Bacterial diseases of dry beans in South Africa with special reference to common bacterial blight and its control

by

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This thesis is dedicated to the late Dr. Dermot P. Coyne for his valuable contribution to bean breeding especially with regard to bacterial diseases. His presence and expertise will be missed.

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BACTERIAL DISEASES OF DRY BEANS IN SOUTH AFRICA WITH SPECIAL REFERENCE TO COMMON BACTERIAL BLIGHT AND ITS CONTROL

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SUMMARY

Bacterial diseases, commonly associated with dry beans, often cause severe yield and seed quality loss. Disease surveys, as reported in chapter 2, indicated that common bacterial blight occurred in 83% and 85% of localities in seed and commercial dry bean production areas, respectively. Halo blight was restricted to cooler production areas and occurred in only 10% of seed production fields and 37% of commercial fields surveyed. Bacterial brown spot was the most widespread bacterial disease of dry bean, occurring in 93% of seed production fields and 100% commercial fields. Although incidences of bacterial diseases were high, severity was generally low. The widespread distribution of bacterial diseases in both seed and commercial production areas raises concern that the production of disease-free seed in South Africa might not represent an effective

control method.

In chapter 3 of this study, 255 *Pseudomonas savastanoi* pv. *phaseolicola* isolates, representative of all the localities and cultivars sampled, were categorized into different races according to their reaction on a set of differential cultivars. Seven races (1, 2, 4, 6, 7, 8 and 9) were identified with race 8, the most prevalent. Races 1, 2, 6 and 8 were widely distributed throughout the production area, while races 4, 7 and 9 were restricted to one or two localities.

In the study presented in Chapter 4, 143 *Xanthomonas axonopodis* pv. *phaseoli* (Xap) and *X. axonopodis* pv. *phaseoli* var. *fuscans* (Xapf) isolates from 44 localities in four countries, were inoculated onto eight *Phaseolus acutifolius* lines that differentiate between pathogenic races. Isolates varied in aggressiveness on cv. Teebus, however, pathogenic reaction on the set of differentials, indicated that all, but one isolate, grouped in what has been reported as race 2. Thus, results based on reaction of the majority isolates, suggest the absence of different races. However, the distinct differential reaction recorded for a single isolate, may prove to represent another, as yet unrecorded, race of this pathogen. Both RAPD and AFLP analyses revealed high frequency of DNA polymorphism among isolates and could distinguish between Xap, Xapf and a non-pathogenic isolate. Differences between Xap and Xapf isolates demonstrate that these are two distinct groups of bacteria. Information gained from this study has enabled us to select the most appropriate isolates to use in a resistance breeding programme.

South African cultivars differed significantly in their susceptibility to bacterial diseases as shown in Chapter 5. Cultivars Teebus, Cerillos, PAN 146 and PAN 159 were the most susceptible to common bacterial blight with Monati and OPS-RS2 exhibiting significantly lower susceptibility. Negative correlations were obtained between disease ratings and yields obtained in the common bacterial blight trial. Cultivars exhibited some levels of resistance to halo blight, with small seeded cultivars generally more resistant than large seeded types. A negative correlation was obtained between halo blight rating and yield. Cultivars differed significantly in their susceptibility to bacterial brown spot. Teebus, Cerillos, Bonus and PAN 159 were the most susceptible cultivars, with Mkuzi exhibiting the highest levels of resistance. The majority of cultivars exhibited acceptable levels of resistance to bacterial brown spot. No significant correlation was obtained between disease rating and yield. Although a number of cultivars exhibited field resistance to halo blight and bacterial brown spot, all cultivars were susceptible to common bacterial blight. This disease is, therefore, considered the most important bean bacterial disease in South Africa. Improvement of common bacterial blight resistance in South African cultivars is thus important to obtain stable yields.

In chapter 7 of this study, backcross breeding was used to improve common bacterial blight resistance in the small white canning bean, cv. Teebus, using resistance in XAN 159 and Wilk 2 sources, respectively. High resistance levels in near-isogenic lines, developed in two independent breeding programmes, indicated successful transfer of resistance from both sources. Presence of SCAR-markers, SU91 and BC420, in 35 of 39 XAN 159 derived Teebus lines and all lines derived from Wilk 2, confirmed successful resistance transfer. AFLP studies conducted to determine genetic

relatedness of two near-isogenic Teebus lines, showed a similarity of 96.2% with the maximum similarity between these lines and Teebus being 93.1%. Material developed in this study has been included a bean breeding programme and seed will be made available to farmers after extensive field testing.

Sequence characterized amplified region (SCAR) markers, linked to four independent quantitative trait loci (QTL) in XAN 159 and GN #1 Nebr. sel. 27, are available for indirect selection of resistance to common bacterial blight in *Phaseolus vulgaris*. In chapter 8, existing SCAR-markers, SU91, BC420, BC409 and SAP6, were evaluated for potential use in the local breeding programme. Segregating populations of progenies developed through backcross breeding with cultivars Teebus and Kranskop as susceptible recurrent parents and XAN 159 and Vax 4 as resistant donor parents were evaluated for presence of existing markers. Presence of all four markers in improved Teebus lines (XAN 159 derived), confirmed successful transfer of resistance in these lines. Marker BC420 was absent in XAN 159 derived Kranskop-lines. These lines were only moderately resistant when tested in the greenhouse, indicating that the QTL linked to this marker is important in order to obtain high levels of resistance. Progenies from first backcrosses with Kranskop as recurrent parent using Vax 4 have exhibited high levels of resistance when tested in the greenhouse and presence of all markers found in Vax 4 confirms transfer of resistance. Results gained from this study indicate that marker assisted selection can successfully be implemented in breeding for common bacterial blight resistance in South Africa.

In chapter 9, I assessed yield losses in South African genotypes, caused by common

bacterial blight. This was determined using one susceptible cultivar (Teebus) and two resistant near-isogenic Teebus-lines (TCBR1 and TCBR2). Different parameters (disease ratings, % leaf area loss and % infection) were used to evaluate disease. Disease incidence was high in plots containing the susceptible cultivar Teebus. Genotypes differed significantly in their susceptibility to common bacterial blight. Copper sprays reduced the percentage leaf area loss and enhanced seed size. Disease free plots, however, were not achieved using copper sprays. Common bacterial blight significantly reduced yield and seed size in the susceptible cultivar, Teebus. Yield losses of 43.5% were observed in diseased Teebus plots after artificial inoculation with common bacterial blight. The resistance introduced, into the near-isogenic lines, upon release in the industry, will contribute to common bacterial blight control in future productions of the small white canning bean.

In the series of studies presented in this thesis, I have clarified a number of issues regarding bacterial diseases of dry beans in South Africa. Information was gained on the incidence and severity of bacterial diseases, pathogenic variation that occurs in two of the three respective pathogen populations, susceptibility of cultivars to bacterial pathogens and deployment of resistance as long term control strategy to the most important disease. Progress that was made in this study, especially with regard to the development of resistant cultivars, will make a significant contribution towards the South African dry bean industry.

CHAPTER 1

GENERAL INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) are among the major food legumes in the world, and are grown on all the continents, except for Antarctica (Singh 1999). Beans represent an important source of protein, B-complex vitamins and minerals (Paradez-López *et al.* 1986) and form the staple food in the diets of many countries (De León *et al.* 1992). World production during 1997 amounted to 11 607 000 mt, produced on an area of 14 302 000 ha (Singh 1999). In South Africa, mean production of 58 000 t on 56 000 ha has been recorded, for the past 10 years (Coetzee 2000). Per capita consumption, in central and eastern Africa, exceeds 40 kg per annum (Singh 1999).

Bacterial diseases are commonly associated with dry beans wherever they are grown and often cause severe yield and seed quality loss (Allen *et al.* 1998). Three major bacterial diseases, common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli)* (Smith) Vauterin *et al.*, halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*) (Burkholder) Gardan *et al.* and bacterial brown spot (*Pseudomonas syringae* pv. *syringae*), van Hall, occur in South Africa. They are widely distributed throughout the bean producing areas (Fourie 2002), but incidence and severity vary annually as a result of biological and climatic factors and management practices.

Bacterial diseases affect foliage, stems, pods and seeds of beans (Yoshii 1980). Common bacterial blight leaf symptoms initially appear as water-soaked spots on the abaxial sides of leaves, which gradually enlarge, become flaccid and later turn brown and necrotic (Yoshii 1980, Saettler 1991). Lesions are often surrounded by a narrow

zone of lemon-yellow tissue (Fig. 1). Pod lesions are water-soaked spots which gradually enlarge, turn red-brown and are slightly sunken (Fig. 2) (Yoshii 1980, Saettler 1991). Lesions usually vary in size and shape, and are frequently covered with bacterial ooze (Saettler 1991). Infected seeds are shrivelled and exhibit poor germination and vigour (Saettler 1991).

Halo blight leaf symptoms initially appear as water-soaked spots that later turn red-brown and necrotic. A lime-green halo frequently develops around the necrotic lesion (Fig. 3) (Schwartz 1989). Symptoms without halos may occur at temperatures exceeding 28°C. Jensen & Livingston (1944), however, identified isolates that produced halo-less lesions at 16°C. Stems may become infected and produce typical greasy spots. Pod symptoms are water-soaked, greasy spots that vary in size and may develop brown margins as they mature (Fig. 4). Infected seeds may rot or appear shriveled and discolored (Schwartz 1989). Internally-infected seed, however, exhibit few symptoms or are symptom-less (Taylor *et al.* 1979). Systemically infected plants exhibit a general lime-green color and plants are often stunted and distorted (Fig. 5) (Allen *et al.* 1998). Systemic chlorosis is more pronounced and uniform at temperatures below 20°C.

Bacterial brown spot leaf symptoms are small, irregular necrotic lesions that are sometimes surrounded by a narrow, pale green chlorotic zone (Fig. 6). Lesions may coalesce, dry out and become brittle, giving leaves a tattered appearance (Watson 1980). Pod lesions are small, dark-brown and deeply sunken, and may serve as a source of infection for seeds. Young infected pods may bend at the point of infection (Fig. 7) (Serfontein 1994).

Effective and economical control of bacterial diseases can only be achieved

using an integrated approach, including cultural practices, chemical sprays and genetic resistance. Planting of pathogen-free seed is the most important primary control method (Gilbertson *et al.* 1990), however, it does not guarantee disease control (Allen *et al.* 1998). Additional cultural practices such as removing, destroying or deep ploughing of debris, effective weed control, crop rotation and minimized movement in fields, especially when foliage is wet, may be effective (Allen *et al.* 1998, Schwartz & Otto 2000). Copper-based bactericides protect foliage against bacterial diseases and secondary pathogen spread. Efficacy of chemical control, however, is limited (Allen *et al.* 1998) and resultant yield increases are minimal (Saettler 1989).

The most important factor of an integrated approach is use of resistant cultivars (Rands & Brotherton 1925). Resistance breeding is, however, a long-term goal and emphasis should be placed on the disease with the highest economical impact on the bean industry. Effective deployment of resistance requires knowledge on pathogen variation, susceptibility of cultivars and resistance available in germplasm. The present study was undertaken to investigate these aspects and to use this knowledge in a breeding programme, especially focusing on common bacterial blight.

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Figure 1. Common bacterial blight on dry bean leaves caused by *Xanthomonas axonopodis* pv. *phaseoli*

Figure 2. Common bacterial blight (*X. axonopodis* pv. *phaseoli)* on dry bean pods

Figure 3. Leaf symptoms of dry bean halo blight caused by *Pseudomonas savastanoi* pv. *phaseolicola*

Figure 4. Halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*) on dry bean pods

Figure 5. Dwarfed bean plant systemically infected with halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*)

Figure 6. Bacterial brown spot (*Pseudomonas syringae* pv. *syringae*) symptoms on dry bean leaves

Figure 7. Pod symptoms of bacterial brown spot (*Pseudomonas syringae* pv. *syringae*)

CHAPTER 2

DISTRIBUTION AND SEVERITY OF BACTERIAL DISEASES ON DRY BEANS (*PHASEOLUS VULGARIS* L.) IN SOUTH AFRICA

ABSTRACT

Disease surveys were conducted during 1995/96 in seed production fields, and 1996/97 and 1997/98 in commercial dry bean producing areas to determine incidence, severity and spread of bacterial diseases in South Africa. Six-hundred-and-eighty-two seed production fields at 31 localities and 81 commercial fields at 24 localities were surveyed. Common bacterial blight occurred in 83% and 85% of localities in seed and commercial production areas, respectively. Halo blight was restricted to cooler production areas and occurred in only 10% of seed production fields and 37% of commercial fields surveyed. Bacterial brown spot was the most widespread bacterial disease occurring in 93% of seed production fields and 100% commercial fields. Although incidences of bacterial diseases were high, severity was generally low. The widespread distribution of bacterial diseases in both seed and commercial production areas questions the effectivity of disease-free seed as primary control method.

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INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) play an important role in crop production systems in Africa and are the second most important plant protein source after groundnuts (Technology Impact Report 1998). Mean production of 58 000 t on 56 000 ha has been recorded in South Africa for the past 10 years (Coetzee 2000). Beans are produced commercially in Mpumalanga (56%), Free State (28%), North West (7%), KwaZulu-Natal (5%) and Northern Cape (4%) provinces. The major areas for small-scale farmer bean production are Mpumalanga, Eastern Cape Province and KwaZulu-Natal. Seed types grown primarily are red speckled sugar (79%), small white (12%) and large white (*P. coccineus* - 4%) beans. Seed types of lesser importance (5%) include carioca, haricot and alubia beans (Coetzee 2000).

Bacterial diseases are a major constraint limiting South African dry bean production. Locally occurring bacterial diseases are common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli,* [Xap] and its fuscans variant *X. axonopodis pv. phaseoli var. fuscans,* [Xapf]), halo blight (*Pseudomonas savastanoi* pv. *phaseolicola,* [Psp]) and bacterial brown spot (*P. syringae* pv. *syringae*, [Pss]). Incidence and severity of these diseases vary annually, being influenced by biological and climatic factors as well as management practices.

Common blight is a worldwide problem in bean production and may be highly destructive during extended periods of warm, humid weather, resulting in yield and seed quality losses (Saettler 1991). Common blight, and fuscous blight are often referred to as separate diseases (Boelema 1967). Xapf differs from Xap in that it produces a brown diffusible pigment in culture media. Although several reports exist that Xapf is more

virulent than Xap (Leakey 1973, Ekpo & Saettler 1976, Bozzano-Saguier & Rudolph, 1994, Opio *et al.* 1996), it has been indicated that the Xapf pigment is not associated with pathogenicity (Gilbertson *et al.* 1991, Tarigan & Rudolph 1996) and could be considered of lesser pathological importance (Schuster & Coyne 1975). The respective common blight bacteria produce identical symptoms on susceptible bean plants and will be discussed as a single disease.

Common blight is usually visible during the crop's reproductive stage. The disease has been reported in 19 of the 20 bean producing countries in Eastern and Southern Africa (Allen 1995) and is one of the five most important biotic constraints of dry bean production in sub-Saharan Africa (Gridley 1994). Although common blight is widely distributed, yield losses have not been well documented, but have been reported to vary between 22% and 45% (Wallen & Jackson 1975, Yoshii 1980).

Halo blight is distributed worldwide and is favoured by cool, wet weather early in the season. Yield losses of 43% have been obtained under experimental conditions. In Africa, serious crop losses have been observed in Lesotho, Rwanda and Zimbabwe (Allen *et al.* 1998).

Bacterial brown spot results in sporadic losses in moderate to hot production areas, especially where plants have been damaged by heavy rain or hail (Serfontein 1994). During 1992, disease incidence was 100% in plantings in Mpumalanga and yield reductions estimated at 55% (Serfontein 1994). Bacterial brown spot occurs throughout bean production areas worldwide and is a serious constraint in snap bean production in the United States of America (USA) (Schwartz 1980). Although widespread in Africa (Allen 1995), bacterial brown spot is considered a disease of minor importance.

Bacterial bean pathogens are seed-borne, this being the primary inoculum source (Allen *et al.* 1998). Planting of disease-free seed is therefore an important primary control method (Zaumeyer & Thomas 1957), and for this reason a disease free seed scheme was introduced in South Africa in 1980 (A.J. Liebenberg, Agricultural Research Council: personal communication). Disease-free seed is produced in Northern Province, northern parts of Mpumalanga and Northern Cape during winter when climatic conditions and rigid quarantine minimize risk of infestation by bacterial pathogens. Seed certification schemes are also successfully implemented in the USA (Copeland *et al.* 1975), Canada (Sutton & Wallen 1970) and Australia (Redden & Wong 1995).

Seed production fields are regularly inspected for presence of disease and fields with visible bacterial infection levels >8% are rejected, whereas fields with <8% infection are regarded acceptable for certified seed. Seed from fields showing no visible disease symptoms are laboratory tested and certified disease free after absence of specific pathogens has been confirmed (W. Havenga, Dry Bean Producers Organization: personal communication).

Despite implementation of the Seed Scheme, damaging epidemics still occur in commercial and seed production areas during wet seasons (D. Fourie: unpublished data). The aim of this survey was to determine incidence, severity and occurrence of bacterial diseases in commercial and seed production fields over three seasons (1995/96 in seed production fields and 1996/97 and 1997/98 in commercial fields). Information obtained from this survey should influence the use of integrated control strategies for effective control of bacterial diseases on dry beans in South Africa.

MATERIAL AND METHODS

Disease survey in seed production fields

Six hundred and eighty two seed production fields at 31 localities were surveyed from March to August (depending on planting date) during 1996 for visual bacterial disease symptoms. Fields were inspected at flowering and full pod set. Two hundred randomly selected plants per field were inspected for presence of typical symptoms. For the seed certification scheme severity was not considered, only percentage disease incidence.

Disease surveys in commercial fields

Bacterial disease surveys of commercial fields were conducted from February to March, during the 1996/97 and 1997/98 growing season, in commercial bean production areas to determine incidence, severity and spread. Eighty-one fields at 24 localities were surveyed prior to flowering, during flowering or at early-pod set, depending on planting-date. Incidence (percentage) of plants showing typical bacterial disease symptoms were assessed in five randomly selected groups of 20 consecutive plants (100 plants/field). Disease severity was evaluated for each plant on a 0-4 scale (0=no symptoms; 1=1-33% foliage affected; 2=34-66% foliage affected; 3=67-100% foliage affected; 4=dead plant) (Bejarano-Alcázar *et al.* 1996). Incidence and severity values were used to calculate a disease index (D_I), using the model: $D_I = (I \times S)/M$ where I=incidence of diseased plants (%), S=mean severity of foliar symptoms and M=maximum severity value (Bejarano-Alcázar *et al.* 1996).

Isolation of bacterial pathogens

Bacteria from seed production fields were isolated by soaking seed samples in a saline solution at 4°C for 24 hr. Serial dilutions were plated on King's B medium (King *et al.* 1954) and yeast-extract-dextrose-calcium-carbonate agar (YDC) (Schaad & Stall 1988) and incubated at 25°C.

Diseased leaves sampled from each commercial field surveyed were used to isolate bacteria. Leaves were rinsed under running tap water for 10 min, surface-sterilized for 3 min in 3,5% sodium hypochlorite and then rinsed twice in sterile water for 1 min each. Leaves were macerated in a droplet of sterile water and macerate streaked onto King's B and YDC agar. Plates were incubated at 25°C.

Identification of bacterial pathogens

Following 72 hr incubation, yellow pigmented colonies typical of *Xanthomonas* spp. were purified on YDC agar by a series of single colony transfers. Production of brown diffusible pigment on YDC differentiated Xapf from Xap isolates (Basu & Wallen 1967). Antiserum specific to Xap and Xapf, obtained from Adgen Agrifood Diagnostics, Auchincruive, Scotland, were used to confirm identity of isolates. Xap and Xapf isolates were inoculated with a multiple needle (Andrus, 1948) onto first trifoliate leaves of the cultivar Teebus to determine pathogenicity.

Fluorescent colonies typical of *Pseudomonas* spp. were selected under UV-light and purified on King's B medium after 48 hr incubation. Isolates were tested for oxidase (-) and levan production (+). Carbon source utilization of sucrose, mannitol, sorbitol and inositol were used to distinguish Pss from Psp isolates (Hildebrand *et al.* 1988).

Agglutination of Psp and Pss antiserum confirmed identity of isolates. Seven- to 10-day-old seedlings of susceptible cultivar Canadian Wonder, were spray-inoculated with Psp isolates (Taylor *et al.* 1996) to confirm pathogenicity. Young attached pods of Teebus plants were inoculated with Pss isolates using the method of Cheng *et al.* (1989).

RESULTS

Distribution of bacterial diseases in seed production and commercial fields

Occurrence of bacterial diseases in dry bean seed production areas and commercial fields is indicated in Tables 1 and 2, respectively. Weather data from surveyed localities are shown in Table 3. Common bacterial blight (Xap, Xapf) occurred in 83% of seed production areas and in 79% commercial fields. Incidences and severities in commercial fields were low, except at Nigel where incidence was 85%. According to laboratory tests, common blight is more widely distributed than indicated in visual field surveys. Although disease symptoms were not noted at Carletonville, Clocolan, Grootpan and Vryheid, the common blight pathogen was isolated from diseased leaves with typical brown spot lesions collected in these areas (Table 2). Petrus Steyn (Free State) was the only locality from which the common blight pathogen was not isolated (Table 2). The fuscans variant, Xapf, was more widespread than Xap in both seed production and commercial fields.

Halo blight (Psp) occurred in only three seed production localities (10%) in the Northern Cape, Northern Province and North West Province. In commercial fields, halo

blight was restricted to cooler production areas and occurred at 37% of localities. Incidence and severity were low with disease indexes ranging from 0,5-24,5.

Bacterial brown spot (Pss) was the most widespread bacterial disease and occurred in 93% seed production and 100% commercial fields, respectively. Although disease incidences were high (up to 100%) severities were generally low (1-2).

DISCUSSION

Planting of disease-free seed is the primary control measure for bacterial diseases of dry beans in South Africa. In addition, copper based bactericides are used to protect foliage against bacterial infestation and secondary spread. However, efficacy of chemical control of bacterial diseases is limited and resultant yield increases have been reported to be minimal (Saettler 1989).

The widespread occurrence of bacterial diseases in seed production areas impacts strongly on the use of disease-free seed as sole local control strategy. Bacterial pathogen infections in seed production areas significantly limited disease-free seed availability during 1996/97. Fifty-one seed production fields (7,5%) were rejected due to infections exceeding 8%. Although visual symptoms were not always observed, pathogens detected during laboratory seed testing resulted in seed rejection. The majority of seed produced (61,8% of fields surveyed) was classified as certified seed with infection levels less than 8%. Only 36 fields surveyed (5,3%) were certified as disease-free.

Local classification of seed as 'certified' and 'certified disease-free', is confusing. Infection levels of 8%, permitted for certified seed, could have serious implications on

commercial production, when considering that 0,5% seed infestation can cause serious outbreaks of bacterial diseases (Sutton & Wallen 1970). On the other hand, zero tolerance in certified disease free seed is impossible to achieve as this would require testing of the entire stock (Taylor *et al.* 1979). Sampling techniques are, therefore, of utmost importance and local seed certification should aim at levels as near to zero percent infestation as possible.

Certification standards in the USA permit 0,005% infected plants during field inspection and no infected seed upon laboratory examination. Infected seed production fields in Idaho are immediately ploughed in and destroyed (Copeland *et al.* 1975). Production under these conditions provides a continuous supply of certified seed that contributes significantly towards disease management of bacterial blight (Sutton & Wallen 1970, Copeland *et al.* 1975).

Isolation of production fields is insufficient in South Africa and problems are encountered with seed production fields which are sometimes in close proximity of commercial fields. New seed production areas need to be obtained. Neighbouring countries such as Zimbabwe and Mozambique could be considered. Although rainfall in seed production areas is low, occurrence of common blight and bacterial brown spot could be favoured by prevailing temperatures. Dissemination of bacterial pathogens between bean fields has been shown to occur by aerosols generated during dry, sunny, windy weather (Hirano *et al.* 1995).

The high incidence of bacterial brown spot and common blight in seed production fields during 1996 was probably responsible for its widespread occurrence in commercial fields during 1996/97 and 1997/98. Although common blight was observed in 79% commercial fields, the causal pathogen was isolated from 96% of localities surveyed.

Common blight is potentially more devastating as no resistant cultivars are available in South Africa.

Incidence and severity of halo blight were low in both seed production and commercial fields. The pathogen was restricted to higher altitudes and favoured by low night temperatures. Although climatic conditions in seed production areas are generally unfavourable for halo blight development, low night temperatures could have contributed to occurrence of disease in Prieska, Messina and Tosca. *P. coccineus* (white large kidney beans), highly susceptible to halo blight, is not included in the seed certification scheme and could have contributed to incidence of disease in commercial fields in Ermelo and Bergville. *P. coccineus* production is restricted to cooler areas that also favour development of halo blight. Although halo blight incidence in commercial and seed production fields was low, this disease has caused considerable yield losses particularly where farmers grow their own seed for two or more years (D. Fourie: unpublished data).

A strong association has been shown to exist between rainfall and onset of epidemic growth in pathogen populations (Hirano *et al.* 1995). These authors reported that *P. syringae* pv. *syringae* populations increased almost 100-fold from 34 to 35 days after planting (DAP) after 26 mm rainfall on 34 DAP. It can be concluded that higher than average rainfall in commercial production areas could have contributed to the occurrence and spread of bacterial diseases.

From information gained from the survey it can be concluded that measures used to control bacterial diseases in South Africa are insufficient. The widespread occurrence of bacterial diseases in South Africa suggest that the use of disease-free seed alone does not guarantee freedom of bacterial diseases. The occurrence of bacterial

diseases, in particular halo blight, has however, decreased significantly since implementation of the disease-free seed scheme. Improvement of local cultivar resistance is important for long term control of bacterial diseases. An integrated disease management system which includes resistant cultivars, disease free seed (produced in more suitable areas), agricultural practices and preventative spraying with copper-based bactericides should reduce occurrence of bacterial pathogens.

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Table 1. Occurrence of bacterial diseases in seed production areas of South Africa during 1996

Locality		Fields surveyed (Ha)	Dry bean cultivars	Bacteria identified in seed samples	R	C	DF
Northern Cape:							
	Hopetown	50 (172)	Helderberg, Kranskop, Donkerberg	Pss, Xapf, Xap	12	20	0
	Prieska	161 (565)	Teebus, Kranskop, Leeukop, Katberg	Pss, Xapf, Xap, Psp	7	135	6
	Kimberley	15 (59)	Katberg, Teebus, Kranskop	Pss, Xapf	0	3	0
	Douglas	96 (399)	Teebus, Kranskop, Katberg, Donkerberg	Pss, Xapf, Xap	2	60	2
	Petrusville	6 (26)	Kranskop	Pss, Xapf	0	6	0
	Perdeberg	9 (32)	Kranskop, Leeukop, Donkerberg	Pss, Xapf	0	9	0
	Modderrivier	25 (92)	Kranskop, Leeukop	Pss, Xapf	0	15	0
	Landanna	7 (20)	Kranskop	Pss	7	0	0
	Hadeson park	26 (93)	Teebus, Katberg	Pss, Xapf	3	7	7
	Koffiefontein	2 (9)	Kranskop	Pss, Xapf	0	1	0
	Jacobsdal	24 (136)	Kranskop, Leeukop	Pss, Xapf	0	10	0
Northern Province:							
	Tzaneen	16 (75)	Helderberg	Pss, Xapf, Xap	0	9	7
	Hoedspruit	12 (136)	Kranskop	Pss, Xapf, Xap	1	9	2
	Dendron	27 (198)	Kranskop, Leeukop, Donkerberg, Kalberg	Pss	5	10	1
	Pietersburg	14 (120)	Teebus, Kranskop	Pss, Xapf	4	6	0
	Vivo	23 (107)	Leeukop, Kranskop	Pss, Xapf, Xap	0	8	0
	Vaalwater	6 (50)	Leeukop	Pss, Xapf, Xap	0	2	0
	Messina	18 (108)	Helderberg, Kranskop	Pss, Psp	0	12	5
Mpumalanga:							
	Lydenburg	35 (337.5)	Kranskop, Bonus, Stormberg	Pss, Xapf, Xap	5	26	4
	Origstad	16 (162.5)	Kranskop, Bonus, Stormberg	Pss, Xap	0	1	0
	Badplaas	8 (181.9)	Kranskop, Stormberg	Pss, Xapf, Xap	0	3	0
	Nelspruit	8 (45.5)	Kranskop	Pss, Xapf, Xap	4	3	0
	Barberton	1 (14.1)	Kranskop	Pss	1	0	0
	Witrivier	10 (55)	Kranskop	Pss, Xapf, Xap	0	10	0
	Burgersfort	4 (37.2)	Stormberg	Xapf, Xap	0	4	0
Free State:							
	Petrusburg	1 (26)	Donkerberg	Pss, Xapf	0	1	0
	Luckhoff	2 (6)	Kranskop	Pss, Xapf, Xap	0	1	0
Northwest:							
	Vryburg	36 (135)	Kranskop	Pss, Xapf, Xap	0	32	2
	Tosca	23 (85)	Kranskop	Pss, Xapf, Xap, Psp	0	18	0
TOTAL:		**681 (3482.7)**			**51**	**421**	**36**

R = number of fields rejected, C = number of fields certified (< 8.0% disease), DF = number fields classified as disease free (0% diseases); Pss = *P. syringae* pv. *syringae*, Psp = *P. savastanoi* pv. *phaseolicola*, Xap = *X. axonopodis* pv. *phaseoli*, Xapf = *X. axonopodis* pv. *phaseoli* var. *fuscans*

Table 2. Incidence and severity of bacterial diseases in the main commercial dry bean production areas of South Africa during 1996/97 and 1997/98

