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RESPONSE OF CABBAGE VARIETIES TO SOFT ROT (*Erwinia carotovora*) AND BLACK ROT (*Xanthomonas campestris*) DISEASES

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ABSTRACT

Soft rot (*Erwinia carotovora*) and black rot (*Xanthomonas campestris* pv. *campestris*) are the main bacterial diseases, which has created severe problems for cabbage (*Brassica oleracea* L. var. *capitata*) cultivation in Sri Lanka. Management of both diseases has increasingly become difficult for farmers due to the existing cropping system, alternate hosts and unavailability of chemical control. Nine commercially available cabbage varieties were screened for resistance under laboratory and field conditions to soft rot, and under field conditions for black rot from 2010 to 2012. Differences in resistance among varieties were observed in the laboratory as well as in the field for both diseases. The stem disk inoculation test of soft rot revealed that all the varieties tested are susceptible to the disease. However, varieties Samurai, Indica and Ninja showed significantly lower susceptibility. In addition, the same varieties showed excellent tolerance to both pathogens in the field screening. Although some of the tested varieties had undesirable head characteristics such as loose heads and head splitting, the above three varieties performed best and found capable of giving consistent yield under open field conditions in the Up Country Intermediate Zone Sri Lanka.

KEYWORDS: Cabbage, Black rot, Soft rot, Varietal screening, Yield and quality

INTRODUCTION

Cabbage (*Brassica oleracea* L. var. *capitata*) is the most important and extensively cultivated crucifer vegetable in the upcountry of Sri Lanka. The approximate extent cultivated in 2010 was 3,677 ha, producing 62,832 mt of fresh cabbage (Anonymous, 2011). The two bacterial pathogens that has continued to be the major disease constraint for sustained production of cabbage in Sri Lanka are *Erwinia carotovora* (Ec) causing the soft rot, and *Xanthomonas campestris* pv. *campestris* (Xcc), causing the black rot. Both pathogens are widely distributed in the soil of agricultural and uncultivated lands worldwide (Perombelon and Kelmann, 1980; Williams, 1980). Although there are no published reports available in Sri Lanka on the actual yield loss of cabbage due to these diseases, heavy losses up to 25-40 % have been experienced by the farmers.

The Ec is also able to cause diseases in potato, tomato and other vegetable crops (Bains *et al.*, 1990; Perombelon and Kelmann, 1980) which are economically important crops in highland farming in Sri Lanka. Contaminated water, soil and some dipterous insects are important sources of inoculums of Ec and has shown a positive correlation with the high disease development in the field (Jorge and Harrison. 1986; Kloepper *et al.*, 1979). In temperate climates, the Ec persists in plant residues left in the fields over winter, and can cause soft rot in the following years (Togashi *et al.*, 2001). Severe attack often results in complete loss of the crop. while late infection leads to poor quality and therefore poor marketability. This pathogen is able to develop during transport and storage (Allefs *et al.*, 1995) and hence, the problem is aggravated throughout the marketing process.

Black rot is characterized by V-shaped, chlorotic to necrotic lesions at the margin of leaves and blackened vascular tissues. Diseased leaves and heads have a poor market value, and are unsuitable for storage as they quickly rot after harvest. The Xcc pathogen survives from season to season as a seed borne inoculum (Schaad *et al.*, 1980) and infection can take place from infested soil (Schaad and White, 1974). Sri Lanka totally depends on imported cabbage seeds, due to the unfavorable environmental conditions for seed production, but these seeds are heavily infected with Xcc (Muthumala, 2006). Use of infected seeds, along with other factors favorable for disease development, has led to a further increase in the disease incidence in the country.

