

**SOURCES OF RESISTANCE TO RACES R0 AND R1 OF *Pseudomonas syringae* PV.
tomato – AGENT OF BACTERIAL SPECK ON TOMATO**

Daniela GANEVA¹ and Nevena BOGATZEVSKA²

¹”Maritsa”Vegetable Crops Research Institute, Plovdiv, Bulgaria

²Institute of Soil Science, Agrotechnologies and Plant Protection “N. Pushkarov”,
Sofia, Bulgaria

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Tomato breeding lines with fruit colour different from the traditional red colour were studied in order to search for sources of resistance to races R0 and R1 of *Pseudomonas syringae* pv. *tomato*. As a result of selection of healthy plants with hypersensitive response (HR), the resistance was stabilized and perspective lines gene-carriers of resistance to bacterial speck were chosen. Lines L1078 and L1083 with brown-red (black) coloured fruits and line L1130 with purple-red fruits possess a complex resistance to races R0 and R1. It was established that two of the lines with rose-coloured tomato fruits (L1088 and L584) were resistant to race 1 of *P. syringae* pv. *tomato*. These lines possessed valuable economic and morphological characters and they could be used in combinative and heterosis breeding for development of resistance to bacterial speck varieties.

Keywords: quality, *Pseudomonas syringae* pv. *tomato* races 0,1, resistance, *Solanum lycopersicum* L.

INTRODUCTION

Bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato* occurs in all major tomato growing areas of the world, which is favoured by cool, moist environmental conditions (POHRONEZNY and VOLIN, 1983; BOGATZEVSKA, 1988, 2002; CAI *et al.*, 2011). The pathogen is kept and disseminated by tomato seeds and weeds. *P. syringae* pv. *tomato* can survive epiphytically on the leaf surface and endophytically in leaf tissues of tomato host plants and in non-host weed associations. Moreover, epiphytic populations of the pathogen persist on symptomless host plants and weeds and can become infective in favourable weather conditions

Corresponding author: Daniela Ganeva, ”Maritsa” Vegetable Crops Research Institute, 32, Brezovsko shosse Str., 4003 Plovdiv, Bulgaria, dganeva@abv.bg

(BOGATZEVSKA, 2002). In addition to a complex biology of the bacterium, the occurrence of new races of the pathogen raises new problems in selection and breeding of resistant tomato cultivars. Two tomato races of the pathogen (R0 and R1) have been described in the world (LAWTON and MACNEIL, 1986; BOGATZEVSKA *et al.*, 1989, 1998, 2000; BUONAURO *et al.*, 1996; MILJAŠEVIĆ *et al.*, 2009; STOYANOVA *et al.*, 2014). Race 0 strains translocate the type III effectors AvrPto and AvrPtoB into the plant cell where they are recognized by *Pto*, whereas race 1 strains either lack these effectors, do not accumulate the proteins, or have variants that are not recognized by *Pto* (LIN *et al.*, 2006; KUNKEAW *et al.*, 2010).

The disease may result in considerable economic losses caused by low fruit quality and plants productivity (POHRONEZNY and VOLIN, 1983; BOGATZEVSKA, 1988, 2002; CAI *et al.*, 2011). Currently, there are no commercial tomato cultivars resistant to bacterial speck grown in Bulgaria (DANAİLOV *et al.*, 2011; ALEKSANDROVA *et al.*, 2013). Development of cultivars with resistance to bacterial speck would be a valuable contribution to growers. Progress, however, depends on the availability of an effective technique to identify resistant germplasm and progeny at the seedling growth stage. Some processing tomato varieties are immune to strains of *P. syringae* pv. *tomato* designated as race 0, but there are no cultivated varieties that are resistant to race 1 types (PEDLEY and MARTIN, 2003). Presence of sources of resistance, knowledge of the genetic control of the resistance and race diversity of the pathogens as well as complex evaluation by economic and biological properties and selection is necessary in order to develop resistant lines and varieties. (KIRYAKOV and SOFKOVA, 2014).

Generally the resistance occurs in wild species and it is attended by low productivity, small fruits with unsatisfactory taste and quality (SOTIROVA and BOGATZEVSKA, 1988, 1990, 1994; ROSE *et al.*, 2005; BAO *et al.*, 2015). At least three other wild tomato species are also known to have the *Pto* gene (ROSE *et al.*, 2005). The increased antioxidative properties of plants led to improved resistance to pathogens in crops (LORENC-KUKUŁA *et al.*, 2007).