Locality	Fields surveyed (No of diseased fields)	Cultivars	Bacteria isolated from diseased leaves	Common Bacterial Blight			Halo Blight			Bacterial Brown Spot		
				I (%)	*S* (1-4)	D_i (%)	*I* (%)	*S* (1-4)	D_i (%)	*I* (%)	*S* (1-4)	D_i (%)
Mpumalanga:												
Delmas	7 (4)	Teebus, Kranskop, Pan 148	Pss, Xapf, Xap	24.0	1.18	7.1	0	-	-	27.5	1.04	7.2
Ermelo	9 (9)	Teebus, Leeukop, Mkuzi, *P. coccineus*	Pss, Xapf, Xap, Psp	38.2	1.06	10.1	15.8	1.28	10.8	17.7	1.00	4.4
Ogies	2 (2)	Cerrillos, Pan 148	Pss, Xapf, Xap	21.0	1.00	5.3	0	-	-	95.0	1.27	30.5
Gauteng:												
Nigel	2 (2)	Kranskop, Cerrillos, Sabie	Pss, Xapf, Xap	85.0	1.16	24.5	0	-	-	3.0	1.00	0.75
Carletonville	1 (1)	-	Pss, Xapf, Xap	0	-	-	0	-	-	100.0	1.70	43.0
Free State:												
Bethlehem	11 (8)	Donkerberg, Teebus, Sabie, Bonus, Stormberg, Kranskop	Pss, Xapf, Xap, Psp	8.6	1.02	2.3	13.9	1.08	4.2	32.6	1.03	8.5
Clarens	5 (5)	Leeukop, Stormberg, Pan 148	Pss, Xapf, Xap, Psp	25.8	1.00	6.5	16.2	1.00	4.1	58.8	1.32	23.4
Harrismith	3 (3)	Kranskop	Pss, Xapf, Xap, Psp	26.3	1.05	7.0	12.3	1.00	3.1	99.3	1.57	39.1
Reitz	2 (2)	Kranskop	Pss, Xapf, Psp	6.5	1.00	1.63	2.0	1.00	0.5	90.5	1.29	28.7
Fouriesburg	1 (1)	Stormberg	Pss, Xapf, Xap, Psp	1.0	1.00	0.25	44.0	1.18	13.0	4.0	1.00	1.0
Petrus Steyn	1 (1)	Kranskop	Pss	0	-	-	0	-	-	90.0	1.00	22.5
Warden	1 (1)	-	Pss, Xapf	11.0	1.00	2.8	0	-	-	94.0	1.00	23.5
Clocolan	4 (4)	Kranskop, Pan 148	Pss, Xapf, Xap, Psp	0	-	-	60.8	1.45	24.6	25.0	2.03	0.5
North West:												
Syferbult	7 (6)	Pan 148, Kranskop, Teebus	Pss, Xapf, Xap	16.9	1.46	8.0	0	-	-	46.4	1.25	16.8
Koster	3 (2)	-	Pss, Xapf, Psp	3.0	1.25	0.9	3.3	1.00	0.8	33.3	2.40	20.0
Derby	1 (1)	-	Pss, Xapf, Xap	17.0	1.00	4.25	0	-	-	17.0	1.00	4.3
Grootpan	3 (3)	Kranskop	Pss, Xapf, Xap, Psp	0	-	-	13.7	1.5	5.1	96.3	1.10	26.6
Coligny	3 (3)	Helderberg	Pss, Xapf, Xap	17.3	1.00	4.3	0	-	-	30.0	1.01	7.6
KwaZulu/Natal:												
Vryheid	5 (4)	Helderberg, Wartburg, Enseleni, Kranskop, Pan 148	Pss, Xapf	0	-	-	0	-	-	50.0	1.00	12.5
Greytown	3 (3)	Mkuzi	Pss, Xapf, Xap	28.6	1.00	7.8	0	-	-	48.7	1.01	12.3
Middelrus	3 (3)	Kranskop	Pss, Xapf, Psp	2.7	1.00	0.7	3.3	1.20	1.0	80.0	1.09	22.2
Newcastle	1 (1)	Sabie	Pss, Xapf	14.0	1.00	3.5	0	-	-	28.0	1.00	7.0
Winterton	1 (1)	Kranskop	Pss, Xapf, Xap	10.0	1.00	2.5	0	-	-	100.0	1.26	31.5
Bergville	2 (2)	Limpopo, *P. coccineus*	Pss, Xapf, Xap, Psp	17.0	1.00	4.3	10.0	1.00	2.5	33.0	1.00	8.3
TOTAL:	**81 (72)**			**19.6**	**1.01**	**5.46**	**17.8**	**1.15**	**6.3**	**54.2**	**1.22**	**16.8**

I = Incidence of plants with foliar symptoms; *S* = Disease severity; D_i = Disease intensity index

Table 3. Weather data from dry bean localities surveyed

Locality	Altitude	[a]Rainfall (mm)	[b]Min T	[c]Max T
Northern Cape:				
Prieska †	-	1.4	4.9	18.5
Kimberley †	1140	1.2	1.3	20.0
Douglas	1082	-	-	-
Petrusville	1143	14.7	6.6	21.5
Perdeberg	1173	16.4	-	-
Koffiefontein	-	19.2	-	-
Jacobsdal	-	29.9	-	-
Northern Province:				
Tzaneen	884	67.7	12.7	22.1
Hoedspruit	550	18.0	12.9	25.4
Dendron	1067	23.6	-	-
Pietersburg	1250	22.6	6.1	21.5
Vivo	1067	23.6	-	-
Vaalwater	1215	15.9	7.6	23.1
Messina	522	-	-	-
Mpumalanga:				
Lydenburg	1644	32.5	7.5	20.9
Orighstad	1525	15.7	5.9	20.3
Badplaas	1100	43.2	9.3	22.3
Nelspruit	660	52.7	10.6	24.0
Barberton	1300	73.9	9.6	19.8
Witrivier	676	44.8	13.3	24.7
Burgersfort	915	-	-	-
Delmas	1623	135.8	13.7	25.5
Ermelo	1765	52.7	13.3	24.3
Ogies	1550	69.3	-	-
Free State:				
Petrusburg	1219	19.8	4.3	21.3
Bethlehem	1631	11.8	13.2	24.9
Clarens	-	-	-	-
Harrismith	1718	97.6	13.6	25.2
Reitz	1615	116.9	13.7	25.9

† Data available for August only; [a] mean rainfall from March to August in seed production fields, February to March in commercial fields;
[b] mean minimum temperatures from March to August in seed production fields, February to March in commercial fields;
[c] mean maximum temperatures from March to August in seed production fields, February to March in commercial fields

CHAPTER 3

CHARACTERIZATION OF HALO BLIGHT RACES ON DRY BEANS IN SOUTH AFRICA

ABSTRACT

Isolates of the halo blight pathogen *Pseudomonas savastanoi* pv. *phaseolicola* were collected in the bean producing areas in South Africa from 1991 to 1996. Of the 1128 isolates, 967 were identified as *P. savastanoi* pv. *phaseolicola*. The majority of these isolates were obtained from a wide range of *Phaseolus vulgaris* cultivars and the rest from *P. coccineus* and *P. lunatus*. Two hundred and fifty five isolates, representative of all the localities and cultivars sampled, were categorized into different races according to their reaction on a set of differential cultivars. Seven races (1, 2, 4, 6, 7, 8 and 9) were identified with race 8 the most prevalent. Races 1, 2, 6 and 8 were widely distributed through the whole production area, while races 4, 7 and 9 were restricted to one or two localities.

Fourie, D. (1998) Characterization of halo blight races on dry beans in South Africa. *Plant Disease* **82**: 307-310.

INTRODUCTION

Halo blight, caused by *Pseudomonas savastanoi* pv. *phaseolicola* (Burkh.) Gardan *et al.*, is an important seed-borne disease of dry beans (*Phaseolus vulgaris* L.) (Buruchara 1983, Beebe & Pastor-Corrales 1991). The disease is a major constraint of dry bean production in South Africa, especially in the moderate to cooler areas of the country. The extent of yield losses has not yet been estimated, but the disease occurs on all commercial cultivars and in many parts of the dry bean production areas.

Several races of *P. savastanoi* pv. *phaseolicola* have been reported worldwide. Races 1 and 2 have originally been described in the United States by Patel & Walker (1965) on their reaction to the cultivar Red Mexican U13. These races have since been reported from several other countries (Wharton 1967, Taylor 1970, Hale & Taylor 1973, Buruchara 1983). A third race from Africa was identified on the basis of its reaction to cv. Tendergreen (Mabagala & Saettler 1992). Recently, Taylor *et al.* (1996) extended the range of differentials to eight cultivars and lines and accordingly identified nine races of *P. savastanoi* pv. *phaseolicola*. Races 1 and 2 have previously been reported in South Africa (Boelema 1984, Edington 1990) on the basis of their reaction to Red Mexican U13. The aim of this study was to identify local races by using the extended range of cultivars, and to determine their geographic distribution.

MATERIALS AND METHODS

Sampling and isolation of bacteria

Leaves and pods of dry beans with halo blight symptoms were collected from the major bean producing areas in South Africa from 1991 to 1996. Samples were taken from various cultivars of *P. vulgaris*, *P. coccineus* L. (large white kidney beans) and *P. lunatus L.* (lima beans) and were collected from 255 disease occurrences. Prior to isolation, pods and leaves were rinsed under running tap water for 10 min and then surface sterilized by soaking material for 3 min in 3.5% sodium hypochlorite and rinsing it twice in sterile water for 1 min each. Bacteria were isolated using the method of Hildebrand *et al.* (1988) and streaked onto King's B medium (King *et al.* 1954). After incubation for 48 hr, fluorescent colonies were selected under UV-light and purified on King's B medium by a series of single colony transfers. Non-fluorescent colonies reminiscent of *Pseudomonas* in culture were additionally selected for further identification. All isolates are maintained at -72°C (Sleesman & Leben 1978) at the ARC-Grain Crops Institute, South Africa.

Identification of isolates

Carbon source utilization of mannitol, sorbitol and inositol, oxidase test and levan production (Hildebrand *et al.* 1988) as well as symptomology on leaves of cv. Canadian Wonder (universal susceptible cultivar) were used to confirm the identity of isolates. Antiserum specific to *P. savastanoi* pv. *phaseolicola* was provided by Dr. Nigel Lyons, of the Horticultural Research International (HRI), Wellesborne, England, during the latter part of the study and was additionally used to confirm the identity of isolates using the method of Taylor (1970).

Race identification

Two hundred and fifty five isolates representative of all the localities and cultivars sampled, were randomly selected for identification of races (Table 1). Seven reference cultures (1302A, 1299A, 2709A, 882, 1281A, 1449B, 2656A) of *P. savastanoi* pv. *phaseolicola* (Teverson 1991), obtained from Dr. Nigel Lyons were included for comparison to the races in South Africa. The isolates were kept on King's B agar slants at 4°C for the duration of the study.

Races were identified according to their reaction on a set of differential dry bean cultivars and lines (Teverson 1991, Taylor *et al.* 1996). Seeds of the differential set were planted in 8-cm-diameter plastic pots in sterile soil and maintained in a greenhouse at a 27°C /19°C day/night cycle of 12 hr each. Seeds from cv. 1072 were treated with 98% sulphuric acid for 30 min and kept overnight in moist paper rolls to germinate. For each isolate, three seeds were planted per pot and three pots used per differential. Pots were randomized prior to inoculation.

Inoculum was prepared by suspending a 24- to 48-hr-old culture in sterile distilled water and adjusting it turbimetrically to contain approximately 10^8 CFU/ml. Seven- to 10-day-old seedlings with fully expanded primary leaves were used for inoculation. Plants were inoculated with a DeVilbiss atomiser by spraying the bacterial suspension in two small areas (0.5 mm diameter) either side of the midrib onto the abaxial surface of the leaves, thereby forcing the bacteria into the leaf tissue (Zaiter & Coyne 1984, Teverson 1991, Taylor *et al.* 1996). The whole leaf area was then sprayed with the bacterial suspension until completely wet. Control plants were inoculated with sterile distilled water. Inoculated plants were kept in a humidity chamber (19°C±1°C,

RH=100%) for 48 hr before being transferred to a greenhouse equipped with a humidifier (18°C night/25°C day, RH=70%). Plants were rated for infection 10 days after inoculation on a 1 to 5 scale (Teverson 1991, Taylor *et al.* 1996) with 1 being highly resistant and 5 being highly susceptible.

Leaf vs pod inoculation

Twenty seven of the isolates selected for race identification were inoculated onto pods to compare whether pods and leaves react similarly to a specific race. Bacterial suspensions (10^8 CFU/ml) were inoculated onto young attached pods of the differential set with a hyperdemic needle using the modified method of Cheng *et al.* (1989). Sterile distilled water was used for control inoculations. Inoculated plants were maintained in a glasshouse (19°C night/27°C day±12 hr day length). Pods were rated for lesion development 10 days after inoculation.

RESULTS

Identification of isolates

A total of 1128 isolates were examined during the study. Nine hundred and sixty seven of the isolates were identified as *P. savastanoi* pv. *phaseolicola*. These isolates tested positive for levan, negative for the oxidase test and did not utilize mannitol, sorbitol and inositol as sole carbon sources. Water-soaked lesions developed when they were inoculated onto cv. Canadian Wonder. Systemic chlorosis as a result of toxin

translocation was also noted. Agglutination was observed with isolates tested with the antiserum specific to *P. savastanoi* pv. *phaseolicola*. A small percentage (2%) of the identified isolates were unable to produce fluorescent pigment on King's B medium and two isolates produced a brown diffusible pigment as described by Mabagala & Saettler (1992) and Taylor *et al.* (1996).

Race identification

Seven races of *P. savastanoi* pv. *phaseolicola* were identified in South Africa (Fig. 1). Of these, race 8 was the most prevalent (46.3%), while races 1, 2 and 6 constituted 27%, 6.3% and 18.6% of the isolates, respectively. Only four isolates of races 7 and 9 and one isolate of race 4 were found. Races 3 and 5 have not been identified from South African isolates of *P. savastanoi* pv. *phaseolicola*. The two isolates which produced a brown diffusible pigment on King's B belonged to race 1 and 6.

Race 8 was widely distributed and occurred in most of the localities sampled (Table 1). Race 1 was initially (during survey of 1991 to 1993) found only in the Mpumalanga Highveld where large white kidney beans are cultivated, but has since spread to new areas. Race 7 was confined to KwaZulu-Natal where it had been isolated from two localities (Cedara and Greytown) and race 9 was only found in KwaZulu-Natal and Mpumalanga. Although the occurrence of races 2 and 6 was low, these races were widespread throughout the production areas (Table 1).

The races identified were isolated from a wide variety of *P. vulgaris* and some *P. coccineus* and *P. lunatus* cultivars (Table 2). All seven races were identified from large seeded *P. vulgaris* cultivars. Five of the seven races were found on Wartburg, four

races on Bonus, SSB 10, 30 and 40 and green beans, while Stormberg, Umlazi and breeding trials hosted three races each. The rest of the large seeded cultivars were associated with one race only. Four races were identified from small seeded *P. vulgaris* cultivars, three from *P. coccineus* and one race from *P. lunatus* (Table 2). All three races of *P.coccineus* (races 1, 6 and 8) were found on cv. SSN1, while race 6 was isolated from SSN1, Bomba and Egyptian Great. Only one isolate, belonging to race 2, was found on *P. lunatus*.

Isolates from race 8 were identified from the majority of cultivars, while isolates belonging to races 1, 2 and 6 were also identified on a wide range of cultivars (Table 2). Race 4 was only found on imported kidney beans, race 7 on cv. Drakensberg and race 9 on cv. Umtata. Eighteen isolates consisting of races 1, 2, 6, 8 and 9 were collected from cultivars of which the names are unknown.

Leaf vs pod inoculation

Seventeen of the 27 isolates inoculated onto pods of the differential set gave similar race identifications as when inoculated onto primary leaves. These included isolates that belong to races 1, 2, 6, 7 and 8. Eight isolates were identified as race 8 when inoculated onto leaves but appeared to be race 6 when inoculated onto pods based on their reaction to cultivar A43. These isolates produced only a trace of water-soaking at the inoculation point on leaves of cv. A43 and were rated 2 on the infection rating scale, but produced water-soaked lesions on pods. Two isolates identified as race 1 on leaves also showed to be race 6 when inoculated onto pods. Only a trace of water-soaking (infection rating=2) was also observed on inoculated leaves of cvs. Red

Mexican and Guatemala 196-B, but water-soaked lesions were clearly visible on pods.

DISCUSSION

Seven races of *P. savastanoi* pv. *phaseolicola* occurring on dry beans in South Africa were identified in this study. Previously only races 1 and 2 have been reported from the country (Edington 1990, Boelema 1994). The increased number of races occurring locally could be contributed either to the introduction of new races into South Africa, or the subdivision of the three previously described races into nine different races by using the extended range of differentials (Teverson 1991, Taylor *et al.* 1996). Also, the current study includes more isolates from more cultivars and a larger geographical area than those reported by Boelema (1984) and Edington (1990).

Race 8 dominated the South African population of *P. savastanoi* pv. *phaseolicola*. This is consistent with the results of Taylor *et al.* (1996) who found race 8 mainly in Lesotho and Southern Africa. It, therefore, appears that this race might have originated from this region. Another possible reason for the extensive occurrence of race 8 in South Africa is that the majority of cultivars planted locally are susceptible to it. Races 1, 2 and 6 also appear to be well established in South Africa, as each of them occurs on a variety of cultivars and in a number of localities. Three races (races 4, 7 and 9) were restricted to one or two localities, and it is likely that they have only recently been introduced. This hypothesis is supported by the fact that the only isolate belonging to race 4 was found on imported seed in a greenhouse trial, and does not occur in dry bean fields in South Africa. *P. savastanoi* pv. *phaseolicola* can be introduced into new areas on infected seed, and breeding programmes or planting of foreign seed can easily

result in the introduction and spread of new races in the country (Mabagala & Saettler 1992).

Various dry bean cultivars planted in South Africa were infected with halo blight in the field. This is of particular concern since the disease can be damaging. One means of controlling halo blight is by the introduction of a Disease Free Seed Scheme in South Africa. The exclusion from the Scheme of large white kidney beans, which is highly susceptible to halo blight, have probably resulted in the spread of race 1 in the country. Race 1 was initially found on large white kidney beans only, but has since been isolated from a number of *P. vulgaris* cultivars (D. Fourie, unpublished data). The planting of a large number of foreign cultivars during the past five years in the South African production areas could also have contributed to the introduction of new races.

Breeding for resistance provides the most effective means of control of halo blight (Beebe & Pastor-Corrales 1991, Mabagala & Saettler 1992). This study showed that a large number of cultivars planted locally are susceptible to *P. savastanoi* pv. *phaseolicola*. It is important that local cultivars and germplasm should be screened for resistance, and identified resistance be introduced into local cultivars. Seven races are present in South Africa and the possibility that new races could be introduced into the country exists. In order to breed for resistance, race non-specific resistance should be incorporated into local cultivars. Edmund and Wisc. HBR 72 are known to have race non-specific resistance and can be considered.

The inconsistent reactions of some isolates of *P. savastanoi* pv. *phaseolicola*, belonging to race 1 and 8, on leaves and pods of differentials A43, Red Mexican U13 and Guatemala 196-B could indicate that different genes are controlling pod and leaf resistance. Similar reactions have been reported by Hale & Taylor (1973). This

phenomena should be further investigated and molecular techniques could assist in confirming the race identification in isolates where indiscrepencies occur.

P. savastanoi pv. *phaseolicola* isolates which produce a brown diffusible pigment have been reported by several authors (Teverson 1991, Mabagala & Saettler 1992, Taylor *et al.* 1996). A similar pigment is often produced by isolates of *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* (Saettler 1991). Two pigment producing bacteria, representing race 1 and race 6, were isolated from material collected from Leslie, situated in the cooler production areas of South Africa. The isolates reported by Mabagala & Saettler (1992) were identified as race 2. It seems as if the pigment production is not limited to a specific race, but its function is still unknown.

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Table 1. Origin of *Pseudomonas savastanoi* pv. *phaseolicola* isolates selected for race identification

Host	Locality		Number of isolates	Races detected
P. vulgaris	Gauteng:	Arnot	14	1,6,8
P. vulgaris		Bapsfontein	1	8
P. vulgaris, P. coccineus		Delmas	41	2,6,8
P. vulgaris, P. coccineus		Nigel	8	1,6,8
P. vulgaris, P. coccineus		Ogies	7	1,8
P. vulgaris		Pretoria	6	2,4,8
P. vulgaris	Free State:	Bethlehem	4	1
P. vulgaris		Bloemfontein	1	1
P. vulgaris		Bothaville	4	6
P. vulgaris		Bervie	1	1
P. vulgaris		Fouriesburg	1	8
P. vulgaris		Reitz	1	8
P. vulgaris		Warden	1	8
P. vulgaris		Kransfontein	4	1,6
P. vulgaris	Mpumalanga:	Burgershall	2	2,8
P. vulgaris, P. coccineus		Ermelo	39	1,2,6,8
P. vulgaris		Groblersdal	1	6
P. vulgaris, P. coccineus		Hendriena	5	1
P. vulgaris		Kendal	1	8
P. vulgaris, P. coccineus		Kriel	12	1,6,8,9
P. vulgaris, P. coccineus		Leslie	4	1,6
P. vulgaris, P. coccineus		Lothair	3	1
P. vulgaris		Lydenburg	1	6
P. vulgaris		Middelburg	5	1,2,8
P. vulgaris		Van Wyksdrif	1	8
P. vulgaris, P. coccineus		Vlakfontein	2	1,6
P. vulgaris		Komatipoort	9	1,6,8
P. vulgaris	Northwest:	Carletonville	5	1,2,8
P. vulgaris, P. coccineus, P. lunatus		Lichtenburg	7	6
P. vulgaris		Potchefstroom	10	1,8
P. vulgaris	KwaZulu\Natal:	Cedara	4	1,7
P. vulgaris		Greytown	12	6,7,8,9
P. vulgaris		Makatini	9	1
P. vulgaris		Normandien	2	6,8
P. vulgaris		Niekershoop	1	8
P. vulgaris	Cape Province:	Douglas	2	6
P. vulgaris		George	4	1,8
P. vulgaris		Klein Karoo	3	1
P. vulgaris		Kokstad	5	8
P. vulgaris		Kimberley	1	2

Table 2. Host range of *Pseudomonas savastanoi* pv. *phaseolicola* races in South Africa

Species	Cultivar	Races 1	2	3	4	5	6	7	8	9	Total
P. vulgaris:											
Large seeded:											
	Allubia Cerrillos								1		1
	And 888								1		1
	Atoki		1						1		2
	Bonus	23	1				5		7		36
	Breeding trials	1					9		16		26
	Broad acres						1				1
	Drakensberg						1	4			5
	Green beans	3	2				1		16		22
	Jenny						2				2
	Kid 27, 28, 35								3		3
	Kidney beans				1						1
	Leeukop								3		3
	Limpopo	1									1
	Montcalm								1		1
	Natal speckled sugar	2									2
	NCM 3031		1								1
	Pan 127								1		1
	Redlands Pioneer								1		1
	Sabie		1								1
	SSB 10, 30, 40	5	2				1		7		15
	Stormberg	4					1		3		8
	Stragonta						1				1
	SUG65,68,70,72								6		6
	Taylor								1		1
	Umlazi	2					2		1		5
	Umtata								1		1
	Wartburg	2	1				2		5	1	11
Small seeded:											
	Arctic		1				1		1		3
	Aurora						1		1		2
	Breeding trials		1						2		3
	CNC						1				1
	Coffee bean								1		1
	CSW 643								1		1
	Heuningberg		1								1
	Kamberg	1	1				3		3		8
	Kosi								2		2
	Mexico 235,309								2		2
	Mkuzi	1					1				2
	Nandi		1								1
	NEP 2								1		1
	Nuweveld								1		1
	Pan 122, 125								3		3
	Teebus						1		4		5
	Yellow haricot								1		1
P.coccineus:	Bomba						1				1
	Egyptian Great						1				1
	SSN1	20					4		5		29
P.lunatus			1								1
	CIAT Trials								4		4
	Mixed beans	1							3		4
	Unknown	3	1				3		8	3	18
TOTAL		69	16		1		43	4	118	4	255

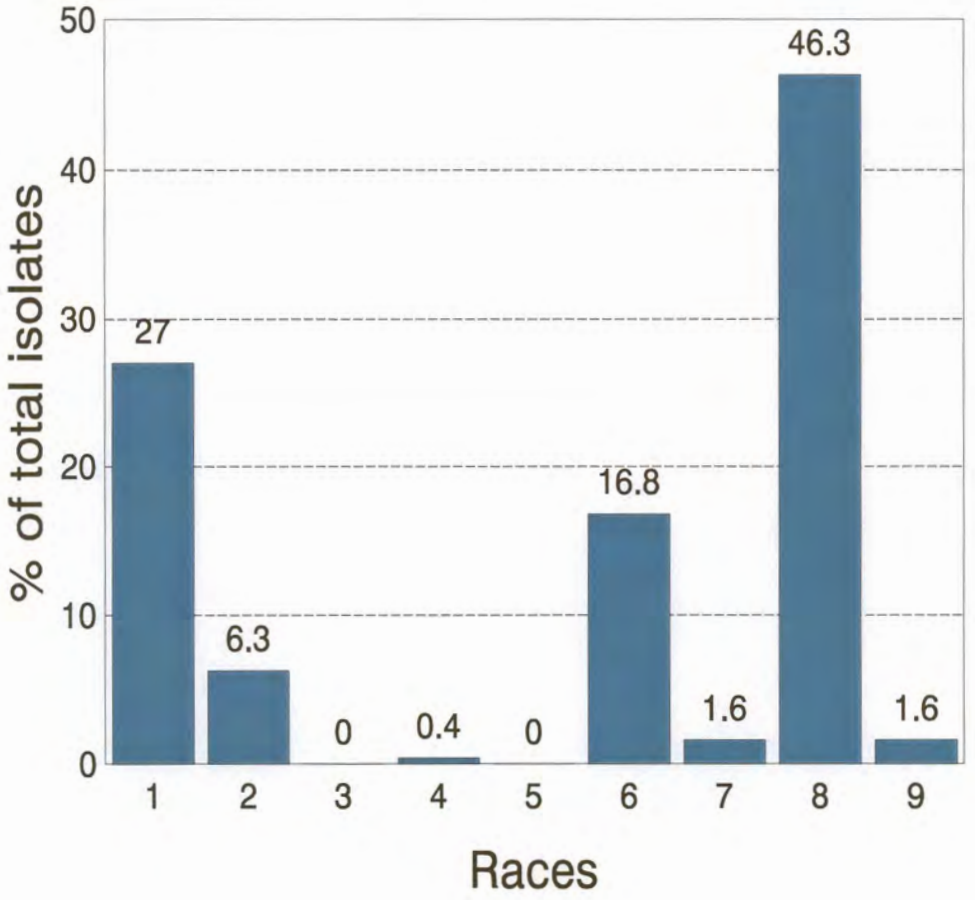

Figure 1. Races of *Pseudomonas savastanoi* pv. *phaseolicola* occurring on dry beans in South Africa

CHAPTER 4

PATHOGENIC AND GENETIC VARIATION IN *XANTHOMONAS AXONOPODIS* PV. *PHASEOLI* AND *X. AXONOPODIS* PV. *PHASEOLI* VAR. *FUSCANS* IN SOUTHERN AFRICA

ABSTRACT

One hundred and forty three common bacterial blight isolates from 44 localities in four countries, were inoculated onto eight *Phaseolus acutifolius* lines that differentiate between pathogenic races. This differential set was expanded to include resistant genotypes, XAN 159, GN #1 Nebr. sel 27, Wilk 2, Wilk 6, Vax 4, Vax 5 and Vax 6 and cv. Teebus as susceptible check. Genetic variation within nine selected Xap and Xapf isolates and a non-pathogenic *Xanthomonas* isolate, was studied using RAPD and AFLP analysis. Genotypes XAN 159, Wilk 2, Wilk 6, Vax 4, Vax 5 and Vax 6 were resistant to all isolates, while GN #1 Nebr. sel 27 and cv. Teebus were susceptible. Isolates varied in aggressiveness on cv. Teebus, however, pathogenic reaction on the set of differentials, indicated that all, but one isolate, grouped in what has been reported as race 2. Thus, results based on reaction of the majority isolates, suggest the absence of different races. However, the distinct differential reaction recorded for a single isolate, may prove to represent another, as yet unrecorded, race of this pathogen. Both RAPD and AFLP analyses revealed high frequency of DNA polymorphism among isolates and could distinguish between Xap, Xapf and a non-pathogenic isolate. Differences between Xap and Xapf isolates demonstrate that these are two distinct groups of bacteria. Information gained from

this study has enabled us to select the most appropriate isolates to use in a resistance breeding programme.

INTRODUCTION

Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) (Smith) Vauterin, Hoste, Kosters & Swings and its fuscans variant, *X. axonopodis* pv. *phaseoli* var. *fuscans* (Xapf), is a devastating seed-borne disease of dry beans (*Phaseolus vulgaris*) in many parts of the world (CIAT 1985). The disease is widespread throughout the South African production areas (Fourie 2002) and is favoured by high temperatures and high relative humidity (Sutton & Wallen 1970). In eastern and southern Africa common blight has been reported in 19 of the 20 bean producing countries (Allen 1995) and is considered one of five most important and widespread biotic constraints to dry bean production in sub-Saharan Africa (Gridley 1994). Genetic resistance is considered the most effective and economical strategy for the control of bean common blight (Rands & Brotherton 1925). However, deployment of resistance without knowledge of variation within a pathogen population could result in costly failure (Taylor *et al.* 1996).

Pathogenic variation in Xap and Xapf isolates has been demonstrated in several reports (Small & Worley 1956, Corey & Starr 1957, Schuster & Coyne 1971, Schuster *et al.* 1973, Yoshii *et al.* 1978, Schuster 1983, Jindal & Patel 1984). Ekpo & Saettler (1976) indicated that Xapf isolates were more pathogenic than Xap. These differences in pathogenicity have been confirmed by other investigators (Leakey 1973, Bozzano-Saguier & Rudolph 1994, Opio *et al.* 1996), but it has been suggested that the brown pigment is not associated with pathogenicity (Gilbertson *et*

al. 1991, Tarigan & Rudolph 1996) and should be considered of lesser pathological importance (Schuster & Coyne 1975).

Gilbertson *et al.* (1991) studied genetic diversity in isolates of Xap and Xapf, using DNA probes isolated from a single Xap isolate genome on isolates from different geographical locations. These studies indicated that there are two distinct groups of bacteria. However, similarities between isolates were revealed when probes were hybridised to DNA from other *X. campestris* pathovars, indicating sufficient similarity to consider Xapf a variety of Xap (Gilbertson *et al.* 1991).

Reports of physiological specialization in *P. vulgaris* have been contradictory. Zapata (1996) indicated *P. vulgaris* genotypes that are useful in differentiation of Xap. However, evidence exists suggesting quantitative interactions between Xap and *P. vulgaris* (Opio *et al.* 1996). Host specialization of Xap reactions on tepary *(P. acutifolius)* lines has been reported (Zapata & Vidaver 1987, Zaiter *et al.* 1989, Opio *et al.* 1996) with eight physiological races identified, suggesting a gene-for-gene relationship (Opio *et al.* 1996). Despite this gene-for-gene interaction, resistance to Xap and Xapf in *P. vulgaris*, derived from *P. acutifolius*, has remained non-specific and durable (Opio *et al.* 1996).

Tepary bean is an excellent source of resistance due to high resistance levels to Xap and Xapf. Variation that may exist in the local pathogen population is important when selecting parents with resistance originating from tepary cultivars. The aim of the study was to determine pathogenic and genetic variation in Xap and Xapf isolates in southern Africa ensuring that appropriate resistance sources are deployed when developing CBB resistant cultivars.