The potential of soft rot development in potato tubers could be reduced with sodium hypochlorite, citric acid (Bartz and Kelmann, 1986) or Kasugamycin (Bartz, 1999). The use of pathogen-free seed and transplants has helped to manage black rot in many developed countries (Taylor *et al.*, 2002; Williams, 1980). Other methods for black rot management include the use of resistant varieties, cultural practices, and physical and chemical treatment of seeds (Kocks and Ruissen, 1996; Kocks and Zadoks, 1996). However, management of these diseases in the prevailing farming systems in Sri Lanka has increasingly become challenging to cabbage growers. The small size of most land holdings in the country, together with an increased number of cabbage or related cropping seasons in a year, curtail the benefits of crop rotation and facilitate dissemination of inoculum across and between farms. The situation has further aggravated by the absence of effective measures, which guarantee that only quality disease-free seeds are sold to farmers. In addition, there are no chemical recommendations available in Sri Lanka for both these pathogens (Anonymous, 2010).

Emphasis on the control of bacterial diseases has always centered on breeding for resistance (Thompson and Kelly, 1957) which is recognized as the best means of control (Yang, 1980). Several cabbage varieties are commercially available in Sri Lanka, however, their response to Ec and Xcc has not been elucidated. The main objective of this study was to evaluate and select commercially available cabbage varieties with resistance to both diseases to generate information for Sri Lankan cabbage growers.

MATERIALS AND METHODS

Two types of experiments were conducted. In the first experiment, a laboratory test was conducted adopting the stem disc inoculation technique (Ho, 1985a) to evaluate the resistance of cabbage varieties to soft rot disease. In the second experiment, cabbage varieties were screened for resistance to black rot and soft rot disease under field conditions in Upcountry Intermediate Zone (UCIZ), and the varietal phenotypic characteristics were assessed at harvest to determine the potential of adoption of the varieties by farmers.

Studies were conducted between November 2010 to February 2012, in the Plant Pathology Laboratory and research field at the Regional Agricultural Research and Development Centre (RARDC), Bandarawela, Sri Lanka. Isolation of *Erwinia carotovora* (Ec) was carried out using diseased specimens collected from the stems of infected cabbage plants. Specimens were washed in running tap water to remove dirt and soil particles. Two cm inner stem tissues showing fresh infection between the healthy and soft rot tissues were cut out using a sterilized scalpel blade. Tissues were surface sterilized in 1 % $Ca(OCl)_2$ (33 % available chlorine) solution for two min followed by three times washing with sterilized distilled water. Thereafter, the tissues were macerated in 10 ml of sterilized distilled water using a sterilized porcelain mortar and pestle. Resulting suspension was streaked on to plates containing modified crystal violate-pectate agar (MCVPA) medium (Ho, 1985b) using a sterilized wire loop. Many MCVPA plates were streaked to ensure successful isolation of relevant colonies. Plates were incubated at room temperature (28 °C) for 4-5 days. The Ec formed large deep cavities in this medium (Perombelon and Burnett, 1991).

In-vitro testing of cabbage varieties to Ec

Nine commercially available cabbage varieties were established in the research field during November 2010. Crop was managed following the recommended procedure of Department of Agriculture (DOA). After two-months of planting, ten plants of each variety were harvested from the field for laboratory evaluation. All leaves and roots of each plant were stripped off using a sterile knife, leaving only 8–10 cm long stem. Stems were surface sterilized in 0.1 % $HgCl_2$

solution for two min and washed in 70 % ethyl alcohol. Then the stems were cut into 5 mm thick discs, weighed and placed in 9 mm glass Petri dishes lined with moistened filter paper. Only one stem piece was placed in each Petri plate. The stem pieces were dried on the upper side by pressing on sterile filter papers. A 50 µl of bacterial suspension (10^9 CRU ml^{-1}), prepared from 48 hrs-old surface cultures on nutrient agar, was dotted in the centre of each disc. Sterile distilled water placed on stem discs was used as control. The extent of disease reaction was assessed after 72 hrs of inoculation by the weighing method, which was based on percentage differences in the weights of the stem discs before and after inoculation (Ho, 1985a). The stem discs were carefully washed in running tap water to remove only the rotted tissues, dried with filter papers, and weighed. Experiment was conducted in the laboratory at 28 °C and treatments were arranged in a Complete Randomize Design with four replicates. Data were subjected to analysis of variance and DART was used to estimate the significant differences between means (p=0.05).