The aim of the study was to find sources of resistance to races of *P. syringae* pv. *tomato* in tomato lines with fruit colour different from the traditional red colour and selection of gene-carriers with high resistance and valuable economical and morphological characters.

MATERIALS AND METHODS

The study was conducted both in laboratory conditions at the Institute of Soil Science, Agro-Technology and Plant Protection “N. Pushkarov” – Sofia and in field conditions at the “Maritsa” Vegetable Crops Research Institute – Plovdiv.

Plant material: Eleven tomato lines with fruit colour different from the traditional red pigmentation. Two-coloured fruits: brown-red (black) – lines L1078 and L1083; purple-red - line L1130; red-yellow – lines L1304 and L1308; orange-yellow - lines L1294, L1207 and L1115. Uni-coloured fruits: rose-coloured – lines L1088, L584 and L525.

Bacterial strains: The plants were tested with the bacterial strains of *P. syringae* pv. *Tomato(Pst)*: **race R0-strain 31t**; **race R1-strain 27t** (personal collection - prof. N. Bogatzevska-ISSAPP)

Inoculation in vivo: from 16 to 44 plants from each line in phase 5-6th leaf were infested by vacuum infiltration method with bacterial suspension adjusted to 10⁴ CFU mL⁻¹ (24h culture)

of *P. syringae* pv. *tomato* (BOGATZEVSKA, 2002). The inoculated plants were grown in nutrient solution in laboratory conditions at room temperature.

Disease estimation: hypersensitive response (HR) on plants was recorded after 24 hours; the number of the diagnostic leaf spots was recorded 4-5 days after the infiltration. The infection with *P. syringae* pv. *tomato* was calculated using the scale of Chambers and Merriman (1975). The classification of the breeding lines in groups was made on the basis of the mean score of infection (ms): immune - 0 (I); resistant: 0.01-0.60 (R); medium sensitive: 0.61-1.49 (MS); sensitive: 1.50-2.99 (S); highly sensitive over 3.00 (SS) (SOTIROVA *et al.*, 1998). Healthy and HR plants were selected and transmitted to be grown on the experimental field at the "Maritsa" VCRI. Seeds were obtained for further screening.

Firstly initial infestation and continuous three - year selection of resistant to *P. syringae* pv. *tomato* R0, R1 were performed using the scheme R0/R0, R1/R1. Then the selected resistant lines were crosswise infiltrated in parallel with: (i) the *P. syringae* pv. *tomato* R0/R1, R1/R0, (ii) a mixture of R0 and R1/ R0,R1, and (iii) a mixture of *Xantomonas vesicatoria* T1,T2,T3 and *X. gardneri* / R0, R1. The average degree of infestation was recorded.

Agro-economical, morphological and phytopathological assessments were performed under three-year comparative field trails. Observations and records were performed for each plant. Vegetation period was estimated from germination phase to the beginning of fruit ripening. Productivity was determined as yield from one plant (kg). The following parameters were recorded at the stage of technological ripeness: average fruit weight (g); fruit shape by the equation $i=h/d$, where i = fruit shape index, h = fruit height (cm), d = fruit diameter (cm); locule number; and fruit colour visually.

The results obtained were processed statistically by analysis of variance (LAKIN, 1990) and multiple analyses of variance (DUNCAN, 1955).

RESULTS AND DISCUSSION

The studied breeding tomato lines demonstrated different degree of infection in artificial infestation with races of *P. syringae* pv. *tomato*. Immunity accessions to *Pst* race 0 were not established in initial test (Table 1).

A resistance response to *P. syringae* pv. *tomato*, race 0, at average degree of infestation (0.01 - 0.60), was shown in two lines (L1078 and L1083). The lines L1130, L1294, L1115, L1088, L584 were slightly susceptible to *P. syringae* pv. *tomato* R0 (0.61 - 1.49). Lines L525, L1207, L1304 and L1308 demonstrated a susceptible response with average degree of infestation from 1.50 to 2.99. Strongly susceptible lines with average degree of infection over 3.00 were not registered. Lines with resistant and slightly susceptible response are of interest for the breeding process and for the practice. Healthy plants with HR reaction after inoculation with *P. syringae* pv. *tomato* R0 were recorded in lines L1078, L1083, L1130, L1088 and L584. Resistant plants were subsequently re-tested and it was established, that the number of the healthy and HR plants, was increased in L1078, L1083 and L1088. After double individual selection of resistance to *P. syringae* pv. *tomato* R0 the value of the average degree of infection was increased in lines L1130 and L584, but the score range which determines the average susceptible response remained unchanged. Individual healthy plants from line L1130 with HR records or score 1 (2 spots per plant) were selected during three sequencing generations for resistance to *P. syringae* pv. *tomato* race 0.

