Screening of Cabbage (Brassica oleracea L.) Germplasm for Resistance to Black Rot

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ABSTRACT  Black rot of Brassica crops is the most devastating disease which causes substantial yield reduction of cabbage throughout the world. The use of resistant cabbage cultivars could be inexpensive and effective measure to combat this destructive disease. We screened cabbage inbred lines for black rot disease resistance through bioassay and identified some novel lines that showed race-specific resistance to Xanthomonas campestris pv. campestris (Xcc) races. The pathogenicity test revealed that out of 27 cabbage lines, one (SCNU-C-4074), six (SCNU-C-3631, SCNU-C-3637, SCNU-C-3639, SCNU-C-4072, SCNU-C-4073 and SCNU-C-3273), two (SCNU-C-3273 and SCNU-C-4118), two (SCNU-C-3270 and SCNU-C-4118), two (SCNU-C-3470 and SCNU-C-41148) and four (SCNU-C-107, SCNU-C-3270, SCNU-C-3470 and SCNU-C-4059) were shown to be resistant to Xcc races 1, 2, 3, 5, 6 and 7, respectively while none of these showed resistance against race 4. Furthermore, these resistant and susceptible lines were evaluated by previously reported molecular markers for black rot resistance. The molecular screening results were also revealed the existence of race-specific resistance in these cabbage lines. This result will help Brassica breeder to develop race-specific black rot resistant cabbage cultivars.

Keywords  Black rot resistance, Xanthomonas campestris pv. campestris, Cabbage, Bioassay, Molecular markers

INTRODUCTION

Cabbage (Brassica oleracea L.) is the most important vegetable among Brassica crops in the world due to its nutritional and health benefits (Lee et al. 2015). Black rot disease, caused by Xanthomonas campestris pv. campestris (Xcc) (Pammel) Dowson, is the most destructive diseases of the crops belonging to the Brassicaceae family (Williams 1980; Lema et al. 2012; Vicente and Holub 2013; Lee et al. 2015). The major host of Xcc is B. oleracea and its subspecies (Vicente et al. 2001). Xcc is mainly a seed born pathogen, but can alive in crop residues and cruciferous weeds and ornamentals (Cook et al. 1952; Roberts et al. 1999; Vicente et al. 2001; Lema et al. 2011). This pathogen can enter into the leaf via insects, wounded tissues and hydathodes (leaf margin) and spreads through vascular tissues (Tonu et al. 2013). Xcc can spread via rain, wind, insects, agricultural equipments and irrigation water (Sharma et al. 2017). The characteristic disease symptoms are V-shaped chlorotic lesions at the leaf margins, necrotic and darkened veins which reduce the quality and production (Williams 1980; Kifuji et al. 2013; Tonu et al. 2013; Vicente and Holub 2013; Lee et al. 2015). In favorable condition, black rot disease can reduce crop yield more than 50% (Williams 1980). However, up to 100% yield loss has been reported in cabbage by farmers in Tanzania (Massomo et al. 2003). Black rot also decreases the market value of cabbage. Like other plant pathogenic...
bacteria, *Xcc* has been separated into different physiological races based on the response of differential cultivars with resistance genes and pathogens. Up to now, 11 physiological races of *Xcc* have been reported (Kamoun et al. 1992; Vicente et al. 2001; Fargier and Manceau 2007; Cruz et al. 2017). Initially, Kamoun et al. (1992) reported five *Xcc* races (0-4). Later, Vicente et al. (2001) reclassified *Xcc* into six races (1-6). Furthermore, Jensen et al. (2010) and Fargier and Manceau (2007) were added race 7 and races 8-9 to Vicente’s classification, respectively. Newly, race 10 and race 11 have been reported in Portugal (Cruz et al. 2017).

The sources of resistance for black rot in *B. oleracea* are very less (Ignatov et al. 1998). However, the existence of black rot resistance was reported in Japanese cabbage (Early Fuji, P143660) as well as in Penca kales (Bain 1952; Dickson and Hunter 1987; Ferreira et al. 1992; Ignatov et al. 1999). Ignatov et al. (1998) reported race-specific resistance to *Xcc* races 1 and 5 in Japanese cabbage lines and Penca kale landraces. The race-specific resistance to *Xcc* in Asian cabbages was said to be inherited from Penca kales (a Portuguese black rot resistant kale landrace) (Ignatov et al. 1998). There were many research reports describing *Xcc* resistant genes in *B. oleracea* and related species. Williams et al. (1972) have been stated that black rot is controlled by one major gene designated as *f* and the heterozygous condition is influenced by one dominant and one modifier gene. They screened 300 cultivars and inbred lines of cabbage for *Xcc* resistance and this *f* gene found only in Early Fuji, a Japanese black rot resistant cabbage cultivar. Dickson and Hunter (1987) reported that black rot resistance is governed by a single recessive gene in cabbage (P1436606, a cabbage line from China). There are some reported quantitative trait loci (QTLs) and associated markers for black rot resistance in different *Brassica* species. For instance, Camargo et al. (1995) described QTLs for black rot resistance based on restriction fragment length polymorphism (RFLP) loci in cabbage. They found that two QTLs on linkage group (LG) 1 and 9 showed resistance against black rot for both young and old plants while two additional QTLs on LG 2 only for young plant resistance. Kifuji et al. (2013) reported a QTL for black rot resistance using 161 EST-SNP markers and found that QTL-1 located on LG C02 is the major QTL in cabbage. A major black rot resistance locus called Xca1bo has been mapped on Chromosome 03 in Indian cauliflower (Saha et al. 2014). Lee et al. (2015) reported a genetic linkage map where they identified four QTLs such as BRQTL-C1_1 and BRQTL-C1_2 on LG C01, BRQTL-C3 on LG C03 and BRQTL-C6 on LG 06. Among these QTLs, BRQTL-C1_2 designates as the most important QTL for black rot in cabbage while remaining three as minor QTLs. Sharma et al. (2016) reported black rot resistance locus Xca1bc on LG B-7 in Indian mustard (*Brassica carinata*). They also reported that black rot resistance in *B. carinata* was controlled by a single dominant gene. Now-a-days, molecular marker based genotyping are extensively used to screen disease resistance. However, the resistance sources in *B. oleracea* (C genome) are limited while major resistance sources have been described in A and B genomes (Taylor et al. 2002; Sharma et al. 2016).

