Studies on host plant resistance to cassava bacterial blight in combination with cultural control measures in ecozones of West Africa

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Zusammenfassung

Maßnahmen zur Entwicklung eines integrierten Bekämpfungsprogramms für den bakteriellen Brand des Maniok (*Manihot esculenta* Crantz) (CBB) wurden einzeln und in Kombination in verschiedenen Ökozonen West-Afrikas getestet. Das wichtigste Element dieser Maßnahmen, die Selektion CBB-resistenter Sorten aus Benin, beinhaltete Untersuchungen zu Wirt-Pathogen und Wirt-Pathogen-Umwelt Interaktionen. Die Charakterisierung der Sorten wurde durch Studien möglicher präformierter Resistenzmechanismen und durch die Identifizierung von genetischen Markern zur Resistenz-Selektion ergänzt. Als weitere Elemente in der integrierten Bekämpfung von CBB wurden agronomische und Kulturmaßnahmen, einzeln und in Kombination, im optimalen System kombiniert mit einer resistenten Sorte, auf ihren Einfluss auf die Symptomentwicklung und den Ertrag untersucht.

Siebenunddreißig Manioksorten aus Benin, darunter Zuchtsorten des International Institute of Tropical Agriculture, Nigeria, wurden auf ihre Anfälligkeit für Bakterienbrand in der Wald-Savannen-Übergangszone, der Feuchtsavanne und der Trockensavanne Benins unter natürlichem Befallsdruck und nach künstlicher Inokulation untersucht. Die Sorten RB92164, RB92022, TMS30572, BEN86004, RB92033 und Dangbo2 (Jahr 1998) bzw. RB92202, RB92151, RB92132 und TMS30572 (Jahr 2000) waren in einer Ökozone resistent. In der Gruppe der resistenteren Sorten zeigten die Sorten CAP94030, BEN86040, RB89509, RB92132 und TMS30572 eine geringe Interaktion mit der Umwelt und waren am stabilsten in ihrer Symptomentwicklung, während die Sorten RB92022 und RB92004 hohe Umweltinteraktionen zeigten und daher unstabil waren. Vier anfällige Sorten zeigten geringe Schwankungen im Befall unter verschiedenen Umweltbedingungen, während Sorte BEN86002 (anfällig) unter allen Bedingungen am stabilsten war. Zehn Sorten mit einem hohen Ertrag wurden identifiziert. In der Gruppe der resistenteren Sorten zeigten nur Sorten TMS30572 und RB89509 einen hohen Ertrag, wobei letztere Sorte in verschiedenen Umwelten keinen stabilen Ertrag erbrachte. Sorten CAP94030, BEN86040, RB92099, TMS30572 und RB92022 waren ertragsstabil.

Die künstlich inokulierten Varianten in der Feuchtsavanne und der Wald-Savannen-Übergangszone zeigten für alle Sorten eine hohen Befall. Diese Umweltbedingungen wären somit für die Selektion resistenter Sorten am besten geeignet. Die höchsten Erträge wurden in der Trockensavanne unter natürlicher Infektion im Jahr 1998 erzielt, während die Feuchtsavanne unter natürlicher Infektion im Jahr 1998 wegen des niedrigen Befalls am besten für die Produktion von Pflanzmaterial geeignet war.

Eine signifikante Beziehung ($r = -0.58$) zwischen Symptomentwicklung (AUSiPC) und Ertrag konnte nur in der nicht inokulierten Variante in der Trockensavanne im Jahr 2000 festgestellt werden. Von den 37 getesteten Sorten (16 Sorten in 2 Jahren und unter 12 verschiedenen Umweltbedingungen, und 21 Sorten in mindestens 1 Jahr unter 6 verschiedenen Umweltbedingungen) kann nur Sorte TMS30572 den Bauern als Sorte mit guter Ertragsstabilität und relativ guter Resistenz in allen drei Ökozonen empfohlen werden.

Bei Untersuchungen der Anzahl und Verteilung der Spaltöffnungen auf der ad- und abaxialen Blattoberfläche von vier anfälligen, mittel-resistenten und resistenten Manioksorten wurden keine signifikanten Unterschiede zwischen den Sorten gefunden, obwohl in den resistenteren Sorten Tendenzen zu geringerer Spaltöffnungszahl auf der adaxialen Blattoberfläche auftraten. Auf dieser Blattoberfläche lagen die Spaltöffnungen entlang der Mittelrippe und der größeren Blattadern. Analysen der Wachsmenge und –zusammensetzung von 7 Sorten ergaben, dass Triterpene die dominierenden Bestandteile des Wachses waren, während Alkane und Säuren in geringeren Mengen vorkamen. Eindeutige Unterschiede in Wachsmengen zwischen anfälligen, mittel-resistenten und resistenten Sorten in drei Ökozonen von Benin konnten nicht festgestellt werden. In rasterelektronenmikroskopischen Untersuchungen der Blattoberflächen einer anfälligen und einer resistenten Sorte zeigte sich eine regelmäßige Verteilung einer großen Wachsmenge auf der abaxialen Oberfläche. Hier waren die Spaltöffnungen durch das Wachs stark verschlossen. Auf der adaxialen Oberfläche hingegen kam Wachs nur in geringen Mengen in Kristallen vor, wodurch die wenigen dort vorhandenen Spaltöffnungen nicht verschlossen wurden. Die nicht durch Wachs verschlossenen Spaltöffnungen der Blattoberseite könnten deshalb, im Vergleich zur Blattunterseite, bevorzugte Eingangspforten für die Bakterien sein. Es wird spekuliert,

dass die tendenziell leicht niedrigere Zahl der Spaltöffnungen in den resistenteren Sorten eine Rolle in der Resistenz durch das Behindern des Eindringens der Bakterien spielen könnte.

Einhundert-elf Linien aus der Rückkreuzung von 5 F1-Individuen und dem weiblichen Elternteil TMS30572 der Maniok-Genom-Kartierungspopulation wurden nach Blatt- und Stängelinokulation auf ihre Reaktion untersucht. Erstmalig wurde in Afrika das Vorkommen von Pathotypen von *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) festgestellt. Pathotypspezifische genetische Resistenz-Marker (QTL) wurden identifiziert. Linien unterschieden sich in ihrer Resistenz, teilweise auch in Bezug auf Blatt- und Stängelebene, in anfällige, mittel-resistente und resistente. Sechzehn Linien waren resistent. Mit Single-Marker-Regressionsanalyse der AUSiPC–Werte nach Stängelinokulation, basierend auf der Maniok-Genom-Kartierung, wurden elf Resistenzmarker identifiziert, die spezifisch für die vier Stämme waren. Dieses Ergebnis bestätigt auch die Existenz von Afrikanischen Pathotypen.

Der Einfluss von (i) Mischkulturanbau von Maniok mit Sorghum oder Augenbohne im Vergleich zu Maniok Monokultur, (ii) Kaliumdüngung oder Mulchen, (iii) Pflanzzeitpunkt, und (iv) die Kombination dieser Maßnahmen auf die Befallsstärke mit CBB wurde in zwei Versuchsorten in zwei Ökozonen unter natürlichem Befall und nach künstlicher Inokulation von Randreihen untersucht. Die Befallsstärke wurde generell durch einen späteren Pflanzzeitpunkt – im letzten Drittel der Regenzeit – reduziert, ohne einen negativen Einfluss auf den Ertrag hervorzurufen. Mischanbau von Maniok mit Sorghum verringerte generell den CBB-Befall um bis zu 80% in den drei Bodenbehandlungsvarianten zu beiden Pflanzzeitpunkten in der Wald-Savannen-Übergangszone, und zum normalen Pflanzzeitpunkt in der Trockensavanne, mit wenigen Ausnahmen. Auch Mischanbau mit Augenbohne reduzierte die Befallsstärke. Obwohl generell kein Einfluss auf das Wurzelgewicht beobachtet wurde, wurde der Ernteertrag doch in der Trockensavanne durch die Kombination von spätem Anbautermin und Mischanbau mit Sorghum in den meisten Varianten reduziert. Mischanbau alleine hatte generell keinen negativen Einfluss auf den Ertrag im Vergleich zu Maniok Monokultur, mit wenigen Ausnahmen an zwei Standorten, während Mischanbau mit Augenbohne den Ertrag in der Trockensavanne um 52% verringerte. Mulchen und Kaliumdüngung hatten keinen Einfluss auf den Befall, aber

erhöhten oder verringerten den Ertrag in einigen Varianten an beiden Standorten (Ökozonen).

Schlussfolgerungen

Nur Sorte TMS30572 war stabil unter verschiedenen Umweltbedingungen – mittel-resistent bis resistent mit einem hohen Ertrag – und kann somit den Bauern empfohlen werden. Ein später Anbauzeitpunkt in der Wald-Savannen-Übergangszone und Mischanbau mit Sorghum in allen Ökozonen kann den Befall zusätzlich weiter reduzieren, ohne dass ein negativer Einfluss auf den Ertrag auftritt. Zur Selektion resistenter Sorten wird zusätzlich zur Bestimmung der Sorte-Umweltinteraktionen eine Stängel- und Blattinokulation mit einem repräsentativen Sortiment von *Xam* Pathotypen unter kontrollierten Bedingungen, sowie die Anwendung Marker-unterstützter Selektion mit den neu identifizierten, pathotypspezifischen Resistenz-Markern (QTLs) empfohlen.

Schlagwörter: Maniok, *Xanthomonas campestris* pv. *manihotis*, integrierten Bekämpfung

Abstract

Elements for an integrated control program for cassava bacterial blight (CBB) were tested single and in combination in different ecozones of West Africa. The most important element, the selection of CBB-resistant cassava genotypes, consisted of studies on genotype-pathogen and genotype-pathogen-environment interactions, using genotypes from Benin. The characterization of genotypes was supported by studies on possible preformed resistance mechanisms and marker-assisted selection for resistance. As further element of integrated control of CBB, agronomic and cultural measures, single and in combination, in the optimal system using a resistant genotype, were evaluated for their effect on CBB and yield in two ecozones of Benin.

Thirty-seven cassava (*Manihot esculenta* Crantz) genotypes from Benin, including advanced breeding lines from the International Institute of Tropical Agriculture, Nigeria, were tested for their reaction to bacterial blight in the forest-savanna transition, wet savanna and dry savanna zones of Benin. In years 1998, genotypes RB92164, RB92022, TMS30572, BEN86004, RB92033 and Dangbo2, and in year 2000, genotypes RB92202, RB92151, RB92132 and TMS30572 were resistant in one ecozone. Among the more resistant genotypes, genotypes CAP94030, BEN86040, RB89509, RB92132 and TMS30572 showed low interaction across environments and were most stable in disease reaction, while genotypes RB92022 and RB92004 showed higher interactions with different environments and were unstable. Four susceptible genotypes also showed low interactions with environments, while genotype BEN86002 (susceptible) was most stable in different environments. Comparing root yield across environments, 10 genotypes were classified as high yielding. Among the more resistant group of genotypes, only TMS30572 and RB89509 were high yielding, with genotype RB89509 being unstable in yield across environments. Genotypes CAP94030, BEN86040, RB92099, TMS30572 and RB92022 had low interactions with environments concerning yield. Inoculated treatments in the wet savanna zone and in the forest-savanna transition zone with stable high symptom severity proved most suitable for screening of genotypes, while the site in the dry savanna zone with natural infection in year 1998 was the best environment for cassava production and the site in the wet savanna zone with natural infection in year 1998 for production of propagation material. The correlation between disease severity expressed as area

under severity index progress curve and root yield in the non-inoculated and inoculated treatments in each ecozone was significant **only** for the non-inoculated treatment in the dry savanna zone in year 2000 (r = -0.58), but not in any other environment. Among the 37 genotypes tested (sixteen repeated in two years and 12 environments, and twenty-one genotypes repeated in at least one year and 6 environments), genotype TMS30572 can be recommended to farmers as relatively stable in disease resistance and in high yield.

Comparing four cassava genotypes susceptible, medium resistant and resistant to bacterial blight, no significant differences in stomatal distributions on abaxial or adaxial leaf surfaces were found though tendencies for fewer stomata in the more resistant genotypes at the adaxial surface were observed. On the adaxial leaf surfaces stomata were located along the midrib and major veins. Analysing cuticular waxes of seven genotypes, triterpenes were the dominant wax constituents and alkanes and acids occurred in minor amounts. Clear differences in wax quantities between susceptible, medium resistant and resistant genotypes from three ecozones of Benin were not observed. Scanning electron-microscopy of a susceptible and a resistant genotype revealed a regular distribution of waxes at the abaxial leaf surface, covering stomatal pores of both genotypes, while on the adaxial leaf surface waxes were in form of crystalloids, did not occlude the rarely observed stomata, and might be portals of entry for the bacteria. Therefore, the slightly lower number of stomata on the adaxial surface in the resistant genotypes might play a role in defence by hindering bacterial entry, but these characteristics were not decisive for resistance.

Hundred-eleven cassava genotypes derived from the backcross of 5 F_1 individuals and the female parent TMS30572 were tested for their reaction to cassava bacterial blight by leaf and stem inoculation, and were used to identify possible *Xanthomonas axonopodis* pv. *manihotis* pathotypes and cassava bacterial blight related genetic markers or involved genes. The genotypes varied in their reaction against four highly virulent strains of *X. axonopodis* pv. *manihotis* from four different geographic origins in Africa. The strains were defined as different pathotypes according to their reactions on leaves and stems. Genotypes with susceptible, medium resistant and resistant reactions were identified for both leaf and stem inoculation methods and partly differed in their reaction on leaves and stems. Sixteen genotypes among the mapping

population showed a resistant reaction. Based on the genetic map of cassava, single-marker regression analysis of area under disease progress curve values from stem-puncture inoculation of each strain was performed. Eleven markers were identified, of which 5 markers on 3 and 1 linkage groups of the female- and male-derived framework of family CM8820, respectively, were significantly linked to disease severity (area under disease progress curve) values with the four strains of *X. axonopodis pv. manihotis*. Based on the segregation of alleles from the female of family CM8873, one marker was found to be associated to resistance to both *X. axonopodis pv. manihotis* strains, GSPB2506 and GSPB2511. Five markers were not linked to any groups. Depending on strain inoculated, specific markers were detected confirming that the 4 African strains belong to 4 different pathotypes.

The effects of (i) intercropping cassava with sorghum or cowpea versus cassava monoculture, (ii) potassium fertilizer application and mulching, (iii) shift of planting date, and (iv) the combination of these measures on the severity of cassava bacterial blight at two sites in two ecozones of West Africa were studied. Disease severity of bacterial blight was generally reduced by late planting - in the last third of the rainy season - with no effect on cassava root yield. Intercropping cassava-sorghum reduced cassava bacterial blight severity significantly up to 80% in the three soil amendment treatments, at normal and late planting time in the forest-savanna transition zone and at normal planting time in the dry savanna zone, with few exceptions. The intercropping of cassava with cowpea also reduced the disease severity. Although generally effects on root yield were not observed, the combination of late planting and intercropping in the dry savanna generally reduced root yield. Cassava-sorghum intercropping generally had no effect on root yield compared to cassava monocropping with few exceptions in two sites (ecozones), while intercropping with cowpea significantly reduced root yield by 52% compared to cassava monocropped, in the dry savanna site. Mulching and potassium treatment had no effect on disease severity, but increased or decreased root yield in some treatments in both sites (ecozones).

Only genotype TMS30572 was stable in different environments - medium resistant to resistant and high-yielding - and could be recommended to farmers. Additionally, late planting in the forest-savanna transition zone, and intercropping in all ecozones, using

the resistant genotype TMS30572, could further reduce CBB development. For breeding for resistance, stem and leaf-inoculation with a representative set of *Xam* pathotypes under glasshouse conditions to identify genotype x pathogen interactions, and use of newly identified markers (QTL) for CBB resistance are recommended to breeders to select resistant genotypes.

Keywords: Cassava, *Xanthomonas campestris* pv. *manihotis*, integrated control

Abbreviations

ANOVA	Analysis of variance
AUDPC	Area under disease progress curve
AUSiPC	Area under severity index progress curve
BC	Backcross
CBB	Cassava bacterial blight
Cfu	Colony forming units
CIAT	Centro Internacional de Agricultura Tropical
Cm	Centimeter
CPD	Critical point dried
DM	Dry matter
Dpi	Days post inoculation
^{o}C	Degree Celsius
DS	Dry savanna
FST	Forest-savanna transition
Fig.	Figure
GLM	General linear model
GSPB	Göttinger Sammlung Phytopathogener Bakterien
Ha	Hectare
IITA	International Institute of Tropical Agriculture
INRAB	Institut National de Recherches Agronomiques du Benin
Kg	Kilogram
LER	Land equivalent ratio
LR	Likelihood ratio
Mg	Milligram
$MgSO_4$	Magnesium sulphate
Ml	Millilitre
Mm	Millimeter
Min	Minute
µl	Microlitre
MR	Medium resistant
P	Probability
QTL	Quantitative trait loci
R	Resistant
RCBD	Randomised complete block design
RFLP	Restriction fragment length polymorphisms
S	Susceptible
Si	Severity index
SSR	Simple sequence repeats
SAS	Statistical Analysis System
Tab.	Table
WS	Wet savanna
Xam	*Xanthomonas axonopodis* pv. *manihotis*
Xcm	*Xanthomonas campestris* pv. *manihotis*

Contents

1. Introduction

Cassava belongs to the dicotyledon family Euphorbiaceae. The genus *Manihot* is reported with about 100 species, among which *Manihot esculenta* Crantz is the only commercially cultivated one. Species considered to be involved in the ancestor of cassava were reviewed more recently (Allem, 1999). *Manihot esculenta* ssp. *flabellifolia* is regarded as the wild progenitor of modern cultivars and becomes part of the primary gene pool of the crop. *Manihot pruinosa* is found as the nearest species to the gene pool of cassava and is difficult to separate from the wild species *M. esculenta* ssp. *flabellifolia* on morphological grounds. *Manihot pilosa* and *Manihot triphylla* are close to cassava and have close vegetative and floral similarities. *M. esculenta* ssp. *peruviana* stands less close to cassava than *M. tripylla* (Allem, 2002). Cassava was domesticated in part of the Amazon, possibly in the Brazilian forest areas. In the 16th century, Portuguese navigators brought cassava from Brazil to the West coast of Africa (Jones, 1959) and later to East Africa through Madagascar and Zanzibar (Jennings, 1976).

Cassava is a shrub with erect and spreading plant types reaching 1-4 m height. The morphological characteristics of cassava are highly variable indicating a high degree of interspecific hybridation. Cassava genotypes are usually characterized on the basis of morphological and agronomic descriptors (Alves, 2002). Recent advances in molecular characterization permitted the use of molecular DNA markers to evaluate genetic diversity of germplasm (Beeching et al., 1993; Fregene et al., 1994). The cassava root is a true root developing a fibrous root system. Few roots bulk and become storage roots. The remaining roots are responsible for water and nutrient absorption. Stems are woody, cylindrical and formed by alternating nodes and internodes. Stem branching is sympodial. Leaves are lobed with palmated veins. Male and female flowers are produced on the same plant and on the same inflorescence, the female flowers open 1-2 weeks before the male flowers. Cassava is cross-pollinated by insects. The fruit is trilocular capsule, ovoid or globular, with a diameter of 1-1.5 cm diameter.

Cassava is adapted to a tropical environment, requiring high temperature and high solar radiation for optimal leaf development and for expression of its photosynthetic potential. Morphologically, cassava leaves combine some characteristics related to high productivity

and drought tolerance. All cassava organs, except seeds, contain cyanogenic glucosides. The most abundant, linamarin, is synthesized in the leaf and, when hydrolysed, releases HCN (Alves, 2002).

Cassava is propagated vegetatively through stem cuttings or stakes. Stakes sufficiently lignified, of at least 20 cm length and minimum 4-5 nodes with viable buds, are cut at a right angle. Soil preparation is necessary to allow good drainage and aeration. Cuttings are planted on flats, ridges, raised beds or mounds. In regions with medium to heavy soils and adequate rainfall (1000-2000 mm $year^{-1}$), stake position is not important, but in areas with sandy soils or erratic rainfall, vertical position is recommended. Generally, stakes are planted at a spacing of 1 m x 1 m, giving 10,000 plants·ha^{-1}, at a depth of 10-15 cm. Weed control is performed using hoes, machetes or sharpened shovels until 4 months after planting. Cassava is commonly intercropped with maize, cowpea, common bean and groundnut, and cassava is also found in intercropping with trees. Significant root increases were observed when mulch and a mineral fertilizer were used. No effect on roots was observed when a strong competitive effect occured (Akondé et al., 1996). The roots are harvested between 8 to 24 months after planting depending on the cultivar and the use. Rotation can restore organic materials and promote biological soil activity with beneficial consequences for structural stability. Cover crops contribute to reduce soil degradation, but their adoption is still compromised by difficulty in obtaining seed or planting material, laborious establishment in the field and their adverse impact on root yield (Leihner, 2002).

Cassava is a major source of dietary energy for low income consumers in many parts of tropical Africa (Berry, 1993; Dahniya, et al., 1994; Nweke, 1994a,b). It takes a greater contribution to total calorie intake in Africa than maize or sorghum. Additionally, leaves are a source of protein. Cassava is a food security crop in areas prone to drought and famine (Hillocks, 2002). Africa produces more cassava than the rest of the world combined. The largest African producers are Nigeria (35% of total African production and 19% of world production), Democratic Republic of Congo (19% of African production), Ghana (8%), Tanzania (7%) and Mozambique (6%) (FAO, 1998).

Shortened fallow periods and declining soil fertility, difficulty to get good quality planting material, lack of well-adapted varieties, and plant pest and diseases are main production constraints. Frequently occurring pests of cassava are: cassava green mites (*Mononychellus*

ssp.), elegant and variegated grasshopper (*Zonocerus elegans* L. and *Z. variegatus* Thumb.), cassava mealybug (*Phenacoccus manihoti* Matile-Ferrero), root mealybug (*Planococcus citri* Risso) and termites. The diseases affecting cassava production are: mosaic disease (Thresh, 1997; Calvert and Thresh, 2002), bacterial blight (Hillocks and Wydra, 2002), anthracnose disease (Hillocks and Wydra, 2002), stem rot (Afouda and Wydra, 1996, 1997), soft rot (Hillocks and Wydra, 2002), dry root rot (Lozano and Booth, 1976; Théberge et al., 1993), *sclerotium* root rot (Afouda et al., 1995) and nematodes (Caveness, 1979; Sikora et al., 1988; Coyne, 1994).

Among the serious diseases of cassava, cassava bacterial blight (CBB) is the second most important disease of cassava after cassava mosaic virus disease. The crop is attacked by *Xanthomonas axonopodis* pv. *manihotis* (Vauterin et al., 1995), former *X. campestris* pv. *manihotis* (Berthet and Bondar, 1915) Dye 1978. Typical symptoms include water-soaked, angular leaf spots, leaf blight, leaf fall and systemic symptoms, resulting in the formation of cankers and shoot die-back, and occur during the rainy season. Yield losses to CBB in the humid lowlands of Africa, where 60% of the area are affected, are estimated to 3.2 million tons. In West Africa, loss of root yield up to 76% was recorded with the highest losses in the dry savanna zone (Wydra et al., 2001a,b; Wydra, 2002; Wydra and Verdier, 2002; Wydra et al., 2003). Strong genotype x environment interactions occurred and losses varied with variety, ecozone and year (Wydra et al., 2001a). Integrated control measures were suggested (Wydra and Rudolph, 1999) including resistant cultivars, crop rotation, burying or burning of infected debris, weeding and avoiding bush fallow around cassava fields (Fanou et al., 1998; Fanou, 1999a; Fanou et al., 2001), mixed cropping associating cassava with maize (Fanou, 1999b), shifting planting date towards the end of the rainy season (Fanou, 1999b), avoiding use of contaminated tools (Lozano, 1986), seed disinfection by heat treatment (Persley, 1979; Lozano et al., 1989; Fanou, 1999b) and serological and molecular methods for detection (Fessehaie, 1997; Fessehaie et al., 1997; Wydra et al., 2003).

Integrated control measures were developed to control CBB (Wydra and Rudolph, 1999; Wydra et al., 2001), but the combined effect of individual measures on the severity of CBB in two ecozones of West Africa has never been studied. Host-plant resistance is the most important element in an integrated management system of CBB. Though selection for resistance has been ongoing since over 20 years, detailed and reliable studies on genotype x environment interactions including determination of yield loss do not exist. The reaction of

local and local improved cultivars from Benin to CBB is not known. Resistance mechanisms of cassava plant are unknown and only few, preliminary studies describe preformed defense mechanisms. Microscopical studies of leaf surfaces showed stomata with extensive occlusion by wax at the abaxial surface on several genotypes under greenhouse conditions (Cooper et al., 2001), but chemical and scanning electron microscopical studies of waxes and stomatal distribution of field plants in relation to resistance to CBB were not conducted. Molecular genetic maps provide a set of landmarks for the complete genome and consequently a high probability of detecting linkages with any gene(s) of interest in genetics or breeding. A genetic map of cassava designed to segregate for traits regarded as priorities for the development of molecular markers and marker-assisted selection was constructed (Fregene et al., 1997). After testing 244 individuals of F1 backcross with Latin American and one African strains, 5 markers were identified (Jorge et al., 2000), but a characterization of the reaction of individuals of the mapping population to a diverse, representative set of highly virulent African strains has not been made. Pathotypes among African strains were to date not described and specific resistance markers to African strains are not known. Further elements of an integrated management system, which support the mostly insufficient resistance found in genotypes, are agronomic and cultural measures. Few, single control measures (see above) were described, but they were never evaluated in combination and tested in different ecozones.

The first part of the studies focussed on host-plant resistance. Genotype x environment interactions in reaction to cassava bacterial blight evaluated in symptom development and yield were investigated in different ecozones of Benin, using local and local improved cultivars from Benin, including advanced breeding lines from the International Institute of Tropical Agriculture, Nigeria. Putative preformed resistance mechanism such as the distribution of stomata and cassava leaf waxes in relation to resistance to CBB were studied. Pathotypes among African strains of *X. axonopodis* pv. *manihotis* and differential genotypes for their identification were found by stem inoculation and leaf infiltration. Evaluating the reaction of 111 individuals of the genome mapping population from the backcross of 5 F1 individuals and the parent TMS30572 to stem and leaf inoculation, pathotype-specific genetic resistance markers and quantitative trait loci involved were investigated.

In the second part of the work, cultural control measures combining (i) intercropping cassava with sorghum or cowpea versus cassava monoculture, (ii) potassium fertilizer application and

mulching, and (iii) planting date shift were studied for their effect on CBB in two ecozones of West Africa.

Finally, recommendations for an integrated control of CBB, suitable (i) to farmers needs and adapted to agroecological conditions and (ii) to breeders for selection of genotypes with resistance to CBB were elaborated.

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2. Genotype x environment interactions in symptom development and yield of cassava genotypes in reaction to cassava bacterial blight in three ecozones of Benin

Abstract: Thirty-seven cassava (*Manihot esculenta* Crantz) genotypes from Benin, including advanced breeding lines from the International Institute of Tropical Agriculture, Nigeria, were tested for their reaction to bacterial blight in the forest-savanna transition, wet savanna and dry savanna zones of Benin. In years 1998, genotypes RB92164, RB92022, TMS30572, BEN86004, RB92033 and Dangbo2, and in year 2000, genotypes RB92202, RB92151, RB92132 and TMS30572 were resistant in one ecozone. Among the more resistant genotypes, genotypes CAP94030, BEN86040, RB89509, RB92132 and TMS30572 showed low interaction across environments and were most stable in disease reaction, while genotypes RB92022 and RB92004 showed higher interactions with different environments and were unstable. Four susceptible genotypes also showed low interactions with environments, while genotype BEN86002 (susceptible) was most stable in different environments. Comparing root yield across environments, 10 genotypes were classified as high yielding. Among the more resistant group of genotypes, only TMS30572 and RB89509 were high yielding, with genotype RB89509 being unstable in yield across environments. Genotypes CAP94030, BEN86040, RB92099, TMS30572 and RB92022 had low interactions with environments concerning yield. Artificially inoculated treatments in the wet savanna zone and in the forest-savanna transition zone with stable high symptom severity proved most suitable for screening of genotypes, while the site in the dry savanna zone with natural infection in year 1998 was the best environment for cassava production, and the site in the wet savanna zone with natural infection in year 1998 for production of propagation material. The correlation between disease severity expressed as area under severity index progress curve and root yield in the non-inoculated and inoculated treatments in each ecozone was significant only for the non-inoculated treatment in the dry savanna zone in year 2000 ($r = -0.58$), but not in any other environment. Among the 37 genotypes tested, genotype TMS30572 can be recommended to farmers as relatively stable in disease resistance and in high yield.

2.1 Introduction

Cassava is a basic component of the farming system in most areas of Sub-Saharan Africa (Nweke et al., 1994). Africa produces 48 million tons cassava roots annually from 7.4 million hectares which provide more than 200 calories per day for 200 million people (Dorosh, 1988). In Benin, cassava is one of the most cultivated root crops and the second food crop after maize (Nago, 1989). Farmers mostly cultivate local varieties (Nweke et al., 1994). The cassava plant suffers from numerous biotic constraints. Among them, cassava diseases of major economic importance in Africa are the African cassava mosaic virus disease, cassava bacterial blight (CBB), cassava anthracnose disease, and root rots (Makambila, 1992; Makambila and Koumouno, 1994; Fokunang et al., 2000; Hillocks and Wydra, 2002; Wydra and Verdier, 2002). CBB caused by *Xanthomonas axonopodis* pv. *manihotis (Xam)* (Vauterin et al., 1995), former *Xanthomonas campestris* pv. *manihotis*, is a major constraint to cassava cultivation worldwide (Lozano, 1986; Wydra and Msikita, 1998; Banito et al., 2001; Wydra and Verdier, 2002).

