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Sources of Resistance to Races of *Xanthomonas vesicatoria* – Causal Agent of Bacterial Spot of Tomatoes

Daniela GANEVA[a], Kamelia ALEKSANDROVA[b], Nevena BOGATZEVSKA[b]
[a]Maritsa"Vegetable Crops Research Institute, 32, Brezovsko shosse Str., 4003 Plovdiv, Bulgaria
[b]Institute of Soil Science, Agrotechnologies and Plant Protection "N. Pushkarov", 7 Shosse Bankya St., 1331 Sofia, Bulgaria.
*Corresponding author: dganeva@abv.bg;

Abstract

Bacterial spot of tomatoes is caused by the bacteria *Xanthomonas vesicatoria* and it is economically significant for Bulgaria, affecting quality and yield of tomato crops, especially in warm and wet seasons. Current investigation aimed to identify sources of resistance to races T1, T2 and T3 of *X. vesicatoria* in 14 tomato breeding lines. A differential reaction of the lines to pathogen races was observed. An individual plant selection of healthy and hypersensitive progenies was made for three generations within the lines with resistant reaction. Redistribution of the percentage of plants by disease rating was observed in the progenies. As a result pure lines with greatest number of healthy and hypersensitive plants were selected. These could be used as sources for resistance to bacterial spot on tomatoes, caused by *X. vesicatoria.*

Key words: *Solanum lycopersicum*, bacterial spot, sensitivity, breeding

Introduction

Bacterial spot caused by *Xanthomonas vesicatoria* is an economically important disease on tomato crops for mid-early and late field production in Bulgaria. Large-scale disseminated species are the ones belonging to tomato (T) and pepper-tomato (PT) pathotypes (Bogatzevska, 2002; Bogatzevska and Sotirova, 1992, 2001-2002). The races T1, T2 and T3 of *X. vesicatoria* have been differentiated (Bogatzevska, 2002; Bogatzevska and Sotirova, 2001-2002).

An important condition for the genetic research of the *host (*tomato plant) - *pathogen* (pathogenic bacteria *X. vesicatoria)* system is the presence of sources of resistance. The level of resistance to *X. vesicatoria* within cultivated species of *L. esculentum* is insufficient (Minsavage et al., 1990, 2003; Somodi et al., 1994, 1996; Scott et al., 1995, 1997, 2001, 2003; Sotirova and Bogatzevska, 1998-1999, 2000). No resistant genotypes to the races of tomato pathotype of *X. vesicatoria* were established in Bulgaria or worldwide (Scott et al., 1997; 2001, 2003). Due to the low level of resistance to the disease in the cultivated tomato sub specie and the lack of resistant varieties, the wild species *L. chilense, L. hirsutum, L. hirsutum f. glabratum* have been used as sources in the breeding programs (Sotirova and Bogatzevska, 2000; Scott et al., 2001). The resistance to *X. vesicatoria* is based on a hypersensitive response that is considered as a monogenic controlling mechanism and as a vertical resistance (Scott et al., 1995, 2001, 2003). High levels of resistance to T1 and T3 of *X. vesicatoria* were established in tomato lines containing three-genome hybrid and (*L. esculentum x L. chilense LA460*) x L. *peruvianum var. humifusum* PI 127*829* and *L. esculentum x L. pimpinellifolium* PI 126925 (Ianova and Bogatzevska, 2006).

The classical breeding approach for development of resistant lines is based on the use of wild species as a source of resistance, backcrosses and selection in the next generation. The development of varieties with durable and multiple resistances is continuous and complicated process that requires extensive and variable immunity researches that precede breeding process.

Every breeding programme concerning the pathogens control depends on the use of appropriate sources of resistance, methods for testing, objective evaluation and selection of the breeding material (Danailov, 2012; Danailov et. al, 2008, Ganeva and Bogatzevska, 2013).

The aim of the present study was to investigate the level of resistance of tomato breeding lines to bacterial spot agent *X. vesicatoria*

and to determine the breeding effect after each cycle of individual selection.