*Xcc* can be retained in seeds, *Brassica* crop residues, and cruciferous weed and ornamentals which act as the sources of inoculum (Schaad and Dianese 1981). Thus, it is very difficult to control black rot disease through disease management practices including agrochemicals. For this reason, exploration of *Xcc* resistant sources is one of the best ways to abate crop loss from black rot disease.

However, new pathogenic races can be evolved due to the intensive cultivation of resistant cultivars that is the main hinder of black rot resistance (Song et al. 2014). Therefore, in this study, we aimed to screen out novel cabbage lines which provide race-specific resistance against *Xcc* races.

**MATERIALS AND METHODS**

**Plant materials**

Twenty seven different cabbage (*Brassica oleracea* L.) inbred lines were tested in this study (Supplementary Table S1). Seeds were obtained from Department of Horticulture, Sunchon National University, Republic of Korea. Initially, seeds were sown in trays containing a nursery pot mixture at plant culture room (25±1°C temperature, 60% Relative humidity and 80-120 μmol/m²/second light intensity). Twenty two days after sowing, the seedlings were
transferred into the pot and kept at the glasshouse. For pathogenicity test, seedlings from each of the above mentioned inbred lines were used for screening against black rot disease.

**Bacterial strains**

A total of seven *Xanthomonas campestris* pv. *campestris* (*Xcc*) races (race 1, 2, 3, 4, 5, 6 and 7) were inoculated in the aforementioned cabbage line for pathogenicity test which are shown in Table 1. The *Xcc* races (race 1-7) were collected from the School of Life Sciences, University of Warwick, UK. All the *Xcc* races were grown on King’s medium B (KB) for 48 hours at 30°C (King et al. 1954).

**Inoculation and disease scoring**

The cabbage inbred lines were inoculated at 35 days after sowing. The *Xcc* races were sub-cultured on KB at 30°C for 48 hours before inoculation. Thereafter, the bacteria were scraped from the culture plates and suspended in 10 mL of sterile water. Finally, three youngest leaves of each plants were inoculated by clipping secondary veins with sterile forceps (maintained at least 10 inoculation points per leaf) followed by dipping into a bacterial suspension (10^8-10^9 CFU/mL) of *Xcc* races and maintained high humidity (Vicente et al. 2001). Three seedlings for each *Xcc* race were used for the inoculation. The disease symptoms of inoculated leaves were evaluated at 2 and 3 weeks after inoculation (WAI). The disease reaction was scored at 2 WAI for each inoculated leaf based on a 0-9 scale (Fig. 1) where 0 = no visible symptoms; 1 = small necrosis or chlorosis near the inoculation point; 3 = typical small V-shaped lesion with black veins; 5 = typical lesion half way to the middle vein; 7 = typical lesion succeeding to the middle vein; and 9 = lesion reaching the middle vein as previously described by Vicente et al. (2002). The highly resistant (HR), resistant (R), susceptible (S) and highly susceptible (HS) lines were characterized based one the scales 0, 2-3, 5-7, and 9, respectively (Fig. 1).

**DNA extraction**

The young leaves of each cabbage lines were collected and immediately frozen in liquid nitrogen. Then, the leaf

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**Table 1. Xanthomonas campestris pv. campestris (Xcc) races used for bioassay-based screening of cabbage lines.**

| Sl. No. | Bacterial race/strains       | Source | Reference       |
|--------|-----------------------------|--------|-----------------|
| 1      | *Xanthomonas campestris* pv. *campestris* Race 1 (B100) | UK     | Vicente et al. 2001 |
| 2      | *Xanthomonas campestris* pv. *campestris* Race 2 (3849A) | US     |                 |
| 3      | *Xanthomonas campestris* pv. *campestris* Race 3 (5212)  | UK     |                 |
| 4      | *Xanthomonas campestris* pv. *campestris* Race 4 (CFBP 5817) | UK     |                 |
| 5      | *Xanthomonas campestris* pv. *campestris* Race 5 (3880) | Australia |                 |
| 6      | *Xanthomonas campestris* pv. *campestris* Race 6 (6181) | Portugal |                 |
| 7      | *Xanthomonas campestris* pv. *campestris* Race 7 (8450A) | UK     |                 |

**Fig. 1.** The disease reaction scoring criteria used in this study for black rot of cabbage. Scales: 0 = no visible symptoms; 1 = small necrosis or chlorosis near the inoculation point; 3 = typical small V-shaped lesion with black veins; 5 = typical lesion half way to the middle vein; 7 = typical lesion succeeding to the middle vein; and 9 = lesion reaching the middle vein (Vicente et al. 2002). 0, high resistant (HR); 1-3, resistant (R); 5-7, susceptible (S); 9, highly susceptible (HS).
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Table 2. List of markers with their primer sequence used in the molecular screening for black rot resistance in cabbage.

| Sl. No. | Markers     | Chromosome | Primer sequence (5’⋯⋯3’) | Tm (°C) | Size (bp) | Marker type | Reference                  |
|--------|-------------|------------|--------------------------|---------|-----------|-------------|-----------------------------|
| 1      | BoESSR291   | C03        | F: AAGCTGGGATGGGAGGAGAT  R: GCACCTAAATCGAACCCCCCTTA | 52      | 114       | SSR         | Izzah et al. 2014           |
| 2      | BoESSR216   | C01        | F: GGTCTTCGCTATGTCGCAAA  R: CGGAGAAGAGCGTTAGGAGG | 52      | 317       | SSR         | Izzah et al. 2014           |
| 3      | BoESSR089   | C01        | F: ATGATCGACGAAACCACCTCC R: TGATACTACCCCCGCTGCTCA | 55      | 259       | SSR         | Izzah et al. 2014           |
| 4      | BoESSR145   | C01        | F: GGGCGAGGAGTGTTAATCCA R: TCATACCCCAAGGCTATTTT | 55      | 240       | SSR         | Izzah et al. 2014           |
| 5      | BoESSR726   | C01        | F: CAATGGTTAAGCAGCTGGGG  R: CGATTGGAAGCAGGCCATTG | 55      | 228       | SSR         | Izzah et al. 2014           |
| 6      | SSR739      | C03        | F: TAGGTTGGAAGGGAGCGCTCA R: CGCTAATAATGGGCCTAAAGG | 52      | 182       | SSR         | Saha et al. 2014            |
| 7      | BnGMS301    | C01        | F: AATATCGAGTGTTAAGGCAAA R: ATCATCTGTGTATGCACACA | 55      | 250       | SSR         | Cheng et al. 2009           |
| 8      | BoGMS0971   | C08        | F: TAAATGCGAAACAGCAGAA  R: CACCCCAAATAGGGATGAG | 52      | 344       | SSR         | Li et al. 2011              |
| 9      | O10G06      | C06        | F: GACAAAGTCCCCTCTGAGTAC R:TGGAATCATCACAACACAGTTTGG | 52      | 109       | SSR         | Li et al. 2011              |
| 10     | Iso0857_371bc | C09     | F: AAAGGAATTTCCCAAGGAGTTC R: TGAGCTACCCCTGAATGCTTTT | 51      | 477       | InDel        | Lee et al. 2015             |

samples were maintained at ~80°C until use. The genomic DNA was isolated using a commercial kit (DNeasy Plant Mini Kit, QIAGEN, Germany) following the manufacturer’s instructions. The integrity and purity of the DNA were assessed by gel electrophoresis (0.8% agarose) and Nanodrop ND-1000 (Nanodrop Technologies Inc., Wilmington, DE, USA), respectively.