First symptoms of CBB appear as dark-green, water-soaked, angular spots on leaves, limited by veinlets and irregularly distributed on the lamina, which extend, coalesce and turn to brown (blight symptom). The water-soaked part becomes surrounded by a chlorotic halo and appears as translucent spot. Small droplets of exudates, first creamy white and later yellow oozing from the lesions, are visible on the lower surface of the leaves under humid conditions. Under conditions of high humidity, large exudation droplets appear in the axis of leaf petioles and the stem, resulting in a loss of turgidity followed by rapid wilting. A heavy infection of the shoot is leading to wilt and plant dieback. Root yield losses of more than 76% were reported in Africa (Fanou, 1999).

Among the proposed control measures (Wydra and Rudolph, 1999), host-plant resistance is one of the most suitable measures for farmers. In West Africa, the major cassava-growing area, a rate of 4.4% of increased yields recorded between 1976 and 1998 was the result from increased planting of improved varieties which can yield nearly 1.5 times more than local varieties (FAO, 2000). Local varieties are one of three main genetic sources of released varieties (Manyong et al., 2000). Selecting local varieties with resistance to CBB could significantly contribute to the increase of genetic diversity in cassava. In Benin, the five improved cultivars with generally good performance BEN86052, RB89509, TMS50395,

TMS30572 and TMS 4(2)1425 were identified by breeders (MDR, 1999). But, generally, the reaction of local and local improved cultivars to CBB is not known. Therefore, the objective of the present studies was to identify genotype x environment interactions among local and local improved genotypes from Benin in their reaction to CBB in different ecozones and to select resistant, high yielding genotypes suitable for farmers.

2.2 Materials and Methods

2.2.1 Experimental sites

The studies were conducted in three field sites located in 3 agro-ecological zones, the forest-savanna transition zone (IITA, Cotonou, South Benin), the wet savanna (Save, INRAB station, Centre Benin), and the dry savanna (Ina, INRAB station, North Benin), in the years 1998-1999, and repeated in 2000-2001. The forest-savanna transition zone has an average annual rainfall of 1,000 to 1,400 mm, which spreads from March to July and from September to October, with a small dry season in August. The long dry period extends from November to March. The mean temperature is about 27 ^{o}C with a low diurnal variation of 7 to 10 ^{o}C (Adam and Boko, 1993). The wet savanna zone has an annual rainfall of 900 to 1,300 mm from April to July and from September to October, followed by a dry season from November to April. The mean temperature is about 29 ^{o}C. The dry savanna zone has an annual rainfall of 700 to 900 mm distributed from April to October, followed by a dry season from November to March. The mean temperature is about 32 ^{o}C (MEHU, 1993).

2.2.2 Planting materials and experimental design

The cassava clones were received from the 'Station de Recherche de Niaouli' in Benin (Tab. 1). Their selection was based on their local ecosystem adaptation and they represent an important part of the widely cultivated clones in Benin. Check genotypes were selected according to their susceptible (BEN86052, TME1) and resistant (TMS30572) reaction to CBB and their good general performance (Boher and Agbobli, 1992; Akparobi et al., 1998; Fanou, 1999; Fokunang et al., 2000). The experiments were conducted during 2 planting seasons in the years 1998 and 2000. An augmented complete randomized block design (RCBD) proposed by Federer (1956) with 3 blocks and 10 to 13 genotypes per block depending on available genotypes was used. This design is appropriate, when large numbers of genotypes cannot be replicated due to insufficient genotype supplies and experimental field area. Standard genotypes used as checks are replicated. Each replicate forms a complete block in the standard design. Additionally, not-assigned plots are created within each replicate, and

not-replicated genotypes for which not sufficient material is available are assigned to those plots in the form of an incomplete block design (Scott and Milliken, 1993; Wolfinger et al., 1997). In the present study, three genotypes formerly identified as susceptible (BEN86052, TME1) and resistant (TMS30572) (Akparobi et al., 1998; Fanou, 1999) served as checks and were replicated three times (three blocks) in an RCBD. Twenty-two cuttings of about 20 cm length of cassava genotypes were planted on two ridges of 10 m at a spacing of 1 m, in June in the forest-savanna transition zone, and in July in the wet savanna and the dry savanna zones in the planting seasons 1998 and 2000. The design was repeated in two treatments - non-inoculated and inoculated - separated by a screen of sorghum of 6 m width.

Table 1: Cassava genotypes planted in forest-savanna, wet savanna and dry savanna zones in Benin during the seasons 1998 and 2000

Genotypes planted in years 1998 and 2000	Genotypes planted only in year 1998	Genotypes planted only in year 2000
1 RB89608	17 RB92033	34 TMS92/0057
2 CAP94030	18 ABC Kologoun	35 TMS91/2327
3 RB92103	21 CAP94034	36 TMS91/2324
4 BEN86052	22 Houekoute	37 CAP92034
5 RB89509	23 RB92052	
6 RB92182	25 CAP94049	
7 CAP94059	26 RB92125	
8 RB92202	27 BEN86018	
9 BEN86002	28 RB92174	
10 TME1	30 Dangbo2	
11 RB92099	31 BEN86016	
12 RB92022	32 RB92164	
13 BEN86040	33 RB92131	
14 TMS30572		
15 RB92004		
16 RB92132		
19 CAP94066[1]		
20 RB92162[1]		
24 RB92151[1]		
29 BEN86004[1]		

1 Genotypes missing in some environments

2.2.3 Inoculation

Three highly virulent strains of *Xanthomonas axonopodis* pv. *manihotis,* GSPB 2506 and GSPB 2510 (Göttinger Sammlung Phytopathogener Bakterien, Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Germany), and Save 10, isolated by K. Wydra at Cotonou, Ina and Save, Benin, respectively, were used for inoculation of field trials. The bacterial suspensions were prepared from 48-hour-old cultures on nutrient glucose agar (nutrient broth 8 g/l, glucose 11 g/l, yeast extract 3 g/l, agar 15 g/l, pH 7.2) with an optical density of 0.06 at 660 nm corresponding to about 10^8 cfu/ml, and further diluted with tap water by 1:10 (10^7 cfu/ml) for inoculation. Few drops of Tween 80 were added to facilitate wetting of sprayed leaves. The inoculum was sprayed with a motorized sprayer (Solo, Germany) onto the lower surface of the leaves in the evening or in the early morning three times at a monthly interval. In the following, these artificially inoculated plots will be named inoculated, while the naturally infected plots will be named non-inoculated.

2.2.4 Symptom assessment

Disease symptoms consisting of leaves with spot, leaves with blight, dropped/wilted leaves and stems with die-back were recorded on ten plants selected randomly per plot one month after the first spraying and at a monthly interval with a gap over the dry season until the harvest at 12 months, using a percentage scale divided in classes: <5%, 5-10%, 10-20%, 20-50%, 50-80% and 80-100%. A leaf with spots and blight was counted as leaf with blight only. For calculations, class values were transformed to mean values for each class. At harvest, the total number of leaves, number of leaves with spots, blight and of wilted leaves were counted only for the first five plants out of the ten plants harvested.

The severity index in days at each evaluation date was calculated according to the following formula: $Si = (1 \times S + 2 \times B + 1 \times W + 2 \times D)/6$, where *S, B, W* and *D* represent the percentage of leaves with spots, blight, wilt and stems with dieback, respectively. The highest possible value is 60 (e.g. evaluation of 80-100% wilt and 80-100% dieback corresponds to (1 x 0 + 2 x 90 + 1 x 0 + 2 x 90)/6). The weight attributed to the symptoms blight and dieback is an estimation resulting from regression analysis of symptom and plant growth data, revealing blight as most important factor influencing root yield, and dieback with highest influence on overall plant growth (leaf and stem weight) (unpublished data). The mean severity index of 10 plants of each genotype at six evaluation dates, with dates 60, 90, 120, 150, 180 and 360 days after planting in each ecozone, was used to calculate the area under severity index progress

curve (AUSiPC) with the calculus method of integration of area under a curve (Genstat for Windows, 1993). The AUSiPC in days over the whole period was then divided by the evaluation period [365 days minus days of dry period (60, 120 and 200 days in the forest-savanna transition, wet savanna and dry savanna zones, respectively, in 1998, and 120, 150 and 200 days in the forest-savanna transition, wet savanna and dry savanna zones, respectively, in 2000)] to receive an average comparable between ecozones. Thus, all AUSiPC values are standardized.

2.2.5 Yield parameter assessment

The weight of storage roots was recorded from 10 plants selected per plot at 12 months after planting. For dry weight determination, roots of each plot were combined, mixed, and a sub-sample was cut into small pieces, weighed and dried in a paper bag in an oven at 105 ^{o}C for 72 h.

2.2.6 Statistical analysis

Analyses of standardized area under severity index progress curve (AUSiPC) and of root dry weight were performed using the Linear Mixed Model ANOVA (Harville, 1988; Littell et al., 1996). The analytical procedures for augmented design using mixed models as implemented in the SAS software (SAS Institute Inc., 1990; 1997) were performed as described by Korie and Okechukwu (2000). The analysis involves estimation of block effects and plot error using the replicated checks. The error derived from the checks was used to obtain valid statistical tests of differences among the other genotypes. Classes of susceptible (S) (75-100%), medium resistant (MR) (50-74.9%), and 'resistant' (R) (0-49.9%) genotypes were formed on basis of the percentage of standardized AUSiPC values in the respective environment in the artificially inoculated treatment, using the highest value as 100%. Data were log-transformed to stabilize the variance for the analysis. Values and standard errors in tables are the real, non-transformed values. Standard errors of real means were calculated for root yield data, but not for AUSiPC, since the latter is based on means per plot and AUSiPC was not calculated on single plant basis. The calculated standardized AUSiPC and root dry weight were subjected to the stability analysis performing the additive main effect and multiplicative interaction (AMMI) model partitioning the interaction between genotypes and environments into principal components, and defining stable genotypes or environments using the MATMODEL software (Gauch, 1993). Pearson correlation analysis was performed to show the relationship

between severity expressed as standardized area under severity index progress curve and root yield using means of genotypes.

2.3 Results

Bacterial blight development of cassava genotypes expressed by the standardized area under severity index progress curve (AUSiPC) values varied considerably within and between ecozones and allowed formation of three groups, susceptible (S), medium resistant (MR) and resistant (R), using data from the inoculated genotypes.

2.3.1 Disease development in the forest-savanna transition zone

In year 1998 in the forest-savanna transition zone, all genotypes were infected in non-inoculated (AUSiPC 3.7-8.8) and inoculated (AUSiPC 4.7-9.6) plots (Tab. 2). Based on the percentages of AUSiPC of genotypes, eighteen genotypes (inoculated treatment) were susceptible (AUSiPC $\geq$ 7.2, 75-100%), fourteen medium resistant (AUSiPC 4.8-7.1, 50-74.9%) and one resistant (genotype RB92164 with AUSiPC $\leq$ 4.7, 0-49.9%) (Tabs. 2, 3).
Comparing both treatments, most susceptible genotypes reacted strongly on the inoculation, while the more resistant genotypes kept the same symptom level as under natural infection. The highest AUSiPC values across ecozones, years and treatments were observed in year 1998 with single values up to 9.6. In year 2000, fourteen genotypes were susceptible (AUSiPC $\geq$ 6.9), nine medium resistant (AUSiPC 4.6-6.8) and one resistant genotype RB92202 with AUSiPC $\leq$ 4.5 (Tabs. 2, 3). In year 2000, natural infection was generally low and susceptible and resistant genotypes could only be differentiated in inoculated plots. Inoculation generally increased AUSiPC by a factor of four, except in the forest-savanna transition zone in year 1998 and the dry savanna zone in year 2000. Genotype RB92202 was resistant in year 2000, but susceptible in year 1998. Without artificial inoculation in year 2000, symptom development of the standard genotypes (BEN86052, TME1, susceptible; TMS30572, resistant) was similar, while after inoculation, less symptoms were observed in the resistant genotype. The disease develops during the rainy season (Fig. 1), while symptoms disappear during the dry season and reappear in the rainy season of the following year.

2.3.2 Disease development in the wet savanna zone

In the wet savanna zone, inoculation increased disease index from AUSiPC 0.8-3.3 to AUSiPC 3.9-7.8 in year 1998, and from AUSiPC 1.7-3.7 to AUSiPC 3.5-7.4 in year 2000 (Tab. 2). In year 1998, 22 genotypes were susceptible (AUSiPC $\geq$ 5.7) and 11 medium

resistant (AUSiPC 3.9-5.6). In year 2000, 15 genotypes were susceptible (AUSiPC ≥ 5.6), eight medium resistant (AUSiPC 3.7-5.5) and one resistant (AUSiPC ≤ 3.6, genotype RB92151).

2.3.3 Disease development in the dry savanna zone

In year 1998, AUSiPC of genotypes ranged between 0.5 and 4.1 (non-inoculated), and 3.7 and 8.3 (inoculated). Ten genotypes were susceptible (AUSiPC ≥ 6.3) seventeen medium resistant (AUSiPC 4.2-6.2), and five resistant (AUSiPC ≤ 4.2) (Tab. 2). In year 2000, AUSiPC of genotypes ranged between 0.7 and 5.2 (non-inoculated), and 2.9 and 6.5 (inoculated). Seven genotypes were susceptible (AUSiPC ≥ 4.9), twelve medium resistant (AUSiPC 3.3-4.8) and two resistant (AUSiPC ≤ 3.2).

2.3.4 Comparison between sites (ecozones)

In both years, in the inoculated treatment the disease was more severe in the forest-savanna transition (total AUSiPC 129 and 123 in years 1998 and 2000, respectively) than in the wet savanna (total AUSiPC 106.5 and 101.9 in years 1998 and 2000, respectively) and the dry savanna zones (total AUSiPC 95.1 and 74.3 in years 1998 and 2000, respectively). High disease severity was generally observed only after inoculation, but not in non-inoculated treatments. Many genotypes, reacting resistant under natural infection were partly highly susceptible after artificial inoculation.

In the forest-savanna transition zone, six genotypes were susceptible in both years, one genotype (RB92202) resistant in one year, but susceptible in the other year, and one genotype (RB92164) resistant, but tested only in one year, while the other genotypes were medium resistant (Tab. 3). No genotype was resistant over two years. In the wet savanna zone, ten genotypes were susceptible in both years and one genotype (RB92151) resistant in one year. Under the generally low disease severity index in the dry savanna zone compared to the other ecozones, three genotypes were susceptible and one genotype TMS30572 was resistant in both years, while genotypes BEN86004, RB92033, Dangbo2 were resistant, but tested only in one year. Different than in other ecozones, the combination resistant and medium resistant in the two years with genotypes (RB92132, RB92022) was observed in the dry savanna zone.

2.3.5 Genotype x environment interactions and disease development

The distribution of 16 genotypes, repeated in each ecozone and year, in inoculated treatments across severity indices varied between years and ecozones revealing high genotype x environment interactions (Fig. 2). The conditions in the forest-savanna transition zone were most favourable for disease development after inoculation, while in the dry savanna, the disease level was generally lower, genotypes were more distributed across disease severity index levels, and genotypes with low disease level occurred.

The standardized AUSiPC in 12 environments (ecozones, years, inoculated and non-inoculated treatments) of those 16 genotypes which had been planted in both years was analysed using the additive main effect and multiplicative interaction (AMMI) model which provided a good description of stability of genotypes and effect of the environment. The environment, genotype, genotype x environment interaction accounted for 82.6%, 4.1% and 13.3% of the treatment sum of squares, respectively, with IPCA1, IPCA2, IPCA3, IPCA4 and IPCA5 contributing to 27.6%, 23.1%, 16.7%, 8.5% and 7.8% of the genotype x environment sum of squares, respectively (see Annex 1). AMMI analyses provided a biplot accounting for 90.4% of the treatment sum of squares (Fig. 3). Genotypes having high AUSiPC values are located on the right side and are more susceptible than genotypes on the left side. Genotypes or environments with high positive or negative IPCA1 scores have high interactions. Thus, genotypes BEN86040 (13), BEN86052 (4), CAP94030 (2), RB92132 (16), RB89509 (5), TMS30572 (14), TME1 (10) and RB92099 (11) in decreasing order showed low interactions with environments, while genotype BEN86002 (9) being on the zero line of the biplot showed no interactions and was the most stable genotype in different environments. Genotypes RB92182 (6), RB92103 (3), RB92202 (8), CAP94059 (7), RB92004 (15), RB89608 (1) and RB92022 (12) were classified as unstable, with the latter three genotypes showing highest interactions with different environments. Among the more resistant genotypes, RB92004 and RB92022 were not stable across environments.

The environments in the forest-savanna transition zone in inoculated and non-inoculated blocks (E2 and E1) in year 1998, in the wet savanna and the dry savanna (E6 and E10) in inoculated blocks in year 1998, and in the forest-savanna transition and the wet savanna in inoculated blocks in year 2000 (E4 and E8, respectively) were most favourable for disease development. In contrast, the non-inoculated blocks in the forest-savanna transition zone in year 2000 and the wet savanna zone in year 1998 (E3 and E5, respectively), were least

favourable for disease development. Among them, the environments E2 and E8 as well as E5, E3 and E9 were stable with high and low symptom severity, respectively, while environments E10, E4 and E11 showed high variability in symptom severity between genotypes.

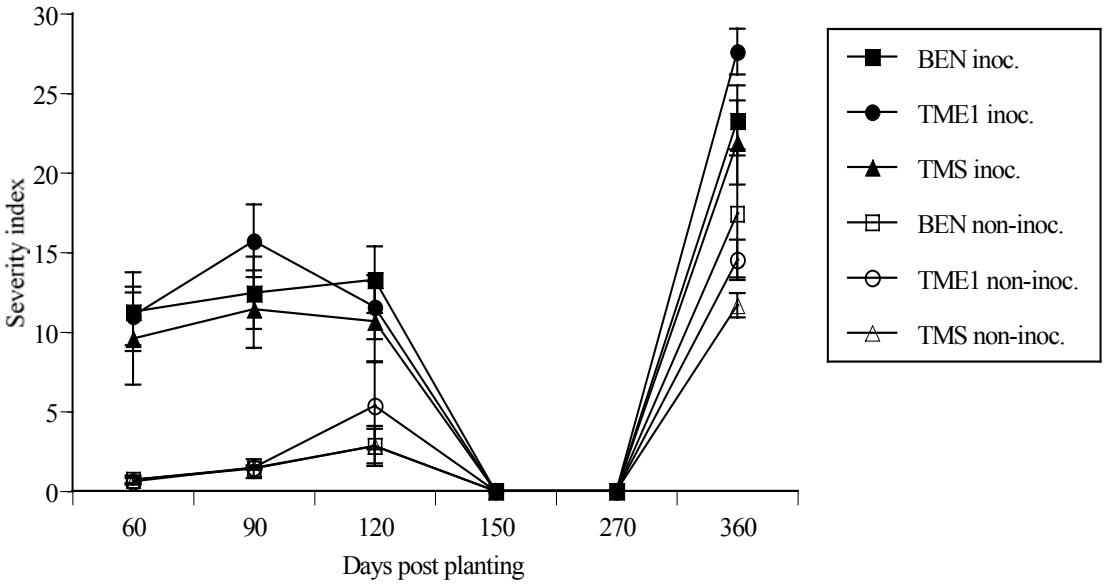

Figure 1: Development of severity index in the susceptible genotypes BEN86052 and TME1, and the resistant genotype TMS30572 in non-inoculated and inoculated treatments in the forest-savanna transition zone in year 2000 (dates of inoculation: 60, 90, 120 days after planting)

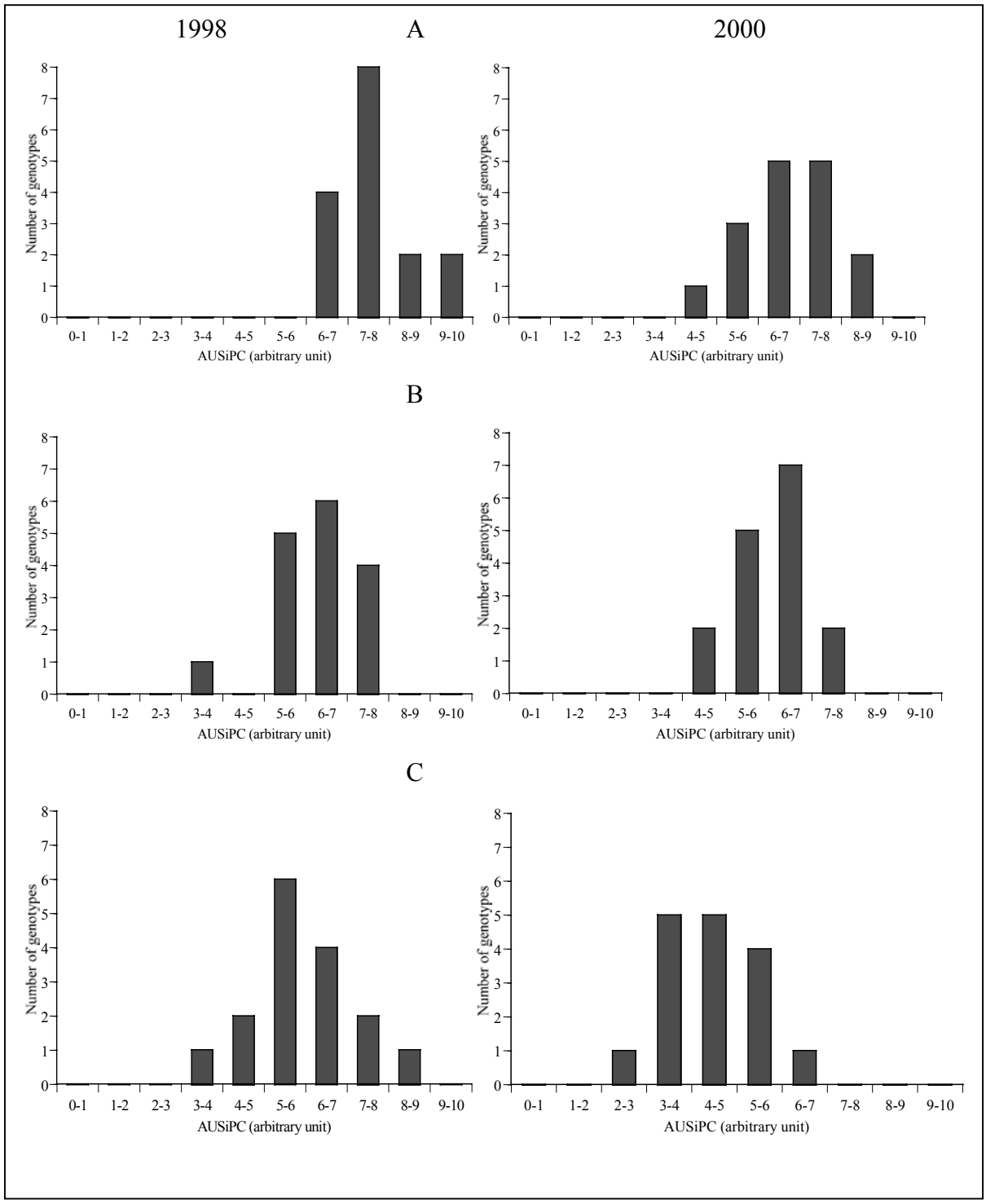

Figure 2: Distribution of 16 genotypes repeated in each ecozone and year according to their disease development expressed as standardized area under severity index progress curve of inoculated treatments in the forest-savanna transition (A), wet savanna (B) and dry savanna (C) zones in year 1998 (left) and year 2000 (right)

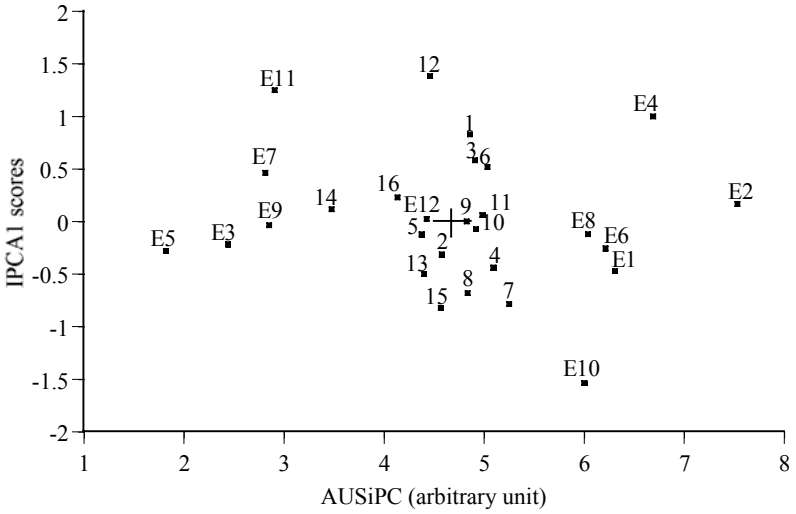

Figure 3: Relation between standardized area under severity index progress curve and IPCA1 scores for 16 genotypes grown in 12 environments

Genotype identification 1: RB89608, 2: CAP94030, 3: RB92103, 4: BEN86052, 5: RB89509, 6: RB92182, 7: CAP94059, 8: RB92202, 9: BEN86002, 10: TME1, 11: RB92099, 12: RB92022, 13: BEN86040, 14: TMS30572, 15: RB92004, 16: RB92132.

Environment: E1, E2: non-inoculated and inoculated blocks, respectively, in the forest-savanna transition zone in 1998; E3, E4: non-inoculated and inoculated blocks, respectively, in the forest-savanna transition zone in 2000; E5, E6: non-inoculated and inoculated blocks, respectively, in the wet savanna zone in 1998; E7, E8: non-inoculated and inoculated blocks, respectively, in the wet savanna zone in 2000; E9, E10: non-inoculated and inoculated blocks, respectively, in the dry savanna zone in 1998; E11, E12: non-inoculated and inoculated blocks, respectively, in the dry savanna zone in 2000.

