Black Rot of Crucifers and Sources of Resistance in Brassica Crops

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Abstract

Since the early 1990s, diseases caused by *Xanthomonas campestris* have been spreading on new host plants and in new regions, that had not been previously affected by the pathogen. Still, vegetable crops of *Brassica oleracea* are the most damaged plants by black rot. Recent achievements in the studies on resistance to black rot were reviewed. For the first time resistance genes were identified based on gene-for-gene interaction with different races of the pathogen. Some East Asian cabbage and Portuguese Penca kale cultivars seemed to carry the homologous genes for race-specific resistance. Their origin in Asian cabbages was traced to the Flat Dutch group of varieties and to heading Mediterranean kale. It is suggested that novel non-specific stem resistance found in Chinese kale, broccoli and cabbage might be an alternative means of genetic protection against the pathogen.

Discipline: Plant disease/Plant breeding
Additional key words: race-specific resistance, race structure, leaf spot diseases

Introduction

Incidence of black rot caused by *Xanthomonas campestris* pv. *campestris* on horticultural brassicas is well recognized worldwide. Periodical epidemics of the disease were usually ascribed to the introduction of susceptible cultivars, careless application of contaminated seeds and seedlings and weather conditions favorable for disease development[37]. Studies on the recent outbreaks caused by *X. campestris* on oilseeds suggested that spreading of new highly aggressive variants of the pathogen was the main reason for these epidemics[21,23]. However, breeding of *B. oleracea* for resistance to black rot has been undertaken without recognition of the existence of pathogenic variants (races). As a result, control of the disease by the introduction of some resistant cultivars may not be effective.

Characteristics of the pathogen

Xanthomonas campestris pv. *campestris* (Xcc) belongs to the genus that causes diseases on at least 124 monocotyledonous and 268 dicotyledonous plant species including all major crop plants[20]. According to a recent reclassification based on DNA analysis[35], Xcc was assigned to the same genetic

Table 1. Diseases produced by the pathovars of *Xanthomonas campestris* on different host plants

Pathovar	Typical symptoms	Race	Reaction of brassicas with genome		
			A	B	C
campestris, aberance	black rot, leaf blight	1, 4	RS[19]	RS[17,19]	RS[17]
campestris	leaf blight, black rot	0	NS[17,19]	NS[17,19]	NS[17]
raphani	leaf spot, black rot	3	RS[17]	na	RS[17]
armoracea	leaf spot	na	na	na	NS[11]

na: No available data, RS: Race-specific response, NS: Non-specific response.
The most common races for each group were designated according to the reaction on the Kamoun et al.[19] differential set of varieties.

group as that of other pathovars infecting a wide range of crucifers as systemic or leaf pathogens (Table 1). The clear difference between leaf spot and black rot symptoms was attributed to the expression of a few genes, present in these pathovars[10]. Factors responsible for the pathogenicity of Xcc include plant-stimulated proteins produced by pathogenicity genes targeted to plant nucleus[39], several enzymes and extra-cellular polysaccharides[10]. No highly toxic compounds were detected in association with the pathogenicity[26].

It was shown that Xcc is composed of genetic and serologically heterogeneous groups of strains[1]. Kamoun et al.[19] reported that isolates of the pathogen could be grouped into 5 races according to the response of B. rapa and B. juncea cultivars. Race 4 was prevalent in Japan and in Portugal, while race 1 in UK, USA and it was also found on seeds of B. oleracea imported to Japan and England[17,25]. Race 0 was found in USA and Portugal (Table 2). Race 2, represented by only isolate 2D520, did not express pathogenicity in particular varieties of B. oleracea, B. napus and Arabidopsis thaliana[6,17]. Race 3, also represented by one isolate, showed the same interaction with differential varieties as isolates of X. campestris pv. raphani did[17]. It is likely that these 2 races were designated by Kamoun et al.[19] based on some other types of interaction with the host plants than avirulence-resistance matching pairs of genes, and further use of these races is questionable.

Evidence of new races was suggested on the basis of interaction between worldwide collection of Xcc and new differential varieties in B. oleracea and B. napus[16,17]. The variability of Xcc continuously endangers cultivars with a narrow genetic base of resistance. The spreading of the disease on new host crops considerably increases the chance of outbreaks on more susceptible vegetables grown in the same locations. Pathotyping of the pathogen populations may be necessary to provide a scientific base for breeding and introduction of resistant varieties in the areas endangered by black rot.