MATERIAL AND METHODS

Isolation and identification of isolates

Diseased plant material was collected from major bean production areas in South Africa and Malawi, Lesotho and Zimbabwe. Leaves were rinsed under running tap water for 10 min, surface-disinfested for 3 min in 3,5% sodium hypochlorite and then rinsed twice in sterile water for 1 min each. Leaf material was macerated in a droplet of sterile water and streaked onto yeast-extract-dextrose-calcium-carbonate (YDC) agar (Schaad & Stall 1988). Plates were incubated at 25°C. Following 72 hr incubation, yellow-pigmented colonies typical of *Xanthomonas* spp. were purified on YDC agar by a series of single colony transfers. Production of brown diffusible pigment on YDC differentiated Xapf from Xap isolates (Basu & Wallen 1967). Agglutination of antiserum specific to Xap and Xapf, obtained from Adgen Agrifood Diagnostics, Auchincruive, Scotland, was used to identify isolates. Pathogenicity tests on susceptible cultivar Teebus were done to confirm identity of isolates.

Pathogenicity tests

Seed from eight tepary lines previously reported to differentiate between Xap and Xapf races (Table 1) (Opio *et al.*, 1996), were obtained from Dr. DP Coyne, University of Nebraska, Lincoln, USA and multiplied from a single seed in a greenhouse to ensure genetically uniform material. The tepary differential set was expanded to include resistant genotypes, XAN 159, GN #1 Nebr. sel 27, Wilk 2, Wilk 6, Vax 4, Vax 5 and Vax 6. Resistance in these lines are all tepary derived. Cultivar Teebus was included as susceptible check. Five seeds of each genotype were planted in 15-cm-diameter plastic pots in sterile soil and maintained in a greenhouse

at 18°C night/28°C day. Seedlings were thinned to four plants per pot after emergence. One pot per differential was used per isolate, each plant representing a replicate. Pots were randomised prior to inoculation. Experiments were repeated to confirm reactions of isolates.

One hundred and forty three isolates from 44 localities in four countries of southern Africa were selected for the study (Table 2). Four isolates received from the International Centre for Agriculture in the Tropics (CIAT) were included as reference cultures. Isolates used for each experiment were regenerated from storage at -72 °C, because loss of pathogenicity was encountered by sub-culturing. Inoculum was prepared by suspending 48- to 72-h-old cultures in sterile distilled water and adjusting it turbidimetrically to contain approximately 10^8 CFU/ml. Fourteen to 20-day-old plants with fully expanded first trifoliate leaves were used for inoculation. Plants were inoculated using the multiple-needle inoculation method (Andrus 1948). Control plants were inoculated with sterile distilled water. Inoculated plants were kept in a greenhouse at 18°C night/28°C day. Plants were rated for infection 14 days after inoculation on a 1 to 9 scale (Aggour *et al.* 1989). Plants, rated 1 to 3, were classified as resistant (incompatible) and ratings of 4 to 9 considered susceptible (compatible).

Isolation of bacterial DNA

Eight isolates (two Xap and six Xapf) from southern Africa, one Xapf isolate from CIAT and a non-pathogenic *Xanthomonas* isolate (Table 3) were used in genetic studies. These isolates were selected based on their geographic origin. Isolates were cultured in 50 ml nutrient broth for 24-48 hr at 25°C prior to DNA isolation.

Bacterial cells were collected by centrifugation at 5 000 rpm for 10 min. Cells were washed three times by resuspending in 5 ml 1 M NaCl and centrifugation at 5 000 rpm for 10 min, followed by two wash steps in 5 ml sterile distilled water. Washed cells were resuspended in 10 ml warm (55°C) extraction buffer containing 0.2 M Tris.HCl (tris (hydroxymethyl) aminomethane), pH 8.0; 10 mM EDTA (ethylenediaminetetraacetate), pH 8.0; 0.5 M NaCl; 1% (w/v) SDS (sodiumdodecylsulfate) and 10 $\mu g.ml^{-1}$ Proteinase K. Resuspended cells were incubated in a water bath at 55°C for one hr and half a volume 7.5 M ammonium acetate was added. The suspension was mixed by gentle inversion and incubated at room temperature for 10 min. Phase separation was enhanced by adding 100 µl TE buffer (10 mM Tris.HCl, pH 8.0; 1 mM EDTA, pH 8.0). Phases were separated by centrifugation at 14 000 rpm for 15 min. The upper aqueous layer was transferred to a fresh tube containing an equal volume of isopropanol, mixed by gentle inversion and incubated at room temperature for a minimum of 2 hr to overnight. DNA was collected by centrifugation at 14 000 rpm for 15 min. The precipitated DNA was washed twice in 1 ml ice-cold 70% (v/v) ethanol, the pellet air-dried at room temperature, and resuspended in 10 µl TE buffer. The DNA was treated with RNase for two hours at 37°C and concentration and purity estimated by measuring absorbances at A_{260} and A_{280}. DNA samples were diluted to a working solution of 200 ng/µl.

RAPD analysis

Arbitrary 10 bp oligonucleotide primers (Operon Technologies, Table 4) were used for the polymerase chain reaction (PCR) based on the protocol of Williams *et al.*

(1990), with minor modifications. Amplification reactions were performed in a 25 µl reaction volume containing Promega (Promega Corporation, Madison, Wisconsin) reaction buffer (500 mM KCl; 100 mM Tris.HCl, pH 9.0 at 25°C; 1% (v/v) Triton X-100), 2 mM $MgCl_2$, 100 µM of each dNTP (dATP, dCTP, dGTP and dTTP), 5 pmol primer, 0.5 units *Taq* DNA polymerase (Promega) and 25 ng template DNA. Reactions were performed using a Hybaid Thermal Cycler (Hybaid Limited, UK) programmed for 5 min at 95°C, 55 cycles of 1 min at 95°C, 1.5 min at 35°C, and 2.5 min at 72°C, followed by one cycle of 5 min at 72.5°C and 5 min at 28°C. The amplification products were analysed by electrophoresis on 1.5% (w/v) agarose gels (Seakem LE) at 80V for 2 hr using UNTAN buffer (0.4 M Trisbase; 0.02 M EDTA, pH 7.4) and detected by staining with 1 $\mu g.ml^{-1}$ ethidium bromide. Gels were photographed under UV light with polaroid 667 film. All reactions were repeated and only reproducible bands were considered in this study.

AFLP analysis

AFLP adapters and primers were designed based on the method of Vos *et al.* (1995). Adapter and primer sequences are given in Table 5. Primers were synthesised by GibcoBRL (Life Technologies, Glasgow, United Kingdom) and oligonucleotides used for the adapters were PAGE (polyacrylamide gel electrophoresis) purified. Adapters were prepared by adding equimolar amounts of both strands, heating for 10 min to 65°C in a water bath and leaving it to cool down to room temperature.

AFLP analysis was performed following the protocol described by Vos *et al.* (1995) and the product manual supplied by Life Technologies Inc. (Glasgow, UK), with minor modifications. Restriction enzymes *Eco*RI and *Mse*I were used to digest

500 ng of isolate genomic DNA for 4 hr and the reaction mixture, without inactivation of the restriction endonucleases, was subjected to the overnight ligation of adapters at 37°C, followed by pre-amplification. The ligation mixture was not diluted prior to pre-amplification and the pre-amplification DNA was diluted only 1:5 prior to selective amplification. The selective amplification was conducted using two primers, and the *Mse*I primers always had three selective nucleotides while the *Eco*RI primers had two, three or four selective nucleotides (Table 5).

Gel electrophoresis

Gel electrophoresis for AFLP analysis was performed using the protocol of Vos *et al.* (1995) but employing a 5% (w/v) denaturing polyacrylamide gel (19:1 acrylamide: bis-acrylamide; 7 M urea; 1x TBE buffer (89 mM Tris-borate; 2.5 mM EDTA)). Electrophoresis was performed at constant power, 80 W for approximately 2 hr.

Silver staining for DNA visualisation

Polyacrylamide gels were silver-stained following the protocol described by the Silver Sequence™ DNA Sequencing System manual supplied by Promega (Madison, WI, USA). The gels were left upright overnight to air dry and photographed by exposing photographic paper (Kodak Polymax II RC) directly under the gel to about 20 sec of dim light. This produced a negative image, exactly the same size of the gel.

Statistical analyses

Data obtained from RAPD and AFLP analysis on ten isolates were used for statistical analysis. DNA bands obtained for each isolate were scored based on their presence (1) or absence (0). Only reliable and repeatable bands were considered. Pair wise genetic distances were calculated between isolates Nei and Li (1979). Cluster analysis was done by the unweighed paired group method using arithmetic averages (UPGMA). All calculations were done with the aid of the programme NTSYSpc version 2.02i.

RESULTS

Identification of isolates

All isolates collected (except Z93) were identified as Xap and Xapf on the basis of their agglutination of specific antiserum and pathogenicity on cv. Teebus (Table 2). Isolate, Z93 did not induce any disease on cv. Teebus and exhibited a weak reaction when tested with the antiserum. The majority of isolates (72%) produced a brown diffusible pigment on YDC agar and were classified as Xapf. Differences in aggressiveness between isolates on the cv. Teebus were detected with mean ratings ranging from moderately to highly susceptible (5-9). The most aggressive isolates included both Xap and Xapf.

Pathogenicity tests

All isolates inoculated onto the tepary differential set induced reaction on genotype Nebr. #21. The majority of isolates (99,3%) exhibited an incompatible reaction

(rating 1-3) on the remaining genotypes, resembling the infection pattern of race 2 (Opio *et al.* 1996) (Table 1). One isolate (X539) induced disease (mean ratings 4-9) on all tepary genotypes and did not resemble any infection pattern previously reported (Table 1). A small percentage of isolates induced a slight reaction on genotypes Nebr. #1 (6.3%; rating=1-2.25), Nebr. #5 (1.4%; rating=1-2.3), Nebr. #8b (9.1%; rating=1-2.0), Nebr. #19 (1.4%; rating=1-1.5), PI 321638 (23.1%; rating=1-2.8) and L242-45 (4.2%; rating=1-1.5). These reactions were not repeatable in further experiments and reactions were, therefore, considered incompatible with mean ratings not exceeding 3. No symptoms developed on Nebr. #22 except when inoculated with isolate X539. Teebus was susceptible to all the isolates tested except for one non-pathogenic isolate (Z93) that did not induce disease on any of the inoculated lines.

Genotypes XAN 159, Wilk 2, Wilk 6, Vax 4, Vax 5 and Vax 6 were generally resistant to all isolates (mean rating=1-3). Six isolates (X563, X573, X121, X295, X561 and X594) induced disease on XAN 159 with a mean rating of 4. GN #1 Nebr. sel 27 were susceptible to all isolates (mean rating=7).

RAPD analysis

RAPD analysis produced between two and ten fragments (Fig. 1), but results were not repeatable. Best results were obtained with primer OPA-02. RAPD analysis revealed a high frequency of DNA polymorphism among isolates and were able to distinguish between Xap, Xapf and the non-pathogenic isolate.

AFLP analysis

The AFLP fingerprinting techniques revealed complex banding patterns that were difficult to interpret (Fig. 2). DNA fingerprinting techniques revealed a high frequency of DNA polymorphism among isolates with a low presence of shared fragments between isolates (Fig. 2). A total of 756 fragments were amplified using 16 primer pair combinations. Only 2.64% of these fragments were shared between all ten isolates. Primer combinations varied in their ability to detect polymorphisms, ranging from 16 to 86 polymorphisms per primer pair, with an average of 47.3 fragments per primer combination. Fragment sizes varied between 100 and 900 base pairs. Selectivity of AFLP analysis, using two restriction enzymes, was enhanced, by using primers containing two, three or four selective nucleotides. This enhancement of primer selectivity did not reduce the complexity of resulting AFLP banding patterns. Best results were obtained when primers containing three selective nucleotides were used in the AFLP analysis.

As with RAPD analysis, the AFLP technique also separated Xap, Xapf and the non-pathogenic isolate into different groups. Fingerprinting techniques, thus, clearly differentiated amongst Xap as well as Xapf isolates. Combined data produced by RAPD and AFLP techniques are shown in Fig. 3. The phenogram drawn using pooled data from the RAPD and AFLP analysis (Fig. 3), showed a maximum similarity between any two isolates of 81% (Xapf isolates Les19 and Xapf180). The minimum similarity between any two isolates was 67.5% (Xap isolates X448 and X590). The Xapf cluster of isolates was linked to the Xap cluster of isolates at a similarity of 45.6% and the non-pathogenic isolate Z93 was linked to the Xapf/Xap cluster with a similarity of 30.6%. Isolates within the Xapf cluster exhibited a similarity of 71%. The obtained cophenetic correlation value of r=0.994 indicated that the

UPGMA cluster analysis was statistically significant.

DISCUSSION

Results of this study, based on pathogenicity and molecular characterizations, showed that diversity exists within Xap(f) populations, in southern Africa. Isolates differed in production of brown pigment as well as aggressiveness on the cv. Teebus. Although it has previously been reported that pigment producing Xapf isolates are more aggressive (Leakey 1973, Ekpo & Saettler 1976, Bozzano-Saguier & Rudolph 1994, Opio *et al.* 1996), the most aggressive isolates in this study included both Xap and Xapf. Isolates with lower levels of aggressiveness, however, belonged to Xap (rating on cv Teebus=5). Gilbertson *et al.* (1991) and Tarigan & Rudolph (1996) reported that pigment is not associated with pathogenicity and should be considered of little pathological importance (Schuster & Coyne 1975). Although no differences in disease reaction were observed, RAPD and AFLP analyses demonstrated that Xap and Xapf represent two distinct groups of bacteria. Although widely distributed in Africa (Opio et al. 1996, Fourie 2002), Xapf isolates do not occur in Costa Rica, Caribbean countries and Spain (CIAT 1992, C. Assensio, MBG-CSIC; personal communication).

All isolates (except X539 and Z93) inoculated on the tepary differential set had an identical infection pattern, similar to race 2 following the classification of Opio *et al.* (1996). Although a number of isolates induced only a mild reaction on some of the tepary lines, these reactions were not always repeatable, which is similar to results obtained by Zaiter *et al.* (1989). The non-pathogenic isolate (Z93) did not induce disease on any of the lines tested.

Except for isolate X539, which exhibited a significantly different infection pattern, no races other than race 2, previously described by Opio *et al.* (1996), could be distinguished. The distinct pattern of differential reaction recorded for this isolate, may represent another, as yet unrecorded, race of Xap. The possibility exists that isolates identical to X539 exist, but may not have been sampled in this study. Continuous monitoring of CBB isolates in future is necessary in order to detect presence of isolates exhibiting differential reactions. Although isolate X539 was pathogenic on the eight tepary lines tested, no disease developed on resistant genotypes used to supplement the differential set, except for GN #1 Nebr. sel. 27. Using these resistant genotypes in a resistance breeding programme would, therefore, not be influenced by the occurrence of this isolate.

Genotypes XAN 159, Wilk 2, Wilk 6, Vax 4, Vax 5 and Vax 6 that were used to supplement the tepary differential set, were generally resistant to all isolates tested. Resistance in all these lines is tepary-derived. XAN 159 was slightly susceptible to a small number of isolates. Resistance instabilities such as these have been reported previously in XAN 159 and its progeny (Beebe & Pastor-Corrales 1991), however, it is still widely used in resistance breeding programmes (Beebe & Pastor-Corrales 1991, Fourie & Herselman 2002, Park *et al.* 1998, Mutlu *et al.* 1999, Singh & Muñoz 1999).

The reportedly resistant line GN #1 Nebr. sel 27 was susceptible to all the isolates used in this study. This line was originally derived from inter-specific crosses between *P. vulgaris* and *P. acutifolius* and has been used in many breeding programmes as a source of resistance (Coyne & Schuster 1974, Mohan & Mohan 1983). Recent molecular studies have, however, indicated that resistance in GN #1 Nebr. sel 27 is derived from *P. vulgaris* and not *P. acutifolius*, as previously described

(Miklas *et al.* 2002). Although susceptible in South Africa, GN #1 Nebr. sel 27 and lines derived from it, have tested resistant in some parts of the USA (Coyne & Schuster 1974) and Spain (C. Assensio, MBG-CSIC: personal communication). Inconsistency in these results could have resulted from the limited distribution of Xapf in some areas of the USA and Spain (R. Gilbertson, University of California-Davis: personal communication).

Results of DNA fingerprinting techniques indicated that genetic diversity exists among isolates of the common blight pathogen. Differences between Xap and Xapf isolates show that these represent two distinct groups of bacteria. Similar distinction between these two groups was also reported by Gilbertson *et al.* (1991), using RFLP's. Non-pathogenic *Xanthomonas* commonly associated with beans could be distinguished from Xap and Xapf using both RAPD and AFLP techniques. These results are similar to those of Gilbertson *et al.* (1990) who distinguished between non-pathogenic and pathogenic isolates using RFLP's.

Although isolate X539 gave a significantly different infection pattern when inoculated onto the tepary lines, no significant difference between this isolate and the others Xapf isolates could be detected using different molecular techniques. It has been reported that strains of Xap and Xapf from similar geographic locations had similar, but not identical RFLP patterns (Gilbertson *et al.* 1991, CIAT 1992). This could not be confirmed in the present, study and is possibly due to the small number of isolates tested.

Results obtained in this study indicate that both pathogenic and genetic variation exist in the CBB pathogen population in southern Africa. However, identical reactions with the majority of isolates on the tepary lines, showed that different CBB races do not occur. Information gained from this study made it possible to select the

most appropriate isolates to use in a resistance breeding programme.

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Table 1. Interaction of *Xanthomonas axonopodis* pv. *phaseoli* and *P. acutifolius* (Opio *et al.* 1996)

Race	Nebr.#1	Nebr.#5	Nebr.#8b	Nebr.#19	Nebr.#21	Nebr.#22	PI321638	L242-45
1	-	-	-	-	-	-	+	-
2	-	-	-	-	+	-	-	-
3	+	-	-	-	-	-	-	-
4	-	+	-	+	+	-	+	-
5	-	-	-	-	+	-	+	-
6	-	+	-	-	+	+	+	-
7	-	-	-	+	+	-	-	-
8	-	-	-	-	-	-	-	-
X539	+	+	+	+	+	+	+	+

-, incompatible reaction (resistant); +, compatible reaction (susceptible)

Table 2. Origin and host range of *Xanthomonas axonopodis* pv. *phaseoli* and *X. axonopodis* pv. *phaseoli* var. *fuscans* isolates used for pathogenicity tests on the dry bean cv. Teebus

Isolate	Locality	Cultivar	Antiserum agglutination	Xap/Xapf	Reaction on Teebus
X6	Cedara	Unknown	+	Xap	9
X78	Kriel	Unknown	+	Xapf	9
X101	M.Hill	Unknown	+	Xapf	9
X102	M.Hill	Unknown	+	Xapf	7
X105	M.Hill	Unknown	+	Xapf	9
X110	M.Hill	Unknown	+	Xapf	9
X111	Unknown	Mixture	+	Xap	9
X117	Unknown	Mixture	+	Xap	9
X119	Unknown	Mixture	+	Xapf	7
X120	Unknown	Mixture	+	Xapf	9
X121	Unknown	Mixture	+	Xap	8
X122	Unknown	Mixture	+	Xap	9
X125	Unknown	Mixture	+	Xapf	9
X130	Ermelo	Kamberg	+	Xapf	8
X138	Kokstad	Helderberg	+	Xapf	9
X147	Carletonville	Redlands Pioneer	+	Xapf	7
X172	Potchefstroom	SSB 30	+	Xapf	9
X176	Potchefstroom	MCM 3031	+	Xap	7
X180	Carletonville	Nep 2	+	Xapf	9
X185	Carletonville	Nep 2	+	Xapf	9
X186	Carletonville	S 1051	+	Xapf	9
X188	Carletonville	S 1051	+	Xapf	9
X189	Carletonville	CNC	+	Xapf	9
X193	Delmas	Breeding material	+	Xap	9
X195	Cedara	Breeding material	+	Xapf	9
X200	Cedara	Breeding material	+	Xapf	9
X206	Delmas	Kamberg	+	Xapf	9
X208	Ogies	Wartburg	+	Xap	9
X214	Ermelo	Teebus	+	Xapf	9
X216	Ermelo	Teebus	+	Xapf	8
X229	Cedara	Breeding material	+	Xapf	9
X231	Bergville	Broad Acres	+	Xap	9
X253	Greytown	Drakensberg	+	Xapf	8
X261	Dundee	Jenny	+	Xapf	9
X269	Lichtenburg	Jenny	+	Xapf	9
X275	Greytown	Drakensberg	+	Xap	7
X277	Delmas	Jenny	+	Xapf	9
X279	Ukulinga	Drakensberg	+	Xapf	6
X280	Ukulinga	Drakensberg	+	Xapf	8
X285	Delmas	Helderberg	+	Xapf	7
X288	Kransfontein	Bonus	+	Xapf	9
X289	Kransfontein	Bonus	+	Xapf	9
X290	Kransfontein	Bonus	+	Xapf	9
X291	Kransfontein	Broad Acres	+	Xapf	9
X292	Kransfontein	Broad Acres	+	Xapf	9
X293	Kransfontein	Broad Acres	+	Xapf	9
X294	Kransfontein	Bonus	+	Xapf	8
X295	Bethlehem	Mixture	+	Xap	5

Isolate	Locality	Cultivar	Antiserum agglutination	Xap/Xapf	Reaction on Teebus
X318	Kransfontein	Bonus	+	Xapf	8
X322	Douglas	Kamberg	+	Xapf	9
X323	Douglas	Kamberg	+	Xapf	9
X324	Douglas	Kamberg	+	Xapf	8
X335	Derby	PAN 143	+	Xapf	8
X337	Dundee	Sabie	+	Xapf	8
X338	Carletonville	Drakensberg	+	Xap	9
X339	Carletonville	Drakensberg	+	Xap	9
X341	Carletonville	Drakensberg	+	Xap	9
X346	Rietgat	SSN 1	+	Xap	9
X350	Kroonstad	Bonus	+	Xapf	9
X359	Reitz	Limpopo	+	Xap	9
X409	Chrissiesmeer	Helderberg	+	Xapf	9
X410	Chrissiesmeer	Helderberg	+	Xapf	8
X414	Chrissiesmeer	Breeding material	+	Xapf	8
X421	Winterton	Kranskop	+	Xapf	8
X423	Winterton	Kranskop	+	Xapf	7
X424	Ermelo	Kranskop	+	Xapf	9
X426	Middelrus	Kranskop	+	Xapf	7
X428	Cyferbult	Kranskop	+	Xapf	8
X443	Carletonville	Unknown	+	Xap	8
X445	Carletonville	Breeding material	+	Xapf	8
X446	Carletonville	Breeding material	+	Xap	9
X447	Amersfoort	Kamberg	+	Xapf	9
X448	Wildebeestfontein	Helderberg	+	Xap	9
X451	Cyferbult	Helderberg	+	Xap	9
X457	Cedara	Breeding material	+	Xapf	8
X458	Cedara	Breeding material	+	Xapf	8
X459	Cedara	Breeding material	+	Xapf	9
X460	Cedara	Breeding material	+	Xapf	9
X462	Vivo	Kranskop	+	Xapf	9
X464	Vivo	Kranskop	+	Xapf	8
X470	Vivo	Kranskop	+	Xapf	8
X471	Vivo	Kranskop	+	Xapf	8
X472	Tom Burke	Kranskop	+	Xapf	8
X473	Pietersburg	Kranskop	+	Xapf	8
X474	Cedara	Breeding material	+	Xapf	7
X476	Lichtenburg	Kranskop	+	Xapf	8
X487	Vivo	Kranskop	+	Xapf	9
X492	Tom Burke	Kranskop	+	Xapf	9
X496	Pietersburg	Kranskop	+	Xap	7
X498	Vivo	Kranskop	+	Xapf	8
X505	Ellisras	Unknown	+	Xap	9
X510	Dendron	Teebus	+	Xapf	8
X513	Dendron	Kranskop	+	Xapf	9
X520	Grootpan	Unknown	+	Xap	5
X521	Koster	Unknown	+	Xapf	9
X522	Greytown	PAN 146	+	Xapf	9
X523	Cedara	Breeding material	+	Xapf	7
X524	Clarens	Unknown	+	Xapf	7
X526	Bethlehem	Leeukop	+	Xapf	8

Isolate	Locality	Cultivar	Antiserum agglutination	Xap/Xapf	Reaction on Teebus
X527	Bethlehem	Bonus	+	Xap	9
X528	Clarens	Unknown	+	Xapf	9
X530	Bethlehem	Bonus	+	Xapf	8
X532	Delmas	Teebus	+	Xap	9
X534	Koster	Unknown	+	Xapf	8
X539	Ermelo	Unknown	+	Xapf	9
X551	Delmas	Kranskop	+	Xapf	9
X552	Delmas	Kranskop	+	Xapf	8
X553	Delmas	Kranskop	+	Xapf	8
X555	Reitz	Kranskop	+	Xapf	8
X559	Bergville	Volunteer beans	+	Xapf	8
X561	Clocolan	PAN 148	+	Xap	7
X562	Clocolan	PAN 148	+	Xap	7
X563	Clocolan	Kranskop	+	Xap	7
X565	Clocolan	Sabie	+	Xapf	9
X569	Greytown	Mkuzi	+	Xapf	8
X573	Delmas	Kranskop	+	Xap	5
X576	Newcastle	Sabie	+	Xapf	8
X578	Clocolan	Sabie	+	Xapf	6
X579	Clocolan	Sabie	+	Xapf	9
X586	Fouriesburg	PAN 148	+	Xap	9
X594	Fouriesburg	Kranskop	+	Xap	7
X598	Fouriesburg	Stormberg	+	Xap	9
X602	Keiskammahoek	Kranskop	+	Xap	9
X604	Keiskammahoek	Kranskop	+	Xap	9
X610	Dohne	Helderberg	+	Xapf	9
X618	Potchefstroom	Unknown	+	Xapf	8
XCP123	CIAT	Unknown	+	Xap	9
XCPF174	CIAT	Unknown	+	Xapf	9
XCPF180	CIAT	Unknown	+	Xapf	9
XCP183	CIAT	Unknown	+	Xap	9
Z93	Zimbabwe	Unknown	(+)	Xanthomonas	1
Z328	Zimbabwe	Unknown	+	Xap	7
Z332	Zimbabwe	Unknown	+	Xap	8
LES2	Lesotho	Unknown	+	Xapf	9
LES6	Lesotho	Unknown	+	Xapf	8
LES11/00	Lesotho	Unknown	+	Xapf	8
LES13	Lesotho	Unknown	+	Xapf	7
LES16/00	Lesotho	Unknown	+	Xapf	7
LES19/00	Lesotho	Unknown	+	Xapf	9
LES54/00	Lesotho	Unknown	+	Xapf	8
MAL13	Malawi	Unknown	+	Xap	8
MAL15	Malawi	Unknown	+	Xapf	7
MAL38	Malawi	Unknown	+	Xap	7
MAL61	Malawi	Unknown	+	Xapf	8

Table 3. Bacterial isolates used for RAPD and AFLP to study genetic variation

Isolate no	Xap/Xapf	Locality
X448	Xap	Wildebeestfontein, SA
X590	Xap	Fouriesburg, SA
Z93	Xanthomonas	Zimbabwe
X279	Xapf	Ukulinga, SA
X462	Xapf	Vivo, SA
X521	Xapf	Koster, SA
X539	Xapf	Ermelo, SA
Les19	Xapf	Lesotho
Mal61	Xapf	Malawi
Xapf180	Xapf	CIAT, Colombia

Table 4. Primer sequences used for RAPD analysis in genetic variation studies of Xap and Xapf

Name	Sequence (5'-3')
OPA-02	TGCCGAGCTG
OPA-07	GAAACGGGTG
OPA-09	GGGTAACGCC
OPA-18	AGGTGACCGT
OPD-01	ACCGCGAAGG
OPD-02	GGACCCAACC
OPD-03	GTCGCCGTCA
OPD-04	TCTGGTGAGG
OPG-08	TCACGTCCAC
OPG-10	AGGGCCGTCT
OPS-01	CTACTGCGCT
OPS-02	CCTCTGACTG

Table 5. Primer sequences used for *Eco*RI/*Mse*I AFLP analysis in Xap and Xapf genetic studies

Name	Type	Sequence (5'-3')
E-A	*Eco*RI Primer+1	AGACTGGTACCAATTCA
E-AA	*Eco*RI Primer+2	GACTGCGTACCAATTCAA
E-AG	*Eco*RI Primer+2	GACTGCGTACCAATTCAG
E-AT	*Eco*RI Primer+2	GACTGCGTACCAATTCAT
E-AAC	*Eco*RI Primer+3	GACTGCGTACCAATTCAAC
E-ACC	*Eco*RI Primer+3	GACTGCGTACCAATTCACC
E-AACA	*Eco*RI Primer+4	GACTGCGTACCAATTCAACA
E-AACC	*Eco*RI Primer+4	GACTGCGTACCAATTCAACC
M-C	*Mse*I Primer+1	GACGATGAGTCCTGAGTAAC
M-CAA	*Mse*I Primer+3	GATGAGTCCTGAGTAACAA
M-CAC	*Mse*I Primer+3	GATGAGTCCTGAGTAACAC
M-CAG	*Mse*I Primer+3	GATGAGTCCTGAGTAACAG
M-CAT	*Mse*I Primer+3	GATGAGTCCTGAGTAACAT
M-CTA	*Mse*I Primer+3	GATGAGTCCTGAGTAACTA
M-CTC	*Mse*I Primer+3	GATGAGTCCTGAGTAACTC
M-CTG	*Mse*I Primer+3	GATGAGTCCTGAGTAACTG
M-CTT	*Mse*I Primer+3	GATGAGTCCTGAGTAACTT

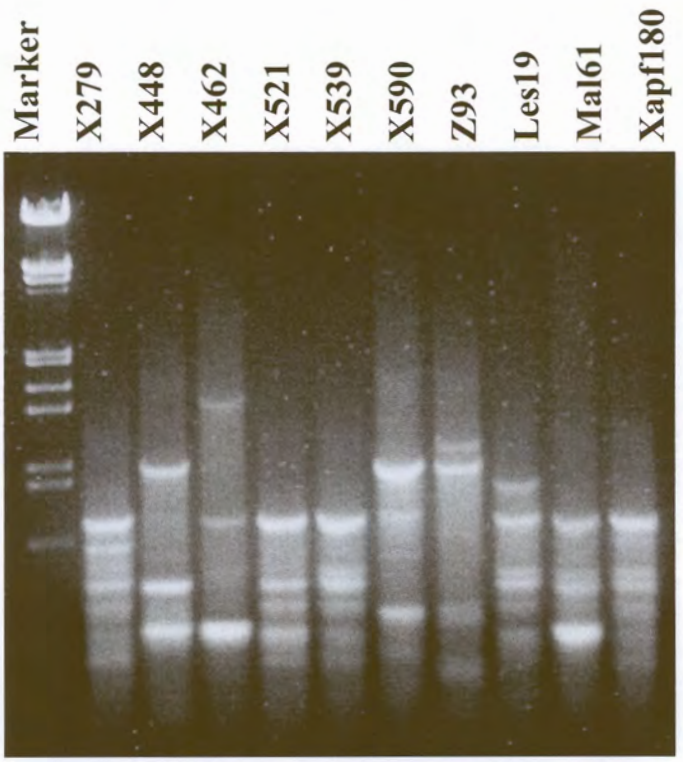

Figure 1. RAPD analysis of 2 Xap (X448 and X590), 7 Xapf (X279, X521, Les 19, Xapf 180, Mal 61, X539 and X462) and 1 non-pathogenic *Xanthomonas* (Z93) isolates