Table 1. Evaluation of resistance to *Pseudomonas syringae* pv. *tomato* race R0

Breeding line	N	0	HR	1	2	3	4	ms
L 1078	25	9	5	11	0	0	0	0.44
L 1078	20	0	16	4	0	0	0	0.20
L 1083	22	2	8	12	0	0	0	0.54
L 1083	18	4	14	0	0	0	0	0.00
L 1130	26	6	4	16	0	0	0	0.62
L 1130	21	1	5	13	2	0	0	0.81
L 1130	22	8	10	4	0	0	0	0.18
L 1304	20	0	0	2	13	5	0	2.15
L 1308	25	0	0	15	10	0	0	1.75
L 1294	21	0	0	12	9	0	0	1.43
L 1207	22	0	0	13	8	1	0	1.54
L 1115	16	0	0	10	6	0	0	1.38
L 1088	30	0	3	21	6	0	0	1.10
L 1088	21	3	9	6	3	0	0	0.57
L 584	20	0	5	13	2	0	0	0.85
L 584	25	2	1	18	4	0	0	1.04
L 525	26	0	0	0	8	12	6	2.92

N—number of plants, HR – hypersensitivity response, *Sotirova and Beleva (1975) degree scale, ms- mean score;

Lines with immune reaction to race R1 of *P. syringae* pv. *tomato* have not been established (Table 2). The lines L1078, L1083, L1130 demonstrated resistance at initial infestation. Slightly susceptible reaction was recorded in the following lines: L1304, L1088 and L584. Breeding lines with susceptible reaction were: L1308, L1294, L1207, L1115 and L525. Strongly susceptible lines were not established. As a result of selection for resistance in two and three sequence generations in lines L1078, L1083 and L1088 and lines L584 and L1130 respectively, it was established that the number of healthy and HR plants considerably increased compared to the initially tested.

The lines with resistant response to either of the two races were of interest but especially valuable were the ones that were resistant to both races. Breeding lines L1078, L1083, L1130 and L1088 possessed complex resistance to both races of *P. syringae* pv. *tomato* after sequence individual selection (Table 3). Line L584 was stabilized for the resistance to *P. syringae* pv. *tomato* race 1, only.

An interesting finding was that among the lines studied with untraditional fruit colour those with resistance to *P. syringae* pv. *tomato* R1 prevailed. Resistance against *P. syringae* pv. *tomato* R0 strains is conferred by the *Pto* protein, which recognizes either of two pathogen effectors: *AvrPto* or *AvrPtoB*. However, current tomato varieties do not possess a resistance to the increasingly common race 1 strains, which lack these effectors (BAO *et al.*, 2015).

Table 2. Evaluation of resistance to *Pseudomonas syringae* pv. *tomato*, race R1

Breeding line	N	0	HR	1	2	3	4	ms
L 1078	44	18	20	6	0	0	0	0.14
L 1078	21	4	6	11	0	0	0	0.52
L 1083	30	2	17	11	0	0	0	0.37
L 1083	16	6	2	8	0	0	0	0.50
L 1130	22	0	14	8	0	0	0	0.36
L 1130	17	10	0	7	0	0	0	0.41
L 1130	24	0	24	0	0	0	0	0.00
L 1308	28	1	0	13	11	3	0	1.57
L 1294	20	0	0	4	10	6	0	2.10
L 1207	30	0	0	7	8	14	1	2.30
L 1115	32	0	0	13	13	6	0	1.78
L 1088	32	4	2	24	2	0	0	0.88
L 1088	21	1	18	2	0	0	0	0.09
L 584	22	6	0	12	4	0	0	0.91
L 584	24	2	6	10	6	0	0	0.92
L 584	20	4	12	4	0	0	0	0.20
L 525	24	0	0	4	4	14	2	2.58

N—number of plants, HR – hypersensitivity response, *Sotirova and Beleva (1975) degree scale, ms- mean score;

Table 3. Evaluation of resistance to *Pseudomonas syringae* pv. *tomato* races R0, R1

