

## RESISTANCE OF *Brassica rapa* L. AND *B. napus* L. TO BLACK ROT AND LEAF SPOT PATHOGENS

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### Summary

The authors estimated the apple varieties and hybrid gene pool created during 1970-2009 years in All-Russian Scientific Research Institute of Orchard Culture the content of sugars, organic acids, ascorbic acid and P-active substances. The peculiarities of varying and inheriting of these substances have been investigated. The prospect of apple varieties selection with improved biochemical composition of fruit was shown.

**Keywords:** race-specific resistance, model «gene-for-gene», interaction plant—pathogen.

Cruciferous plants (the family *Brassicaceae*) are susceptible to many phytopathogens, the most harmful of which is the bacterium *Xanthomonas campestris* Pam. (Dow.). *X. campestris* variants cause diseases with different symptoms - vascular bacteriosis (*X. campestris* pv. *campestris* - Xcc) and leaf blotch (*X. campestris* pv. *raphani* - Xcr). Physiological races are detected by reaction of cultivars possessing race-specific resistance genes (1, 2). Three genetically close diploid species of the genus *Brassica* (*B. rapa*, *B. nigra* and *B. oleracea*) are distinct in type of resistance to bacterioses caused by *X. campestris*. In forms carrying B-genome, this feature is determined by the gene *Rxa1* (previously designated as *Rb*) (1), in A-genome - *Rxa4*, and in C-genome - *Rxa3* encoding the most common type of resistance (2). Along with it, there are allelic variants of three major loci and other types of resistance to *X. campestris* (3, 4). Breeding for pathogen resistance is limited by a small number of available donors (especially in *B. rapa* and *B. napus*), while the existence of more than 9 pathogen races (4) complicates interpretation of literature data on resistance of these species. Resistance to vascular bacteriosis was also found in several cultivars of *B. rapa* (5).

The purpose of study – to assess geographical distribution and rate of occurrence of race-specific resistance upon the analysis of *Brassica rapa* and *B. napus* collection samples by their response to strains of five pathogen races that cause vascular bacteriosis and one race of the leaf blotch pathogen.

**Technique.** *B. rapa* and *B. napus* samples were obtained from collections of the N.I. Vavilov All-Russia Research and Development Institute of Plant Growing (VIR), National Research Institute of Vegetables, Ornamental Plants and Tea (NIVOT, Japan), the All-Russia Research and Development Technology Institute of Rape (Lipetsk) and several breeding companies. The object of study were 134 samples of eight cultivated subspecies of *B. rapa* representing different geographic regions of Western Europe, Russia, Central Asia, China, Korea and Japan, and 10 wild populations from Russia and Western Europe (Great Britain and Germany). Some of these samples had been previously analyzed by molecular methods to determine the level of genetic diversity, phylogenetic relations of subspecies and taxonomic status of certain forms of a species (6, 7). Along with it, there were tested 56 samples of oilseed rape (*B. napus* var. *oleifera* L.) originated from the area Western Europe - Japan.

The plants were grown in pots of 10 cm diameter in a greenhouse at 20/16 °C (day/night) and 16-hour daylight before inoculation performed when the stage of 3-4 true leaves. The plants were inoculated with strains of five races Xcc (2): B-32 (race 6), Cal54-3 (race 5), HRI1279a (race 4), NCPPB528T (race 3), PHW231 (race 1) and the strain 5001a of race Xcr 1 (8). Bacteria were stored at –84 °C; the 2-days culture grown on the modified King B medium was used to prepare the inoculum by dilution to the titer 10<sup>6</sup> cells/ml. In the variant with Xcc races, plants were infected by pinching 3-4 leaves, in the variant of Xcr, plants were sprayed with bacterial suspension (10<sup>7</sup> cells/ml). Inoculated plants were kept in a moist chamber for 24 h, then grown in a greenhouse during 2 weeks at 24 °C. For plants infected by pinching, the results were recorded in 2 weeks using the 3-point scale: 0 – no reaction recorded or necrosis only around the point of inoculation (hypersensitivity reaction - HSR), 1 - necrosis around the point of inoculation and chlorosis of areas 0,5 cm diameter, 2 - the development of V-shaped necroses. The lesion of plants infected by spraying was assessed in 1 week by the 3-point scale: 0 - no reaction or HSR (necroses of less than 0,1 mm diameter), 1 - necroses occupy up to 5% leaf area, necrotic spots of 1-2 mm diameter; 2 – necroses greater than 2 mm diameter cover more than 10% leaf area. Three independent tests were performed on 50 plants of each sample; resistant plants were re-inoculated to confirm the result. The obtained data on three tests were used to determine average rate of resistant individuals (pathogen lesion - 0 or 1 point) as a percentage from the total number of plants in each sample. The number of tested plants was sufficient for detection of 99% resistant individuals whose frequency exceeded 3%.