Polymerase chain reaction (PCR)

Previously reported markers were used to screen the cabbage inbred lines for black rot resistance (Table 2). The PCR amplification was performed in 20 μL reaction mixture, containing of 2X Prime Taq premix (Genet Bio, Republic of Korea), 10 pmole of each forward and reverse primers (Macrogen Inc., Seoul, Republic of Korea) and 100 ng genomic DNA as template. The thermal cycle was set with an initial denaturation at 95°C for 5 minutes, followed by 30-35 cycles of denaturation at 95°C for 30 seconds, annealing at specific Tm to respective primer sets (Table 2) between 52 and 61°C for 30 seconds, extension at 72°C for 30 seconds and a final extension at 72°C for 5 minutes. Then, the PCR products were subjected to agarose gel electrophoresis to visualize the bands. The concentration of agarose gel was varied based on amplicon size. Finally, results were compared with the bioassay results.

Cloning and sequencing

The polymorphic markers for black rot disease (BoGMS0971, BnGMS301, BoESSR291, BoESSR726 and O10G06) were amplified by PCR using Phusion® High-Fidelity DNA Polymerase (New England Biolabs) from resistant (R) and susceptible (S) cabbage lines. The polymorphic bands were extracted from the gel and purified using the Wizard SV gel and PCR cleanup system (Promega, Madison, WI, USA) from both resistant and susceptible lines for each of the above mentioned markers. Then, cloning was done using TOP cloner blunt kit (Enzynomics, Daejeon, Republic of Korea) according to the manufacturer’s instruction. Three independent PCR positive clones were sequenced with the universal primers (M13FpUC and M13RpUC) by ABI 3730XL DNA sequencer (Macrogen Inc., Seoul, Republic of Korea). Subsequently, the cloned sequences of R and S lines for each marker were aligned using the Clustal Omega online
RESULTS

Screening of cabbage inbred lines for the black rot resistance

The disease reaction results of 27 cabbage inbred lines for black rot disease resistance are shown in Table 3 and Fig. 2. The tested cabbage lines provide race-specific resistance against different *Xanthomonas campestris* pv. *campestris* (*Xcc*) races. The result showed that cabbage line SCNU-C-4074 was resistant (Fig. 2) against *Xcc* race 1. A total of six cabbage lines were highly resistant to *Xcc* race 2 (SCNU-C-3273, SCNU-C-3631, SCNU-C-3637, SCNU-C-3639, SCNU-C-4072 and SCNU-C-4073). Similarly, two lines SCNU-C-3273 and SCNU-C-4118 were resistant to *Xcc* race 3. Likewise, lines SCNU-C-3270 (highly resistant) and SCNU-C-4118 (resistant) were resistant to *Xcc* race 5. Two cabbage lines (SCNU-C-3470 and SCNU-C-41148) were shown to be resistant against *Xcc* race 6. A total of four cabbage lines (SCNU-C-107, SCNU-C-3270, SCNU-C-3470 and SCNU-C-4059) were resistant to *Xcc* race 7 of which three lines (SCNU-C-3270, SCNU-C-3470 and SCNU-C-4059) were highly resistant. On the other hand, all of the tested lines were susceptible for *Xcc* race 4. However, some cabbage lines were resistant to more than one *Xcc* races. For example, line SCNU-C-3270 shown to be resistant against race 5 and 7 (highly resistant), line SCNU-C-3273 against race 2 (highly resistant) and 3, and SCNU-C-4118 against race 3, 5 and 6.

Molecular screening cabbage inbred lines for the black rot resistance

A total of 10 previously published markers (9 SSR and 1 InDel) for black rot resistance were selected for this study based on reported QTL information. Among those reported markers, only five showed polymorphic amplification and were able to distinguish resistant (R) and susceptible (S) cabbage lines against black rot disease (Table 3 and Supplementary Fig. S1). Thereafter, the bioassay and molecular screening results were compared and those having more than 60% adaptability for each race were shown in Table 3. The outcome of the comparison showed that the OI10G06 marker separates R and S lines with 83.3% adaptability, respectively for *Xcc* race 1 followed by BnGMS301 (79.2%), BoESSR726 (79.2%) and BoESSR291 (66.7%). Similarly, BoESSR291 and OI10G06 can differentiate R and S lines against *Xcc* race 2 with 73.9% adaptability followed by BoESSR726 (65.2%) and BnGMS0971 (60.9%). In case of race 3, BnGMS301 can differentiate R and S lines with 81.5% adaptability followed by BoESSR291 (77.8%), BoESSR726 (74.1%) and OI10G06 (70.1%). For race 4, BnGMS301 showed 79.2% match with the bioassay results followed by BoESSR726 (75%), OI10G06 (70.8%) and BoESSR291 (66.7%). In case of race 5, BnGMS301 showed 91.3% match with the phenotypic screening results followed by BoESSR726 (78.3%), OI10G06 (73.9%) and OI10G06 (69.6%). For race 6, BnGMS301 and BoESSR726 can separated R and S cabbage lines with 81.5% adaptability with the bioassay results followed by BoESSR291 (70.4%) and OI10G06 (66.7%). In case of race 7, BnGMS301, BoESSR291, OI10G06 and BoESSR726 showed 72.0%, 68.0%, 68.0% and 64.0% match with the bioassay results, respectively.