Breeding line	<i>P. syringae</i> pv. <i>tomato</i> , R0								<i>P. syringae</i> pv. <i>tomato</i> , R1							
	N	0	HR	1	2	3	4	ms	N	0	HR	1	2	3	4	ms
L 1078 R0									19	7	4	8	0	0	0	0.42
L 1078 R1	21	0	6	15	1	0	0	0.81								
L 1078 R0, R1	21	5	16	0	0	0	0	0.00	18	0	18	0	0	0	0	0.00
L 1078 Xv, Xg	23	9	14	0	0	0	0	0.00	28	4	24	0	0	0	0	0.00
L 1083 R0									17	3	13	1	0	0	0	0.06
L 1083 R1	19	0	16	3	0	0	0	0.16								
L 1083 R0, R1	29	17	12	0	0	0	0	0.00	20	0	20	0	0	0	0	0.00
L 1083 Xv, Xg	22	5	17	0	0	0	0	0.00	20	20	0	0	0	0	0	0.00
L 1130 R0									22	8	14	0	0	0	0	0.00
L 1130 R1	22	0	14	8	0	0	0	0.36								
L 1130 Xv, Xg	21	0	18	3	0	0	0	0.14	26	0	22	4	0	0	0	0.15
L 1294 Xv, Xg	18	0	9	9	0	0	0	0.50	25	8	14	3	0	0	0	0.12
L 1088 R0									26	0	26	0	0	0	0	0.00
L 1088R1	20	0	13	7	0	0	0	0.35								
L 1088 Xv, Xg	22	0	10	12	0	0	0	0.55	28	4	24	0	0	0	0	0.00
L 584 R0/R1									21	1	14	6	0	0	0	0.29
L 584 R1	20	0	0	6	10	4	0	1.90								
L 584 Xv, Xg	22	0	18	4	0	0	0	0.18	20	0	20	0	0	0	0	0

N—number of plants, HR – hypersensitivity response, *Sotirova and Beleva (1975) degree scale, ms- mean score; Xv - *Xanthomonas vesicatoria* ; Xg - *Xanthomonas gardneri*

The resistance to both races of *P. syringae* pv. *tomato* is possible to be stabilized in breeding lines only provided that it has been stabilized prior to each race individually. The lines L1078, L1083, L584, L1130 and L1088 that were resistant to *X. gardneri* and the races of *X. vesicatoria*, retained with mean scores of infestation either unchanged or decreased after infiltration with the races of *P. syringae* pv. *tomato*.

Stabilized resistant response to both races of *P. syringae* pv. *tomato* was achieved in L1078 and L1083 resistant to *X. gardneri* and the races of *X. vesicatoria*. Line L1294 showed susceptible reaction to both races of *P. syringae* pv. *tomato* in the initial tests. However, an increase of the number of healthy plants and those with HR to *P. syringae* pv. *tomato* after screening and selection of plants with resistant reaction to *X. gardneri* and the races of *X. vesicatoria* was observed (Table 3). This result was recorded in L1294, only. Further investigations are needed to prove the statement that selection for resistance to bacterial spot leads in an increase of the resistance to the races of *P. syringae* pv. *tomato* as well. Genetic studies indicated that single dominant genes controlled resistance to bacterial speck. Four genes, from *Pto-1* to *Pto-4*, have been reported so far (PITBLADO and MACNEILL, 1983; PILOWSKY and ZUTRA, 1986; STOCKINGER and WALLING, 1994). *Pto-1* is also generally referred to as *Pto* and has been cloned. The *Pto* gene for resistance is widely deployed in both fresh market and processing tomato varieties (MARTIN *et al.*, 1993). Genetic analysis of disease resistance in tomatoes system has revealed that resistance to race 0 strains of *P. syringae* pv. *tomato* is controlled by a single resistance locus, *Pto*. *Pto* was derived from the sexually compatible wild species *Lycopersicon pimpinellifolium* and mapped to the short arm of chromosome 5 at map position (PEDLEY and MARTIN, 2003). Strains of avirulent race 0 elicited resistance on *Pto*-expressing tomatoes whereas strains of virulent race 1 are not recognized by *Pto* and therefore cause disease on *Pto*-expressing lines. An avirulence gene, *avrPto*, eliciting resistance on *Pto*-expressing tomato lines was isolated from a race 0 strain in 1992 (RONALD *et al.*, 1992). Introduction of the cloned *avrPto* gene into a race 1 strain and inoculation of the strain onto leaves of a pair of near-isogenic lines differing at the *Pto* locus demonstrated that *Pto* and *avrPto* define a gene-for-gene interaction that is the basis for resistance to bacterial speck disease in tomato. Typical of many gene-for-gene interactions, *Pto*-mediated resistance to *avrPto*-expressing *P. syringae* pv. *tomato* strains is associated with localized cell death, termed the HR. *AvrPto* was found to be present in all the examined *P. syringae* pv. *tomato* R0 strains and absent in *Pst* R1 strains (PEDLEY and MARTIN, 2003).