Field experiments

Two field experiments were conducted between April 2011 and February 2012 at the RARDC, Bandarawela. Nine cabbage varieties, consisting "Green coronet", the popular variety as a control, were evaluated. Seeds were obtained from seed importing companies namely Messrs Opex Holdings (Pvt) Ltd. and Onesh Trading (Pvt) Ltd., Sri Lanka. Cabbage seedlings were raised in nurseries for four weeks. Seedlings were then transplanted on 8.4 m^2 experimental plots with a row spacing of 50 cm and inter row spacing of 40 cm. The trial was irrigated, to supplement rainfall, and kept weed-free by hand hoeing. Experiments were laid out in a Randomized Complete Block Design with four replicates and 48 plants per plot.

Data collection

Disease incidence was scored weekly from the onset of symptoms to harvest. The number of plants with either soft rot or black rot symptoms was counted and percentage disease incidence was calculated at the end of cropping season. The weekly scores were used to calculate area under the disease progress curve (AUDPC) (Equation 1; Campbell and Madden, 1990);

$$A_k = \sum_{i=1}^{n_i-1} \frac{(y_i - y_{i-1})}{2} (t_{i-1} - t_i) \qquad \text{..........................Equation 1}$$

where "n" is the number of evaluations, "y" the percentage disease incidence, and "t" the number of days between two assessments.

RESULTS AND DISCUSSION

Cabbage varieties responded differently to the soft rot pathogen in the laboratory evaluation and statistical analysis showed two groups among nine varieties (Figure 1). The varieties KK cross, KK cross premium, Autumn queen, and Summer autumn recorded the highest weight loss due to Ec infection. Other five varieties showed lower weight loss revealing their ability to resist the infection. As the soft rot bacterium always severely attack the soft tissues of the core region and other soft delicate tissues of the stem, the weighing method is useful in evaluating the extent of such infections. Ho (1985a) suggested that weighing method is the most sensitive and accurate way to assess host-pathogen interaction of soft rot, as this method took into account the depth and extent of internal infection by the pathogen. Experimental results of *in-vitro* test indicated that all the nine cabbage varieties tested were susceptible to soft rot in the laboratory evaluation. However, Samurai, Ninja, Indica, Green coronet and Green top were significantly less susceptible ($p<0.05$) than the rest. The popular cabbage variety Green coronet also recorded lower disease susceptibility without significant differences ($p>0.05$) between aforementioned varieties (Figure 1).

The bacteria in phyllosphere soil enter plants through wounds caused by adverse weather, other diseases or insect damage. The initial infection usually occurs on the petioles of the bottom leaves, which are in close contact with soil when the plant has reached its wrapping stage (Ren *et al.*, 2001), and then rapidly spreads into the main stem causing the entire plant to collapse within a few days. Hence, assessing stem resistance to the pathogen is an important feature in understanding host resistance. In addition, the stem resistance phenomenon may have an influence on the ability of the pathogen to move upwards to the cabbage head and affect the quality and quantity of the final product.

