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.

REFERENCES

Adams, M.W., Kelly, J.D. & Saettler, A.W. (1988) A gene for resistance to common blight (*Xanthomonas campestris* pv. *phaseoli*). *Annual Report of the Bean Improvement Cooperative* **31**: 73-74.

Aggour, A.R., Coyne, D.P. & Vidaver, A.K. (1989) Comparison of leaf and pod

disease reactions of beans (*Phaseolus vulgaris* L.) inoculated by different methods with strains of *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye. *Euphytica* **43**: 143-152.

Allen, D.J., Buruchara, R.A. & Smithson, J.B. (1998) Diseases of common bean. In *The pathology of food and pasture legumes* (D.J. Allen & J.M. Lenné,eds) : 179-235.. CAB International, Wallingford.

Andrus, C.F. (1948) A method of testing beans for resistance to bacterial blights. *Phytopathology* **38**: 757-759.

Arnaud Santana, E., Coyne, D.P., Steadman, J.R., Eskridge, K.M. & Beaver, J.S. (1994) Heritabilities of seed transmission, leaf and pod reactions to common blight, leaf reaction to web blight and plant architecture and their associations in dry beans. *Annual Report of the Bean Improvement Cooperative* **37**: 46-47.

Arnaud-Santana, E., Mmbaga, M.T., Coyne, D.P. & Steadman, J.R. (1993) Sources of resistance to common bacterial blight and rust in elite *Phaseolus vulgaris* L. germplasm. *Horticultural Science* **28**: 644-646.

Beebe, S.E. & Pastor-Corrales, M.A. (1991) Breeding for disease resistance. In *Common Beans, Research for Crop Improvement* (A. Van Schoonhoven & O. Voysest, eds) : 561-610. CAB International, Wallingford.

Coyne, D.P. & Schuster, M.L. (1973) *Phaseolus* germplasm tolerant to common blight bacterium (*Xanthomonas phaseoli*). *Plant Disease Reporter* **57**: 111-114.

Coyne, D.P. & Schuster, M.L. (1974a) Breeding and genetic studies of tolerance to several bean (*Phaseolus vulgaris* L.) bacterial pathogens. *Euphytica* **23**: 651-656.

Coyne, D.P. & Schuster, M.L. (1974b) Differential reaction of pods and foliage of

beans (*Phaseolus vulgaris*) to *Xanthomonas phaseoli*. *Plant Disease Reporter* **58**: 278-282.

Coyne, D.P., Schuster, M.L. & Shaughnessy, L. (1966) Inheritance of reaction to halo blight and common blight bacteria in a *Phaseolus vulgaris* variety cross. *Plant Disease Reporter* **50**: 29-32.

Drijfhout, E. & Blok, W.J. (1987) Inheritance of resistance to *Xanthomonas campestris* pv. *phaseoli* in tepary bean (*Phaseolus acutifolius*). *Euphytica* **36**: 803-808.

Eskridge, K.M. & Coyne, D.P. (1996) Estimation and testing hypothesis about the number of genes in using inbred-backcross data. *Journal of Hereditary* **87**: 410-412.

Finke, M.L., Coyne, D.P. & Steadman, J.R. (1986) The inheritance and association of resistance to rust, common bacterial blight, plant habit and foliar abnormalities in *Phaseolus vulgaris* L. *Euphytica* **35**: 969-982.

Fourie, D. (2002) Distribution and severity of bacterial diseases on dry beans (*Phaseolus vulgaris* L.) in South Africa. *Journal of Phytopathology* **150**: 220-226.

Freytag, G.F., Bassett, M.J. & Zapata, M. (1982) Registration of XR-235-1-1 bean germplasm. *Crop Science* **22**: 1268-1269.

Graham, G.C., Mayers, P. & Henry, R.J. (1994) Simple and rapid method for the preparation of fungal genomic DNA for PCR and RAPD analysis. *Biotechniques* **16**: 48-50.

Jung, G., Coyne, D.P., Scroch, P.W., Nienhuis, J., Arnaud-Santana, E., Bokosi, J., Ariyarathne, H.M., Steadman, J.R., Beaver, J.S. & Kaeppler, S.M. (1996) Molecular markers associated with plant architecture and resistance to

common blight, web blight, and rust in common beans. *Journal of the American Society for Horticultural Science* **121**: 794-803.

McElroy, J.B. (1985) Breeding for dry beans, *P. vulgaris* L., for common blight resistance derived from *Phaseolus acutifolius* A. Gray. PhD. Dissertation, Cornell University, Ithaca, New York.

Miklas, P.N., Beaver, J.S., Grafton, K.F. & Freytag, G.F. (1994) Registration of TARS VCI-4B multiple disease resistant dry bean germplasm. *Crop Science* **34**: 1415.

Miklas, P.N., Smith, J.R., Riley, R., Grafton, K.F., Singh, S.P., Jung, G. & Coyne, D.P. (2000) Marker-assisted breeding for pyramiding resistance to common bacterial blight. *Annual Report of the Bean Improvement Cooperative* **43**: 39-40.

