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Field evaluation for resistance to the black rot pathogen *Xanthomonas campestris* pv. *campestris* in cabbage (*Brassica oleracea*)

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Abstract

Black rot, caused by *Xanthomonas campestris* pv. *campestris*, *(Xcc)*, is one of the most serious diseases of crucifers world-wide. Forty-nine genotypes were evaluated for resistance under field conditions in Tanzania after artificial inoculation with *Xcc* race 1. Open pollinated white cabbage cultivars were generally susceptible, while Portuguese and pointed cabbages exhibited partial resistance. Some F1 white cabbage cultivars were highly susceptible, whereas others exhibited a high level of partial resistance. The most promising of the hybrid cultivars were T-689 F1, Gianty F1, No. 9690 F1, N 66 F1, and SWR-02 F1. Breeding line Badger I-16 exhibited the highest level of resistance of all genotypes. The genotypes accounted for 72.9–75.5% of the variation of the disease severity when assessed on the leaves, and 71.4% of the variation when assessed as internal black rot in heads at harvest. High correlations (equal to or above 0.7) were found between disease severities assessed on leaves three times during the growing season and also with the amount of internal black rot in heads. Leaf loss also was correlated with disease severity. The high genetic determination of the trait and the high correlations between disease assessments indicate that selection for resistance to black rot will be efficient when field screenings are carried out. Evaluation of genotypes for disease severity on leaves during the growing season combined with evaluations of head resistance in the most promising genotypes may be a simple method to select resistant cultivars.

Introduction

Black rot, caused by *Xanthomonas campestris* pv. *campestris*, (*Xcc)*, is considered one of the most serious diseases of crucifers world-wide (Williams, 1980; CAB International, 2002). The most economically important hosts of *Xcc* are the cole crops, forms of the polymorphic species *Brassica oleracea* (including cabbage, cauliflower, broccoli, Brussels sprouts, and kale) (Rubatzky and Yamaguchi, 1997), but *Xcc* is also reported on a number of other cruciferous crops, weeds, and ornamentals (Bradbury, 1986). Black rot has been reported as a major constraint in cabbage production in several countries including Nepal (Adhikari and Basnyat, 1999), Tanzania (Black et al., 2000; Massomo et al., 2003), Zimbabwe (Mguni et al., 1999) and in cauliflower production in India (Sharma et al., 1977; Sharma et al., 1995). In Tanzania, black rot is widespread in most of the

cabbage growing areas, especially during the rainy seasons (Mgonja and Swai, 1998), where individual fields may succumb to heavy crop losses.

Xcc thrives especially well in warm and humid climates (Williams, 1980) and survives from season to season in infected seed (Walker and Tisdale, 1920) and in soil and even longer in plant debris in soil (Schaad and White, 1974), and it spreads readily to nearby plants by rain splash (Williams, 1980). Leaf invasions depend on water accumulating at natural openings, primarily hydathodes, where guttation droplets are formed during high humidity (Cook et al., 1952; Robeson et al., 1989). Typical disease symptoms are V-shaped lesions starting from leaf margins with blackened veins and chlorosis of surrounding tissue (Sutton and Williams, 1970; Williams, 1980).

Besides crop diversification and rotation, important ways to control black rot are the use of pathogen-free seeds, seed treatment, sound sanitation practices including elimination of potential inoculum sources, such as infected crop debris (Williams, 1980; Kocks et al., 1998) and weeds (Williams, 1980; Schaad and Dianese, 1981), and use of resistant cultivars (Williams, 1980).

Kocks and Ruissen (1996) showed that increased field resistance reduced the development of black rot in time and space in cabbage. Resistance to *Xcc* has been identified in different genotypes of *B. oleracea* (Bain, 1952; Williams et al., 1972; Hunter et al., 1987; Henz and Melo, 1994; Sharma et al., 1995; Taylor et al., 2002; Griesbach et al., 2003), including commercially available cultivars of cabbage (Williams et al., 1972, Griesbach et al., 2003), and a series of inbreds, Badger I-14 to I-20, released for breeding programmes (Williams et al., 1972; Williams, 1980) with resistance introduced from cv. Early Fuji (Bain, 1952; Bain, 1955). Furthermore, related Brassica species (Bain, 1952; Guo et al., 1991; Westman et al., 1999; Taylor et al., 2002; Tonguç and Griffiths, 2004) have also been found to exhibit resistance to *Xcc*.

