International Journal of Agriculture and Crop Sciences.
Available online at www.ijagcs.com
IJACS/2015/8-5/723-731
ISSN 2227-670X ©2015 IJACS Journal

Evaluation Of Resistance On Cabbage Varieties Resistance Against Xanthomonas campestris pv. Campestris in Mozambique

Leonel Abel Alberto

College of Agronomy and Forest Engineering, Zambeze University

Corresponding author email: leonelabel1@yahoo.com.br

ABSTRACT: Black rot is a devastating bacterial disease causing greatly yield and quality losses in Cabbage. This research was conducted to evaluate the resistance cabbage varieties resistance against black rot in Mozambique. A previous research showed high incidence of black rot on Copenhagen Market (70%), Starke 3308 (67.9%), and Glory F1 (67.3%) but seed sellers and importers continue providing these seeds. In order to urge these agents to provide new black rot resistant varieties, samples of infected plants were collected in Mahotas, Boane and Chóckwè. The bacteria were isolated and purified. Four varieties, Glory of Enkhuizen, Copenhagen Market, Glory F1 and Starke 3308, were transplanted in randomized block design and evaluated its resistance against Xanthomonas campestris pv. campestris (Xcc) in Greenhouse. Inter-varietal differences were observed for external (leaf) and internal symptoms (inside the stem). Starke 3308 and Glory F1 exhibited EBR resistance for all inoculums. The maximum index of resistance in Starke 3308 was 2 and 3.75 in Glory F1, both expressing resistance against Xcc. Glory F1, Starke 3308 and Copenhagen Market exhibited IBR index less than 3, showing Vascular discoloration progresses up to less than half of the steam without symptoms in inner leaves. Starke 3308 and Glory F1 also expressed less AUDPC ranging from 14.29-17.50 and 19.25-32-67, respectively. In this research revealed that Starke33008 and Glory F1 express resistance against Xcc which can contribute to the decreased cost of cabbage crop management and resistance of Xcc.

***Key words**:* Xanthomonas campestris pv. campestris, black rot, brassica, Mozambique

INTRODUCTION

Black rot has become the major disease constraint to smallholder cabbage (Brassica oleracea var. capitata) growers in Africa where substantial crop losses are experienced especially during the warm and wet seasons (Massomo, 2002).

The production of cabbage (Brassica oleracea L. var. Capitata L.) in Mozambique occupies a prominent place in horticulture, ranking third, after the tomato and onion. Potential areas of cabbage production in the country are the valleys of the Incomati, Limpopo and Umbelúzi River in the south; the plateau regions of Manica and Angónia and Lichinga in the center, and the region will Lichinga in the north (INE, 2002). In Mozambique cabbage is grown throughout the year. However, due to its high susceptibility to attack by bacteria Xcc causing black rot and Cabbage Moth (Plutela xylostela) in the hot and rainy season, usually done in the cool and dry season, between March and August. In general, during the nurseries watering is through manual sprinkler, while in the field is through gravity (Bila et al., 2012).

The bacterial cells infect cabbage through hydathodes at the leaf margins, causing V-shaped lesions, or through stomata, causing round lesions. Another study shows that Xcc normally gains entry into plants via hydathodes. Once inside the plant, Xcc colonizes the vascular system where it produces an extracellular polysaccharide (EPS) called xanthan, which can obstruct the xylem vessels, causing tissue necrosis and severe leaf wilting (Williams, 1980; Onsando, 1992). Dissemination in the field occurs in wind-driven rain, and insects are considered vectors. The disease is severe under conditions of high rainfall and humidity, with maximum development around 28°C; disease intensity is not reduced by high K and P levels. Using resistant varieties can provide durable and sustainable way to reduce crop losses (Lee & Hong, 2015). Losses caused by Black rot range from quality reduction to complete crop loss under favorable condition to the pathogen (Shimelis & Swart, 2004).