Mutlu, N., Coyne, D.P., Park, S.O., Steadman, J.R., Reiser, J. & Jung, G. (1999) Backcross breeding with RAPD molecular markers to enhance resistance to common bacterial blight in pinto beans. *Annual Report of the Bean Improvement Cooperative* **42**: 7-8.

Nei, M. & Li. W.H. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Science, USA* **76**: 5269-5273.

Nodari, R.O., Tsai, S.M., Guzman, P., Gilbertson, R.L. & Gepts, P. (1993) Towards an integrated linkage map of common bean. III. Mapping genetic factors controlling host-bacteria interactions. *Genetics* **134**: 341-350.

Park, S.J. & Dhanvantari, B.N. (1987) Transfer of common blight *(Xanthomonas campestris* pv. *phaseoli*) resistance from *Phaseolus coccineus* Lam. to *P. vulgaris* L. through interspecific hybridization. *Canadian Journal of Plant*

Science **67**: 685-695.

Park, S.O., Coyne, D.P. & Jung, G. (1998) Gene estimation, associations of traits, and confirmation of QTL for common bacterial blight resistance in common bean. *Annual Report of the Bean Improvement Cooperative* **41**: 145-146.

Rands, R.D. & Brotherton, W. (1925) Bean varietal tests for disease resistance. *Journal of Agricultural Research* **31**: 110-154.

Schuster, M.L. & Coyne, D.P. (1981) Biology, epidemiology, genetics and breeding for resistance to bacterial pathogens of *Phaseolus vulgaris*, L. *Horticultural Reviews* **3**: 28.

Schuster, M.L., Coyne, D.P., Behre, T. & Leyna, H. (1983) Sources of *Phaseolus* species resistance and leaf and pod differential reactions to common blight. *Horticultural Science* **18**: 901-903.

Schwartz, H.F. & Otto, K.L. (2000) Enhanced bacterial disease management strategy. *Annual Report of the Bean Improvement Cooperative* **43**: 37-38.

Silva, L.O., Singh, S.P. & Pastor-Corrales, M.A. (1989) Inheritance of resistance to bacterial blight in common bean. *Theoretical and Applied Genetics* **78**: 619-624.

Singh, S.P. & Muñoz, C.G. (1999) Resistance to common bacterial blight among *Phaseolus* species and common bean improvement. *Crop Science* **39**: 80-89.

Tsai, S.M., Nodari, R.O., Moon, D.M., Camargo, L.E.A., Vencovsky, R. & Gepts, P. (1998) QTL mapping for nodule number and common bacterial blight in *Phaseolus vulgaris*, L. *Plant and Soil* **204**: 135-145.

Valladarez-Sanchez, N.E., Coyne, D.P. & Schuster, M.L. (1979) Differential reaction of leaves and pods of *Phaseolus* germplasm to strains of *Xanthomonas phaseoli* and transgressive segregation for tolerance from crosses of

susceptible germplasm. *Journal of the American Society for Horticultural Science* **104:** 648-654.

Van Schoonhoven, A. & Pastor-Corrales, M.A. (1987) Standard system for the evaluation of bean germplasm. CIAT, Cali, Colombia.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van Der Lee, T., Hornes, M., Freijters, A., Pot, J., Peleman, J., Kuiper, M. & Zabeau, M. (1995) AFLP: a new concept for DNA fingerprinting. *Nucleic Acids Research* **21**: 4407-4414.

Wallen, V.R. & Jackson, H.R. (1975) Model for yield loss determination of bacterial blight of field beans utilizing aerial infrared photography combined with field plot studies. *Phytopathology* **65**: 942-948.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. & Tingey, S.V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**: 6531-6535.

Yoshii, K., Gálvez-E, G.E. & Alvarez-A, G. (1978) Screening bean germplasm for tolerance to common blight caused by *Xanthomonas phaseoli* and the importance of pathogenic variation to varietal improvement. *Plant Disease Reporter* **62**: 343-347.

Yoshii, K. (1980) Common and Fuscous blights. In *Bean Production Problems* (H.F. Schwartz & G.E. Gálvez, eds) : 157-172. Centro Internacional de Agricultura Tropical (CIAT). Cali, Colombia.

