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Variation in the Bacterial Blight Pathogen (*Xanthomonas campestris* pv. *malvacearum)* and its Implications on Cotton Breeding in Uganda

[1]Akello, B. and [2]Hillocks R. J.
[1] Serere Agricultural and Animal Production Research Institute (SAARI)
P. O. Soroti, Uganda.
[2]Natural Resources Institute, University of Greenwich,
Chatham, Maritime, Kent MR4 4TB, U. K.

Abstract

Bacterial blight caused by *Xanthomonas Campestris* pv. *malvacearum* is an important disease found throughout cotton growing areas in Uganda. Its symptoms known as seedling blight, angular leaf spot, black-arm and bacterial boll rot are all found in Uganda. A set of 8 upland cotton cultivars developed by Hunter *et al.* (1968), i.e. differential varieties, were used to determine the variants or races of the blight pathogen found in Uganda. Results indicated race 10 or 18 as the commonest, followed by race 7. Races 6 and 16 were also identified. About 23% of the isolates caused symptoms on all differential varieties indicating the presence of a new virulent race of the pathogen, which may be race 20. The existence of several races and appearance of new ones has implications on breeding resistant cotton lines and some of these are discussed in this paper.

Key words: Races, *Xanthomonas campestris pv. malvacearum,* bacterial blight, cotton breeding

Introduction

Bacterial blight of cotton also known as black-arm caused by *Xanthomonas campestris* pv. *malvacearum (Xcm)* is widespread occurring in almost all cotton growing areas. It attacks all parts of the cotton plant (*Gossypium spp.*) above the ground and is evident in all growth stages from seedling to boll stage.

It exists in different forms in different places throughout Africa (Wickens, 1953) and was one of the main factors limiting the development of the crop when cotton cultivation was being promoted in Uganda in the late 1920's to early 1930's. At that time losses of 25-33% were attributed to the disease countrywide (Hansford, 1933).

The first bacterial blight resistant upland cottons *(G. hirsutum*) were developed in Uganda by selection from a cultivar called Allen obtained from Nigeria after it was introduced to Africa from the USA (Knight and Hutchinson, 1950). Selections from Allen led to developments of Albar (Allen Black-arm resistant) cultivars which formed the basis for development of blight resistant lines throughout Eastern and Southern Africa for much of the second half of the last century (Innes and Jonnes, 1972; Hillocks and Chinodya 1988).

The existence of pathogenic specialization in *Xcm* was first noted when resistant material developed in Uganda and Sudan had severe symptoms when grown in India (Knight, 1946). Consequently, Balasubramanyan and Raghavan (1950) concluded that biological races of the pathogen existed that could break

down resistance based on the genes B_2 and B_3 which were believed to be the genetic basis for blight resistance in Albar cultivars. A little later a similar situation occurred in the USA where Stoneville 20, a blight resistant line, became severely affected by the disease. The virulent strain was isolated and later the original strain was designated as race 1 and the new strain as race 2 (Bird and Hunter, 1955). Hunter et al. (1968) developed a set of eight upland lines having different resistance genes and similar genetic backgrounds with the identified 9 races of *Xcm*.

The set of differential cultivars assembled by Hunter et al, (1968) has been used in different countries to determine races and more than one race has been found to be present in most countries (Hillocks, 1992).

Recent observations in Uganda suggest that bacterial blight symptoms are more prevalent on the current commercial varieties BPA 85, BPA 89 and BPA 95 than was the case during the 1970s when wide spread studies were undertaken (Akello, 1999; Akello and Hillocks, 2002). In view of the apparent increasing importance of bacterial blight, a study was undertaken to determine, for the first time, the races of *Xcm* present in Uganda. This information is useful in developing varieties that are resistant, which will result in better cotton yields, thereby reducing poverty among the farmers.

This paper presents the findings of the study and discusses the implications of those findings on breeding disease resistant varieties in Uganda.

Materials and methods

Assessment of races in the screen house

A total of 40 isolates of *Xcm* were collected from sites in the four districts included in the survey. Of these, 33 were pathogenic and were isolated from leaf lesions and single colony isolates maintained on nutrient agar. A set of cotton cultivars constituted by Hunter *et al.* (1968) as the host differentials for distinguishing races of *Xcm* were collected at Namulonge Research Institute in Uganda (courtesy Dr. R. J. Hillocks). The eight differential cultivars growing in pots were inoculated with each of the isolates using a syringe containing inoculum prepared by mixing the contents of one Petri plate culture grown for 72 hours with 10 ml of sterile water. The syringe needle was used to pierce the hypocotyl of two-week old seedlings and a drop of inoculum introduced into the wound (Wickens, 1953). The seedlings were maintained in a humid atmosphere for two weeks. Lesions were scored from 1 – 5, the scores representing lesion lengths from 1 = < 5mm to 5 = >20mm. Scores 1 and 2 were regarded as resistant reactions and 3 and over as susceptible.