The present studies were conducted to evaluate and to identify Brassica oleracea var. capitata varieties resistant to Xcc strains. In resource-limited cabbage growing fields in developing countries Jensen et al. (2005) have observed that cabbage F1 hybrids constitute a considerable share of the production in areas where seeds can be purchased in nearby cities. Nevertheless, the use of cabbage cultivars having resistance is not habit. Planting resistant varieties farmers would decrease the application of chemical pesticides for protecting environments, increasing the food security and stable yields in cabbage production. This can play important role in Mozambique since there are no local breeding companies producing cabbage seeds. The greenhouse was useful to exclude infection from other pathogens. Results showed that only Glory F1 and Starke 3308 were more resistant than Glory of Enkhuizen and Copenhagen Market. So, seed sellers and importers have to provide exotic hybrids of head cabbage or other vegetables with adequate level of disease resistance.

MATERIAL AND METHODS

In 2011, Samples with clearly expressed symptoms of “V” shaped leaf edge necrosis were collected in Mahotas, Boane and Chóckwè, from four B. oleracea varieties: Glory of Enkhuizen, Copenhagen Market, Glory F1 and Starke 3308. Isolation and identification of Xcc were carried out at Laboratory of Plant Pathology at Faculty of Agronomy and Forest engineering from Eduardo Mondlane University.

The bacterium was isolated from the leaves, petioles and stem tissues. Plant material was rinsed under running water and dried, and then cut into small fragments which were macerated in sterilized distilled water and left for 15 minutes to enable diffusion of the bacteria. The suspension was drained on yeast extract, glucose and $CaCO_3$

medium (YDC) in Petri dishes. The inoculated plates were kept at 28°C for 72 h. Observations were made for the development of light yellow, convex small bacterial colonies on nutrient agar medium and pure cultures were obtained by picking out the individual colonies and transferring them onto the slanting YDC media in test tubes. To ensure vitality of the strains the tubes containing the bacteria were mantained priodically in a fridge and storing at temperatures below 4°C.

To prepare the inoculum strains were harvested and adjusted to 10^8 cfu/ml in 0.85% saline solution. Types of symptoms induced by spray inoculation were recorded 14–21 days after inoculation (DAI).

The experimental design was a completely randomized block an arrangement in subdivided plots (split-plot) having as the main plot three inocula (Xcc-Mahotas, Xcc-Boane, Xcc-Chóckwè) and three controls (negative 0.85% NaCl, positive control Xcc 3207 and water), and four varieties (Glory of Enkhuizen, Copenhagen Market, Glory F1 and Starke 3308) were regarded as sub-factors. The experiment consisting of 24 treatments and 3 replications and 16 plants were used per treatment. The inoculation was done 3 weeks after transplant by the injection process through the syringe on the leaf stalks. Readings were made in all plants in the subplot. To obtain the data for disease severity was used the creiteria created by Williams (1985):

$$SD = [(W_1X_1 + W_2X_2 + ... + W_kX_k) / nPT]$$

Where nPT = Number of plants observed; SD = disease severity; W_1, W_2,..., W_k = severity index; X_1, X_2, ..., X_k = number of plants with index X.

Data for disease severity (SD), which resulted in 3 weekly readings taken from the time the disease was manifested in non-inoculated leaves in plants which underwent the injection of solution containing Xcc, were used to calculate the area under the curve progress of the disease (AUDPC) according to the equation Campbell and Madden (1990):

$$AUDPC = \sum_{i}^{n-1} [\frac{(y_1 + y_{i+1})}{2}] X (t_{i+1} - t_i)$$

Where n is the number of evaluations, y the disease severity indices (EBRI), and tthe number of days after Xcc inoculation when evaluation is done; (t,y)=(0,0) is included as the first evaluation. The AUDPC values, rather than single EBRI values, were used in order to reflect disease progress throughout the whole growing season.