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

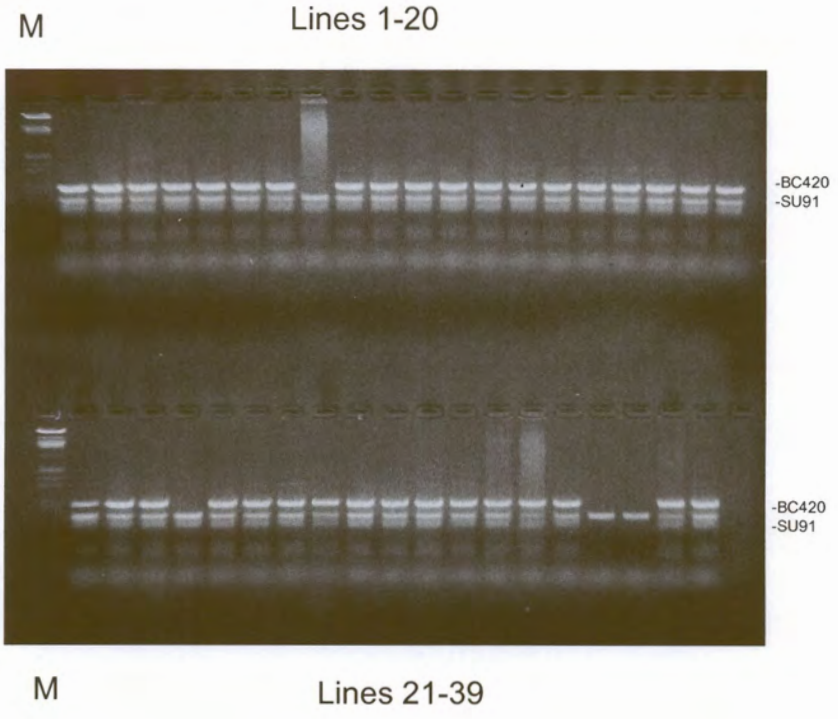

Figure 2. Screening of advanced XAN 159 derived Teebus lines with improved CBB resistance for presence of SCAR markers SU91 and BC420

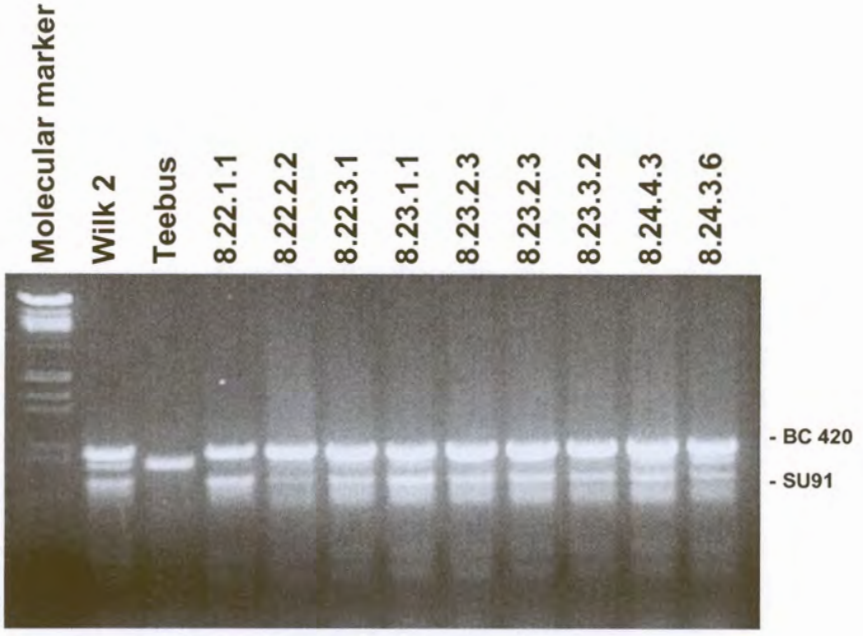

Figure 3. Screening of advanced Wilk 2 derived Teebus lines with improved CBB resistance for presence of SCAR markers SU91 and BC420