Table 2: Standardized AUSiPC of non-inoculated and inoculated genotypes with *Xam* strains GSPB2506, Save 10 and GSPB2510 in forest-savanna transition, wet savanna and dry savanna zones in two years (in order of high to low AUSiPC in the forest-savanna transition zone, 1998, inoculated treatment)

	Forest savanna transition				Wet savanna				Dry savanna			
	1998		2000		1998		2000		1998		2000	
Genotypes	AUSiPC Non-inoc.	AUSiPC Inoc.	AUSiPC Non-inoc.	AUSiPC Inoc.	AUSiPC Non-inoc.	AUSiPC Inoc.	AUSiPC Non-inoc.	AUSiPC Inoc.	AUSiPC Non-inoc.	AUSiPC Inoc.	AUSiPC Non-inoc.	AUSiPC Inoc.
2 CAP94030	6.6[1]	9.6	2.4	5.9	1.3	6.4	2.5	5.2	2.7	6.3	2.3	3.7
1 RB89608	5.8	9.4	2.0	7.1	2.1	6.9	2.6	4.0	2.3	5.5	5.2	5.6
17 RB92033	8.8	9.1	nd[2]	nd	1.6	4.7	nd	nd	2.4	3.7	nd	nd
18 ABC Kologoun	5.3	8.7	nd	nd	1.5	7.6	nd	nd	1.7	4.7	nd	nd
19 CAP94066	6.7	8.5	1.9	7.6	1.5	5.9	2.8	4.6	nd	nd	1.7	3.5
3 RB92103	5.8	8.1	3.4	7.1	2.0	6.8	3.2	6.2	2.9	4.7	3.5	5.2
4 BEN86052	7.4	8.1	2.6	6.6	2.7	6.8	2.6	5.6	3.2	7.2	3.2	5.1
20 RB92162	6.8	7.9	3.0	9.1	1.2	6.1	3.0	7.0	2.4	4.5	nd	nd
23 RB92052	5.0	7.9	nd	nd	2.0	6.1	nd	nd	2.9	6.4	nd	nd
8 RB92202	6.9	7.8	1.2	4.5	3.3	6.8	3.1	6.8	2.7	6.9	3.1	4.9
10 TME1	7.2	7.8	2.6	7.3	1.9	5.4	3.0	6.7	3.8	6.3	2.9	4.4
33 RB92131	7.6	7.8	nd	nd	0.8	5.4	nd	nd	0.5	4.4	nd	nd
7 CAP94059	7.1	7.7	3.4	6.3	2.4	7.1	2.3	6.4	3.1	8.3	3.5	5.4
16 RB92132	4.5	7.7	1.9	6.3	2.3	5.6	2.9	5.9	2.2	5.1	2.3	2.9
21 CAP94034	7.4	7.6	nd	nd	2.9	5.8	nd	nd	2.0	4.8	nd	nd
11 RB92099	6.0	7.6	4.0	5.7	1.3	5.6	3.7	7.2	2.8	5.9	3.5	6.5
22 Houekoute	4.0	7.4	nd	nd	1.4	6.1	nd	nd	2.9	6.9	nd	nd
6 RB92182	6.7	7.4	2.2	8.1	1.7	7.5	2.8	5.7	4.1	5.7	4.1	4.4
5 RB89509	6.1	7.1	2.1	6.9	1.1	6.0	2.7	6.6	1.6	6.1	2.1	4.1
9 BEN86002	6.9	7.1	2.2	7.3	1.5	7.2	3.3	7.4	2.8	5.4	2.2	4.4
25 CAP94049	5.6	7.1	nd	nd	1.8	6.0	nd	nd	2.6	6.5	nd	nd
26 RB92125	6.5	7.0	nd	nd	2.1	6.1	nd	nd	1.8	6.1	nd	nd

Table 2: continued

	Forest savanna transition				Wet savanna				Dry savanna			
	1998		2000		1998		2000		1998		2000	
Genotypes	AUSiPC Non-inoc.	AUSiPC Inoc.	AUSiPC Non-inoc.	AUSiPC Inoc.	AUSiPC Non-inoc.	AUSiPC Inoc.	AUSiPC Non-inoc.	AUSiPC Inoc.	AUSiPC Non-inoc.	AUSiPC Inoc.	AUSiPC Non-inoc.	AUSiPC Inoc.
13 BEN86040	6.7	6.8	3.5	6.1	1.8	5.7	1.7	6.5	2.9	5.8	1.7	3.6
24 RB92151	6.5	6.6	3.8	8.3	1.1	5.9	2.4	3.5	2.3	5.1	nd	nd
12 RB92022	5.8	6.3	1.4	8.5	0.9	5.7	3.7	6.7	2.7	3.9	3.6	4.3
28 RB92174	5.1	6.3	nd	nd	1.3	5.0	nd	nd	2.4	7.2	nd	nd
27 BEN86018	4.8	6.1	nd	nd	1.1	4.8	nd	nd	1.0	4.9	nd	nd
31 BEN86016	5.4	6.1	nd	nd	1.3	5.2	nd	nd	2.7	5.5	nd	nd
14 TMS30572	5.6	6.0	1.9	5.9	1.2	3.9	2.2	4.9	1.6	4.2	1.2	3.1
15 RB92004	5.8	6.0	2.2	7.7	1.2	7.8	3.0	5.5	3.2	7.8	0.7	3.9
29 BEN86004	5.1	5.9	1.6	8.1	1.4	6.2	3.1	5.1	1.8	3.8	nd	nd
30 Dangbo2	3.7	4.8	nd	nd	0.8	5.0	nd	nd	1.9	4.1	nd	nd
32 RB92164	3.7	4.7	nd	nd	1.6	4.4	nd	nd	2.2	4.9	nd	nd
34 TMS92/0057	nd	nd	3.5	6.1	nd	nd	3.3	5.9	nd	nd	2.4	4.9
35 TMS91/2327	nd	nd	1.4	7.4	nd	nd	3.2	6.7	nd	nd	2.7	3.7
36 TMS91/2324	nd	nd	2.0	8.4	nd	nd	3.0	5.3	nd	nd	1.7	3.9
37 CAP92034	nd	nd	2.2	5.7	nd	nd	2.4	5.4	nd	nd	2.4	3.9
SE[3]	C[4] 0.1	C 0.4	C 0.3	C 0.8	C 0.2	C 0.8	C 0.2	C 0.5	C 0.8	C 0.5	C 0.5	C 0.7
SE	X[5] 0.2	X 0.8	X 0.5	X 0.9	X 0.3	X 1.3	X 0.4	X 0.5	X 1.3	X 0.9	X 0.6	X 1.2
Range	3.7-8.8	4.7-9.6	1.2-4.0	4.5-9.1	0.8-3.3	3.9-7.8	1.7-3.7	3.5-7.4	0.5-4.1	3.7-8.3	0.7-5.2	2.9-6.5
Σ[6]	107.6	129	40.9	123	30.8	106.5	48.1	101.9	44.6	95.1	43.3	74.3

1 Real mean values
2 nd = not determined due to non- availability of genotypes
3 SE = standard error
4 C = check genotypes BEN86052, TME1 and TMS30572; repeated in each block
5 X = other genotypes than check; not repeated in each block
6 Σ = Total standardized AUSiPC of 16 genotypes which were repeated in each ecozone and year

Table 3: Reaction of cassava genotypes to bacterial blight in three ecozones in the inoculated treatments during the planting seasons 1998 and 2000

	FST[1]		WS		DS	
Genotypes	1998	2000	1998	2000	1998	2000
7 CAP94059	S[2]	MR	S	S	S	S
3 RB92103	S	S	S	S	MR	S
4 BEN86052	S	MR	S	S	S	S
8 RB92202	S	R	S	S	S	S
10 TME1	S	S	MR	S	S	MR
6 RB92182	S	S	S	S	MR	MR
9 BEN86002	MR	S	S	S	MR	MR
11 RB92099	S	MR	MR	S	MR	S
2 CAP94030	S	MR	S	MR	S	MR
15 RB92004	MR	S	S	MR	S	MR
1 RB89608	S	S	S	MR	MR	S
5 RB89509	MR	S	S	S	MR	MR
12 RB92022	MR	S	S	S	R	MR
13 BEN86040	MR	MR	S	S	MR	MR
16 RB92132	S	MR	MR	S	MR	R
14 TMS30572	MR	MR	MR	MR	R	R
20 RB92162	S	S	S	S	MR	nd[3]
22 Houekoute	S	nd	S	nd	S	nd
23 RB92052	S	nd	S	nd	S	nd
19 CAP94066	S	S	S	MR	nd	MR
18 ABC Kologoun	S	nd	S	nd	MR	nd
25 CAP94049	MR	nd	S	nd	S	nd
35 TMS91/2327	nd	S	nd	S	nd	MR
34 TMS92/0057	nd	MR	nd	S	nd	S
33 RB92131	S	nd	MR	nd	MR	nd
21 CAP94034	S	nd	S	nd	MR	nd
26 RB92125	MR	nd	S	nd	MR	nd
28 RB92174	MR	nd	MR	nd	S	nd
24 RB92151	MR	S	S	R	MR	nd
29 BEN86004	MR	S	S	MR	R	nd
36 TMS91/2324	nd	S	nd	MR	nd	MR
17 RB92033	S	nd	MR	nd	R	nd
27 BEN86018	MR	nd	MR	nd	MR	nd
31 BEN86016	MR	nd	MR	nd	MR	nd
37 CAP92034	nd	MR	nd	MR	nd	MR
30 Dangbo2	MR	nd	MR	nd	R	nd
32 RB92164	R	nd	MR	nd	MR	nd

1 FST: Forest Savanna transition, WS: Wet Savanna, DS: Dry Savanna 2 S: Susceptible (75-100%), MR: medium resistant (50-74.9%), R: resistant (0-49.9%), percentages calculatedaccording to genotype with highest AUSiPC value in ecozone and year 3 nd: not determined

2.3.6 Yield evaluation of cassava genotypes in 3 ecozones

In the forest-savanna transition zone in years 1998 and 2000, yield differences were not significant between check genotypes BEN86052 (S, MR in 1998 and 2000, respectively; disease reaction was only classified for inoculated treatments), TME1 (S in both years) and TMS30572 (MR in both years) in the inoculated and non-inoculated treatments (Tab. 4). The maximum value of 21.5 t·ha^{-1} of dry root yield was observed for genotype RB89509 (MR), and the minimum value of 0.7 t·ha^{-1} for genotype BEN86016 (MR) in the inoculated treatment in year 1998, while the maximum value of 17.6 t·ha^{-1} of dry root yield was observed for genotype RB89608 (MR), and the minimum value of 5.7 t·ha^{-1} for genotype BEN86004 (S) in the inoculated treatment in year 2000.

In the wet savanna zone in year 1998, yield differences between the check genotypes TME1 and BEN86052, BEN86052 and TMS30572, and TME1 and TMS30572 were significant in the non-inoculated treatment ($p = 0.0147$, $p = 0.0437$, $p = 0.0001$, respectively). The maximum dry root yield of 14.5 t·ha^{-1} and the minimum of 1.4 t·ha^{-1} were observed with genotypes TME1 and BEN86004, respectively (non-inoculated treatment). In year 2000, yield difference between the check genotypes TME1 (S) and TMS30572 (MR) was significant in the inoculated treatment ($p = 0.001$). The maximum dry root yield of 21.3 t·ha^{-1} was observed with genotype TMS91/2324 (MR) and the minimum dry root yield of 3.4 t·ha^{-1} with genotype BEN86040 (MR) in the inoculated and non-inoculated treatment, respectively.

In the dry savanna zone in years 1998 and 2000, yield differences between the check genotypes were not significant in the inoculated, nor the non-inoculated treatments. In year 1998, the maximum value of 17.6 t·ha^{-1} of dry root yield was recorded for genotype RB92182 (MR) and the minimum value of 1.2 t·ha^{-1} for genotype RB92164 (MR) (non-inoculated treatment). In year 2000, the maximum value of 12.2 t·ha^{-1} of dry root yield was produced by genotype TMS91/2324 (MR) and the minimum value of 2.6 t·ha^{-1} by genotype RB89509 (MR) in the non-inoculated and inoculated treatment, respectively.

2.3.7 Genotype x environment interactions and root yield

The dry root yield in 12 environments (ecozones, years, inoculated and non-inoculated treatments) of those 16 genotypes, which were repeated in both years, was analysed by the additive main effect and multiplicative interaction (AMMI) model. The environment, genotype and genotype x environment interaction accounted for 28.5%, 24.5% and 47% of

the treatment sum of squares, respectively, with IPCA1, IPCA2, IPCA3 and IPCA4 contributing to 36.6%, 19.7%, 11.9% and 9.9% of the genotype x environment sum of squares, respectively (see Annex 2). AMMI analysis provided a biplot accounting for 70.2% of the treatment sum of squares (Fig. 4). Genotypes on the right side have higher yields than genotypes on the left side. Genotypes or environments with high positive or negative IPCA1 scores have high interactions. Genotypes CAP94030 (2), BEN86040 (13), RB92099 (11), TMS30572 (14) and RB92022 (12) in decreasing order showed low interactions with environments, while genotypes CAP94059 (7), TME1 (10), BEN86052 (4), RB92103 (3), RB92202 (8), RB89608 (1), RB92132 (16), RB92182 (6), RB89509 (5) and RB92004 (15) in increasing order were classified as unstable because of higher absolute IPCA 1 scores and, thus, showed interactions with different environments.

The environments in the forest-savanna transition zone in inoculated blocks (E2) in year 1998, in the forest-savanna transition zone in non-inoculated blocks and wet savanna zone in inoculated blocks in year 2000 (E3 and E8, respectively) were most favourable for high yield. In contrast, the non-inoculated blocks in the wet savanna zone in year 1998 and the inoculated blocks in the dry savanna zone in year 2000 (E5 and E12, respectively), were least favourable for high yield. Among the environments with favourable conditions for higher yield, the sites in the wet savanna zone artificially inoculated and with natural infection in year 2000 (E8 and E7, respectively), showed highest stability across genotypes, while in the sites in the forest-savanna transition zone artificially inoculated in year 1998 and with natural infection in year 2000 (E2 and E3, respectively) high variability between genotypes was observed.

2.3.8 Relation between the standardized area under severity index progress curve and root yield

Based on the mean of AUSiPC and root yield across 12 environments, 16 genotypes were ranked in decreasing order (Tab. 5). Among genotypes with lower AUSiPC (AUSiPC $\leq$ 4.5), only genotypes TMS30572 and RB89509 were high yielding, while the other genotypes had a lower yield. Genotype RB89509 belonged to the group classified as more stable in disease reaction across environments (Fig. 3), but was medium resistant and susceptible in 3 environments, respectively, while TMS30572 was resistant in two environments and medium resistant in 4 environments. Most of genotypes with high AUSiPC (> 4.6) were high-yielding, and among the high yielding genotypes (yield $\geq$ 10 t·ha^{-1}), only genotype TMS30572 was stable in yield across environments. In the site in the dry savanna zone with natural infection

in year 1998 (E9), genotypes were stable in high yield and low symptom severity. In the artificially inoculated site in the wet savanna zone in year 2000 (E8), genotypes were stable in high yield and high symptom severity, while in the artificially inoculated site in the wet savanna zone in year 1998 (E6), they were stable on a high symptom level at a lower yield. The correlation between disease severity expressed as standardized area under severity index progress curve and root yield in the non-inoculated and inoculated treatments in each ecozone was significant for the non-inoculated treatment in the dry savanna zone in year 2000 (r = -0.58), but not in year 1998 and not in any other environment (Tab. 6).

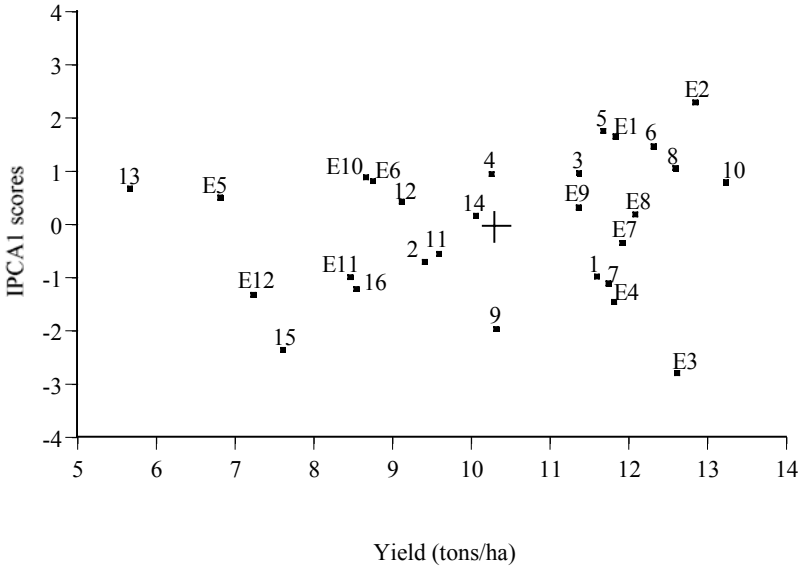

Figure 4: Relation between root yield and IPCA1 scores for 16 genotypes grown in 12 environments

Genotype identification 1: RB89608, 2: CAP94030, 3: RB92103, 4: BEN86052, 5: RB89509, 6: RB92182, 7: CAP94059, 8: RB92202, 9: BEN86002, 10: TME1, 11: RB92099, 12: RB92022, 13: BEN86040, 14: TMS30572, 15: RB92004, 16: RB92132.

Environment: E1, E2: non-inoculated and inoculated blocks, respectively, in the forest-savanna transition zone in 1998; E3, E4: non-inoculated and inoculated blocks, respectively, in the forest-savanna transition zone in 2000; E5, E6: non-inoculated and inoculated blocks, respectively, in the wet savanna zone in 1998; E7, E8: non-inoculated and inoculated blocks, respectively, in the wet savanna zone in 2000; E9, E10: non-inoculated and inoculated blocks, respectively, in the dry savanna zone in 1998; E11, E12: non-inoculated and inoculated blocks, respectively, in the dry savanna zone in 2000.

Table 4: Root yield (t·ha^{-1}) of 37 genotypes in inoculated and non-inoculated treatment in forest-savanna, wet savanna and dry savanna zones in two years (order of genotypes corresponds to Tab. 2)

		Forest savanna transition				Wet savanna				Dry savanna			
		1998		2000		1998		2000		1998		2000	
	Genotypes	Non-Inoc.	Inoc.	Non-Inoc.	Inoc.	Non-Inoc.	Inoc.	Non-Inoc.	Inoc.	Non-Inoc.	Inoc.	Non-Inoc.	Inoc.
1	RB89608	9.4±2.3[1]	13.3±1.8	17.2±3.1	17.6±4.4	7.8±1.0	12.6±2.0	10.7±1.5	9.5±1.8	12.6±1.9	9.8±1.3	11.1±1.0	9.3±1.7
17	RB92033	7.1±1.2	8.4±1.1	nd[2]	nd	6.3±0.7	6.6±0.6	nd	nd	11.0±2.5	12.5±2.6	nd	nd
18	ABC Kologoun	5.2±0.9	6.1±1.3	nd	nd	2.7±0.3	4.8±0.5	nd	nd	4.6±0.7	5.1±1.0	nd	nd
19	CAP94066	19.7±2.5	10.9±1.4	13.9±4.2	12.8±2.4	8.2±1.0	14.3±2.5	13.3±3.5	9.7±2.8	nd	nd	4.5±0.5	5.5±1.0
2	CAP94030	13.2±1.4	8.9±1.0	14.6±1.7	13.1±1.6	5.3±1.0	6.9±1.1	14.3±2.9	9.9±1.6	7.7±2.1	6.7±1.2	7.7±1.5	4.6±0.6
3	RB92103	17.6±2.4	12.9±1.8	8.8±2.9	8.4±2.0	10.9±1.6	6.3±1.2	14.1±4.5	10.4±0.8	16.1±2.3	10.0±1.3	11.6±1.2	9.3±1.7
20	RB92162	19.2±2.3	15.0±2.2	11.5±2.1	10.1±1.2	6.4±0.5	9.0±1.0	13.8±2.6	8.9±1.2	7.7±0.9	5.7±0.8	nd	nd
21	CAP94034	4.1±0.6	5.4±0.6	nd	nd	3.0±0.4	4.7±0.6	nd	nd	12.2±2.4	6.4±0.8	nd	nd
4	**BEN86052**[3]	13.9±1.8	14.3±2.3	9.8±2.4	9.1±1.8	6.8±0.4	9.1±1.8	11.1±1.7	11.2±1.5	10.5±2.3	11.4±0.9	10.6±0.8	5.3±1.1
22	Houekoute	4.7±0.5	2.4±0.9	nd	nd	2.5±0.5	2.6±0.6	nd	nd	1.2±0.2	2.7±0.6	nd	nd
5	RB89509	15.2±2.6	21.5±1.9	13.3±4.6	11.7±2.8	9.3±1.8	14.3±2.0	15.0±5.5	13.6±1.3	6.1±0.4	11.9±1.3	5.6±1.1	2.6±0.5
6	RB92182	20.0±1.6	17.6±1.9	11.5±3.2	12.0±3.9	4.8±0.9	12.5±2.0	11.0±2.1	17.5±2.7	17.6±2.8	8.6±1.1	5.6±1.2	8.9±0.6
23	RB92052	6.0±1.1	6.6±0.4	nd	nd	3.4±0.5	4.3±0.8	nd	nd	3.5±0.6	4.3±0.8	nd	nd
7	CAP94059	9.9±1.5	15.0±2.5	14.3±1.1	14.2±5.3	7.4±1.4	9.2±2.0	16.4±3.8	17.6±3.2	12.2±2.6	8.4±1.2	11.2±1.8	7.2±0.4
8	RB92202	12.0±1.5	18.4±1.6	9.0±3.4	15.3±2.7	7.8±1.3	10.1±2.0	13.7±3.2	18.2±6.2	15.5±2.6	13.5±1.4	8.9±2.7	8.7±2.1
9	BEN86002	10.6±1.3	7.8±1.2	20.3±3.7	11.2±1.9	4.1±0.9	6.2±1.1	9.4±1.3	13.6±1.9	11.3±2.2	8.3±1.1	9.3±1.7	11.7±2.5
10	**TME1**	13.1±1.3	16.6±2.3	13.0±3.4	13.7±3.8	14.5±3.1	11.9±1.9	15.9±6.6	16.9±3.6	14.8±2.7	12.4±1.2	8.5±1.7	7.5±1.4
24	RB92151	6.5±1.2	14.2±2.0	13.7±3.8	11.2±3.0	3.8±0.7	7.4±1.8	11.6±0.7	14.6±3.5	10.9±1.9	2.5±0.5	nd	nd
11	RB92099	7.0±0.9	8.6±1.2	11.0±2.3	9.4±0.9	3.6±0.6	10.5±2.0	9.6±1.6	11.9±2.4	14.8±2.5	9.2±1.3	10.9±1.9	8.6±1.1
12	RB92022	11.3±1.5	10.6±1.8	8.2±2.2	6.4±1.4	6.4±1.0	8.2±1.8	11.6±2.3	11.5±0.8	11.2±2.1	6.7±1.0	8.8±3.3	8.5±1.4
13	BEN86040	7.9±1.6	11.8±1.7	5.8±0.9	8.0±4.3	4.4±0.3	4.9±0.6	3.4±3.4	3.6±2.7	5.5±0.6	5.2±0.5	4.5±0.7	3.0±0.5
25	CAP94049	10.3±2.5	11.4±1.4	nd	nd	5.6±0.7	3.2±0.6	nd	nd	6.2±1.4	2.4±0.5	nd	nd
26	RB92125	7.7±1.2	10.4±2.6	nd	nd	4.0±0.8	9.8±1.3	nd	nd	9.9±1.4	8.3±1.1	nd	nd
27	BEN86018	4.7±0.6	5.4±2.4	nd	nd	6.0±1.2	3.9±0.6	nd	nd	13.9±2.5	2.6±0.5	nd	nd

Table 4: continued

	Forest savanna transition				Wet savanna				Dry savanna			
	1998		2000		1998		2000		1998		2000	
Genotypes	Non-Inoc.	Inoc.	Non-Inoc.	Inoc.	Non-Inoc.	Inoc.	Non-Inoc.	Inoc.	Non-Inoc.	Inoc.	Non-Inoc.	Inoc.
14 TMS30572	14.4±1.8	14.9±3.4	15.5±3.8	12.3±3.2	5.8±0.3	8.5±2.0	8.1±0.7	7.6±0.7	11.1±1.7	9.9±1.8	7.1±0.6	5.5±0.6
28 RB92174	13.4±3.5	15.0±3.4	nd	nd	6.7±1.0	6.4±1.0	nd	nd	7.3±2.2	5.6±1.2	nd	nd
15 RB92004	4.5±0.5	7.2±0.7	17.3±5.2	14.8±5.8	2.9±0.5	2.4±0.5	11.0±1.4	9.5±0.9	6.2±1.4	3.0±0.8	7.0±1.8	5.5±1.4
29 BEN86004	6.6±0.5	9.1±1.3	7.7±1.7	5.7±1.7	1.4±0.3	2.0±0.4	nd	nd	1.3±0.3	2.9±0.4	nd	nd
30 Dangbo2	5.8±1.8	12.0±2.6	nd	nd	3.6±0.6	2.4±0.5	nd	nd	nd	nd	nd	nd
16 RB92132	9.3±1.5	6.1±1.7	12.2±3.4	11.8±2.3	7.2±1.8	6.4±1.2	10.2±2.1	10.8±2.1	8.4±1.6	3.6±0.5	7.0±2.7	9.5±2.8
31 BEN86016	3.7±0.6	0.8±0.6	nd	nd	1.8±0.4	2.1±0.5	nd	nd	5.5±0.5	4.9±0.5	nd	nd
32 RB92164	3.8±0.6	0.7±0.6	nd	nd	3.4±0.6	2.9±0.5	nd	nd	1.2±0.3	2.2±0.4	nd	nd
33 RB92131	10.5±2.5	14.7±2.0	nd	nd	6.5±1.0	2.8±0.6	nd	nd	16.4±2.6	8.4±1.5	nd	nd
34 TMS92/0057	nd	nd	11.5±2.7	11.7±3.8	nd	nd	10.2±1.5	9.8±1.9	nd	nd	10.6±1.7	7.7±2.5
35 TMS91/2327	nd	nd	28.7±4.2	14.6±2.6	nd	nd	17.3±4.5	16.9±2.0	nd	nd	9.6±1.8	8.6±1.6
36 TMS91/2324	nd	nd	22.8±2.2	10.6±1.7	nd	nd	18.1±0.8	21.3±5.1	nd	nd	12.2±0.4	9.5±1.6
37 CAP92034	nd	nd	16.1±2.5	12.0±2.0	nd	nd	3.8±0.3	3.7±1.1	nd	nd	8.8±0.6	7.0±0.2

1 Original mean of dry root yield in $t \cdot ha^{-1}$ with original standard error
2 nd: not determined due to non-availability of genotypes
3 Check genotypes repeated in each block, in bold

Table 5: Means of 16 genotypes for the standardized area under disease severity index progress curve (AUSiPC) and dry root yield ($t \cdot ha^{-1}$) calculated over 12 environments. Decreasing order of the means for AUSiPC (left) and yield (right).

Genotype	AUSiPC	Genotype	Dry root yield
7 CAP94059	5.2	10 TME1	13.2
4 BEN86052	5.1	8 RB92202	12.6
6 RB92182	5.0	6 RB92182	12.3
11 RB92099	4.9	7 CAP94059	11.9
10 TME1	4.9	1 RB89608	11.7
3 RB92103	4.9	5 RB89509	11.6
1 RB89608	4.8	3 RB92103	11.3
8 RB92202	4.8	9 BEN86002	10.3
9 BEN86002	4.8	4 BEN86052	10.2
2 CAP94030	4.5	14 TMS30572	10.0
15 RB92004	4.5	11 RB92099	9.6
12 RB92022	4.4	2 CAP94030	9.4
13 BEN86040	4.4	12 RB92022	9.1
5 RB89509	4.3	16 RB92132	8.5
16 RB92132	4.1	15 RB92004	7.6
14 TMS30572	3.4	13 BEN86040	5.6

Table 6: Correlation coefficients between disease development expressed as standardized area under severity index progress curve and root yield calculated over 16 genotypes in 12 environments

		Non-inoculated genotypes	Inoculated genotypes
FST	1998	+0.20	+0.02
FST	2000	-0.20	-0.23
WS	1998	+0.26	-0.03
WS	2000	+0.30	+0.37
DS	1998	+0.41	+0.04
DS	2000	-0.58*	-0.31

FST: forest-savanna transition, WS: wet savanna, DS: dry savanna
* significant at 5% probability level.

2.4 Discussion

Thirty-seven cassava genotypes, of which 16 were repeated in all environments, were evaluated for their reaction to cassava bacterial blight and yield in three sites located in the forest-savanna transition, wet savanna and dry savanna zones in years 1998 and 2000 under natural infection and in an artificially inoculated treatment. Most genotypes showed a susceptible and in some environments a medium resistant reaction to bacterial blight, while resistance was rarely observed.

No genotype revealed stable resistance in year 1998 across ecozones, while one genotype (RB92164) was resistant in the forest-savanna transition zone and five genotypes (RB92022, TMS30572, BEN86004, RB92033 and Dangbo2) were resistant in the dry savanna zone. In year 2000, one genotype (RB92202) was resistant in the forest-savanna transition zone, one genotype (RB92151) was resistant in the wet savanna zone, and two genotypes (RB92132, TMS30572) were resistant in the dry savanna zone. Only genotype TMS30572 was resistant in the dry savanna zone in both years. Genotypes identified as resistant varied with ecozones and years, probably due to factors such as infection pressure and temporal and ecozonal fluctuations in environmental conditions. The differences observed among environments in distribution of genotypes according to their disease index were mainly due to the low rainfall after the dry season in year 2001 compared to year 1999.

The influence of rainfall on disease severity was also reported by Fanou (1999) selecting improved cassava genotypes for resistance in different ecozones in Benin and Nigeria. The observed diversity in disease reactions of genotypes could be due to differential responses of the same set of genes to changes in environment or by expression of different sets of genes in different environments (Cockerham, 1963; Falconer, 1990). Some diseases are difficult to score reliably, since they are highly sensitive to the environment, and a crop cultivar with an adequate resistance in one location may be unacceptably susceptible in another (Bai and Shaner, 1994). Disease development expressed as area under severity index progress was generally higher in inoculated treatments than in naturally infected ones. Genotypes evaluated as resistant under low inoculum pressure could be susceptible under high, artificial inoculum pressure, thus proving that screening for disease resistance should be conducted only under high disease pressure (Hillocks and Wydra, 2002; Wydra, 2002). Since a homogeneous, high infection in repeated trials over 2 or more years is hardly to be achieved, genotypes should be artificially inoculated and not only evaluated under natural conditions, as performed by other

authors (Fokunang et al., 2000; Restrepo et al., 2000). Thus, screening under natural, low disease pressure could result in false selection of resistant genotypes.

In the additive main effect and multiplicative interaction (AMMI) analysis, genotypes and environments with low or near zero Y axis (IPCA1 scores) have small or nil interactions with environments and are considered stable (Crossa et al., 1991). Among the more resistant genotypes, five genotypes (CAP94030, BEN86040, RB89509, RB92132 and TMS30572) showed low interaction across environments and were most stable, while genotypes RB92022 and RB92004 showed higher interactions with different environments and were unstable. Three susceptible genotypes also showed negligible interactions with environments and genotype BEN86002 (susceptible) was most stable in different environments. Comparing root yields across environments, four genotypes (RB92022, RB92099, CAP94030 and TMS30572) had negligible interactions with environments, among which only the latter genotype was among the high yielding ones. Among the more resistant genotypes across environments, only genotype TMS30572 and RB89509 belonged to the high yielding group. But, regarding reaction in specific environments, genotype RB89509 was susceptible in 3 of 6 environments and can therefore not be recommended to farmers.

The site in the dry savanna zone with natural infection in year 1998 (E9), in which yield was high and symptom severity low across genotypes would be most suitable for production of cassava, while the artificially inoculated site in the wet savanna zone in year 2000 (E8) with high yield and high symptom severity across genotypes would be most suitable for screening for resistance. The artificially inoculated site in the wet savanna zone in year 1998 (E6) with higher symptom severity and lower yield was most unsuitable for cassava production and, therefore, good for selecting genotypes, which still perform well under harsh conditions. A highly significant genotype x environment interaction for fresh root yield, cassava bacterial blight and other cassava diseases was also reported from other studies in Africa (Otoo et al., 1994; Dixon and Nukenine, 2000). But, as our data show, an evaluation of genotype performance in one year as described by some authors (Fokunang et al., 2000) and without artificial inoculation (Otoo et al., 1994; Dixon and Nukenine, 2000; Fokunang et al., 2000; Restrepo et al., 2000) is not sufficient to discriminate between genotypes.

Some susceptible genotypes recovered from infection without any or with low reduction of yield, e.g. genotypes RB92202, RB92103, BEN86052, CAP94059, BEN86002, TME1,

RB92182 and RB89608. Wydra (2002) stated that some cassava varieties are able to compensate the negative effect of the disease under favourable growth conditions and therefore a symptom threshold for yield loss cannot be determined, and thus, loss remains unpredictable. Genotype BEN86052 was identified by her as tolerant and also in the present studies it yielded high in spite of high AUSiPC. Genotypes with a high tolerance to stress were found showing specific adaptation to stress environments or little variation between environments (Lin et al, 1986; Simmonds, 1991).

Some genotypes showed higher dry root yield in the inoculated treatment than in the non-inoculated treatment which could be due to their ability to develop quickly new leaves on stems with dieback (own observation) and thus, developing more assimilation area. The medium resistant or resistant genotypes CAP94066, TMS30572, RB89509 and BEN86040 in the dry savanna in year 2000 showed the lowest dry root yield in the inoculated treatment. This may be due to the occurrence of a greatly increased biosynthetic activity due to increased expression of resistance mechanisms at the expense of stored host energy, which may ultimately limit yield. This was demonstrated by the results of Smedegaard-Petersen and Tolstrup (1985) in incompatible interactions between barley and the pathogen barley powdery mildew.