Since the pathogen can remain in soil even in plant debris only for 1 or 2 growing seasons[29], survival in contaminated seeds and on weed crucifers is considered to be most essential for the cycle of the disease. In Southeast Asia, although pak-choi, pet-tsai and other oriental brassicas are less damaged by black rot than vegetables of the B. oleracea group, they could become a source of inoculum.

Resistance to black rot in brassicas

The term "resistance" has been sometimes applied to very distinctive events, which results in a reduced damage of plants under natural conditions, including specific morphological characteristics and disease escape due to environmental factors[27]. Several studies have clearly revealed the ability of plants to decrease their susceptibility to black rot after inoculation with some micro-organisms[8]. Plant morphology and life cycle may play a very important role in the degree of black rot development in the field. The rate of guttation plays a major role in the difference in disease development between susceptible cultivars[28]. Here, the word "resistance" will be used here only for the genetic factors specific to host-pathogen interaction.

Ability of the pathogen to multiply in the vascular system of plant plays a major role in the expression of black rot symptoms. Vein plugging in plants infected with Xcc seems to be due to the accumulation of fibrillar material in vessels to prevent pathogen spreading inside the vascular system. Due to water deficiency, wilt and death of leaf segments between affected veins are the main reasons for the V-shaped lesions, typical of black rot[5]. It is less

Table 2. Geographical distribution of *Xanthomonas campestris* pv. *campestris* races

Country	No. tested isolates	Race frequency (%)				
		0	1	2	3	4
Japan[17]	65	0	35.0	0	0	65.0
UK[25]	100	0	72.0	0	0	28.0
Portugal[37]	61	31.0	18.0	0	0	51.0
USA[19]	na	present	present	present	present	present
Europe, Russia[17]	25	0	42.0	0	0	58.0
Total	251	7.8	51.2	na	na	41.0

na: Not available.
Races were designated based on the Kamoun et al.[19] set of differential varieties.

known that Xcc can produce some other symptoms. Under common nursery conditions, the disease can appear as yellowing of cotyledons and deformation of first true leaves on seedlings. At low temperatures, after the fall of diseased leaves, plants may remain infected but symptomless until the occurrence of warm weather[37]. Alternatively, they could recover under cool temperature[4]. Systemic invasion often causes symptoms of "chlorotic spotting" or "pale mottle" on susceptible plants[1,9] — local whitish deformation of the epidermis on parts of leaves affected by the compounds produced by the bacteria. The proposed toxic nature of these compounds[9] has not been confirmed experimentally and other factors like pathogenicity proteins or bacterial hormones should be considered.

Area of pathogen spreading in plant vessels is much more extensive than the area of visual symptoms[9] and sometimes, typical V-shaped marginal lesions can appear as a result of systemic infection as well as after hydathode infection. Under wet and cool weather conditions, when intercellular spaces in leaves are soaked with water, the bacteria can penetrate into plants through stomata and induce extensive leaf spot lesions without systemic symptoms[9]. In several cases, Xcc caused rapidly expanding lesions referred to as "blight"[1,15]. Vascular spreading of the bacteria in some other crucifers can be symptomless[34].

The resistant reaction in plant occurs in hydathodes, the natural gateway for the pathogen penetration into plant[9], and in the vascular system, where the pathogen spreads and multiplies[5]. In early studies, a difference between leaf and stem susceptibility of cabbages was noticed. With the same leaf reaction as in European cultivars, the Japanese cultivars exhibited a lower stem susceptibility[3,32]. This novel stem resistance can be represented as the arrest of the pathogen in the stem vascular system. It was observed that in the progeny of a cross between stem-resistant Chinese kale and leaf-resistant cabbage, these types of plant reaction were controlled by different genes and could be evaluated separately[17]. Routine observation of visual symptoms of black rot produced by conventional inoculation methods had ignored the difference between the resistance types and gave data that did not enable to distinguish the different types of resistance. Thus, selection for one type can result in the loss of another.

Race-specific resistance in brassicas was normally associated with hypersensitive response (HR) at the site of inoculation with incompatible race of Xcc[19], but sometimes only partial expression was observed[16]. The distribution of the resistance to Xcc among *Brassica* species displays some interesting similarities (Table 3). The dominant single gene in plants with B genome[13,14] conferred the highest level of resistance to all races except for race 0. The high frequency of dominant resistance to race 4 was found in *B. rapa* of Japanese and Central Asian groups as well as in cultivars of oilseed *B. napus*[16,17].