The significance of differences between samples in share of plants resistant to certain pathogen races were determined by analysis of variance (9). Correlations between samples' responses to different races were estimated by the Pearson's method of correlation analysis (10) using the program STATISTICA 6.0 (StatSoft, USA)

**Results.** It was revealed a diversity of plant reactions in different samples, subspecies and geographic groups. Most of the samples exhibited test-reproducible responses. The response of *B. rapa* varied depending on pathogen race, subspecies and region of plant origin (Tables 1, 2 and 3). On average, 47,3% individuals of the sample manifested resistance to race Xcr 1, 11,1% - to Xcc 1, 18,7% - to Xcc 3, 49,1% - to Xcc 4, 17,0% - to Xcc 5 and 2,0% were resistant to Xcc 6. Only two samples of *B. rapa* (rosette cabbage

Ta-gu-tsai VIR-129 and Chinese cabbage Local VIR-108 originated from Southern China) were resistant to all pathogen races including Xcc 6. *B. napus* samples showed more uniform response – about 92,7% plants were resistant to race Xcr 1, 37,2% - to Xcc 1, 30,8% - to Xcc 3, 95,5% - to Xcc 4, 30,0% - to Xcc 5, and no plants resistant to Xcc 6.

Plant responses to infection with races Xcr 1 and Xcc 4 were found to be highly correlated ( $r = 0,96$  for *B. rapa* and  $r = 0,915$  for *B. napus*), as well as responses to Xcc 3 and Xcc 5 ( $r = 0,98$  for *B. rapa* and  $r = 0,99$  for *B. napus*). Along with it, there was a statistically significant correlation ( $r = 0,53$ ) between responses of *B. rapa* to races Xcc 1 and Xcc 5.

### 1. Geographical origin and resistance of *Brassica rapa* and *B. napus* samples to several races of the pathogen *Xanthomonas campestris* Pam. (Dow.)