Disease development expressed as area under severity index progress curve was negatively correlated to root yield only in the non-inoculated treatment in the dry savanna zone in year 2000, indicating that the disease may cause losses under not clearly identifiable conditions. In other environments no significant correlations were found. Also, Fokunang et al. (2000) did not find a significant correlation between CBB severity and tuber root yield evaluating a cassava germplasm collection under natural infection conditions in the forest savanna transition zone in Nigeria in a one year trial. An evaluation of genotype reactions to CBB should thus be only performed under artificial inoculation in repeated years in several locations per ecozone to identify genotypes with stable resistance. Additionally, inoculation with different pathotypes (Zinsou, 2001) under controlled conditions has to be performed in order to give a final evaluation of resistance of genotypes.

In conclusion, difficulties to recommend suitable genotypes to farmers reside in high genotype x environment interactions for cassava bacterial blight and root yield. Considering disease reaction and root yield across environments, only genotype TMS30572 among the

thirty-seven genotypes tested (sixteen repeated in two years and 12 environments, and twenty-one genotypes repeated in at least one year and 6 environments) was stable in different environments - medium resistant to resistant and high-yielding - and could be recommended to farmers. This genotype with a resistant reaction in the dry savanna in both years seemed to be specifically suitable to this ecozone. Nevertheless, for confirmation and further selection of resistant, high-yielding genotypes, continuous and intensive evaluation in repeated years in several locations per ecozone, and inoculation with different pathotypes under controlled conditions should be performed. Genotypes should be evaluated for their resistance in various environments, which favour stable, high symptom severity [e.g. E2, the artificially inoculated site in the forest-savanna transition zone (in year 1998); E8, the artificially inoculated site in the wet savanna zone (in year 2000)], including those with most unsuitable conditions for high yield in combination with high infection pressure [e.g. E6, the artificially inoculated site in the wet savanna zone (in year 1998)]. For production of cassava stems for propagation, E5 [e.g. the site in the wet savanna zone with natural infection (in year 1998)] would be most suitable, while E9 [the site in the dry savanna zone with natural infection (in year 1998)] was the best environment for cassava production.

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Annex 1: AMMI analysis of standardized AUSiPC of 16 cassava genotypes grown in 12 environments

Source	DF	SS	MS	Probability
Treatment	191	851.7	4.4	1NA
Genotype (G)	15	34.7	2.3	1NA
Environment (E)	11	703.9	63.9	1NA
GxE	165	113.0	0.68	1NA
IPCA1	25	31.2	1.2	1NA
IPCA2	23	26.1	1.1	1NA
IPCA3	21	18.9	0.9	1NA
IPCA4	19	9.7	0.5	1NA
IPCA5	17	8.8	0.5	1NA
Residual	60	18.1	0.3	1NA

Annex 2: AMMI analysis of dry root yield of 16 cassava genotypes grown in 12 environments

Source	DF	SS	MS	Probability
Treatment	191	2945.3	15.4	1NA
Genotype (G)	15	722.1	48.1	1NA
Environment (E)	11	839.1	76.2	1NA
GxE	165	1383.9	8.3	1NA
IPCA1	25	506.3	20.2	1NA
IPCA2	23	273.5	11.8	1NA
IPCA3	21	164.4	7.8	1NA
IPCA4	19	137.0	7.2	1NA
Residual	77	302.4	3.9	1NA

3. Stomatal distribution and leaf waxes of cassava (*Manihot esculenta* Crantz) in relation to resistance against cassava bacterial blight

Abstract. Comparing four cassava genotypes susceptible, medium resistant and resistant to bacterial blight, no significant differences in stomatal distributions on abaxial or adaxial leaf surfaces were found though tendencies for fewer stomata in the more resistant genotypes at the adaxial surface were observed. On the adaxial leaf surfaces stomata were located along the midrib and major veins. Analysing cuticular waxes of seven genotypes, triterpenes were the dominant wax constituents and alkanes and acids occurred in minor amounts. Clear differences in wax quantities between susceptible, medium resistant and resistant genotypes from three ecozones of Benin were not observed. Scanning electron-microscopy of a susceptible and a resistant genotype revealed a regular distribution of waxes at the abaxial leaf surface, covering stomatal pores of both genotypes, while on the adaxial leaf surface waxes were in form of crystalloids, did not occlude the rarely observed stomata, and might be portals of entry for the bacteria. Therefore, the slightly lower number of stomata on the adaxial surface in the resistant genotypes might play a role in defence by hindering bacterial entry, but these characteristics were not decisive for resistance.

3.1 Introduction

Cassava (*Manihot esculenta* Crantz) belongs to the family Euphorbiaceae, to which all the cultivated forms are related. It is a major staple food for more than 400 million people in Africa, South America and Asia (El-Sharkawy, 1993). The crop is attacked by cassava bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) Vauterin et al. (1995), former *Xanthomonas campestris* pv. *manihotis* Bondar (Bondar, 1915), representing one of the economically most important cassava diseases in Africa (Hillocks and Wydra, 2002; Wydra and Msikita, 1998). Typical disease symptoms include angular leaf spots, blight and wilt, exudation and dieback. The disease cycle is characterized by a parasitic phase occurring during the rainy season and a survival phase during the dry season (Daniel, 1991). In the latter phase, bacteria survive in apparently healthy stems and on leaf surfaces. When the rainy season starts again, epiphytic *Xam* populations are able to colonize intercellular spaces of the leaf mesophyll after penetrating through stomata and wounds, and to invade the vascular system.

Structural and induced defense mechanisms contribute to the cassava plant's strategies for resistance to microorganisms (Wydra, 2002; Wydra et al., 2002). After infection with *Xam*, resistant genotype reacted with lignin and callose deposits, and the production of phenolic compounds associated with suberin within the infected vessels (Kpémoua et al., 1996). Tyloses were also observed which may limit the spread of the pathogen in the vascular system of resistant plants. An expressed sequence tag library of latex revealed that about 26% of genes expressed after infection encoded for pathogenesis related (PR) or stress-related proteins such as phenylalanine ammonia lyase (PAL) and peroxidase (Day et al., 1997; Cooper et al. 1997; Kemp et al., 2001). A role of latex, produced abundantly on wounding, in defense is suggested by its rapid coagulation and its components such as lysozyme, chitinase, glucanase and protease (Flood et al., 1995; Cooper et al., 2001). In addition, a role of pectin in the resistance reaction was suggested since pectin extracted from cell walls of young cassava leaves caused a synergistic interaction with *Xam* lipopolysaccharides and inhibited a haemagglutinin of *Xam*, while pectin from old, less susceptible leaves and pectins from other sources were not active (Wydra et al., 2002). Typical oxidative burst was observed in cassava cell suspension cultures in response to elicitors and to *Xam* (Beeching et al., 1998; Gomez-Vasquez et al., 1999). Similarities to 78 differentially expressed genes involved in hypersensitive reaction (HR) in cassava leaves including glucanase, catalase, 1-

aminocyclopropane-1-carboxylate (ACC) oxidase and a WRKY transcription factor have been described after infection with the incompatible *Pseudomonas syringae* pv. *tomato* (Kemp et al., 2001). Horizontal resistance in form of preformed mechanisms, such as latex and leaf pectins, were suggested to be involved in resistance of cassava against bacterial blight (Wydra et al., 2002).

Genetics of resistance to CBB in cassava and genes governing resistance were investigated by inoculation with Colombian and one African strain of *X. axonopodis* pv. *manihotis* (Jorge et al., 2000; Jorge et al., 2001). Among African strains, pathotypes and specific quantitative trait loci for resistance to pathotypes from four different geographic origins in Africa were identified (Zinsou et al., 2002).

In field trials, in Benin similarly high numbers of leaves with typical angular leaf spots were observed in a susceptible as well as a resistant genotype, under high infection pressure after artificial inoculation, while the incidence of spots was significantly lower in the resistant than in the susceptible genotype (BEN86052, TMS30572, respectively) under the lower, natural inoculum pressure (Wydra, unpublished). This phenomenon suggested a preformed resistance mechanism inhibiting the entry of bacteria into leaves when they are present in low numbers, however, allowing the infection by overcoming the preformed structural resistance when they are present in high numbers. In microscopical studies of greenhouse grown cassava plants numerous stomata were observed on the abaxial leaf side, which were occluded with extensive wax on most genotypes (Cooper et al., 2001). Epicuticular waxes or lipids occurring on the surface of leaves as well as terpenoids and flavonoids were reported to inhibit growth of epiphytic bacteria and fungi (Harborne et al., 1975; Swain, 1977; Barthlott and Wollenweber, 1981). The wax layer makes the abaxial surface non-wettable and may make this leaf side unsuitable as route of entry for *Xam* (Cooper et al., 2001). Thus, stomata on the adaxial leaf sides might serve as portals for entry of *Xam*. But, stomata and leaf waxes of field-grown genotypes have never been investigated, nor have waxes been analysed quantitatively and qualitatively. Therefore, the objectives of the present study were (i) to analyse the distribution of stomata on both leaf sides of cassava genotypes, and (ii) to investigate the possible role of cassava leaf surface waxes in the resistance reaction to cassava bacterial blight by qualitative and quantitative analysis of abaxial leaf waxes of susceptible, medium resistant and resistant cassava genotypes grown in three ecozones of Benin, as well as by electron-microscopical analyses of both leaf surfaces.

3.2 Materials and Methods

3.2.1 Distribution of stomata

Plants of the four genotypes BEN86052 (Benin landrace), TMS30572 (58308 x Branca de santa Caterina), O88/01454 (59 x IAC7-127)op and O88/01043 [(58308 x oyaruba fufu) x (58308 x 56198)], identified in former field trials as susceptible, resistant, and medium resistant (the latter two), respectively, (Fanou, 1999) were grown from cuttings of 20 cm length received from apparently healthy field plants, in pots containing sandy soil, at the International Institute of Tropical Agriculture (IITA) station in Cotonou, Benin. Plants were transferred to the glasshouse (28^{o} C and 65% relative humidity) for the subsequent studies, 5 weeks after planting of cuttings.

Both surfaces of leaves of five plants per genotype were sprayed with a clear acrylic solution of a resin adhesive (Sprayway INC, Addison, IL 60101, USA). The coating film obtained after drying was peeled off with a clear tape and viewed on a slide under a microscope (R. Cooper, personal communication). Frequency of stomata in the adaxial and abaxial leaf epidermis was determined by observation under a microscope with 40x10 magnification, standardized as a unit area for uniform comparison. Counts were made in ten parts per mature leaflet of both leaf surfaces of 15 leaflets per genotype, in each the distal, central, and proximal parts of the leaf and between the principal veins of the abaxial surface and along the major veins for the adaxial surface, where no stomata occurred in the area between principal veins.

3.2.2 Cassava leaf waxes

3.2.2.1 Collection sites

Cassava leaves were collected from 11 months-old plants from experimental fields located in three agro-ecological zones of Benin, the forest-savanna transition zone (IITA, Cotonou, South Benin), the wet savanna zone (Save, INRAB station, Centre Benin) and dry savanna zone (Ina, INRAB station, North Benin) during the first rainy season in April 2001. The forest-savanna transition zone has an average annual rainfall of 1000 to 1400 mm, which spreads from March to July and from September to October, with a small dry season in August. The long dry period extends from November to March. The mean temperature is about 27 ^{o}C with a low diurnal variation of 7 to 10 ^{o}C (Adam and Boko, 1993). The wet savanna zone has an annual rainfall of 900 to 1300 mm varying from April to July and from September to October followed by a dry season from November to April. The mean

temperature is about 29 ^{o}C. The dry savanna zone has an annual rainfall of 700 to 900 mm distributed from April to October followed by a dry season from November to March. The mean temperature is about 32 ^{o}C (MEHU, 1993).

3.2.2.2 Plant material

The fourth leaf from the top of 11-months-old cassava plants of genotypes BEN86052 and TMS30572 identified as susceptible and resistant to CBB, respectively (Akparobi et al., 1998; Fanou, 1999; Wydra et al., 1999; Zinsou et al., 2000, Zinsou, 2001), was selected randomly in the field in three ecozones, and collected for wax extraction. Additionally, leaves from genotypes RB92132, CAP92034, CAP94030, RB92004 and BEN86040 (medium) resistant to cassava bacterial blight (Zinsou, 2002; chapter) were collected from the three ecozones. In previous studies, these genotypes were evaluated for numbers of leaves with spots or blight, dropped/wilted leaves and stems with die-back symptoms at a monthly interval with a gap over the dry season until the harvest at 12 months, and the severity index in days over 12 months was calculated according to the following formula:

$S_i = (1 \times S + 2 \times B + 1 \times W + 2 \times D)/6$

where *S, B, W* and *D* represent the percentage of leaves with spots, blight, wilt and stems with dieback, respectively. The mean severity index of 10 plants of each genotype at six evaluation dates, with dates 60, 90, 120, 150, 180 and 360 days after planting in each ecozone, was used to calculate the area under severity index progress curve (AUS_iPC) with the calculus method of integration of area under a curve. The AUS_iPC in days over the whole period was then divided by the evaluation period [365 days minus days of dry period (120, 150 and 200 days in the forest-savanna transition, wet savanna and dry savanna zones, respectively)] to receive an average comparable between ecozones. Thus, all AUSiPC values are standardized. Classes of susceptible (S) (75-100%), medium resistant (MR) (50-74.9%), and 'resistant' (R) (0-49.9%) genotypes were formed on basis of the percentage of standardized AUS_iPC values in the respective environment in the artificially inoculated treatment, using the highest value as 100%. The reaction of the cassava genotypes to bacterial blight in three ecozones was summarized in Table 1.

Table 1: Reaction of seven cassava genotypes to bacterial blight in three sites in the forest savanna transition, wet savanna and dry savanna zones in the inoculated treatments during the planting season 2000

Genotypes	FST[1]	WS	DS
BEN86052	MR[2]	S	S
BEN86040	MR	S	MR
CAP94030	MR	MR	MR
RB92004	S	MR	MR
RB92132	MR	S	R
CAP92034	MR	MR	MR
TMS30572	MR	MR	R

1 FST: forest savanna transition zone, WS: wet savanna zone, DS: dry savanna zone

2 S: susceptible (75-100%), MR: medium resistant (50-74.9%), R: resistant (0-49.9%), percentages calculated according to genotype with highest AUS_iPC value in ecozone

3.2.2.3 Wax isolation and analysis

Glass flasks (20 mm diameter, 57 mm length) containing 5ml chloroform were covered with the abaxial side of the cassava leaves and turned for 30 seconds to extract the wax from the abaxial side. Five leaf samples per genotype were extracted in 1 bottle, with 3 replicates (bottles) per genotype and per ecozone. Samples were analysed at the Institut für Biowissenschaften, Lehrstuhl für Botanik II, University Würzburg, Germany and at the Institute of Botany, Department of Ecophysiology, University of Bonn, Germany. Wax extracts were filtered and C_{24} alkane (Fluka) was added to the sample as internal standard (about 100 µg of alkane to 1 mg wax extract). After evaporation of the solvent, 20 µl BSTFA (N,N, bistrimethylsilyltrifluoroacetamide, Machery-Nagel) were added to the wax samples in the presence of 10 µl pyridine at 70 ^{o}C for 30 min converting free hydroxyl and carboxyl groups into their corresponding trimethylsilyl derivatives. Quantitative analyses of the samples were carried out by injecting 1µl of the prepared samples into a gas chromatograph (HP5890 II, Hewlett Packard) using hydrogen as a carrier gas (Hauke and Schreiber, 1998). The following temperatures and pressures were used by step: 50 ^{o}C (2 min), 50-200 ^{o}C (40 ^{o}C min^{-1}), 200-320 ^{o}C (3 ^{o}C min^{-1}), 320 ^{o}C (20 min) and 40 kPa (40 min), 40-150 kPa (10 kPa min^{-1}), 150 kPa (30 min). Quantitative analysis of the samples was carried out using gas chromatography (HP5890 II, Hewlett Packard) coupled to mass spectrometry (HP MSD 5971, Hewlett Packard) with helium as carrier gas (Hauke and Schreiber, 1998).

3.2.2.4 Scanning electron microscopy of cassava leaves

Specimens of about 5 mm^2 of both leaf sides of the two cassava genotypes BEN86052 and TMS30572 were treated using the standard method described by Ensikat and Barthlott (1993). Samples were dehydrated in an ascending series of aqueous solutions of glycerol in 10% steps to a final concentration of 80 to 100% pure glycerol, mounted on scanning electron microscopy (SEM) stubs and stored in a dessicator to evaporate remaining water. For comparison with glycerol-treated samples, air-dried and critical-point-dried (CPD) samples were investigated. After fixation with glutaraldehyde, CPD specimens were gradually dehydrated with ethanol and critical-point dried with CO_2 at 40 ^oC. The specimens were coated with gold and analyzed in a Cambridge Stereoscan S-200.

3.3 Results

3.3.1 Distribution of stomata on leaves

On the abaxial surfaces, the number of stomata was similar in the proximal, central and distal leaf parts for all investigated genotypes (Tab. 2). The number of stomata between the principal veins of the abaxial leaf surfaces tended to be higher for the susceptible genotype BEN86052 compared to the more resistant genotypes (Fig. 1), but the difference was not statistically significant. On the adaxial surfaces of leaves of the four genotypes, the number of stomata was slightly higher at the proximal ends of the leaves compared to the distal ends, and slightly reduced in the resistant genotype TMS30572 and the medium resistant genotype O88/01454 compared to the susceptible genotype. However, the differences observed between genotypes were not significant. Stomata of the adaxial surfaces were mainly located along the major veins (Fig. 1).

Table 2: Average number of stomata on abaxial and adaxial surfaces of cassava leaves of 4 genotypes on proximal, central, distal leaf areas, and the area between the principal veins (only for abaxial surface)

Genotypes	Frequency of stomata per area unit						
	Abaxial surface				Adaxial surface		
	Proximal	Central	Distal	Between[1]	Proximal	Central	Distal
BEN86052	46a [2,3]±1.9	46a±2.0[4]	45a±2.7	58a±4.0	7a±1.1	6a±1.0	6a±1.9
O88/01043	48a±2.0	50a±2.2	48a±1.9	51a±3.8	7a±1.1	6a±1.5	5a±1.0
TMS30572	48a±1.7	46a±1.5	47a±1.2	49a±4.2	5a±1.1	5a±1.4	4a±1.1
O88/01454	50a±2.9	46a±0.9	50a±2.1	52a±3.1	4a±2.1	4a±1.4	3a±1.9

1 Between principal veins

2 Average of 10 repeated counts of 15 leaflets

3 Values are not significantly different between genotypes at p = 0.05

4 Standard error of the means

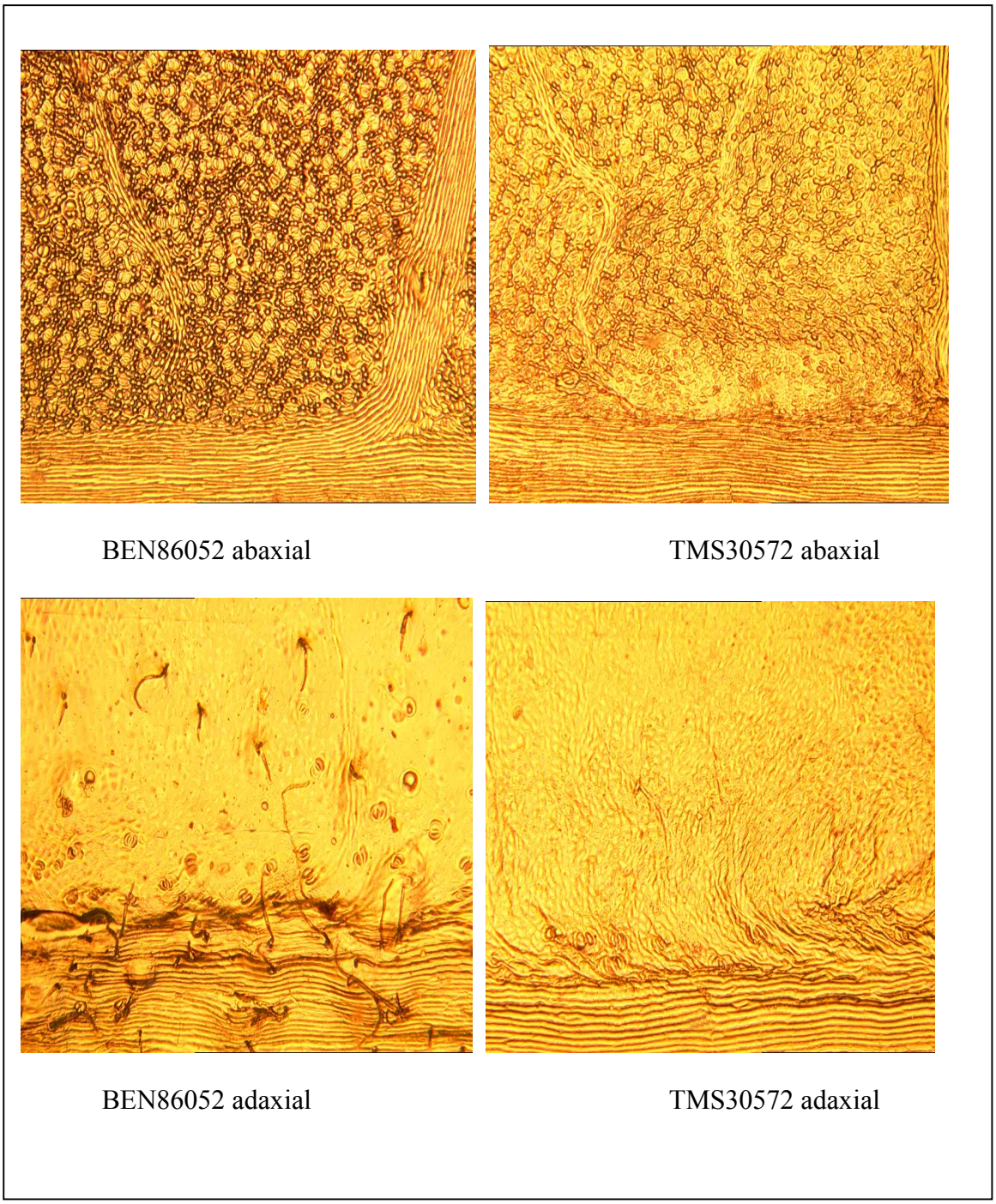

Figure 1: Stomata on the abaxial and adaxial surfaces (40x10) along the major vein in BEN86052 (susceptible) and TMS30572 (resistant)

3.3.2 Cassava leaf waxes

In cuticular waxes extracted from abaxial leaf surfaces of 2 genotypes, triterpenes (beta amyrins, epi-taraxerol, taraxeron, taraxerol) were dominant, but alkanes (C_{25}-C_{33}) and acids (C_{30} and C_{32}) also occurred (Fig. 2). Qualitative differences between the susceptible genotype BEN86052 and the resistant genotype TMS30572 across the forest-savanna transition, wet savanna and dry savanna ecozones were not observed. The quantity of triterpenes was higher in the resistant genotype than in the susceptible genotype in the three ecozones.

Comparing samples from leaves from seven genotypes, including the formely analysed susceptible and resistant genotypes collected in three sites (ecozones), no qualitative differences were found in the wax composition, and quantitative differences in triterpenes were generally not related to the degree of resistance or susceptibility (Fig. 3). Alkanes were generally higher in the ‘susceptible’ genotype BEN86052 and the ‘resistant’ genotype TMS30572 than in other genotypes, while no clear differences in acid amounts were found between genotypes across the three sites. Comparing sites (ecozones), triterpenes quantities were generally higher in the wet savanna than in the forest-savanna site, while in the dry savanna site, high and low quantities were observed. No significant correlation between disease development expressed as area under severity index progress curve and triterpenes was observed in inoculated and non-inoculated treatments across sites (Tab. 2).

By scanning electron microscopy waxes were demonstrated to be homogeneously distributed on the abaxial leaf surface (Fig. 4). Waxes covered and occluded stomata of both a susceptible and a resistant genotype. On adaxial leaf surfaces wax crystallides could be observed, whereas stomata were rarely observed.

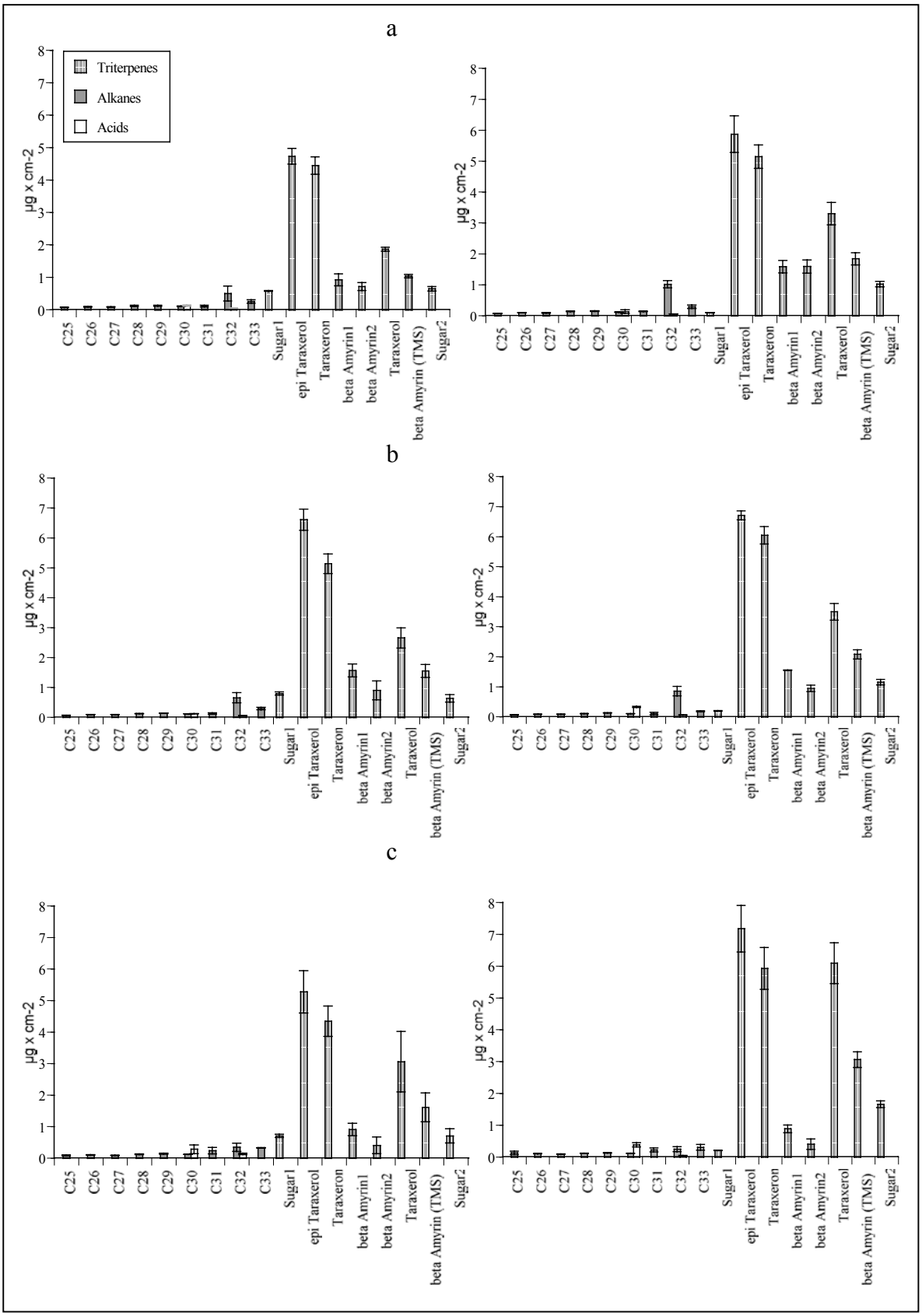

Figure 2: Wax composition of the abaxial leaf surface of the susceptible genotype BEN86052 (left) and the resistant genotype TMS30572 (right) in the forest-savanna transition (a), wet savanna (b) and dry savanna (c) zones

Beta Amyrin3 was found in BEN86052 in the forest-savanna transition and in TMS30572 in the wet savanna zone

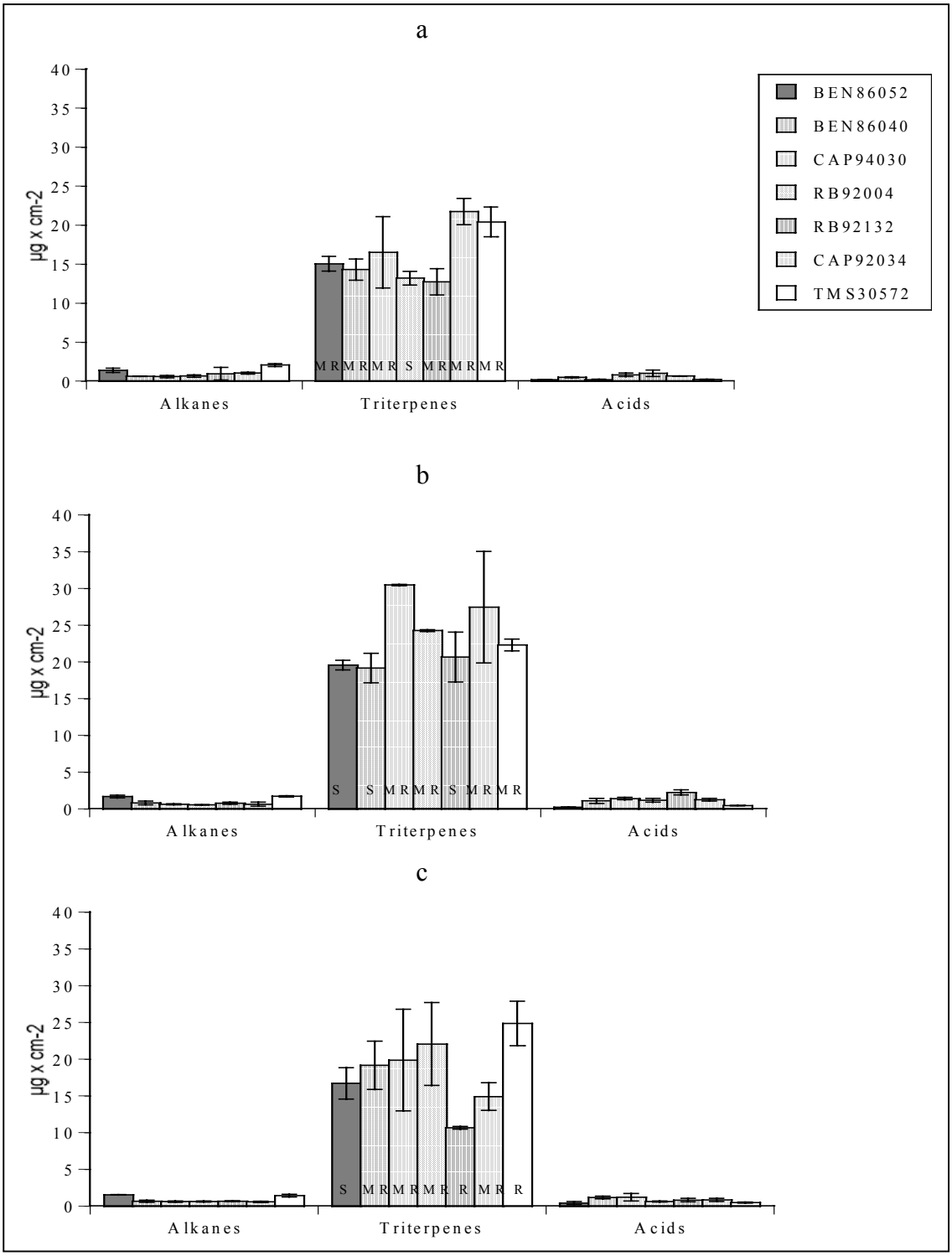

Figure 3: Leaf wax composition of the abaxial surface of cassava genotypes BEN86052, CAP92034, RB92132, CAP94030, RB92004, BEN86040 and TMS30572 varying in reaction to cassava bacterial blight in (a) Cotonou (forest-savanna transition zone), (b) Save (wet savanna zone), and (c) Ina (dry savanna zone)