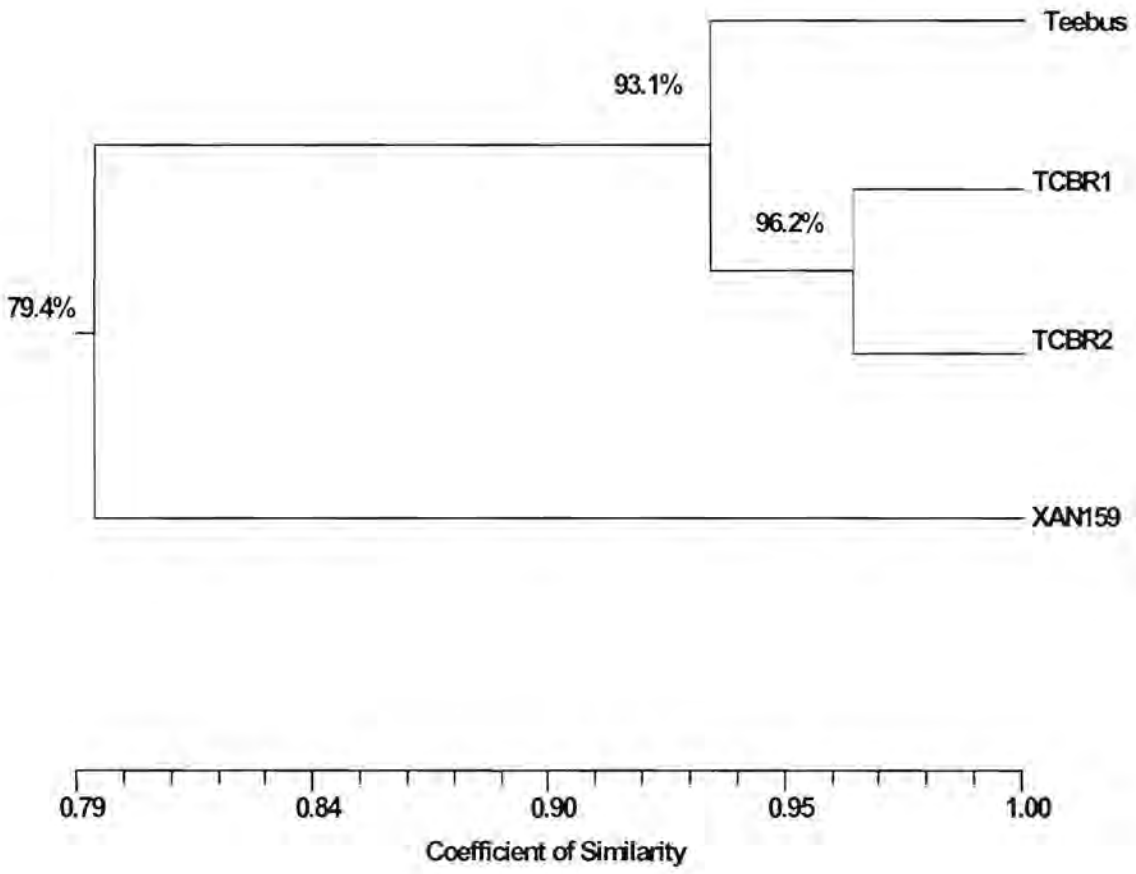

Figure 4. Dendogram of near-isogenic lines derived from backcrosses with Teebus as recurrent susceptible parent and XAN 159 as resistant donor parent

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.

REFERENCES

Aggour, A.R., Coyne, D.P. & Vidaver, A.K. (1989) Comparison of leaf and pod disease reactions of beans (*Phaseolus vulgaris* L.) inoculated by different methods with strains of *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye. *Euphytica* **43**: 143-152.

Andrus, C.F. (1948) A method of testing beans for resistance to bacterial blights. *Phytopathology* **38**: 757-759.

Bai, Y., Michaels, T.E. & Pauls, K.P. (1996) Identification of RAPD markers linked to bacterial blight resistance genes in *Phaseolus vulgaris* L. *Annual Report of the Bean Improvement Cooperative* **39**: 164-165.

Beattie, A., Michaels, T.E. & Pauls, K.P. (1998) An efficient, reliable method to screen for common bacterial blight (CBB) resistance in *Phaseolus vulgaris* L. *Annual Report of the Bean Improvement Cooperative* **41**: 53-54.

Coyne, D.P. & Schuster, M.L. (1974) Breeding and genetic studies of tolerance to several bean (*Phaseolus vulgaris* L.) bacterial pathogens. *Euphytica* **23**: 651-656.

Dursun, A., Coyne, D.P., Mohamed, M.F. & Jung, G. (1995) Inheritance of resistance to common bacterial blight in tepary bean. *Annual Report of the Bean Improvement Cooperative* **38**: 162-163.

Graham, G.C., Mayers, P. & Henry, R.J. (1994) Simple and rapid method for the preparation of fungal genomic DNA for PCR and RAPD analysis. *Biotechniques* **16**: 48-50.

Kelly, J.D. & Miklas, P.N. (1999) Marker-assisted selection. In *Common Bean Improvement in the Twenty-First Century* (S.P. Singh, ed) : 93-123. Kluwer Academic Publishers, London.

Miklas, P.N., Smith, J.R., Riley, R., Grafton, K.F., Singh, S.P. Jung, G. & Coyne, D.P. (2000) Marker-assisted breeding for pyramiding resistance to common bacterial blight. *Annual Report of the Bean Improvement Cooperative* **43**: 39-40.

Mohan, S.T. & Mohan, S.K. (1983) Breeding for common bacterial blight resistance in beans. *Annual Report of the Bean Improvement Cooperative* **26**: 14-15.

Park, S.O., Coyne, D.P., Mutlu, N., Jung, G & Steadman, J.R. (1999) Confirmation of molecular markers and flower colour associated with QTL for resistance to common blight in common beans. *Annual Report of the Bean Improvement Cooperative* **42**: 5-6.

Singh, S.P. & Muñoz, C.G. (1999) Resistance to common bacterial blight among *Phaseolus* species and common bean improvement. *Crop Science* **39**: 80-89.

Stavely, J.R. (2000) Pyramiding rust and viral resistance genes using traditional and marker techniques in common bean. *Annual Report of the Bean Improvement Cooperative* **43**: 1-4.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. & Tingey, S.V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**: 6531-6535.