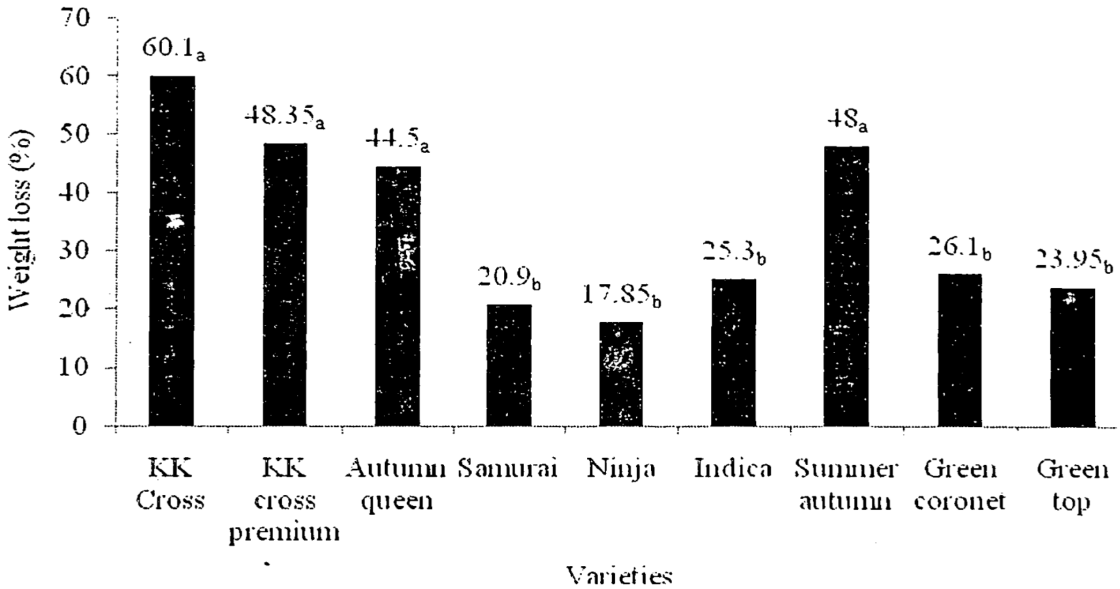

Figure 1. Percentage weight loss observed in cabbage varieties after 72 hrs of incubation with *Erwinia carotovora* (Ec) inoculation. Means followed by the same letter are not significantly different by the DMRT at p=0.05. Values are means of four replicates

The main criterion used to assess the reaction of varieties to soft rot and black rot in open field was AUDPC, which indicated the extent of diseases development in the foliage and stem. In open field evaluation, under natural disease pressure, variation in AUDPC for both diseases was noted among varieties (Table 1). The highest AUDPC for soft rot was observed in KK cross. Although, Autumn queen recorded the highest AUDPC for black rot, its response to soft rot was comparatively less. Samurai, Indica and Ninja are the cabbage varieties that recorded significantly lower AUDPC for both diseases in this study compared to other tested varieties. Disease incidence (%) for Ec and Xcc, recorded at the time of harvesting, showed a similar trend as AUDPC (Table 2). Samurai, Ninja and Indica showed the lowest disease incidence, under natural disease pressure, in both seasons.

All tested varieties showed certain amount of disease symptoms, indicating varying degree of susceptibility to the tested pathogens. Black rot resistance in crucifers is known to operate at the hydathode region (Alvarez, 2000). However, another form of resistance to black rot, controlled by different genes, operates in the stems and is responsible for the arrest of the pathogen in the stem vascular system (Bain, 1955).

Table 1. Disease reaction of nine cabbage varieties to black rot and soft rot in field trials conducted during *yala* 2011 and *maha* 2011/2012

Treatments	*Yala 2011*		*Maha 2011/2012*	
	Mean AUDPC Erwinia*	*Mean AUDPC Xanthomonas*	*Mean AUDPC Erwinia*	*Mean AUDPC Xanthomonas*
KK cross	1216.17^{a}	881.66^{c}	1582.8^{a}	748.33^{c}
KK cross premium	881.97^{b}	676.20^{d}	698.6^{bc}	636.20^{de}
Autumn queen	46.87^{f}	4369.14^{a}	99.6 ^{de}	4102.47^{a}
Samurai	6.74^{fg}	5.22^{e}	43.5^{e}	13.46^{f}
Ninja	3.44^{g}	8.36^{e}	31.8^{e}	13.62^{f}
Indica	29.58^{fg}	20.71^{e}	136.3^{de}	17.38^{f}
Summer autumn	657.00^{c}	689.93^{d}	857.00^{b}	563.27^{e}
Green coronet	373.50^{d}	1445.54^{b}	606.8^{bc}	1245.54^{b}
Green top	139.19^{e}	839.66^{c}	412.5^{cd}	706.33^{dc}
CV %	6.1	4.52	25.85	5.42

*AUDPC: Area under the disease progress curve from time of planting to harvest. Within a column, the means followed by the same letter are not significantly different by the Duncan's Multiple Range Test at p=0.05. Values are means of four replications.