S: susceptible, MR: medium resistant, R: resistant

Table 2: Correlation coefficients between disease development expressed as standardized area under severity index progress curve and amounts of triterpenes, forming the major substance class of leaf surface waxes of seven cassava genotypes[1] in three sites (ecozones)

Site	Non-inoculated	Inoculated
Cotonou, FST[2]	-0.27	-0.64
Save, WS	+0.20	-0.57
Ina, DS	-0.65	-0.004

1 Genotypes BEN86052, BEN86040, CAP94030, RB92004, RB92132, CAP92034, TMS30572

2 Ecozones FST = forest-savanna transition, WS = wet savanna, DS = dry savanna

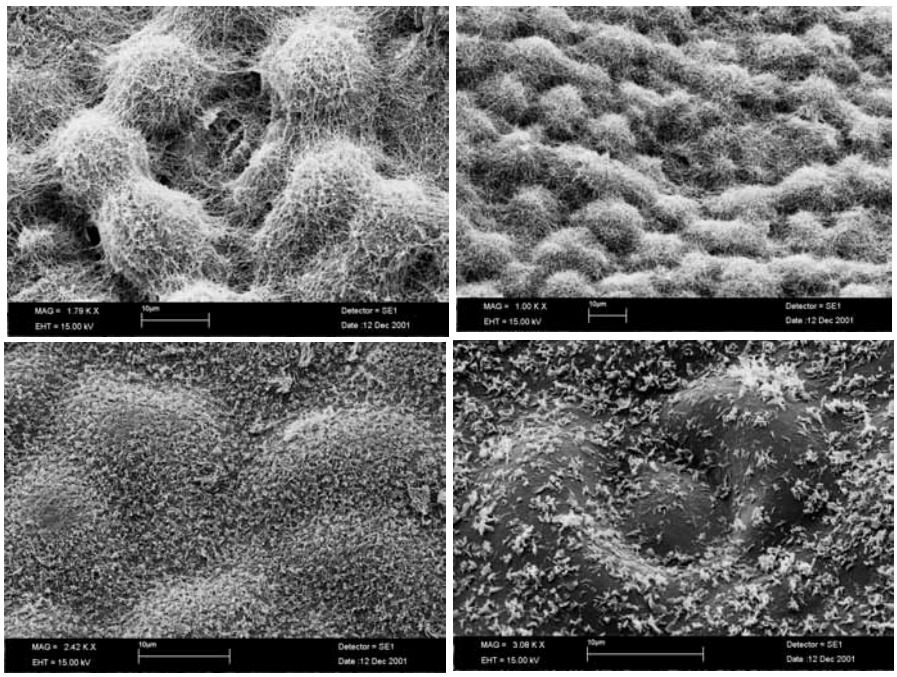

Figure 4: Scanning electron microscopy of the abaxial and adaxial leaf surfaces (upper and lower photos) of cassava genotypes BEN86052 (left) and TMS30572 (right)

3.4 Discussion

Bacterial entry in leaf tissues is mainly via stomata, and stomatal frequency and morphology were shown to be associated with resistance to bacterial diseases, e.g. bacterial leaf spot of tomato (Ramos et al., 1992). Significant differences in stomatal distribution were not found on the abaxial nor on the adaxial surface comparing four susceptible and resistant genotypes of cassava. There was only a slight trend towards higher numbers of stomata between veins on the abaxial surface and a tendency of higher stomate numbers at the proximal end of the adaxial surface in the susceptible genotype. Also Cooper et al. (2001) observed fewer stomata on adaxial surfaces of a field resistant compared to a susceptible genotype. Thus, differences in the number of stomata and their distribution could have an influence on the significantly lower incidence of spots observed in the resistant genotype TMS30572 compared to the susceptible genotype BEN86052 under low natural inoculum pressure in field trials (unpublished data). Nevertheless, scanning electron microscopy (SEM) observations which only cover small surface areas did not reveal differences in stomata numbers on adaxial surfaces. Therefore, leaf waxes which may occlude stomata and, thus, eventually prevent bacterial entry were characterized by chemical analysis of abaxial leaf surface waxes isolated from resistant, medium resistant and susceptible cassava genotypes, and by scanning electron microscopy of abaxial and adaxial surfaces of a resistant and a susceptible genotype.

For the first time, cassava leaf waxes were quantitatively and qualitatively analysed. However, no clear differences in wax quantities, specifically in triterpenes forming the most abundant wax fraction, were observed between susceptible, medium resistant and resistant genotypes in the forest-savanna transition, wet savanna and dry savanna zones. Thus, other resistance mechanisms must be involved in pathogen defence. SEM revealed that waxes covered stomata on the abaxial leaf surfaces of both a susceptible and a resistant genotype, while the adaxial surfaces were not covered by wax, but wax was in crystalloid form. Also Cooper et al. (2001) found adaxial stomata not being occluded by wax, but they did not analyse nor quantify the wax layers of abaxial and adaxial leaf surfaces. It is suggested, that bacterial penetration into intercellular spaces could be favoured in genotypes or on leaf sides with lower amounts of surface waxes, while higher wax quantities observed in other genotypes could reduce rates and numbers of bacteria invading into the mesophyll. The observed tendencies of lower stomate numbers on adaxial surfaces of the more resistant genotypes than of the susceptible genotype might therefore contribute to the resistance,

especially considering their location along the midrib and the major veins, which retain longer a water film (Cooper et al., 2001), necessary for bacterial penetration.

Stomatal anatomy itself, e.g. a very narrow entrance and broad, elevated guard cells, was suggested to confer resistance to some varieties against certain of their bacterial pathogens (Agrios, 1997). Differences in thickness and permeability of cuticles, stomata, hydathodes and trichomes have been described by Schönherr and Baur (1996). Additionally, anti-microbial effects of epicuticular wax compounds such as terpenoides and flavonoides against bacteria or fungi were described (Harborne et al., 1975; Swain, 1977). After longer periods of rain these anti-microbial components on the leaf surface may be released and washed off and make plants more susceptible to their pathogens (Barthlott and Wollenweber, 1981). Generally, higher triterpene quantities were observed in the wet savanna than in the other zones. Thus, it may be speculated that differences in environmental conditions may have an influence on wax quantities and, thereby, contribute to the high genotype x environment interactions in cassava.

Bacteria, which infect plants either through stomata, lenticels, nectarines, hydathodes or wounds, require the presence of liquid water on the leaf surface in order to enter the leaf interior using their own mobility (Manners, 1982). Hydrophobic waxes' deposits on leaf surfaces form a water-repellent surface and thereby prevent the formation of a water film, in which pathogens may multiply. Cooper et al. (2001) stated that the adaxial leaf surface of cassava is non-wettable and seems unlikely as route of entry for *Xam*. Knoll and Schreiber (1998) reported that an increased availability of water on the leaf surface favoured microbial growth and further epiphytic colonization of the phylloplane. Therefore, water films on leaf surfaces enable the spread of motile bacteria, which can move in the water film by means of flagella and thus colonize new habitats in the phyllosphere (Elstner et al., 1996; Hoffmann et al., 1985). Furthermore, water films on the leaf surface increase rates of foliar leaching of solutes diffusing across the cuticle, which in turn result in a better nutrient supply of leaf surface microorganisms. Thus, differences in nutrient availability or direct effects of wax components influence microbial populations on leaf surfaces. But, in cassava a high level of epiphytic *Xam* populations on leaf surfaces of various, resistant as well as susceptible genotypes (Persley, 1978; Daniel and Boher, 1985) in different ecozones (Fanou, 1999) suggested that cassava leaf waxes may have no significant effect on epiphytic bacterial populations. Similarly, recovery of high bacterial numbers of *Pseudomonas syringae* pv. *syringae* on resistant and susceptible bean cultivars were reported (Upper and Hirano, 1996).

In conclusion, the number of adaxial leaf stomata and cassava leaf surface wax might play a role in defence against bacterial blight as a structural barrier, but seem not to be decisive for the resistance of genotypes which is known to be polygenic (Hahn et al., 1979). Lower stomata numbers and high wax quantities may be involved in reducing the number of bacteria invading leaves, but variations in wax quantities and the number of stomata in the tested genotypes were not or only tendentiously related to the described resistances and, thus, the differences observed in spot symptoms comparing susceptible and resistant genotypes are thought to be related to further defence mechanisms.

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4. Identification of pathotypes of *Xanthomonas axonopodis* pv. *manihotis* in Africa and detection of specific quantitative trait loci (QTL) for resistance to cassava bacterial blight

Abstract. Hundred-eleven cassava genotypes derived from the backcross of 5 F_1 individuals and the female parent TMS30572 were tested for their reaction to cassava bacterial blight by leaf and stem inoculation, and were used to identify possible *Xanthomonas axonopodis* pv. *manihotis* pathotypes and cassava bacterial blight related genetic markers or involved genes. The genotypes varied in their reaction against four highly virulent strains of *X. axonopodis* pv. *manihotis* from four different geographic origins in Africa. The strains were defined as different pathotypes according to their reactions on leaves and stems. Genotypes with susceptible, medium resistant and resistant reactions were identified for both leaf and stem inoculation methods and partly differed in their reaction on leaves and stems. Sixteen genotypes among the mapping population showed a resistant reaction. Based on the genetic map of cassava, single-marker regression analysis of area under disease progress curve values from stem-puncture inoculation of each strain was performed. Eleven markers were identified, of which 5 markers on 3 and 1 linkage groups of the female- and male-derived framework of family CM8820, respectively, were significantly linked to disease severity (area under disease progress curve) values with the four strains of *X. axonopodis pv. manihotis*. Based on the segregation of alleles from the female of family CM8873, one marker was found to be associated to resistance to both *X. axonopodis pv. manihotis* strains, GSPB2506 and GSPB2511. Five markers were not linked to any groups. Depending on strain inoculated, specific markers were detected confirming that the 4 African strains belong to 4 different pathotypes.

4.1 Introduction

Cassava is a basic component of the farming system in most areas of Sub-Saharan Africa (Nweke et al., 1994). It provides more than 60% of the daily calorie intake of some 500 million people in the Sub-Saharan region of Africa (FAO, 1997). The crop is attacked by cassava bacterial blight (CBB), caused by *Xanthomonas campestris* pv. *manihotis* Bondar (Bondar 1915), renamed *Xanthomonas axonopodis* pv. *manihotis (Xam)* Vauterin et al. (1995), one of the most important cassava diseases in Africa (Wydra and Msikita, 1998; Banito et al., 2002; Wydra and Verdier, 2002). Symptoms include angular leaf spots, blight, wilting, and stem dieback after systemic infection. Among the proposed control measures (Wydra and Rudolph, 1999), host-plant resistance is one of the most suitable measures for farmers. Resistance of African cassava cultivars to bacterial blight originated from interspecific-cross breeding with the wild species *Manihot glaziovii* and is assumed to be polygenic (Hahn et al., 1979). Resistance in cassava is considered to be quantitative (Kpémoua et al., 1996), and a hypersensitive reaction has not been reported in cassava cultivars in the interaction with *Xam* strains. Race-cultivar combinations have never been reported between cassava cultivars and *Xam* strains (Boher and Agbobli, 1992), although pathotypes and haplotypes were evidenced among Latin American strains (Assigbétsé et al., 1998; Verdier et al., 1998). The variability of *Xam*, based on pathogenicity and on physiological, biochemical, and molecular characterization, revealed greater genetic diversity in Latin America than in Africa (Grousson et al., 1990; Fessehaie, 1997; Restrepo and Verdier, 1997; Assigbétsé et al., 1998; Verdier et al., 1998; Wydra et al., 1999a).

Horizontal and vertical resistance are involved in host plant resistance of crops (Heath, 2000). Molecular genetic mapping of host-plant resistance to bacterial blight in rice revealed that a single locus confers vertical resistance to a specific race of the pathogen (Song et al., 1996; Yoshimura et al., 1996). In quantitative or horizontal resistance to *Phytophthora infestans* in potato, only 5 of the 11 genomic regions showed no specificity against just two races tested, while the others were significant against just one (Leonards-Schippers et al., 1994). Against bacterial wilt of tomato several quantitative trait loci (QTL), with loci on chromosome 6 playing the predominant role, controlled resistance to *Ralstonia solanacearum* (Danesh et al., 1994; Thoquet et al., 1996a; Thoquet et al., 1996b; Mangin et al., 1999; Wang et al., 2000). Horizontal resistance in form of preformed mechanisms, such as latex and leaf pectins, were suggested to be involved in resistance of cassava against bacterial blight (Wydra et al., 2003).

A genetic linkage map of cassava was constructed from 90 individuals of the F_1 mapping population of a cross of TMS30572 (female parent) and CM2177-2 (male parent) at the Centro Internacional de Agricultura Tropical (CIAT). Thirty-six markers with unique alleles from the female and male parents were employed to identify 20 male- and female-derived linkage maps (Fregene et al., 1997). The map was saturated with the characterization of 172 additional simple sequence repeats (SSRs) markers (Mba et al., 2001). Cassava genotypes used in the QTL analysis were the two largest families of BC_1 populations, CM8820+B (= CM8820 + CM8820B) and CM8873+B (= CM8820 + CM8820B) derived from the backcross of two F_1 genotypes (CM7857-4 and CM7857-77) with TMS30572, for which the genetic map was built (Jorge, 2000). Fifty-eight restriction fragment length polymorphisms (RFLPs) and sixty-three SSRs from 17 of the 20 linkage groups were chosen to analyse these two largest families. Sixty-seven and 56 markers identified for the female and male map of families CM8820+B, respectively, were associated in 18 linkage groups. Also, 50 and 45 markers identified for the female and male map of families CM8873+B, respectively, were associated in 18 linkage groups.

Genetics of resistance to CBB in cassava and genes governing resistance were investigated by inoculation with Colombian and one African strain of *X. axonopodis* pv. *manihotis* (Jorge et al., 2000; Jorge et al., 2001), but analysis had up to now not included more African strains.

After testing 244 genotypes of F_1 backcross with one African and Latin American strains, 5 markers were identified (Jorge, 2000), but a characterization of the reaction of genotypes of the mapping population to a diverse, representative set of highly virulent African strains has not been made.

Therefore, the objectives of the present studies were to

- **(i)** test 111 genotypes of the mapping population for their reaction to CBB using leaf and stem inoculation methods,
- **(ii)** identify possible *Xanthomonas axonopodis* pv. *manihotis* pathotypes, and to
- **(iii)** contribute to the identification of CBB resistance-related genetic markers.

4.2 Materials and Methods

4.2.1 Cassava genotypes

The cassava mapping population comprised 90 F_1 plants from an intraspecific cross between TMS30572 (female parent), an elite cassava cultivar developed at the International Institute of Tropical Agriculture (IITA), Nigeria, and CM2177-2 (male parent), a successful cassava cultivar resulting from breeding at the Centro Internacional de Agricultura Tropical (CIAT) in Colombia, and 60 supplementary individuals, giving a total of 150 individuals. Genotype TMS30572 is partly resistant (Akparobi et al., 1998; Wydra et al., 1999b; Zinsou et al., 2000) and genotype CM2177-2 is 'tolerant' to cassava bacterial blight (CBB) (Fregene et al., 1997). Hundred-eleven genotypes derived from the backcross of five F_1 individuals (CM7857-4, CM7857-10, CM7857-51, CM7857-77, CM7857-115) and the female parent TMS30572 were used. These genotypes were grouped in 7 populations, [CM8820 and CM8820B (reciprocal cross), CM8870, CM8872, CM8873 and CM8873B (reciprocal cross), CM8877], by artificial pollinization of female flowers by pollen from the male parent (Tab. 1).

Table 1: List of 111 genotypes of the mapping population derived from a F_1 backcross of cassava genotypes TMS30572 (female parent) and five F_1 individuals[1]

Population	Genotype code	Total number
CM8820	1, 2, 4, 5, 9, 11, 12, 13, 16, 19, 23, 27, 30, 31, 32, 33, 34, 38, 40, 43, 46, 47, 48, 50, 53, 56, 58, 61, 64, 67, 70, 72, 75	33
CM8820B	3, 6, 7, 8, 10, 12, 13, 14, 17, 20, 21, 23, 25	13
CM8870	1, 3, 6, 13, 14, 15, 17	7
CM8872	3, 13, 14, 15, 16	5
CM8873	1, 7, 11, 12, 13, 14, 18, 25, 28, 30, 31, 35, 38, 39, 40, 42, 44, 48, 54, 58, 63, 64, 67, 69, 71, 74, 77, 78, 80, 81, 84, 86	32
CM8873B	1, 3, 5, 7, 9, 14, 15, 16, 17, 20, 25, 31, 35, 36	14
CM8877	6, 8, 9, 11, 13, 15, 52	7

1 F_1 individuals: CM7857-4, CM7857-10, CM7857-51, CM7857-77, CM7857-115

Stem cuttings of 20 cm length from apparently healthy plants were received from IITA, Nigeria. They were planted in plastic pots of 16 cm diameter containing sandy soil at IITA, station in Cotonou, Benin, in 6 repetitions per inoculated bacterial strain. After 6 weeks, plants were transferred to the glasshouse (26 ^{o}C, 70% relative humidity) for inoculation and the subsequent studies. Six plants per bacterial strain and per genotype were used.

4.2.2 Bacterial strains and their origin

The three highly virulent strains of *Xam* GSPB2506, GSPB2507, and GSPB2511 (Göttinger Sammlung Phytopathogener Bakterien, Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Germany) isolated by K. Wydra, IITA, in Cotonou, Benin, and Ibadan and Onne, Nigeria, respectively, and the strain Uganda 12, isolated by B. Boher, IRD France, in Uganda were used for stem inoculation. In addition, strains GSPB2506, GSPB2511 and Uganda 12 were also inoculated by leaf infiltration.

4.2.3 Stem inoculation and symptom evaluation

Four week-old plants of 111 genotypes (6 plants per genotype) were stem-inoculated at the third leaf axil from the top by inserting a sharp tooth-pick, contaminated with about 10^7 cfu/ml by passing through a 48 hour-old culture of *Xam* (G. Sanchez, S. Restrepo and V. Verdier, "unpublished data" cited in Restrepo et al., 2000) grown on nutrient glucose agar (nutrient broth 8g/l, glucose 11g/l, yeast extract 3g/l, agar 15 g/l, pH 7.2). Genotypes BEN86052 (susceptible) and TMS30572 (partly resistant) (Fanou, 1999; Akparobi et al., 1998; Fokunang et al., 2000; Zinsou et al. 2001) were used as references. Plants were evaluated 5, 10, 15, 20, 25 and 30 days after inoculation in symptom classes: class 0 = no symptom, class 1 = 1 leaf wilted, class 2 = 2-4 leaves wilted, class 3 = more than 4 leaves wilted, class 4 = dieback of plant or shoot. Additionally, the area under the disease progress curve (AUDPC) was calculated for each inoculated plant from the disease reaction scores 5, 10, 15, 20, 25 and 30 days after inoculation by using the following formula:

$$AUDPC = \Sigma_i.[(D_i + D_{i-1}) * (t_i - t_{i-1})]/2$$

with D_i = disease score at time t_i using the 0-4 symptom classes and t_i = observation dates measured in days after inoculation (Shaner and Finney, 1977). AUDPC was calculated to compare the resistance of each genotype to the 4 strains and the aggressiveness of strains towards the cassava genotypes after stem inoculation. On the basis of the percentage of AUDPC of each strain, - the reaction on the highly susceptible genotype CM8820-47 being set as 100% -, groups of S (stem)-AUDPC resistant (0-33.2%), medium resistant (33.3-

49.9%) and susceptible genotypes (50-100%), were formed. After adding the AUDPC values of the 4 strains, [total stem (TS-) AUDPC], groups of resistant, medium resistant and susceptible genotypes were defined using the same percentage ranges as above. Genotypes showing reverse differential reactions with percentages of AUDPC values comparing strains differing by ≥ 33% in the reverse direction of GSPB2507 > GSPB2511 > Uganda 12 > GSPB2506 were selected. Additionally, genotypes showing striking differential reactions with percentages of AUDPC values comparing strains differing by ≥ 43% were defined as differential genotypes. The two-dimensional biplot of principal component analysis using 29 identified differential genotypes and 14 genotypes with lowest or highest reaction in the three resistance groups based on the stem-area under disease progress curve (S-AUDPC) of 4 strains was established to confirm the grouping of differential genotypes.

4.2.4 Leaf infiltration and symptom evaluation

Eight genotypes identified as resistant in the previous stem puncture test (CM8873-64, 69, CM8820-30, 34, 40, CM8877-11, CM8820B-2, CM8870-1) were used. The abaxial surfaces of the first two nearly fully expanded leaves were carefully infiltrated with a bacterial suspension of *Xam* (10^6 cells/ml) in 0.01 M $MgSO_4$ obtained from 48-hour grown strains on NGA agar by means of a glass atomizer (Hokawat and Rudolph, 1991). Infiltration was carried out until a temporary water-soaking appeared without leaf damage. Two leaves per plant and 5 plants per genotype and bacterial strain were used. Control plants were inoculated with sterile 0.01 M $MgSO_4$ solution. The area of spots (water-soaked and necrotic spots) on leaves was determined each third day starting 14 days post inoculation (dpi) using a transparent plastic sheet on which the areas were reproduced and calculated. The leaf-area under disease progress curve (L-AUDPC) was calculated from the spot area measures 14, 17, 20, 23, 26 and 29 days after inoculation by using the formula above, with ‘D’ being the spot area on leaves, ‘t’ corresponding to days after inoculation, and ‘i’ to evaluation days. Groups of resistant (AUDPC 0-16.6%), medium resistant (16.7-33.2%) and susceptible (33.3-100%) genotypes were formed. The two-dimensional biplot of principal component analysis on 10 leaf infiltrated genotypes based on the leaf-area under disease progress curve (L-AUDPC) of 3 strains was established to show grouping of genotypes.

4.2.5 Quantitative trait loci analysis of cassava bacterial blight resistance

A simple linear regression between molecular markers and AUDPC of *Xam* strains was performed per marker by the QTL cartographer program (Basten et al., 1994; Basten et al., 1999). The program Mapmarker, which analyses marker data by calculating recombination frequencies between markers was used. Pairs of markers, that, in the same linkage, are supposed to have less than 0.25 of recombination frequencies between them, are in the same group. If two markers have more than 0.25 of recombination frequency, they are in different groups. A significant association between DNA marker and CBB resistance was declared when the probability obtained from regression was equal to or less than 0.05 and the likelihood ratio (LR) statistic (Fischer value from analysis of variance) was more or equal to 6 in order to minimize the detection of false positives. The amount of phenotypic variance explained by each marker was derived from analysis of variance.

4.3 Results

4.3.1 Stem inoculation

Among the 111 genotypes derived from a backcross of cassava genotypes TMS30572 (female parent) and 5 F_1 individuals (CM7857-4, CM7857-10, CM7857-51, CM7857-77, CM7857-115), sixteen genotypes showed a resistant reaction against stem inoculation with four highly virulent strains from diverse origin, 26 a medium resistant reaction and 69 a susceptible reaction (data not shown, Annex). The reference genotypes BEN86052 and TMS30572 were susceptible and resistant, respectively. Strain GSPB2507 appeared to be the most virulent, followed by GSPB2511, Uganda 12 and GSPB2506 (Tab. 2).

Analysing strain x genotype interactions, 19 groups of 29 differential genotypes, which could be useful for pathotype detection, were identified between the 111 genotypes (Tab. 3). The susceptible (S) and resistant reactions of genotypes to the four strains revealed their differentiation in four different pathotypes. The two-dimensional biplot of principal component analysis provided a good description of the differential genotypes, and revealed the major underlying sources of variation and allowed the data to be plotted (Fig. 1). The first principal component axis (IPCA 1) is related to the TS-AUDPC. Genotypes with high TS-AUDPC are placed on the right side of the midpoint, while resistant genotypes with low TS-AUDPC are on the left side. Thus, twelve genotypes showing low TS-AUDPC, with lowest TS-AUDPC for genotypes CM8877-11 (42) and CM8873B-25 (43), belonged to the resistant

group. Genotypes CM8820-38 (23), CM8820B-7 (24), CM8873B-20 (25), CM8873B-36 (26), CM8820-4 (27), CM8873-25 (28), CM8873B-16 (29), CM8820-64 (30) and CM8820-40 (41) were medium resistant. Twenty-two genotypes with high TS-AUDPC, with highest values for genotypes CM8820-47 (1), CM8873-44 (2), CM8873-74 (3) belonged to the susceptible group. Eleven genotypes with high differential reactions (IPCA 2 > 0.5) and nine genotypes with low differential reactions (IPCA 2 < -0.5) were identified.