Yu, K., Park, S.J. & Poysa, V. (1999) Application of molecular markers to assist selection of common beans resistant to common bacterial blight *(Xanthomonas campestris* pv. *phaseoli*). *Annual Report of the Bean Improvement Cooperative* **42**: 3-4.

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

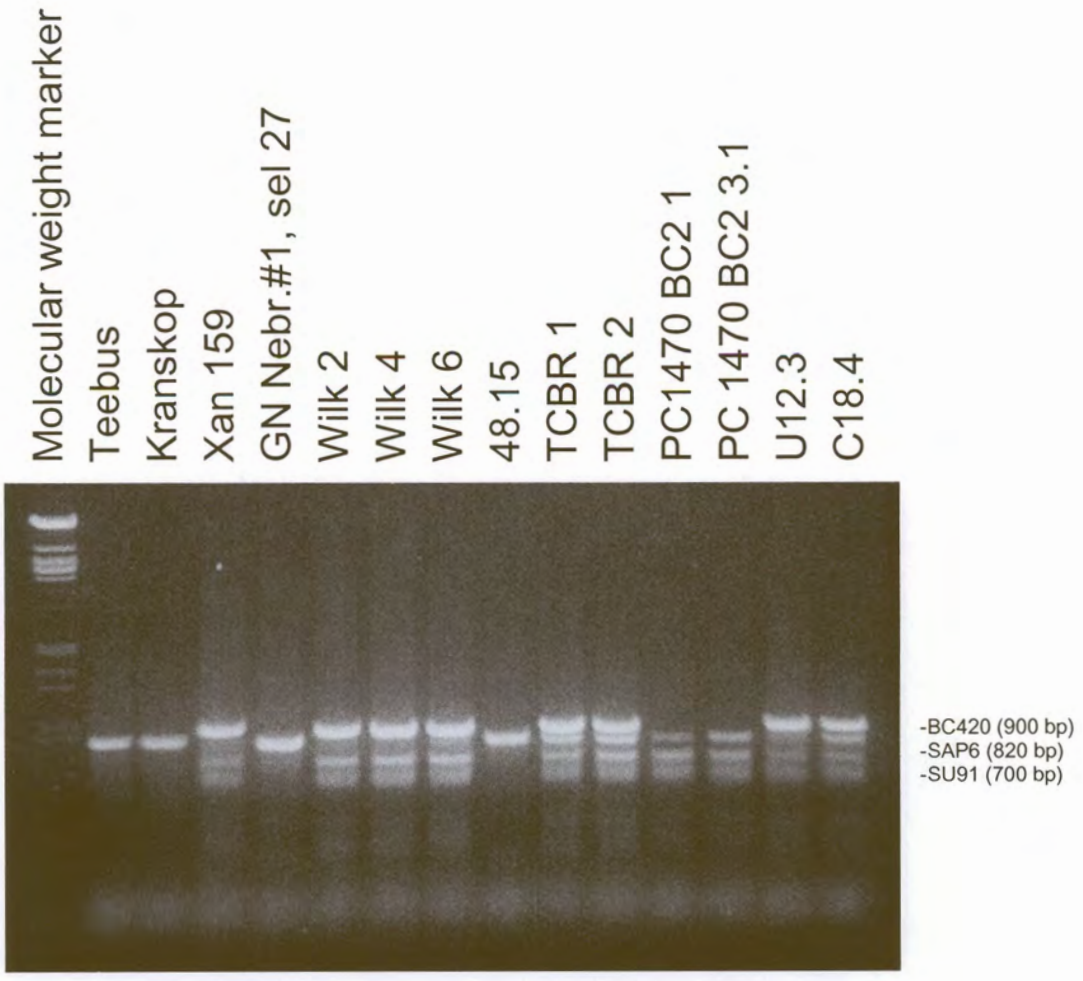