Table 3. Some reported accessions of brassicas resistant to black rot

Species, subspecies	Genome	Accession	Resistance type
B. oleracea var. *capitata*	CC	Fujiwase (Early Fuji)[2]	rs
		PI436606[11]	rs
		Singapura (F₁)[17]	S
var. *costata*		Penca kales[12,17]	rs
var. *botrytis*		SN455[30]	na
var. *italica*		Marathon (F₁)[17]	S
var. *alboglabra*		S1[17]	S, rs
B. rapa var. *rapifera*	AA	Just Right (F₁)[19]	rs
		Tokyo Hybrid (F₁)[19]	rs
		Seven Top Green[19]	rs
var. *parachinensis*		RCBr[17]	rs
B. napus	AACC	Cobra[16]	rs
		CrGC–5[16]	rs
		Giant English[16]	rs
B. carinata	BBCC	PI199947[13]	rs
B. juncea	AABB	Florida Broad Leaf[19]	rs

rs: Race-specific resistance, S: Stem resistance, na: Not available.

Presently, plants of the *B. oleracea* group, especially cabbage and cauliflower, are most severely damaged by the disease[37]. The frequency of black rot resistance in *B. oleracea* is very low. Although, there are no true resistant accessions among several hundreds of varieties and landraces of cabbage and cauliflower[30,32,38], many additive genes were responsible for the reduced damage by black rot in the field in diallel crosses between varieties or breeding lines of cabbages[33] and cauliflower[25]. Attempts to introduce into *B. oleracea* the dominant monogenic resistance from *B. carinata* PI199947 (previously designated as *B. napus*) were made via protoplast fusion, but the progeny obtained was more susceptible than the resistant parent, suggesting that some other genes were important as well[14].

Since the first report of resistant cabbage cultivars by Bain[2], the existence of resistance has been detected in several other *Brassica oleracea* plants[11,12,18,30] (Table 3). Since plant defense includes several biochemical events, such as synthesis of receptor-like kinase (SRK), defense proteins, chitinase and enzymes of phenylpropanoid metabolism[24], several genes should be involved in the phenotypic expression of the resistance. Although, single resistance genes in cabbage varieties Huguenot and cauliflower SN455 were reported, the participation of a larger number of genes could not be ruled out[3,18].

Two accessions of *B. oleracea* var. *capitata* Fujiwase and PI436606 have been widely used to breed commercial cultivars. Unfortunately, the resistance of these cultivars showed complex relationships with the genetic background and race-specific action[11,17,38]. The black rot resistance of variety Fujiwase was controlled by a single recessive factor affected by 2 gene-modifiers[38], as well as the resistance of plants selected from line PI436606[11]. Recently, by the use of quantitative trait loci (QTL) mapping, the resistance in Fujiwase progeny has been found to be associated with several additive loci in different linkage groups and one of them, responsible for the resistance in both adult and young stages was dominant, while the other was recessive[7].

In the progenies of Chinese kale, cabbage line PI436606 and the Penca kale, a homologous recessive gene was responsible for the race-specific resistance to the newly designated race 5 of Xcc and a dominant gene provided resistance to race 1. Several cabbage lines of Japanese origin and Penca kale landraces displayed similar patterns of race-specific reaction and inheritance of resistance to races 1 and 5 of Xcc[17] (Table 4).

Cabbages have been introduced to Japan relatively recently and in most of them the pedigree included some varieties of the Flat Dutch group[31]. This group was selected from heading Mediterranean kale related to the landrace Penca de Mirandella, which is also resistant to black rot[12,22]. As tested by Bain[2], all open-pollinated varieties related to Flat Dutch contained a large number of resistant plants in bulk. In the same experiments, suggesting a similar race composition of the applied inoculum, the frequency of resistance in the Fujiwase stock was about 95%.

Table 4. *Brassica oleracea* accessions grouped according to their postulated resistance genes[17]

Subspecies	Accession	Postulated resistance genes (R)
alboglabra	S1	1
tronchuda	ISA55, ISA454	1, 2
capitata	Badger Inbred 16, Kinkei DH01, Reiho DH01, Fujiwase 1; Fujiwase DH01, 02, 05; Harukei DH01	1, 2
	PI436606; DH M9606; Aichi dai Bansei DH01, 02	2
	Reiho DH01, 03; Fujiwase DH03, 04; Matsunami DH22	2, 4
	Matsunami DH77; DH M9602, 9603	4

If an accession showed a variable reaction, single plant selection was made prior to the test;
DH, doubled haploid lines, obtained from Japanese cultivars resistant to black rot.
The genes of resistance were proposed on the basis of both resistance-avirulence matching pairs in the gene-for-gene interaction between the resistant varieties and Xcc races and the resistance inheritance. Gene R1 conferred the resistance to race 1, gene R2 – to race 5, gene R4 – to race 4.