Table 1. Reaction of differential cultivars to inoculation with 33 isolates of Xanthomonas campestris pv. malvacearum.

No. of	Reactions differential cultivars*								
Isolates	A	B	C	D	E	F	G	H	Race
17	+	+	+	+	+	+	-	+	10/18
9	+	+	+	+	+	+	+	+	unknown
4	+	+	-	+	+	+	-	+	7
2	+	+	+	+	-	+	-	+	16
1	+	+	-	+	+	-	-	+	6

Differential cultivars: A. Acala 44; B, Stoneville 2B; C, Stoneville 20: D, Mebane; E, 1 –10B; F, 20-3; G, 101-102B; H, Gregg

Table 2. Reaction of differential cultivars to natural infection and leaf inoculation at Namulonge in 1995/96 and 1996/97

Cultivar	Disease severity score (1- 5) and disease reaction[1]			
	Natural leaf infection		noculation	
	1995/96	1996/97	1995/96	1996/97
Acala 44	**2.1**	**2.6**	**3.4 S**	**2.8 S**
Stoneville 2B	**2.0**	**2.6**	**3.3 S**	**3.2 S**
Stoneville 20	**1.6**	**2.8**	**2.7 S**	**2.2 S**
Mebane	**1.7**	**3.0**	**3.2 S**	**3.1 S**
1-10B	**1.5**	**3.0**	**3.4 S**	**2.8 S**
20-3	**2.4**	**2.9**	**3.5 S**	**3.3 S**
101-102B	**1.9**	**2.7**	**1.5 R**	**1.5 R**
Gregg	**2.0**	**2.8**	**3.6 S**	**3.5 S**
BPA 85	**2.1**	**3.0**	**2.7 S**	**3.4 S**
BPA 89	**1.9**	**3.1**	**3.5 S**	**3.6 S**
BPA 95	**2.1**	**3.4**	**3.1 S**	**2.9 S**
SE	+0.30(NS)	+0.28(NS)	+ 0.54(*)	+0.45(*)

[1]S=Susceptible, R =Resistant
Significance level for SE: NS = non significant, *(P = 0.05)*

Table 3: Reaction of differential cultivars to natural infection and leaf inoculation at Masindi in 1995/96 and 1996/97

Cultivar	Disease severity score (1-5) and disease reaction[1]			
	Natural leaf infection		Inoculation	
	1995/96	1996/97	1995/96	1996/97
Acala 44	2.6 S	3.4 S	3.0	3.3 S
Stoneville 2B	2.2 S	3.3 S	2.5	3.2 R
Stoneville 20	2.0 S	3.7 S	2.7	1.5 R
Mebane	2.6 S	3.6 S	3.0	2.9 S
1-10B	2.4 S	3.7 S	2.7	2.2 S
20-3	2.7 S	3.7 S	2.9	2.6 S
101-102B	2.7 S	2.9 S	1.9	1.8 R
Gregg	2.5 S	3.4 S	2.7	2.9 S
BPA 85	2.5 S	3.5 S	3.4	3.3 S
BPA 89	3.1 S	3.6 S	2.7	3.5 S
BPA 95	2.7 S	3.4 S	2.5	2.3 S
SE	+0.25 (**)	+0.20(*)	+0.43(NS)	+0.57(*)

[1]S=Susceptible, R= Resistant
Significance levels for SE; NS =Non significant; (*P = 0.05); (**P = 0.01)

Assessment of races in the field trials

A second method of assessment was used in which the set of host differentials was planted at four locations in 1995/96 and 1996/97, i.e. field stations in Masindi and Kasese districts and at Namulonge and Serere Research Institutes. These trials were planted as randomized blocks with three replications and two 6m row plots. Bacterial blight incidence and severity was assessed under natural conditions on the first row and on leaf inoculation by high pressure spray onto the under surface of the uppermost fully expanded leaf of each plant in the second row. Inoculum was prepared by soaking in rain water infected leaf trash collected in the vicinity of the trial.

Results

In the inoculation tests with fourty different isolates 17% were non pathogenic and 33 were pathogenic. The majority of the isolates (42%) gave a susceptible reaction on all cultivars except 101 – 102B, indicating race 10 or 18 in the system of Hunter et al. (1968). Four isolates (10%) gave a susceptible reaction on all cultivars except 101-102B and Stoneville 20, indicating race 7. Two isolates (5%) gave a susceptible reaction on all but 101-102B and 1-10B, indicating race 16. A single isolate (3%) gave a resistant reaction on three differential cultivars, 101-102B, Stoneville 20 and 20-3, indicating race 6. The remaining isolates (23%) gave a susceptible reaction on all cultivars, a reaction for which there was no known race when the system was devised (Table 1).