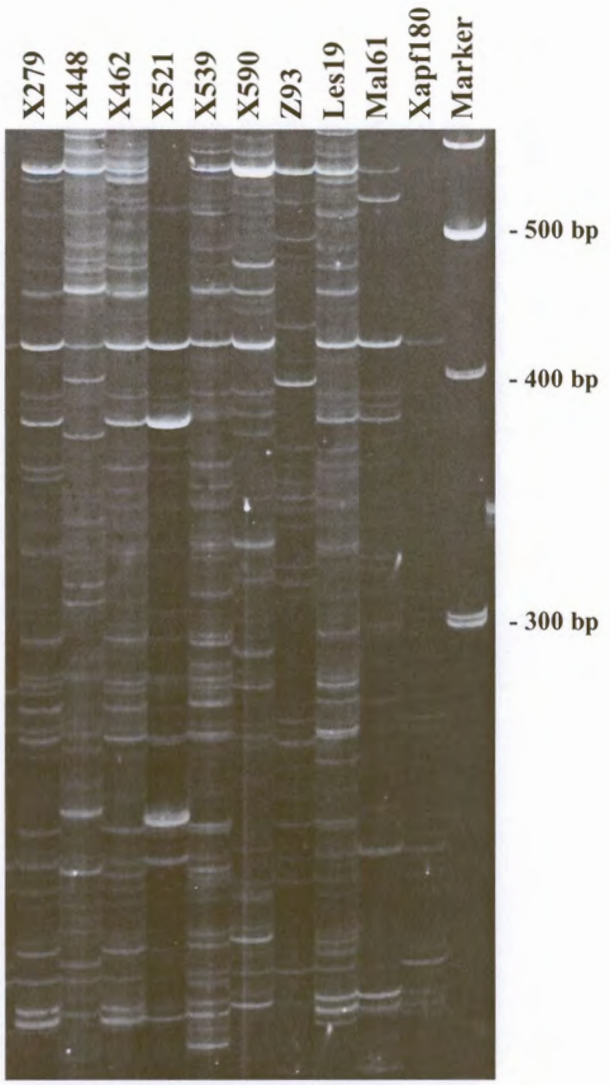

Figure 2. AFLP analysis of 2 Xap(X448 and X590), 7 Xapf (X279, X521, Les 19, Xapf 180, Mal 61, X539 and X462) and 1 non-pathogenic *Xanthomonas* (Z93) isolates

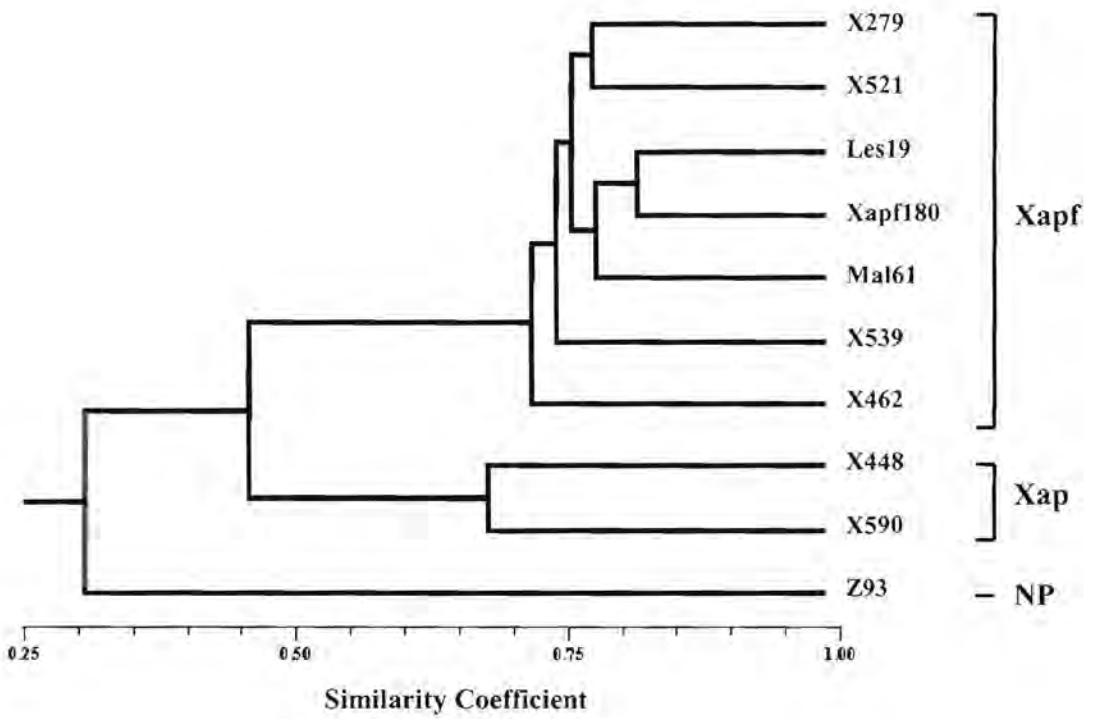

Figure 3. Genetic relationship of 2 Xap, 7 Xapf and 1 non-pathogenic *Xanthomonas* isolates based on combined RAPD and AFLP data

CHAPTER 5

SUSCEPTIBILITY OF SOUTH AFRICAN DRY BEAN CULTIVARS TO BACTERIAL DISEASES

ABSTRACT

Twenty-one locally grown commercial dry bean cultivars were evaluated at Potchefstroom during the 1998/1999 and 1999/2000 seasons for resistance to common bacterial blight, halo blight and bacterial brown spot. Results indicated that South African cultivars differed in their susceptibility to bacterial diseases. Cultivars Teebus, Cerillos, PAN 146 and PAN 159 were most susceptible to common bacterial blight with Monati and OPS-RS2 having low levels of resistance. Negative correlations between disease ratings and yields were obtained in the common bacterial blight trial. Levels of resistance to halo blight were observed with small seeded cultivars generally being more resistant than large seeded types. A negative correlation was obtained between halo blight rating and yield. Cultivars differed regarding susceptibility to bacterial brown spot with the majority having adequate resistance. Teebus, Cerillos, Bonus and PAN 159 were most susceptible, with Mkuzi exhibiting highest resistance. No correlation was obtained between disease rating and yield. Although a number of cultivars exhibited field resistance to halo blight and bacterial brown spot, all cultivars were more or less susceptible to common bacterial blight. Common bacterial blight can be considered the most important bean bacterial disease in South Africa. Improvement of common bacterial blight resistance in South African cultivars is necessary for yield stability.

INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) represent an important leguminous food crop grown in South Africa, with approximately 50 000 tons being produced annually by commercial and small scale farmers. Bacterial diseases, e.g. common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*, Xap) (Smith) Vauterin *et al.*, halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*, Psp) (Burkholder) Gardan *et al.* and bacterial brown spot (*Pseudomonas syringae* pv. *syringae*, Pss), van Hall, limit dry bean production in many international bean producing areas (CIAT 1985). Pathogens responsible are all seed-borne infecting beans at different stages of maturity. Their relative importance varies annually depending on biological and climatic factors and management practices.

Common bacterial blight (CBB) is widespread throughout the South African bean production areas (Fourie 2002). It can also be highly destructive during extended periods of warm, humid weather, resulting in yield and seed quality loss (Saettler 1991). Typical blight symptoms are visible during the crop's reproductive stage. Yield losses have been poorly documented, but vary from 22% to 45% (Wallen & Jackson 1975, Yoshii 1980).

Halo blight (HB) is restricted to cooler production areas at higher altitudes and typical symptoms are visible from seedling the stage to crop maturity. Serious yield losses have been observed, particularly where farmers grow their own seed for a number of seasons (D.Fourie: unpublished data). Yield losses of 43% have been obtained under experimental conditions (Allen *et al.* 1998). Pathogenic variation

within Psp isolates exist, with seven (races 1, 2, 4, 6, 7, 8 & 9) of the described nine races (Taylor *et al.* 1996) occurring in South Africa (Fourie 1998).

Bacterial brown spot (BBS), the most widespread bacterial disease in South Africa, occurs in all seed and commercial production areas (Fourie 2002). Sporadic losses occur in moderate to hot climatic areas, particularly where plants have been damaged by heavy rain or hail (Serfontein 1994). Yield reduction, as high as 55%, were reported (Serfontein 1994).

Bacterial bean pathogens are seed-borne and this is the primary inoculum source (Allen *et al.* 1998). Planting of pathogen-free seed is the most important primary control method (Gilbertson *et al.* 1990). Use of pathogen-free seed, however, does not guarantee disease control, as other inoculum sources exist (Allen *et al.* 1998). Additional cultural practices, such as removing, destroying or deep ploughing of debris, effective weed control, crop rotation and minimizing movement within fields when foliage is wet, may be also effective in controlling the disease (Allen *et al.* 1998, Schwartz & Otto 2000).

Copper based bactericides protect foliage against infestation and secondary pathogen spread (Oshima & Dickens 1971, Weller & Saettler 1976, Opio 1990, Schwartz *et al.* 1994). Efficacy of chemical control is limited (Allen *et al.* 1998) and resultant yield increases are minimal (Saettler 1989).

The most effective and economic bacterial control strategy in dry beans, is this use of cultivars with stable resistance (Rands & Brotherton 1925). The aim of the study was to determine susceptibility of local commercial cultivars to CBB, HB and BBS and thus to direct breeding strategies towards resistance against important bacterial diseases in South Africa.

MATERIAL AND METHODS

Twenty-one South African dry bean cultivars (Table 1) were evaluated for resistance to CBB, HB and BBS. Three field trials, one for each disease, were conducted at Potchefstroom during the 1998/1999 and 1999/2000 seasons. Cultivars were hand planted in 2 row plots, 5 m in length with 750 mm inter-row and 75 mm intra-row spacing. Trials were planted in a complete randomised block design with three replications, each surrounded by two border rows. Weed, insect and fungal control measures were applied, following standard agricultural practices.

Two Xap isolates (X6 and Xf105) were used, in a mixture to inoculate the common blight trial. A mixture of Psp isolates representing local races (races 1, 2, 6, 7, 8 & 9) was used to inoculate the halo blight trial. Race 4 isolates were not included as this race has only been identified locally from greenhouse grown seedlings. A highly aggressive Pss isolate (BV100) was used for the bacterial brown spot inoculum.

Inoculum was prepared from 48 h cultures grown on King's B medium (Psp and Pss) and yeast-extract-dextrose-calcium-carbonate agar (YDC) medium (Xap), respectively. Bacterial cells were suspended in tap water and adjusted to 10^8 CFU/ml water. Trials were irrigated prior to inoculation and repeated weekly to enhance disease development. Each trial was inoculated in the late afternoon using a motorized backpack sprayer at 21, 29 and 36 days after planting. First disease evaluations were done 10-14 days after the first inoculations on a 1-9 scale (Van Schoonhoven & Pastor-Corrales 1987) with 1 being resistant and 9 susceptible.

Evaluations were repeated at flowering and at full pod set. At maturity, two row plots of all cultivars were harvested manually and yield data recorded.

Data were analysed using a factorial analysis of variance (Statgraphics Plus 5.0) with disease ratings and yield as variables. Cofficients of linear correlation were used to determine the relationships between the measured variables.

RESULTS

Susceptibility of South African cultivars, to CBB, HB and BBS, are shown in Tables 2, 3 and 4, respectively. All cultivars screened were susceptible to CBB (Table 2). Cultivars, Teebus, Cerillos, PAN 146 and PAN 159 were susceptible differing from the other cultivars, with ratings of 7 and higher. Less disease developed on cultivars Monati and OPS-RS2 with mean ratings of 4.7 and 4.8, respectively. Small seeded cultivars were generally more susceptible to CBB than large seeded red speckled sugars. Lowest yields were recorded on Cerillos, and PAN 159, while OPS-RS3 was the highest yielding cultivar (Table 2).

Cultivars exhibited higher levels of resistance to HB than to CBB (Table 3). Teebus, PAN 150 and Mkuzi were the most resistant cultivars with PAN 182 most susceptible. Large seeded cultivars were more susceptible to HB than small seeded cultivars, with mean disease ratings averaging 4 and 5. Yields in the HB trial were generally higher than those in the CBB and BBS trials (Table 3). Lowest yielding cultivars were OPS-RS1 and PAN 159 while PAN 150 was the highest yielding cultivar. Yields of the HB trials differed significantly over the two seasons.

Cultivars differed in susceptibility to BBS (Table 4). Teebus, Cerillos, Bonus and PAN 159 were most susceptible, with Mkuzi exhibiting highest levels of resistance. The majority of cultivars had acceptable levels of resistance to BBS. Significant yield differences were obtained for cultivars in the BBS trial (Table 4). Kranskop was the lowest yielding cultivar with highest yields recorded for PAN 178. Significant differences were observed in disease rating and yield over both seasons.

DISCUSSION

Results indicated significant differences in susceptibility of South African cultivars to the economically important bacterial diseases. All cultivars were susceptible to CBB, with Teebus, Cerillos, PAN 146 and PAN 159 being most susceptible. Teebus is, currently, the only cultivar approved by the canning industry, with an acceptable canning quality. Improvement of resistance within this cultivar is extremely important.

Yields recorded for PAN 146 and PAN 159 were significantly lower than the majority of red speckled sugar cultivars. Yield reduction could be attributed to high susceptibility. Lowest yield was recorded in Cerillos, which was highly susceptible to CBB. High levels of susceptibility to CBB in Teebus, could have contributed to the reduction in yield. Negative correlations (P=-0.48) between disease ratings and yields indicate yield reduction due to CBB. No seasonal variation in disease rating and yields obtained was recorded indicating that CBB incidence and severity was not significantly influenced by the environmental conditions over the two seasons.

Acceptable levels of resistance to HB were identified in commercial cultivars. Large seeded cultivars were generally more susceptible than small seeded cultivars. Thus, attempts should be made to improve HB resistance in these cultivars.

Yields recorded in the HB trial were generally higher than those obtained in the CBB and BBS trials. A negative correlation (P=-0.56) existed between HB disease rating and yield. This disease could seriously affect yield under conducive conditions, particularly when plants are systemically infected (D. Fourie: unpublished data). Yields differed significantly over the two seasons, indicating that prevailing environmental conditions influenced yield.

Although cultivars differed significantly in their susceptibility to BBS, the majority of cultivars exhibited acceptable levels of resistance. Disease ratings and yield were, however, influenced by prevailing environmental conditions over the two seasons. Screening of cultivars for BBS resistance should, therefore, be conducted in multi-locational trials, over seasons. Although field resistance to BBS exists, this disease is the most widespread bean bacterial disease (Fourie 2002) and is a serious threat, particularly in the disease-free seed scheme. BBS is a relatively new disease in South Africa (Serfontein 1994) and studies on pathogenic variation and epidemiology of Pss need to be conducted. This could influence future screening for resistance. No significant correlation (P=-0.08) was, however, obtained between BBS rating and yield.

Although a number of cultivars exhibited field resistance to HB and BBS, all cultivars were moderately to highly susceptible to CBB. This disease is, therefore, considered the most important bean bacterial disease in South Africa. Improvement of CBB resistance in South African cultivars would largely contribute to obtain stable

yields. Improving CBB resistance in Teebus should be a priority because of its high commercial value.

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Table 1. Characteristics of 21 commercial South African dry bean cultivars screened for resistance to bacterial diseases

CV Name	Bean type	Growth habit*	Mean growing season (days)	Seed size (seeds 30g)
Teebus	Small white canning	I	92	127
Helderberg	Small white canning	II	99	180
OPS-KW1	Small white canning	II	96	156
PAN 182	Small white canning	II	90	183
PAN 185	Small white canning	II	96	183
Cerillos	Alubia	I	91	57
Kranskop	Red speckled sugar	II	97	63
OPS-RS1	Red speckled sugar	II	96	63
OPS-RS2	Red speckled sugar	I	100	61
OPS-RS3	Red speckled sugar	II	97	65
Jenny	Red speckled sugar	II	96	57
Bonus	Red speckled sugar	III	97	69
Monati	Red speckled sugar	II III	97	55
PAN 146	Red speckled sugar	I	86	70
PAN 148	Red speckled sugar	II	96	72
PAN 159	Red speckled sugar	I	85	74
PAN 178	Red speckled sugar	II	97	76
Stormberg	Red speckled sugar	III	97	70
Leeukop	Red speckled sugar	III	99	69
PAN 150	Carioca	II	95	123
Mkuzi	Carioca	II	96	143

* Type I: Determinate growth habit: flowers at end of branches stop stem growth
Type II: Intermediate growth habit: few short and upright branches, grow after flowering
Type III: Intermediate growth habit: long and low trailing branches

Table 2. Common bacterial blight reaction and yield of 21 South African dry bean cultivars in artificially inoculated field trials at Potchefstroom

Cultivar	Mean disease rating (1-9)		Yield kg.ha^{-1}	
Teebus	7.8	g	702	abcd
Helderberg	6.0	ef	645	abc
OPS-KW1	5.8	de	752	abcde
PAN 182	6.5	f	696	abcd
PAN 185	6.0	ef	983	defg
Cerillos	7.8	g	477	a
Kranskop	5.8	de	905	cdef
OPS-RS1	5.8	de	930	cdef
OPS-RS2	4.8	ab	1096	fg
OPS-RS3	5.3	bcd	1283	g
Jenny	5.2	abc	1009	defg
Bonus	5.7	cde	1077	efg
Monati	4.7	a	1000	defg
PAN 146	7.5	g	567	ab
PAN 148	5.2	abc	1001	defg
PAN 159	7.3	g	504	a
PAN 178	5.3	bcd	1053	efg
Stormberg	5.3	bcd	1080	fg
Leeukop	5.8	de	843	bcdef
PAN 150	5.8	de	1008	defg
Mkuzi	5.7	cde	1081	fg

Means followed by different letters differ significantly according to LSD (P=0.05)

Table 3. Halo blight reaction and yield of 21 South African dry bean cultivars in artificially inoculated field trials at Potchefstroom during the 1998/1999 and 1999/2000 seasons

Cultivar			Yield (kg.ha-1)			
	Mean disease rating (1-9)		1998/1999		1999/2000	
Teebus	3.0	a	2137	ef	3356	s
Helderberg	3.5	b	2703	mno	2729	no
OPS-KW1	3.2	ab	2137	ef	3103	r
PAN 182	5.3	f	1831	c	2031	de
PAN 185	4.0	c	2307	gh	3129	r
Cerillos	5.0	def	1933	cd	1956	cd
Kranskop	4.8	def	3031	qr	2636	lmn
OPS-RS1	5.0	def	1204	a	2836	op
OPS-RS2	4.7	def	2457	ik	2831	op
OPS-RS3	5.0	def	2275	fg	2347	ghi
Jenny	5.0	def	2556	klm	3103	r
Bonus	5.0	def	2723	no	2729	no
Monati	5.0	def	2627	lmn	2364	ghi
PAN 146	5.2	ef	1916	cd	1956	cd
PAN 148	5.0	def	1667	b	3636	t
PAN 159	4.8	def	1307	a	2249	fg
PAN 178	5.0	def	2516	kl	2943	pq
Stormberg	4.8	def	1884	cd	2617	lmn
Leeukop	5.0	def	1813	bc	2431	hik
PAN 150	3.0	a	4031	u	3049	qr
Mkuzi	3.0	a	3631	t	2756	no

Means followed by different letters differ significantly according to LSD (P=0.05)

Table 4. Bacterial brown spot reaction and yield of 21 South African dry bean cultivars in artificially inoculated field trials at Potchefstroom during the 1998/1999 and 1999/2000 seasons

Cultivar	Mean disease rating (1-9)				Yield (kg.ha^{-1})			
	1998/1999		1999/2000		1998/1999		1999/2000	
Teebus	6.7	l	6.0	j	840	fg	791	ef
Helderberg	3.0	c	3.0	c	929	h	1096	kl
OPS-KW1	3.0	c	3.0	c	577	ab	985	hi
PAN 182	2.7	b	3.0	c	947	hi	779	ef
PAN 185	2.7	b	3.0	c	1103	kl	767	ef
Cerillos	6.3	k	5.0	h	947	hi	1113	kl
Kranskop	4.3	g	3.0	c	543	a	1529	q
OPS-RS1	3.3	d	3.0	c	1231	mn	1291	no
OPS-RS2	2.3	a	3.0	c	631	bc	1369	op
OPS-RS3	4.0	f	3.0	c	680	cd	1359	op
Jenny	3.7	e	3.0	c	792	ef	1332	o
Bonus	6.0	j	5.0	h	920	gh	1104	kl
Monati	2.7	b	3.0	c	1076	jk	1333	o
PAN 146	4.0	f	3.0	c	1160	lm	611	ab
PAN 148	3.3	d	3.0	c	1217	mn	1724	r
PAN 159	6.0	j	5.3	i	991	hi	1168	lm
PAN 178	3.7	e	3.0	c	1587	q	2020	s
Stormberg	3.0	c	3.0	c	813	ef	1425	p
Leeukop	2.7	b	3.0	c	783	ef	1021	ij
PAN 150	2.7	b	3.0	c	1423	p	825	ef
Mkuzi	2.3	a	3.0	c	1209	m	745	de

Means followed by different letters differ significantly according to LSD (P=0.05)

CHAPTER 6

COMMON BACTERIAL BLIGHT: A DEVASTATING DISEASE OF DRY BEANS IN AFRICA

INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) are an important source of protein, B-complex vitamins and minerals (Paradez-López *et al.* 1986) and a staple food in the diet of many Latin American countries (De León *et al.* 1992). In central America, they provide between 20% and 30% of the dietary protein and are second only to maize as a staple food (Bressani *et al.* 1963). In Africa, beans are the second most important protein source after groundnuts (Technology Impact Report 1998) and production amounts to 2 049 000 t, of which 373 000 t is produced in Uganda, 332 000 t in Ethiopia, 309 000 t in Angola and 217 500 t in Tanzania. Mean annual production in South Africa over the last ten years is 58 000 t (Coetzee 2000).

Diseases are one of the most important factors reducing bean yields in most bean producing countries (Beebe & Pastor-Corrales 1991). Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Vauterin, Hoste, Kosters & Swings and its fuscans variant, *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* is a major disease limiting dry bean production in South Africa (Technology Impact Report 1998) and is considered one of the most important bean diseases worldwide (CIAT 1985). The disease is widespread throughout South African production areas (Fourie 2002) and is favoured by high temperatures and high relative

humidity (Sutton & Wallen 1970).

CBB was first reported in the USA by Beach in 1892. The same year Halsted described a bacterial disease, based on lesions on dry bean pods and seeds, and obtained similar lesions after inoculations (Zaumeyer & Thomas 1957). Smith (1897) first described the organism associated with this disease and named the bacterium *Bacillus phaseoli* E.F.Smith. After describing the cultural characteristics of the organism in 1901 he transferred it to the genus *Pseudomonas* (Zaumeyer & Thomas 1957). The name was again changed in 1905 to *Bacterium phaseoli* and later classified as *Phytomonas phaseoli* (E.F. Smith) by Bergey *et al.* (Zaumeyer & Thomas 1957). Dowson (1943) created the genus *Xanthomonas* and renamed the CBB bacterium, *Xanthomonas phaseoli*. The genus *Xanthomonas* was subdivided into five species and the causal organism renamed, *Xanthomonas campestris* pv. *phaseoli* (E.F Smith) Dye (Dye *et al.* 1980).

A similar bacterium to *Bacterium phaseoli* was isolated from bean plants, but differed in that it produced a brown diffusible pigment in culture media. The bacterium produced identical symptoms when inoculated onto bean plants and was named *Xanthomonas campestris* pv. *phaseoli* var. *fuscans* (Burkh.) Starr & Burkh. The disease was referred to as fuscous blight (Zaumeyer & Thomas 1957). Although this varietal form is often not recognized (Sutton & Wallen 1967, Leakey 1973), studies have revealed considerable genetic variation between these organisms (Birch *et al.* 1997, Toth *et al.* 1998) supporting proposals that they retain distinct taxonomic status (Chan & Goodwin 1999).

Based on DNA-DNA hybridization studies, Vauterin *et al.* (1995) suggested that the CBB organism and the fuscans variant should be reclassified as *Xanthomonas*

axonopodis pv. *phaseoli* and *X. axonopodis* pv. *phaseoli* var. *fuscans* respectively. Throughout this document, these will be referred to as Xap and Xapf. Schaad *et al.* (2000), however, rejected the transfer to *X. axonopodis* pv. *phaseoli* and recommended that it should be retained as a pathovar of *X. campestris*.

SYMPTOMOLOGY

CBB affects foliage, stems, pods and seeds of beans (Yoshii 1980). Leaf symptoms initially appear as water-soaked spots on the abaxial sides of leaves, which gradually enlarge, become flaccid and later turn brown and necrotic (Yoshii 1980, Saettler 1991). Lesions are often surrounded by a narrow zone of lemon-yellow tissue (Yoshii 1980, Saettler 1991). Lack of chlorotic zones on leaves of pompadour germplasm have, however, been reported (Beaver *et al.* 1992).

Bacteria enter leaves through natural openings such as stomata and hydathodes or through wounds (Yoshii 1980) from where they multiply and spread (Saettler 1991). Bacteria may also enter the stem and reach the vascular system of bean plants. The bacteria rapidly increase and fill xylem vessels that result in wilting of plants (Burkholder 1921). Burkholder (1921) also found bacteria in the root system of vascularly infected plants, however, no lesions have been observed below the soil surface. Systemically infected plants are in the minority (Burkholder 1921) and the pathogen does not systemically infect all *P. vulgaris* cultivars (Haas 1972).

Pod lesions are water-soaked spots which gradually enlarge, turn red-brown and are slightly sunken (Yoshii 1980, Saettler 1991). Lesions usually vary in size and shape, and are frequently covered with bacterial ooze (Saettler 1991). Infected seeds are

shrivelled and exhibit poor germination and vigour (Saettler 1991). Planting of infected seed may result in lesion development on seedling stems resulting in "snake head" symptoms, which occur (Burkholder 1921) when the plant growing tip is destroyed and only the cotyledons remain. Lesions on older stems are water-soaked spots that enlarge, discolour and may extend or girdle up the stem if infection occurs at a node. These lesions weaken stems which may break in windy conditions (Allen *et al.* 1998).

DISTRIBUTION AND ECONOMIC IMPORTANCE

CBB occurs in temperate, subtropical and tropical regions (Singh 1991) and causes severe damage under favourable environmental conditions. In Latin America it is particularly widespread in northwestern Argentina, south-central Brazil, Venezuela, central Cuba and coastal Mexico (Singh & Muñoz 1999). Although CBB was first considered a disease of minor importance in the United States of America, it was reported during 1919 to occur in all the important bean-producing states (Burkholder 1921).

In eastern and southern Africa CBB has been reported in 19 of the 20 bean producing countries (Allen 1995). It is thus considered one of five most important and widespread biotic constraints in dry bean production in sub-Saharan Africa (Gridley 1994). CBB was reported in South Africa prior to 1931 (Doidge & Bottomley 1931) while fuscous blight was first noted in 1962 (Boelema 1967). Both common and fuscous blight are widespread throughout the South African bean production area (Fourie 2002).

Other countries in which CBB occurs are Canada (Wallen *et al.* 1963, Wallen & Galway 1976, Huang *et al.* 1996), Australia (Wimalajeewa & Nancarrow 1978),

Germany (Tarigan & Rudolph 1996), France (J.J. Serfontein: personal communication), Hungary (Velich *et al.* 1991), Italy (Calzolari 1997), Bulgaria (Kiriakov *et al.* 1993), Dominican Republic (Mmbaga *et al.* 1992), India (Khandale & Kore 1979), Russia (Russkikh 1999) and New Zealand (Watson 1970). Distribution of the *X. axonopodis* pv. *phaseoli* var. *fuscans* (Xapf) seems to be more limited and does not occur in Costa Rica or Caribbean countries (CIAT 1992).

Although CBB is widely distributed, yield losses have not been well documented. Estimated losses of up to 38% have been reported in field trials in Ontario, Canada by Wallen & Jackson (1975). In Colombia, estimated yield losses of 22% and 45% have been documented after natural and artificial infections, respectively (Yoshii 1980). Moffet & Middleton (1979) obtained significant yield differences between inoculated and uninoculated plots of navy beans, despite the fact that CBB was observed in both plots. CBB in Uganda was associated with yield depression in beans and losses varied depending on susceptibility of varieties, developmental stage of crop at time of infection and climatic conditions during the season (Opio *et al.* 1992).

THE PATHOGEN

Cultural and morphological characteristics

Xap and Xapf can be easily isolated from CBB symptoms on leaves and pods using general isolation media (Schaad & Stall 1988). On media such as sucrose peptone agar (SPA), colonies are circular, smooth and mucoid with a yellow pigment referred to as xanthomonadin. Intensity of this yellow colour varies with medium used (Moffet &

Croft 1983). Corey & Starr (1957) described four colony types of Xap which had identical nutritional patterns and growth rates, but differed in amount of polysaccharide produced and ability to produce lesions. Differences in lesion development and morphology were correlated with polysaccharide production (Corey & Starr 1957).

Xanthomonas are non-sporing, gram-negative, aerobic rods, which are motile by means of a single polar flagellum (Moffet & Croft 1983). Characteristics are that they do not reduce nitrates, are catalase positive, asparagine is not used as a sole carbon and nitrogen source and they are weak producers of acids from carbohydrates (Schaad & Stall 1988). The organism also causes proteolysis of milk and starch hydrolysis (Saettler 1989) and does not induce a hypersensitive reaction on tobacco (Gilbertson et al. 1990).

Isolation media containing tyrosine differentiates between Xap and Xapf in that the latter produces a brown diffusible pigment (Basu & Wallen 1967). Goodwin & Sopher (1994) found this pigment to be produced due to secretion and subsequent oxidation of homogentisic acid rather than tyrosine activity.

Selective media are more effective for isolating specific bacteria from diseased material when selective at species level (Claflin, *et al.* 1987). A number of semi-selective media have been developed and improved to isolate Xap and Xapf (Kado & Heskett 1970, Schaad & White 1974, Trujillo & Saettler 1979, Claflin *et al.* 1987, Mabagala & Saettler 1992, Dhanvantari & Brown 1993, Jackson & Moser 1994, Gozczynska & Serfontein 1998).

