“By crawling, a child learns to stand”

~ African Proverbs

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Genetic diversity and blast resistance in African cultivated rice (*Oryza glaberrima* Steud.)

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Summary

Blast (*Magnaporthe oryzae,* family: *Magnaporthaceae*, Couch) is a very important rice disease. It is the most widespread one causing significant economic losses worldwide. Outbreaks of this disease are often very destructive, especially in intensive rice cultivation systems or when new locally susceptible varieties are planted. The spread of damage caused by rice blast has been widely reported in Africa including Benin where blast threats could become more important in the future, because of the popularity of certain susceptible cultivars and the spontaneous development of recently documented new pathogenic races. Blast disease management involves several methods among which the use of genetic resistance has been, and will continue to be, the most sustainable one. There is evidence that the West African indigenous rice species, *Oryza glaberrima*, would be a potential source of resistance genes to various diseases including blast. However, this potential as a resistance gene donor has not been yet fully exploited in breeding programs like that of the Asian rice, *O. sativa*. In the past, breeders have paid more attention to the high-yielding character of *O. sativa* for improving rice varieties. We, therefore, initiated characterization studies of selected *O. glaberrima* accessions for exploring their genetic diversity, blast resistance, and yield performance. It is expected the obtained results to be used in forthcoming rice breeding programs, especially those including *O. glaberrima*.

Field experiments aiming at identifying blast-resistant germplasm were performed in Benin on a collection of 345 *O. glaberrima* and 5 *O. sativa* rice accessions. Two screening trials were conducted during the two subsequent rainy seasons, from April to July and from August to October in 2015. Similar blast resistance patterns were found during both experiments. We also observed a wide range of rice responses to blast attacks among the germplasm studied. Several rice accessions were found resistant; 12% were shown to be highly resistant, whereas 24% were moderately resistant. Genetic variation in our germplasm collection assessed with 77 AFLP (amplified fragment length polymorphism) markers revealed that some of them were significantly correlated with blast resistance (R = 0.61). In addition, different AFLP marker

combinations were tested to better examine the link between genetic diversity in the germplasm with blast resistance. Stepwise regression analysis showed that 13 out of 77 AFLPs were the better indicators of disease resistance. The study further found that 90% of rice accessions differed from each other for genetic diversity depending on their affection by blast disease. In highly resistant rice accessions, marker CTA22- 366 (with a size of 366 base pairs) was particularly present which opens the perspective for marker-assisted selection of blast-resistant accessions. Finally, a subset of 37 *O. glaberrima* and 5 *O. sativa* rice accessions (representing 12% of total rice accessions tested) was selected to represent variation of the initial germplasm collection and this selection was based on the geographical origin, pairwise genetic distances (revealed by 77 AFLP markers aiming at maximized distances) and differential reactions to blast disease.

To better understand population structure and genetic diversity of the rice accessions tested, the latter rice subset mentioned above was examined using a panel of 20 SSR (simple sequence repeat), 77 AFLP markers and 17 morphological traits. The polymorphic information content of the SSR markers ranged from 0.58 to 0.91 with an average of 0.78 indicating that they are highly informative. Germplasm structure analysis by using SSR data also revealed the presence of three different populations that correspond to the three genetic groups identified by phylogenetic analysis without any correlation to geography. Results of AMOVA (Analysis of MOlecular VAriance) indicated extensive between-individuals variation (80%) which shows that a high genetic differentiation exists between our rice accessions that belong to three populations. However, no significant alleles differences (Fst = 0.001, P = 0.48) were found between five *O. glaberrima* rice and all *O. sativa* rice accessions (population 1) at 18 loci out of 20 SSR markers. This information thus reveals an intense gene flow has occurred between *O. glaberrima* and *O. sativa*, possibly resulting from the close coevolution of both rice species in West Africa. We also observed important morphological similarities between those accessions and *O. sativa*, especially for the ligule shape, panicle form, an abundance of secondary branching, which corroborates this gene flow. Gene flow in these rice accessions

is a source of genetic variability that could be used in breeding programs to generate new rice varieties adapted to West Africa's environmental conditions. Mantel test analysis based on AFLPs found a strong correlation (R = 0.72) between genetic distance and geographical origin. However, the amount of genetic diversity revealed by AFLPs (PIC = 0.35) was relatively low, so that no population structure was found among all of the rice accessions in our subset when AFLP markers were used.