Table 2. Incidence of soft rot and black rot (%) of cabbage varieties under natural disease pressure during *yala* 2011 and *maha* 2011/2012 at RARDC, Bandarawela

Varieties	*Disease incidence (%)*			
	Yala 2011		*Maha 2011/2012*	
	Ec	*Xcc*	*Ec*	*Xcc*
KK cross	77.34^{a}	68.07^{c}	79.04^{a}	56.15^{c}
KK cross premium	57.40^{b}	53.61^{d}	69.35^{b}	46.43^{d}
Autumn queen	45.15^{bc}	89.34^{a}	20.76^{f}	77.15^{a}
Samurai	7.78^{e}	8.94^{f}	12.65^{gh}	4.58^{g}
Ninja	4.72^{e}	9.59^{f}	6.73^{h}	6.95^{g}
Indica	9.6^{e}	15.82^{e}	14.70^{fg}	14.76^{f}
Summer autumn	45.16^{bc}	52.06^{d}	47.05^{c}	38.62^{e}
Green coronet	28.21^{d}	82.30^{b}	38.05^{d}	64.94^{b}
Green top	25.97^{d}	69.20^{c}	30.42^{e}	54.65^{c}
CV %	26.25	6.40	10.60	8.97

Ec - *Erwinia carotovora*; Xcc - *Xanthomonas campestris* pv *campestri*). Disease incidence (%) was calculated at the time of harvesting for each plot (48 plants plot^{-1}). Within a column, the means followed by the same letter are not significantly different by the Duncan's Multiple Range Test at p=0.05. Values are means of four replications.

Since the first recognition of Xcc races by Kamoun *et al.* (1992), several studies analyzed black rot resistance in relation to race types (Ignatov *et al.*, 19989; Vicente *et al.*, 2000; Taylor *et al.*, 2002). Recently, six races were defined according to their reaction on a *Brassica* differential assortment and based on the gene-for-gene relationship between pathogen and host plants (Ignatov *et al.*, 1999; Vicente *et al.*, 2001). There are now a number of black rot resistance studies, but sources with a sufficiently high level of resistance for breeding purposes are limited (Taylor *et al.*, 2002).

Massomo *et al.*, (2004) reported that black rot pathogen was able to cause severe damage when applied through the roots, compared to the usual leaf hydathode route. This implies that roots could be an important infection court for Xcc, especially when the concentration of the bacterium in the soil is high during transplanting. Most cabbage growers in Sri Lanka practice repeated cropping of cabbage using inappropriate methods of disposal of infected crop debris. As the inoculum levels of the bacterium in the soil at time of transplanting play a critical role in black rot development (Kocks *et al.*, 1998), contaminated crop debris may be a very important potential source of inoculum in Sri Lanka. The black rot pathogen is capable of surviving from a few months up to two years in un-decomposed infected crop residues (Schaad and White, 1974; Kocks *et al.*, 1998).

A significant genotypic variation, in terms of yield potential, was observed among the cabbage varieties tested (Table 3). However, yields were lower in the *yala* season 2010 compared to that of the *maha* season 2010/2011. Although there were no significant differences observed ($p>0.05$) in per head weight, the marketable yield showed significant variations among varieties ($p<0.05$). The highly susceptible varieties for soft rot such as KK cross and KK cross premium either yielded smaller heads in both seasons or were completely macerated after heading. Consistently high yields were observed in varieties Samurai and Ninja, which lost very few heads due to the diseases.