Table 1. Index for external black rot extent (EBRI)

score	Plant status/Index of resistance
1	tiny lesions in hidatodes showing marginal necrotic panels with black edges/HR
3	Small lesions V-shaped, narrow, with 2 cm, sometimes with necrotic panels with diffuse chlorotic margins/R
5	Small to medium V-shaped lesions with necrotic centers, diffuse chlorotic margins, denigrated ribbed internally and externally and lesions rarely progress to the midrib/MR
7	A V-shaped lesion progresses and reaches the main rib, the ribs become blackened/S
9	Marginal lesions tend to coalesce, giving a dry leaf appearance; systemic invasion accompanied by severe wilting and plant death occurs/HS

Note: HR= high resistance; MR= Moderate resistance; S= susceptibility; HS= high susceptibility

At the end of the cycle, plants were harvested and assessed for internal black rot (IBR) severity taking into account the extent of black rot development within the stem according to the scale described by Wulff et al. (2002):

Table 2. Index for internal black rot extent (IBRI)

score	Plant status/Index of resistance
1	Healthy plant/HR
2	Vascular discoloration progresses up less than half of the steam without symptoms in inner leaves/R
3	Vascular discoloration progresses up to more than half of the steam without symptoms in inner leaves/MR
4	Vascular discoloration progresses up to more than half of the steam, 1 to 3 leaves with symptoms/S
5	Vascular discoloration progresses up more than half of the steam, more than 3 leaves with symptoms/HS

statistical analyses

Analyses of variance was carried out using statistical package version 5.0 SAEG. Data from EBR and IBR were transformed aiming at homogeneity of variance.

DISCUSSION AND DISCUSSION

Symptoms of black rot may differ widely among species of brassicas. On cauliflower, the bacteria infection through stomates produce brown or black specks on leaves, black veins, extended leaf margins and also discolored curds. In addition, the severity of Xcc symptoms and aggressiveness of black rot varies between different strains of the bacteria (Alvarez, 2000). The isolates of Xcc can be differentiated into races according on the reaction of several crop lines after inoculation. The race structure which include 5 races (0 to 4) was initially proposed in 1992 (Kamoun et al., 1992); a revised classification model including 6 races was presented in 2001(Vicente et al., 2001) and, today, the model was expanded to confine nine races (Jensen et al., 2010; Fargier & Manceau, 2007).

Copenhagen Market and Glory of Enkhuizen were the first to present black rot symptoms two weeks after inoculation as characteristics faint V-shaped lesions beginning at the margin of the leaf. In these varieties small chlorosis associated with mildew on the leaves have appeared after 10 days of inoculation.

As can be seen in the table 3, the disease severity on leaves was significantly different as effect of variety and type of inoculum ($p<0.05$). The Scott-Knott test revealed significant differences of EBR index highest and was lower in Starke 3308 followed by Glory F1. The Inoculum of Mahotas and Boane were those that originated highest EBR index (Table 4).

Table 3. ANOVA for EBR

Source of Variation	Df	Sum of squares	Average square	F.	Sig
Block	2	0.3370016E-01	0.1685008E-01	1.55	0.2257
Inoculum (a)	5	11.02818	2.205635	203.11	0.0000
Error (a)	10	0.1694498	0.1694498E-01	1.56	0.1587
Variety	3	3.391235	1.130412	104.09	0.0000
Inoculum*variety	15	2.752335	0.1834890	16.90	0.0000
Error (b)	36	0.3909428	0.1085952E-01		
Total	71	17.76584			

coef. of variation = 6.1801

Table 4. Means of EBR index

	Inoculum			
Variety	Mahotas	Boane	Chokwe	Xcc 3207
C.Market	5.993333 Aa	5.333333 Bb	2.833333 Ac	2.250000 Bc
Gloria F1	3.750000 Ba	3.500000 Ca	2.333333 Ab	2.083333 Bb
G.Enkhuizen	6.666667 Aa	7.000000 Aa	2.750000 Ac	3.416667 Ab
Starke3308	1.583333 Ca	2.000000 Da	1.083333 Bb	1.583333 Ba