Sample	Source	Subspecies	Region	Share of resistant plants, %					
				Xcr 1	Xcc 1	Xcc 3	Xcc 4	Xcc 5	Xcc 6
Just Right F1 <sup>a</sup>	Takii Seeds	<i>rapifera</i>	J	100	0	0	100	0	0
Tokyo Cross F1 <sup>a</sup>	Takii Seeds	<i>rapifera</i>	J	100	0	0	100	0	0
Seven Top Green <sup>a</sup>	Sakata Seeds	<i>rapifera</i>		100	0	0	100	0	0
Suwan	NIVOT	<i>rapifera</i>	J	70	2	10	62	12	0
Hijiore	NIVOT	<i>rapifera</i>	J	66	0	12	70	10	0
Shogoin oomaru	NIVOT	<i>rapifera</i>	J	90	0	10	100	12	4
Local 3091	NIVOT	<i>dichotoma</i>	A	60	0	30	64	24	0
Local 3096	NIVOT	<i>dichotoma</i>	A	52	0	34	50	30	0
Local 3124	NIVOT	<i>dichotoma</i>	A	44	0	36	50	40	0
Local 3159	NIVOT	<i>dichotoma</i>	A	50	0	56	70	60	0
Local 3161	NIVOT	<i>dichotoma</i>	A	68	0	80	100	70	0
Local 3166/1	NIVOT	<i>dichotoma</i>	A	44	0	50	84	50	0
Local 3166/2	NIVOT	<i>dichotoma</i>	A	80	0	70	100	70	0
Local 3167	NIVOT	<i>dichotoma</i>	A	70	0	44	100	34	0
Local 3170	NIVOT	<i>dichotoma</i>	A	100	0	54	50	40	0
Local 3171	NIVOT	<i>dichotoma</i>	A	100	0	56	50	50	0
Local 3172	NIVOT	<i>dichotoma</i>	A	80	0	40	80	30	0
Local 3177	NIVOT	<i>dichotoma</i>	A	30	0	70	42	46	0
Local 3181	NIVOT	<i>dichotoma</i>	A	44	0	74	84	48	0
Local 3188	NIVOT	<i>dichotoma</i>	A	100	0	50	100	40	0
Local 3103	NIVOT	<i>dichotoma</i>	A	100	100	100	100	100	0
Naeshubaekno									
baechu VIR-308	NIVOT	<i>pekinensis</i>	K	84	76	0	90	0	0
Ta-gu-tsai VIR--129	NIVOT	<i>rosularis</i>	Ch	100	100	100	100	100	100
Local VIR--108	NIVOT	<i>pekinensis</i>	Ch	100	100	100	100	100	100
Len-sin-dzon VIR--122	NIVOT	<i>pekinensis</i>	Ch	78	0	68	80	74	0
Ducre VIR--312	NIVOT	<i>pekinensis</i>	K	76	100	0	100	0	0
Kasin VIR--132	NIVOT	<i>pekinensis</i>	J	68	0	80	76	80	0
Siao-bai-kou VIR--74	NIVOT	<i>pekinensis</i>	Ch	100	100	100	100	100	0
Okuta Osaka		<i>pekinensis</i> ½							
shirona VIR--217	NIVOT	<i>chinensis</i>	J	70	0	62	72	56	0
Bansei mana VIR--372	NIVOT	<i>pekinensis</i> ½							
Local VIR--53	NIVOT	<i>chinensis</i>	J	56	0	68	60	60	0
Hikoshima spring	NIVOT	<i>dichotoma</i>	A	64	0	50	58	50	0
VIR--100	NIVOT	<i>pekinensis</i> ½ <i>narinosa</i>	J	45	70	75	68	50	0
Bansei Nagasaki	NIVOT	<i>pekinensis</i> ½ <i>narinosa</i>	J	69	100	100	100	100	0
VIR--212	NIVOT								
Nagoya Market VIR--238	NIVOT	<i>pekinensis</i> ½ <i>narinosa</i>							
Dou early VIR--89	NIVOT	<i>pekinensis</i>	J	0	46	60	0	54	0
Osaka Market VIR--98	NIVOT	<i>pekinensis</i>	Ch	60	54	53	45	48	0
Dunganskaya VIR--139	NIVOT	<i>pekinensis</i> ½ <i>chinensis</i>	J	80	85	75	90	100	0
Hiroshimana VIR--335	NIVOT	<i>pekinensis</i>	A	100	100	100	100	100	0
Bi ce VIR--58	NIVOT	<i>pekinensis</i>	J	100	78	60	100	62	0
Piorbai VIR--75	NIVOT	<i>chinensis</i>	J	52	0	50	52	48	0
Tai na VIR--46	NIVOT	<i>chinensis</i>	Ch	24	0	10	30	8	0
Nicanme Juki Jiro	NIVOT	<i>chinensis</i>	R	30	0	10	34	0	0
Taisai VIR--214	NIVOT	<i>chinensis</i>	J	65	45	0	70	0	0
Ching Pang Ju Tsai	NIVOT								
VIR--203	NIVOT	<i>chinensis</i>	J	75	0	100	80	60	0
Hae Yu Tatsai VIR--84	NIVOT	<i>rosularis</i>	Ch	100	0	23	100	10	0
Mibuna VIR--115	NIVOT	<i>nipposinica</i>	J	100	100	0	100	0	0
Local VIR--163	NIVOT	<i>rapifera</i>	Ch	80	20	25	90	20	0
Goseki Late VIR--242	NIVOT	<i>rapifera pervidis</i>	J	80	50	50	60	50	0
Gurin Debyu VIR--302	NIVOT	<i>chinensis</i> ½ <i>narinosa</i>							
Shelgam	VIR	<i>B. napus</i>	J	40	0	10	40	10	0
t-161	VIR	<i>B. napus</i>	A	100	0	94	100	85	0
Murasaki natane	VIR	<i>B. napus</i>	A	70	0	100	100	80	0
Ergly	VIR	<i>B. napus</i>	J	100	0	100	100	100	0
Jiho	VIR	<i>B. napus</i>	E	100	0	70	100	75	0
Bronowski	VIR	<i>B. napus</i>	E	100	100	100	100	100	0
Haplona	VIR	<i>B. napus</i>	E	100	0	82	100	70	0
Galaksi	VIR	<i>B. napus</i>	E	95	0	70	90	74	0
Starleit	VIR	<i>B. napus</i>	E	100	0	100	100	100	0
Zolotosevski	VIR	<i>B. napus</i>	E	100	0	100	100	100	0
Galant	VIR	<i>B. napus</i>	E	100	0	100	100	100	0
Vesreo	VIR	<i>B. napus</i>	E	100	0	100	100	100	0
417	VNIPTIR	<i>B. napus</i>	R	80	100	0	100	0	0