Material and Methods

Plant material **–** Fourteen tomato breeding lines from genotype collection of the Maritsa Vegetable Crops Research Institute in Plovdiv were an object of the study. The lines differed by their origin, economical, morphological, chemical, technological and sensory characteristics. Four lines (L1076, L1080, L1042 and L1033) were with homogenic advanced progenies obtained as a result of interspecific hybridization. Three lines - L1168, L1227 and L1260 were of local origin with large fruits and good taste properties. Seven lines of high productivity and good morphological, chemical and technological properties were also included: L640 - indeterminate, L757 and L503 determinate, large fruited, and L 273, L446, L525, L520 - determinate ones suitable for processing as peeled tomatoes.

Bacterial pathogen - *X. vesicatoria*: T1 strain № 24t; T2 strain № 53t and T3 strain № 30t (strains belong to pathology collection of the Plant protection department of Institute of soil science, agrotechnologies and plant protection "N. Pushkarov", Sofia).

Inoculation in vivo – plants from each variety (over/or 20 numbers) in growth stage of the 5-6^{-th} leaf were infected with bacterial suspension of *X. vesicatoria* (races T1, T2, T3) from 36h culture in concentration 10^8 cfu/ml by vacuum-infiltration method. The infected plants from each race were grown individually in nutrient solution of Knop at 20-25^0 C (Bogatzevska, 2002).

Evaluation of the disease – the development of hypersensitive reaction (HR) was registered after 24h; the number of the spots necessary to diagnose bacterial spot disease on the leaves was registered 4-5 days after the infiltration. The mean score of infestation was calculated (ms) based on the scale of Sotirova and Beleva (1975). The classification of the breeding lines in groups was made on the basis of the mean score of infestation (ms): immune (I): 0; resistant (R): 0,01 - 0,6; moderately sensitive (MS): 0,61 - 1,49; sensitive (S): 1,50 - 2,99; highly sensitive over 3 (SS).

After the artificial inoculation with races T1, T2 and T3 to *X.vesicatoria* in laboratory conditions, a selection of health plants with hyper sensitive or resistant reaction with 1-5 spots was made. The individual plants were grown according to all agro-technical conditions and requirements of mid-early field production of tomato up to fruit ripening. Seeds were obtained and then they were used for another cycle of artificial screening and selection. The experiment cycle were conducted for the period of 3 years. As criteria for evaluation of lines were used the records from degree scale from 0 to 4 and the mean scale of infestation. It was studied the breeding effect on the resistance to races T1, T2 and T3 to *X. vesicatoria* after each cycle of individual selection. The estimation rates were calculated in percentage for better comparison of the data to be made.

Results and Discussion

In the first year of study it was performed a preliminary test of 14 tomato breeding lines in order to identify the desirable genetic factor – resistance to races T1, T2, T3 of *X. vesicatoria* (Table.1). Different degree of infestation was established. Accessions with immune response were not registered. Resistant and highly susceptible lines to races T1 and T3 were not established in 2011. The number of slightly susceptible is significant as the greatest one is in T1, followed in T3 and the smallest – in T2. The susceptible lines in T2 were more than the slightly susceptible. High degree of infestation to race T2 was recorded in line L757 (rate 3). The average degree of infestation in the studied tomato lines was the greatest in race T2, followed by T3 and the lowest was in T1. This tendency was kept during the next years on the basis of the performed individual selection of healthy plants with having hypersensitive response. Seven lines were resistant to race T1, one to T2 and four to T3. Line 1168 could be offered as a new source of resistance to the three races of *X. vesicatoria.* The line was obtained as a result of multiple individual selection in local population. The plants were indeterminate, with average fruit weight of 250-280 g, pink colour, and very good morphological and sensory characters. Line 1076 – indeterminate with mid-large, red coloured fruits and line 503 – high yielding, determinate, large fruited, with red round fruits and good taste quality were resistant to races T1 and T2. Resistant to T1 were the following lines: L1080 – indeterminate, with mid-large to small fruits, L273 – simultaneous ripening, determinate, small fruited, with oval fruits, suitable for industrial processing and L1260 – with indeterminate growth habit, large cordate shaped, red fruits and very good taste. Lines L1227 – with indeterminate growth habit, very large, oblate-circulate red fruits and good taste was resistant to T3.

Table. 1. Resistance of tomato breeding lines to race T1, T2, T3 of *X.vesicatoria*.