Sequence analysis of amplified DNA fragments using markers

The amplified polymorphic markers were further cloned and sequenced from R and S lines. Through sequencing, the genetic variations including insertion/deletions (InDels), single nucleotide polymorphisms (SNPs) between R and S lines for the polymorphic markers were detected (Supplementary Fig. S2). The BoGMS0971 marker amplified a 351-bp DNA fragment for R lines and 330-bp for S lines. The sequence alignment results showed that there was a total 21-bp deletions from S lines compared to R lines. This marker located on the intergenic region between two genes (Bo8g092870 and Bo8g092880) (Table 4). The BnGMS301 amplified 238-bp DNA fragment for S lines but 215-bp with total 27-bp deletion for R lines, which are located between two genes (Bo1g053300 and Bo1g053310). In case of the BoESSR291, the tested cabbage lines showed three different types of PCR bands. These bands were
Table 3. Comparison of bioassay and molecular screening results for black rot resistance in cabbage.

| Sl. No. | Lines       | Race 1 | Race 2 | OI10G06 | BnGMS301 | BoESSR726 | BoESSR291 | OI10G06 | BnGMS301 | BoESSR726 | BoESSR291 |
|---------|-------------|--------|--------|---------|-----------|------------|-----------|---------|-----------|------------|-----------|
|         |             | P<sup>i</sup> | G<sup>j</sup> |         |           |            |           | P<sup>i</sup> | G<sup>j</sup> |            |           |
| 1       | SCNU-C-106  | HS<sup>i</sup> | +       | +       | +         | +         | HS        | +       | +         | +          | +         |
| 2       | SCNU-C-107  | n/a<sup>i</sup> | -       | +       | -         | +         | HS        | -       | +         | +          | +         |
| 3       | SCNU-C-3122 | S<sup>i</sup> | +       | -       | +         | +         | HS        | +       | +         | +          | +         |
| 4       | SCNU-C-3270 | S      | +       | -       | +         | S         | +         | +       | +         | +          | +         |
| 5       | SCNU-C-3273 | S      | +       | +       | -         | HR<sup>i</sup> | -         | +       | +         | -          | -         |
| 6       | SCNU-C-3305 | HS     | +       | +       | +         | +         | S         | +       | +         | +          | +         |
| 7       | SCNU-C-3328 | S      | +       | -       | -         | +         | HS        | +       | +         | -          | -         |
| 8       | SCNU-C-3412 | S      | +       | +       | +         | +         | HS        | +       | +         | +          | -         |
| 9       | SCNU-C-3414 | S      | -       | +       | +         | -         | n/a       | -       | -         | +          | -         |
| 10      | SCNU-C-3449 | HS     | +       | +       | +         | +         | HS        | +       | +         | +          | +         |
| 11      | SCNU-C-3470 | n/a    | +       | +       | -         | +         | n/a       | +       | -         | +          | -         |
| 12      | SCNU-C-3631 | S      | -       | +       | +         | -         | HR<sup>i</sup> | -       | -         | +          | -         |
| 13      | SCNU-C-3637 | S      | +       | +       | +         | -         | HR<sup>i</sup> | -       | +         | +          | -         |
| 14      | SCNU-C-3639 | S      | +       | +       | -         | HR<sup>i</sup> | +         | +       | -         | +          | -         |
| 15      | SCNU-C-4059 | S      | +       | +       | +         | +         | S         | +       | +         | +          | -         |
| 16      | SCNU-C-4066 | S      | +       | +       | -         | S         | -         | +       | +         | +          | -         |
| 17      | SCNU-C-4071 | S      | -       | +       | +         | S         | +         | -       | +         | +          | -         |
| 18      | SCNU-C-4072 | n/a    | -       | +       | +         | +         | HR<sup>i</sup> | +       | -         | +          | -         |
| 19      | SCNU-C-4073 | S      | +       | +       | +         | +         | HR<sup>i</sup> | +       | +         | +          | -         |
| 20      | SCNU-C-4074 | R<sup>i</sup> | -       | -       | -         | +         | n/a       | +       | -         | -          | -         |
| 21      | SCNU-C-4085 | S      | +       | +       | +         | S         | +         | +       | +         | -          | -         |
| 22      | SCNU-C-4105 | S      | +       | +       | -         | S         | +         | +       | -         | +          | -         |
| 23      | SCNU-C-4118 | S      | +       | -       | -         | S         | -         | +       | -         | +          | -         |
| 24      | SCNU-C-4161 | HS     | +       | +       | +         | +         | S         | +       | +         | -          | -         |
| 25      | SCNU-C-4168 | S      | -       | +       | +         | -         | S         | -       | -         | +          | -         |
| 26      | SCNU-C-4302 | HS     | +       | +       | -         | +         | HS        | +       | +         | +          | -         |
| 27      | SCNU-C-4320 | HS     | +       | +       | +         | +         | HS        | +       | +         | +          | -         |

Matched 20.0 19.0 19.0 16.0 17.0 17.0 15.0 14.0
Not matched 4.0 5.0 5.0 9.0 6.0 6.0 8.0 9.0
% Adaptability 83.3 79.2 79.2 66.7 73.9 73.9 65.2 60.9
Table 3. Continued 1.