This subset of 42 rice accessions was also evaluated for 15 agronomic traits in order to examine opportunities for combining high-yield performance and blast resistance. This study was carried out under both irrigated upland and lowland conditions in Benin using these 42 rice accessions along with seven upland (ARICA 4, ARICA 5, CG 14, NERICA 1, NERICA 2, NERICA 4 (*O. sativa* x *O. glaberrima*) and Moroberekan (*O. sativa*)) and seven lowland varieties (ARICA 1, ARICA 2, ARICA 3, NERICAL 14, NERICAL 19 (*O. sativa* x *O. glaberrima*), TOG 5681 (*O. glaberrima*) and IR 841, (*O. sativa*)), which were included as varieties of reference for scientific studies on rice breeding in Africa. Results showed there were significant differences between lowland and upland trials for seven agronomic traits (percentage of fertile tillers, the total number of spikelets, spikelet fertility, panicle secondary branching, days to 80% heading, days to 80% maturity and grain yield). In upland growing conditions, three highly-blast resistant *O. glaberrima* accessions (WAB0002143, WAB0029182, and WAB0029194) out-yielded all the seven reference varieties used with yields of 540, 573, and 603 g/m^2, respectively. In irrigated lowland conditions, *O. glaberrima* rice accessions, WAB0019882, WAB0008956, and WAB0015043 showed superior levels of grain yield (567.78, 698.90 and 600.05 g/m^2, respectively) compared to the seven lowland reference varieties used. Also, three *O. glaberrima* rice accessions (the highly blast-resistant WAB0008956 and WAB0015043, and the highly susceptible WAB0029342) were found to have better agronomic performance in lowland conditions than all *O. sativa* rice accessions that originated from Benin. The earliest maturing accession (less than 108 days) was the highly blast-susceptible *O. glaberrima* rice accession WAB0030263. Resistant accessions in the first field experiments

tend to produce a relatively higher number of secondary panicle branching compared to susceptible ones in both lowland (R = 0.52) and upland (R = 0.44) growing conditions. Our study thus reveals a set of blast-resistant *O. glaberrima* rice accessions that have good yield potential and which Benin national research institutes can use directly without any necessary further improvement before implementing them for cultivation at farmer level. However, whether consumers will accept their grain quality needs to be further researched.

Blast resistance was initially identified in several rice accessions on-station at AfricaRice in Cotonou. However, it was unclear whether resistance would be efficient or not throughout Benin as rice accessions were only exposed to blast pathotypes locally present on AfricaRice research station. It would have been difficult and costly to screen all 350 rice accessions for strong resistance to blast. Therefore, some accessions of the rice subset of 42 rice accessions were screened under controlled conditions with selected isolates for the putative source of strong resistance to blast disease. We used a set of 9 Beninese isolates that represents the African blast pathotypic diversity to screen 21 selected rice accessions (18 *O. glaberrima* and 3 *O. sativa* rice accessions) that were resistant in the field and three *O. glaberrima* derived F2 populations. The majority (66.67%) of these rice accessions were shown to be resistant against at least five isolates. The potential for strong resistance was confirmed in six *O. glaberrima* accessions (WAB0029182, WAB0029194, WAB0032298, WAB0032497, WAB0015703, WAB0002143, and WAB0008956) that resisted all nine isolates. These resistant accessions would be appropriate to grow rice especially in environments where blast attacks are severe; they also have a lot of agronomically important traits and suit Benin environments. Chi-square analyses showed that the strong resistance to blast pathotypes of Benin is most likely controlled by a single or several linked genes (exhibiting a simple Mendelian pattern of inheritance). Genetic analyses also revealed that the strong blast resistance was not governed by Pi2 or Pi9 R gene. Because blast isolates used to cover most known blast resistance genes (R genes), these accessions might harbor new or a combination of known R genes that have potential for rice blast resistance breeding. Moreover, in our study, drought (DTY3.1) and flood

tolerance genes were identified in *O. glaberrima* rice accessions WAB0030263 and WAB0032298, respectively. The identification of genes that increase *O. glaberrima* rice blast resistance is urgently required. The identification of candidate DNA markers linked to these newly identified resistance genes will allow an effective introgression of these R genes into currently farmer-adopted blast-susceptible rice varieties through marker-assisted selection (MAS). They could be combined with genes for high yield, flood, and drought tolerance to develop more productive and blast-resistant varieties that are resilient to environments including those of Benin.