Detection and identification

Apart from using selective media, techniques such as bacteriophage typing (Katznelson *et al.* 1954, Sutton & Wallen 1967), serology testing (Trujillo & Saettler 1979), host inoculation (Saettler 1971), ELISA (Wong 1991) and immunofluorescent staining (Malin *et al.* 1983), can be used to detect and identify Xap and Xapf. These techniques are time consuming and labourious. More sensitive, rapid and specific detection of the pathogen is often needed. This is particularly important when identification is complicated by epiphytic Xap strains (Gilbertson *et al.* 1989, Audey *et al.* 1994), that may confuse seed certification (Wong 1991, Audey *et al.* 1994).

Gilbertson *et al.* (1989) developed a plasmid DNA probe for rapid detection of pathogenic Xap strains which may be used in a breeding programme to select CBB resistant genotypes (Constabel *et al.* 1996). Based on this probe, another highly specific PCR probe, for Xap detection, was developed to detect as few as 10 colony forming units (CFU), using ethidium bromide-stained agarose gel (Audey *et al.* 1994). Audey *et al.* (1996) developed a rapid, sensitive PCR assay for detection of seedborne Xap in large bean seed samples containing as few as one infected in 10 000 healthy seeds. Birch *et al.* (1997) used RAPD-PCR to differentiate between Xap and Xapf. Toth *et al.* (1998) used primers which amplified a DNA fragment from all Xapf-isolates used, while no amplification products were obtained from Xap-isolates. These primers, therefore, provide a rapid, improved method to differentiate between these two variants.

Taxonomy and host range

The genus *Xanthomonas* consists of five species, each currently subdivided into a number of pathovars. These subdivisions remain controversial as pathovar demarcation

is often criticised as they are differentiated by inoculating host plants of that specific pathovar (Dye 1959, Lazo & Gabriel 1987), without determining the extent of host specificity (Starr 1983). Burkholder (1944) isolated *Xanthomonas* from diseased cowpeas, which were pathogenic to both beans and cowpeas. Infection was not obtained on cowpeas when inoculated with bean Xap isolates. It was suggested that the bacterium be named *X. vignicola* sp. nov. Vakili *et al.* (1975) confirmed these findings.

Schuster and Coyne (1977b) reported *X. vignicola* to be pathogenic on beans and cowpeas and that Xap, in some cases, showed a moderate degree of virulence when inoculated onto cowpeas, while *X. phaseoli* var. *sojense* was pathogenic on beans and cowpeas. Sabet (1959) found that Xap, *X. phaseoli* var. *sojense*, *X. alfalfa* and *X. vignicola* were all pathogenic on beans and suggested that all these be considered forms of Xap. Restriction fragment length polymorphisms (RFLP's) have been used to study the taxonomy of *X. campestris* (Gilbertson 1987, Lazo & Gabriel 1987, Lazo *et al.* 1987, Gabriel *et al.* 1989, Gilbertson *et al.* 1991) and results support pathovar classification.

The host range of Xap includes common bean (*Phaseolus vulgaris* L.), scarlet runner bean (*P. coccineus* L.), *P. lunatus*, urd bean (*Vigna mungo* (L.) Hepper), mung bean (*V. radiata* (L.) Wilczek var. *radiata*), tepary bean (*P. acutifolius* A. Gray var. *acutifolius*), *V. aconitifolia* (Jacq.) Maréchal, *V. angularis* (Willd.) Ohwi *et* Ohasi, *V. umbellata*, *Lablab purpureus* (L.) Sweet, *Strophostyles helvola* (L.) Elliot, soybean (*Glycine max* (L.) Merril), *Mucuna deeringiana* (Bort.) Merrill, *Lupinus polyphyllus* Lindl., cowpea (*V. unguiculata* (L.) Walp. ssp. *unguiculata*), *Macroptilum lathyroides*, *Pisum sativum*, *Strophostyles helvola* and *Mucuna deeringiana* (Saettler 1989, Allen et al.

1998).

Pathogenic and genetic diversity

Differences in virulence among pathogenic *Xanthomonas* bean strains have been confirmed in several reports (Yoshii *et al.* 1978, Schuster 1983). Small & Worley (1956) indicated that virulence differences of bacteria may be detected on culture media. Virulent Xap and *P. syringae* pv. *phaseoli* colonies were red in colour, while weakly virulent isolates were light in colour or remain white. Schuster & Coyne (1975), however, were unable to detect these visual differences. Colony types have also been used to differentiate degrees of virulence (Corey & Starr 1957, Jindal & Patel 1984).

Schuster & Coyne (1971) isolated Xap strains from Colombian seed more virulent than a Nebraskan isolate when inoculated onto three *Phaseolus* species. An equally virulent Xap strain was obtained from Uganda (Schuster *et al.* 1973). Ekpo & Saettler (1976) confirmed the observed variation in Xap and found that Xapf was more aggressive than Xap.

Several reports support the observed virulence differences between Xap and Xapf (Leakey 1973, Bozzano-Saguier & Rudolph 1994, Opio *et al.* 1996), and reports indicate that the Xapf pigment is not associated with pathogenicity (Gilbertson *et al.* 1991, Tarigan & Rudolph 1996) and considered of negligible pathological importance (Schuster & Coyne 1975). Pectolytic *Xanthomonas* associated with, but not pathogenic to beans can be distinguished from Xap and Xapf by RFLP's (Gilbertson *et al.* 1990).

Gilbertson *et al.* (1991) studied genetic diversity between Xap and Xapf, using DNA probes isolated from the genome of a single Xap strain. This was tested on a

diverse strain collection from various geographical locations. Genetic differences, based on RFLP patterns, indicated that two distinct bacterial groups exist. Similarities were revealed that were not observed when probes were hybridized to DNA from other *X. campestris* pathovars. This indicates sufficient similarities between Xap and Xapf, to consider Xapf a variety of Xap. Strains of Xap and Xapf from similar geographical locations had similar, but not identical RFLP patterns (Gilbertson *et al.* 1991). Similar results were obtained by CIAT (1992).

Although differences in isolate virulence are evident, physiological specialization on *P. vulgaris* is unknown. Zapata (1996) indicated that *P. vulgaris* genotypes exist which are useful in differentiation of Xap. Evidence suggests that interaction between Xap and *P. vulgaris* is quantitative (Opio *et al.* 1996). Host specialization of Xap based on reactions on *P. acutifolius* lines has been reported (Zapata & Vidaver 1987, Zaiter *et al.* 1989, Opio *et al.* 1996), with eight distinct physiological races identified, suggesting a gene-for-gene relationship. Different races could not be distinguished in studies conducted in South Africa (*vide* Chapter 4).

DISEASE DEVELOPMENT

CBB develops under warm, humid temperatures, causing greater damage to plants at 28°C than at lower temperatures (Saettler 1989). Bacteria enter leaves through stomata or wounds where they invade intercellular spaces causing gradual dissolution of the middle lamella (Zaumeyer & Thomas 1957). Bacteria enter stems through stomata of the hypocotyl and epicotyl, or vascular elements leading from leaves or infected cotyledons.

Plant wilting is caused by plugging of vessels or cell wall disintegration (Zaumeyer & Thomas 1957). Bacteria enter via pod sutures from the vascular system of the pedicle and pass into the funiculus through the raphe, into the seed coat where it remains until seed germination. Once the pathogen is in the seed area, the micropyle may also serve as a point of entry. Direct penetration through the seed coat has not been observed (Zaumeyer & Thomas 1957). Upon seed germination rifts are formed in the cotyledon epidermis and bacteria pass through these openings into intercellular spaces and may invade the entire cotyledon. Vascular bundles may also be invaded and hence plant wilting (Zaumeyer & Thomas 1957).

EPIDEMIOLOGY

Dissemination and survival

The most effective survival mechanism for Xap, is infected bean seed (Cafati & Saettler 1980b, Gilbertson *et al.* 1990, Arnaud-Santana *et al.* 1991, Opio *et al.*1993), within which bacteria may survive for up to thirty six years (Allen *et al.* 1998). Seed contamination may be internal or external (Saettler 1989, Allen *et al.* 1998) and even symptomless (Thomas & Graham 1952, Weller and Saettler 1980a), having serious implications for seed certification schemes.

Conflicting reports exist on the ability of Xap to survive in infested soil and plant debris (Schuster & Coyne 1976, Saettler *et al.* 1986, Gilbertson *et al.* 1990). Gilbertson *et al.* (1990) found Xap populations to overwinter in bean debris on no-tillage plots. Non-pathogenic pectolytic strains of *X. campestris* were also consistently isolated.

Experiments conducted in the Dominican Republic indicated that Xap survived up to 7 months on infected debris on the soil surface, but not in buried debris after 30 days (Arnaud-Santana *et al.* 1991). Xap survival studies conducted over ten years in Michigan indicated that infected crop debris is not the primary inoculum source for CBB (Saettler *et al.* 1986). Infected bean debris may be more important as an inoculum source in tropical and sub-tropical than in temperate areas (Gilbertson *et al.* 1990).

Survival of Xap is greater under dry conditions (Schuster & Coyne 1977a) as bacteria decline rapidly under moist conditions (Allen *et al.* 1998). Sabet & Ishag (1969) reported that Xap survived in press-dried bean leaves for more than 18 months in the laboratory, while Gilbertson *et al.* (1988) found Xap to remain viable in dry-leaf inoculum after 6 years. The longer survival under laboratory conditions as opposed to that in the field could be attributed the presence of antagonists, such as protozoa, in the soil (Habte & Alexander 1975).

Xap also survives on weeds and other host plants (Cafati & Saettler 1980c, Angeles-Ramos *et al.* 1991, Opio *et al.* 1995). Certain weed species may harbor the pathogen for up to 6 months (Opio *et al.* 1995). Angeles-Ramos *et al.* (1991) isolated epiphytic, pectolytic *Xanthomonads* from symptomless weeds where pathogenic strains were isolated from within infected fields. Epiphytic colonies survive on a wide range of plant species in families *Amaranthaceae*, *Commelinaceae*, *Compositae*, *Cruciferae*, *Gramineae*, *Oxalidaceae* and *Portulaceae* in addition to various legumes (Allen *et al.* 1998). Epiphytic Xap populations are important in the epidemiology of CBB on dry beans (Ishimaru *et al.* 1991) and are differentially affected in hosts of different genotypes (Cafati & Saettler 1980a).

The mechanisms of CBB dissemination over long distance (from one part of the

country to another), or plant to plant or field to field (Zaumeyer & Thomas 1957) vary. Seed transmission primarily disseminates CBB over international boundaries (Saettler & Perry 1972). Infections as low as 0,2% and 0,5% result in field epidemics under favourable conditions (Ednie & Needham 1973, Opio *et al.* 1993). Seedborne inoculum introduces the pathogen randomly to a field providing a number of primary infection foci. Spread from such foci is more effective than field margins (Mabagala 1997). Inoculum levels of 10^3-10^4 bacteria per seed were the minimum required to result in bacterial transfer from seed to seedling (Weller & Saettler 1980a). In Uganda even lower bacterial populations per seed (10^2 CFU/seed) were found to incite field infections (Opio *et al.* 1993).

Genotypes differ in their ability to transmit Xap from seed to seedlings (Schuster *et al.* 1979, CIAT 1994, Opio *et al.* 1994b, Mabagala 1997). Bacterial populations in resistant varieties are less than in susceptible ones, however, CBB may be transmitted through seed of resistant bean cultivars. Systemic invasion, however, does not occur in resistant varieties (Schuster *et al.* 1979).

Secondary spread of CBB depends on the number of infection foci, presence of vectors, crop growth stage, environmental conditions and cultural practices (Allen *et al.* 1998). Insects that disseminate Xap include grasshoppers (*Melanoplus* spp.), Mexican bean beetle (*Epilachna varivestis* Muts.), borers (*Diapreps abbrevialus* Boh.), *Ceratoma ruficornis* and white flies (*Bemisia tabaci*) (Zaumeyer & Thomas 1957, Sabet & Ishag 1969, Kaiser & Vakili 1978).

Wind-blown soil and debris not only disseminate bacterial plant pathogens, but also wound host plants allowing bacterial penetration (Claflin *et al.* 1973). CBB incidence in 2-week-old bean plants was 25 and 55% after exposure to soil blown 13,9

m/sec for 3 and 5 minutes respectively (Claflin *et al.* 1973). Wind disseminated Xap bacterial infections may be restricted by the pathogen's inability to survive in soil (Burke 1957). Rain, dew, hail and irrigation water are also important factors in disease dissemination (CIAT 1992) as is mechanical dissemination by means of implements, animals and humans.

Growth stage

Appearance of CBB in bean fields is closely related to plant developmental stage (Weller & Saettler 1980b). Although blight symptoms sometimes appear on seedlings, symptoms are generally not seen during the vegetative growth stage. Under field conditions, symptoms usually occur during the reproductive stage, initially observed on the lower, older leaves. Secondary pathogen spread occurs rapidly following primary infection.

Inoculation of plants under controlled conditions, indicated that leaf age affects Xap responses (Patel & Walker 1963). Susceptibility to Xap increases with leaf age (Goss 1940), however, Patel & Walker (1963) found younger leaves to be more susceptible. These plants were in the vegetative stage and infections did not simulate natural field infection.

Environmental influences

Temperature

CBB is generally regarded a high-temperature disease with greatest damage occurring at 28°C (Goss 1940, Patel & Walker 1963). Goss (1940) found that CBB symptoms appeared on inoculated plants within 6 days at 32°C, 10 days at 28°C, 14 days at 24°C and no visible symptoms after 17 days at 20°C and 16°C respectively. Symptoms were most severe at 28°C which agrees with Patel & Walker (1963) and Arnaud Santana *et al.* (1993a). *In vitro* bacterial growth is greatest at 28° and 32°C, gradually decreasing as temperatures are reduced with little growth at 16°C (Patel & Walker 1963).

Although classified a high-temperature disease, CBB infections may occur at relatively low temperatures but the incubation period is prolonged. This explains disease outbreaks under conditions generally unfavourable for infection (Goss 1940).

Humidity

High humidity is preferable for CBB development (Goss 1940, Sutton & Wallen 1970), however, CBB was also reported to spread rapidly during dry weather (Goss 1940). After artificial inoculation of bean plants, Goss (1940) found infections were more severe on plants kept at low-relative humidity. Plant pathogenic bacteria do not form spores, but may tolerate dessication and survive under extended dry conditions. Xap can survive for relatively long periods under varied environmental conditions, in an extracellular polysaccharide it produces in culture (Leach *et al.* 1957).

Photoperiod

Photoperiod affects expression of common bean reactions to Xap, which have serious

implications in resistance breeding. Disease reactions in growth chamber studies were more severe under short photoperiod and high temperatures than under long photoperiod and low temperatures (Arnaud Santana *et al.* 1993a). No significant interactions were detected. Short photoperiod increased disease severity in the field (Arnaud-Santana 1993a). Schuster *et al.* (1985) found lines adapted to temperate zones did not increase in susceptibility under short daylight, however, two tropical lines increased in susceptibility. Similarly Webster *et al.* (1983), found lines with moderate resistance in temperate zones were susceptible in the tropics.

DISEASE MANAGEMENT

CBB remains a major dry bean production constraint as it is difficult to control. An integrated disease management approach, including cultural practices, chemical sprays and resistant varieties, is needed to adequately control disease.

Cultural practices

Xap contaminated seed is considered the primary inoculum source. Planting of pathogen-free seed is the most important primary control method (Gilbertson *et al.* 1990). Disease-free seed is generally produced in areas where climatic conditions and rigid quarantine minimize infestation risk and has been successfully implemented in the USA, Canada (Copeland *et al.* 1975), Australia (Redden & Wong 1995) and South Africa (D. Fourie: unpublished data). Apart from field inspections, success of seed certification programmes depends on accurate pathogen detection in seed (Audey *et*

al. 1996). Several methods for bacterial detection in seed have been reported (Ednie & Needham 1973, Lachman & Schaad 1985, Venette *et al.* 1987, Aggour *et al.* 1988, Roth 1988, Redden & Wong 1995, Audey *et al.* 1996).

Use of disease-free seed does not guarantee disease control as other inoculum sources exist (Allen *et al.* 1998). Additional cultural practices such as removing, destroying or deep ploughing of debris, effective weed control, crop rotation and minimized movement in fields, especially when foliage is wet, may be effective (Allen *et al.* 1998, Schwartz & Otto 2000). Intercropping with maize decrease incidence and severity of CBB (Fininsa 1996). Crop rotation may be less effective if epiphytic bacteria survive on non-host rotation plants.

Chemical control

Copper based bactericides protect foliage against Xap and secondary pathogen spread and include copper sulphate, copper ammonium carbonate (Oshima & Dickens 1971), copper hydroxide, potassium (hydroxymethyl) methyldithiocarbamate (Weller & Saettler 1976), cupric carbonate, cupric sulphate (Opio 1990), and cupric hydroxide (Schwartz *et al.* 1994). Efficacy of CBB chemical control is limited (Allen *et al.* 1998) and resultant yield increases are minimal (Saettler 1989).

Early season disease detection can improve efficacy of bactericide applications (Schwartz *et al.* 1994). Schwartz *et al.* (1994) effectively controlled bacterial diseases by applying cupric hydroxide early in the season, thereby reducing bacterial populations before they establish within diseased tissue. An average of three applications provided average yield increases of between 5% and 9%.

No methods are available to eradicate internal seed populations, however, external contamination may be controlled by streptomycin sulphate and sodium hypochlorite (Liang *et al.* 1992). Liang *et al.* (1992) investigated the potential of osmotic conditioning in reducing internal Xap populations from seeds, using polyethylene glycol (PEG) and glycerol as antibiotic carriers. They found that tetracycline and chlorotetracycline in PEG solutions effectively reduced Xap, but were phytotoxic. PEG solutions containing streptomycin reduced, but did not eradicate internal bacterial populations from naturally infected seeds with few phytotoxic effects.

Streptomycin is rapidly absorbed into bean stems and translocated to leaves but there is no indication that antibiotics are translocated downward through stems, trifoliate leaves or peduncle into the pod (Mitchell *et al.* 1954). Antibiotics should not be applied to leaves as resistant mutants may develop (Saettler 1989), which is the major reason why antibiotic use is prohibited in South Africa. Development of resistance to chemicals (Romeiro *et al.* 1998), costs involved and efficacy limit use of chemical control which may be feasible under certain circumstances, such as seed production or as a component of an integrated control strategy (Allen *et al.* 1998).

Biological control

Resistance in susceptible plants induced by inoculation with avirulent isolates does exist. Bean leaf extract with avirulent isolates, evaluated at CIAT (1989) significantly reduced CBB under field conditions. Mabagala (1999) identified two *Bacillus* spp. and a *Pseudomonas fluorescens* isolate that exhibited *in vitro* and *in vivo* antagonism to Xap.

Genetic resistance

The most effective and economic bean CBB control strategy is use of genetic resistance (Rands & Brotherton 1925). CBB resistance breeding has been extensively researched (Beebe & Pastor-Corrales 1991). Rands & Brotherton (1925) identified lines with resistance to CBB. Subsequent efforts only yielded moderate levels of resistance (Yoshii *et al.* 1978) with no immunity in *P. vulgaris*. Wild populations of *P. vulgaris* also gave intermediate Xap resistance reactions (Navarrete-Maya & Acosta-Gallegos 1997). Higher levels of resistance were found in scarlet runner bean (*P. coccineus*) while highest levels were identified in tepary beans (*P. acutifolius*) (Singh & Muñoz 1999).

Honma (1956) made interspecific crosses between *P. vulgaris* and *P. acutifolius* to derive the resistant line GN #1 Nebr. sel. 27 (Coyne & Schuster 1974a). This line has been used many breeding programmes as a resistance source (Coyne & Schuster 1974a, Mohan & Mohan 1983) and resulted in development of resistant lines such as Jules (Coyne & Schuster 1970), Harris (Coyne *et al.* 1980), Tara, Valley (Coyne & Schuster 1974b) and Starlight (Coyne *et al.* 1991).

Another resistance source commonly used is PI 207262 which was developed in Colombia (Coyne & Schuster 1973). GN #1 Nebr. sel. 27 and PI 207262 have limited use as GN #1 Nebr. sel. 27 is susceptible to isolates from Colombia and Uganda (Schuster *et al.* 1973, Yoshii *et al.* 1978). Both lines and derivates are poorly adapted to tropical conditions (Webster *et al.* 1983). XAN 112, developed from crosses between Jules and PI 207262, had greater resistance and was better adapted to tropical conditions (Schuster & Coyne 1981, Silva *et al.* 1989). XAN 112 has been extensively evaluated as a resistance source in many countries (Argentina, Brazil, Colombia, Cuba,

Guatemala, France and USA) (CIAT 1987).

Germplasm is continuously screened at CIAT to find more suitable resistance sources. From approximately 15 000 lines screened, only a few lines with moderate resistance levels were identified (CIAT 1988). Hybridization between common (*P. vulgaris*) and tepary beans (*P. acutifolius*) was initiated at CIAT in 1989 where they used congruity backcrossing to overcome hybridization barriers such as genotype incompatibility, early embryo abortion, hybrid sterility and lower frequencies of hybridization (Mejía-Jiménez *et al.* 1994).

Near-immune lines (XAN 159, XAN 160, XAN 161 and OAC 88-1) were derived from crosses between *P. acutifolius* and *P. vulgaris* (Beebe & Pastor-Corrales 1991). Although resistance instabilities were reported in XAN 159 and its progeny (Beebe & Pastor-Corrales 1991), it is still widely used in resistance breeding programmes (Beebe & Pastor-Corrales 1991, Fourie & Herselman 2002, Park *et al.* 1998a, Mutlu *et al.* 1999, Singh & Muñoz 1999). Resistant varieties were also developed from interspecific crosses between *P. vulgaris* and *P. coccineus* (Freytag *et al.* 1982, Park & Dhanvantari, 1987, Miklas *et al.* 1994).

New resistant lines (Vax 1 Vax 2, Vax 3, Vax 4, Vax 5 and Vax 6) were recently developed at CIAT from interspecific hybridization of *P. vulgaris* and *P. acutifolius* and gene pyramiding (Singh & Muñoz 1999). These lines showed high resistance when tested against isolates from various geographical origins (Zapata *et al.* 1998, Jara *et al.* 1999). Vax 1 and Vax 2 were susceptible when evaluated in Uganda (R. Buruchara, CIAT: personal communication) and South Africa (D. Fourie: unpublished data). Resistance levels in Vax 3, Vax 4 and Vax 6 are as high as those found in *P. acutifolius* (Singh & Muñoz 1999). Substantial progress has been made through gene pyramiding.

Lines developed through pyramiding are often not of suitable commercial seed type and resistance must be transferred to cultivars of different market classes (Singh & Muñoz 1999). Sources of CBB resistance are shown in Table 1.

Adams *et al.* (1988) reported that a single major recessive gene confers resistance in a snap bean line, A-8-40. Eskridge & Coyne (1996) found CBB resistance in common bean to be controlled by one to five genes. Genetic markers indicated CBB resistance to be linked from two to six quantitative trait loci (QTL) (Nodari *et al.* 1993, Jung *et al.* 1996, Miklas *et al.* 1996, Jung *et al.* 1997, Park *et al.* 1998b, Tsai *et al.* 1998).

Depending on resistance sources used and evaluation methodology, one to three genes appear to confer resistance in *P. acutifolius* to CBB (McElroy 1985, Drijfhout & Blok 1987, Silva *et al.* 1989). Based on resistance of F1, segregation in F2 and reaction of F3 plants and lines, Drijfhout & Blok (1987) concluded that resistance was governed by a single dominant gene which was confirmed by Silva *et al.* (1989). McElroy (1985) indicated that resistance in XAN 159, XAN 160, and XAN 161 is controlled by one major and a few minor genes. A single QTL explained 62% of the total phenotypic variation in a line derived from XAN 159, confirming that one major gene control blight resistance (Yu *et al.* 1999).

Welsh & Grafton (1997) concluded that resistance derived from *P. coccineus* is conferred by one recessive gene. Range of reaction varied in susceptible plants indicating presence of minor genes modifying expression of CBB resistance. Yu *et al.* (1998), however, detected two resistance genes in the line XR-235-1-1which carries *P. coccineus*-derived CBB resistance.

Kolkman & Michaels (1994) found that PI 440 795 and PI 319 443 from which

XAN 159, XAN 161 and OAC 88-1 were derived, carried identical genes for CBB resistance. Segregation for susceptibility in F2 generations obtained from crosses between these lines suggested that more than one resistance gene is transferred from the tepary parent and these genes should be pyramided to confer durable resistance (Michaels 1992). Resistance in XAN 159 and OAC 88-1 is, however, linked to the same RAPD marker (Singh & Muñoz 1999).

CBB resistance is quantitatively inherited with dominance for susceptibility (Coyne *et al.* 1966, Coyne *et al.* 1973, Finke *et al.* 1986). Although gene action is primarily additive, dominance and epistatic effects have been observed (Beebe & Pastor-Corrales 1991). Low estimates of narrow sense heritability have been reported (Coyne & Schuster 1974a, Arnaud-Santana *et al.*1994). Selection for resistance in advanced lines should therefore be conducted in replicated trials under uniform disease pressure (Arnaud-Santana *et al.* 1994).

Differential reactions of pods and leaves to Xap have been reported (Coyne & Schuster 1974c, Valladarez-Sanchez *et al.* 1979, Schuster *et al.* 1983, Park & Dhanvantari 1987, Aggour *et al.* 1989). Pod susceptibility in large seeded bean types (Andean origin) seems to be more problematic (Beebe & Pastor-Corrales 1991). From 18 *P. vulgaris* germplasm lines evaluated against four Xap strains, XAN 159, BAC 6 and XAN 112 had the best combined leaf and pod resistance (Arnaud-Sanata *et al.* 1993b). Lack of association between leaf and pod disease reactions, indicates the importance of evaluating both reactions to develop a resistant plant.

Coyne & Schuster (1974c), found genes controlling late maturity and resistance to be linked in crosses with GN #1 Nebr. sel. 27, and that susceptibility increased with onset of plant maturity. Adams *et al.* (1988) indicated that reaction to Xap was not

associated with flower colour or with days to flower. Purple flower colour (V gene) and RAPD markers, however, have been reported to be associated with QTL affecting leaf and pod resistance in a bean cross (Jung *et al.* 1997, Mutlu *et al.* 1999, Park *et al.*, 1999).

Assessment of resistance

Different inoculation techniques described to evaluate CBB resistance include aspersion (inoculum sprayed under pressure onto leaves) and wounding of leaves using scissors, razor blades, needles, surgical blades etc. (Andrus 1948, Schuster 1955, Pastor-Corrales *et al.* 1981, Opio *et al.* 1994a). Vacuum infusion of bean seed with a bacterial suspension gave significantly higher incidence and severity scores than spraying of bacterial suspension on plants (Bett & Michaels 1992). Gilbertson *et al.* (1988) successfully used infected dry leaves as a source of inoculum and suggested it to be an effective inoculation method where laboratory facilities are limited.

Opio *et al.* (1994a) indicated that inoculum concentrations between 10^6 and 10^8 CFU/ml water, were adequate for disease development using several inoculation techniques. Aggour *et al.* (1988) found a significant interaction between methods of inoculation, inoculum concentration and genotype. Saettler (1977) indicated that bacterial concentrations ranging from 3-6x10^7 CFU/ml gave reactions that correlated with those in the field.

Mohamed *et al.* (1993) developed a detached leaf technique for bioassay of Xap reaction over a wide range of bean genotypes and environmental conditions. Navarrete-Maya *et al.* (1995) however, found that spray inoculation of detached leaves did not

produce reliable results. Detached pods (Ariyarathane *et al.* 1996) and detached seedling stem inoculation assays (Lienert & Schwartz 1994) can also be used effectively for evaluation of resistance against CBB.

Various rating scales have been developed for evaluating and quantifying disease reaction on leaves and pods (Saettler 1977, Yoshii *et al.* 1978, Valladarez-Sanchez *et al.* 1983, Park & Dhanvantari 1987, Van Schoonhoven & Pastor Corrales 1987, Mohamed *et al.* 1993, Arnaud-Santana *et al.* 1994). Rating scales should be standardized and utilized uniformly when comparing lines with CBB resistance (Saettler 1977).

Marker assisted selection (MAS)

Evaluation of field reactions is costly in terms of time and space. Molecular markers linked to resistance were developed for indirect selection in breeding for resistance (Bai *et al.* 1996, Beattie *et al.* 1998, Park *et al.* 1999, Yu *et al.* 1999). Yu *et al.* (1999) screened 138 F5 lines derived from HR67 (resistance derived from XAN 159), using a SCAR-marker and subsequently tested it for CBB resistance in the greenhouse. Based on marker information, 28 of the 138 lines had the SCAR band present and were predicted to be resistant. On comparing SCAR results with field inoculation test data, 23 of 28 plants gave a resistant phenotypic reaction (DSI<2.0) indicating an accuracy of 82%. Only 3.6% of the lines were mis-classified as resistant plants. Cost estimates further indicated that use of marker assisted selections costed approximately one third less than greenhouse testing (Yu *et al.* 1999).

Expression of QTL may differ over environments or populations in various crops

and only one QTL affecting resistance to Xap was consistently expressed in four common bean populations (Park *et al.* 1999). Marker-QTL associations need to be confirmed in a breeding programme, particularly for traits like CBB resistance that have complex inheritance patterns, low narrow-sense heritabilities and a number of genes involved (Park *et al.* 1999).

Pyramiding of resistance genes into a single cultivar is necessary to achieve stable resistance. Use of marker assisted selection can contribute considerably when pyramiding genes (Kelly & Miklas 1999, Sing & Muñoz 1999, Dursun *et al.* 1995). Independence of resistance genes to be combined, however, need to be closely monitored as many lines and cultivars have common sources of CBB resistance (Kelly & Miklas 1999). Use of SCAR-markers linked with three independent QTL derived from XAN 159 and GN #1 Nebr. sel. 27, has resulted in advanced cranberry, pinto and snap bean germplasm with combined resistance to CBB. MAS should therefore expedite improvement of blight resistance in other market classes of bean (Miklas *et al.* 2000).

CONCLUSION

Although CBB has been studied extensively, it continues to be a major constraint in dry bean production in many parts of the world. Many contradictory results have been reported and work confirming various aspects are required. Disease management is complicated by the pathogen being seed borne and that widely adapted sources of resistance are limited. Good progress, however, has been made recently to improve resistance to CBB by combining genes from different *Phaseolus* species into a common bean type. Lines obtained from gene pyramiding (i.e. Vax 3, Vax 4 and Vax 6) possess

levels of CBB resistance that are as high as those found in *P. acutifolius* accessions (Singh & Muñoz 1999). QTL mapping contributed significantly to understanding the genetic control of a trait as complex as CBB resistance. Continued efforts in finding new sources of resistance and improvement of current levels of resistance in cultivars are needed.

It is indicated in the review that a number of different rating scales are being used in disease assessment. An internationally accepted scale needs to be standardized to allow meaningful comparison of results over time and in different parts of the world.