| Sl. No. | Lines  | Race 3 | BnGMS301 | BoESSR291 | BoESSR726 | O110G06 | Race 4 | BnGMS301 | BoESSR726 | O110G06 | BoESSR291 |
|---------|--------|--------|----------|------------|------------|---------|--------|----------|------------|---------|------------|
| 1       | SCNU-C-106 | HS     | +        | +          | +          | +       | n/a    | +        | +          | +        | +          |
| 2       | SCNU-C-107 | HS     | +        | -          | +          | -       | S      | +        | +          | -        | -          |
| 3       | SCNU-C-3122| HS     | -        | +          | +          | +       | HS     | -        | +          | +        | +          |
| 4       | SCNU-C-3270| HS     | -        | +          | +          | +       | S      | -        | +          | +        | +          |
| 5       | SCNU-C-3273| R      | +        | -          | +          | +       | S      | +        | +          | +        | -          |
| 6       | SCNU-C-3305| S      | +        | +          | +          | +       | HS     | +        | +          | +        | +          |
| 7       | SCNU-C-3328| HS     | -        | +          | -          | +       | HS     | -        | -          | +        | +          |
| 8       | SCNU-C-3412| HS     | +        | +          | +          | +       | S      | +        | +          | +        | +          |
| 9       | SCNU-C-3414| S      | +        | -          | +          | -       | S      | +        | +          | -        | -          |
| 10      | SCNU-C-3449| HS     | +        | +          | +          | +       | HS     | +        | +          | +        | +          |
| 11      | SCNU-C-3470| S      | +        | +          | -          | +       | S      | +        | -          | +        | +          |
| 12      | SCNU-C-3631| S      | +        | -          | +          | -       | S      | +        | +          | -        | -          |
| 13      | SCNU-C-3637| S      | +        | -          | +          | +       | S      | +        | +          | +        | -          |
| 14      | SCNU-C-3639| S      | +        | +          | -          | +       | S      | +        | -          | +        | +          |
| 15      | SCNU-C-4059| S      | +        | +          | +          | +       | S      | +        | +          | +        | +          |
| 16      | SCNU-C-4066| HS     | +        | -          | +          | +       | HS     | +        | +          | -        | -          |
| 17      | SCNU-C-4071| HS     | +        | +          | +          | -       | S      | +        | +          | -        | -          |
| 18      | SCNU-C-4072| HS     | +        | +          | +          | -       | n/a    | +        | +          | -        | -          |
| 19      | SCNU-C-4073| HS     | +        | +          | +          | +       | S      | +        | +          | +        | +          |
| 20      | SCNU-C-4074| HS     | -        | +          | -          | -       | HS     | -        | -          | -        | -          |
| 21      | SCNU-C-4085| HS     | +        | +          | +          | +       | HS     | +        | +          | +        | +          |
| 22      | SCNU-C-4105| HS     | +        | +          | -          | +       | S      | +        | -          | +        | +          |
| 23      | SCNU-C-4118| R      | -        | -          | +          | +       | S      | -        | +          | -        | -          |
| 24      | SCNU-C-4161| HS     | +        | +          | +          | n/a    | n/a    | +        | +          | +        | +          |
| 25      | SCNU-C-4168| HS     | +        | -          | +          | -       | S      | +        | +          | -        | -          |
| 26      | SCNU-C-4302| HS     | +        | +          | -          | +       | S      | +        | -          | +        | +          |
| 27      | SCNU-C-4320| HS     | +        | +          | +          | +       | HS     | +        | +          | +        | +          |

Matched: 22.0 21.0 20.0 19.0 19.0 18.0 17.0 16.0
Not matched: 5.0 6.0 7.0 8.0 5.0 6.0 7.0 8.0
% Adaptability: 81.5 77.8 74.1 70.1 79.2 75.0 70.8 66.7
### Table 3. Continued 2.

| Sl. No. | Lines         | Race 5 P<sup>j</sup> | Race 5 G<sup>k</sup> | Race 6 P<sup>j</sup> | Race 6 G<sup>k</sup> |
|---------|---------------|----------------------|----------------------|----------------------|----------------------|
|         |               | BnGMS301 | BoESSR726 | BoESSR291 | O110G06 | BnGMS301 | BoESSR726 | BoESSR291 | O110G06 |
| 1       | SCNU-C-106    | HS       | +        | +        | +        | HS       | +        | +        | +        |
| 2       | SCNU-C-107    | HS       | +        | +        | -        | HS       | +        | +        | -        |
| 3       | SCNU-C-3122   | S        | -        | +        | +        | HS       | -        | +        | +        |
| 4       | SCNU-C-3270   | R        | -        | +        | +        | S        | -        | +        | +        |
| 5       | SCNU-C-3273   | n/a      | +        | +        | -        | HS       | +        | +        | -        |
| 6       | SCNU-C-3305   | S        | +        | +        | +        | HS       | +        | +        | +        |
| 7       | SCNU-C-3328   | HS       | -        | -        | +        | HS       | -        | +        | +        |
| 8       | SCNU-C-3412   | S        | +        | +        | +        | S        | +        | +        | +        |
| 9       | SCNU-C-3414   | n/a      | +        | -        | +        | HS       | +        | -        | -        |
| 10      | SCNU-C-3449   | HS       | +        | +        | +        | S        | +        | +        | -        |
| 11      | SCNU-C-3470   | n/a      | +        | -        | +        | R        | +        | -        | +        |
| 12      | SCNU-C-3631   | S        | +        | +        | -        | HS       | +        | +        | -        |
| 13      | SCNU-C-3637   | HS       | +        | +        | -        | HS       | +        | +        | -        |
| 14      | SCNU-C-3639   | HS       | +        | -        | +        | S        | +        | -        | +        |
| 15      | SCNU-C-4059   | S        | +        | +        | +        | S        | +        | +        | +        |
| 16      | SCNU-C-4066   | HS       | +        | -        | +        | HS       | +        | +        | -        |
| 17      | SCNU-C-4071   | HS       | +        | +        | -        | HS       | +        | +        | -        |
| 18      | SCNU-C-4072   | HS       | +        | +        | -        | HS       | +        | +        | -        |
| 19      | SCNU-C-4073   | HS       | +        | +        | +        | HS       | +        | +        | -        |
| 20      | SCNU-C-4074   | n/a      | -        | -        | +        | S        | -        | -        | +        |
| 21      | SCNU-C-4085   | S        | +        | +        | +        | HS       | +        | +        | +        |
| 22      | SCNU-C-4105   | HS       | +        | -        | +        | S        | +        | -        | +        |
| 23      | SCNU-C-4118   | R        | -        | -        | +        | R        | -        | -        | +        |
| 24      | SCNU-C-4161   | S        | +        | +        | +        | HS       | +        | +        | +        |
| 25      | SCNU-C-4168   | HS       | +        | +        | -        | HS       | +        | +        | -        |
| 26      | SCNU-C-4302   | HS       | +        | -        | +        | S        | +        | -        | +        |
| 27      | SCNU-C-4320   | HS       | +        | +        | +        | HS       | +        | +        | +        |

Matched 21.0 18.0 17.0 16.0 22.0 22.0 19.0 18.0
Not matched 2.0 5.0 6.0 7.0 5.0 5.0 8.0 9.0
% Adaptability 91.3 78.3 73.9 69.6 81.5 81.5 70.4 66.7
Table 3. Continued 3.