Lines	Mean score (ms)																	
	XvT1						XvT2						XvT3					
	2011		2012		2013		2011		2012		2013		2011		2012		2013	
L1076	1.36	MS	0.84	MS	0.47	R	2.00	S	1.15	MS	0.77	MS	1.23	MS	1.30	MS	0.50	R
L1080	1.20	MS	0.68	MS	0.22	R	1.19	MS	1.24	MS			1.15	MS	1.23	MS	1.32	MS
L1042	1.29	MS	1.40	MS			1.38	MS	1.70	S			0.86	MS	1.10	MS		
L1433	1.50	S	1.08	MS			1.70	S	1.47	MS			1.39	MS	1.31	MS		
L503	1.46	MS	0.77	MS	0.00	RR	2.13	S	0.60	R	1.07	MS	1.58	MS	1.40	MS	0.36	R
L273	1.25	MS	1.04	MS	0.53	R	1.77	S	1.40	MS	1.17	MS	1.89	S	1.54	S	1.00	MS
L446	1.43	MS	1.23	MS			2.00	S	1.50	MS			2.21	S	1.53	S		
L525	1.45	MS	1.40	MS			1.86	S	1.48	MS			2.10	S	1.30	MS		
L520	1.78	S	1.48	MS			1.81	S	1.42	MS			1.58	S	1.00	MS		
L1168	1.13	MS	0.55	R	0.41	R	1.25	MS	0.73	MS	0.44	R	0.86	MS	0.60	R	0.10	R
L1227	0.72	MS	0.62	MS	0.60	R	1.46	MS	1.14	MS	0.65	MS	1.04	MS	0.52	R	0.21	R
L1260	1.00	MS	0.76	MS	0.12	R	1.94	S	0.72	MS	0.64	MS	2.00	S	1.00	MS	0.68	MS
L640	1.18	MS	1.14	MS			1.46	MS	0.83	MS			1.00	MS	1.17	MS		
L757	2.00	MS	1.42	MS			3.00	SS	1.71	S			2.80	S	1.85	S		
$\bar{x}$	1.34		1.03		0.32		1.78		1.22		0.79		1.57		1.20		0.60	

Regardless of the performed individual selection of healthy plants along with ones with hypersensitive reaction to each of the races of *X. vesicatoria* in 2012 and 2013 segregation in the lines was still observed. During the years all the lines demonstrated a decrease of the infestation degree to the three races except of the L1042 where an increase was observed.

A redistribution of the percentage share of the respective ratings was observed in formation of the mean score of infestation degree as a result of the individual selection.

Number of healthy and HR plants was 6% to race T1 of *X. vesicatoria* during the first year of test (figure 1). Plants with rates 3 and 4 were not recorded. Healthy and HR plants were increased up to 66.9% as a consequence of the selection made. There were no plants with rate 2 while the plants with rate 1 decreased with 55.0%. The highest percentage of plants with hyper sensitive reaction to race T1 was recorded in the lines L1080 (72.5%), L273 (46.7%), L1260 (44.0%) and L1227 (40.0%).

Number of healthy and HR plants to race T2 in the first year of the study were insignificant (1.7%) (figure 2). Susceptible and highly susceptible plants were recorded (rates 3 and 4). The highest percentage of plants was with rate 2 (52.2%). Number of healthy and HR plants increased to 31.1% as a result of the selection, as the number of plant with hyper sensitive response was twice more than the healthy ones. Plants with rates 3 and 4 were not recorded. Number of plants with rate 2 has decreased with 18%, while the ones with rate 1 have increased with 82.2%. The highest percentage of plants with hyper sensitive response after three –cycle of individual selection was recorded in the lines L1168 (32.0%), L1260 (31.8%), L1227 (26.9%). There was no breeding line obtained in which the plants with hyper sensitive reaction to exceed 50.0%. Line 1168 could be defined as a resistant to race T2 by mean score of infestation (T2-0.44) and number of healthy and HR plants (56.0%). It is necessary to continue selection for resistance for achieving of durable resistance.

Number of healthy and HR plants to race T3 were the greatest (8.3%) compared to T1 and T2 in the first year (figure 3). The number of plants with rates 3 and 4 is almost equal to the number of susceptible and highly susceptible plants to T2. After two-time selection, the number of plants with rates 3 and 4 decreased considerably and after the third –time selection there were no plants recorded with susceptible and highly susceptible reaction. As a result of three -time cycle of individual selection, the healthy and HR plants increased up to 51 %. Number of the recorded plants with hyper sensitive reaction in L1227 was 75%, in L1076 – 50% and in L503-45.5%. The number of healthy plants with hyper sensitive reaction in L1168 was 90 %.