Figure 1. Screening lines for presence of SCAR markers BC420, SAP6 and SU91

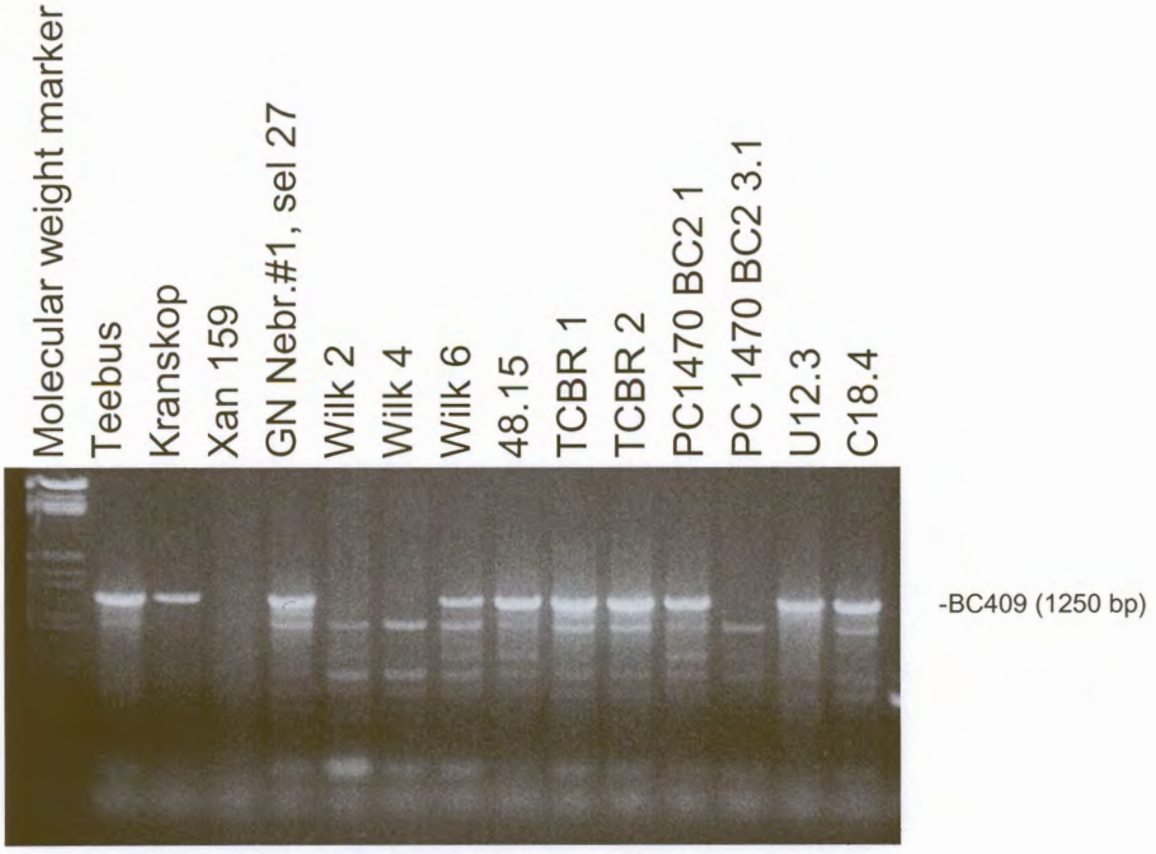

Figure 2. Screening of lines for presence of SCAR marker BC409

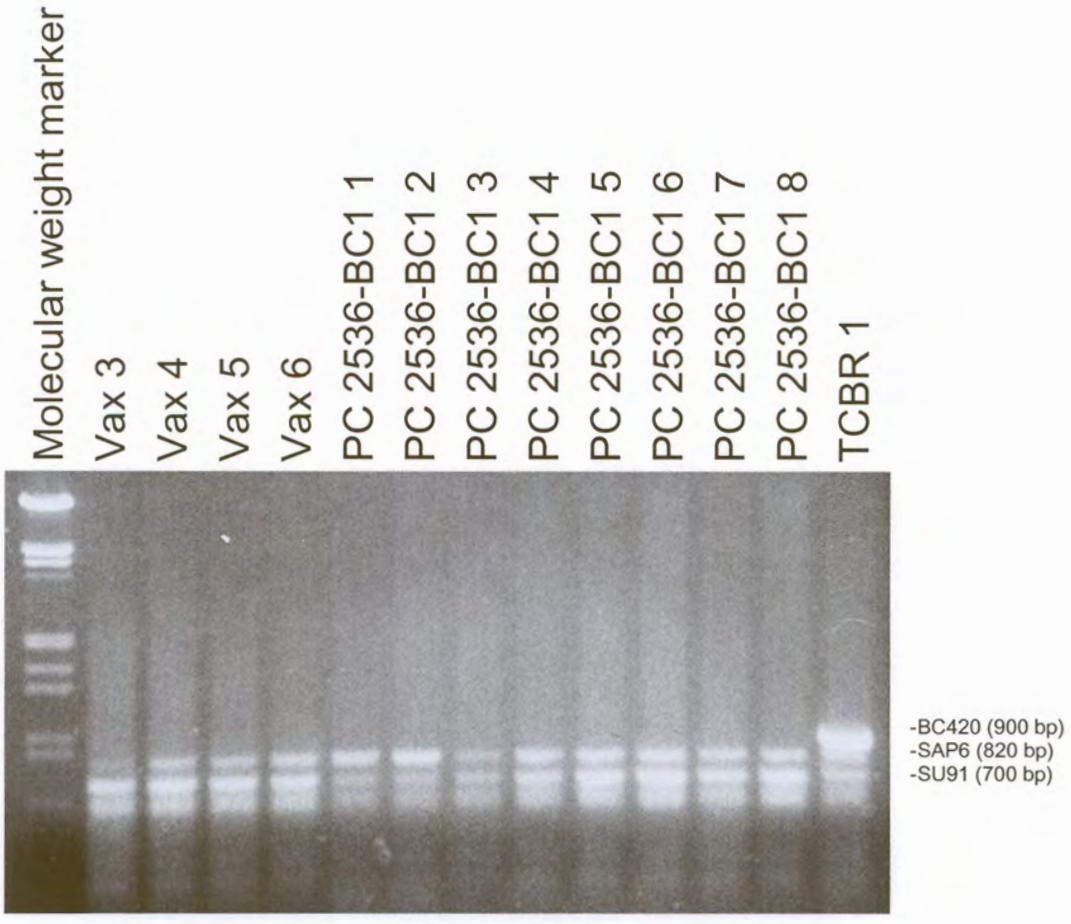

Figure 3. Screening of lines for presence of SCAR markers SU91, BC420 and SAP6

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.