Most studies to identify useful resistance sources have been carried out at the seedling stage under greenhouse conditions, but a few have also included testing during the adult plant stage under field conditions (Williams et al., 1972; Hunter et al., 1987; Massomo et al., 2004). In resource-limited cabbage growing areas in developing countries, such as Tanzania, we have observed that cabbage F1 hybrids constitute a considerable share of the production in areas where seeds can be purchased in nearby cities. However, the deployment of cabbage cultivars possessing resistance is not routine. The goal of this study was therefore to compare the level of field resistance in cabbage F1 hybrids with that of open-pollinated cultivars and the breeding line 'Badger I-16', with a view to identify correlations between disease severity on leaves and internal black rot in heads, and to determine the overall genetic resistance component that can be expected in field disease assessments as a means to identify simple guidelines for resistance evaluations of genotypes under field conditions.

Materials and methods

Trial site

The genotypes were tested for resistance in two artificially inoculated field trials at the Asian Vegetable Research and Development Center – Africa Regional Program (Madiira Vegetable Research and Training Station), Tengeru, Arusha, Tanzania (03′22 S, 36′48 E, altitude 1267 m). The soil type was clay (sand, silt and clay 24:27:49) (Mlahagwa, 2000).

Plant material and experimental designs

The evaluation included 49 cabbage genotypes from several seed suppliers (listed in Tables 4 and 5). Seed of twenty-seven F1 hybrids of white cabbage (*Brassica oleracea* convar *capitata* var. *alba*) were acquired from seed companies and included cultivars, which on the basis of their experience exhibit resistance to *Xcc*. Two cultivars, Gloria F1 and Amigo F1, were already grown in Tanzania. During the evaluation period, three of the hybrid cultivars were found to be duplicates (Table 4). Eleven open-pollinated white cabbage cultivars were included and comprised Copenhagen Market types, grown in Tanzania, as well as other similar early cultivars, obtained from seed suppliers, and the Nordic Gene Bank, Sweden. Two locally available pointed headed cabbage (*B. o.* convar. *capitata* var. *conica*) cultivars and four Portuguese cabbage (*B. o.* var. *tronchuda*) cultivars (Soares and Rebello, Portugal) were also included. The breeding line Badger Inbred 16 (I-16) was obtained from Crucifer Genetics Cooperative, USA.

Cabbage seeds were lightly surface sterilised for 2 min in 2.5% sodium hypochlorite (NaOCl) (modified after Babadoost et al., 1996, using a stronger solution and no heat), rinsed in tap water and sown in propagation trays, unit size 5.5×5.5 cm (Vefi, VP 54, Hamax-Vefi, Norway) in a sieved soil mixture of local forest soil, sand, and cattle manure (1:1:1), previously treated with 400 g m^{-3} basamid (Dazomet, Chemical Industries Ltd., Dar-es-Salaam, Tanzania) at least 2 weeks prior to use. Seedlings were raised outdoors. The seedlings did not exhibit any signs of *Xcc* infection while in the propagation trays.

One–month-old seedlings were transplanted into the field in rows established on ridges, allowing for irrigation in the furrows during the growing season (planting distance 60 cm between rows, 45 cm within rows). Two successive trials including all 49 genotypes were carried out, experiment I during a rainy season, and experiment II during a slightly drier period (November 2000–March 2001). Each trial was a 7×7 partially balanced lattice design with four replications (Gomez and Gomez, 1984). Each genotype was represented by a single row of 13 plants in each replication.

Inoculation

Three (exp. I) and two weeks (exp. II) after transplanting, the plants were inoculated with a saline bacterial suspension, prepared from a local *Xcc* strain SM19 isolated from *B. oleracea* var. *capitata* (Massomo et al., 2003). This strain has a Biolog profile, XccA, which is common in Tanzania, and a BOX-PCR fingerprint profile shared by 65.7% out of 108 tested Tanzanian strains (Massomo et al., 2003). The strain has been race typed to race 1, which is one of the common races worldwide (Vicente et al., 2001). Race typing was done by S. J. Roberts and J. G. Vicente, Horticulture Research International, Wellesbourne, UK, according to Vicente et al. (2001), and acceded to their collection as HRI-W 8387.

For inoculum production, strain SM19 was cultured in 9 cm Petri dishes at 28 °C for 2 days on nutrient agar (NA: 5 g peptone; 3 g meat extract; 15 g agar; 1000 ml distilled water), followed by 2 days on yeast extract-dextrose-$CaCO_3$ agar (YDC: 10 g yeast extract; 10 g dextrose; 20 g calcium carbonate; 15 g agar; 1000 ml distilled water). The bacterial growth was washed off the medium using 5 ml of saline (8.5 g NaCl per l in distilled water) per Petri dish, and further diluted in saline, amended with 20 μl detergent per 10 l, to give 10^8 cfu ml^{-1} (OD 0.2 at 600 nm). Ten litres bacterial suspension were applied per replication in the field trial using a back-pack sprayer (Solo, Germany: with piston pump, hollow cone nozzle, droplet size approximately 250–400 μm). Inoculation was carried out in the evening at twilight.