Table 2: Reaction of 29 identified differential genotypes and 14 genotypes with lowest or highest reaction in the three resistance groups, selected among the BC_1 mapping population (111 genotypes) to stem puncture inoculation with 4 highly virulent strains of *X. axonopodis* pv. *manihotis*

Genotypes	S-AUDPC[1] of strains				TS-AUDPC	Reaction
	GSPB2507[2]	GSPB2511	Uganda12	GSPB2506	Total	
1 CM8820-47	60.0±0.0	60.0±0.0	60.0±0.0	60.8±2.7[3]	240.8	S[4,6]
2 CM8873-44	36.6±5.9	56.0±2.2	60.0±0.0	57.5±0.7	210.1	S[5]
3 CM8873-74	50.8±9.4	52.9±1.3	37.5±1.4	60.0±0.0	201.2	S[5]
4 CM8873-48	36.5±3.0	58.3±1.9	50.0±8.8	22.5±3.3	167.3	S[5]
5 CM8873-81	30.0±0.0	60.0±3.8	42.5±2.2	30.0±0.0	162.5	S[5]
6 CM8820-61	48.3±0.9	34.5±1.8	20.0±0.0	52.5±0.0	155.3	S[5]
7 CM8820B-17	28.7±3.6	49.0±3.3	35.0±0.0	40.0±2.2	152.7	S[5]
8 BEN86052[7,8]	37.5±5.0	40.8±2.2	42.9±2.7	33.7±5.4	154.9	S
9 CM8820-16	50.0±0.0	26.2±6.3	25.0±0.0	47.5±2.4	148.7	S[5]
10 CM8877-9	34.0±7.1	52.5±2.2	40.0±0.0	13.2±1.8	139.7	S[5]
11 CM8873-39	29.1±4.7	48.7±2.0	30.8±2.1	30.4±3.7	139.0	S[5]
12 CM8820-46	23.3±5.4	41.2±1.9	42.0±1.7	30.6±8.6	137.1	S[5]
13 CM8873-7	35.8±1.2	27.9±4.1	48.7±4.5	24.1±4.9	136.5	S[5]
14 CM8873B-1	27.9±4.3	39.1±6.7	51.2±4.6	16.2±4.0	134.4	S[5]
15 CM8873B-7	45.4±4.8	32.5±9.1	42.5±3.8	13.7±4.2	134.1	S[5]
16 CM8820-56	34.1±9.7	54.5±9.4	32.0±1.2	12.5±0.0	133.1	S[5]
17 CM8820B-25	44.5±2.2	44.5±0.0	0.0±0.0	42.0±1.9	131.8	S[5]
18 CM8873-35	19.1±2.5	32.0±1.7	40.0±0.0	35.8±5.0	126.9	S[5]
19 CM8820-13	22.5±5.2	23.3±4.8	52.5±0.0	28.3±9.5	126.6	S[5]
20 CM8873B-31	50.0±0.0	20.8±7.0	33.7±4.4	20.8±7.7	125.3	S[5]
21 CM8820-9	36.2±6.8	32.9±6.3	32.9±6.2	22.0±3.2	124.0	S

Table 2: continued

Genotypes	S-AUDPC[1] of strains				TS-AUDPC	Reaction
	GSPB2507[2]	GSPB2511	Uganda12	GSPB2506	Total	
22 CM8877-52	30.0±3.6	46.0±7.9	24.1±0.9	37.0±5.5	122.6	S
23 CM8820-38	24.0±3.3	30.0±0.0	46.6±1.2	18.5±2.7	119.1	MR[5]
24 CM8820B-7	25.8±4.3	37.5±1.4	50.4±2.7	0.0±0.0	113.7	MR[5]
25 CM8873B-20	22.0±5.9	44.1±10.2	35.4±7.6	9.5±3.1	111.0	MR[5]
26 CM8873B-36	31.6±6.4	0.0±0.0	35.8±2.0	27.5±3.9	94.9	MR[5]
27 CM8820-4	37.5±0.0	27.5±0.0	26.2±0.6	0.0±0.0	91.2	MR[5]
28 CM8873-25	32.9±2.2	27.5±0.7	2.5±0.0	27.9±3.0	90.8	MR[5]
29 CM8873B-16	18.7±1.6	31.6±4.2	17.9±2.7	16.5±2.6	84.7	MR
30 CM8820-64	25.0±3.0	45.0±5.2	11.8±3.1	0.0±0.0	81.8	MR[5]
31 CM8820-30[8]	30.4±0.4	21.5±4.4	18.6±5.9	11.2±1.7	81.7	MR
32 CM8873-86	15.4±0.4	37.9±5.6	15.8±0.6	9.5±2.2	78.6	R[5]
33 CM8873-69[8]	25.0±4.1	21.5±4.4	17.5±4.3	12.5±0.0	76.5	R
34 CM8873-78	17.5±1.8	45.0±2.0	0.0±0.0	13.3±2.9	75.8	R[5]
35 CM8870-1[8]	16.2±0.6	16.2±2.0	16.6±2.2	15.0±1.9	64.0	R
36 CM8873-64[8]	34.1±7.5	22.9±7.7	6.8±1.3	0.0±0.0	63.8	R[5]
37 CM8870-17	40.0±8.6	0.0±0.0	5.0±0.0	17.0±3.8	62.0	R[5]
38 TMS30572[7,8]	13.0±1.3	18.3±2.4	16.6±1.2	10.0±1.4	57.9	R
39 CM8820B-23[8]	14.5±0.4	21.8±8.2	15.0±0.0	3.1±0.4	54.4	R
40 CM8820-34[8]	25.0±2.9	15.6±2.1	11.6±2.1	0.0±0.0	52.2	R
41 CM8820-40[8]	19.0±2.2	12.5±0.0	11.6±0.4	6.8±0.4	49.9	R
42 CM8877-11[8]	10.0±0.0	6.8±1.3	0.0±0.0	0.0±0.0	16.8	R
43 CM8873B-25	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0	R
Total[9]	4,151.6	4,146	3,713.5	3,184.9		

1 S-AUDPC = area under disease progress curve after stem inoculation

2 *Xanthomonas axonopodis* pv. *manihotis* strains from Ibadan (GSPB2507), Onne (GSPB2511), Uganda (Uganda 12), and Cotonou (GSPB2506)

3 Highest S-AUDPC value used as 100% to determine the reaction group (see footnote 6)

4 Genotype with the highest reaction, T(total)-AUDPC set as 100%

5 Differential genotypes (see text for explanation)

6 S = susceptible 50-100% T(total)-AUDPC (120.4-240.8), MR = medium resistant 33.3-49.9% T-AUDPC (80.2-120.3), R = resistant 0-33.2 T-AUDPC (0-80.1)

7 BEN86052 and TMS30572 as susceptible and resistant standard

8 Genotype selected for leaf inoculation

9 Total of AUDPC of 111 individuals of the mapping population

Table 3: Pathotypes of *X. axonopodis* pv. *manihotis* identified according to their reaction after stem puncture inoculation of 111 genotypes, and groups of differential genotypes [grouping according to the percentage of the area under disease progress curve into resistant (R), medium resistant (MR) and susceptible (S)]

Genotypes	Reaction on strain				Groups of diff. gt[1].
	1 GSPB2507	2 GSPB2511	3 Uganda12	4 GSPB2506	
2 CM8873-44 3 CM8873-74	S[2]	S	S	S	1[3]
4 CM8873-48	S	S	S	MR	2
7 CM8820B-17 11 CM8873-39 12 CM8820-46	MR	S	S	S	3
13 CM8873-7 20 CM8873B-31	S	MR	S	MR	4
9 CM8820-16	S	MR	MR	S	5
5 CM8873-81	MR	S	S	MR	6
10 CM8877-9 15 CM8873B-7 16 CM8820-56	S	S	S	R	7
17 CM8820B-25 6 CM8820-61	S	S	R	S	8
18 CM8873-35	R	S	S	S	9
19 CM8820-13	MR	MR	S	MR	10
14 CM8873B-1 24 CM8820B-7 25 CM8873B-20	MR	S	S	R	11
26 CM8873B-36	S	R	S	MR	12
27 CM8820-4	S	MR	MR	R	13
28 CM8873-25	S	MR	R	MR	14
23 CM8820-38	MR	MR	S	R	15
36 CM8873-64	S	MR	R	R	16
30 CM8820-64	MR	S	R	R	17
37 CM8870-17	S	R	R	R	18
32 CM8873-86 34 CM8873-78	R	S	R	R	19

1 Diff. gt.= differential genotypes

2 S = susceptible S(stem)-AUDPC 30.4-60.8, MR = medium resistant S-AUPDC 20.3-30.3,

R = resistant S-AUDPC 0-20.2

3 Genotypes 2 and 3 showed susceptible reaction with all the strains, but differ in reaction to strain GSPB2507 and Uganda 12, respectively, by more than 33% from the highest symptom value caused by these strains (Tab. 2) and are therefore grouped under differential genotypes (differentials' group 1)

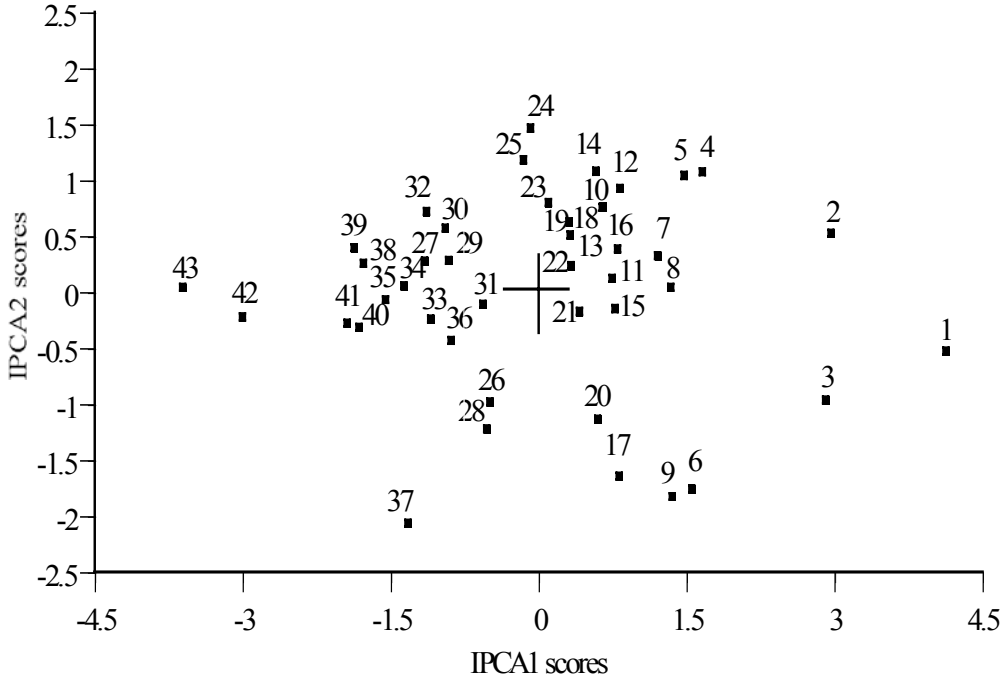

Figure 1: Principal component analysis of area under disease progress curves (S-AUDPC) after stem inoculation of 43 genotypes (identification of genotypes in Tab. 2) with four *X. axonopodis* pv. *manihotis* strains, with genotypes 1-22 (susceptible), 23-31 (medium resistant) and 32-43 (resistant)

4.3.2 Leaf inoculation

When 8 cassava genotypes identified as resistant after stem inoculation (Tab. 2) and, in addition, the susceptible genotype BEN86052 and the resistant TMS30572 were leaf-infiltrated with 3 highly virulent strains of *Xam* (GSPB2506, GSPB2511 and Uganda 12), considerable differences in spot areas and day of shed of leaves were observed (Tab. 4). Some genotypes still increased in total spot areas of inoculated leaves after the first leaf drop, while a reduction was observed with others (data not shown). Thus, the resistant genotypes developed low symptom areas with early, late or no leaves' shed (CM8820-40, CM8820-30, CM8873-69, respectively). The susceptible genotypes developed high symptom areas and shed leaves very late. Genotypes CM8873-69, CM8820-30, and CM8820-40 were identified as resistant, and, generally, the susceptibility of the standard variety BEN86052 and resistance of TMS30572 were confirmed. In contrast to the stem inoculation, strain GSPB2506 was most virulent, followed by Uganda 12 and GSPB2511. Differential reactions indicating three different pathotypes were observed with genotypes CM8877-11 and CM8870-1 and CM8873-64 (Tab. 5).

In the two-dimensional biplot of the principal component analysis, genotypes TMS30572 (38), CM8820-40 (41), CM8820-30 (31) and CM8873-69 (33) had low TL (total leaf) - AUDPC and belonged to the resistant group (Fig. 2). Genotypes BEN86052 (8) and CM8877-11 (42) with high TL-AUDPC, were susceptible, while genotypes CM8870-1 (35), CM8873-64 (36), CM8820B-23 (39), and CM8820-34 (40) were medium resistant. Genotypes with high differential reactions BEN86052 (8), CM8870-1 (35), CM8873-69 (33) or TMS30572 (38), CM8873-64 (36) had high or low IPCA 2 scores, respectively.

4.3.3 Comparison of reaction of genotypes to stem and leaf inoculation

Among the resistant genotypes selected after stem inoculation, genotype CM8877-11 was identified as susceptible and genotypes CM8870-1, CM 8873-64, CM8820B-23 and CM8820-34 as medium resistant, respectively, after leaf inoculation (Tab. 5). Genotypes TMS30572, CM8820-40, CM8820-30 and CM8873-69 were resistant with both inoculations methods.

4.3.4 Comparison of virulence of *Xam* strains after stem and leaf inoculations

The four strains GSPB2507, GSPB2511, Uganda 12 and GSPB2506 belong to different pathotypes according to their reaction on leaves and stems (Tab. 5). Strain GSPB2507 was most virulent, followed by GSPB2511, Uganda 12 and GSPB2506 after stem inoculation (Tab. 2), while the contrary was observed after leaf inoculation with three of the strains, with GSPB2506 being highest virulent (Tab. 4). The differential genotype CM8873-64 showed no symptom with strain GSPB2511 after stem inoculation, but higher symptoms after leaf infiltration than caused by strains Uganda 12 and GSPB2507.

Table 4: Spot areas expressed as area under disease progress curve (L-AUDPC) and day of first leaf drop of 8 genotypes of the mapping population (selected as resistant after stem inoculation) and of one susceptible (BEN86052) and one resistant (TMS30572) reference genotypes, after leaf infiltration with 3 highly virulent strains of *X. axonopodis* pv. *manihotis*

Genotypes	GSPB2511		Uganda 12		GSPB2506			
	L-AUDPC[1] (mm^2)	drop[2] (dpi)	L-AUDPC (mm^2)	drop (dpi)	L-AUDPC (mm^2)	drop (dpi)	TL-AUDPC[3]	Reaction
8 BEN86052	8800.2	23	33261.4	17	41926.1[4]	20	83987.8	S[5]
42 CM8877-11	8904.6	23	5314.2	17	25944.3	17	40163.1	S
35 CM8870-1	2684.3	23	4659.7	17	20030.6	17	27374.6	MR
36 CM8873-64	15699.1	23	6162.3	17	5140.3	17	27001.7	MR
39 CM8820B-23	6901.0	17	8479.1	17	8532.3	20	23912.4	MR
40 CM8820-34	3344.4	20	8719.1	20	9420.7	20	21484.3	MR
38 TMS30572	7048.5	23	2601.5	26	2530.0	23	12180.0	R
41 CM8820-40	2885.8	17	2664.3	17	2414.6	17	7964.8	R
31 CM8820-30	1421.6	23	2658.0	23	2885.8	23	6965.4	R
33 CM8873-69	0	–	252.4	–	233.9	–	486.3	R
Total	57689.5		74772		119058.6			

1 Infiltration of 2 leaves per plant with 10^6 cfu/ml; spot area measured in mm^2

2 Day of first leaf drop

3 TL-AUDPC = total leaf-AUDPC, sum of reaction (spot area) of 3 strains

4 Highest value used as 100%

5 S = susceptible 33.3-100% (TL-AUDPC 30,000-83,988), MR = medium resistant 16.7-33.2% (TL-AUDPC 15,000-29,999), R = resistant 0-16.6% (TL-AUDPC 0-14,999)

Table 5: Reaction of 9 genotypes, selected as resistant after stem inoculation and 1 susceptible reference genotype, to stem and leaf inoculations

Genotypes	GSPB2507	GSPB2511		Uganda 12		GSPB2506		Overall reaction	
	Stem[1]	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf
BEN86052[2]	S[3]	S	MR[4]	S	S	S	S	S	S
CM8877-11[5]	R	R	MR	R	R	R	S	R	S
CM8870-1[5]	R	R	R	R	R	R	S	R	MR
CM8873-64[5]	S	MR	S	R	R	R	R	R	MR
CM8820B-23	R	MR	R	R	MR	R	MR	R	MR
CM8820-34	MR	R	R	R	MR	R	MR	R	MR
CM8820-30	S	MR	R	R	R	R	R	MR	R
CM8873-69	MR	MR	R	R	R	R	R	R	R
TMS30572	R	R	MR	R	R	R	R	R	R
CM8820-40	R	R	R	R	R	R	R	R	R

1 Strain GSPB2507 not used for leaf inoculation due to insufficient number of cuttings

2 Susceptible standard variety

3 S = susceptible 50-100% [S(stem)-AUDPC 30.4-60.8], MR = medium resistant 33.3-49.9% (S-AUPDC 20.3-30.3), R = resistant 0-33.2% (S-AUDPC 0-20.2)

4 S = susceptible 33.3-100% (L-AUDPC 15,000-41,927), MR = medium resistant 16.7-33.2% (L-AUDPC 7,000-15,000), R = resistant 0-16.6% (L-AUDPC 0-7,000)

5 Differential genotypes identified by leaf inoculation (genotypes with R and S)

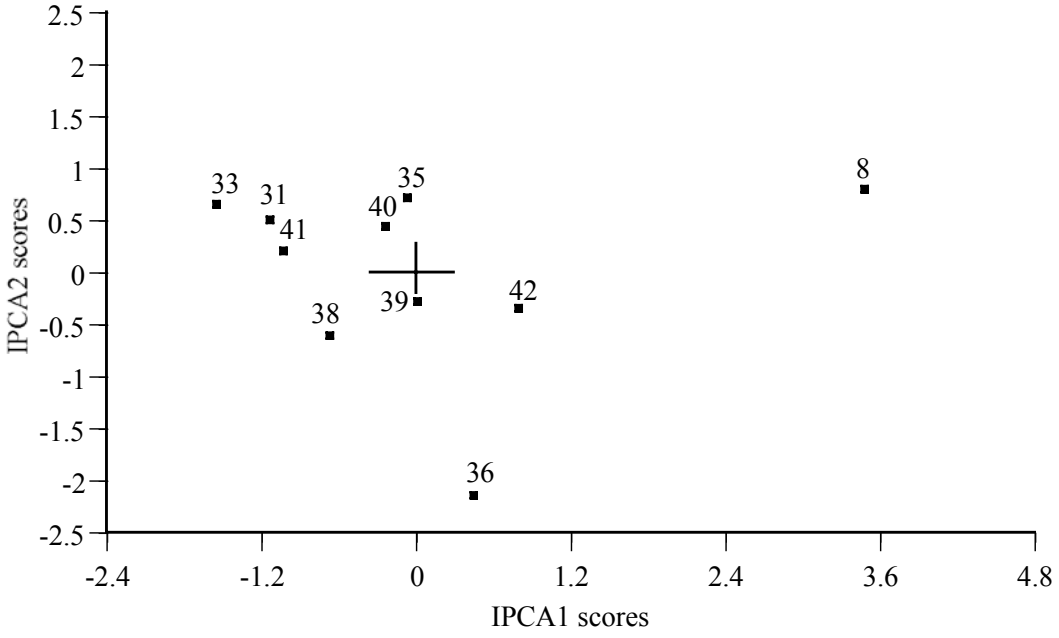

Figure 2: Principal component analysis of area under disease progress curve (L-AUDPC) after leaf inoculation of 10 genotypes (identification of genotypes in Tabs. 2, 4) with four *X. axonopodis* pv. *manihotis* strains, with genotypes 8 and 42 (susceptible), 35, 36, 39 and 40 (medium resistant) and 31, 33, 38 and 41 (resistant)

4.3.5 Quantitative trait loci analysis for cassava bacterial blight resistance

Single-marker regression analysis of AUDPC values from stem-puncture inoculation of each strain, based on the genetic linkage map of cassava, revealed 5 markers on 3 and 1 linkage groups of the female- and male-derived framework of family CM8820, respectively, which were significantly linked to disease reaction caused by the four strains of *Xam* (Tab. 6). Based on the segregation of alleles from family CM8873, one marker (SSRY83) in the female-derived framework was found to be associated to resistance, to *Xam* strains GSPB2506 and GSPB2511. Among the 11 markers associated to the four strains, six markers were identified as related to 5 linkage groups (quantitative trait loci, QTLs), while 5 markers were not linked to any group. The position of the 6 markers is shown on linkage groups of genetic maps of families CM8820+B and CM8873+B, developed by Jorge (2000) (Fig. 3).

Groups 2 and 7 (QTLs 3 and 4) were found to be associated with response to *Xam* strain Uganda 12 explaining 18.1% and 23.2%, respectively, of the variance. Two regions within group 11 (QTLs 1 and 2) were found to be associated to *Xam* strains GSPB2507 and GSPB2511, explaining 16% and 37.7%, respectively, of disease phenotype variance. One

region of group 1 (QTL 5) explained 21.8% and 33.3% of the phenotypic variance associated with the response of strains Uganda 12 and GSPB2507, respectively. Group 14 (QTL6) was linked to strains GSPB2511 and GBPB2506, explaining 19.6% and 18.2% of the phenotypic variance, respectively. These results underlined, that the 4 African strains belong to 4 different pathotypes. Among the 10 genotypes inoculated by leaf infiltration, only 6 have marker data. This small number of genotypes does not allow analysis of linkage between leaf resistance and markers.

Table 6: Markers showing most significant likelihood ratio (LR) values (threshold = 6) after simple linear regression (QTL cartographer) for each map (female- and male-derived) and for each strain inoculated, and identified quantitative trait loci (QTL)

Family	Map	Strain	Linkage group	Marker	LR	P[1]	% Variance explained	QTLs
CM8820	female	GSPB2507	11	SSRY6	7.9	0.010	16	QTL1
		GSPB2511	11	SSRY6	6.7	0.011	18	QTL1
				SSRY170	6.5	0.012	19.7	QTL2
		Uganda 12	2	SSRY55	7.4	0.027	18.1	QTL3
			7	SSRY93	6.7	0.011	23.2	QTL4
	male	GSPB2507	1	GY12	6.1	0.015	21.8	QTL5
		Uganda 12	1	GY12	6.0	0.015	33.3	QTL5
			not linked	SSRY17	6.7	0.011	ND[2]	
CM8873	female	GSPB2507	not linked	SSRY104	6.1	0.015	ND	
		GSPB2511	14	SSRY83	15.4	0.000	19.6	QTL6
		Uganda 12	not linked	SSRY104	7.6	0.007	ND	
		GSPB2506	14	SSRY83	9.5	0.002	18.2	QTL6
			not linked	SSRY104	8.0	0.005	ND	
	male	GSPB2507	not linked	SSRY7	8.2	0.005	ND	
		GSPB2511	not linked	SSRY84	8.6	0.004	ND	
		GSPB2506	not linked	SSRY157	7.9	0.005	ND	

1 Significance
2 Not determined

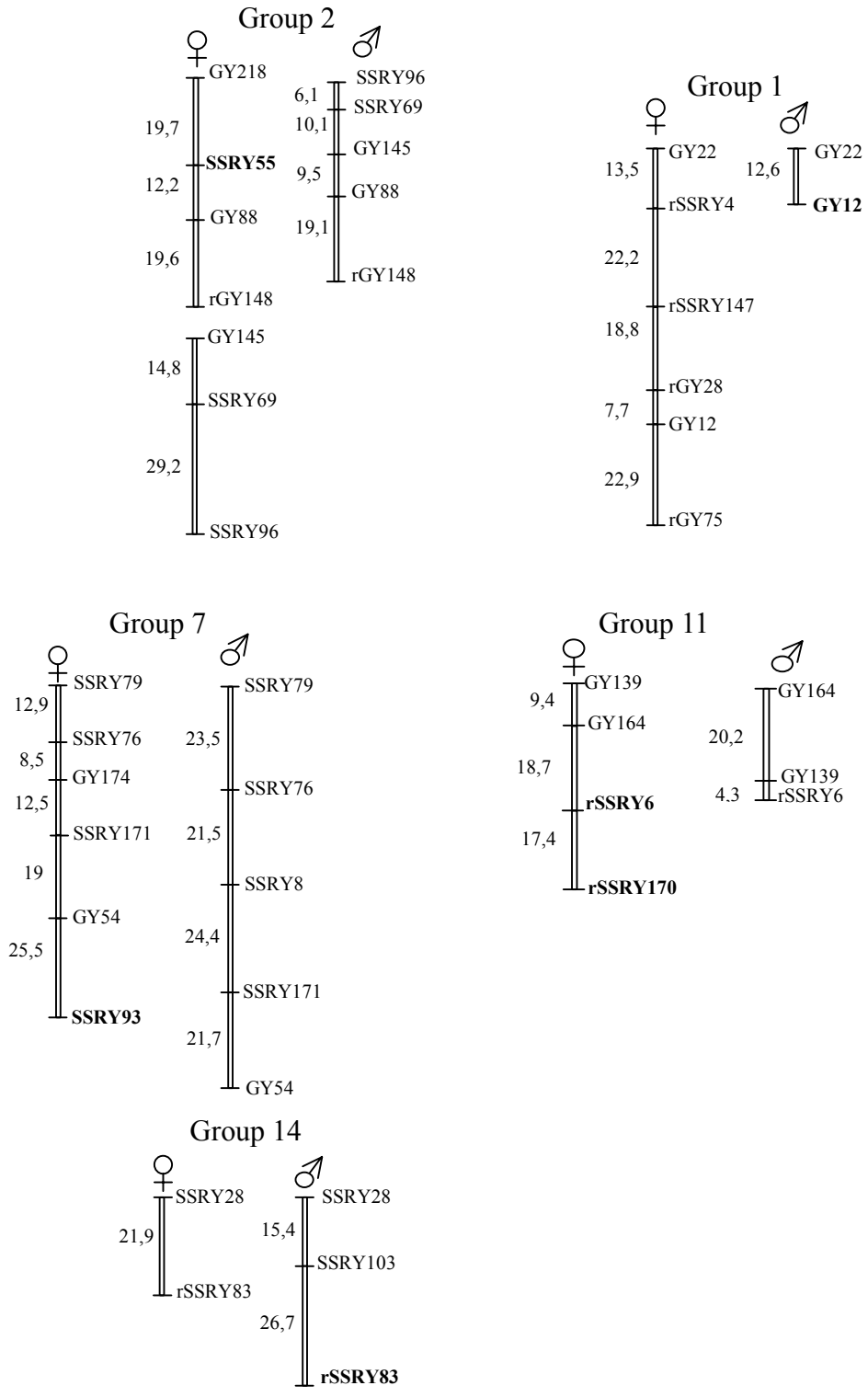

Figure 3: Markers on 4 linkage groups of the genetic map developed by Jorge (2000), based on marker segregation RFLP (GY) and microsatellites (SSRY) in parent gametes TMS30572 (♀) and CM7857-4 (♂) of BC_1 populations CM8820+CM8820B (groups 1, 2, 7, 11), and in parent gametes TMS30572 (♀) and CM7857-77 (♂) of BC_1 populations CM8873+CM8873B (group 14). Markers with "r" are linked in repulsion. Distances at left are in cM. Newly identified markers to African strains are in bold.

4.4 Discussion

Among hundred-eleven genotypes derived from the backcross of 5 F_1 individuals and the female parent TMS30572, 19 differential genotypes were identified by stem inoculation with *X. axonopodis* pv. *manihotis* and 3 differential genotypes by leaf inoculation. Four *X. axonopodis* pv. *manihotis* pathotypes were described and 11 specific genetic markers associated to resistance to these pathotypes, of which 6 QTLs, were found.

Host x pathogen-interactions between a large number of genotypes of a cassava mapping population and four strains of *X. axonopodis* pv. *manihotis* were studied using stem puncture and leaf infiltration methods. Although not all the 111 genotypes of the mapping population were compared by both, stem and leaf infiltration, the results revealed obvious differences between genotypes and strains after inoculation with each of the two methods.

Both stem inoculation and leaf infiltration revealed to be suitable and necessary to screen for resistance to bacterial blight. Different strain x genotype interactions occurred with both methods. Although stem inoculation is more rapid than leaf infiltration, both methods are recommended to identify sources of resistance. The leaf infiltration was also recommended to determine the resistance of genotypes against bacterial diseases in glasshouse trials (Hokawat and Rudolph, 1991; Flood et al., 1995; Cooper et al., 1997). Khatri-Chhetri (1999), Wydra et al. (2002) and Sikirou (1999) revealed interactions of cowpea genotypes with strains of *X. axonopodis* pv. *vignicola* using the leaf infiltration method. In contrast, leaf inoculation was not recommended by Restrepo et al. (2000), who did not find resistance reactions in the leaf of cassava. These contradictory observations may be due to the leaf inoculation methods used by the latter authors, e.g. inoculation by placing 10µl of a bacterial suspension in a small hole previously punched out with a cork borer without detailed symptom observation, compared to the leaf infiltration and symptom measurement in our studies.

After stem inoculation, the genotypes varied in their reaction against four highly virulent strains of *Xam* from four different geographic origins in Africa. Sixteen genotypes among the mapping population showed a resistant reaction. After leaf infiltration, the resistant genotypes developed small symptom areas with no, early or very late leaves' shed. The resistant genotype CM8820-40, which dropped leaves early, was also resistant after stem inoculation. Thus, an early leaf drop of a genotype, which develops only weak symptoms, may indicate a type of resistance mechanism. These results suggest the existance of resistance mechanisms in

leaves and stems, and are supported by the results of Zinsou (2001) on the multiplication of *Xam* in these organs in a resistant variety compared to a susceptible variety. A similar observation was reported only for the stem by Restrepo et al. (2000). Also in the stem, Kpémoua et al. (1996) found that parenchyma cells in the phloem or adjacent to the xylem which synthesized callose and lignin, play an important role in resistance. These authors observed bacterial lysis pockets in the xylem limiting or stopping bacterial extension. In contrast, due to the lack of correlation between leaf and stem reactions, Restrepo et al. (2000) did not suggest the existance of resistance mechanism in the leaf. Only recently, possible resistance mechanism on leaf level were described in form of plant cell wall pectins (Wydra et al., 2002) and, in cell culture studies, latex production and PR-proteins (Kemp et al., 2001; Cooper et al., 2001).

The four strains GSPB2507, GSPB2511, Uganda 12 and GSPB2506 belong to different pathotypes according to their reactions on leaves and stems. Additionally, strain x genotype interactions on stem and also leaf level were observed. Some authors reported strain x genotype interactions on stem level (Restrepo and Verdier, 1997; Restrepo et al., 2000). Verdier et al. (1998) and Restrepo et al. (1999) related this variation to the possible presence of pathotypes in Latin America, as also shown by our results for African strains. The results obtained by both inoculation methods indicated the existance of resistance mechanisms in the leaf and stem and may contribute to the identification of resistance related markers and genes. The ranking of genotypes based on the percentages of the total AUDPC for both inoculation methods were similar to those of the principal component analysis and confirmed the effectiveness of the method.

Six QTLs that explained 16% to 55.1% of the phenotypic variance were characterized using the 4 African strains, suggesting that several genes are involved in resistance to CBB. This finding is supported by the microscopic observations of Kpémoua et al. (1996), who found, that lignin and callose deposits and the occurrence of phenolic compounds within the infected vessels associated with suberin, and tyloses may limit disease extension in the resistant genotype. Thus, quantitative or ‘horizontal’ resistance, associated with numerous genes having smaller effects, but acting against a broad spectrum of pathogenic races, seems to be the basis of the resistance. This type of resistance may also be strain-specific in cases in which QTL are responsible for the resistance (Kreike et al. 1994; Leonards-Schippers et al., 1994; Concibido et al., 1996). Our data revealed strain-specific resistance with corresponding QTLs,

and also support the 'new' concept of a combination of two forms of resistance, multigenic and monogenic resistance (Heath, 2000).

In the F_1 population, Jorge et al. (2001) identified 12 QTLs for resistance to five different strains. For the African strain ORST X-27 and one Colombian strain, resistance QTLs appeared to be introgressions from a wild *Manihot* species and are located on one linkage group of the female-derived map, which has a large number of polymorphic markers and shows much lower recombination frequency than the rest of the genome (Jorge et al., 2000). Using the same BC_1 population as in the present study, Jorge (2000) identified 5 QTLs linked to CBB resistance, against 4 Colombian and 1 African strains. Groups 2 and 11 were found associated to resistance of Colombian and African strains, but the other groups could be related to a pathogenic specialization. The few numbers of QTL detected in the case of the BC_1 population compared to the F_1 population (Jorge et al., 2001) could be either due to the number of markers selected for the BC_1 mapping (121) compared to those selected for the F_1 population (142), the homozygous regions occurring during backcross giving recessive QTL, or specific interactions which affected resistance.

In barley, three QTL accounting for nearly 30% of the phenotypic variation, detected on chromosomes 3 and 7, are involved in partial resistance to bacterial leaf streak (*X. axonopodis* pv. *hordei*) (El Attari et al., 1998). In tomato, resistance to *Ralstonia solanacearum* is controlled by 4 QTLs on chromosomes 6, 7, 10 and 12 (Danesh et al. 1994; Wang et al. 2000). In bean, 7 QTLs are involved in resistance to common bean bacterial blight (*X. axonopodis* pv. *phaseoli*) (Nodari et al., 1993), while in rice 10 QTL were detected correlated to resistance against rice bacterial blight (Li et al., 1999). Regions of the cassava genome involved in resistance to *Xam* differ according to the strain inoculated, indicating that specific resistance factors may be involved with respect to the different *Xam* strains. The QTL results also support the observation that the 4 strains belong to 4 different pathotypes.