| Sl. No. | Lines       | Race 7  | BnGMS301 | BoESSR291 | OI10G06 | BoESSR726 |
|---------|-------------|---------|----------|-----------|----------|-----------|
|         |             | P       | G        |           |          |           |
| 1       | SCNU-C-106  | HS      | +        | +         | +        | +         |
| 2       | SCNU-C-107  | R       | +        | -         | -        | +         |
| 3       | SCNU-C-3122 | HS      | -        | +         | +        | +         |
| 4       | SCNU-C-3270 | HR      | -        | +         | +        | +         |
| 5       | SCNU-C-3273 | n/a     | +        | -         | +        | +         |
| 6       | SCNU-C-3305 | S       | +        | +         | +        | +         |
| 7       | SCNU-C-3328 | HS      | -        | +         | +        | -         |
| 8       | SCNU-C-3412 | HS      | +        | +         | +        | +         |
| 9       | SCNU-C-3414 | n/a     | +        | -         | -        | +         |
| 10      | SCNU-C-3449 | S       | +        | +         | +        | +         |
| 11      | SCNU-C-3470 | HR      | +        | +         | +        | -         |
| 12      | SCNU-C-3631 | HS      | +        | -         | -        | +         |
| 13      | SCNU-C-3637 | HS      | +        | -         | +        | +         |
| 14      | SCNU-C-3639 | S       | +        | +         | +        | -         |
| 15      | SCNU-C-4059 | HR      | +        | +         | +        | +         |
| 16      | SCNU-C-4066 | S       | +        | -         | +        | +         |
| 17      | SCNU-C-4071 | HS      | +        | +         | -        | +         |
| 18      | SCNU-C-4072 | HS      | +        | +         | -        | +         |
| 19      | SCNU-C-4073 | S       | +        | +         | +        | +         |
| 20      | SCNU-C-4074 | HS      | -        | +         | -        | -         |
| 21      | SCNU-C-4085 | HS      | +        | +         | +        | +         |
| 22      | SCNU-C-4105 | HS      | +        | +         | +        | -         |
| 23      | SCNU-C-4118 | HS      | -        | -         | +        | -         |
| 24      | SCNU-C-4161 | HS      | +        | +         | +        | +         |
| 25      | SCNU-C-4168 | HS      | +        | -         | -        | +         |
| 26      | SCNU-C-4302 | S       | +        | +         | +        | -         |
| 27      | SCNU-C-4320 | HS      | +        | +         | +        | +         |

Matched 18.0 17.0 17.0 16.0
Not matched 7.0 8.0 8.0 9.0
% Adaptability 72.0 68.0 68.0 64.0

*P, phenotype based on disease reaction; *G, genotype on molecular marker screening; *HR, high resistant; *R, resistant; *S, susceptible; *HS, high susceptible; *n/a, bioassay data not available.

114-bp DNA fragment for R lines and another two with InDels, where 21-bp deletions and 16-bp insertions from R line resulted in being susceptible to black rot disease. However, BoESSR291 showed high identity with a genomic fragment where putative promoter sequence of a gene encoding enzyme cystathionine gamma-synthase (Bo3g055310) was located. The BoESSR726 marker amplified a 127-bp DNA fragment for S lines and one with 18-bp deletions for R lines (109-bp), which are located in the intergenic region of two genes (Bo6g095520 and Bo6g095530) (Table 4).

**DISCUSSION**

Black rot is considered as one of the main threats of cabbage production in the world and it is very difficult to control. The race-specific resistance in *Brassica* crops is
Fig. 2. The resistant and susceptible cabbage lines against different races of *Xanthomonas campestris* pv. *campestris* (*Xcc*) at 2 weeks after inoculation. The letters a, b, c, d, e, f and g, represents *Xcc* race 1, 2, 3, 4, 5, 6 and 7, respectively.
linked to the hypersensitive response at inoculated area with incompatible race, nonetheless occasionally partial symptom appeared (Kamoun et al. 1992; Ignatov et al. 1997). However, recent reports described Xcc race 1, 4 and 6 as the major races while remaining as the minor races in B. oleracea (Ignatov et al. 1998; Vicente et al. 2001; Taylor et al. 2002; Chidamba and Bezuidenhout 2012; Lema et al. 2012; Rouhrazi and Khodakaramian 2014). In B. oleracea, Taylor et al. (2002) reported that 43% of the tested accessions were resistant to one or more of the minor Xcc races (2, 3, 5, and 6) and only one showed partial resistance to Xcc races 1, 3, 5, and 6. They also noted that resistance is more common to Xcc race 3 and 5 whereas very rare to race 1 in B. oleracea. Moreover, Xcc race 6 was predominant in B. rapa (Lema et al. 2015). But recently Cruz et al. (2017) reported the existence of races 4, 6 and 7 (encompassing 21 out of 33 isolates) in Portugal. In this study, we identified novel cabbage lines with race-specific hypersensitive response against different races of Xcc. The disease reaction results revealed that there were 1, 6, 2, 2, 2, and 4 cabbage inbred lines exhibiting resistance against Xcc race 1, 2, 3, 5, 6 and 7, respectively while none of tested lines were resistant to race 4 (Fig. 2 and Table 3). This result is consistent with previous report as described the race-specific resistance against Xcc in Brassica species by Ignatov et al. (1998). Besides, Tonu et al. (2013) also reported black rot resistance in B. oleracea in particular to Xcc race 3 and race 5. It should be noted that the phenotypic screening results revealed SCNU-C-4059 as resistant lines against race 7 but none of the tested markers correlated with the phenotype except BoGMS0971 which showed only 36% adaptability with bioassay results (Table 3).

The genetic inheritance of major black rot resistance genes has been elucidated in different Brassica species including B. rapa, B. carinata and B. napus containing A, BC and AC genome, respectively (Guo et al. 1991; Ignatov et al. 2000; Vicente et al. 2002). However, the strong resistance source for Xcc race 4 was reported to be originated from A genome while resistance to both races 1 and 4 from B genome, and B. juncea (AB genome) was the most resistant species to races 1 and 4 (Taylor et al. 2002).

To date, several QTLs for black rot resistance have been described in B. oleracea and other B. species (Camargo et al. 1995; Cheng et al. 2009; Kifuji et al. 2013; Izzah et al. 2014; Saha et al. 2014; Lee et al. 2015; Sharma et al. 2016). In this study, five reported SSR markers showed polymorphism in banding pattern for R and S lines of the tested cabbage lines. By using these markers, we were able to separate R and S lines which were consistent with bioassay based phenotypic screening results (Table 3 and Fig. 2), nevertheless, none of them perfectly match with phenotypic data. These published markers were located on four different chromosomes such as BnGMS301 and BoESSR726 on chromosome C01, BoESSR291 on chromosome C03, OI10G06 on chromosome C06, and BoGMS0971 on chromosome C08 (Fig. 3). Previous reports described several QTLs for black rot resistance on those chromosomes. For instance, Lee et al. (2015) described four QTLs on three different chromosomes where BRQTL-C1_2 on chromosome C01 was the main QTL for black rot resistance in cabbage. Interestingly, the tested polymorphic marker BnGMS301 located on chromosome C01. Tonu et al. (2013) found XccBo(Reiho)2 on chromosome C08 as the major locus for black rot resistance in cabbage and the SSR marker BoGMS0971 was closely linked to it. The remaining two SSR markers namely BoESSR291 and OI10G06 were linked to the minor QTLs (BRQTL-C3 and BRQTL-C6, respectively).