Field operations

Fertilizer application and insect pest control were carried out according to local practices. At transplanting, cattle manure was applied to each planting hole, (app. 20×20×10 cm) at an estimated rate of 18,000 kg ha^{-1}. Urea, 46% (Norsk Hydro, East Africa, Nairobi, Kenya) was applied in the field at a rate of 100 kg a.i ha^{-1} three times during the growing season. Rigorous control of insect pests (diamond back moth, *Plutella xylostella* in exp. I, and cabbage head worm, *Crocidolomia pavonana* syn. *binotalis* in exp. II) was accomplished to reduce the risk of *Xcc* entry through insect created wounds using the following insecticides: Carbofuran, 5% (Furadan 5G, Rhone-Poulenc, Dar-es-Salaam, Tanzania) was applied at transplanting at a rate of 1 kg a.i. ha^{-1}, pirimiphos-methyl (Actellic 50EC, Zeneca Agrochemicals, Fernhurst, Surrey, England) was applied at a rate of 0.5 kg a.i. ha^{-1} twice (exp. I) and once (exp. II) before transplanting of seedlings, and profenofos (Selecron 720 EC, Novartis AG, Basel, Switzerland) was applied at a rate of 0.7 kg a.i. ha^{-1} three times (exp I) after transplanting of seedlings and once (exp. II) before transplanting of seedlings according to the manufacturers' instructions. Treatment with seed extract of Neem (*Azadirachta indica*) (Dreyer and Hellpap, 1991; Saucke et al., 2000) was carried out three times before transplanting, and five times during the cultivation period in both experiments. Crushed Neem seed (250 g l^{-1} water) was soaked for 24 h filtered, diluted and applied at a rate of 25 kg ha^{-1}. All foliar applications were sprayed over the plants with a back-pack sprayer using 1000 l water ha^{-1}.

Except in rainy weather, furrow irrigation was carried out twice a week to favour disease development. Precipitation was recorded by a rain gauge in the field and temperature data were collected at

HORTI-Tengeru Meterological Station, Tengeru, approx. 1 km from the field.

Plant and disease assessments

Plant assessments included days to maturity (harvest) from transplanting, head shape, weight of heads, and number of leaf scars at harvest, indicative of leaf shed. Disease severity was assessed visually on leaves of individual plants twice during the growing season and at harvest, using a scale from 0 to 9, modified after Williams et al. (1972) and Williams (1985): 0 = no visible attack, 1 = highly resistant: minute necrotic zones, 1–3 mm in diameter, at the hydathodes, or light brown leaf margins, 2 = marginal chlorotic, necrotic lesions, 0.5–3.0 cm in diameter, often at hydathodes, 3 = small to medium V-shaped lesions with distinct marginal chlorosis and blackened veins within the lesion, 1–5 cm in diameter, 4 = medium V-shaped lesions extending to the midrib with distinct marginal chlorosis and blackened veins within the lesion, more than 5 cm in diameter, 5 = large V-shaped lesions coalescing and expanding beyond the midrib, leaves appear scorched with coalescing lesions, 6 = many, 50–75%, of wrapper leaves exhibit symptoms, and a few are necrotic, 7 = almost all, 75–100%, of wrapper leaves exhibit symptoms, many wrapper leaves are necrotic, 8 = all wrapper leaves exhibit symptoms, many are necrotic, 9 = all wrapper leaves are necrotic. At harvest, the heads were cut in half and internal black rot (IBR) attack was assessed using a scale from 0 to 4, modified after Wulff et al. (2002): 0 = no visible symptom, 1 = vein discolouration of internal stem, up to 2 cm long, 2 = vein discolouration of internal stem more than 2 cm long, 3 = vein discolouration of internal stem and heart leaves in the lower part of leaves, 4 = vein discolouration of internal stem and heart leaves in the lower and upper part of leaves.

Statistical analyses

Analyses of variance using PROC GLM (SAS/STAT version 6.12; SAS Institute Inc., Cary, NC, USA) were first carried out on data from the lattice design. However, as no major effects of incomplete blocks within replications were detected, the data were finally analysed as a randomised complete block design. Data for IBR were transformed by Arcsin(sqrt(IBR/4)) before analysis aiming at homogeneity of variance. Components of variance for effects of genotypes and environments were estimated from expectation of the corresponding mean squares from the analysis of variance. The genetic determination was calculated as the percentage of total variation explained by the genotype component of variance (Falconer, 1989).

Results

The first symptoms of black rot appeared in the field 14 days after inoculation as characteristic faint V-shaped lesions beginning at the margin of the leaf. As the disease progressed, lesions became more pronounced and exhibited black veins. These symptoms were observed on all genotypes, except for breeding line Badger I–16, on which dark brown to black spots at the hydathodes of leaf margins only rarely developed into small, confined chlorotic lesions. At harvest, internal black rot (IBR) symptoms were detected in heads as dark streaks along the veins of internal stems and heart leaves. Disease severity was homogenous within genotypes, except for the two pointed cabbage cultivars, which varied somewhat in susceptibility among individual plants.