Evaluation of cabbage head characteristics at harvest showed that almost all the varieties had semi round to round shaped heads (height: width ratio >0.7), with the exception of variety Ninja, which is a flat head type (ratio <0.7). Leaf compactness scores in the cabbage heads were the lowest in varieties KK cross and KK cross premium. Samurai and Ninja had very compact heads, which can be considered as a good character for packing and transportation. Leaf scars sometimes play a role in disease susceptibility, because they can act as entry points to both pathogens (Black, 2001). However, tested varieties did not show significant differences ($p<0.05$) for number of leaf scars in the stem. In addition, a tendency of head splitting, especially during the *yala* season 2011, was observed in varieties Autumn queen and Summer autumn.

Table 3. Yield and head characteristics of cabbage varieties under field conditions in the *yala* 2010 and *maha* 2010/2011 seasons

Varieties	*Days taken to maturity*	*Head weight (kg)*		*Marketable yield ($t\ ha^{-1}$)*		*Number of leaf scars $plant^{-1}$*		*Head characteristics*			
								shape (H/D)*		*Color*	*Compactness***
		Y	*M*	*Y*	*M*	*Y*	*M*	*Y*	*M*		
KK cross	55-60	1.38^{b}	1.36^{b}	20.64^{b}	22.07^{e}	10.03^{ab}	15.23^{a}	0.71^{a}	0.75^{a}	Light Green	1
KK cross premium	55-60	1.26^{b}	1.34^{b}	30.95^{ab}	29.32^{d}	9.07^{b}	11.9^{b}	0.74^{a}	0.79^{a}	Light Green	1
Autumn queen	60-65	1.57^{b}	1.86^{a}	35.32^{ab}	37.51^{abc}	10.60^{ab}	09.03^{de}	0.81^{a}	0.85^{a}	Dark green	3
Samurai	65-70	1.46^{b}	1.65^{ab}	38.89^{a}	41.41^{a}	9.97^{ab}	07.77^{e}	0.76^{a}	0.79^{a}	Dark green	4
Ninja	70-75	2.02^{a}	1.70^{ab}	38.10^{a}	40.16^{ab}	11.67^{a}	10.93^{bc}	0.65^{a}	0.63^{a}	Green	4
Indica	60-65	1.51^{b}	1.69^{ab}	36.51^{ab}	31.50^{cd}	10.37^{ab}	09.9^{cd}	0.76^{a}	0.77^{a}	Green	3
Summer autumn	60-65	1.59^{b}	1.44^{ab}	37.70^{a}	38.56^{ab}	10.50^{ab}	08.53^{de}	0.79^{a}	0.83^{a}	Green	3
Green coronet	60-65	1.36^{b}	1.65^{a}	30.95^{ab}	37.42^{abc}	10.27^{ab}	08.33^{de}	0.81^{a}	0.85^{a}	Green	3
Green top	60-65	1.49^{b}	1.67^{ab}	31.12^{ab}	34.95^{bcd}	09.10^{b}	08.9^{de}	0.72^{a}	0.75^{a}	Green	3
CV%		15.97	13.59	25.35	14.60	9.93	8.76	12.36	15.16		

Y= *yala* 2010; M= *maha* 2010/2011. Within a column, the means followed by the same letter are not significantly different by the Duncan's Multiple Range Test at p=0.05. Values are means of four replications. *H/D= Height of the head divided by its diameter. ** Compactness rated in a 1–4 scale where: 1=very loose, 2=loose, 3=compact, 4=very compact.

CONCLUSIONS

There is a huge potential of managing soft and black rots in cabbage by host resistance. Partially resistant cabbage varieties such as Ninja, Indica and Samurai with good attributes are likely to be adopted by farmers, and thus, replace black rot and soft rot susceptible cabbage variety currently grown in UCIZ Sri Lanka.

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