The variations observed in the degree of resistance in the studied breeding lines during the tree-year cycle revealed that the presence of resistance even in the individual healthy and HR plants could be increased and stabilized as a result of multiple screening and selection procedure. The selected resistant lines in the current study could be used in breeding programme as sources of

resistance to bacterial spot caused by *X. vesicatoria.*

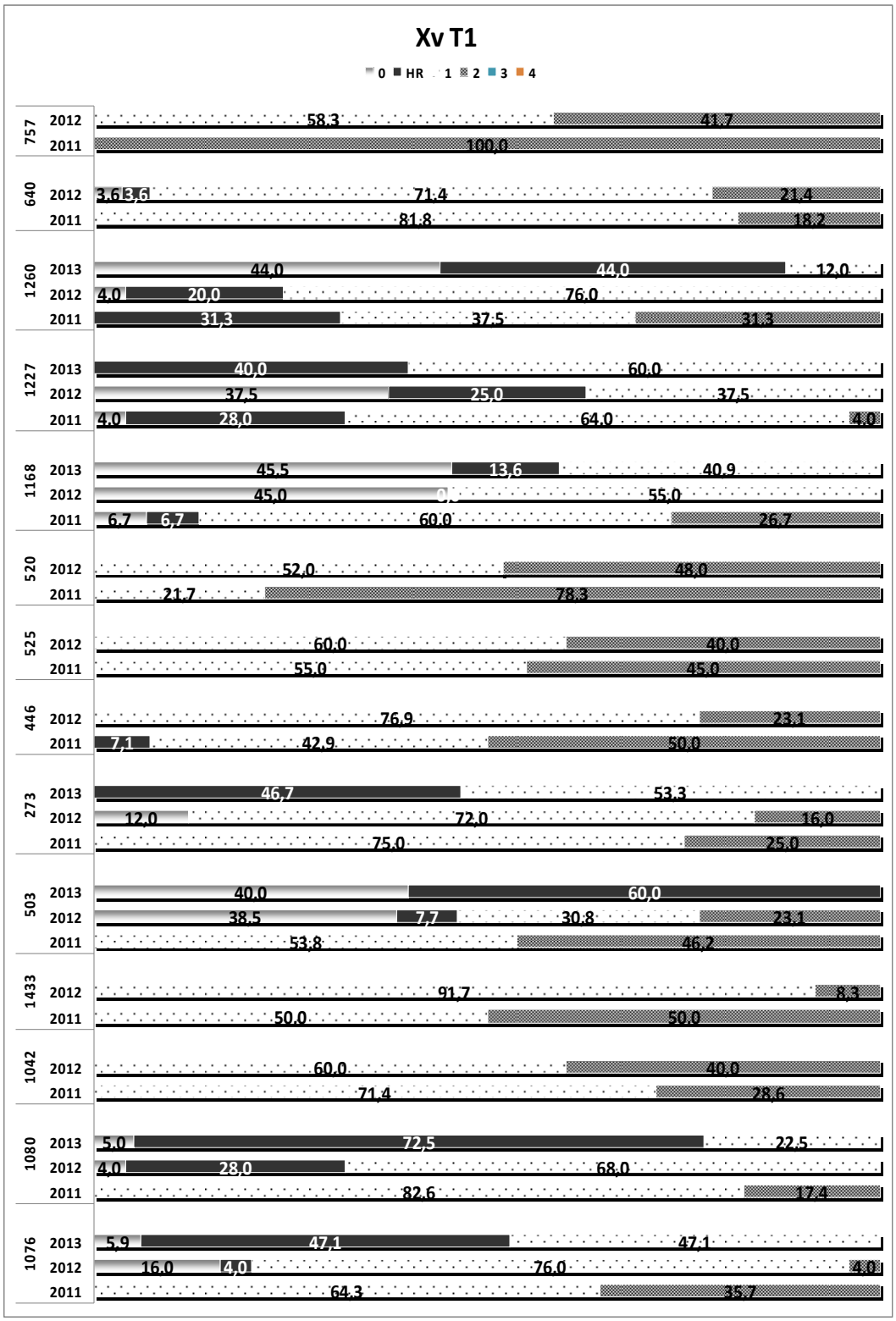

Figure 1. Distribution of the plants according to the rate of infestation to race T1, in %