Results of the statistical analyses of plant and disease assessment data are shown in Table 1. The disease severity on leaves at the three disease assessment dates, as well as in heads at harvest, was significantly different for the two experiments ($P \leq 0.0001$). The disease severity was highest in experiment II at the first assessment date, but highest in experiment I at the following two assessments (Table 2). This coincided with an earlier inoculation in experiment II after transplanting than in experiment I, and conversely with higher precipitation in experiment I, in particular the first 25 days after inoculation (137 mm out of 401 mm from transplanting to harvest) than in experiment II (61 mm out of 284 mm from transplanting to harvest).

The genotypes exhibited significantly different levels of susceptibility with respect to leaf symptoms at the three assessment dates ($P \leq 0.0001$) as well as significantly different IBR ($P \leq 0.0001$). The genotypes accounted for between 72.9 and 75.5% of the variation of the disease severity when assessed on the leaves, and 71.4 % of the variation when disease

Table 1. Mean squares, divided into types and groups within white cabbage for disease assessments, leaf scars and head weight for 49 cabbage genotypes evaluated for resistance to *Xanthomonas campestris* pv. *campestris*

Source of variation	df	Disease assessment (severity)[a]			Leaf scars	df	IBR[b]	Head weight
		Dis1	Dis2	Dis3			(Transf.)	(kg)
Experiment	1	9.302***	77.042***	305.270***	4.639	1	3.547***	3.581***
Replication (Experiment)	6	1.853***	3.545***	2.066***	2.016	6	0.067***	0.454***
Genotype[c]	48	3.094***(75.5)	8.125***(75.0)	7.153***(72.9)	11.575***(39.8)	44	0.325***(71.4)	1.130***(80.6)
Among types	2	0.864***	9.375***	5.411***	4.898*	1	0.095**	0.227**
Portuguese cabbage[d]	3	0.128	0.032	0.548	5.640**	–	–	–
Pointed cabbage	1	0.001	0.005	0.007	0.198	1	0.002	0.020
White cabbage	42	3.486***	8.836***	7.841***	12.672***	42	0.339***	1.178***
Among groups (white cab)	2	45.561***	110.127***	103.786***	71.358***	2	5.488***	17.284***
Hybrids	30	1.674***	2.493***	2.964***	9.168***	30	0.054***	0.473***
Open pollinated	10	0.504***	7.608***	3.281***	11.447***	10	0.162***	0.074**
Breeding line Badger I-16	0	–	–	–	– 0		–	–
Experiment x Genotype	48	0.208***	1.143***	0.778***	4.978*** 44	44	0.041***	0.078***
Error	284	0.105	0.195	0.243	1.330	257	0.012	0.027
Corrected total	387					352		

The genetic determination for the assessments (percent) is noted in brackets after mean squares for genotypes.
*,**, *** significant at $P = 0.05$, 0.01 and 0.001 probability levels, respectively.
[a]Disease assessments were carried out on leaves three times during the growing season, three (Dis1) and five (Dis2) weeks after inoculation as well as at harvest (Dis3) on a scale from 0 to 9.
[b]Internal Black Rot in heads at harvest on a scale from 0 to 4. Data were transformed by Arcsin(sqrt(IBR/4)) before analysis.
[c]The effect of genotypes was also tested against significant interactions (experiment×genotype), and were significant for all evaluated parameters at $P \leq 0.001$, except for leaf scars, $P = 0.0019$.
[d]Portuguese cabbage genotypes did not form heads during the experiments and were therefore not included in analyses of Internal Black Rot (IBR) and head weight.

severity was assessed as IBR (Table 1). The number of leaf scars, indicative of the number of wrapper leaves shed during the growing season, as well as head weight was also significantly different among genotypes ($P \leq 0.0001$).

When the variation among genotypes was partitioned and analysed by type (Portuguese, pointed and white cabbage) there was a significant difference among types at the three disease evaluations ($P \leq 0.001$) (Table 1). At the first disease assessment, the white cabbage cultivars exhibited a lower disease severity than the Portuguese and pointed cabbage cultivars, but at the two later assessments, this was reversed (Table 2). The three types also exhibited significantly different numbers of leaf scars ($P \leq 0.05$), IBR ($P \leq 0.01$) and head weight ($P \leq 0.01$) (Tables 1 and 2).