Resistance against *P. syringae* pv. *tomato* R0 strains is conferred by the *Pto* protein, which recognizes either of two pathogen effectors: *AvrPto* or *AvrPtoB*. However, current tomato varieties do not have resistance to the increasingly common race 1 strains, which lack these effectors (BAO *et al.*, 2015). Initial characterization of the *Pto* gene revealed that it is a member of a gene family, present in both resistant and susceptible tomato lines (PEDLEY and MARTIN, 2003). Members of the *Pto* gene family are transcribed in both resistant and susceptible tomato leaves, and there is no evidence that any of them are induced by pathogen infection. In tomatoes, the *Pto* kinase (product of the *Pto* R gene) confers resistance to strains of the bacterial speck pathogen, *P. syringae* pv. *tomato*, that carry the corresponding avirulence gene *avrPto*. Resistance to bacterial speck disease is initiated by a mechanism involving the physical interaction of the *Pto* kinase and the *AvrPto* protein (GU and MARTIN, 2016).

The tomato genotypes studied differed in habitus, pigmentation, average weight, fruit shape and locule number (Table 4). Growth type of eight of the lines was indeterminate and in

the remaining three accessions it was determinate. Fruits without green shoulder were observed in two of the accession only - L1088 and L525. Diversity by fruit shape among the lines was also observed. Fruit shape of the line L1083 was flat-round; L1088, L1115 and L525 were with oval fruits; L1294 – with oval-elongated and with round fruit shape were the remaining ones. The number of locules of the fruits also varied – from 2-3 to multi-locular.

Table 4. Characteristic of plants and fruits of tomato lines

Breeding line	Growth type	Fruit				
		Green shoulders before maturity	Colour at ripeness phase	Fruit shape I=h/d	Shape	Locule, number
L1078	indeterminate	present	brown-red	0.97	round	2-3
L1083	indeterminate	present	brown-red	0.69	flat-round	>5
L1130	indeterminate	present	red-purple	0.90	round	5-7
L1304	indeterminate	present	red-yellow	0.94	round	3-4
L1308	indeterminate	present	red-yellow	0.95	round	>5
L1294	indeterminate	present	orange-yellow	1.40	oval	2-3
L1207	determinate	present	orange-yellow	0.84	round	5-7
L1115	indeterminate	present	orange-yellow	1.02	round	>5
L1088	indeterminate	absence	rose	1.20	oval	2-3
L584	determinate	present	rose	0.99	round	4-6
L525	determinate	absence	rose	1.26	oval	2-3

To combine disease resistance with high yield and fruit quality is a problem for every breeding process (SOFKOVA *et al.*, 2010). The lines included in the study differed significantly by biological earliness – from early to mid-early lines (Table 5). The recorded resistant lines belonging to the early group were L584 and L1088 and to mid-early group - L1130, L1078 and L1083. There were significant differences by the character productivity. Accessions L1083 and L1130 were resistant and high yielding. The average fruit weight was from 41.9 to 183.7g (Table 5). Line L1083 was described with the largest fruits, with average fruit weight 183.7 g and with the highest productivity. The lines with rose-coloured fruits, L584 and L1088, showed the lowest productivity. The variation coefficient in nine of the studied lines was with values up to 10%, giving information about uniformity by productivity and average fruit weight.