Existence of Xap races remains controversial. Races have been identified in some bean growing areas. Pathogenic variation may have serious implications in development of blight resistant varieties. An attempt was made during the First International Workshop on CBB (Coyne *et al.* 1996) in which minimum standards for race designation were proposed. During the Second International Workshop on CBB held in South Africa in 2002, it was, however, decided that there is a greater need to have differentials in *P. vulgaris*. The investment in time and resources does not justify working with a tepary system and *P. vulgaris* does not appear to have that degree of specificity (Steadman et al. 2002).

CBB, however, can only be effectively managed if a comprehensive integrated management strategy is developed. Studies on epidemiology and control of this devastating disease have been well documented and these technologies need to be transferred to producers and resource poor farmers.

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Table 1. Sources of resistance to common bacterial blight in dry beans

Variety	Origin	Reference
GN Nebr. # 1 Sel.27	UNL	Coyne & Schuster 1983
GN Tara	UNL	Coyne & Schuster 1983
GN Jules	UNL	Singh & Muñoz 1999
OAC 88-1	UGC	Singh & Muñoz 1999
XAN 159	CIAT	Mc Elroy, CIAT 1985
XAN 112	CIAT	CIAT 1984
XAN 91	CIAT	CIAT 1983
PI 207262	Colombia	Coyne & Schuster 1983
BAC 5	IAPAR	Arnaud-Santana *et al.* 1993
BAC 6	IAPAR	Arnaud-Santana *et al.* 1993
IAPAR 14	IAPAR	Beebe & Pastor-Corrales 1991
IAPAR 16	IAPAR	Beebe & Pastor-Corrales 1991
Tamaulipa 9-B (G 04399)	CIAT	Arnaud-Santana *et al.* 1993
MSU 183 (G 06700)	CIAT	Arnaud-Santana *et al.* 1993
Calima 9 (G 06772)	CIAT	Arnaud-Santana *et al.* 1993
PI 209.481 (G 16836	CIAT	Arnaud-Santana *et al.* 1993
RKN (G 18443)	CIAT	Arnaud-Santana *et al.* 1993
ODCSJ (G 18168)	CIAT	Arnaud-Santana *et al.* 1993
G 19195A	CIAT	Arnaud-Santana *et al.* 1993
PC 50	Dominican Republic	Schuster et al. 1983
ICA L-23	ICA, Colombia	Beebe & Pastor-Corrales 1991
Guama 23	ICA, Colombia	Beebe & Pastor-Corrales 1991
WBB-20-	UPR	CIAT 1997
G17341	CU	CIAT 1997
XAN 263	CIAT	CIAT 1997
XAN 309	CIAT	CIAT 1997
XAN 328	CIAT	Singh & Muñoz 1999
XAN 330	CIAT	Singh & Muñoz 1999
XAN 332	CIAT	CIAT 1997
Wilk 2	CU	Singh & Muñoz 1999
VAX 1	CIAT	Singh & Muñoz 1999
VAX 2	CIAT	Singh & Muñoz 1999
VAX 3	CIAT	CIAT 1997
VAX 4	CIAT	Singh & Muñoz 1999
VAX 5	CIAT	CIAT 1997
VAX 6	CIAT	CIAT 1997

UNL = University of Nebraska, Lincoln; UGC = University of Guelph; CIAT = Centro Internacional de Agricultura Tropical; IAPAR = Instituto Agronômico do Paraná; ICA = Instituto Clombiano Agropecuario; UPR = University of Puerto Rico; CU = Cornell University

CHAPTER 7

IMPROVEMENT OF COMMON BACTERIAL BLIGHT RESISTANCE IN THE SOUTH AFRICAN DRY BEAN CULTIVAR TEEBUS

ABSTRACT

Backcross breeding was used to improve CBB resistance in the small white canning bean, cv. Teebus, using resistance in XAN 159 and Wilk 2 sources, respectively. High resistance levels in near-isogenic lines, developed in two independent breeding programmes, indicated successful transfer of resistance from both sources. Presence of SCAR-markers, SU91 and BC420, in 35 of 39 XAN 159 derived Teebus lines and all lines derived from Wilk 2, confirmed successful resistance transfer. AFLP studies conducted to determine genetic relatedness of two near-isogenic Teebus lines, showed a similarity of 96.2% with the maximum similarity between these lines and Teebus being 93.1%. Material developed in this study has been included a bean breeding programme and seed will be made available to farmers after extensive field testing.

INTRODUCTION

One of the most important dry bean (*Phaseolus vulgaris* L.) diseases in South Africa is common bacterial blight (CBB), caused by the bacterium*Xanthomonas axonopodis* pv. *phaseoli* (Xap) (Smith) Vauterin, Hoste, Kosters & Swings and i's fuscans variant, *X. axonopodis* pv. *phaseoli* var. *fuscans* (Xapf). The disease is widespread

worldwide and occurs in all the major South African bean producing areas (Fourie 2002). Yield losses have been poorly documented but are reported to vary between 22% and 45% (Wallen & Jackson 1975, Yoshii 1980). Infected seed is the primary inoculum source and planting of pathogen-free seed is an important means of disease avoidance. Other control measures include preventative spraying with copper based bactericides, removing, destroying or deep ploughing of debris, effective weed control, crop rotation and minimized movement of humans and implements in fields (Allen *et al.* 1998, Schwartz & Otto 2000). However, the most effective and economic CBB control strategy is the use of genetic resistance (Rands & Brotherton 1925).

CBB resistance breeding has been extensively researched (Beebe & Pastor-Corrales 1991). Rands & Brotherton (1925) first identified lines with CBB resistance. Subsequent efforts yielded moderate levels of resistance (Yoshii *et al.* 1978) with no immunity in *P. vulgaris*. Higher levels of resistance were found in scarlet runner bean (*P. coccineus*), with highest levels identified in tepary beans (*P. acutifolius*) (Singh & Muñoz 1999).

Interspecific crosses between *P. vulgaris* and *P. acutifolius* resulted in development of resistant lines such as GN #1 Nebr. sel. 27, XAN 112, XAN 159, XAN 160, XAN 161 and OAC 88-1 (Coyne & Schuster 1974a, Schuster & Coyne 1981, Silva *et al.* 1989, Beebe & Pastor-Corrales 1991). Resistant varieties were also developed from interspecific crosses between *P. vulgaris* and *P. coccineus* (Freytag *et al.* 1982, Park & Dhanvantari 1987, Miklas *et al.* 1994). Most of these are considered exotic germplasm and are poorly adapted to local conditions, but are suitable as donor parents in a breeding programme.

Depending on resistance source used and evaluation methodology, one to six

genes appear to confer CBB resistance in bean (McElroy 1985, Drijfhout & Blok 1987, Adams et al. 1988, Silva *et al.* 1989, Eskridge & Coyne 1996). Genetic markers have indicated that CBB resistance is linked to between two and six quantitative trait loci (QTL) (Nodari *et al.* 1993, Jung *et al.* 1996, Park *et al.* 1998, Tsai *et al.* 1998).

CBB resistance is quantitatively inherited with dominance for susceptibility (Coyne *et al.* 1966, Coyne & Schuster 1973, Finke *et al.* 1986). Although gene action is primarily additive, dominance and epistatic effects have been observed (Beebe & Pastor-Corrales 1991). Low estimates of narrow sense heritability have also been reported (Coyne & Schuster 1974a, Arnaud-Santana *et al.* 1994).

All locally grown commercial dry bean cultivars are susceptible to CBB (*vide* Chapter 5) and improvement of resistance in local cultivars is important for the control of CBB. Thus, the aim of this study was to identify sources of CBB resistance in exotic germplasm that could be used in a backcross breeding programme. In this study emphasis is placed on improving resistance of the highly susceptible small white canning bean, cv. Teebus.

MATERIAL AND METHODS

Evaluation of germplasm for CBB resistance

Eighteen CBB resistance sources (Table 1), obtained from CIAT (International Centre for Agriculture in the Tropics), were screened, under field and greenhouse conditions, for resistance to local isolates of Xap and Xapf. BAT 41, BAT1297, obtained from CIAT, and a South African cultivar, Teebus were included as

susceptible checks.

Greenhouse screening

Twenty-five seeds of each genotype were planted in 20 litre plastic bags (5 seeds per bag) in sterile soil and maintained in a greenhouse at 18°C night/28°C day. Seedlings were thinned to four plants per pot after emergence. A mixture of two local aggressive isolates (X6 and Xf105) were used for inoculation. Inoculum was prepared by suspending 48- to 72-h-old cultures in sterile distilled water, which was adjusted to 10^8 CFU/ml. Fourteen to 20-day-old plants with fully expanded first trifoliate leaves were inoculated using the multiple-needle inoculation method (Andrus 1948). Control plants were inoculated with sterile distilled water. Plants were maintained in a greenhouse at 18°C night/28°C day and rated, on a 1 to 9 scale (Aggour *et al.* 1989), 14 days after inoculation, with 1 being resistant and 9 susceptible.

Young, detached pods from each genotype were inoculated with one Xap isolate (X6) using the method of Aggour *et al.* (1989). Disease reactions were recorded 10 days after inoculation on a 1-9 scale (Aggour *et al.* 1989) with 1 being resistant and 9 susceptible.

Field screening

Two 5 m rows (65 seeds per row) of each genotype were planted in an unreplicated trail in the field and evaluated for CBB resistance. Inoculum was prepared similar to that for the greenhouse trials with the exception that non-sterile tap water was used.

A motorised backpack sprayer was used for inoculating plants in the field at 25, 32 and 39 days after planting. Rows were evaluated for disease reaction from the time when first symptoms appeared until the crop matured. Evaluations were based on CIAT 1-9 scale with 1 being resistant and 9 susceptible (Van Schoonhoven & Pastor-Corrales 1987).

Breeding for resistance

Genotypes exhibiting highest levels of resistance to local isolates under greenhouse and field conditions were selected to improve resistance of a local cultivar, Teebus, in a backcross breeding programme (Table 2). Teebus was selected based on its commercial value and preference by the canning industry.

Crosses were made, in the greenhouse, between the resistant donor (pollen) parent, and the recurrent susceptible parent (Teebus). First trifoliate leaves of plants from F1-generations were inoculated with a bacterial suspension containing approximately 10^8 CFU/ml water, using the multiple needle puncture method (Andrus 1948). Leaves were rated for infection 14 days after inoculation on a 1 to 9 scale with 1 being highly resistant and 9 being highly susceptible. Teebus plants were inoculated as susceptible controls. Susceptible plants were discarded (plants rated >3-9) and resistant plants (rated 1-3) retained for backcrossing. Backcrossing to the recurrent parent was continued for five generations and approximately 94% of the recurrent parent was recovered with addition of the resistance gene(s).

Segregating BC5F2 populations were planted in field trials at Potchefstroom during the 1999/2000 season and evaluated for resistance. Plots consisted of unreplicated single rows of 5 m each with 30 seeds planted per row. Teebus was

planted every sixth row throughout the plot as a susceptible check (Fig. 1). First or second trifoliate leaves of each plant in a 5 m row were inoculated using the multiple needle method (Andrus 1948), which was followed by spray inoculating plants with motorized backpack sprayer. Spray inoculations were repeated weekly until adequate disease developed on susceptible checks. Each plant was rated separately and single plants with high levels of resistance (rating 1-2) were marked. Spray-inoculated canopies of selected single plants were evaluated periodically from when first symptoms appeared on the susceptible checks until the crop matured.

Single plant progeny rows (F3 generation) were planted during winter (May, 2000) at Makhatini Research Station, KwaZulu-Natal, inoculated and similarly rated. Single plants were again selected and F4 generations planted in progeny rows at Potchefstroom the following summer (2000/2001). The process continued until homozygous single rows from F6 generations, with uniform high levels of resistance, could be selected during the 2001/2002 season.

Field selections judged to be homozygous for important properties were included in the main bean breeding programme, where they are tested for canning quality. Selective lines will be entered into yield trials that run for three seasons prior to cultivar release. Successful varieties will ultimately be entered in the National Cultivar Trials and seed will be made available to farmers. Results of this study are limited to the confirmation of improvement of resistance of Teebus and do not include further evaluation and release of varieties.

Confirmation of resistance using SCAR markers

Thirty nine near-isogenic resistant Teebus lines (BC5F4), derived from backcrossing

with XAN 159 as donor parent, and 8 lines derived from backcrossing with Wilk 2 (BC5F2), were evaluated for presence of two independent CBB resistant QTL from XAN 159, using existing SCAR markers SU91 and BC420 (Miklas *et al.* 2000). Total genomic DNA was extracted from lyophilised leaf tissue (Graham *et al.* 1994). SCAR primers SU91 and BC420 (Table 3) were synthesized by GibcoBRL (Life Technologies, Glasgow, United Kingdom), based on the primer sequences obtained from Miklas *et al.* (2000). Primers were suspended in TE buffer to a concentration of 200 pmol/µl and a work solution of 10 pmol/µl was prepared. SCAR markers, for the polymerase chain reaction (PCR), were based on the protocol of Williams *et al.* (1990) with minor modification. Reactions were performed using a PCR Sprint Thermal Cycler (Hybaid Limited, UK) programmed for 5 min at 94°C, 30 cycles of 1 min at 94°C, and 1.5 min at 72°C, followed by one cycle of 5 min at 72°C.

Amplification products were analysed by electrophoresis in 1.5% (w/v) agarose gels (Seakem LE) at 80 V for 2 hr using UNTAN buffer (0,4 M Trisbase, 0,02 M EDTA, pH 7.4) and detected by staining with 1 µg/ml ethidium bromide. Gels were photographed under UV light with Polaroid 667 film.

Determination of genetic relatedness of near-isogenic Teebus lines

Extracted DNA from Teebus, XAN 159 and two near-isogenic Teebus lines (TCBR1 and TCBR2) were subjected to amplified fragment length polymorphism (AFLP) analysis to determine genetic distances between these lines. AFLP adapters and primers (Table 4) were designed based on the methods of Vos *et al.* (1995). Primers were synthesised by GibcoBRL (Life Technologies, Glasgow, United Kingdom) and oligonucleotides used for adapters were PAGE (polyacrylamide gel electrophoresis)

purified. Adapters were prepared by adding equimolar amounts of both strands, heated for 10 min to 65°C in a water bath and left to cool at room temperature.

Gel electrophoresis for AFLP analysis was performed (Vos *et al.* 1995) using a 5% (w/v) denaturing polyacrylamide gel [19:1 acrylamide: bis-acrylamide; 7 M urea; 1x TBE buffer (89 mM Tris-borate; 2.5 mM EDTA)]. Electrophoresis was carried out at constant power, 80 W for approximately 2 hr. Polyacrylamide gels were silver-stained following the protocol described by Silver Sequence™ DNA Sequencing System manual supplied by Promega (Madison, WI, USA). Gels were left upright overnight to air dry and photographed by exposing photographic paper (Kodak Polymax II RC) directly under the gel to about 20 sec of dim light. This produced a negative image, exactly the same size as the gel.

AFLP data were scored based on presence (1) or absence (0) of DNA bands obtained for each line. Only reliable and repeatable bands were considered. Pair wise genetic distances were calculated between isolates Nei and Li (1979). Cluster analysis was done using the unweighted paired group method using arithmetic averages (UPGMA). Statistical analyses were performed using NTSYSpc version 2.02i.

RESULTS

Germplasm evaluation for CBB resistance

Reaction of genotypes to local Xap and Xapf isolates are shown in Table 1. Four lines, XAN 159, Wilk 2, Wilk 4 and Wilk 6 exhibited good combined leaf, pod and field resistance. P.I.196932 was resistant when tested in the greenhouse but was

moderately susceptible in the field. The susceptible checks (BAT 41, BAT 1297 and Teebus) were susceptible under both greenhouse and field conditions. Thirteen lines were lost due to peanut mottle virus and could, therefore, not be evaluated in the field for CBB resistance. Two lines, XAN 155 and OAC 88-1 were moderately susceptible when inoculated on first trifoliate leaves but were moderately to highly resistant when inoculated on pods.

Breeding for resistance

XAN 159 and Wilk 2 were selected for their high levels of resistance to local isolates, for use in two independent backcross programmes to improve resistance of cv. Teebus. Five backcrosses have been completed in both breeding programmes and approximately 94% of Teebus has been recovered. Phenotypic disease reactions indicated that lines developed had high levels of resistance (rating 1-2), confirming transfer of resistance from both XAN 159 and Wilk 2.

A total of 1972 single plant field selections were made from advanced Teebus lines (BC5F2-F5) with resistance from XAN 159. Six hundred and forty three single plant progeny rows were evaluated and 136 homozygous (BC5F6) lines with high levels of resistance (rating 1-2) were passed on to a breeder for further evaluation. A total of 401 single plants were selected from Wilk 2 derived Teebus lines (BC5F2-F5), from which 146 single plant progeny rows were evaluated and 11 homozygous resistant lines selected for further evaluation. Lines (F6 generation) segregating for resistance are being evaluated further.

Confirmation of resistance using SCAR markers

SCAR-marker SU91 were present in all (39) XAN 159 derived Teebus lines tested, while BC420 was present in 35 of 39 lines (Fig. 2). Both SU91 and BC420 markers were present in all (8) lines derived from Wilk 2 backcrosses with Teebus as recurrent parent (Fig. 3).

Determination of genetic relatedness of near-isogenic Teebus lines

The dendrogram (Fig. 4), drawn from AFLP data, resulted in two groups, one containing the resistant donor parent (XAN 159) and the other containing near-isogenic lines and the recurrent susceptible parent (Teebus). The Teebus cluster was linked to the XAN 159 cluster at a similarity of 79.4%. Near-isogenic lines (TCBR1 and TCBR2) exhibited a similarity of 96.2%. Similarity between the two near isogenic lines and Teebus was 93.1%. The obtained cophenetic correlation value of r=0.973 indicated that the UPGMA cluster analysis was statistically significant.

DISCUSSION

Adequate levels of resistance were identified in XAN 159 and Wilk 2 to use in a backcross breeding programme to improve resistance of cv. Teebus. XAN 159 was developed at CIAT through interspecific crosses between *P. vulgaris* and *P. acutifolius*, which exhibited combined leaf and pod resistance to local isolates. Similar resistance in XAN 159 was obtained by Arnaud-Sanata *et al.* (1993), when evaluating 18 lines for combined leaf and pod resistance in the USA. Resistance

instabilities have been reported in XAN 159 and its progenies (Beebe & Pastor-Corrales 1991), however, it is still used widely in resistance breeding programmes (Beebe & Pastor-Corrales 1991; Park *et al.* 1998, Mutlu *et al.* 1999, Singh & Muñoz 1999). Wilk 2 has combined resistance genes from *P. vulgaris*, *P. coccineus* and *P. acutifolius*, including XAN 159 or its sister lines (Singh & Muñoz 1999) and was developed at Cornell University, USA.

Differential reactions of pods and leaves in a number of genotypes screened indicated the importance of evaluating both these plant parts when developing resistant plants. Similar differential reactions of pods and leaves to Xap have been reported previously (Coyne & Schuster 1974b, Valladarez-Sanchez *et al.* 1979, Schuster *et al.* 1983, Aggour *et al.* 1989).

Phenotypic disease reaction of advanced lines, in greenhouse and field evaluations, indicated that resistance in cv. Teebus was successfully improved. Homozygous resistant lines were selected and resistant varieties, from these lines, will be released. A number of lines, however, continued to segregate and these need to be evaluated further. Resistance stability is a concern in CBB resistance breeding. Segregation may occur even after more than a dozen generations of selfing (Singh & Muñoz 1999).

PCR studies indicated that both existing SCAR-markers, SU91 and BC420, were present in XAN 159 and Wilk 2 derived Teebus lines tested. This confirms successful transfer of resistance in these advanced lines. Greenhouse results indicated these lines had resistance levels superior to that of XAN 159, which could be attributed to the presence of additional resistance gene(s) being present in these lines. Presence of XAN 159 markers in Wilk 2 derived lines confirms that XAN 159 or similar source was used in developing Wilk 2. Since Wilk lines were of the first

with pyramided resistance genes from various sources, additional CBB resistance genes might be present in advanced Teebus lines. A combination of XAN 159 and Wilk 2 derived Teebus lines may result in stable CBB resistance. Markers linked to additional resistance genes in Wilk 2 are necessary when gene pyramiding is attempted.

High genetic relatedness between Teebus and near-isogenic lines as indicated in AFLP studies indicated that characteristics of cv. Teebus have been recovered with the addition of the resistance gene(s) from XAN 159. Improvement of CBB resistance was thus successfully accomplished in this study. Breeding for resistance in canning beans, however, should always progress within the boundaries set by the industry for canning quality. It is, therefore, important to maintain, as far as possible, the sought-after quality of the original cultivar. Negative correlation, with regard to quality, has been reported where XAN 159 was used as resistance source (J.D. Kelly, Michigan State University: personal communication). Preliminary results indicated that canning quality of improved Teebus lines compared well with that of Teebus (D. Fourie: unpublished data). A final decision on release of these cultivars, however, is taken once improved lines fulfill all criteria such as yield, quality and adaptation.

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Table 1. Reaction of dry bean accessions to a mixture of Xap and Xapf isolates under greenhouse and field conditions (1=resistant; 9=susceptible)

Cultivar	Greenhouse		Field
	Leaves	Pods	
XAN 112	5.9	5.3	3.3
XAN 155	5.7	3.6	-
XAN 199	5.9	5.2	-
XAN 159	2.3	1.3	1.3
OAC 88-1	4.8	1	-
XAN 91	7	7.1	-
IAPAR 14	5.9	5.3	3.2
WILK 85-36	5.2	5	-
WILK 2	1.1	1.3	1.5
WILK 4	1.4	1	1.3
WILK 6	3.7	1	1.5
XAN 266	5.7	5.2	-
XAN 272	7	5.2	-
NY 79-3776-1	5.1	5.4	-
NY 79-3755-2	7.3	5.9	-
P.I. 207262	7.3	5.1	-
TAMAULIPAS 9-3	7.1	5.5	-
P.I. 196932	1.6	1	5
BAT 41 (susceptible)	7.6	7.1	-
BAT 1297 (susceptible)	9	7.4	-
Teebus (susceptible)	9	7.5	9

- Lines not evaluated as result of peanut mottle virus

Table 2. Scheme of backcross programme used to improve common bacterial blight resistance in cv. Teebus

Step	Action
1	Recurrent parent (Teebus) x Donor
2	Test - Backcross 1
3	Test - Backcross 2
4	Test - Backcross 3
5	Test - Backcross 4
6	Test - Backcross 5
7	Test - select resistant F1 plants
8	F2 single plant progeny rows, identify homozygous rows
9	Increase seed - evaluate resistance
10	Compare lines: yield and adaptation, select best
11	Replicated trials: compare with recurrent parent
12	Further evaluation or release

Table 3. SCAR markers used to screen segregating populations for indirect selection of resistant progeny of Teebus and XAN 159 crosses

Primer	Sequence (5'-3')	PCR product size	Resistance source	Linkage group
SU91-1	CCACATCGGTTAACATGAGT	700 bp	XAN159	B8
SU91-2	CCACATCGGTGTCAACGTGA			
BC420-1	GCAGGGTTCGAAGACACACTGG	900 bp	XAN159	B6
BC420-2	GCAGGGTTCGCCCAATAACG			

Table 4. Primer sequences used for *Eco*RI/*Mse*I AFLP analysis to study genetic relatedness between Teebus and near-isogenic Teebus lines (TCBR1 and TCBR2)

Name	Type	Sequence (5'-3')
E-AAC	*Eco*R1	GACTGCGTACCAATTCAAC
E-AAG	*Eco*R1	GACTGCGTACCAATTCAAG
M-CAG	*Mse*I	GATGAGTCGTGAGTAACAG
M-CAT	*Mse*I	GATGAGTCGTGAGTAACAT
M-ACA	*Mse*I	GATGAGTCGTGAGTAAACA
M-ACC	*Mse*I	GATGAGTCGTGAGTAAACC
M-CTT	*Mse*I	GATGAGTCGTGAGTAACTT

Figure 1. Cultivar Teebus planted as susceptible check every sixth row with segregating breeding lines with improved resistance in between

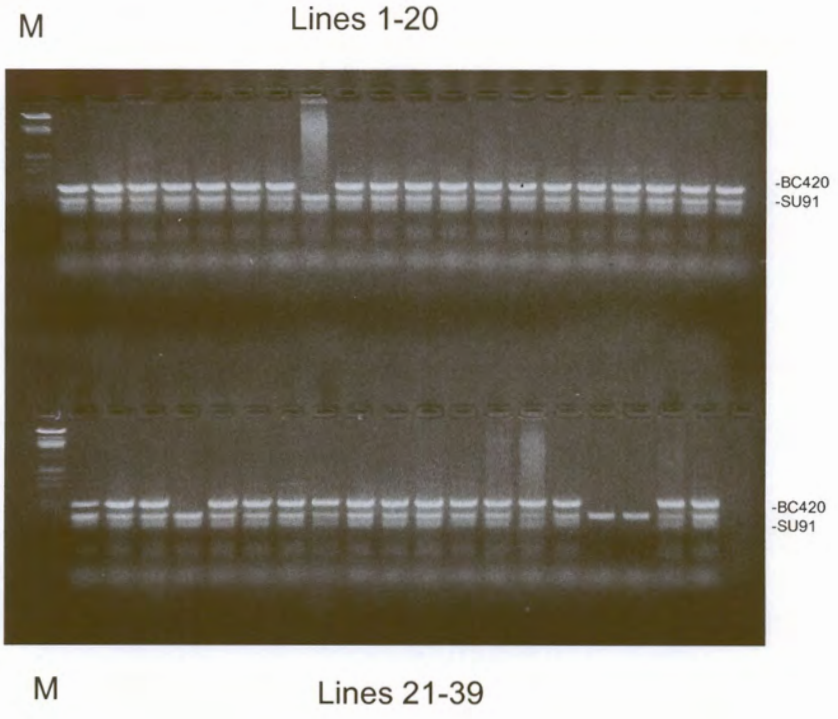

Figure 2. Screening of advanced XAN 159 derived Teebus lines with improved CBB resistance for presence of SCAR markers SU91 and BC420

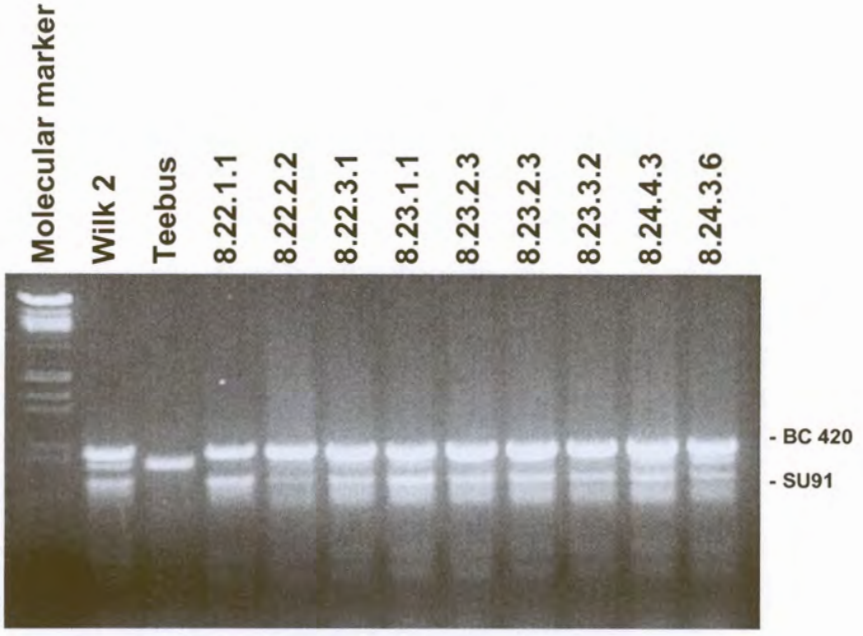

Figure 3. Screening of advanced Wilk 2 derived Teebus lines with improved CBB resistance for presence of SCAR markers SU91 and BC420

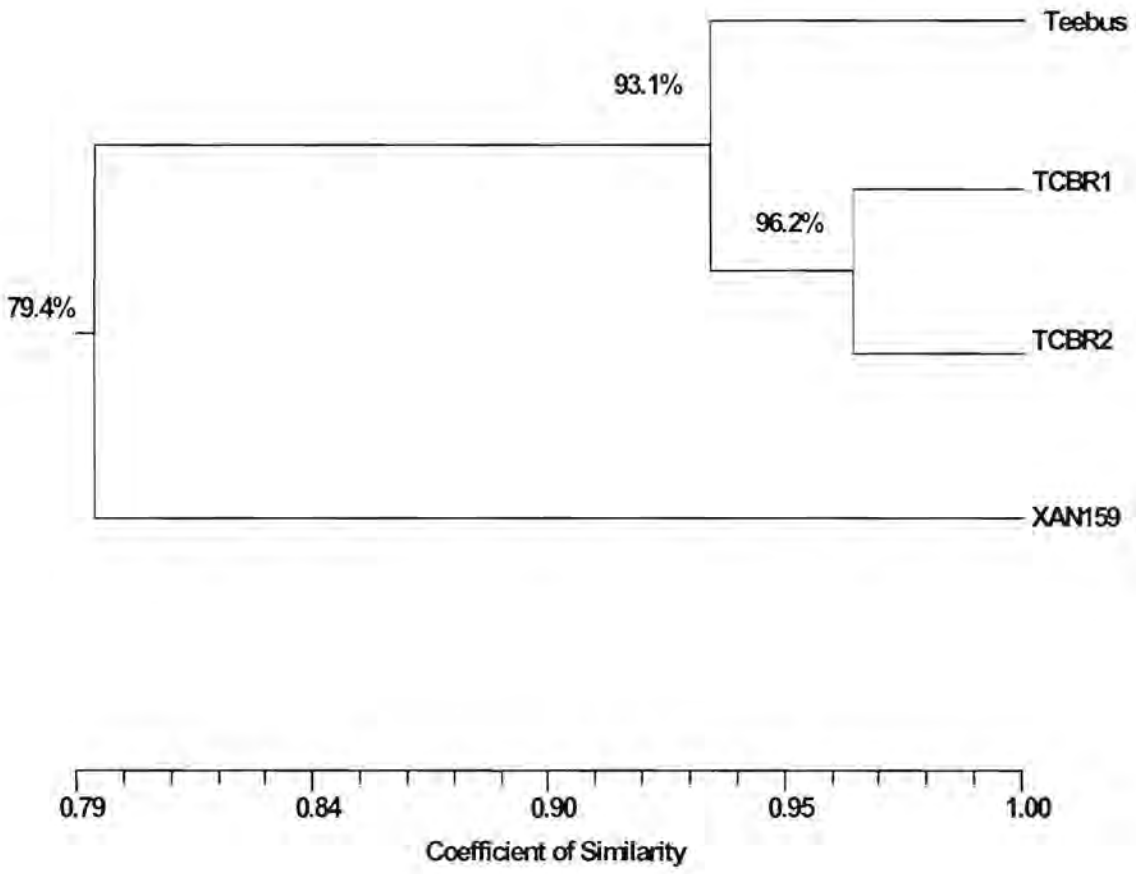

Figure 4. Dendogram of near-isogenic lines derived from backcrosses with Teebus as recurrent susceptible parent and XAN 159 as resistant donor parent

CHAPTER 8

APPLICATION OF MOLECULAR MARKERS IN BREEDING FOR BEAN COMMON BLIGHT RESISTANCE IN SOUTH AFRICA

ABSTRACT

Sequence characterized amplified region (SCAR) markers, linked to four independent quantitative trait loci (QTL) in XAN 159 and GN #1 Nebr. sel. 27, are available for indirect selection of resistance to common bacterial blight in *Phaseolus vulgaris*. Existing SCAR-markers, SU91, BC420, BC409 and SAP6, were evaluated for potential use in the local breeding programme. Segregating populations of progenies developed through backcross breeding with cultivars Teebus and Kranskop as susceptible recurrent parents and XAN 159 and Vax 4 as resistant donor parents were evaluated for presence of existing markers. Presence of all four markers in improved Teebus lines (XAN 159 derived), confirmed successful transfer of resistance in these lines. Marker BC420 was absent in XAN 159 derived Kranskop-lines. These lines were only moderately resistant when tested in the greenhouse, indicating that the QTL linked to this marker is important in order to obtain high levels of resistance. Progenies from first backcrosses with Kranskop as recurrent parent using Vax 4 have exhibited high levels of resistance when tested in the greenhouse and presence of all markers found in Vax 4 confirms transfer of resistance. Results gained from this study indicate that marker assisted selection can successfully be implemented in breeding for common bacterial blight resistance in South Africa.