Since Fujiwase has a documented origin from Flat Dutch, it is highly probable that its resistance was inherited from Flat Dutch or from heading Mediterranean kale. We can assume that the race-specific resistance to black rot in Asian cabbages did not appear *de novo* under the pressure of the disease, as suggested by some researchers[37], but probably was inherited from the plants related to heading Portuguese kales. Only the use of genetic markers in the analysis of race-specific HR will enable to determine whether the genes studied in one cultivar are the same as in others.

After these recent achievements, sources of race-specific leaf and non-specific stem resistance among *B. oleracea* became available for plant breeding. The discovery of the race structure of Xcc populations for the first time enabled to design a breeding program based on the recognition of different genes and different mechanisms of resistance to black rot.

References

1) Alvarez, A. M. et al. (1994): Serological, pathological, and genetic diversity among strains of *Xanthomonas campestris* infecting crucifers. *Phytopathology*, **84**, 1449–1457.

2) Bain, D. (1952): Reaction of brassica seedlings to blackrot. *Phytopathology*, **42**, 316–319.

3) Bain, D. (1955): Resistance of cabbage to black rot. *Phytopathology*, **45**, 35–37.

4) Bain, D. (1955): Disappearance of blackrot symptoms in cabbage seedlings. *Phytopathology*, **45**, 55–56.

5) Bretschneider, K. E., Gonella, M. P. & Robeson, D. J. (1989): A comparative light and electron microscopical study of compatible and incompatible interactions between *Xanthomonas campestris* pv. *campestris* and cabbage. *Physiol. Molec. Plant Pathol.*, **34**, 285–297.

6) Buell, R. & Sommerville, S. C. (1995): Expression of defense-related and putative signaling genes during tolerant and susceptible interaction of *Arabidopsis* with *Xanthomonas campestris* pv. *campestris*. *Molec. Plant Mic. Interact.*, **8**, 435–443.

7) Camargo, L. E. A., Williams, P. H. & Osbourn, T. C. (1995): Mapping of quantitative trait loci controlling resistance of *Brassica oleracea* to *Xanthomonas campestris* pv. *campestris* in field and greenhouse. *Phytopathology*, **85**, 1296–1300.

8) Cook, D. & Robeson, D. J. (1986): Active resistance of cabbage (*Brassica oleracea*) to *Xanthomonas campestris* pv. *campestris* against the causal agent of black rot by coinoculation. *Physiol. Molec. Plant Pathol.*, **28**, 41–52.

9) Cook, A. A., Walker, J. C. & Larson, R. H. (1952): Studies on the disease cycle of black rot of crucifers. *Phytopathology*, **42**, 162–167.

10) Daniels, M. J. et al. (1993): Plant and bacterial genes involved in interactions between *Xanthomonas* and crucifers. Advances in molecular genetics of plant microbe interactions. Seattle, Washington, USA, 433.

11) Dickson, M. D. & Hunter, J. E. (1987): Inheritance of resistance in cabbage seedlings to black rot. *HortScience*, **22**, 108–109.

12) Ferreira, M. E. et al. (1993): Screening of Portuguese cole landraces (*Brassica oleracea* L.) with *Leptosphaeria maculans* and *Xanthomonas campestris* pv. *campestris*. *Euphytica*, **65**, 219–227.

13) Guo, H., Dickson, M. H. & Hunter, J. E. (1991): *Brassica napus* sources of resistance to black rot in crucifers and inheritance of resistance. *HortScience*, **26**, 1545–1547.

14) Hansen, L. N. & Earle, E. D. (1995): Transfer of resistance to *Xanthomonas campestris* pv. *campestris* into *Brassica oleracea* L. by protoplast fusion. *Theor. Appl. Genetics*, **91**, 1293–1300.

15) Ho, B. L. (1986): Screening of cabbage varieties for resistance to bacterial blight disease. *MARDI Research Bull.*, **14**, 35–38.

16) Ignatov, A. et al. (1997): Identification of *Xanthomonas campestris* pv. *campestris* races and sources of resistance. *In* ISHS Symposium on brassicas. 10th Crucifer Genetics Workshop. 23–27 Sept. Rennes-France, 215.

17) Ignatov, A., Hida, K. & Kuginuki, Y. (1998): Pathotypes of *Xanthomonas campestris* pv. *campestris* and resistance inheritance in *Brassica* species. *Sel'scokhozaistvennaya Biologiya* [In Russian] (in press).