Comparison along the column is with a capital letter

Comparison along the line is made with a lowercase letter

At the inicial stage of disease the symptoms were similar to fusarium. According Williams (1980) symptoms of black rot of crucifers may be masked by other diseases such as mildew when conditions are unfavorable. This factor may have contributed to the late onset of the characteristic symptoms of the disease. As result of disease progress, lesions became more expressed showing black veins. Star3308 showed diminished symptoms and Gloria F1 showed symptoms in "V" shaped ranging from small to medium while C. Market and G. Enkhuizen had Small to medium V-shaped lesions with necrotic centers and regard a particular strain, the V-shaped lesion progressed and reached the main rib. These symptoms were less expressed in Glory F1, ranging from 2.08-3.75, and Starke3308, 1.58-2.0, but more pronounced exhibiting black veins in Copenhagen Market, 2.25-5.99, and Glory of Enkhuizen, 2.75-7.0.

The results of the ANOVA revealed a significant interaction between variety and type of inoculum and also a significant effect of variety and type of inoculums (Table 5). The (Scott-Knott) test revealed a significant difference of IBRI in different varieties for a particular type of inoculum. Significant differences were also observed in a certain variety taking into account different inoculum. IBRI values were higher in Gloria of Enkhuizen and lower in the other varieties. Inocula from Mahotas and Boane showed higher pathogenicity. In general, all varieties showed vascular discoloration progressed to less than half of the stem and no symptoms observed in the inner leaves, with exception of Enkhuizen G. in which the vascular discoloration progressed to more than half of the stem and symptoms reached up to three inner leaves (Table 6).

Table 5. ANOVA for IBR

Source of Variation	Df	Sum of squares	Average square	F.	Sig
Block	2	0.1428744	0.7143721E-01	3.74	0.0334
Inoculum (a)	5	1.640287	0.3280573	17.17	0.0000
Error (a)	10	0.4150000E-01	0.4150000E-02	0.22	******
Variety	3	1.508192	0.5027307	26.32	0.0000
Inoculum*variety	15	0.9294764	0.6196510E-01	3.24	0.0019
Error (b)	36	0.6876981	0.1910272E-01		
Total	71	4.950028			

coef. of variation = 7.9714; obs.: ****** mean the value is to high

Table 6. Means of IBR index

	Inoculum			
Variety	Mahotas	Boane	Chokwe	Xcc 3207
C.Market	2.550000 Bb	2.700000 Ba	2.400000 Bb	2.700000 Ca
Gloria F1	2.550000 Bb	2.700000 Bb	2.100000 Cc	3.150000 Ba
G.Enkhuizen	4.950000 Aa	4.800000 Aa	3.450000 Ac	4.200000 Ab
Starke 3308	2.550000 Ba	1.950000 Cc	1.800000 Dc	2.250000 Db

Comparison along the column is with a capital letter

Comparison along the line is made with a lowercase letter

At the harvest stage, internal black rot (IBR) symptoms were detected in heads as dark streaks in a line with veins of internal stems and heart leaves. Copenhagen Market and Glory of Enkhuizen exhibited susceptibility expressing high IBR index, 2.4-2.7 and 3.45-4.95, respectively. Similar results for EBR and IBR in susceptible varieties were observed by Jensen et al. (2005) while carrying out field evaluation for resistance to the black rot pathogen Xcc in cabbage. Symptom development and characteristics were similar to those previous reported for susceptible and resistant genotypes (Staub and Williams, 1972). The pathogen virulence and variety resistance may contribute to the qualitative and quantitative yield of cabbage crop (Vicente, 2004). According to McElhaney et al. (1998) in susceptible varieties the resistance imposed on pathogen entry through the vascular system and hidatodes is lower leading to rapid disease development on the leaves and increased seizure of the pathogen in the vascular system. In the Brassica-Xcc interation system, a separation is made between stem resistance, which prevent the plantlets from a quick spread of Xcc in the vascular system of plant stem, and leaf resistance (Ignatov et al. 1998, 1999b). During his research, Massomo et al. (2003) noted that in varieties with high external resistance showed lower levels of disease progression.