INTRODUCTION

Reliable field and greenhouse screening methods are important in resistance breeding for phenotypic selection of resistant plant progenies. Screening for resistance may be limited when breeders are challenged with mixed races of a pathogen or when many resistance genes are present in the host (Kelly & Miklas 1999). Climatic and environmental conditions can influence disease development in the field and the possibility of escapes also exists. Indirect selection for resistance using molecular markers offers breeders a viable alternative to confirm presence of favourable gene combinations in new breeding lines (Kelly & Miklas 1999).

Molecular markers linked to common bacterial blight (CBB) resistance in dry beans (*Phaseolus vulgaris*) have been developed for indirect selection of resistance to *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Vauterin, Hoste, Kosters & Swings, (Bai *et al.* 1996, Beattie *et al.* 1998, Park *et al.* 1999, Yu *et al.* 1999). Resistance sources, GN #1 Nebr. sel 27 and XAN 159 each contribute two independent quantitative trait loci (QTL) with major effects on CBB resistance (Miklas *et al.* 2000). Sequence characterized amplified region (SCAR) markers linked to these four QTL are available for DNA marker-assisted breeding (Miklas *et al.* 2000). Advanced cranberry, pinto and snap bean germplasm with combined resistance to common blight has been developed in the USA using these markers in the selection process (Miklas *et al.* 2000).

Yu *et al.* (1999) screened 138 F5 lines derived from HR67 (resistance derived from XAN 159), using SCAR-marker BC420, and subsequently tested these lines for CBB resistance in the greenhouse. Based on marker information, 28 of the 138 lines had the SCAR band present and were predicted to be resistant. Comparison of

SCAR results with field data showed that 23 of 28 plants gave a resistant phenotypic reaction (rating<2.0), indicating an accuracy of 82%. Only 3.6% of the lines were misclassified as resistant. Cost estimates indicated that use of marker-assisted selections costs approximately one third less than greenhouse testing (Yu *et al.* 1999).

Expression of QTL may differ over environments or populations in various crops (Park *et al.* 1999). Marker-QTL associations need to be confirmed in a breeding programme, particularly for traits such as CBB resistance that have complex inheritance patterns, low narrow-sense heritabilities and multiple genes involved (Park *et al.* 1999).

Pyramiding of genes, expressing resistance to the same pathogen in a single cultivar, is necessary to achieve stable resistance. Use of marker-assisted selection can contribute considerably when pyramiding of genes is attempted (Kelly & Miklas 1999, Sing & Muñoz 1999, Dursun *et al.* 1995). Independence of resistance genes to be combined, however, needs to be closely monitored as many lines and cultivars have common sources of CBB resistance (Kelly and Miklas 1999).

Development of resistant cultivars is important in controlling CBB in South Africa. The aim of the present study was to determine whether SCAR-markers linked to four independent QTL derived from XAN 159 (SU91 and BC420) and GN #1 Nebr. sel 27 (SAP6 and BC409), could be used for indirect selection of resistance in the local breeding programme.

MATERIAL AND METHODS

Segregating populations of progenies from backcrosses were used for indirect selection of resistance using available SCAR markers SU91, BC420 (XAN 159 derived) and BC409 and SAP6 (GN # 1 Nebr. sel. 27 derived) (Tables 1 & 3). Teebus and Kranskop were included as suceptible checks. Genotypes XAN 159, GN #1 Nebr. sel. 27, Wilk 2, Wilk 4, Wilk 6, Vax 3, Vax 4, Vax 5 and Vax 6 were used as resistant checks. A resistant line (48.15), developed in South Africa through interspecific crosses between *P. vulgaris* and *P. acutifolius,* as well as segregating populations with combined rust and CBB resistance (U 12 and C 18) were also included (Table 3).

Phenotypic screening

Ten seeds of each line were planted in 20 litre plastic bags (4 seeds per bag) in sterile soil and maintained in a greenhouse at 18°C night/28°C day. Inoculum was prepared by suspending 48- to 72-h-old cultures (isolates X6 and Xf105) in sterile distilled water, and adjusting it to 10^8 CFU/ml using a Shimadzu UV-260 spectrophotometer. Fourteen to 20-day-old plants with fully expanded first trifoliate leaves were inoculated using the multiple needle inoculation method (Andrus 1948). Inoculated plants were kept in a greenhouse at 18°C night/28°C day. Plants were rated for infection 14 days after inoculation on a 1 to 9 scale (Aggour *et al.* 1989) with 1 being resistant and 9 susceptible.

Isolation of genomic plant DNA

Young leaves from resistant plants and susceptible checks were harvested and washed with sterile distilled water. Washed leaves were freeze-dried separately for each plant and kept at -20°C until further use. DNA was isolated from sampled leaves using a modified version of the method described by Graham *et al.* (1994). Freeze-dried leaves were ground to fine powder for DNA extraction and a volume of 750 µl CTAB buffer (100 mM Tris [tris(hydroxymethyl) aminomethane], pH 8.0; 20 mM EDTA (ethylenediaminetetraacetate), pH 8.0; 1.4 mM NaCl; 2% (w/v) CTAB (hexadecyltrimethylammonium bromide); 0.2% (v/v) ß-mercaptho-ethanol added to approximately 250 µl of the fine leaf powder in a 1.5 ml microfuge tube. The suspension was thoroughly mixed and the tube incubated at 65°C for one hour. A 500 µl volume of chloroform: isoamyl alcohol (24:1) was added and the suspension mixed by gentle inversion. After centrifugation at 14 000 rpm for 3 min, the upper aqueous layer was transferred to a fresh tube containing 500 µl isopropanol, mixed by gentle inversion and incubated at room temperature for 20 min. The suspension was centrifuged at 14 000 rpm for 5 min, 500 µl 70% (v/v) ethanol added and incubated at room temperature for 20 min. DNA was precipitated at 14 000 rpm for 5 min, the pellet air-dried for 1 hr, and resuspended in TE buffer (10 mM Tris-Cl, pH 8.0; 1 mM EDTA, pH 8.0). Resuspended DNA was extracted with 1/10 volume 7.5 M ammonium acetate and an equal volume of chloroform: isoamyl alcohol (24:1). The aqueous layer was transferred to a fresh tube containing two volumes of cold absolute ethanol. Precipitated DNA was washed three times in cold 70% (v/v) ethanol, the pellet air-dried, and resuspended in TE buffer. DNA was treated with RNase for 2 hr at 37°C, after which concentration and purity were estimated by

measuring absorbances at A_{260} and A_{280}. DNA samples were diluted to a working solution of 200 ng/µl.

SCAR markers and PCR reactions

SCAR primers, BC409, SAP6, SU91 and BC420 (Table 2), were synthesized by GibcoBRL (Life Technologies, Glasgow, United Kingdom), based on primer sequences obtained from Miklas *et al.* (2000). Primers were suspended in TE buffer to a concentration of 200 pmol/µl. A work solution of 10 pmol/µl was prepared. SCAR markers were used for the polymerase chain reaction (PCR) based on the protocol of Williams *et al.* (1990) with minor modifications. Amplification reactions were performed in a 25 µl reaction volume containing Promega (Promega Corporation, Madison, Wisconsin) reaction buffer (500 mM KCl; 100 mM Tris-HCl, pH 9.0; 1% (v/v) Triton X-100), 2 mM $MgCl_2$, 100 µM of each dNTP (dATP, dCTP, dGTP, dTTP), 10 pmol primer, 1 unit *Taq* DNA polymerase (Promega) and 25 ng template DNA. Reactions were performed using a PCR Sprint Thermal Cycler (Hybaid Limited, UK) programmed for 5 min at 94°C, 30 cycles of 1 min at 94°C, 1 min at 58°C for SAP6, SU91 and BC420 primers and 50°C for BC409 primers, and 1.5 min at 72°C, followed by one cycle of 5 min at 72°C.

Amplification products were analysed by electrophoresis in 1.5% (w/v) agarose gels (Seakem LE) at 80V for 2 h using UNTAN buffer (0.4 M Trisbase, 0.02 M EDTA, pH 7.4) and detected by staining with 1 µg/ml ethidium bromide. Gels were photographed under UV light with Polaroid 667 film.

RESULTS

Phenotypic disease reaction

Phenotypic reactions of lines are shown in Table 1. Genotypes XAN 159, Wilk 2, Wilk 4, Wilk 6, Vax 3, Vax 4, Vax 5 and Vax 6 were resistant to local Xap and Xapf isolates (ratings ranging from 1-2.7). GN #1 Nebr. sel. 27, Teebus and Kranskop were susceptible with mean disease ratings of 8.0, 9.0 and 7.5, respectively.

Breeding lines developed through backcross breeding were segregating and produced both susceptible and resistant plants. Susceptible plants were discarded and resistant plants used for molecular studies. Small white breeding lines (TCBR1, TCBR2, U12 and C18) were highly resistant (rating = 1.0). Large seeded red speckled sugar beans (PC 1470 BC2 1 and PC 1470 B2 3.1) were moderately susceptible (rating = 4.3-4.9), while 48.1 and PC 2536-BC1 were resistant.

Indirect screening using molecular markers

SCAR markers SAP6 and BC409 (GN #1 Nebr. sel. 27) were present in all the lines tested except for XAN 159, Wilk 2 and Wilk 4. Marker BC409 was absent in the breeding line PC 1470 BC2 3.1, while SAP6 was present in this line (Figures 1-3; Table 3). The Vax lines were not tested for marker BC409. Marker SU91 (XAN 159 derived) were present in all the lines except Teebus, Kranskop, GN #1 Nebr. sel. 27 and 48.15 (Figures 1-3; Table 3). The other XAN 159 derived marker (BC420) was only present in XAN 159, Wilk 2, Wilk 4, Wilk 6, TCBR 1, TCBR 2, U12 and C18 (Figures 1-3; Table 3).

DISCUSSION

Results of this study indicate that existing markers for indirect selection of CBB resistance can be successfully used in the South African breeding programme. Presence of markers SAP6 and BC409 (GN #1 Nebr. Sel.27), in local cultivars Teebus and Kranskop, were most likely inherited from parents used to develop these cultivars. GN #1 Nebr. sel. 27 was derived from interspecific crosses between *P. vulgaris* and *P. acutifolius* and has been used in many breeding programmes as a resistance source (Coyne & Schuster 1974, Mohan & Mohan 1983). Although susceptible in South Africa, GN #1 Nebr. sel 27 and lines derived from it, have resistance in the USA (Coyne and Schuster 1974) and Spain (C. Assensio, MBG-CSIC: personal communication).

The Wilk lines were developed at Cornell University, USA and although the exact pedigree and germplasm used are not known, combined resistance genes from *P. vulgaris*, *P. coccineus* and *P. acutifolius*, including XAN 159 or its sister lines, were used in developing these lines (Singh & Muñoz 1999). Presence of markers SU91 and BC420 in Wilk lines confirms that resistance in XAN 159 (or the same source) was used in developing these lines. Resistance in these lines was superior to that of XAN 159 when inoculated with local isolates, thus confirming the presence of additional CBB resistance genes.

Presence of four existing markers, SU91, BC420, SAP6 and BC409, in small white canning bean lines (TCBR1, TCBR2, U12 and C18), developed through backcross breeding with XAN 159, confirmed successful transfer of resistance. Greenhouse results indicated that these lines had higher levels of resistance than XAN 159. This could be attributed to the combined resistance from GN Nebr. #1 sel.

27 and XAN 159 present in these lines. Although GN #1 Nebr. sel. 27 is susceptible to local isolates when tested on its own, it seems to contribute to higher levels of resistance when combined with XAN 159 resistance. Near-isogenic lines were also developed through backcross breeding with Teebus as susceptible recurrent parent and Wilk 2 as resistant donor parent in a separate breeding programme (*vide* Chapter 7) and these lines also have both markers (SU91 and BC420) present (D. Fourie, unpublished data). In order to combine these lines with near-isogenic Teebus lines derived from XAN 159, it is necessary to develop markers linked to additional QTL in Wilk lines.

Marker BC420 was absent in XAN 159 derived Kranskop-lines (PC 1470 BC2 1 and PC 1470 BC2 3.1) with acceptable seed colour (red speckled seed). These lines were only moderately resistant when tested in the greenhouse, indicating that the QTL linked to this marker is important in order to obtain high levels of resistance. Resistance of Kranskop lines, however has been improved and this can be attributed to presence of the QTL linked to the SU91 marker. The BC420 marker is located near the *V*-locus conditioning purple flower colour (Miklas *et al*. 2000). High levels of resistance have been identified in some Kranskop derived lines (D. Fourie, unpublished data) but highly resistant plants all had purple flowers resulting in these plants producing seed with unacceptable colour.

The BC420 marker was also absent in the Vax lines. These lines were recently developed at CIAT (International Centre for Agriculture in the Tropics) and are highly resistant to Xap and Xapf. Absence of marker BC420 could indicate that the linkage between the *V*-locus and the resistance gene has been broken and that although the resistant gene may be present, the marker is absent (P.N. Miklas, USDA: personal communication). The possibility also exists that other untagged

genes could contribute to the high levels of resistance present in these lines, as resistant genes from different sources have been pyramided into these lines (Singh & Muñoz 1999). Resistance from Vax lines should be used in improving resistance in large seeded (red speckled sugar) bean varieties. Progenies from first backcrosses with Kranskop as recurrent parent using Vax 4 exhibited high levels of resistance when tested in the greenhouse and presence of all markers found in Vax 4 confirms transfer of resistance.

The locally developed line 48.15, developed through interspecific crosses between *P. vulgaris* and *P. acutifolius*, was highly resistant when tested in the greenhouse. PCR studies indicated that resistance was not the same as XAN 159 (markers absent) and attempts should be made to combine this resistance in XAN 159 derived Kranskop lines.

XAN 159 derived CBB resistant Teebus lines have been successfully combined with rust resistant Teebus lines developed in an independent breeding programme. Markers are also available to confirm rust resistance (Stavely 2000). The use of markers is especially advantageous when combining resistance to different diseases into one cultivar.

Results gained from this study show that marker-assisted selection can successfully be implemented in breeding for common bacterial blight resistance in South Africa. The use of molecular markers alone, however, has not resulted in lines with resistance superior to that of XAN 159 in the USA (R. Riley, Syngenta, USA: personal communication). This suggesets that some minor genes contributing to CBB resistance are lost when relying on markers only. The combined use of both phenotypic screening and molecular markers is, therefore, important in developing CBB resistant lines.

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Table 1. Phenotypic disease reaction of dry bean lines artificially inoculated in the greenhouse using the multiple needle method (Andrus 1948)

Cultivar/Line	Mean disease rating (1-9 scale)
Teebus	9.0
Kranskop	7.5
XAN 159	2.3
GN #1 Nebr. sel.27	8.0
Wilk 2	1.3
Wilk 4	1.4
Wilk 6	2.7
Vax 3	2.8
Vax 4	1.3
Vax 5	2.4
Vax 6	2.1
PC 2536- BC1(1-8)	1.0
48.15	1.3
TCBR 1	1.0
TCBR 2	1.0
PC 1470 BC2 1	4.3
PC 1470 BC2 3.1	4.9
U12	1.0
C18	1.0

Table 2. SCAR markers used to screen segregating populations

Primer	Sequence (5'-3')	PCR product size	Resistance source	Linkage group
BC409-1	TAGGCGGCGGCGCACGTTTTG	1 250 bp	GN#1 sel 27	B10
BC409-2	TAGGCGGCGGAAGTGGCGGTG			
SAP6-1	GTCACGTCTCCTTAATAGTA	820 bp	GN#1 sel 27	B10
SAP6-2	GTCACGTCTCAATAGGCAAA			
SU91-1	CCACATCGGTTAACATGAGT	700 bp	XAN159	B8
SU91-2	CCACATCGGTGTCAACGTGA			
BC420-1	GCAGGGTTCGAAGACACACTGG	900 bp	XAN159	B6
BC420-2	GCAGGGTTCGCCCAATAACG			

Table 3. Presence and absence of molecular markers in dry bean genotypes as indicated in Figures 1, 2 and 3

Cultivar/Line	Description	SCAR Markers			
		XAN 159		GN #1 Nebr. sel.27	
		SU91	BC420	SAP6	BC409
Teebus	Small white canning bean (*Phaseolus vulgaris*, susceptible parent)	-	-	+	+
Kranskop	Red speckled sugar (*P. vulgaris*, susceptible parent)	-	-	+	+
XAN 159	Resistant line (*P. acutifolius* x *P. vulgaris*)	+	+	-	-
GN #1 Nebr. sel.27	Resistant line (*P. vulgaris* x *P. acutifolius*)	-	-	+	+
Wilk 2	Resistant line (sources unknown)	+	+	-	-
Wilk 4	Resistant line (sources unknown)	+	+	-	-
Wilk6	Resistant line (sources unknown)	+	+	+	+
Vax 3	Resistant line (*P. vulgaris* x *P. acutifolius* + gene pyramiding)	+	-	+	nt
Vax 4	Resistant line (*P. vulgaris* x *P. acutifolius* + gene pyramiding)	+	-	+	nt
Vax 5	Resistant line (*P. vulgaris* x *P. acutifolius* + gene pyramiding)	+	-	+	nt
Vax 6	Resistant line (*P. vulgaris* x *P. acutifolius* + gene pyramiding)	+	-	+	nt
PC 2536- BC1(1-8)	BC 1 lines (Kranskop / Vax 4)	+	-	+	nt
48.15	Resistant line developed locally through interspecific crosses	-	-	+	+
TCBR 1	Backross inbred line (Teebus / XAN 159)	+	+	+	+
TCBR 2	Backross inbred line (Teebus / XAN 159)	+	+	+	+
PC 1470 BC2 1	BC2 line (Kranskop / XAN 159)	+	-	+	+
PC 1470 BC2 3.1	BC2 line (Kranskop / XAN 159)	+	-	+	-
U12	Segregating lines with combined rust and CBB resistance	+	+	+	+
C18	Segregating lines with combined rust and CBB resistance	+	+	+	+

+ = marker present; - = marker absent; nt = not tested

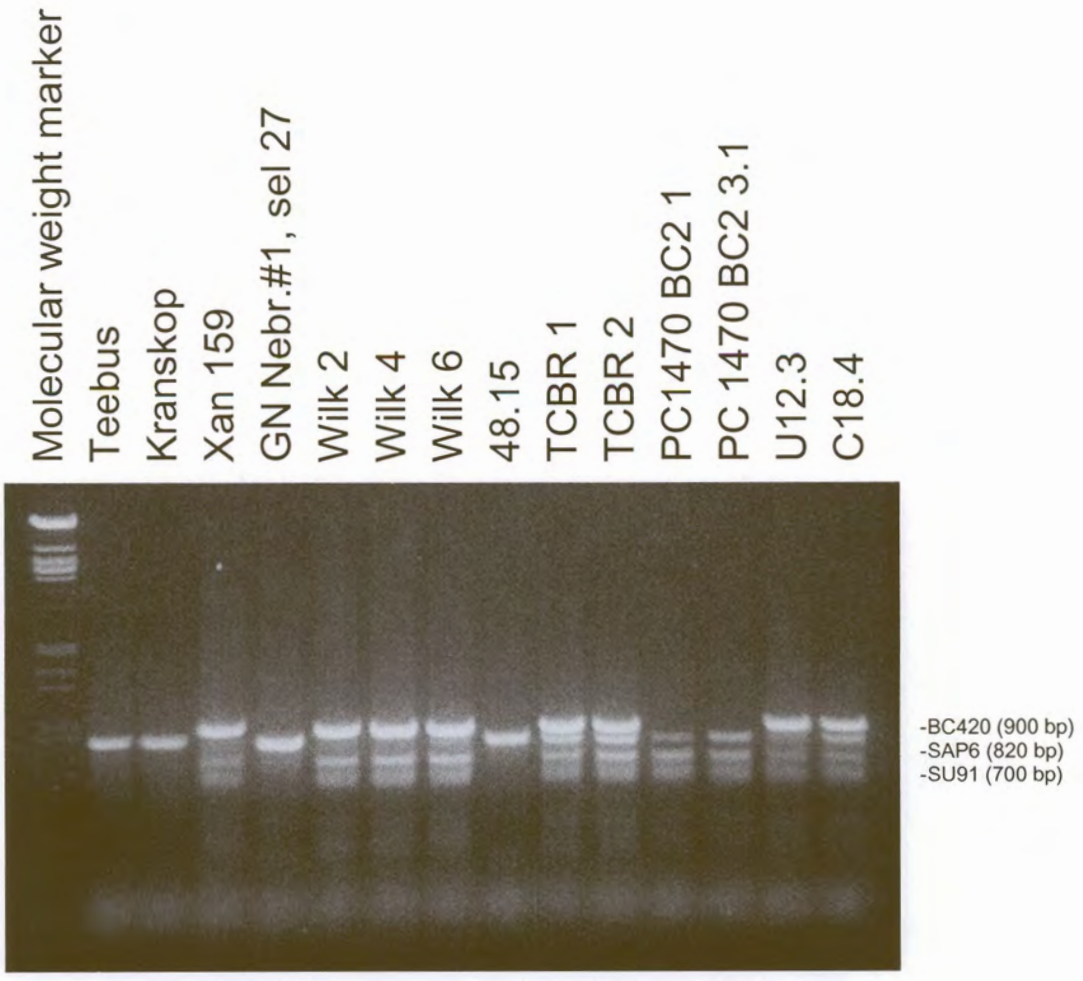

Figure 1. Screening lines for presence of SCAR markers BC420, SAP6 and SU91

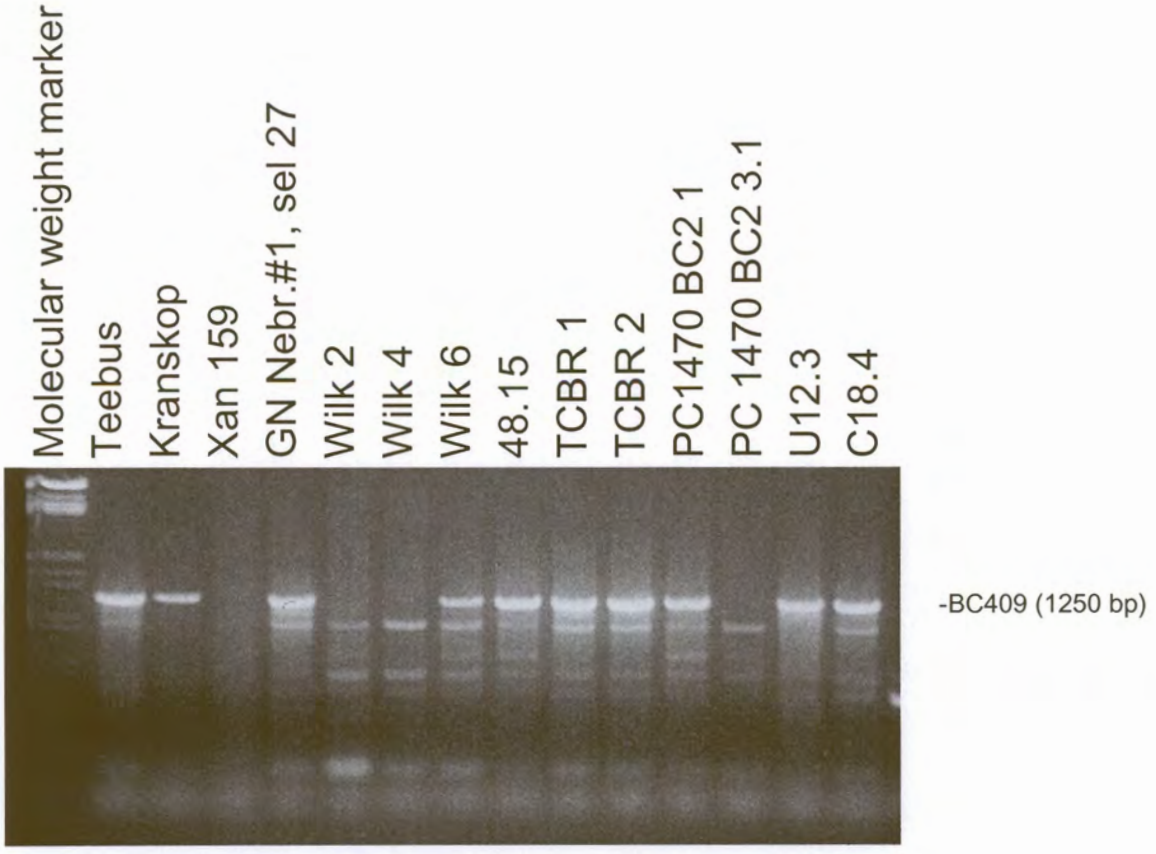

Figure 2. Screening of lines for presence of SCAR marker BC409

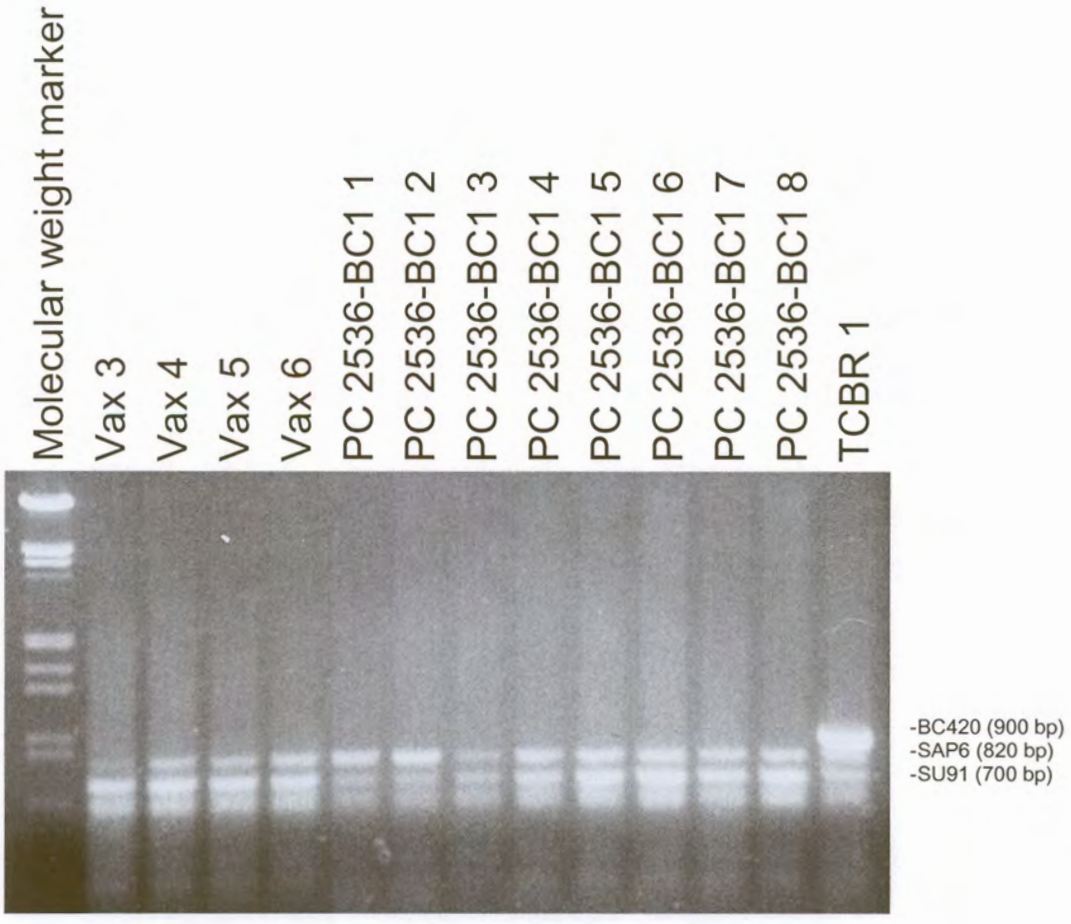

Figure 3. Screening of lines for presence of SCAR markers SU91, BC420 and SAP6

CHAPTER 9

YIELD LOSS ASSESSMENT IN SOUTH AFRICAN DRY BEAN GENOTYPES CAUSED BY COMMON BACTERIAL BLIGHT

ABSTRACT

Trials were conducted in Potchefstroom (Northwest Province), Delmas (Mpumalanga) and Cedara (KwaZulu/Natal) during the 2001/2002 season to assess yield loss in dry bean genotypes caused by common bacterial blight. The effect of genotype and environment on this disease was determined using one susceptible cultivar (Teebus) and two resistant near-isogenic Teebus-lines (TCBR1 and TCBR2). Different parameters (disease ratings, % leaf area loss and % infection) were used to evaluate disease. Disease incidence was high in plots containing the susceptible cultivar Teebus. Genotypes differed significantly in their susceptibility to common bacterial blight. Copper sprays reduced the percentage leaf area loss and enhanced seed size. Disease-free plots, however, were not maintained using copper sprays. Common bacterial blight significantly reduced yield and seed size in the susceptible cultivar, Teebus. Yield losses of 43.5% were observed in diseased Teebus plots after artificial inoculation with the common bacterial blight pathogen. The resistance introduced, into the near-isogenic lines, upon release in the industry, will contribute to reducing the impact of common bacterial blight in future production of the small white canning bean.

INTRODUCTION

Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) (Smith) Vauterin, Hoste, Kosters & Swings and its fuscans variant, *X. axonopodis* pv. *phaseoli* var. *fuscans* (Xapf) is considered one of the most important bean diseases worldwide (CIAT 1985). This seed-borne disease is widespread and occurs in temperate, subtropical and tropical regions (Singh 1991). CBB affects foliage, stems, pods and seeds of beans and causes severe damage under favourable environmental conditions (Yoshii 1980). An integrated disease management approach, including cultural practices, copper-based chemical sprays and resistant varieties, is needed to adequately control CBB (Allen *et al.* 1998, Schwartz & Otto 2000).

