and London: John Wiley & Sons, Inc., (Chapter 14).

Candilo, M. di., & Calzolari, A. (1992). Characteristics of new processing tomato lines resistant to bacterial speck. *Advances in Horticultural Science,* 6, 167-171.

Carland, F. M., & Staskawicz, B. J. (1993). Genetic characterization of the *Pto* locus of tomato: semi-dominance and cosegregation of resistance to *Pseudomonas syringae* pathovar *tomato* and sensitivity to the insecticide fenthion. *Molecular and General Genetics,* 239, 17-27.

Chambers, S. C., & Merriman, P. R. (1975). Perennation and control of *Pseudomonas syringae* pv. *tomato* in Victoria. *Australian Journal of Agricultural Research*, 26, 657-663.

Eenink, A. H. (1981). Partial resistance in lettuce to downy mildew (*Bremia lactucae*). 1. Search for partially resistant genotypes and influence of certain plant characters and environments on the resistance level. *Euphytica,* 30, 619-628.

Jones, J. P., & Jones, J. B. (1989). Field control of target spot and bacterial speck of tomato. *Proceedings of The Florida State Horticultural Society,* 101, 358-361.

Klement, Z. (1963). Rapid detection of pathogenicity of phytopathogenic pseudomonads. *Nature*, 199, 299-300.

Kozik, E. U. (2002). Studies on resistance to bacterial speck (*Pseudomonas syringae* pv. *tomato*) in tomato cv. Ontario 7710. *Plant Breeding*, 121, 526-530.

Kozik, E. U., & Sobiczewski, P. (2000). Response of tomato genotypes to bacterial speck (*Pseudomonas syringae* pv. *tomato*). *Acta Physiologiae Plantarum*, 22(3), 243-246.

Louws, F. J., Wilson, M., Campbell, H. L., Cuppels, D. A., Jones, J. B., Shoemaker, P. B., Sahin, F., & Miller, S. A. (2001). Field control of bacterial spot and bacterial speck of tomato. *Plant Disease*, 85, 481-488

Macias, W. (1996). Bakteryjna cętkowatość pomidora. *Ochrona Roślin*, 10, 6-7 [in Polish].

Macias, W. (1999). Wpływ terminów inokulacji pomidora bakterią (*Pseudomonas syringae* pv. *tomato*) na rozwój i szkodliwość bakteryjnej cętkowatości pomidora. *Mat. I Konf. Grupy Roboczej Bakteryjnych Chorób Roślin Komitetu Ochrony Roślin PAN, ISiK, Skierniewice*, 9 grudnia 1998, 47-57 [in Polish].

Pilovsky, M., & Zutra, D. (1982). Screening wild tomatoes for resistance to bacterial speck pathogen (*Pseudomonas tomato*). *Plant Disease,* 66(1), 46-47.

Pitblado, R. E., & MacNeill, B. H. (1983). Genetic basis of resistance to *Pseudomonas syringae* pathovar *tomato* in field tomatoes. *Canadian Journal of Plant Pathology*, 5, 251-255.

Preston, G. M. (2000). *Psedomonas syringae* pv. *tomato*: The right pathogen, of the right plant, at the right time. *Molecular Plant Pathology*, 1, 263-275.

Ramos, R. S., Sinigaglia, C., & Chiba, S. (1989). Chemical control of bacterial speck (*Pseudomonas syringae* pv. *tomato*) of tomato. *Biologico*, 55, 1-3.

Silva, V. L., da, & Lopes, C. A. (1995). *Pseudomonas syringae* pv. *tomato* resistant to copper-sprayed tomato fields. *Fitopatologia-Brasileira*, 20, 85-89.

Table 1. Distribution of seedlings of parents and progenies from crosses between the bacterial speck resistant 'Ontario 7710' and susceptible cvs. Luban and Rumba by disease rating

Population	No. of plants in infection classes					Total number of tested plants	DSI*
	0	1	2	3	4		
Luban				24	6	30	3.2 d
Bc_5	6	14	10	12	18	60	2.4 c
Bc_4S_1	52	24	10	8	24	118	1.4 b
Bc_3S_2	32	34	32	22		120	1.4 b
Bc_2S_3	86	54	28	46	18	232	1.4 b
Bc_1S_4	120					120	0.0 a
Rumba				16	14	30	3.5 d
Bc_5	4	13	13	13	17	60	2.4 c
Bc_4S_1	46	16	16	24	18	120	1.6 b
Bc_3S_2	120					120	0.0 a
Bc_2S_3	48	26	4	28	2	108	1.2 b
Bc_1S_4	120					120	0.0 a
Ontario 7710	30					30	0.0 a

*DSI = disease severity index,

Means followed by the same letter are not significantly different at alfa = 0.05

Table 2. Yield components in populations from a cross between the bacterial speck resistant 'Ontario 7710' and susceptible cvs. Luban and Rumba

Population	Fruit fractions in total yield in %				
	marketable yield	nonmarketable yield			
		small <3.5cm	irregular	cracked	spoiled
Luban	**92.3 b**	**0.5 g**	**0.2 g**	**0.0**	**6.9 b**
Bc_5	95.2 a	1.2 e	0.0 h	0.0	3.6 cd
Bc_4S_1	95.6 a	1.0 e	1.0 d	0.0	2.5 de
Bc_3S_2	95.2 a	1.2 e	1.7 c	0.0	2.0 e
Bc_2S_3	94.4 a	0.6 fg	1.8 c	0.0	3.3 cd
Bc_1S_4	95.4 ab	0.8 e-g	0.8 e	0.0	2.9 c-e
Rumba	**85.4 d**	**3.4 c**	**2.2 b**	**1.0**	**8.0 a**
Bc_5	88.2 c	2.9 d	0.6 f	0.0	6.2 b
Bc_4S_1	88.5 c	4.2 b	0.5 f	0.0	6.9 b
Bc_3S_2	83.8 d	6.3 a	2.2 b	0.0	7.8 ab
Bc_2S_3	91.1 b	3.2 c	1.7 c	0.1	3.9 c
Bc_1S_4	90.5 b	0.9 fg	4.1 a	0.0	4.2 c
Ontario 7710	**88.5 c**	**2.6 d**	**0.0 h**	**0.0**	**8.8 a**

Means followed by the same letter are not significantly different at alfa = 0.05

Table 3. Fruit characteristics in populations from a cross between the bacterial speck resistant 'Ontario 7710' and susceptible cvs. Luban and Rumba

Population	Weight of marketable fruit (g)	Shape coefficient	Number of locules	Hardness*
Luban	**107.7**	**0.8**	**4.7**	**2.6**
Bc_5	102.3	0.8	4.6	2.9
Bc_4S_1	101.5	0.8	4.7	2.8
Bc_3S_2	103.1	0.8	4.6	2.8
Bc_2S_3	112.3	0.9	4.7	2.6
Bc_1S_4	102.9	0.9	4.4	2.9
Rumba	**92.1**	**0.8**	**4.9**	**1.9**
Bc_5	91.9	0.8	4.4	3.3
Bc_4S_1	94.8	0.9	4.2	2.6
Bc_3S_2	104.4	0.8	5.0	3.0
Bc_2S_3	100.4	0.8	4.2	2.6
Bc_1S_4	105.9	0.9	4.4	2.6
Ontario 7710	**45.6**	**1.2**	**2.2**	**3.4**

* hardness after 2 weeks of storage on a 1-5 scale with 1 = soft fruits, 3 = medium hard, 5 = hard fruits

Means followed by the same letter are not significantly different at alfa = 0.05