### Table 4. The tested molecular markers and their location on Brassica oleracea genome.

| Marker name | Location | Description |
|-------------|----------|-------------|
| BoGMS0971   | C8:3175431..31754753 | Intergenic region (Bo8g092870 and Bo8g092880) |
| BnGMS301    | C1:15386406..15386643 | Intergenic region (Bo1g053300 and Bo1g053310) |
| BoESSR291   | C3:21846613..21846644 | Promoter region of a gene cystathionine gamma-synthase (Bo3g055310) |
| BoESSR726   | C1:15967972..15968174 | Intergenic region (Bo1g054960 and Bo1g054970) |
| OI10G06     | C6:29898028..29898121 | Intergenic region (Bo6g095520 and Bo6g095530) |

*The chromosomal locations are shown according to the ENSEMBL-based Brassica oleracea genome database.*
Fig. 3. Chromosomes location of five reported polymorphic markers on Brassica oleracea.

Table 5. Genes around the tested polymorphic molecular markers in Brassica oleracea genome.

| Makers      | Gene ID     | Description                                      | NCBI\(^z\) | TAIR\(^x\) | Bolbase\(^x\) |
|-------------|-------------|--------------------------------------------------|------------|------------|--------------|
| BoGMS0971   | Bo8g092870  | V-type proton ATPase subunit D-like              |            |            |              |
|             | Bo8g092880  | Citrate synthase 2, peroxisomal-like             |            |            |              |
| BnGMS301    | Bo1g053300  | (E)-beta-ocimene synthase, chloroplastic         |            |            |              |
|             | Bo1g053310  | (E)-beta-ocimene synthase, chloroplastic         |            |            |              |
| BoESSR291   | Bo3g055310  | Cystathionine gamma-synthase                     |            |            |              |
| BoESSR726   | Bo1g054960  | Uncharacterized                                  |            |            |              |
|             | Bo1g054970  | Uncharacterized                                  |            |            |              |
| OII10G06    | Bo6g095520  | Uncharacterized                                  |            |            |              |
|             | Bo6g095530  | Protein argonaute 7-like                         |            |            |              |

\(^z\)The National Center for Biotechnology Information (NCBI); \(^x\)The Arabidopsis Information Resource (TAIR); \(^y\)The Brassica oleracea Genome Database (Bolbase).

(Lee et al. 2015). Plant disease resistance governed by an interaction between specific disease resistance gene (R) of the host plant and avirulence gene (avr) the pathogen and also known as “gene-for-gene” model for plant disease resistance (Flor 1971; Dangl and Jones 2001). The R genes codes for only five classes of proteins and those encoded “nucleotide-binding site and leucine-rich-repeat” (NBS-LRR) proteins are the major R genes in “gene-for-gene” plant resistance (Van der Biezen and Jones 1998; Dangl and Jones 2001). The NBS-LRR type R genes were further subdivided into two classes based on N-terminal structures such as those having the intracellular signaling domains of
the *Drosophila* Toll and mammalian interleukin (IL)-1 receptors (TIR-NB-LRR), and others having coiled-coil domains (CC-NB-LRR) (Dangl and Jones 2001). None of the tested markers were located in neither the *R* genes nor any other annotated genes (Table 5). Therefore, it is crucial to identify *R* genes responsible for race-specific resistance in cabbage. Taking this into consideration, we performed crossing between *R* and *S* cabbage lines for each *Xcc* race (race 1, 2, 3, 5, 6 and 7) to develop mapping population. Thereafter, with molecular mapping, the specific *R* genes responsible for race-specific resistance could be elucidated.

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**REFERENCES**

Bain D. 1952. Reaction of brassica seedlings to blackrot. Phytopathology 42: 316-319.

Camargo L, Williams P, Osborn T. 1995. Mapping of quantitative trait loci controlling resistance of *Brassica oleracea* to *Xanthomonas campestris pv. campestris* in the field and greenhouse. Phytopathology 85: 1296-1300.

Cheng X, Xu J, Xia S, Gu J, Yang Y, Fu J, *et al.* 2009. Development and genetic mapping of microsatellite markers from genome survey sequences in *Brassica napus*. Theor. Appl. Genet. 118: 1121-113.

Chidamba L, Bezuidenhout CC. 2012. Characterisation of *Xanthomonas campestris pv. campestris* isolates from South Africa using genomic DNA fingerprinting and pathogenicity tests. Eur. J. Plant Pathol. 133: 811-818.

Cook A, Walker J, Larson R. 1952. Studies on the disease cycle of black rot of crucifers. Phytopathology 42: 162-167.

Cruz J, Tenreiro R, Cruz L. 2017. Assessment of diversity of *Xanthomonas campestris* pathovars affecting cruciferous plants in portugal and disclosure of two novel *X. campestris pv. campestris* races. J. Plant Pathol. 99: 403-414.

Dangl JL, Jones JD. 2001. Plant pathogens and integrated defence responses to infection. Nature 411: 826-833.

Dickson M, Hunter J. 1987. Inheritance of resistance in cabbage seedlings to black rot. HortScience 22: 108-109.

Fargier E, Manceau C. 2007. Pathogenicity assays restrict the species *Xanthomonas campestris* into three pathovars and reveal nine races within *X. campestris pv. campestris*. Plant Pathol. 56: 805-818.

Ferreira M, Dias J, Mengistu A, Williams P. 1992. Screening of Portuguese cole landraces (*Brassica oleracea L.*) with *Leptosphaeria maculans* and *Xanthomonas campestris pv. campestris*. Euphytica 65: 219-227.

Flor HH. 1971. Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. 9: 275-296.

Guo H, Dickson M, Hunter J. 1991. *Brassica napus* sources of resistance to black rot in crucifers and inheritance of resistance. HortScience 26: 1545-1547.

Ignatov A, Kuginuki Y, Hidam K. 2000. Distribution and inheritance of race-specific resistance to *Xanthomonas campestris pv. campestris* in *Brassica rapa* and *B. napus*. J. Russ. Phytopathol. Soc. 1: 89-94.

Ignatov A, Hida K, Kuginuki Y. 1999. Pathotypes of *Xanthomonas campestris pv. campestris* in Japan. Acta. Phytopathol. Entomol. Hung. 34: 177-182.