Although CBB is widely distributed, yield losses have not been well documented. In Colombia, estimated yield losses of 22% and 45% have been documented after natural and artificial infection, respectively (Yoshii 1980). Estimated losses of up to 38% have been reported in field trials in Ontario, Canada by Wallen & Jackson (1975). They indicated that yield loss is primarily due to defoliation early in the season, as a result of severe leaf infection. In addition to reduction in number of seeds, CBB also causes reduction in seed size (Wallen & Jackson 1975). Common blight in Uganda has been associated with yield depression in beans and losses varied depending on susceptibility of varieties, developmental stage of crop at the time of infection, and climatic conditions during the season (Opio *et al.* 1992).

Moffet & Middleton (1979) obtained significant yield differences between inoculated and un-inoculated common blight plots of navy beans. Although strict measures were taken to maintain disease-free un-inoculated plots, seed transmission and spread from inoculated seedlings, resulted in CBB development in these plots. Yield differences were, therefore, measured between plots with different disease levels rather than between diseased and healthy plots. Depression of yield and seed quality loss was clearly related to the disease level in the crop (Moffet & Middleton 1979).

CBB yield loss assessment studies are difficult to conduct because maintenance of disease free plots in these experiments verges on the impossible. Disease-free plots can, however, be obtained using resistant varieties, if these are available. The aim of the study was, therefore, to assess yield losses in bean caused by CBB using a local commercial cultivar Teebus and two near-isogenic Teebus lines (TCBR1 and TCBR2) with improved CBB resistance. The effect of genotype and environment on CBB was also considered.

MATERIAL AND METHODS

Trials were conducted in Potchefstroom (Northwest Province), Delmas (Mpumalanga) and Cedara (KwaZulu/Natal) during the 2001/2002 season, to assess yield losses as a result of CBB and to determine the effect of genotype and environment on this disease. Three genotypes, cv. Teebus (susceptible, small white canning bean) and two resistant near-isogenic Teebus-lines, TCBR1 and TCBR2, were randomly planted in four row plots, 5 m in length with 750 mm inter-row and

75 mm intra-row spacing. Resistant lines, TCBR1 and TCBR2, were developed through backcross breeding (*vide* Chapter 7) using XAN 159 as resistant donor parent and cv. Teebus as recurrent susceptible parent. Plots were arranged in a split-plot design consisting of three replications with genotypes as main plots and spray treatments as sub-plots. Herbicide (flumetsulam/sulfonanilide, 1 $l.ha^{-1}$) was applied directly after planting. Trials were sprayed with a systemic fungicide (flusilazol/carbendazim, 350 $ml.ha^{-1}$), after seedling emergence and before flowering, to control rust [*Uromyces appendiculatus* (Pers.) Ung.].

A mixture of Xap and Xapf isolates was used to inoculate two rows (sub-plots) of each main plot. Inoculum was prepared from 48- to 72 h cultures grown on yeast-extract-dextrose-calcium-carbonate agar (YDC) (Schaad & Stall 1988). Bacterial growth was suspended in tap water and adjusted to 10^8 CFU/ml water. Trials conducted in Potchefstroom were irrigated prior to inoculation, and thereafter, at weekly intervals, to enhance disease development.

Plots were artificially inoculated in the late afternoon using a motorized backpack sprayer at 21, 29 and 36 days after planting. The trial in Delmas received one spray of inoculum at 29 days after planting. Trials at Delmas and Cedara were not irrigated and were treated as dry land production units. Copper ammonium acetate (500 $ml.ha^{-1}$) was applied to the remaining two un-inoculated rows of each plot, using a knap-sack sprayer. Different parameters (disease ratings, % leaf area loss and % infection) were used to evaluate disease at full pod set stage. Plots were rated for CBB using a 1-9 scale (Van Schoonhoven & Pastor-Corrales 1987) with 1 being resistant (no disease present) and 9 being susceptible (dead plants). Percentage leaf area loss was visually determined on 15 randomly selected plants

within each sub-plot. Each leaf on six randomly selected plants per sub-plot was categorized into different classes (0=no symptoms; 1=1-20% of leaf affected; 2=20-40% of leaf affected; 3=40-60% of leaf affected; 4=60-80% of leaf affected; 5=80-100% of leaf affected) and percentage infection calculated using the formula [($\sum$n.v / i.N) x 100] where n=number of leaves per class, v=class value, i=highest class value and N=total number of leaves (Townsend & Heuberger 1943).

At maturity, the number of pods per sub-plot was calculated, harvested and yield recorded. Data were analysed using a multi-factorial analysis of variance (Statgraphics Plus 5.0) with genotype (whole plot) and treatment (sub-plot) as factors. Coefficients of linear correlations were used to determine the relationships between variables measured. Relationships between yield loss, disease (% infection) and percentage leaf area loss were determined using linear regression analysis.

RESULTS

Genotype by disease reactions at Potchefstroom and Cedara are given in Table 1. No disease developed at Delmas and this locality was, therefore, not included. Lines TCBR1 and TCBR2 were resistant at both localities (rating 1.8-2.7) and differed significantly from Teebus, which was susceptible (rating 6.7-7.8). In general, TCBR1 was the most resistant line and differed significantly from TCBR2, when rated for CBB resistance. Incidence of CBB was significantly higher in Potchefstroom (78.6%) than in Cedara (53.4%). Copper sprays were not effective in preventing plants from becoming infected in un-inoculated sub-plots. Differences in loss of leaf area,

however serves as an indication of expected loss under higher levels of inoculum pressure (Table 2).

Genotypes differed significantly in the number of pods produced (Table 3). TCBR2 yielded the largest number of pods and differed significantly from TCBR1 and Teebus.

Yields obtained from TCBR1 and TCBR2 differed significantly from Teebus in Potchefstroom and Cedara (Table 4). Teebus differed significantly from TCBR2 in Delmas but not from TCBR1.

Genotypes differed significantly with regard to seed size (100 g seed mass) in inoculated plots at all localities (Table 5). Copper-sprayed un-inoculated treatments significantly increased seed size of TCBR1 and TCBR2 at all localities. However this increase in seed size was only significant in Teebus at Delmas.

Correlations between variables are shown in Table 6. The percentage infection correlated positively with leaf area loss (P=0.97) and rating (P=0.98), and had a negative correlation with yield (P=-0.91), seed size (P=-0.62) and number of pods (P=-0.89). Positive correlations existed between percentage leaf area loss and rating (P=0.94). Percentage leaf area loss, however correlated negatively with yield (P=-0.94), seed size (P=-0.75) and number of pods (P=-0.81). Negative correlations were found between disease rating and yield (P=-0.88) and between rating and number of pods (P=-0.91). Positive correlations existed between seed size and yield (P=0.67) and number of pods and seed size (P=0.83). Seed size did not correlate with disease rating or number of pods.

Leaf area loss, disease (% infection) and yield loss relationships are presented in Figure 1. R^2-values (R^2=0.88 and R^2=0.83, respectively) indicated

stable linear relationships between disease parameters and yield loss. Losses of up to 43.5% were observed in cv. Teebus as a result of high CBB (89.6%) incidence.

DISCUSSION

Results of this study showed significant genotype x environment interactions as well as yield loss due to CBB on dry beans. Differences in CBB incidence between localities could have resulted from differences in environmental factors. No disease developed in Delmas and this could be due to the prevailing warm, dry conditions at that locality (P.J. Koen, ARC-GCI: personal communication) as well as inadequate inoculations. Although disease incidence in plots of resistant lines TCBR1 and TCBR2, was low, these plots were not free of disease. In these plots, the majority plants did not exhibit any symptoms but a few plants were diseased and this could have resulted from segregation still occurring in these lines. Resistance stability is a concern in CBB resistance breeding because segregation has been recorded in populations after more than twelve generations of selfing (Singh & Muñoz 1999).

Copper sprays were not effective in maintaining plots free of disease. Although this chemical had an effect on the percentage leaf area loss and seed size, no yield increases were obtained in un-inoculated copper sprayed plots. Efficacy of chemical control has been shown to be limited (Allen *et al.* 1998) and resultant yield increases minimal (Saettler 1989). Yield of the susceptible cv. Teebus was significantly reduced in Potchefstroom, where disease incidence was high. Yield differences of approximately 1000 kg were recorded in cv. Teebus, when compared to yields obtained from resistant lines TCBR1 and TCBR2. Such yield reductions

could have serious financial implications for the producer. The highest yields recorded were in Delmas where no disease occurred. Although yields in TCBR2 differed significantly from that of Teebus, yields between the genotypes were not as profound at Delmas as at the other localities. TCBR2 was the highest yielding line in Potchefstroom and Delmas and also produced the largest number of pods. This line, however, was slightly more susceptible than TCBR1.

CBB caused a significant reduction in seed size of genotypes. Seed size of cv. Teebus harvested at Potchefstroom was significantly reduced (19.0 g.100seed^{-1}) compared normal seed size (24 g.100seed^{-1}) under conditions of low disease pressure. The high percentage leaf area loss (85.0%), which occurred in this locality, could have contributed to the reduction in seed size recorded at this locality. Reduction in seed size as a result of CBB has also previously been recorded in field trials in Canada (Wallen & Jackson 1975).

Positive correlations between different disease parameters suggest that any of these can be used to quantify disease. Negative correlation between disease parameters, yield and seed size confirmed the effect of disease on yield and seed size. Linear regressions indicated a stable relationship between increased infection of CBB and yield loss. Yield losses of 43.5% that were observed in diseased plots, emphasise the economic threat of this disease for commercial dry bean producers.

Although disease-free plots were not achieved in the study, the use of lines with improved resistance, enabled us to quantify the effect of CBB on yield and seed size. Low disease incidence and superior yields, obtained from plots with resistant lines, illustrate the positive contribution that resistant cultivars can have on the South African dry bean industry.

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Table 1. Disease parameters for common bacterial blight assessment of three lines at two localities in inoculated common bacterial blight plots

Genotype	Rating (1-9 scale)		% Infection		% Leaf area loss	
	Potchefstroom	Cedara	Potchefstroom	Cedara	Potchefstroom	Cedara
Teebus	7.8 d	6.7 c	78.6 d	53.4 c	85.0 c	37.9 b
TCBR1	1.4 a	1.8 a	1.0 a	0.9 a	0.4 a	4.1 a
TCBR2	2.7 b	1.8 a	10.6 b	1.9 a	2.6 a	1.4 a

Means followed by different letters differ significantly according to LSD (P=0.05)

Table 2. Percentage leaf area loss of three lines in inoculated vs un-inoculated copper sprayed plots

Genotype	CBB inoculated plots	Un-inoculated copper sprayed plots
Teebus	71.1 c	51.7 b
TCBR1	1.8 a	2.7 a
TCBR2	2.2 a	1.8 a

Means followed by different letters differ significantly according to LSD (P=0.05)

Table 3. Total number of pods harvested from Teebus, TCBR1 and TCBR2 at Potchefstroom and Cedara

Genotype	Number of pods
Teebus	1746 a
TCBR1	2553 b
TCBR2	2776 c

Means followed by different letters differ significantly according to LSD (P=0.05)

Table 4. Yield data of Teebus, TCBR1 and TCBR2 recorded at Potchefstroom, Cedara and Delmas

Genotype	Yield kg.ha^{-1}		
	Potchefstroom	Cedara	Delmas
Teebus	1668 a	2165 b	3187 d
TCBR1	2653 c	2609 c	3364 de
TCBR2	2680 c	2511 c	3425 e

Means followed by different letters differ significantly according to LSD (P=0.05)

Table 5. 100g Seed mass of Teebus, TCBR1 and TCBR2 assessed from yields obtained at Potchefstroom, Cedara and Delmas

Genotype	Treatment	Locality		
		Potchefstroom	Cedara	Delmas
Teebus	Inoculated	19.0 a	23.7 hi	22.3 c
	Un-inoculated copper sprayed	19.0 a	23.9 ij	24.2 j
TCBR1	Inoculated	21.6 b	22.8 de	25.7 lm
	Un-inoculated copper sprayed	23.6 ghi	23.3 fg	25.0 k
TCBR2	Inoculated	22.5 cd	23.4 gh	25.8 m
	Un-inoculated copper sprayed	23.0 ef	23.0 ef	25.4 l

Means followed by different letters differ significantly according to LSD (P=0.05)

Table 6. Correlation coefficients of % infection, % leaf area loss, rating, yield, 100g seed mass and number of pods

Parameter	% Infection	% Leaf area loss	Rating	Yield	g.100 Seed^{-1}	No. pods
% Infection	-	0.97** (12)	0.98** (12)	-0.91** (12)	-0.62* (12)	-0.89** (12)
% Leaf area loss		-	0.94** (12)	-0.94** (12)	-0.75** (12)	-0.81** (12)
Rating			-	-0.88** (12)	-0.54 (12)	-0.91** (12)
Yield				-	0.67** (18)	0.83** (12)
g.100 Seed^{-1}					-	0.34 (12)
No. pods						-

* Correlations significant at P<0.05; ** Correlations significant at P<0.01

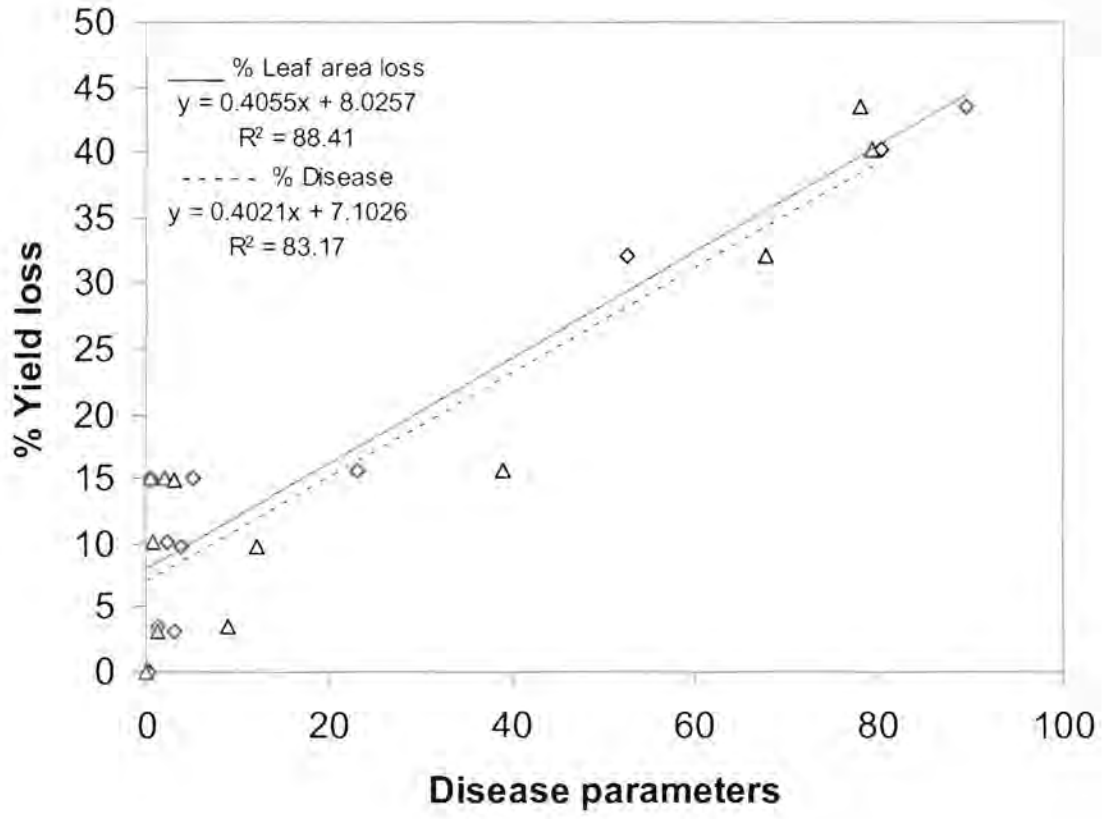

Figure 1. Relationship between yield loss, disease and leaf area loss in Teebus, TCBR1 and TCBR2 plots artificially inoculated with common bacterial blight at Potchefstroom and Cedara

CHAPTER 10

GENERAL DISCUSSION

This study has clarified a number of issues regarding bacterial diseases of dry beans (*Phaseolus vulgaris* L.) in South Africa. Information was gained on the incidence and severity of bacterial diseases, pathogenic variation that occurs in two of the three respective pathogen populations, susceptibility of cultivars to bacterial pathogens and deployment of resistance as long term control strategy to the most important disease.

Disease surveys conducted, to determine incidence, severity and occurrence of bacterial diseases in South Africa, indicated that common bacterial blight (CBB) and bacterial brown spot (BBS) were widespread and occurred in the majority of commercial and seed production areas. Incidence and severity of halo blight (HB) were low in both seed production and commercial fields. The widespread occurrence of bacterial diseases in seed production areas impacts strongly on the use of disease-free seed as sole local control strategy. Isolation of production fields is insufficient in South Africa and problems are encountered with seed production fields which are sometimes in close proximity of commercial fields. New seed production areas need to be obtained.

Use of disease free seed is an important primary control strategy, however, it does not guarantee freedom of bacterial diseases. Genetic resistance is considered the most effective and economic strategy for the control of bacterial diseases (Allen et al. 1998). Effective deployment of resistance requires knowledge of variation within a pathogen population (Taylor *et al.* 1996). Studies on pathogenic variation of *P. savastanoi* pv. *phaseolicola* (Burkholder) Gardan *et al.* indicated that seven (races 1,

2, 4, 6, 7, 8 & 9) of the internationally reported nine races (Taylor et al. 1996) occurred on dry beans in South Africa. Previously only races 1 and 2 have been reported from the country (Boelema 1994, Edington 1990). The increased number of races occurring locally could be contributed either to the introduction of new races into South Africa, or the international subdivision of the three previously described races into 9 different races by using the extended range of differentials (Taylor *et al.* 1996, Teverson 1991). Race 8 dominated the South African population of *P. savastanoi* pv. *phaseolicola*. This is consistent with the results of Taylor *et al.* (1996) who found race 8 mainly in Lesotho and Southern Africa. It, therefore, appears that this race might have originated from this region.

Pathogenicity and molecular characterization studies of *Xanthomonas axonopodis* pv. *phaseoli* (Xap) (Smith) Vauterin *et al.* and *X. axonopodis* pv. *phaseoli* var *fuscans* (Xapf), showed that diversity exists within these populations, in southern Africa. However, no races other than race 2, previously described by Opio *et al.* (1996) could be distinguished. A distinct differential reaction recorded for a single isolate (X539), may prove to represent another, as yet unrecorded, race of this pathogen. Continuous monitoring of CBB isolates in future is necessary to detect presence of isolates exhibiting differential reactions. DNA fingerprinting techniques revealed differences between Xap and Xapf isolates, indicating that these represent two distinct groups of bacteria. Similar distinction between these two groups, was also reported by Gilbertson *et al.* (1991) using RFLP's. Results obtained in this study indicate that both pathogenic and genetic variation exist in the CBB pathogen population in southern Africa. However, identical reactions with the majority of isolates on the tepary lines, showed that different CBB races do not occur. Information gained from this study

made it possible to select the most appropriate isolates to use in a resistance breeding programme.

Susceptibility of locally grown commercial cultivars to CBB, HB and BBS, were conducted to direct breeding strategies towards obtaining resistance to the most important bacterial disease in South Africa. Results indicated significant differences in susceptibility of South African cultivars to bacterial diseases. All the cultivars were susceptible to CBB, with Teebus, Cerillos, PAN 146 and PAN 159 being the most susceptible. Teebus is, currently, the only cultivar approved by the canning industry with acceptable canning quality. Improvement of resistance of this cultivar is thus important. Acceptable levels of resistance to HB were identified in commercial cultivars. Large seeded cultivars were generally more susceptible than small seeded cultivars and attempts should be made to improve HB resistance in some of these cultivars. Although cultivars differed significantly in their susceptibility to BBS, the majority of cultivars exhibited acceptable levels of resistance. Disease ratings and yield were, however, influenced by prevailing environmental conditions over the two seasons. Screening of cultivars for BBS resistance should, therefore, be conducted in multi-locational trials over seasons. Although a number of cultivars exhibited field resistance to HB and BBS, all cultivars were moderately to highly susceptible to CBB. This disease is, therefore, considered, the most important bean bacterial disease, in South Africa. Improvement of CBB resistance in South African cultivars would largely contribute to obtain stable yields. Improving of CBB resistance in Teebus was considered a priority because of its high commercial value.

Backcross breeding was used to improve CBB resistance in cv. Teebus, using resistance in XAN 159 and Wilk 2 sources, respectively. Phenotypic disease reaction

of advanced lines from this breeding programme indicated that resistance in cv. Teebus was successfully improved. High genetic relatedness between Teebus and near-isogenic lines, as shown in AFLP studies, indicated that characteristics of cv. Teebus has been recovered with the addition of the resistance gene(s) from XAN 159. Improvement of CBB resistance was thus, successfully accomplished in this study. Breeding for resistance in canning beans, however, should always progress within the boundaries set by the industry for canning quality. It is, therefore, important to maintain, as far as possible, the sought-after quality of the original cultivar. A final decision on release of advanced material, developed in this breeding programme, is taken once improved lines fulfill all criteria such as yield, quality, etc.

Sequence characterized amplified region (SCAR) markers linked to four independent QTL, derived from XAN 159 (SU91 and BC420) and GN #1 Nebr. sel 27 (SAP6 and BC409) (Miklas *et al.* 2000), were evaluated for possible use for indirect selection of CBB resistance in the local breeding programme. Presence of all four markers in improved Teebus lines, developed through backcross breeding with XAN 159, confirmed successful transfer of resistance. Greenhouse results indicated that these lines had higher levels of resistance than XAN 159. This could be attributed to the combined resistance from GN Nebr. #1 sel. 27 and XAN 159 present in these lines. XAN 159 derived CBB resistant Teebus lines have been successfully combined with rust resistant Teebus lines developed in an independent breeding programme. Markers are also available to confirm rust resistance (Stavely 2000). The use of markers is especially advantageous when combining resistance to different diseases into one cultivar.

All markers except for BC420 was present in XAN 159 derived Kranskop-lines

developed in another breeding programme (Fourie & Herselman 2002). These lines were only moderately resistant when tested in the greenhouse, indicating that the QTL linked to BC420 is important to obtain high levels of resistance. This marker is located near the *V*-locus conditioning purple flower colour (Miklas *et al.* 2000) and presence of it in large seeded breeding lines, results in resistant plants having purple flowers and therefore producing seed with unacceptable colour. Resistance from Vax 4 is currently used in improving resistance in large seeded (red speckled sugar) bean varieties. Absence of marker BC420 in Vax 4 could indicate that the linkage between the *V*-locus and the resistance gene has been broken and that although the resistant gene may be present, the marker is absent (P.N. Miklas, USDA: personal communication). Progenies from first backcrosses with Kranskop as recurrent parent using Vax 4 have exhibited high levels of resistance when tested in the greenhouse and presence of all markers found in Vax 4 confirms transfer of resistance.

Results gained from this study indicate that marker assisted selection can successfully be implemented in breeding for CBB resistance in South Africa. Advanced cranberry, pinto and snap bean germplasm with combined resistance to CBB has been developed in the USA using these markers in the selection process (Miklas *et al.* 2000).

Trials conducted to assess yield loss in Teebus and two resistant near-isogenic Teebus-lines (TCBR1 and TCBR2), indicated significant genotype, environmental and yield loss effects on CBB of dry beans. Linear regressions indicated a stable relationship between increased infection of CBB and yield loss. Yield losses of 43.5% were recorded in diseased Teebus plots. CBB also caused significant reduction in seed size. Yield losses varying between 22% and 45% have been documented by previous reporters (Wallen & Jackson1975, Moffet & Middleton 1979, Yoshii 1980, Opio *et al.*

1992). Yield differences were, however, measured between plots with different disease levels rather than between diseased and healthy plots. Although disease free plots were not obtained in the study, by using copper sprays, the use of lines with improved resistance enabled us to quantify the effect of CBB on yield and seed size. Low disease incidence and superior yields, obtained from plots with resistant lines, illustrates the positive contribution the use of resistant cultivars can make to the South African dry bean industry.

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BACTERIAL DISEASES OF DRY BEANS IN SOUTH AFRICA WITH SPECIAL REFERENCE TO COMMON BACTERIAL BLIGHT AND ITS CONTROL

by

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SUMMARY

Bacterial diseases, commonly associated with dry beans, often cause severe yield and seed quality loss. Disease surveys, as reported in chapter 2, indicated that common bacterial blight occurred in 83% and 85% of localities in seed and commercial dry bean production areas, respectively. Halo blight was restricted to cooler production areas and occurred in only 10% of seed production fields and 37% of commercial fields surveyed. Bacterial brown spot was the most widespread bacterial disease of dry bean, occurring in 93% of seed production fields and 100% commercial fields. Although incidences of bacterial diseases were high, severity was generally low. The widespread distribution of bacterial diseases in both seed and commercial production areas raises concern that the production of disease-free seed in South Africa might not represent an effective

control method.

In chapter 3 of this study, 255 *Pseudomonas savastanoi* pv. *phaseolicola* isolates, representative of all the localities and cultivars sampled, were categorized into different races according to their reaction on a set of differential cultivars. Seven races (1, 2, 4, 6, 7, 8 and 9) were identified with race 8, the most prevalent. Races 1, 2, 6 and 8 were widely distributed throughout the production area, while races 4, 7 and 9 were restricted to one or two localities.

In the study presented in Chapter 4, 143 *Xanthomonas axonopodis* pv. *phaseoli* (Xap) and *X. axonopodis* pv. *phaseoli* var. *fuscans* (Xapf) isolates from 44 localities in four countries, were inoculated onto eight *Phaseolus acutifolius* lines that differentiate between pathogenic races. Isolates varied in aggressiveness on cv. Teebus, however, pathogenic reaction on the set of differentials, indicated that all, but one isolate, grouped in what has been reported as race 2. Thus, results based on reaction of the majority isolates, suggest the absence of different races. However, the distinct differential reaction recorded for a single isolate, may prove to represent another, as yet unrecorded, race of this pathogen. Both RAPD and AFLP analyses revealed high frequency of DNA polymorphism among isolates and could distinguish between Xap, Xapf and a non-pathogenic isolate. Differences between Xap and Xapf isolates demonstrate that these are two distinct groups of bacteria. Information gained from this study has enabled us to select the most appropriate isolates to use in a resistance breeding programme.

South African cultivars differed significantly in their susceptibility to bacterial diseases as shown in Chapter 5. Cultivars Teebus, Cerillos, PAN 146 and PAN 159 were the most susceptible to common bacterial blight with Monati and OPS-RS2 exhibiting significantly lower susceptibility. Negative correlations were obtained between disease ratings and yields obtained in the common bacterial blight trial. Cultivars exhibited some levels of resistance to halo blight, with small seeded cultivars generally more resistant than large seeded types. A negative correlation was obtained between halo blight rating and yield. Cultivars differed significantly in their susceptibility to bacterial brown spot. Teebus, Cerillos, Bonus and PAN 159 were the most susceptible cultivars, with Mkuzi exhibiting the highest levels of resistance. The majority of cultivars exhibited acceptable levels of resistance to bacterial brown spot. No significant correlation was obtained between disease rating and yield. Although a number of cultivars exhibited field resistance to halo blight and bacterial brown spot, all cultivars were susceptible to common bacterial blight. This disease is, therefore, considered the most important bean bacterial disease in South Africa. Improvement of common bacterial blight resistance in South African cultivars is thus important to obtain stable yields.

In chapter 7 of this study, backcross breeding was used to improve common bacterial blight resistance in the small white canning bean, cv. Teebus, using resistance in XAN 159 and Wilk 2 sources, respectively. High resistance levels in near-isogenic lines, developed in two independent breeding programmes, indicated successful transfer of resistance from both sources. Presence of SCAR-markers, SU91 and BC420, in 35 of 39 XAN 159 derived Teebus lines and all lines derived from Wilk 2, confirmed successful resistance transfer. AFLP studies conducted to determine genetic

relatedness of two near-isogenic Teebus lines, showed a similarity of 96.2% with the maximum similarity between these lines and Teebus being 93.1%. Material developed in this study has been included a bean breeding programme and seed will be made available to farmers after extensive field testing.

Sequence characterized amplified region (SCAR) markers, linked to four independent quantitative trait loci (QTL) in XAN 159 and GN #1 Nebr. sel. 27, are available for indirect selection of resistance to common bacterial blight in *Phaseolus vulgaris*. In chapter 8, existing SCAR-markers, SU91, BC420, BC409 and SAP6, were evaluated for potential use in the local breeding programme. Segregating populations of progenies developed through backcross breeding with cultivars Teebus and Kranskop as susceptible recurrent parents and XAN 159 and Vax 4 as resistant donor parents were evaluated for presence of existing markers. Presence of all four markers in improved Teebus lines (XAN 159 derived), confirmed successful transfer of resistance in these lines. Marker BC420 was absent in XAN 159 derived Kranskop-lines. These lines were only moderately resistant when tested in the greenhouse, indicating that the QTL linked to this marker is important in order to obtain high levels of resistance. Progenies from first backcrosses with Kranskop as recurrent parent using Vax 4 have exhibited high levels of resistance when tested in the greenhouse and presence of all markers found in Vax 4 confirms transfer of resistance. Results gained from this study indicate that marker assisted selection can successfully be implemented in breeding for common bacterial blight resistance in South Africa.

In chapter 9, I assessed yield losses in South African genotypes, caused by common

bacterial blight. This was determined using one susceptible cultivar (Teebus) and two resistant near-isogenic Teebus-lines (TCBR1 and TCBR2). Different parameters (disease ratings, % leaf area loss and % infection) were used to evaluate disease. Disease incidence was high in plots containing the susceptible cultivar Teebus. Genotypes differed significantly in their susceptibility to common bacterial blight. Copper sprays reduced the percentage leaf area loss and enhanced seed size. Disease free plots, however, were not achieved using copper sprays. Common bacterial blight significantly reduced yield and seed size in the susceptible cultivar, Teebus. Yield losses of 43.5% were observed in diseased Teebus plots after artificial inoculation with common bacterial blight. The resistance introduced, into the near-isogenic lines, upon release in the industry, will contribute to common bacterial blight control in future productions of the small white canning bean.

In the series of studies presented in this thesis, I have clarified a number of issues regarding bacterial diseases of dry beans in South Africa. Information was gained on the incidence and severity of bacterial diseases, pathogenic variation that occurs in two of the three respective pathogen populations, susceptibility of cultivars to bacterial pathogens and deployment of resistance as long term control strategy to the most important disease. Progress that was made in this study, especially with regard to the development of resistant cultivars, will make a significant contribution towards the South African dry bean industry.