In conclusion, stem and leaf-inoculation with a representative set of *Xam* pathotypes under glasshouse conditions to identify genotype x pathogen interactions are recommended to breeders to select resistant genotypes. Regions of the cassava genome involved in resistance to *Xam* differed according to the inoculated pathotype, and new markers specific to African strains were identified. The existence of pathotype-specific genes or groups of genes (vertical resistance) suggest that the polygenic resistance of cassava may be based on a continuum of

monogenic resistances. The newly identified markers for CBB resistance can be used to increase the efficiency of selecting resistant genotypes in Africa by selecting particular alleles from the linkage groups.

4.5 References

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5. Effect of soil amendments, intercropping and planting time in combination on the severity of cassava bacterial blight in two ecozones of West Africa

Abstract. The effects of (i) intercropping cassava with sorghum or cowpea versus cassava monoculture, (ii) potassium fertilizer application and mulching, (iii) shift of planting date, and (iv) the combination of these measures on the severity of cassava bacterial blight at two sites in two ecozones of West Africa were studied. Disease severity of bacterial blight was generally reduced by late planting - in the last third of the rainy season -, with no effect on cassava root yield. Intercropping cassava-sorghum reduced cassava bacterial blight severity significantly up to 80% in the three soil amendment treatments, at normal and late planting time in the forest-savanna transition zone and at normal planting in the dry savanna zone, with few exceptions. The intercropping of cassava with cowpea also reduced the disease severity. Although generally effects on root yield were not observed, the combination of late planting and intercropping in the dry savanna generally reduced root yield. Cassava-sorghum intercropping generally had no effect on root yield compared to cassava monocropping with few exceptions in two sites (ecozones), while intercropping with cowpea significantly reduced root yield by 52% compared to cassava monocropped, in the dry savanna site. Mulching and potassium treatment had no effect on disease severity, but increased or decreased root yield in some treatments in both sites (ecozones).

5.1 Introduction

Cassava is the most dominant root crop in West Africa (Dapaah, 1994) and accounts for over 60% of the daily calorie intake of some 500 million people in the Sub-Saharan region of Africa (FAO, 1997). Major constraints to stable production of cassava in Africa are diseases and pests, besides low soil fertility and unfavourable climatic and socio-economic factors. Among the economically important diseases, cassava bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) (Vauterin et al., 1995), former *Xanthomonas campestris* pv. *manihotis* (Berthet-Bondar) Dye, is one of the most devastating ones (Wydra and Msikita, 1998). Host plant resistance is an important measure in an integrated control system of CBB suitable for farmers (Wydra and Rudolph, 1999; Wydra et al., 2003). But resistance in cassava against CBB is polygenic, partial (Hahn et al. 1979) and highly dependent on the environment and inoculum pressure (Wydra, 2002). Sustainable cassava production depends on the use of genotypes adapted to the environment (Zinsou et al., 2002) combined with efficient cultural practices (Okogbenin et al., 1999; Wydra and Rudolph, 1999; Wydra et al., 2002). Thus, only an integrated approach could contribute to crop management (Kranz and Hau, 1980).

Traditional cassava production is characterized by intercropping and cultivar mixtures (Okigbo and Greenland, 1976). Several short-duration crops such as maize, yam, sorghum, assorted vegetables and cowpea are usually intercropped with cassava in the humid tropics of West Africa (Okoli, 1996). In Benin, cassava is intercropped with maize (South and Centre), sorghum or millet (North), and legumes (MDR, 1999). Intercropping was widely studied as a means to reduce pests and diseases (Ofuya, 1991; Trenbath, 1993; Ahohuendo and Sarkar, 1995; Sikirou, 1999; Fininsa and Yuen, 2001), but not always with positive effect. Thus, intercropping was not suitable to suppress bean anthracnose and bean web blight in a maize-bean intercrop system (Woolley and Davis, 1991). Cassava-maize intercropping was recommended for the forest-savanna transition (Fanou, 1999) and the dry savanna zones of Nigeria to reduce CBB (Tabot, 1995). In Benin, intercropping to reduce bacterial blight of cassava was never investigated in the forest-savanna transition and the dry savanna zone.

Cassava is known to require N and K fertilizers for maximum growth and root yields (Howeler, 1991; Olasantan et al., 1994). Potassium fertilizer in an appropriate dose was reported to reduce CBB incidence and severity under greenhouse conditions (Adeniji and Obigbesan, 1976; Arene and Odurukwe, 1979). Another often recommended cultural measure

to reduce the incidence and severity of plant diseases by reduction or prevention of soil splashing is mulching (Galindo et al., 1983; Moreno and Mora, 1984; Fitt and McCartney, 1986). Mulches may also prevent direct contact of the foliage, fruit, or vines with the soil and thus prevent diseases transmitted from the soil (Thurston, 1992). In Benin, low fertility soils such as acrisols, acrenosols and luvisols reduce productivity, but the use of means to maintain and to improve soil fertility such as green manure are rarely observed (Maliki et al. 1997; Gaiser et al., 2000; von Oppen et al., 2000).

Among the agronomic measures to reduce disease epidemics, the shift of planting date to avoid the peak time of inoculum pressure during a susceptible stage of a crop is recommended. Also for control of cassava bacterial blight, the shift to a late planting date was observed to reduce disease incidence and severity (Ambe, 1993; Fokunang et al., 2000), but detailed symptom and yield evaluations and observations over two or more years were not conducted.

To develop an integrated control system for bacterial blight, control measures have to be optimised and adapted to ecozones and farmers' conditions, and finally combined in a deliverable and feasible package. Therefore, the optimal combination of measures such as planting date, application of fertilizers, mulching, and intercropping, to reduce CBB severity and increase root yield, has to be identified for each ecozone.

The objectives of this study were to analyse the effects of (i) intercropping cassava with sorghum or cowpea versus cassava monoculture, (ii) potassium fertilizer application and mulching, (iii) shift of planting date and (iv) the combination of these measures on the severity of CBB and root yield in two sites (ecozones) of West Africa.

5.2 Materials and Methods

Three field experiments were conducted from 1998 to 1999 and repeated from 2000 to 2001 at the International Institute of Tropical Agriculture (IITA), Cotonou station, South Benin and at Ina, 'Institut National de la Recherche Agronomique du Benin' (INRAB) station, North Benin. IITA is located in the forest-savanna transition zone with total rainfall averages of 1200 mm per annum during the rainy seasons from March to July and from September to October. The long dry period extends from November to March. The mean temperature is about 27°C with a low diurnal variation of 7 to 10°C. Ina is located in the dry savanna zone with an annual rainfall ranging from 700 to 900 mm during the rainy season from April to October, followed by a dry season from November to March. The mean temperature is about 32°C. Soils in Cotonou are arenosols or acrisols, and luvisols in Ina, which have good physical characteristics, but a low nutrient level (Maliki et al. 1997; Gaiser et al., 2000; von Oppen et al., 2000). The nutrient status of the soils is given in Table 1.

Table 1: Characteristics of soils (0-15 cm) of the experimental fields at IITA, Cotonou, South Benin (forest-savanna transition zone) and Ina, North Benin (dry savanna zone)

Ecozones	O. M. (%)[1]	P (Bray I, ppm)[2]	C%	N%	C/N	K^+ (meq/100g)
Forest-savanna transition	2.62	5	1.52	0.155	13.6	0.77
Dry savanna	1.06	1	0.61	0.045	9.8	0.28

1 O.M. = organic matter

2 Assimilate phosphor

5.2.1 Fertilizer and mulching application and planting date variation in two ecozones

The experiment was arranged in a split plot design fitted to a randomised complete block design with three replicates. The main plots, with 3 m alleys between the plots, corresponded to 4 treatments: KCl 0 kg·ha^{-1} (control), 80 kg·ha^{-1}, 120 kg·ha^{-1}, and 2t·ha^{-1}of dry matter (DM) of *Cassia siamea* (Caesalpiniaceae) as mulch. *Cassia siamea* is a commonly grown tree, which is recommended to farmers in Benin as fuel wood and green manure (Leihner et al., 1999). The subplots of 1 m x 10 m, with 2 m alleys between the plots, were planted with genotypes BEN86052 (Benin landrace) and TMS30572 (58308 x Branca de santa Caterina), an improved local genotype, susceptible to CBB and an improved 'resistant' genotype (Wydra et al., 1999), respectively. Mature stems from apparently healthy plants of both genotypes were obtained from the International Institute of Tropical Agriculture (IITA), Benin. Cassava was planted in the first week of June (normal planting) and in the first week of July (late planting, in the last third of the rainy season) in both sites (ecozones) in 1998 and 2000. In year 2000, plants were poorly established in Ina (dry savanna) at the late planting date due to climatic conditions, so that this part of the trial was abandoned. Cassava genotypes were planted at 1 m x 1 m (10,000 plants·ha^{-1}) on ridges in cassava plots with two rows of 10 m each per plot. Fertilizer (80 and 120 kg·ha^{-1} of KCl) was drilled in a furrow at 0.20 m distance from cassava rows and covered with soil one week before inoculation of *Xam*. Cassava rows of each plot were sprayed with the highly virulent *Xam* strains GSPB2506 and GSPB2510 (Göttinger Sammlung Phytopathogener Bakterien, Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Germany) isolated in Cotonou and Ina, respectively, at the concentration of 10^7 cfu/ml, one month after planting. Some drops of Tween 20 were added to the spraying suspension to increase adhesion of the bacteria to leaf surfaces. Inoculation was repeated 3 times in monthly intervals.

5.2.1.1 Disease assessment

Disease symptoms were recorded on the two cassava rows of each subplot one month after the first inoculation and at monthly intervals during the rainy and the dry season until harvest at 12 months. On 10 plants selected randomly per plot the percentage of leaves with typical watersoaked-spots, leaves with blight, wilted leaves, and additionally shoot tips with dieback were recorded in classes (Wydra et al., 1999): <5%, 5-10%, 10-20%, 20-50%, 50-80%, 80-100%. A leaf with spots and blight was counted as leaf with blight only. For calculations, class values were transformed to mean values for each class. At harvest, the total number of leaves, number of leaves with spot, blight and of wilted leaves were counted for the first five plants

out of the ten plants harvested. The severity index at each evaluation date was calculated according to the following formula: $Si = (1 \times S + 2 \times B + 1 \times W + 2 \times D)/6$, where S, B, W and D represent the percentage of leaves with spots, blight, wilt and of stems with dieback, respectively. The highest possible value is 60 (e.g. evaluation of 80-100% blight and 80-100% dieback corresponds to [1 x 0 + 2 x 90 + 1 x 0 + 2 x 90]/6). The weight attributed to the symptoms blight and dieback is an estimation resulting from regression analysis of symptom and plant growth data, revealing blight as most important factor influencing root yield, and dieback influencing mostly overall plant growth (leaf and stem weight) (unpublished data). The mean severity index of subplots at the evaluation dates 60, 90, 120, 150, 180 and 360 days after planting in each ecozone was calculated and used to determine the area under severity index progress curve (AUSiPC) of subplots using the 6 evaluation dates according to the following formula:

$$AUSiPC = \Sigma_i.[(S_i + S_{i-1}) * (t_i - t_{i-1})]/2$$

where Si is the mean of severity index at time t_i, t corresponds to days after inoculation with i: 60, 90, 120, 150, 180, 360 (Shaner and Finney, 1977; Jeger and Viljanen-Rollinson, 2001). The AUSiPC in days over the whole period was then divided by the evaluation period [365 days minus days of dry period (60, 120 and 200 days in the forest-savanna transition and dry savanna zones, respectively, in 1998; 120, 150 and 200 days in the forest-savanna transition and dry savanna zones, respectively, in year 2000)] to receive an average comparable between ecozones. Thus, all AUSiPC values are standardized.

5.2.1.2 Yield assessment

Storage root weight was recorded on 10 plants per subplot at the harvest 12 months after planting. Root samples were combined from each plot, mixed, and a subsample was cut into small pieces, weighed and dried in a paper bag in an oven at 105 ^{o}C for 72 h to obtain the dry weights.

5.2.2 Intercropping trial

The susceptible cassava genotype BEN86052 was intercropped with sorghum (UCS111) or cowpea genotype IT84E-124 (IITA Ibadan, Nigeria). Cassava and cowpea were planted the same day (first week of June) in Ina (dry savanna) in year 1998. Cassava was planted at 1 m x 1 m spacing (10,000 plants·ha^{-1}) on the flat, both in mono- and intercropped cassava plots, in four rows of 10 m per plot. Each plot measured 3 m x 10 m, with 2 m alleys between the plots. Cowpea was planted at 62,500 plants·ha^{-1} in rows at 40 cm left and right from the two

inner cassava lines and at 20 cm in the cowpea row giving in total 4 rows of cowpea. The design was a randomised complete block, replicated three times giving 9 plots. Rows of cowpea were sprayed thrice against insect pests, at 30 days after planting (dap) with Cymbush (Cypermethrin) (2 ml·l^{-1}) and on 40 and 50 dap with Cymbush Dimethoate (2 ml·l^{-1}). Weeding was carried out when necessary. No fertilizers were applied to any of the treatments. Disease scoring data and yield parameters were collected as described above, an additional harvest of cassava roots was conducted at 6 months. Sorghum was harvested at 4 months and cowpea at 3 months after planting.

5.2.3 Combined trial: intercropping, fertilizer and mulch application, and planting date variation in two ecozones

The experiment was arranged in a split plot design fitted to a randomized complete block design with three replicates. The main plots were a monocrop of cassava (sole cassava) and sorghum-cassava intercropped, with 3 m alleys between the plots. The subplots of 3 m x 10 m, with 2 m alleys between the plots, comprised 2 fertilizer doses, 0 kg·ha^{-1} of KCl (control cassava), 120 kg·ha^{-1} of KCl, and a third treatment of 2t·ha^{-1}of dry matter (DM) as mulch of *Cassia siamea* a tree commonly growing in Benin, used as fodder and fire wood.

Mature stems from apparently healthy plants of cassava genotype BEN86052 were obtained from IITA, Benin. Cassava was planted in the first week of June (normal planting) and in the first week of July (late planting) at IITA Cotonou and INRAB station Ina in years 1998 and 2000. In year 1998, the main plot cassava monocropped was included in the design of the first experiment (control, fertilizer and mulching application). Cassava genotypes were planted at 1 m x 1 m spacing (10,000 plants·ha^{-1}) on ridges both in mono- and intercropped cassava plots with four rows of 10 m each per plot. Sorghum (UCS111) was planted at the same time at a spacing of 0.5 m x 1 m, giving 3 rows alternating with cassava rows. Fertilizer (120 kg·ha^{-1} of KCl) was drilled in a furrow at 0.20 m distance from cassava rows and covered with soil one week before inoculation of *Xam*. Cassava border rows of each plot were sprayed with the highly virulent *Xam* strains GSPB2506 and GSPB2510 isolated in Cotonou and Ina, respectively, as described above.

Disease symptoms were recorded on 10 randomly selected plants of the two cassava rows inside the border rows of each sub-plot one month after the first inoculation and at monthly intervals during the rainy and the dry season until harvest at 12 months. Disease scoring data

and yield parameters were collected and calculations performed as described above. Sorghum was harvested at 4 months.

5.2.4 Statistical analysis

ANOVA were performed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA) (SAS, 1990; SAS, 1997) on standardized AUSiPC and root yield data, which were log-transformed to stabilise the variance. The Student-Newmann-Keuls test was used to compare mean values of standardized AUSiPC and of root yield, measuring planting time, potassium fertilizer and mulching, and cropping system effects ($p \leq 0.05$). Values given in tables are real means with corresponding standard errors.

5.3 Results

5.3.1 Effect of potassium and mulching at two planting dates in two ecozones on symptom expression and root yield

5.3.1.1 Disease development at two planting dates

Comparing normal and late planting dates in Cotonou (forest-savanna transition zone) in year 1998, the disease development expressed as area under severity index progress curve (AUSiPC) was significantly reduced by 15-23% in genotype BEN86052 at late planting in the control and the KCl (120 kg•ha^{-1}) and mulching treatments, and significantly reduced by 13-23% in genotype TMS30572 at late planting in the control and the treatment KCl (80 kg•ha^{-1}) (Tab. 2). In year 2000, disease development was significantly reduced by 38-58% at late planting in genotype BEN86052 and by 45-63% genotype TMS30572 in the controls and treatments except for the KCl (120 kg•ha^{-1}) treatment in both genotypes. In Ina (dry savanna), CBB severity was significantly reduced by about 55-58% at late planting in genotype BEN86052 in all the treatments, and by about 58-59% in genotype TMS30572 in the control and KCl (120 kg•ha^{-1}) and mulching treatments.

5.3.1.2 Disease development in fertilizer and mulching treatments

In Cotonou (forest-savanna transition zone) in year 1998, the effect of potassium and mulching on disease development in both susceptible and resistant genotypes was not significantly different from the control at both planting times, except a reduction of 14-23% in the fertilizer and mulching treatments for genotype TMS30572 at the normal planting time

(Tab. 2). In year 2000, at the normal planting time, the disease was significantly reduced by 29% in genotype BEN86052 and by 40% in genotype TMS30572 in the KCl (120 kg·ha^{-1}) treatment. At late planting, a significant disease increase (33%) was observed in genotype TMS30572 with mulching treatment. In Ina (dry savanna), a significant increase (23%) of disease severity was only observed in the KCl (120 kg·ha^{-1}) treatment at normal planting time in the resistant genotype in year 2000.

5.3.1.3 Root yield at two planting times

Comparing normal and late planting times, in Cotonou (forest-savanna transition zone) in year 1998, no significant effect of planting time on root yield 12 months after planting was observed, except for a reduction in genotype BEN86052 in the mulching treatment at late planting ($p = 0.001$) (Tab. 3). In year 2000 at the late planting date, root yield was significantly reduced by 44% in genotype BEN86052 in the mulching treatment and in genotype TMS30572 in the KCl (80 kg.ha^{-1}) treatment by 54% (Tab. 3), while a significant root yield increase of 45% occurred in genotype TMS30572 in the control, compared to normal planting. In Ina (dry savanna), yield was significantly reduced in both genotypes in the treatment KCl (120 kg·ha^{-1}) at the late planting date, while in other treatments no effect of planting time occurred.

5.3.1.4 Root yield in fertilizer and mulching treatments

In Cotonou (forest-savanna transition zone) in year 1998, potassium application and mulching increased root yield significantly in the susceptible genotype BEN86052 except in the mulching treatment at late planting date (Tab. 3). No significant effect of any treatment was observed for TMS30572. In year 2000, only at the normal planting time in genotype BEN86052, a significant ($p = 0.02$) yield increase occurred in the mulching treatment. A significant yield depression was observed in genotype TMS30572 with KCl (80 kg·ha^{-1}) at the late planting in Cotonou in year 2000. In Ina (dry savanna) in year 1998, yield was significantly increased at the normal planting date in genotype TMS30572 in the KCl (120 kg·ha^{-1}) treatment ($p = 0.01$), and at the late planting date in genotype BEN86052 in the mulching treatment ($p = 0.012$) (Tab. 3).

Table 2: Cassava bacterial blight expressed as standardized area under severity index progress curve (AUSiPC) of genotypes BEN86052 (susceptible to CBB) and TMS30572 (resistant) in two fertilizer (KCl 80 kg·ha^{-1}, 120 kg·ha^{-1}) and a mulching treatments at two planting times in Cotonou (forest-savanna transition zone) and Ina (dry savanna zone) in two years

			Cotonou (Forest-savanna transition)		Ina (Dry savanna)	
			Planting date		Planting date	
Year	Treatment	Genotypes	Normal	Late	Normal	Late
1998	Control	BEN86052	6.0± 0.1[1]	**4.7± 0.4**	6.2± 0.8	**2.6± 1.0**
	KCl-80		5.4± 0.6	4.6± 0.3	6.5± 0.3	**2.7± 0.9**
	KCl-120		6.2± 0.7	**4.8± 0.2**	5.2± 1.3	**2.4± 0.5**
	Mulching		5.5± 0.4	**4.2± 0.4**	5.6± 0.7	**2.5± 0.6**
	Control	TMS30572	5.5± 0.4	**4.2± 0.2**	3.1± 0.6	**1.3± 0.5**
	KCl-80		4.4± 0.2*	**3.8± 0.2**	2.7± 0.3	1.9± 0.5
	KCl-120		4.2± 0.4*	4.2± 0.7	3.1± 0.5	**1.3± 0.1**
	Mulching		4.7± 0.2*	4.5± 0.2	3.2± 0.4	**1.3± 0.3**
2000	Control	BEN86052	4.5± 0.7	**2.1± 0.1**	4.7± 0.7	nd
	KCl-80		5.0± 0.2	**2.1± 0.1**	4.1± 0.7	nd
	KCl-120		3.2± 0.2*	2.6± 0.5	4.9± 0.2	nd
	Mulching		4.2± 0.7	**2.6± 0.4**	4.6± 0.4	nd
	Control	TMS30572	2.0± 0.2	**1.1± 0.3**	2.7± 0.3	nd
	KCl-80		2.2± 0.8	**0.8± 0.2**	2.7± 0.4	nd
	KCl-120		1.2± 0.1*	1.6 ±0.9	3.5± 0.7*	nd
	Mulching		1.6± 0.2	**2.4± 0.0***	2.6± 0.3	nd

1 Original means with original standard errors, in bold: significant comparison between normal and late planting dates at $p \leq 0.05$, with star: significant comparison between (fertilizer, mulching) treatments and control at $p \leq 0.05$

nd: Not determined due to crop failure under unfavourable climatic conditions

Table 3: Dry root yield (t·ha^{-1}) 12 months after planting of genotypes BEN86052 (susceptible to CBB) and TMS30572 (resistant) in two fertilizer (KCl 80 kg·ha^{-1}, 120 kg·ha^{-1}) and a mulching treatments at two planting times in Cotonou (forest savanna transition zone) and Ina (dry savanna zone) in two years

			Cotonou (Forest-savanna transition)		Ina (Dry savanna)	
			Planting date		Planting date	
Year	Treatment	Genotypes	Normal	Late	Normal	Late
1998	Control	BEN86052	13.6± 5.8[1]	11.4± 1.9	13.1± 1.6	13.6± 3.2
	KCl-80		22.2± 4.1*	18.1± 4.0*	15.4± 2.2	18.4± 2.4
	KCl-120		24.0± 8.5*	19.5± 7.8*	15.1± 3.2	**11.3± 1.8**
	Mulching		15.8± 1.5*	**10.9± 0.5**	16.2± 0.8	18.9± 1.3*
	Control	TMS30572	17.8± 6.6	19.6± 7.5	13.4± 1.5	12.1± 3.1
	KCl-80		16.4± 7.0	14.8± 5.7	15.2± 3.8	12.3± 1.0
	KCl-120		15.8± 7.7	19.3± 9.4	18.5± 5.0*	**11.1± 1.2**
	Mulching		20.2± 8.2	20.7± 8.9	15.5± 1.2	14.7± 1.3
2000	Control	BEN86052	10.8± 2.5	14.6± 1.2	7.8± 1.5	nd
	KCl-80		18.5± 5.5	18.4± 2.7	12.7± 1.0	nd
	KCl-120		10.1± 3.1	12.1± 3.0	6.6± 1.3	nd
	Mulching		20.1± 5.9*	**11.3± 1.0**	8.5± 1.0	nd
	Control	TMS30572	10.6± 3.2	**19.2± 8.0**	6.9± 1.2	nd
	KCl-80		20.9± 10.3	**9.5± 1.4***	5.6± 0.9	nd
	KCl-120		20.3± 5.9	14.3± 1.3	14.3± 7.6	nd
	Mulching		14.0± 4.6	15.4± 4.5	7.8± 1.9	nd

1 Original means with original standard errors, in bold: significant comparison between normal and late planting dates at $p \leq 0.05$, with star: significant comparison between (fertilizer, mulching) treatments and control at $p \leq 0.05$

nd: Not determined due to crop failure under unfavourable climatic conditions

5.3.2 Effect of intercropping on disease development and root yield in the dry savanna zone

A significant reduction of disease development expressed as standardized AUSiPC of 26.2% and 32.7% was observed in the cassava-sorghum and cassava-cowpea intercropping, respectively, compared to the sole cassava planting (Tab. 4) ($p = 0.001$). The effect of intercropping on root yield was significant, with a reduction of 60% in cassava-sorghum intercropping and 48.4% in cassava-cowpea compared to sole cassava at the harvest at 12 months ($p < 0.0001$).

Table 4: Standardized area under severity index curve (AUSiPC) and dry root yield ($t \cdot ha^{-1}$) in sole cassava, cassava-sorghum and cassava-cowpea in Ina (dry savanna) at normal planting time in 1998

Cropping system	AUSiPC	Dry root yield (6 months)	Dry root yield (12 months)
Sole cassava	6.1± 0.5a[1]	2.0± 0.5a	16.5± 2.5a
Cassava-sorghum	4.5± 0.6b	1.8± 0.5a	6.6± 0.7b
Cassava-cowpea	4.1± 0.4b	1.9± 0.2a	8.5± 2.0b

1 Original means with original standard errors; means with the same letter are not significant at $p = 0.001$

5.3.3 Effect of combination of intercropping, fertilizer/mulching treatments and two planting dates on symptom expression and root yield in two ecozones

5.3.3.1 Disease development at two planting times

Disease development in the control, KCl (120 $kg \cdot ha^{-1}$) and mulching treatments of cassava monocropped and intercropped with sorghum at the normal planting time in 1998 at Cotonou (forest-savanna transition zone), is shown in (Fig. 1, a, b, c). Generally, the disease severity index was lower in the intercropping system, except at 120 days after planting. The disease developed during the rainy season. Symptoms disappeared during the dry season and reappeared in the rainy season of the following year. The severity index was significantly lower in cassava intercropped compared to monocropped at 2 and 12 months after planting in the mulching, and at 2, 3 and 12 months in the KCl treatment and the control. The disease

severity in intercropping increased at 4 months after planting when the intercrop was harvested.

In Cotonou in year 1998, disease development was significantly reduced in the monocropping system with all the treatments at the late planting compared to normal planting (Tab. 5). In year 2000, a significant disease reduction was observed at the late planting in both cropping systems. In Ina (dry savanna), disease development was significantly reduced in both cropping systems with the different treatments at the late planting in year 1998, except in the intercropping system with the KCl and mulching treatments, while the trial failed due to climatic conditions in year 2000.

5.3.3.2 Disease development in fertilizer and mulching treatments

The effect of fertilizer and mulching on disease development at both planting times and in both sites (ecozones) in both years was not significant (Tab. 5).

5.3.3.3 Disease development in two cropping systems

Disease development was significantly reduced by 25 to 45% in the intercrop cassava-sorghum compared to the sole cassava in all treatments only at normal planting time in Cotonou, while at late planting the effect was observed only in all treatments in year 2000 (Tab. 5). In Ina, disease reduction in intercropping compared to sole cassava was significant at the normal planting time in KCl treatment in year 1998 and in all treatments in year 2000.

5.3.3.4 Root yield at two planting times

In Cotonou, root yield was not significantly reduced in both years at the late planting except for the mulching treatment in cassava monocropping in the two years, where a yield reduction was observed, and the control in cassava monocropping in year 2000, where a yield increase occurred at the late planting (Tab. 6). In Ina, in year 1998, root yield was significantly reduced at the late planting except for the control in cassava monocropping, and the mulching treatment in cassava monocropping, where a significant yield increase was observed.

5.3.3.5 Root yield in fertilizer and mulching treatments

In Cotonou in year 1998, root yield significantly increased compared to the control only in KCl treatment in cassava monocropping at both planting times (Tab. 6). In year 2000, only at the normal planting time a significant yield increase occurred in the mulching treatment in

cassava monocropping. In the mulching treatment in cassava-sorghum at the normal planting time, a significant yield reduction was observed. In Ina in year 1998, yield significantly increased at the normal planting date in the KCl and mulching treatments in cassava-sorghum, and at the late planting date in the mulching treatment in cassava monocropping, while in year 2000 yield significantly increased only in the KCl treatment in cassava monocropping (Tab. 6). Yield decreased and increased in Ina in year 1998 at the late planting in KCl and mulching treatment, respectively, in monocropping.

5.3.3.6 Root yield in two cropping systems

In Cotonou in year 1998, root yield was significantly higher in cassava monocropping in the KCl treatment at both planting times (Tab. 6). In year 2000, a high, significant yield increase occurred in the mulching treatment at the normal planting time and in the control at the late planting time in cassava monocropping. In Ina in year 1998, yield significantly increased at the normal planting date in the control in cassava monocropping and at the late planting date with all the treatments in cassava monocropping. In year 2000, yield significantly increased in KCl treatment in cassava monocropping.