REFERENCES

Allen, D.J., Buruchara, R.A. & Smithson, J.B. (1998) Diseases of common bean. In *The pathology of food and pasture legumes* (D.J. Allen & J.M. Lenné, eds) : 179-235. CAB International, Wallingford.

CIAT. (1985) Bean Programme Annual Report for 1985. Centro Internacional de Agricultura Tropical, Cali, Colombia.

Moffet, M.L. & Middleton, K.J. (1979) Effect of common blight on yield of navy beans. *Australian Plant Pathology* **8**: 19.

Opio, A.F., Teri, J.M. & Allen, D.J. (1992) Assessment of yield losses caused by common bacterial blight of beans in Uganda. *Annual Report of the Bean Improvement Cooperative* **35**: 113-114.

Saettler, A.W. (1989) Common bacterial blight. In *Bean Production Problems in the Tropics 2nd ed* (H.F. Schwartz & M.A. Pastor-Corrales, eds) : 261-283. CIAT, Cali, Colombia.

Schaad, N.W. & Stall R.E. (1988) Xanthomonas. In *Laboratory Guide for Identification of Plant Pathogenic Bacteria 2nd ed* (N.W. Schaad, ed) : 81-93. The American Phytopathological Society, St. Paul, MN, USA.

Schwartz, H.F. & Otto, K.L. (2000) Enhanced bacterial disease management strategy. *Annual Report of the Bean Improvement Cooperative* **43**: 37-38.

Singh, SP. (1991) Bean Genetics. In *Common Beans, Research for Crop Improvement* (A. Van Schoonhoven & O. Voysest, eds) : 199-286. CAB International, Wallingford.

Singh, S.P. & Muñoz, C.G. (1999) Resistance to common bacterial blight among *Phaseolus* species and common bean improvement. *Crop Science* **39**: 80-89.

Townsend, G.R. & Heuberger, J.W. (1943) Methods for estimating losses caused by disease in fungicide experiments. *Plant Disease Reporter* **27**: 340-343.

Van Schoonhoven, A. & Pastor-Corrales, M.A. (1987) Standard system for the evaluation of bean germplasm. 53p. CIAT, Cali, Colombia.

Wallen, V.R. & Jackson, H.R. (1975) Model for yield loss determination of bacterial blight of field beans utilizing aerial infrared photography combined with field plot studies. *Phytopathology* **65**: 942-948.

Yoshii, K. (1980) Common and fuscous blights. In *Bean Production problems in the Tropics* (H.F. Schwartz & M.A. Pastor-Coralles, eds) : 157-172. CIAT, Cali, Colombia.