18) Jamwal, R. S. & Sharma, P. P. (1986): Inheritance of resistance to black rot (*Xanthomonas campestris* pv. *campestris*) in cauliflower. *Euphytica*, **35**, 941–943.

19) Kamoun, S. et al. (1992): Incompatible interaction between crucifers and *Xanthomonas campestris* involves a vascular hypersensitive response, role of the hrpX locus. *Molecular Plant-Microbe Interactions*, **5**, 22–33.

20) Leyns, F. et al. (1984): The host range of the genus *Xanthomonas*. *Botanical Review*, **50**, 308–356.

21) Leite, R., Ruano, O. & Komori, N. (1994): Characterization of *Xanthomonas campestris* pv. *campestris* isolated from canola. *Summa Phytopath.*, **20**, 35–38.

22) Lizgunova, T. V. (1984): Crop plants of USSR; Cabbage. Kolos. Leningrad [In Russian].

23) Mihail, J. D. et al. (1993): Bacterial blight of *Crambe abyssinica* in Missouri caused by *X. campestris*. *Plant Disease*, **77**, 569–574.

24) Pastuglia, M. et al. (1997): Rapid induction by wounding and bacterial infection of an S gene family receptor-like kinase gene in *B. oleracea*. *Plant Cell*, **9**, 49–60.

25) Redstone, S. et al. (1997): Review of *Xanthomonas campestris* pv. *campestris* in the cauliflower crop in West Cornwall, UK. *In* ISHS Symposium on brassicas. 10th Crucifer Genetics Workshop, 23–27 Sept. Rennes-France, 216.

26) Robeson, D. J. & Cook, D. R. (1985): Production of low molecular weight carboxylic acid by *X.*

campestris pv. *campestris* in relation to the amino acid composition of the medium and their possible involvement in pathogenesis. *Physiol. Molec. Plant Pathol.*, **26**, 219–230.

27) Robinson, R. A. (1969): Disease resistance terminology. *Rev. Appl. Mycol.*, **48**, 593–605.

28) Ruissen, M. A. & Gielink, A. J. (1994): The development of black rot in cabbage as a result of differences in guttation between cultivars and the relation of guttation to infectiousness. *In* Proc. of 8th International conference on plant pathogenic bacteria, June 9–12. Versailles, France, 767–777.

29) Schaad, N. W. & White, W. G. (1974): Survival of *Xanthomonas campestris* in soil. *Phytopathology*, **64**, 1518–1520.

30) Sharma, B. R., Swarup, V. & Chatterjee, S. S. (1977): Resistance to black rot disease (*Xanthomonas campestris*) in cauliflower. *Scientia Horticulturae*, **7**, 1–7.

31) Shinohara, S. (1959): Cabbage. Sangyo-Tosho, Tokyo [In Japanese].

32) Studentsov, O. V. (1971): Resistance of some types and varieties of coles to bacterial and fungal diseases. *Appl. Bot. Genet. Plant Breed.*, **45**, 165–179 [In Russian].

33) Tewari, R. N., Chatterjee, S. S. & Swarup, V. (1979):

Inheritance of resistance to black rot (*Xanthomonas campestris*) in cabbage. *Vegetable Science*, **6**, 27–36.

34) Tsuji, J. & Somerville, S. C. (1992): First report of natural infection of *Arabidopsis thaliana* by *Xanthomonas campestris* pv. *campestris*. *Plant Diseases*, **76**, 539.

35) Vauterin, L. et al. (1995): Reclassification of *Xanthomonas*. *Int. J. Syst. Bacteriol.*, **45**, 472–489.

36) Vicente, J. G., Dias, J. S. & Taylor, J. D. (1997): Occurrence and distribution of *Xanthomonas campestris* pv. *campestris* races in Portugal. *In* ISHS Symposium on brassicas. 10th Crucifer Genetics Workshop, 23–27 Sept. Rennes-France, 214.

37) Williams, P. H. (1980): Black rot; a continuing threat to world crucifers. *Plant Disease*, **64**, 736–742.

38) Williams, P. H., Staub, T. & Sutton, J. C. (1972): Inheritance of resistance in cabbage to black rot. *Phytopathology*, **62**, 247–252.

39) Yang, Y. & Gabriel, D. W. (1995): *Xanthomonas* avirulence/pathogenicity gene family encodes functional plant nuclear targeting signals. *Molecular Plant–Microbe Interaction*, **8**, 627–631.