Ignatov A, Vicente J, Conway J, Roberts S, Taylor J. 1997. Identification of *Xanthomonas campestris pv. campestris* races and sources of resistance. ISHS Symposium on *Brassicas*. 10th Crucifer Genetics Workshop. pp. 23-27.

Izakah NK, Lee J, Jayakodi M, Perumal S, Jin M, Park B, *et al.* 2014. Transcriptome sequencing of two parental lines of cabbage (*Brassica oleracea L. var. capitata L.*) and construction of an EST-based genetic map. BMC Genomics 15: 149.

Jensen BD, Vicente JG, Manandhar HK, Roberts SJ. 2010. Occurrence and diversity of *Xanthomonas campestris pv. campestris* in vegetable *Brassica* fields in Nepal. Plant Dis. 94: 298-305.

Kamoun S, Kamdar HV, Tola E, Kado CI. 1992. Incompatible interactions between crucifers and *Xanthomonas campestris* involve a vascular hypersensitive response:
role of the \textit{hrpK} locus. Mol. Plant-Microbe Interact. 5: 22-33.

Kifuji Y, Hanzawa H, Terasawa Y, Nishio T. 2013. QTL analysis of black rot resistance in cabbage using newly developed EST-SNP markers. Euphytica 190: 289-295.

King EO, Ward MK, Raney DE. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44: 301-307.

Lee J, Izzah NK, Jayakodi M, Perumal S, Joh HJ, Lee HJ, \textit{et al.} 2015. Genome-wide SNP identification and QTL mapping for black rot resistance in cabbage. BMC Plant Biol. 15: 32.

Lema M, Soengas P, Velasco P, Francisco M, Cartea M. 2011. Identification of sources of resistance to \textit{Xanthomonas campestris pv. campestris} in \textit{Brassica napus} crops. Plant Dis. 95: 292-297.

Lema M, Cartea ME, Francisco M, Velasco P, Soengas P. 2015. Screening for resistance to black rot in a Spanish collection of \textit{Brassica rapa}. Plant Breeding 134: 551-556.

Lema M, Cartea ME, Sotelo T, Velasco P, Soengas P. 2012. Discrimination of \textit{Xanthomonas campestris pv. campestris} races among strains from northwestern Spain by \textit{Brassica} spp. genotypes and rep-PCR. Eur. J. Plant. Pathol. 133: 159-169.

Li H, Chen X, Yang Y, Xu J, Gu J, Fu J, \textit{et al.} 2011. Development and genetic mapping of microsatellite markers from whole genome shotgun sequences in \textit{Brassica oleracea}. Mol. Breed. 28: 585-596.

Massomo SM, Nielsen H, Mabagala RB, Mansfeld-Giese K, Hockenhull J, Mortensen CN. 2003. Identification and characterisation of \textit{Xanthomonas campestris pv. campestris} strains from Tanzania by pathogenicity tests, biolog, rep-PCR and fatty acid methyl ester analysis. Eur. J. Plant Pathol. 109: 775-789.

Roberts S, Hiltunen L, Hunter P, Brough J. 1999. Transmission from seed to seedling and secondary spread of \textit{Xanthomonas campestris pv. campestris} in \textit{Brassica} transplants: effects of dose and watering regime. Eur. J. Plant Pathol. 105: 879-889.

Rouhazi K, Khodakaramian G. 2014. Genetic fingerprinting of Iranian \textit{Xanthomonas campestris pv. campestris} strains inducing black rot disease of crucifers. Eur. J. Plant Pathol. 139: 175-184.

Saha P, Kalia P, Sonah H, Sharma TR. 2014. Molecular mapping of black rot resistance locus Xca1bo on chromosome 3 in Indian cauliflower (\textit{Brassica oleracea var. botrytis} L.). Plant Breeding 133: 268-274.

Schaad N, Di Pace J. 1981. Cruciferous weeds as sources of inoculum of \textit{Xanthomonas campestris} in black rot of crucifers. Phytopathology 71: 1215-1220.

Sharma BB, Kalia P, Singh D, Sharma TR. 2017. Introgredion of black rot resistance from \textit{Brassica carinata} to cauliflower (\textit{Brassica oleracea botrytis} Group) through embryo rescue. Front. Plant Sci. 8: 1255.

Sharma BB, Kalia P, Yadava DK, Singh D, Sharma TR. 2016. Genetics and molecular mapping of black rot resistance locus Xca1bc on chromosome B-7 in Ethiopian mustard (\textit{Brassica carinata} A. Braun.). PLoS ONE 11: e0152290.

Song E, Kim S, Noh T, Cho H, Chae S, Lee B. 2014. PCR-based assay for rapid and specific detection of the new \textit{Xanthomonas oryzae pv. oryzae} K3a race using an AFLP-derived marker. J. Microbiol. Biotechnol. 24: 732-739.

Taylor J, Conway J, Roberts S, Astley D, Vicente JG. 2002. Sources and origin of resistance to \textit{Xanthomonas campestris pv. campestris} in \textit{Brassica} genomes. Phytopathology 92:105-111.

Tonu NN, Doullah MA, Shimizu M, Karim MM, Kawanabe T, Fujimoto R, \textit{et al.} 2013. Comparison of Positions of QTLs Conferring Resistance to \textit{Xanthomonas campestris pv. campestris} in \textit{Brassica oleracea}. Am. J. of Plant Sci. 4: 11-20.

Van der Biezen EA, Jones JD. 1998. Plant disease-resistance proteins and the gene-for-gene concept. Trends Biochem. Sci. 23: 454-456.

Vicente JG, Holub EB. 2013. \textit{Xanthomonas campestris pv. campestris} (cause of black rot of crucifers) in the genomic era is still a worldwide threat to \textit{Brassica} crops. Molecular Plant Pathology 14: 2-18.

Vicente JG, Taylor J, Sharpe A, Parkin I, Lydiate D, King G. 2002. Inheritance of race-specific resistance to \textit{Xanthomonas campestris pv. campestris} in \textit{Brassica} genomes. Phytopathology 92: 1134-1141.

Vicente JG, Conway J, Roberts S, Taylor J. 2001. Identification and origin of \textit{Xanthomonas campestris pv. campestris} races and related pathovars. Phytopathology 91: 492-499.

Williams PH.1980. Black rot: a continuing threat to world crucifers. Plant Dis. 64: 736-742.

Williams P, Staub T, Sutton J. 1972. Inheritance of resistance in cabbage to black rot. Phytopathology 62: 247-252.