419	VNIPTIR	<i>B. napus</i>	R	100	100	0	100	0	0
420	VNIPTIR	<i>B. napus</i>	R	80	100	0	100	0	0
422	VNIPTIR	<i>B. napus</i>	R	75	100	0	100	0	0
423	VNIPTIR	<i>B. napus</i>	R	82	100	0	100	0	0
425	VNIPTIR	<i>B. napus</i>	R	90	100	0	100	0	0
428	VNIPTIR	<i>B. napus</i>	R	100	100	0	100	0	0
	430	VNIPTIR	R	100	100	0	100	0	0
431	VNIPTIR	<i>B. napus</i>	R	73	100	0	100	0	0
433	VNIPTIR	<i>B. napus</i>	R	86	100	0	100	0	0
434	VNIPTIR	<i>B. napus</i>	R	100	100	0	100	0	0
437	VNIPTIR	<i>B. napus</i>	R	93	100	0	100	0	0
441	VNIPTIR	<i>B. napus</i>	R	100	100	0	100	0	0
443	VNIPTIR	<i>B. napus</i>	R	100	100	0	100	0	0
Masora	VIR	<i>B. napus</i>	E	100	100	100	100	100	0
424	VNIPTIR	<i>B. napus</i>	R	100	100	100	100	100	0
426	VNIPTIR	<i>B. napus</i>	R	100	100	100	100	100	0
438	VNIPTIR	<i>B. napus</i>	R	100	100	100	100	100	0
439	VNIPTIR	<i>B. napus</i>	R	100	100	100	100	100	0
440	VNIPTIR	<i>B. napus</i>	R	100	100	100	100	100	0

Note. a – differentiating varieties according to J.G. Vicente et al., 2002; A – Central Asia, including Middle Asia, India and Pakistan, E – Western or Eastern Europe, Ch – China, J – Japan, K – Korea, R – Russia; NIVOT, VIR, VNIPTIR – respectively, National Research Institute of Vegetables, Ornamental Plants and Tea (Japan), N.I.Vavilov All-Russia Research and Development Institute of Plant Growing (St.Petersburg), All-Russia Research and Development Technology Institute of Rape (Lipetsk).

There were found reliable differences in distribution of resistance in *B. rapa* subspecies (Table 2) and samples of different geographical origin (Table 3). The highest resistance to races Xcc 4 and Xcr 1 was shown by the samples *B. rapa nipposinica*, *B. rapa dichotoma* and, respectively, by samples originated from Central Asia and Japan. Some individuals of this group exhibited resistance to races Xcc 1, Xcc 3 and Xcc 5. Several samples originated from India and Middle Asia were resistant to all races except Xcc 6.

## 2. Resistance of *Brassica rapa* subspecies to different races of the pathogen *Xanthomonas campestris* Pam. (Dow.)

Subspecies	Pathovar, race						Number of samples
	Xcr 1	Xcc 1	Xcc 3	Xcc 4	Xcc 5	Xcc 6	
<i>campestris</i>	2,7 C	2,0 B	0,7 B	1,7 B	1,0 B	0,25 A	8
<i>narinosa + rosularis</i>	10,3 BC	0 B	0 B	9,3 B	0 B	0 A	6
<i>pekinensis</i>	25,4 B	19,8 AB	20,4 A	26,0 AB	20,0 AB	3,2 A	31
<i>chinensis</i>	26,2 B	4,6 B	20,5 A	27,6 AB	14,4 AB	0 A	14
<i>parachinensis</i>	35,0 B	0 B	0 B	43,2 AB	0 B	0 A	8
<i>rapifera + perviridis</i>	67,8 A	3,3 B	4,2 B	66,4 A	4,0 B	0,2 A	32
<i>dichotoma</i>	74,1 A	7,7 B	44,0 A	79,5 A	37,8 A	0 A	22
<i>nipposinica</i>	76,3 A	23,9 A	24,8 A	81,1 A	25,2 A	0 A	11
Total							132 <sup>a</sup>

Note. a – the rest of samples had unclear taxonomic status or their number was insufficient for statistical analysis; A, B, C – groups of means whose differences are reliable at 95 % according to Duncan's criterion (11).

Resistance to race Xcc 1 was most frequently observed in samples of *B. rapa nipposinica* and *B. rapa pekinensis*, to Xcc 3 and Xcc 5 - in *B. rapa dichotoma*. On the contrary, majority of plants the subspecies *campestris*, *narinosa*, *chinensis* originated from Russia, Western Europe and China were susceptible to the pathogen.