Among the two pointed cabbages and the four Portuguese cabbage cultivars, no significant effects of genotype were detected for the assessed disease severities, but the number of leaf scars among the non-heading Portuguese cabbage cultivars was significantly different ($P \leq 0.01$) (Table 1). There was a significant effect of all assessed parameters among the white cabbages ($P \leq 0.001$). When the group of white cabbage was further partitioned into hybrids, open-pollinated cultivars and the breeding line, a significant difference was detected among the three groups for all assessed parameters ($P \leq 0.001$), and this effect was also significant for the assessed parameters within the groups of hybrids and open-pollinated cabbages, respectively ($P \leq 0.001$, except for head weight among open-pollinated cultivars $P \leq 0.01$) (Table 1). Badger I-16 exhibited the highest level of resistance in the three disease assessments, followed by the F1 hybrids, while the open-pollinated cultivars in general were the most susceptible, with notably high IBR disease scores (Table 2).

Table 2. Means for disease assessments, head weight and leaf scars at harvest for experiments, cabbage types and white cabbage groups evaluated for resistance to *Xanthomonas campestris* pv. *campestris*

	Disease assessment (severity)[a]					Head weight (kg)	Leaf scars (no.)
	Dis1	Dis2	Dis3	IBR[b]			
				(Transf.)	(Orig.)		
Experiment 1	2.42 (0.02)[3]	4.86 (0.03)	6.37 (0.04)	0.318 (0.008)	0.78	1.00 (0.01)	11.70 (0.08)
Experiment 2	2.71 (0.02)	3.93 (0.03)	4.59 (0.04)	0.113 (0.008)	0.28	0.81 (0.01)	11.49 (0.08)
Cabbage – type:							
Portuguese	2.71 (0.06)	3.67 (0.08)	4.89 (0.09)	–	–	–	11.86 (0.20)
Pointed	2.83 (0.08)	4.09 (0.11)	5.31 (0.12)	0.137 (0.027)	0.34	0.82 (0.04)	12.03 (0.29)
White	2.54 (0.02)	4.47 (0.02)	5.54 (0.03)	0.219 (0.006)	0.54	0.92 (0.01)	11.55 (0.06)
White cabbage – group:							
Badger I-16	0.53 (0.11)	1.48 (0.16)	1.78 (0.17)	0.005 (0.039)	0.01	0.31 (0.06)	9.09 (0.41)
F.1 hybrid	2.34 (0.02)	4.14 (0.03)	5.31 (0.03)	0.116 (0.007)	0.27	1.12 (0.01)	11.31 (0.07)
OP	3.30 (0.03)	5.66 (0.05)	6.52 (0.05)	0.531 (0.012)	1.35	0.40 (0.02)	12.46 (0.12)

[a]Disease assessments were carried out on leaves three times during the growing season, three (Dis1) and five (Dis2) weeks after inoculation as well as at harvest (Dis3).
[b]Internal Black Rot in heads at harvest. Data were transformed by Arcsin(sqrt(IBR/4)) before analyses. Original data are also presented.
[c]Standard errors in brackets.

Correlations between the three leaf disease severity assessments were high and all above or equal to 0.86 (Table 3). The correlations between leaf disease severities and IBR were also high, equal to or above 0.70, whereas correlations between the number of leaf scars and the disease assessments were lower, 0.47–0.63.

Descriptive characteristics of the individual genotypes are presented in Table 4 (open pollinated genotypes) and Table 5 (hybrid genotypes). Most genotypes, with the exception of the four Portuguese cabbage cultivars, produced heads. The genotypes were harvested between 52 and 75 days after transplanting, and the shape of the heads varied from high round to flat round (Tables 4 and 5). The least susceptible open-pollinated cultivars, Romenco and Copenhagen Market LD, had a lower disease score on leaves than the most susceptible F1 hybrids, Gloria F1 and SWG-01 F1 (the latter identified to be synonymous to Gloria F1 at harvest), and SWD-06 F1, but similar scores for IBR as these hybrids (Table 4 and 5). The most promising of the hybrid cultivars exhibiting partial resistance were T-689 F1 and Gianty F1 (Takii, Japan), No. 9690 F1 (Kyowa, Japan), N 66 F1 (Nozaki, Japan), and SWR-02 F1 (Kenya Seed) (Table 5).

Table 3. Pearson correlation coefficients for disease assessments of 49 cabbage genotypes evaluated for resistance to *Xanthomonas campestris* pv. *campestris* in field trials

	Disease assessment (severity)[a]			Leaf scars
	Dis2	Dis3	IBR[b] (Transf.)	
Dis1	0.87	0.86	0.77	0.63
Dis2		0.93	0.73	0.52
Dis3			0.70	0.51
IBR				0.47

[a]Disease assessments were carried out on leaves three times during the growing season, three (Dis1) and five (Dis2) weeks after inoculation as well as at harvest (Dis3) on a scale from 0 to 9.
[b]Internal Black Rot in heads at harvest on a scale from 0 to 4. Data were transformed by Arcsin(sqrt(IBR/4)) before analysis.