When the differentials were grown in the field at Namulonge, the reaction to natural infection was variable and differences between the cultivars in bacterial blight severity were not statistically significant (Table 2). At least some blight symptoms occurred on all cultivars. Significant differences were obtained in response to leaf inoculation with 101-102B showing a resistant reaction, indicating that the inoculum included race 10 and/or race 18. At Masindi, significant differences in blight reaction were obtained with natural infection in both years and in response to inoculation in 1996/97 (Table 3). All cultivars showed a susceptible response to natural infection, indicating that a new race was present. However, there was no evidence of a new race in the inoculum used as 101-102B. Stoneville 20 gave a resistant reaction, indicating race 7. Results from Serere and Kasese were similar to those of Namulonge and are not presented.

Discussion and conclusion

Results from this study showed that race 10 and/or 18 were present and had the widest virulence range, infecting all the host differentials except 101 – 102B which carries gene combination B_2, B_3 Bsm. In addition, there was a race causing symptoms on all the differentials. When the host differentials were developed none of the known races attacked the variety 101-102B. Later on however, in some countries, races were found that attacked all differentials including previously immune cottons. This new race was designated race 20 and has been reported in Sudan, Chad and Upper Volta (Hillocks, 1992). The race attacking all differentials in this study was therefore likely to be 20 since Uganda shares a common border with Sudan where race 20 has been confirmed present.

The liberalization of the cotton industry puts Uganda at a higher risk of importing new races of the seed from border countries and may get mixed with our local varieties during the ginning of seed cotton. The evidence presented indicates that severe symptoms of bacterial blight occurring in Uganda may be due to a new race of the blight pathogen. This, however, does not rule out other possible explanations for more severe bacterial blight symptoms than was common before the 1980s.

From the late 1970s to early 1990s, cotton research was disrupted making it difficult to rigorously select and breed bacterial blight resistant varieties. Cotton breeders are faced with a challenge of keeping varieties pure and therefore free from new races. In the liberalised cotton industry, some cotton gets into Uganda from neighbouring countries, and in the process new races may find their way in. This, therefore, means breeders have to work with the knowledge of the races present. Furthermore, varieties with resistance to races with wide virulence range need to be developed. Cultivars like S 295 with resistance to race 20 have been developed elsewhere and its resistance is designated B_{12} (Wallace and El-Zik, 1989). Steps to broaden the genetic base of the BPA varieties in Uganda to include genes for resistance to race 20 need to be taken.

To develop varieties with resistance to multiple races, it is necessary that the inoculum of the bacterial blight pathogen used for screening cotton lines for resistance contain a mixture of isolates to include those with a wide virulence range. Furthermore, screening should be done across many locations.

Although breeding resistant varieties is the long term and most effective method of managing bacterial blight, it must be used in combination with other management options. It has been pointed out that African countries have a high probability of evolution of new strains/races of *Xcm* because its populations are exposed to high selection pressure during the growing seasons and in off-seasons when plants are left in the field after harvest (Hillocks, 1992). For example, in Sudan it was reported that a race appeared which was capable of overcoming resistance of all single and combination of major B genes (Verma, 1986). Similarly, the wide spread losses due to bacterial blight in Chad from 1988 to 1992 were attributed to appearance of new races in 1985 (ICAC ,1994). In Uganda, opportunities exist for evolution of new strains because plants are often left in the field especially in Bukonjo, Kasese. The enforcement of closed season activities like burning trash must be reactivated to reduce development of new races. Alongside breeding, other methods of bacterial blight control like seed dressing should be advocated for as part of integrated disease management.

For a long time, the commercial crop of Uganda has been BPA (Bukalasa Pedigree Albar) and until very recently SATU (Serere Albar Type Uganda) series. Their genetic resistance was based on the major gene B_2 reinforced by polygenes (Innes, 1963). Further selection for bacterial blight has continued as a routine procedure, and this can be modified with further knowledge of the factors governing the expression of host resistance and virulence of the pathogen. The severe symptoms of bacterial blight found in Bukonjo, Kasese, can be termed as a break down of resistance, probably due to appearance of new races. It would have been useful to compare the recent BPA releases with the original BPA material but this was not possible in this study.

It should also be noted that at the time of their release, BPA and SATU were not completely resistant or immune to bacterial blight. Some varieties like Reba had a higher resistance, almost immunity (Innes and Brown, 1968) and was stable under a wide range of environmental conditions. Reba's resistance was attributed to the presence of the major gene B_{9L}. This attribute can be useful in breeding for resistance. Some lines known to have resistance to a wide range of biotic and abiotic factors (Multi Adversity Lines) have been imported into Uganda and are being evaluated for use in the breeding programme (Serungjogi, personal communication). This is one of the ways of overcoming new races.

This study has given insight into the races present in Uganda. Breeding resistant varieties is a long and expensive process. This knew knowledge will contribute to a more focused approach to breeding of resistant varieties thereby hastening the process. The study has made a big contribution to improvement in cotton productivity and therefore to poverty reduction.

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