Over 90% rape varieties manifested resistance to Xcc 4 and Xcr 1, but all plants of this species were susceptible to Xcc 6 and more susceptible to Xcc 1, Xcc 3 and Xcc 5 than *B. rapa*. The oilseed rape varieties of different geographical origin were not suitable for comparison owing to the widespread use in breeding work of genetic material from Western Europe. In general, rape samples showed higher uniformity of response than *B. rapa*; only two cultivars (Hanna and Capricorn) were susceptible to all strains of the pathogen. Only 7 samples of *B. napus* (mainly spring rape) manifested a complex resistance to races Xcc 1, Xcc 3 and Xcc 5.

## 3. Geographical distribution of resistance to the pathogen *Xanthomonas campestris* Pam. (Dow.) races in *Brassica rapa*

Region	Pathovar, race						Number of samples
	Xcr 1	Xcc 1	Xcc 3	Xcc 4	Xcc 5	Xcc 6	
Central Asia <sup>a</sup>	73,40 A	8,00 A	40,00 A	77,52 A	35,36 A	12,00 A	25
China	25,30 B	7,40 A	14,90 AB	26,30 B	13,03 AB	3,57 A	56
Western Europe	0,25 B	2,70 A	0 B	0,25 B	0 B	0,25 A	10
Japan and Korea	74,70 A	15,90 A	17,40 AB	76,30 A	16,80 AB	0,09 A	46
Russia	6,50 B	2,00 A	2,50 B	6,00 B	1,50 B	0,25 A	8
Total							145

Note. a – including India and Pakistan; A, B, C – groups of means whose differences are reliable at 95 % according to Duncan's criterion (11).

Races Xcc 1, Xcc 3, Xcc 4, Xcc 5 and Xcc 6 used in this study are known to be represented by 3 of 4 avirulence genes described in studies of "gene-for-gene" relationship between differentiating cultivars and pathogen (1-3).

Differentiating varieties *B. rapa* Just Right F<sub>1</sub> and Tokyo Cross F<sub>1</sub> – descendants of a common ancestor (cv Shogoin oomaru) resistant to the race Xcc 4 – showed the similar reaction to inoculation with many Xcc isolates collected in different regions (1, 2, 12, 13). The role of a single dominant gene providing resistance to Xcc 4 has been confirmed by results of assessment of populations obtained by crosses between Seven Top Green (heterozygous plant) ½ Just Right F<sub>1</sub>, Just Right F<sub>1</sub> ½ Just Right S4 (susceptible line S4) and the sample 3177 ½ Just Right S4. All of these plants most likely carry homologous resistance genes (14). The presence of different alleles of one resistance locus *Rxa4* has been recently established in these differentiators and in the cultivar Seven Top Green (3). Though, it's still an open question about the homology of dominant genes for resistance to race Xcc 4 in *B. rapa* and *B. napus*.

Significant correlation between plant responses to races Xcr 1 and Xcc 4 confirms the data about identical racial structure of these two pathovars (8, 15). The correlation between resistance to races Xcc 3 and Xcc 5 possibly indicates the presence of a homologous *Rxa3* gene in A-genomes of plants.

Highest rates of resistance to vascular bacteriosis were established in *B. rapa* subpopulation from Central Asia known to be of the largest genetic diversity among the gene pool of this species (16) and in the genetically close subpopulation from Japan (17). These samples effectively combated the race Xcc 4 widespread in Japan, Russia, Great Britain and Portugal (1, 2).

The population of *B. rapa* includes genetically isolated groups of vegetable crops of Chinese origin, oilseeds from Central Asia and turnip from Europe and Russia resulting the selection in contrast climate and soil conditions (16, 18). Recent studies based on molecular analysis showed the trend to classify samples by geographical origin – European, Indian (or united Eurasian) and East Asian groups (19, 20). Distribution of resistance to the race Xcc 4 sharply contrasts in groups separated by a small distance (Japan and China, or Central Asia and China). The studied samples of Chinese rosette cabbage and leaf cabbage resistant to all six pathogen races can be close relatives (6). Resistance to Xcc 4 in *B. napus* can be inherited from *B. rapa* (possibly, a diploid ancestor). However, it's not clear whether the resistance genes for particular races are homologous in all studied samples.

Thus, highest rates of *Brassica rapa* subspecies resistant to *Xanthomonas campestris* Pam. (Dow.) race Xcc 4 were established in samples originated from Central Asia and Japan. For the first time the authors have detected samples-donors of complex resistance to six pathogen races and sources of resistance to races Xcc 1 and Xcc 3. These findings will help the creation of *B. rapa* and *B. napus* cultivars and hybrids resistant to vascular bacteriosis and leaf blotch caused by phytopathogenic *Xanthomonas*.

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