Table 5. *Biological earliness, productivity and average fruit weight*

Breeding line	Vegetation period, days			Productivity kg/plant			Average fruit weight (g)		
	\bar{X} \pm sd		CV%	\bar{X} \pm sd		CV%	\bar{X} \pm sd		CV%
L 1078	109,5 \pm 1.3	b	1.2	1445.0 \pm 146.6	fg	10.2	93.2 \pm 5.7	f	6.2
L 1083	110.8 \pm 1.5	ab	1.4	2940.0 \pm 134.9	a	4.6	183.7 \pm 20.1	a	10.9
L 1130	108.0 \pm 2.2	b	2.0	2927.5 \pm 270.4	a	9.2	115.9 \pm 17.1	d	14.7
L 1304	105.0 \pm 2.9	c	2.8	1412.5 \pm 51.9	fg	3.7	66.2 \pm 5.1	g	7.7
L 1308	110.8 \pm 1.0	ab	0.9	1747.5 \pm 166.6	d	9.5	157.5 \pm 16.7	b	10.6
L 1294	108.0 \pm 1.7	b	1.6	1497.5 \pm 141.3	f	9.4	100.5 \pm 6.1	ef	6.1
L 1207	113.5 \pm 1.3	a	1.1	2382.5 \pm 231.9	c	9.7	141.6 \pm 8.8	c	6.2
L 1115	113.3 \pm 1.3	a	1.1	2692.5 \pm 123.1	b	4.6	160.4 \pm 8.8	b	5.5
L 1088	100.3 \pm 1.7	d	1.7	1313.8 \pm 149.4	fg	11.4	41.9 \pm 3.2	h	7.7
L 584	98.8 \pm 2.2	d	2.5	1218.8 \pm 82.3	g	6.8	108.5 \pm 5.5	de	5.1
L 525	110.8 \pm 1.5	ab	1.4	1450.0 \pm 101.3	fg	6.9	51.4 \pm 2.4	h	4.6

a,b.. Duncan`s multiple range test ($p < 0,05$)

The lines L1083 and L1130 have been found to possess resistance to *P. syringae* pv. *tomato*, high productivity, untraditional fruit colour (brown-red, black, red-purple) and have been considered as an attraction and challenge for the consumers. Therefore, these lines are of interest for the breeding process.

Lines L1083, L1078, L1130, L1088 and L584 with resistant response to the races of *P. syringae* pv. *tomato* are valuable donors of resistance in the combinative and heterosis selection and genetic studies but they could not be developed as a direct varieties.

CONCLUSIONS

Tomato lines with untypical fruit pigmentation studied in this experiment were described with different degree of resistance to agent of the bacterial speck- *P. syringae* pv. *tomato* races R0 and R1.. Immune response was not recorded among the lines studied. The resistance was stabilized to both R0 and R1 of *P. syringae* pv. *tomato* as a result of performed successive individual selection of healthy and HR plants. Lines L1078, L1083 and L1130 were found to have complex resistance to both races of *P. syringae* pv. *tomato*. Lines L 1088 and L584 were resistant to race 1 of *P. syringae* pv. *tomato*, only. The resistant lines with valuable economic properties and untraditional colour of the fruits are valuable sources of resistance in combinative and heterosis selection of new improved varieties with complex disease resistance.

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**IZVORI OTPORNOSTI PREMA RASAMA R0 I R1 *Pseudomonas syringae* PV. *tomato* –
PROUZROKOVACIMA BAKTERIOZNE PEGAVOSTI**Daniela GANEVA¹ i Nevena BOGATZEVSKA²¹”Maritsa” Istraživački institut za povrće, Plovdiv, Bugarska²Institut za zemljište, Agrotehnologiju i zaštitu bilja, “N. Pushkarov”,
Sofija, Bugarska

Izvod

Selekzione linije paradajza koje nemaju tradicionalnu crvenu boju ploda, ispitane su kao izvor otpornosti prema rasama R0 i R1 *Pseudomonas syringae* pv. *tomato*. Kao rezultat selekcije zdravih biljaka sa hipersenzibilnom reakcijom (HR), stabilne otpornosti, odabrane su perspektivne linije koje su nosioci gena otpornosti prema bakterioznoj pegavosti koja je izazvana ovim patogenom. Linije L1078 i L1083 sa crveno-braon (crnom) bojom i linija L1130 sa purpurno crvenom bojom ploda poseduju kompleksnu rezistentnu reakciju prema rasama R0 i R1. Utvrđeno je da su dve linije roze obojenosti ploda paradajza (L1088 i L584) otporne prema rasi 1 *P. syringae* pv. *tomato*. Ove linije poseduju visoke ekonomske i morfološke osobine, te se mogu kombinovati i očekivati heterotični efekti za dobijanje sorti/hibrida otpornih na bakterioznu pegavost izazvane rasama R0 i R1 *Pseudomonas syringae* pv. *tomato*.

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