Discussion

Symptom development and characteristics were comparable to those previously described for resistant and susceptible genotypes (Staub and Williams, 1972). All 11 open pollinated white cabbage cultivars exhibited high levels of susceptibility. Many open-pollinated cultivars, including Copenhagen Market types, have also been reported previously as highly susceptible (Bain,

Table 4. Mean disease severity, head weight and leaf scars at harvest for 17 open pollinated *Brassica oleracea* genotypes and one breeding line evaluated for resistance to *Xanthomonas campestris* pv. *campestris*

Genotype	Seed supplier[a]	Disease assessment (severity)[b]					Leaf scars (no)	Days to harvest[d]	Head	
		Dis1	Dis2	Dis3	IBR[c] (Transf.)	(Orig.)			Shape[e]	Weight (kg)
Portuguese cabbage:										
Penca Espanhola	S&R	2.67	3.68	4.55	–		12.02	73	No head	–
Murciana	S&R	2.76	3.71	4.88	–		12.55	73	No head	–
Glória de Portugal	S&R	2.85	3.72	4.94	–		10.64	73	No head	–
Penca de Chaves	S&R	2.56	3.58	5.18	–		12.22	73	No head	–
Pointed headed cabbage:										
Sugar Loaf (PV)	PV	2.83	4.11	5.29	0.125	0.30	12.14	73	Conical	0.85
Sugar Loaf (Mf)	Mf	2.82	4.07	5.33	0.148	0.38	11.92	73	Conical	0.78
White cabbage:										
Breeding line:										
Badger I-16	CrGC	0.53	1.48	1.78	0.005	0.01	9.09	75	HR	0.31
Open pollinated:										
Romenco	RS	2.93	4.44	5.47	0.404	0.99	11.74	64	R	0.44
Copenhagen Market (LD)	LD	3.26	4.81	5.69	0.254	0.60	13.05	57	R	0.35
Copenhagen Market (Nepal)	Np	3.18	4.82	5.94	0.367	0.90	12.32	57	R	0.40
Glory of Enkhuizen	PV	2.97	4.43	6.35	0.613	1.59	11.98	68	R	0.60
Golden Acre (Nepal)	Np	3.50	6.14	6.39	0.668	1.72	13.23	52	R	0.38
Copenhagen Market 'Opus'	NGB	3.46	6.49	6.54	0.548	1.41	13.85	52	R	0.27
Ditmarsker 'Midi'	NGB	3.17	5.82	6.95	0.512	1.28	11.53	57	R	0.53
Copenhagen Market 'Biro'	NGB	3.11	5.44	7.01	0.523	1.36	9.72	57	R	0.39
Copenhagen Market (PV)	PV	3.40	6.02	7.06	0.585	1.48	13.67	57	R	0.45
Early Ditmarsker 'Ega'	NGB	3.54	6.70	7.23	0.736	1.90	11.62	52	R	0.30
Early Ditmarsker 'Special'	NGB	3.74	7.16	7.36	0.625	1.61	14.30	52	R	0.31
Standard error		0.11	0.16	0.17	0.039	–	0.41	–	–	0.06

Within types the genotypes are ranked firstly according to disease severity on leaves at harvest (Dis3), and secondly according to Internal Black Rot (IBR).

[a]Seed supplier: CrGC = Crucifer Genetics Cooperative, USA; HM = Harris Moran Seed Company, USA; KS = Kenya Seed Company, Ltd. Kenya; Kw = Kyowa Seed Co., Ltd., Japan; LD = L. Dæhnfeldt A/S, Denmark; Mf = Mayford Seeds (Pty.) Ltd., South Africa; from market, Nepal; NGB = Nordic Gene Bank, Sweden; NN = Nippon Norin Seed Co., Japan; Nz = Nozaki Seed, Japan; PV = Pop Vriend Seeds B.V., Holland; RS = Royal Sluis, Holland/Regina Seeds, Kenya; S&G = S&G/Novartis Seeds B.V., Holland; S&R = Soares & Rebello Lda., Portugal; Tk = Takii & Company, Ltd., Japan.

[b]Disease assessments were carried out on leaves three times during the growing season, three (Dis1) and five (Dis2) weeks after inoculation as well as at harvest (Dis3).

[c]Internal Black Rot in heads at harvest. Data were transformed by Arcsin(sqrt(IBR/4)) before analysis.

[d]Mean of days from transplanting to harvest in experiment I and II.

[e]HR = high round, R = round, R-FR = round-flat round, F = Flat round.