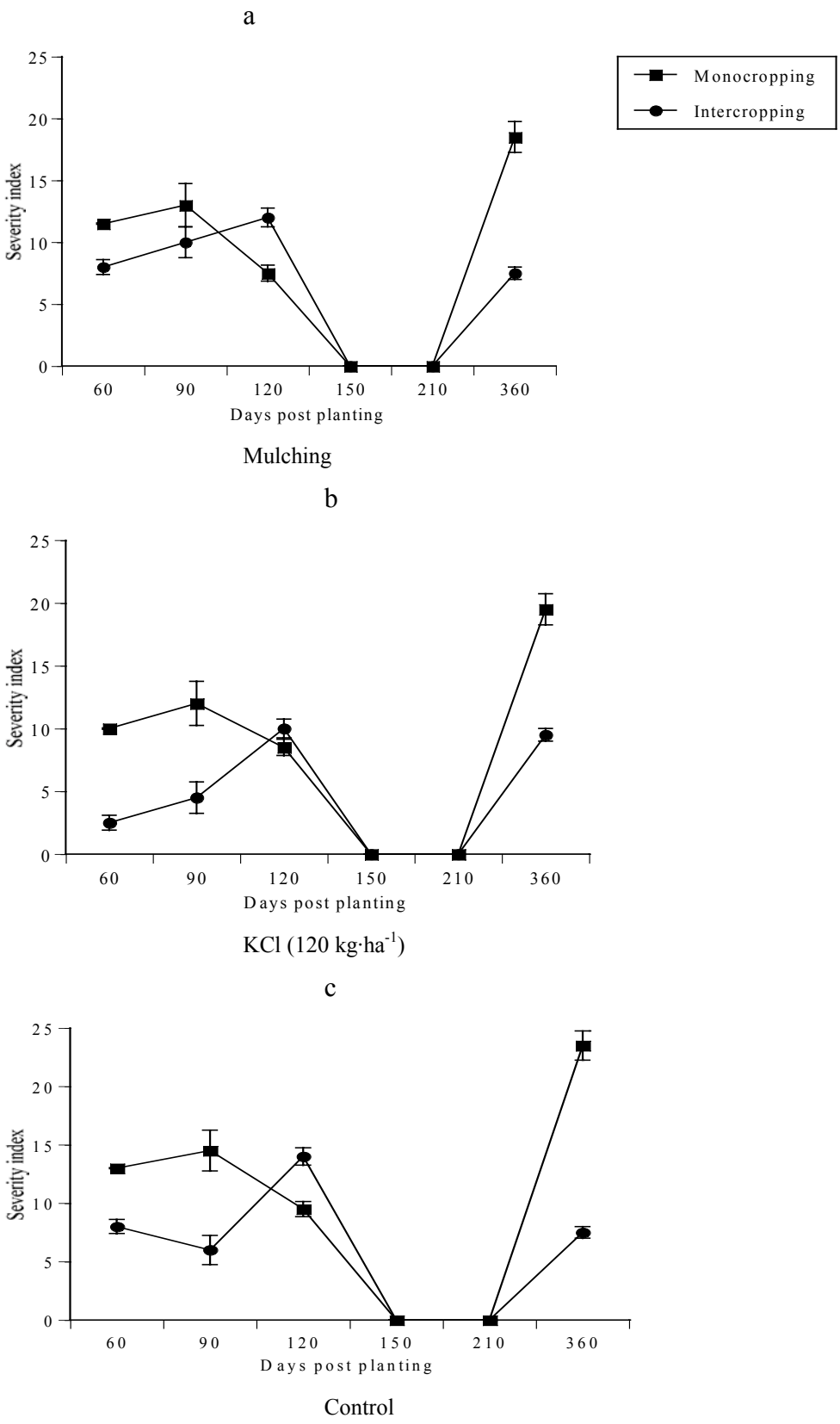

Figure 1: Development of disease severity index in mulching (a), KCl (b) and control (c) treatment of cassava monocropped and intercropped with sorghum at the normal planting time in Cotonou (forest-savanna transition zone) in year 1998

Table 5: Cassava bacterial blight expressed as standardized area under severity index progress curve (AUSiPC) in cassava-sorghum and sole cassava (genotype BEN86052) in a fertilizer (KCl 120 kg·ha^{-1}) and a mulching treatments at two planting times in Cotonou (forest-savanna transition zone) and Ina (dry savanna zone) in two years

Year	Cropping system	Treatment	Cotonou (Forest-savanna transition)		Ina (Dry savanna)	
			Planting time		Planting time	
			Normal	Late	Normal	Late
1998	C[1]	Control	6.0± 0.1[2]	**4.7± 0.4**	6.2± 0.8	**2.6± 1.0**
	CS	Control	*4.6± 0.9*	4.4± 0.2	5.1± 0.5	**2.1± 0.2**
	C	KCl[1]	6.2± 0.7	**4.8± 0.2**	5.2± 1.3	**2.4± 0.5**
	CS	KCl	*4.6± 0.5*	4.7± 0.1	*3.1± 0.7**	2.4± 0.5
	C	Mulching	5.5± 0.4	**4.2± 0.4**	5.6± 0.7	**2.5± 0.6**
	CS	Mulching	*4.4± 0.3*	4.0± 0.9	4.6± 1.3	3.2± 0.1
2000	C	Control	4.2± 0.0	**2.6± 0.3**	3.2± 0.0	nd
	CS	Control	*2.7± 0.0*	***0.8± 0.2***	*2.7± 0.3*	nd
	C	KCl	4.2± 0.1	**2.1± 0.3**	3.1± 0.0	nd
	CS	KCl	*2.6± 0.1*	***0.8± 0.4***	*2.6± 0.3*	nd
	C	Mulching	4.4± 0.3	**2.5± 0.1**	3.1± 0.0	nd
	CS	Mulching	*2.5± 0.1*	***0.5± 0.3***	2.7± 0.3	nd

1 C= monocropped cassava, CS = intercropped cassava with sorghum

2 Original means with original standard errors, in bold: significant comparison between normal and late planting dates at $p \leq 0.05$, with star: significant comparison between (fertilizer, mulching) treatments and control for each cropping system at $p \leq 0.05$, and in italic: significant comparison between cropping system for each treatment at $p \leq 0.05$

nd: Not determined due to crop failure under unfavourable climatic conditions

Table 6: Dry root yield (t·ha^{-1}) after 12 months in cassava-sorghum and sole cassava in a fertilizer (KCl 120 kg·ha^{-1}) and a mulching treatments at two planting times in Cotonou (forest-savanna transition zone) and Ina (dry savanna zone) in two years

Year	Cropping system	Treatment	Cotonou (Forest-savanna transition)		Ina (Dry savanna)	
			Planting time		Planting time	
			Normal	Late	Normal	Late
1998	C[1]	Control	13.6± 5.8[2]	11.4± 1.9	13.1± 1.6	13.6± 3.2
	CS	Control	9.1± 1.6	10.9± 0.7	*9.6± 0.6**	***6.3± 2.3***
	C	KCl[1]	24.0± 8.5*	19.5± 7.8*	15.1± 3.2	**11.3± 1.8***
	CS	KCl	10.4± 2.7	12.1± 1.8	15.9± 2.1	***6.8± 0.4***
	C	Mulching	15.8± 1.5	**10.9± 0.5**	16.2± 0.8	**18.9± 1.3***
	CS	Mulching	13.9± 2.5	12.4± 3.1	14.2± 0.9	***7.2± 2.2***
2000	C	Control	11.1± 2.5	**15.9± 2.3**	15.4± 0.4	nd
	CS	Control	10.8± 1.6	*7.8± 1.7*	14.8± 3.5	nd
	C	KCl	10.8± 3.1	7.8± 0.6*	28.2± 1.5*	nd
	CS	KCl	9.1± 4.4	6.3± 0.3	*11.4± 2.4*	nd
	C	Mulching	19.9± 5.9*	**9.6± 1.2***	15.1± 0.9	nd
	CS	Mulching	*3.6± 0.8**	7.0± 0.6	10.4± 2.6	nd

1 CS = intercropped cassava with sorghum, C= monocropped cassava

2 Original means with original standard errors, in bold: significant comparison between normal and late planting dates at $p \leq 0.05$, with star: significant comparison between (fertilizer, mulching) treatments and control for each cropping system at $p \leq 0.05$, and in italic: significant comparison between cropping system for each treatment at $p \leq 0.05$

nd: Not determined due to crop failure under unfavourable climatic conditions

5.4 Discussion

A combination of cultural and agronomic measures such as intercropping cassava with sorghum or cowpea versus cassava monoculture, potassium fertilizer application and mulching, and shift of planting date were evaluated for their effect in reduction of CBB in two sites (ecozones: forest-savanna transition, dry savanna). Generally, shifting planting to a later date and intercropping cassava with sorghum had a suppressive effect on CBB, while KCl fertilizers and mulching had no effect.

A disease reduction was observed at the **late planting** time - in the last third of the rainy season -, compared to normal planting in the susceptible genotype BEN86052 in 13 of 15 treatments in monocropping in both sites (ecozones) and years. Late planting also reduced disease development in the resistant genotype in 8 of 12 treatments, but the effect was less pronounced than in genotype BEN86052, also due to a generally lower disease level in the resistant genotype. During the late planting period compared to the normal planting, weather conditions such as temperature, relative humidity and rainfall were not favourable for disease development (Annex). A disease reduction of cassava mosaic disease, cassava bacterial blight and cassava leaf spot at late planting was also reported in Cameroon (Ambe, 1993).

No significant effects of **potassium and mulching treatments** on disease development were observed, except in 5 of 38 and 2 of 24 treatments, respectively. Thus, these results can generally not confirm the reports on the disease reducing effect of potassium, found in a greenhouse trial (Arene, 1977; Arene and Odurukwe, 1979). In those studies, NPK fertilizer, among them potassium as most important element with application of 90 and 180 $kg \cdot ha^{-1}$ of K_2O reduced CBB incidence and severity. The effect of mulching on CBB was never studied before, but in others crops, positive and negative effects on diseases were observed. In bean cultivation, mulch of selected weeds prevented soil splashing, which was the most important source of inoculum causing web blight of beans (*Thanatephorus cucumeris*, anamorph *Rhizoctonia solani*) (Galindo et al., 1983). Muimba-Kankolongo et al. (1989) found that mulches reduced the incidence of a cassava stem tip dieback of unknown etiology in Zaire. On the contrary, incidence and severity of *Stenocarpella* leaf spot of maize, caused by *S. macrospora*, was increased by soil treatments that included mulching, compared to those including the removal of residues (Mora and Moreno, 1984). Also, mulching with the grass *Panicum maximum,* keeping rice plants green for a longer period, increased fungal attacks (no fungus was specified) in the Peruvian Amazon (Bandy and Sanchez, 1986).

Generally, intercropping with sorghum and cowpea reduced disease development compared to cassava monocropping. **Intercropping cassava-sorghum** reduced CBB development significantly in 14 of 22 treatments, among them intercropping treatments in the forest-savanna transition zone at normal planting time in both years and in all intercropped plots in both ecozones in year 2000. Cassava-cowpea also reduced the disease severity. Sorghum plants always dominated simultaneously-planted cassava plants, acting as physical barriers which reduce rain splash and the total rain received by cassava. Under these conditions, the bacterial multiplication and movement as well as the inoculum concentration could be reduced contributing to a reduced infection rate. Besides the barrier effect suppressing pathogen spread, intercropping reduces weeds, which may serve as an inoculum source, since weeds in cassava fields were shown to harbour epiphytic *Xam* for at least 30 days under field conditions (Fanou, 1999). The low rainfall in Ina (dry savanna) in year 2000 was generally not favourable to disease spread. The suppressive effect of intercropping on CBB was also reported in cassava intercropped with maize in the forest-savanna transition (Fanou, 1999) and in dry savanna zones in Nigeria (Tabot, 1995), but never in cassava-sorghum intercropped in combination with different treatments. Variability in the effect between years could be due to differences in climatic conditions. Additionally, competition between cassava and sorghum for nutrients could explain variations. Intercropping cassava with maize, melons, or others crops significantly decreased the severity of cassava bacterial blight in Nigeria, probably by reducing soil splashing by rain (Ene, 1977). Intercropping bean with maize or sorghum was reported to delay bean common bacterial blight epidemics in Ethiopia (Fininsa and Yuen, 2001). But, intercropping does not always have a disease and or pest reducing effect. Thus, Sikirou (1999) did not observe a clear effect on cowpea bacterial blight when cowpea was intercropped with maize or cassava in the forest-savanna transition zone in West Africa, and a bean-cassava association showed no effect on scab, rust and *Cercospora* leaf spots of cassava (Moreno, 1979). By intercropping tomato with cowpea, soybean or Welsh onion to control tomato bacterial wilt caused by *Ralstonia solanacearum* only a slight or no reduction of the disease was recorded (Michel et al., 1997). Cassava-maize intercropping increased the severity of powdery mildew (Moreno, 1979), and anthracnose and web blight (*Thanatephorus cucumeris*) was increased in maize-bean intercrops (Wolley and Davis, 1991).

A significant effect of planting time on root yield was generally not observed except a reduction and an increase in 9 and 3 of 36 treatments, respectively. The reduced root yield in Ina at the late planting time could be due to competition between cassava and sorghum for

nutrients during the short growing season in the dry savanna. Nembozanga Sauti (1984) obtained in Malawi the highest yield at normal planting and the lowest at late planting in Baka, while no significant difference in yield with different planting time was found in Bvumbwe. Ambe (1993) recorded lower yields at late planting time resulting from reduced soil water capacity and slow plant establishment. In contrast, some authors reported that late planting resulted in highest dry matter yield (Okigbo, 1971; Ezedinma et al., 1981).

Potassium (KCl 80 or 120 kg·ha^{-1}) treatment increased yield in 7 and decreased yield in 2 of 38 treatments. Potassium (K_2O) fertilizer at the rates 90 and 180 kg·ha^{-1} increased significantly root yield in a greenhouse trial (Arene and Odurukwe, 1979). But, these authors did not investigate a potassium effect in the field at different planting times and under variable environmental influences. The chloride ion of KCl increased plant growth and carbohydrate accumulation in roots under favourable climatic conditions (Ngongi et al., 1976). The reported positive effect of potassium on root yield, could generally not be confirmed by our studies and seems to depend highly on environment and genotype. Also, Wydra (2002), reported variability in root yield explained by genotype x environment interaction in five ecozones of West Africa. Mulching treatment had generally no significant effect on root yield except a reduction and an increase in 2 and 5 of 24 treatments, respectively. Mulching generally favours nutrient release, weed suppression, root development and soil moisture. No effect of mulching on yield was also observed on maize in Southern Benin (Maliki et al., 1997), on rice, soybean, peanut, and cowpea in Peru (Bandy and Sanchez, 1986), while a yield increase was recorded on maize (Okigbo and Lal, 1982; Bandy and Sanchez, 1986; Mugendi et al., 1997) and on taro (Miyasaka et al., 2001).

Cassava-sorghum intercropping reduced root yield compared to cassava monocropping in 4 of 12 treatments in Cotonou and 6 of 10 treatments in Ina. Cassava-cowpea intercropping reduced significantly root yield by 52% at the normal planting time in Ina (dry savanna). Thus, yield reductions occurred mostly in the dry savanna site. Intercropping leads to competition for water, nutrients, and sunlight and may thus reduce yield. But, the additional yield of sorghum or cowpea should be taken into account. Thus, cowpea-cassava in alternate rows showed the highest benefit of land equivalent ratio (LER>1) compared to monoculture (Sikirou, 1999). Also, Fanou (1999) found that growth parameters and root yield of cassava were generally not significantly different comparing cropping systems, and no significant effect on cassava root yield occurred when intercropping normal or late maturing maize

varieties with cassava in South Nigeria (Ezumah et al., 1988). Nevertheless, yield reductions were reported, e.g. a reduction in dry matter yield of cassava in intercropping (Okoli, 1996), yield reductions from 15 to 35% and 10 to 40%, when cassava was intercropped with cowpea and groundnut, respectively (CIAT, 1979), and lower root yield in cassava intercropped with groundnut, cowpea and melon (Zuofa et al., 1992). In contrast, Tijani and Akinnifesi (1997) stated that tuberous root yield of cassava was improved by intercropping of soybean irrespective of the stage of development.

Considering disease severity and root yield, bacterial blight was reduced in 24 of 36 treatments by late planting, generally without effect on root yield. Since a non-infected control is not achievable under field conditions, the effect of CBB on root yield can hardly be determined. Yield reductions of up to 76% comparing two infection levels were observed (Fanou, 1999, Wydra, 2002, Zinsou, 2002). Nevertheless, the reduction in CBB development may have increased yield, so that an expected reduction due to late planting occurred only in 9 of 36 treatments. Also, a compensation capacity of the cassava plant such as BEN86052, considered as a tolerant variety due to its ability to produce high root yields in spite of high disease severity under favourable growth conditions, influences the relation between disease severity and root yield (Wydra, 2002). Additionally, a disease reduction on cassava leaves at the late planting increases number of healthy cassava leaves in areas where young leaves of cassava are consumed as vegetable.

Mulching and KCl fertilizer treatments reduced disease development in 1 of 24 and 6 of 38 treatments, respectively, but only rarely increased root yield under certain environmental conditions (planting time, ecozone), probably due to climatic conditions favourable to growth. Thus, both mulching and KCl fertilizers treatments could not be recommended to farmers as means to reduce CBB and to increase root yield.

Intercropping cassava with sorghum reduced CBB at both planting dates in both sites (ecozones) in 14 of 22 treatments, but reduced significantly root yield in 10 of 22 treatments. Late planting reduced disease development of bacterial blight, but occasionally climatic conditions may lead to poor growth of plants, while soil amendments had generally no effect on CBB. Thus, late planting date in the forest-savanna transition zone and intercropping in all ecozones could be recommended to farmers to reduce CBB development. Additionally, intercropping will yield another harvest from the additional crops.

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6. Conclusions

To select cassava genotypes suitable in an integrated control program for cassava bacterial blight (CBB) and to better understand genotype-pathogen and genotype-pathogen-environment interactions, genotypes from Benin were tested for their reaction to CBB in different ecozones. Putative preformed resistance mechanisms such as the distribution of stomata and cassava leaf waxes were characterized in order to possibly identify quick screening methods for resistance. Selection of resistant genotypes can be enhanced by marker-assisted selection. Therefore, the reaction of 111 genotypes of a F_1 backcross of the genome mapping population to CBB as well as the existance of *Xam* pathotypes, and specific genetic markers and quantitative trait loci involved in resistance to pathotypes were investigated. As further element of integrated control of CBB, agronomic and cultural measures, single and in combination, were studied for their effect on CBB.

For selection of genotypes from Benin, including advanced breeding lines from the International Institute of Tropical Agriculture, Nigeria, with resistance to CBB, thirty-seven cassava genotypes, of which 16 were repeated in all environments, were evaluated for their reaction to CBB and yield in three sites located in the forest-savanna transition, wet savanna and dry savanna zones in years 1998 and 2000 under natural infection and in an artificially inoculated treatment. Most genotypes showed a susceptible and in some environments a medium resistant reaction to bacterial blight, while resistance was rarely observed. Disease development expressed as area under severity index progress curve was generally higher in inoculated treatments than in naturally infected ones. Genotypes evaluated as resistant under low inoculum pressure were identified as susceptible under high, artificial inoculum pressure. Therefore, screening for disease resistance should be conducted only under high disease pressure. Since a homogeneous, high infection in repeated trials over two or more years is hardly to be achieved under natural conditions, genotypes should be artificially inoculated for evaluation of their resistance.

Among the more resistant genotypes across environments, only genotype TMS30572 and RB89509 belonged to the high yielding group. But, regarding reaction in specific environments, genotype RB89509 was susceptible in 3 of 6 environments and can therefore not be recommended to farmers. The dry savanna zone site with natural infection (in year 1998), in which yield was high and symptom severity was low across genotypes, would be

most suitable for production of cassava, while the artificially inoculated site in the wet savanna zone (in year 2000) with high yield and high symptom severity across genotypes would be most suitable for screening for resistance. The artificially inoculated site in the wet savanna zone (in year 1998) with higher symptom severity and lower yield was most unstable for cassava production and, therefore, good for selecting genotypes, which still perform well under harsh conditions. Disease development expressed as area under severity index progress curve was negatively correlated to root yield **only** in the non-inoculated treatment in the dry savanna zone in year 2000, indicating that the disease may cause unpredictable losses under not clearly identifiable conditions.

In conclusion, difficulties to recommend suitable genotypes to farmers reside in high genotype x environment interactions for cassava bacterial blight and root yield. Considering disease reaction and root yield across environments, only genotype TMS30572 among the thirty-seven genotypes tested (sixteen repeated in two years and 12 environments, and twenty-one genotypes repeated in at least one year and 6 environments) was stable in different environments - medium resistant to resistant and high-yielding - and could be recommended to farmers. This genotype with a resistant reaction in the dry savanna in both years seemed to be specifically suitable to this ecozone. Nevertheless, for confirmation and further selection of resistant, high-yielding genotypes, continuous and intensive evaluation in repeated years in several locations per ecozone, and inoculation with different pathotypes under controlled conditions should be performed. Genotypes should be evaluated for their resistance in various environments which favour stable, high symptom severity [e.g. the artificially inoculated site in the forest-savanna zone (in year 1998), the artificially inoculated site in the wet savanna zone (in year 2000)], including those with most unsuitable conditions for high yield in combination with high infection pressure [e.g. the artificially inoculated site in the wet savanna zone (in year 1998)]. For production of cassava stems for propagation, the site in the wet savanna zone with natural infection (in year 1998) would be most suitable, while the site in the dry savanna zone with natural infection (in year 1998) was the best environment for cassava production.

Preformed defense mechanisms contribute to the plant's strategy for resistance to microorganisms. But, significant differences in stomatal distribution were not found on the abaxial nor on the adaxial surface comparing four susceptible and resistant genotypes of cassava. Only a slight trend towards higher numbers of stomata between veins on the abaxial

surface and a tendency of higher stomate numbers at the proximal end of the adaxial surface was observed in the susceptible genotype. Thus, differences in the number of stomata and their distribution might contribute to the significantly lower incidence of spots observed in the resistant genotype TMS30572 compared to the susceptible genotype BEN86052 under low natural inoculum pressure in field trials (unpublished data). Additionally, leaf waxes which may occlude stomata and, thus, eventually prevent bacterial entry were characterized by quantitative and qualitative analysis of abaxial leaf surface waxes isolated from resistant, medium resistant and susceptible cassava genotypes, and by scanning electron microscopy (SEM) of abaxial and adaxial surfaces of a resistant and a susceptible genotype. However, no clear differences in wax quantities, specifically in triterpenes forming the most abundant wax fraction, were observed between susceptible, medium resistant and resistant genotypes in the forest-savanna transition, wet savanna and dry savanna zones. Thus, other resistance mechanisms must be involved in pathogen defence. SEM revealed, that waxes covered stomata on the abaxial leaf surfaces of both a susceptible and a resistant genotype, while the adaxial surfaces were not covered by wax, and wax was in crystalloid form.

In conclusion, lower stomata numbers and high wax quantities may be involved in reducing the number of bacteria invading leaves, but variations in wax quantities and the number of stomata in the tested genotypes were not or only tendentiously related to the described resistances and, thus, differences observed in spot symptoms comparing susceptible and resistant genotypes are thought be related to further defence mechanisms.

Host x pathogen interactions among a large number of genotypes of a cassava mapping population and four strains of *Xanthomonas axonopodis* pv. *manihotis* were studied using stem puncture and leaf infiltration methods. Although not all the 111 genotypes of the mapping population were compared by both, stem and leaf infiltration, the results revealed obvious differences between genotypes and strains after inoculation with each of the two methods. Thus, both stem and leaf inoculations are necessary to identify sources of resistance to bacterial blight. Different strain x genotype interactions occurred with both methods. The four strains of *X. axonopodis* pv. *manihotis* belong to different pathotypes according to their reaction on leaves and stems, e.g. strain x genotype interactions on stem and leaf level were observed. Among hundred-eleven genotypes derived from the backcross of 5 F_1 individuals and the female parent TMS30572, 19 differential genotypes for pathotype identification were found by stem inoculation with *X. axonopodis* pv. *manihotis* and 3 differential genotypes by

leaf inoculation. Eleven specific genetic markers associated to resistance to these pathotypes, among them six quantitative trait loci (QTLs), were found. Our data revealed strain-specific resistance with corresponding QTLs and also support the new concept of combination of two forms of resistance, multigenic and monogenic resistance (Heath, 2000). Regions of the cassava genome involved in resistance to *Xam* differ according to the strain inoculated indicating that specific resistance factors may be involved with respect to the different *Xam* strains. The QTL results also confirm that the 4 strains belong to 4 different pathotypes.

In conclusion, stem and leaf-inoculation with a representative set of *Xam* pathotypes under glasshouse conditions to identify genotype x pathogen interactions are recommended to breeders to select resistant genotypes. Regions of the cassava genome involved in resistance to *Xam* differed according to the inoculated pathotype, and new markers specific to African strains were identified. The existance of pathotype-specific genes or groups of genes (vertical resistance) suggest that the polygenic resistance of cassava may be based on a continuum of monogenic resistances. The newly identified markers for CBB resistance can be used to increase the efficiency of selecting resistant genotypes in Africa by selecting particular alleles from the linkage groups.

A combination of cultural and agronomic measures such as intercropping cassava with sorghum or cowpea versus cassava monoculture, potassium fertilizer application and mulching, and shift of planting date were evaluated for their effect in reduction of CBB in two sites (ecozones: forest-savanna transition, dry savanna). Generally, shifting planting to a later date - the last third of the rainy season - and intercropping cassava with sorghum had a suppressive effect on CBB, while KCl fertilizers and mulching had no effect. Considering disease severity and root yield, bacterial blight was reduced in 24 of 36 treatments by late planting generally without effect on root yield. Since a non-infected control is not achievable under field conditions, the effect of CBB on root yield can hardly be determined. Yield reductions of up to 76% comparing two infection levels were observed (Fanou, 1999; Wydra, 2002; Zinsou, 2002). Nevertheless, the reduction in CBB development may have increased yield, so that an expected reduction due to late planting occurred only in 9 of 36 treatments. Also, a compensation capacity of the cassava plant such as BEN86052, considered as a tolerant variety due to its ability to produce high root yields in spite of high disease severity under favourable growth conditions, influences the relation between disease severity and root yield (Wydra, 2002). Additionally, a disease reduction on cassava leaves at the late planting

increases the number of healthy cassava leaves in areas where young leaves of cassava are consumed as vegetable. Mulching and KCl fertilizer treatments reduced disease development in 1 of 24 and 6 of 38 treatments, respectively, but only rarely increased root yield under certain environmental conditions (planting time, ecozone), probably due to climatic conditions favourable to growth. Thus, both mulching and KCl fertilizers treatments could not be recommended to farmers as means to reduce CBB and to increase root yield. Intercropping cassava with sorghum reduced CBB at both planting dates in both sites (ecozones) in 14 of 22 treatments, but reduced significantly root yield in 10 of 22 treatments. Late planting reduced disease development of bacterial blight, but occasionally climatic conditions may lead to poor growth of plants in the dry savanna zone, while soil amendments had generally no effect on CBB.

In conclusion, a late planting date in the forest-savanna transition zone and intercropping could be recommended to farmers to reduce CBB development. Additionally, intercropping will yield another harvest from the additional crops.

Recommendations

Only genotype TMS30572 was stable in different environments - medium resistant to resistant and high-yielding - and could be recommended to farmers. Additionally, late planting in the forest-savanna transition zone, and intercropping in all ecozones, using the resistant genotype TMS30572, could further reduce CBB development. For breeding for resistance, stem and leaf-inoculation with a representative set of *Xam* pathotypes under glasshouse conditions to identify genotype x pathogen interactions, and use of newly identified markers (QTL) for CBB resistance are recommended to breeders to select resistant genotypes. Environments suitable for cassava production, production of planting material or for selection for resistance were identified.

Annex: Rainfall (mm) and temperature (°C) in Cotonou (forest-savanna transition zone), Save (wet savanna zone) and Ina (dry savanna zone) from year 1998 to 2001

Rainfall (mm)

	Cotonou				Save				Ina			
Month	1998	1999	2000	2001	1998	1999	2000	2001	1998	1999	2000	2001
Jan.	4.1	28.1	0	0	37.8	0		0	2.2	0		4.5
Feb.	1.6	138.7	0	0	8.2	34		10.8	2.8	32.5		0
Mar.	25.4	42.1	43.2	27.9	5.1	99.7		25.3	4	7.5		6.6
Apr.	27.4	169	86.2	158.9	158.5	190			64.7	28.5		
May	192.6	109.9	192.9	58.5	104.9	89.1			147.3	37.2		
June	250.1	217.8	65.7		95.6	137.7	108.7		273.8	130.8	149.9	
July	18	424	51.3		61.6	402.8	147.1		224.3	228.7	282.1	
Aug.	0	56.3	103		123.8	237.2	111.4		299.3	350	352.4	
Sep.	55.4	56.4	119.9		141.1	102.3	200.2		106.6	0	249.2	
Oct.	74.7	157.3	57.3		132.4	149.6	148.5		126.1	0	95.9	
Nov.	124.3	23.7	32.9		14	4.6	0		0	0	0	
Dec.	1.4	2	0		2.6	0	0		0	0	0	

Temperature (°C)

	Cotonou				Save				Ina			
Month	1998	1999	2000	2001	1998	1999	2000	2001	1998	1999	2000	2001
Jan.	24.9	26.8	27.4	26.8	29	28.9		28.6	25	24.4		23.4
Feb.	28.5	27.1	25.9	28.5	30.6	29.7		30.1	30.8	25.8		28.9
Mar.	29.2	28	28.9	29.3	32.4	28.9		31	29.3	30.5		
Apr.	28.9	27.1	28.1	27.9	30.4	28.8			31.1	29.2		
May	27.5	26.6	27.7	27.9	28.6	28.4			28.7	28.3		
June	26.2	25.6	25.9		27.3	27	27.4		26.3	26.5	26.4	
July	25.1	24.8	25.2		26.1	25.7	25.8		25.7	24	25	
Aug.	24.5	24.9	24.9		25.3	25.6	25.1		24.3	24.5	24.4	
Sep.	25.5	25.4	25.8		26.1	25.8	26.1		25.8		25.4	
Oct.	25.8	28.6	26.6		27.1	26.5	27.1		26.1		26.7	
Nov.	27.2	26.6	27.4		29.1	28.5	29		27.3		25.2	
Dec.	26.6	26.9	27.3		28.6	28.5	28.3		27.3		23.6	

Curriculum vitae

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Eidesstattliche Erklärung

Hiermit versichere ich an Eides statt, das ich die vorliegende Dissertation eigenständig verfasst habe und sie weder an der Universität Hannover noch an anderen Universitäten als Diplomarbeit oder andere Prüfungsarbeit verwendet oder eingereichet habe. Die zur Erstellung der Arbeit benutzen Hilfen oder Hilfsmittel habe ich vollständig aufgeführt.

Hannover, den 29. November 2002

(Amégnikin Valerien Zinsou)