Results of Sutton & Williams (1970) showed that plants inoculated with highly virulent strains of Xcc have more material to clog the vessels and larger lesions than plants inoculated with less aggressive strains. Jensen et al. (2005) showed that the IBR in the heads is highly correlated with susceptibility of leaves to black rot and a severe attack on the leaves results in progress of the diseases into the heads reducing the quality of marketable heads.

The AUDPC is used to express the progress of disease severity based on the number of times that severity was assessed taking into consideration the time duration of the disease (Agrios, 2004). The ANOVA showed a significant interaction ($p<0.05$) between variety and inoculum. These two factors separately, also exhibited significantly different numbers of total amount of disease (Table 7).

Table 7. Means of AUDPC

	Inoculum			
Variety	Mahotas	Boane	Chokwe	Xcc 3207
C.Market	54.27333 Ba	41.41667 Bb	23.91667 Ac	18.95833 Bd
Gloria F1	32.66667 Ca	28.58333 Cb	19.25000 Bc	18.95833 Bc
G.Enkhuizen	56.87500 Aa	56.58333 Aa	24.50000 Ac	29.16667 Ab
Starke 3308	16.04167 Db	17.50000 Da	14.29167 Cc	16.04167 Cb

Comparison along the column is with a capital letter

Comparison along the line is made with a lowercase l

The test of Scott-Knott grouping showed a significant difference ($p<0.05$) in the amount of disease to both effect of the variety as for the inoculum. Therefore, the amount of disease for the same type of inoculum was higher in the Gloria-Enkhuizen and Market Copenhagen varieties and lowest in Gloria F1 and Star 3308. For different varieties, the inoculants Mahotas and Boane were also more virulent giving greater amount of disease in the Gloria-Enkhuizen and lower amount of disease in Gloria F1 and Star 3308 (Table 8). Starke 3308 and Glory F1 also expressed less AUDPC ranging from 14.29-17.50 and 19.25-32-67, respectively. Similar results were obtained by Massomo et al. (2003), who found greater amount of disease in Gloria F1 and Gloria of Enkhuizen while studying resistance in different varieties of cabbage. Tables 4, 6 and 7 show that at least in some resistant genotypes, viz. Gloria F1 and Starke3308, the bacteria was hampered in distribution in the host tissue. These findings demonstrate that there is a mechanism of resistance acting in these plants.

Table 8. Means of amount of disease expressed through AUDPC

	Inoculum			
Variety	Mahotas	Boane	Chokwe	Xcc 3207
C.Market	54.27333 Ba	41.41667 Bb	23.91667 Ac	18.95833 Bd
Gloria F1	32.66667 Ca	28.58333 Cb	19.25000 Bc	18.95833 Bc
G.Enkhuizen	56.87500 Aa	56.58333 Aa	24.50000 Ac	29.16667 Ab
Star 3308	16.04167 Db	17.50000 Da	14.29167 Cc	16.04167 Cb

Comparison along the column is with a capital letter

Comparison along the line is made with a lowercase letter

This research was carried out to identify varieties resistant to black rot to help commercial farmers to achieve more benefits. Gloria F1 Star and 3308 were more resistant to black rot compared to Copenhagen Market and Gloria of Enkhuizen. The progression of black rot of crucifers in the stem is proportional to external index of severity. The study revealed also that the strains from Boane and Mahotas where more virulent.

ACKNOWLEDGEMENT

Acknowledges are addressed to Department of Vegetal protection of Eduardo Mondlane University for financial support. I thank Ana Maria Monjane (PhD) and Joao Bila (MSc) for constructive comments for the manuscript. I also thank the lab technicians Lucrecia and Xadreque for their help in the lab and greenhouse works.