Table 5. Mean disease severity, head weight and leaf scars at harvest for 31 hybrid white cabbage genotypes evaluated for resistance to *Xanthomonas campestris* pv. *campestris*

Genotype	Seed supplier[a]	Disease assessment (severity)[b]					Leaf scars (no)	Days to harvest[d]	Head	
		Dis1	Dis2	Dis3	IBR[c] (Transf.)	(Orig.)			Shape[e]	Weight (kg)
T-689 F.1	Tk	1.30	3.04	4.14	0.021	0.05	9.69	64	R–FR	1.56
Gianty F.1	Tk	1.75	3.58	4.41	0.010	0.02	11.53	68	F	1.48
No. 9690 F.1	Kw	1.76	3.30	4.42	0.066	0.15	10.98	71	F	1.50
N 66 F.1	Nz	1.76	3.53	4.60	0.057	0.11	10.79	70	R–FR	1.39
SWR-02 F.1	KS	2.38	3.80	4.63	0.101	0.27	9.84	71	R–FR	0.99
N 578 F.1	Nz	1.78	3.47	4.81	0.100	0.22	10.33	68	FR	1.27
NN 3130 F.1	NN	1.91	3.65	4.83	0.108	0.26	10.36	70	FR	1.36
Fortress F.1	HM	2.43	4.17	4.86	0.016	0.04	9.54	68	R	0.79
N 564 F.1	Nz	2.34	3.90	4.98	0.081	0.17	11.37	65	R–FR	0.84
NN 3106 F.1	NN	1.81	3.87	5.03	0.045	0.10	11.05	66	FR	1.52
JK-1 F.1	S&G	2.48	3.86	5.06	0.101	0.23	12.47	68	F	1.19
KK Cross F.1	Tk	2.05	3.97	5.16	0.025	0.05	13.22	64	FR	1.27
T-676 F.1	Tk	2.05	4.04	5.20	0.078	0.16	10.10	64	R–FR	0.95
Rotan F.1	LD	2.27	4.17	5.24	0.099	0.21	10.52	65	R–FR	0.95
Wakamine F.1	Tk	2.05	3.82	5.27	0.086	0.20	10.09	66	F	1.14
Resist Crown F.1	Tk	2.35	4.44	5.29	0.093	0.21	11.24	64	FR	1.22
Gospel F.1	LD	2.45	4.11	5.29	0.147	0.33	13.55	64	R–FR	0.75
Maja F.1	LD	2.47	3.82	5.35	0.137	0.33	12.24	66	F	1.32
Bravo F.1	HM	2.68	4.31	5.36	0.040	0.08	10.97	64	R–FR	0.89
SWR-03 F.1 (= Rotan F.1)	KS	2.43	4.22	5.46	0.162	0.35	10.81	64	R–FR	0.82
NN 3135 F.1	NN	2.63	4.04	5.51	0.210	0.49	12.52	68	F	1.37
Talisman F.1	HM	2.44	4.46	5.59	0.066	0.16	10.75	66	R	0.88
Amigo F.1	RS	2.33	4.29	5.59	0.120	0.26	10.66	68	FR	1.11
SWP-04 F.1	KS	2.81	4.56	5.61	0.129	0.27	12.45	68	R	0.94
Blue Thunder F.1	HM	2.49	4.47	5.62	0.154	0.37	11.82	70	R	1.09
SWM-05 F.1 (= Maja F.1)	KS	2.45	4.15	5.80	0.198	0.44	12.04	68	F	1.32
Globot F.1	S&G	2.74	4.94	5.95	0.053	0.10	11.12	67	FR	0.93
KWC 101 F.1	S&G	2.47	4.52	6.04	0.160	0.38	10.94	68	FR	1.13
SWG-01 F.1 (=Gloria F.1)	KS	3.32	5.04	6.33	0.288	0.72	12.29	66	R	0.92
Gloria F.1	LD	3.32	5.30	6.44	0.357	0.92	12.57	66	R	0.87
SWD-06 F.1	KS	3.05	5.56	6.83	0.277	0.65	12.80	64	FR	0.91
Standard error		0.11	0.16	0.17	0.039	–	0.41	–	–	0.06

Genotypes are ranked according to disease severity on leaves at harvest (Dis3).
[a, b, c, d, e] For comments, see Table 4.

1952; Williams et al., 1972). The four Portuguese cabbage genotypes did not form heads during the growing season, presumably because of lack of adaptation to the prevailing climatic conditions, as they have been reported previously to form heads under other climatic conditions (Monteiro and Williams, 1989). Previously, Monteiro and Williams (1989) found that all Portuguese cole genotypes were susceptible to *Xcc* when tested during the seedling stage. The study here indicates that a certain level of partial resistance was exhibited under field conditions, in particular towards the end of the growing season. The two cultivars of pointed cabbage exhibited some variation among plants in the level of susceptibility, which indicates that the resistance level of these two cultivars may be elevated through recurrent selection for resistance.