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

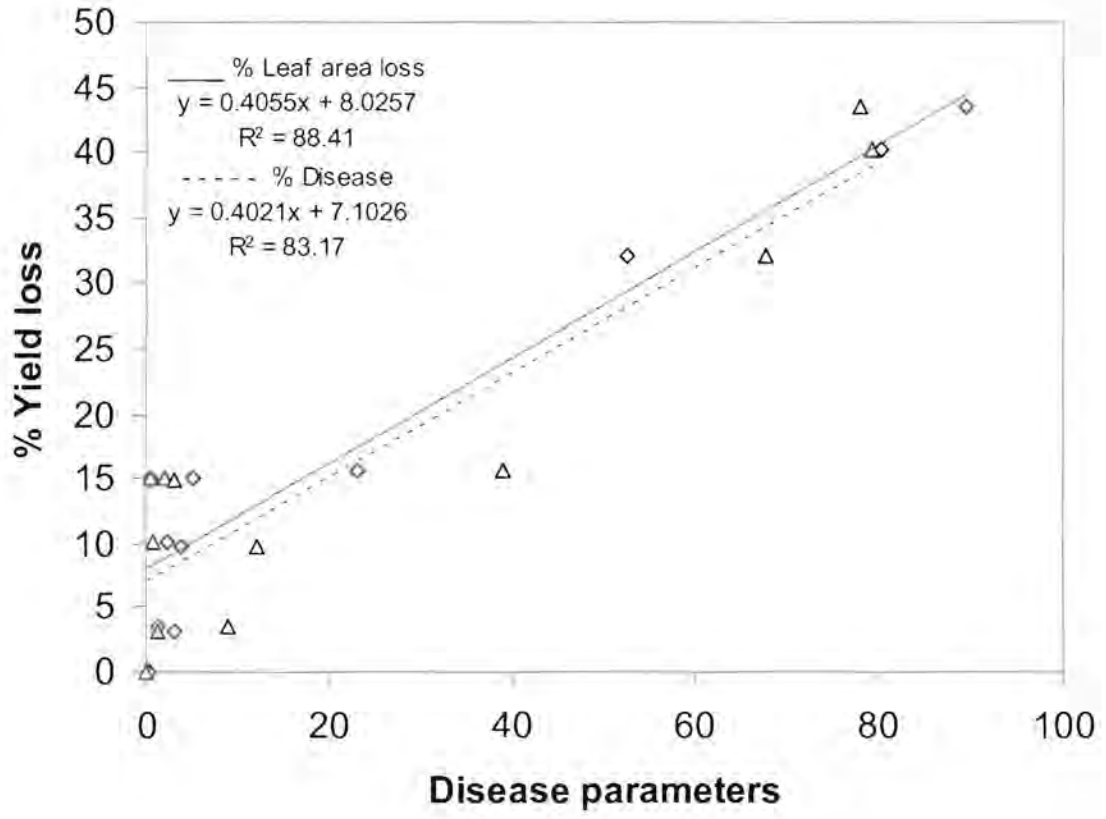

Figure 1. Relationship between yield loss, disease and leaf area loss in Teebus, TCBR1 and TCBR2 plots artificially inoculated with common bacterial blight at Potchefstroom and Cedara

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.

REFERENCES

Allen, D.J., Buruchara, R.A. & Smithson, J.B. (1998) Diseases of common bean. In *The pathology of food and pasture legumes* (D.J. Allen & J.M. Lenné,eds) :179-235. CAB International, Wallingford.

Boelema, B.H. (1984) Infectivity titrations with race 2 of *Pseudomonas syringae* pv. *phaseolicola* in green beans (*Phaseolus vulgaris*). *Phytophylactica* **16**: 327-329.

Edington, B.R. (1990) The identification of race 1 of *Pseudomonas syringae* pv. *phaseolicola* in South Africa. *Annual Report of the Bean Improvement Cooperative* **33**: 171.

Fourie, D. & Herselman, L. (2002) Breeding for common blight resistance in dry beans in South Africa. *Annual Report of the Bean Improvement Cooperative* **45**: 50-51.

Gilbertson, R.L., Otoya, M.M., Pastor-Corrales, M.A. & Maxwell, D.P. (1991) Genetic diversity in common blight bacteria is revealed by cloned repetitive DNA sequences. *Annual Report of the Bean Improvement Cooperative* **34**: 37-38.

Miklas, P.N., Smith, J.R., Riley, R., Grafton, K.F., Singh, S.P., Jung, G. & Coyne, D.P.

(2000) Marker-assisted breeding for pyramiding resistance to common bacterial blight. *Annual Report of the Bean Improvement Cooperative* **43**: 39-40.

Moffet, M.L. & Middleton, K.J. (1979) Effect of common blight on yield of navy beans. *Australian Plant Pathology* **8**: 19.

Opio, A.F., Teri, J.M. & Allen, D.J. (1992) Assessment of yield losses caused by common bacterial blight of beans in Uganda. *Annual Report of the Bean Improvement Cooperative* **35**: 113-114.

Opio, A.F., Allen, D.J. & Teri, J.M. (1996) Pathogenic variation in *Xanthomonas campestris* pv. *phaseoli*, the causal agent of common bacterial blight in *Phaseolus* beans. *Plant Pathology* **45**: 1126-1133.

Stavely, J.R. (2000) Pyramiding rust and viral resistance genes using traditional and marker techniques in common bean. *Annual Report of the Bean Improvement Cooperative* **43**: 1-4.

Taylor, J.D., Teverson, D.M., Allen, M.A. & Pastor-Corrales, M.A. (1996) Identification and origin of races of *Pseudomonas syringae* pv. *phaseolicola* from Africa and other bean growing areas. *Plant Pathology* **45**: 469-478.

Teverson, D.M. (1991) Genetics of pathogenicity and resistance in the halo-blight disease of beans in Africa. Ph.D. diss. University of Birmingham, Birmingham.

Wallen, V.R. & Jackson, H.R. (1975) Model for yield loss determination of bacterial blight of field beans utilizing aerial infrared photography combined with field plot studies. *Phytopathology* **65**: 942-948.

Yoshii, K. (1980) Common and fuscous blights. In *Bean Production problems in the Tropics* (H.F. Schwartz & M.A. Pastor-Coralles, eds) : 157-172. CIAT, Cali, Colombia.

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

by

DEIDRÈ FOURIE

SUPERVISOR: Dr. P.S.van Wyk

CO-SUPERVISOR: Prof. M.J. Wingfield

DEPARTMENT: Microbiology and Plant Pathology

DEGREE: PhD

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.