REFERENCES

Alvarez AM. 2000. Black rot of crucifers. In: Slusarenko AJ, Fraser RSS, van Loon LC (Eds.) Mechanisms of Resistance to Plant Diseases. Dordrecht, The Netherlands: Kluwer Academic Publishers. pp 21-52

Bila J, Mondjana AM, Mortensen CN, Lund OS. 2012. Podridão negra de repolho em Moçambique: Estratégias para o manejo sustentável da doença. DSHC. Maputo, Mozambique.

Day LA, Feldman S, Minja R, Wien C. 1992. Vegetable production in Tanzania, a four case study in Arumeru District. Special Survey Report. Cornell University, United States of America and Sokoine University of Agriculture, Tanzania. 1–46 pp

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 Pathology 56 (2007): 805-818

Ignatov A, Kuginuki Y, Hida K. 1998. Race specific reaction of resistance to black rot in Brassica oleracea. – Eur. J. Pl. Pathol. **104,** 821–827

Ignatov, A., K. Hida, Y. Kuginuki. 1999a. Phytopathotypes of Xanthomonas campestris pv. campestris in Japan. – Acta Phytopathol. Entomol. Hung. 34, 177–184

INE. 2002. Censo Agro-pecuário 1999 – 2000. Maputo

Jensen BD, Vicente JG, Manandhar HK, Roberts SJ. 2010. “Occurrence and diversity of Xanthomonas campestris pv. campestris in vegetable brassica fields in Nepal.” Plant Disease 94 (2010): 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 hrpX locus. Molecular Plant-Microbe Interactions 5 (1992): 22-33

Lee YH, Hong JK. 2015. Differential defence responses of susceptible and resistant kimchi cabbage cultivars to anthracnose, black spot and black rot disease. Plant Pathology, 64: 406-415

Massomo SMS, Mabagala RB, Mortensen CN. 2003a. Black rot of cabbage in Tanzania: Guidelines for management of the disease. Technical bulletin, Kandrup, Copenhagen, Denmark. 19 pp

Massomo SMS. 2002. Black rot of cabbage in Tanzania: Characterisation of Xanthomonas campestris pv. Campestris and disease management strategies. Ph.D. Diss., The Royal Veterinary and Agricultural University, 2002. Copenhagen, Denmark. 215 pp

McElhaney R, Alvarez AM, Kado CI. 1998. Nitrogen limits Xanthomonas campestris pv. campestris invasion of the host xylem. Physiological and Molecular Plant Pathology, 15-24

Onsando JM. 1992. Black rot of crucifers. In: Chaube, H.S., U.S. Singh, A.N. Mukhopadyay, and J. Kumar, (eds) Plant diseases of international importance. Diseases of vegetables and oil seed crops. Prentice Hall, Englewood Cliffs, New Jersey, United States of America, 243–252

Shimelis HA, Swart WJ. 2004. Black rot development in introduced cabbage hybrids in Ethiopia. African Plant Protection, 10(1): 43-52

Sutton JC, Williams PH. 1970. The use of aureomycin as a treatment of swede seed for the control of black rot (Xanthomonas campestris). Plant diseases Rep., 38: 547-552

Vicente JG, Conway J, Roberts SJ, Taylor JD. 2001. Identification and origin of Xanthomonas campestris pv. campestris races and related pathovars. Phytopathology 91 (2001): 492-499

Vicente JMGN. 2004. A podridão negra das Crucíferas. Instituto Superior de Agronomia. Universidade Técnica de Lisboa. 3-43 pp

Williams PH. 1980. Black rot: A continuing threat to world crucifers. Plant Disease, 64: 736-742

Williams PH. 1985. Black rot (Xanthomonas campestris pv. campestris) (Pammel) Down. Crucifer genetic cooperative departament of plant pathology. Madison