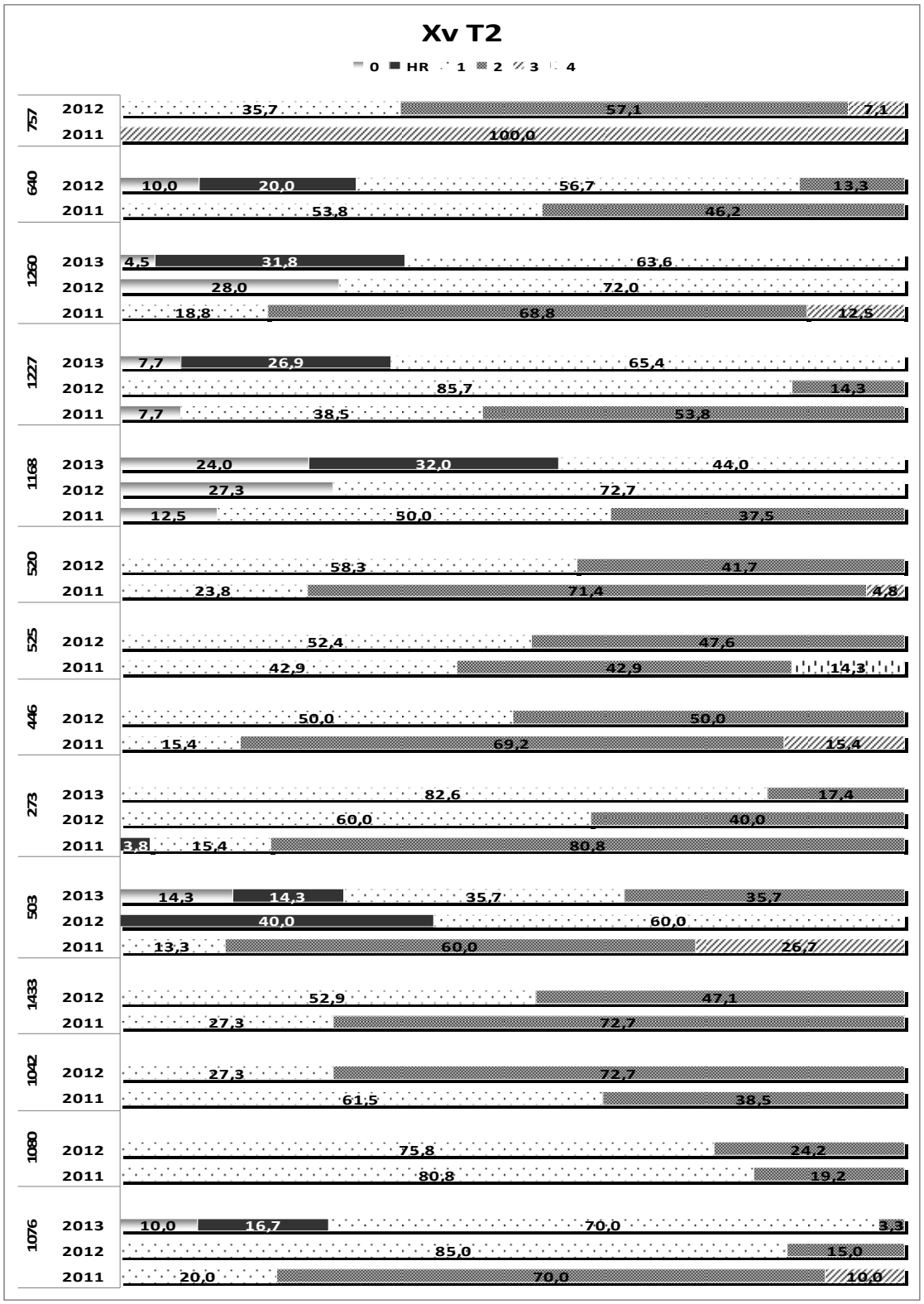

Figure 2. Distribution of the plants according to the rate of infestation to race T2, in %