The higher resistance in general of F1 hybrids compared with that of open pollinated cultivars, demonstrates that resistance to *Xcc* is a trait, which to some extent has been selected for and incorporated into F1 hybrids via breeding programmes. Most of the partially resistant F1 hybrids were of Japanese origin. Interestingly, Williams et al. (1972) also concluded that Japanese hybrids generally were more resistant than cultivars from the USA. The level of partial resistance exhibited in some of the tested hybrids may be adequate to control black rot in farmers' fields as part of an integrated disease management strategy. Specifically, the most resistant cultivars in this study are candidates justifying further resistance testing in Tanzania.

The resistance level in Badger I-16 was comparable to previous earlier dated results (Staub and Williams, 1972) and higher in its expression than the resistance exhibited in any of the other cultivars tested in this study, suggesting that resistance in the F1 hybrids may be based on other sources of resistance than that of Badger or the resistance is less expressed in the hybrids because of heterozygous genetic conditions. Alternatively, the resistance trait from Badger has not been completely exploited and incorporated into these cultivars.

Another strain of race 1 from the US (*Xcc* strain PHW 1205), race typed by Vicente et al. (2001), has been used previously to evaluate resistance in Badger I-16 (Camargo et al., 1995). Together with our results this confirms that Badger I-16 still has potential as a useful resistance source to obtain *Xcc* resistance to race 1, one of the most common races world-wide (Vicente et al., 2001). This is despite the fact that this line was developed some 30 years ago (Williams, 1980) on the basis of a resistance source identified in the 1950s (Bain, 1952, 1955), and that resistance to *Xcc* race 1 is rare (Taylor et al., 2002). Through conventional crossings it may be worthwhile to introgress the *Xcc* resistance trait into local, open-pollinated, susceptible cultivars. This may lead to alternative sources of *Xcc* resistant material from which seed in some areas can even be produced locally and sold more cheaply than imported hybrid seed. This may be a sound alternative for resource-limited farmers in remote areas, where accessibility to hybrid seed is limited. However, the search for other resistance sources with different genetic backgrounds must continue in order to deal with the issue of *Xcc* race specificity in this line, which has been indicated in testing of 4-week-old plants using a clipping inoculation method (Taylor et al., 2002). However, further testing of the specificity of the resistance in Badger under field conditions would be of interest, for instance with race 4, which also is widespread worldwide (Vicente et al., 2001).

The results confirm the existence of genetic variation in terms of resistance to *Xcc* in cultivated cabbage material. The resistance is obviously quantitative in nature, probably affected by several genes and interactions with environments. For such quantitative traits the genetic determination provides valuable information on the efficiency of selection (Falconer, 1989; Hill et al., 1998) of resistant cultivars for cultivation or continued breeding purposes. The rather high genetic determination for *Xcc* resistance found during this study of 71–75% indicates that resistant cultivars for local areas can be selected with high efficiency under field conditions.

However, the existence of pathogenic variants (races) in *Xcc* (Kamoun et al., 1992; Vicente et al., 2001) suggests that such testing of cultivars should be performed in several localities to ensure that cultivars are exposed to a range of strains before selection.

The high correlation between susceptibility of leaves to black rot and IBR in heads indicates that a severe attack on the leaves results in progress of the disease into the heads, and hence has a negative effect on the quality of marketable heads.

A high correlation between leaf susceptibility and internal stem susceptibility has previously been reported when young plants were inoculated under greenhouse conditions (Henz and Melo, 1994), and was also indicated by Massomo et al. (2004) under field conditions, although a few exceptions were identified in that study. In this way the genotypes evaluated in this study, did not allude to the existence of a differential kind of resistance in the stem that may be governed by genes different from those operating in the hydathodes, as earlier reported by Ignatov et al. (1999). Investigations involving marker tagged *Xcc* strains to study pathogen progress within tissue (Dane and Shaw, 1993; Mochizuki and Alvarez, 1996; So et al., 2002) may be used to study resistance mechanisms further.

The positive correlation between the number of leaf scars at harvest and the disease assessments suggests that severe disease attack may have caused premature leaf shed of wrapper leaves. However, the senescence reaction leading to leaf loss seems to occur too late to be an important mechanism to limit the development of IBR, as there was a clear positive correlation between initial leaf attack and IBR at harvest.

The high genetic determination for the resistance trait using visual scoring of disease severity under field conditions, and the high correlation between disease assessments of leaves during the growing season, suggest that breeders or local extension services in collaboration with farmers may carry out resistance screenings for black rot under field and weather conditions favouring black rot development to select the most resistant cultivars based on one single disease assessment on leaves when symptoms are readily visible. Cultivars exhibiting resistance in leaf assessments may finally be examined for internal black rot at harvest. This is an efficient and simple method to identify resistant genotypes before introduction into a new area, or before selection of genotypes for participation in breeding programmes.

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