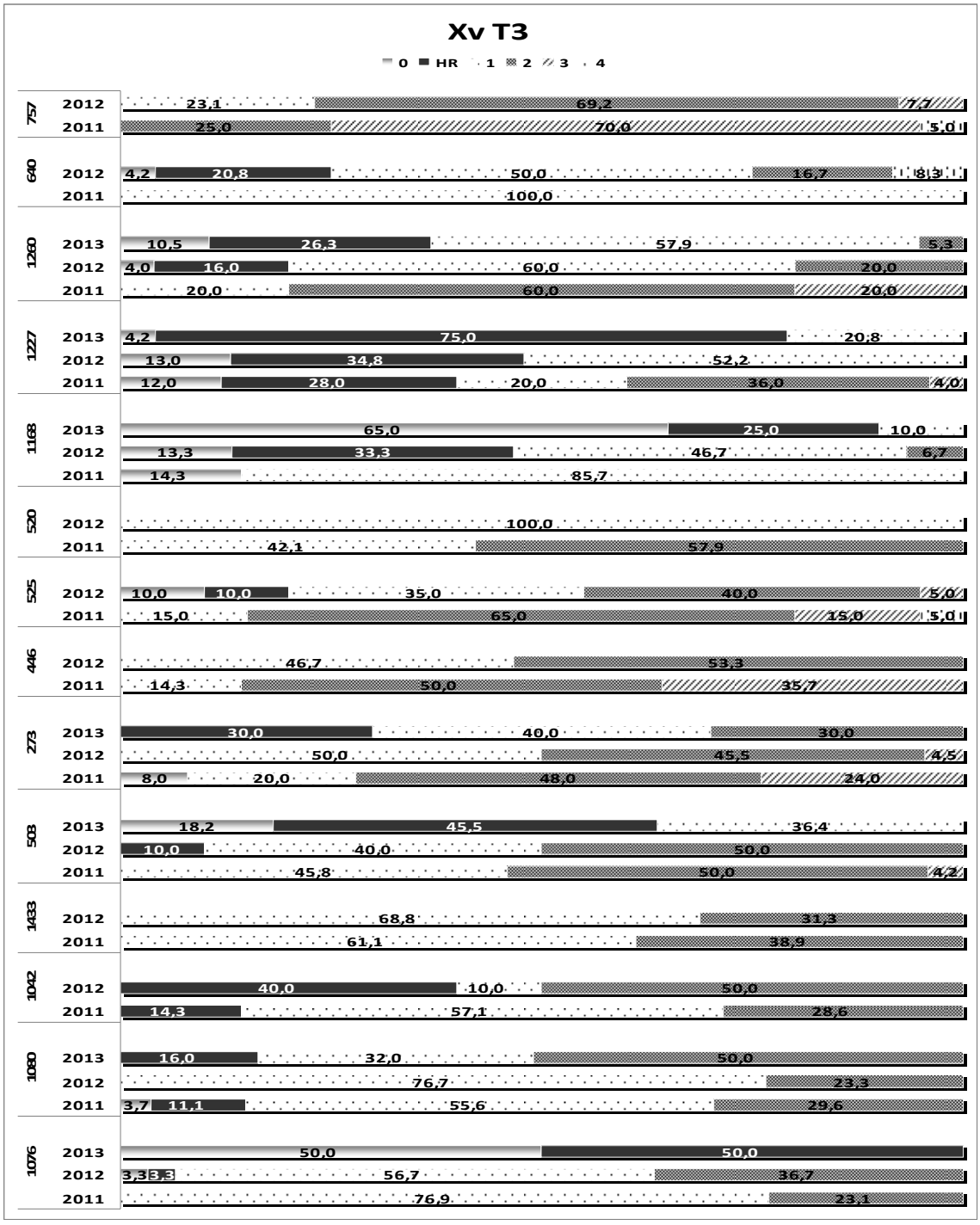

Figure 3. Distribution of the plants according to the rate of infestation to race T3, in%

Conclusions

Seven tomato lines with resistance to race T1, one line – to T2 and four lines– to T3 were identified. Line 1168 was resistant to the three races of *X. vesicatoria.* It is with indeterminate growth habit, very large fruits (average weight 250-280 g), pink colour, with very good morphological and sensory characters. Line 1076 and L503 were resistant to both races T1 and T2. Resistant to race T1 were the following lines L1080, L273 and L1260; resistant to T3 was L1227. The lines demonstrated resistance could be used in the tomato breeding as a new source of resistance to *X. vesicatoria.*

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