Finally, this Ph.D. research provided novel insights into the genetic diversity and potential of *O. glaberrima* for improving rice varieties which will help a better promotion of *O. glaberrima* cultivars in the farmers and consumers' communities. Wider use of these cultivars will also sustain their conservation efforts. The results of this study contribute to global and sustainable rice production, especially under environmental stress conditions.

Samenvatting

Pyriculariose (*Magnaporthe oryzae*, familie Magnaporthaceae, Couch) is een wijdverspreide rijstziekte die wereldwijd aan de basis ligt van belangrijke opbrengstverliezen. Uitbraken van pyriculariose zijn vaak heel destructief, zeker in intensieve rijstteeltsystemen of wanneer nieuwe, vatbare variëteiten worden aangeplant. De ziekte komt veelvuldig voor in Afrika, ook in Benin, waar *M. oryzae* in de toekomst wel eens meer schade zou kunnen aanrichten als gevolg van de populariteit van bepaalde vatbare cultivars en het recent gedocumenteerde opduiken van nieuwe pathogene rassen. Verschillende methodes kunnen worden gebruikt om pyriculariose te bestrijden. Genetische resistentie was en zal ook in de toekomst de meest duurzame methode zijn om de ziekte onder controle te houden. Er zijn aanwijzingen dat de inheemse West-Afrikaanse rijstsoort *Oryza glaberrima*, een potentiële bron is van resistentiegenen tegen deze ziekte. Dit potentieel wordt momenteel echter onvoldoende gebruikt in veredelingsprogramma's van bv. de Aziatische rijst, *O. sativa.* In het verleden hebben rijstveredelaars meer aandacht besteed aan opbrengstverhoging dan aan ziekteresistentie. Om die reden hebben wij de genetische diversiteit, de resistentie tegen *M. oryzae*, en het opbrengstpotentieel onderzocht van geselecteerde *O. glaberrima* accessies. De resultaten van deze studie kunnen nuttig zijn in toekomstige rijstveredelingsprogramma's, vooral dan die van *O. glaberrima*.

We verrichtten veldonderzoek op een collectie van 345 *O. glaberrima* and 5 *O. sativa* rijst accessies met oog op de identificatie van ziekteresistent genetisch plantenmateriaal. Er werden twee proeven verricht in twee opeenvolgende regenseizoenen (april tot juli en augustus tot oktober 2015). In beide proeven werden gelijkaardige resistentiepatronen vastgesteld. We vonden een grote resistentievariatie in de onderzochte planten. Twaalf % was uiterst resistent, terwijl 24 % matige resistentie vertoonde. We onderzochten de genetische variatie in de geselecteerde rijstpopulatie aan de hand van 77 AFLP (*amplified fragment length polymorphism*) merkers. Van een aantal merkers kon significantie correlatie (R = 0.61) met resistentie tegen *M. oryzae* worden aangetoond. De link tussen genetische diversiteit en

ziekteresistentie werd verder onderzocht door het testen van verschillende AFLP-merkercombinaties. Door stapsgewijze regressieanalyse konden de 13 beste ziekteresistentiemerkers van de 77 AFLP-merkers worden geïdentificeerd. Er werd verder aangetoond dat 90 % van de rijstaccessies genetisch van elkaar verschilde voor wat betreft hun gevoeligheid voor *M. oryzae*. In de meest resistente accessies was merker CTA22-366 (met een grootte van 366 baseparen) nadrukkelijk aanwezig. Dit opent perspectieven voor het gebruik van deze merker bij de selectie van ziekteresistente accessies. Er werd ook een subset van 37 *O. glaberrima* en 5 *O. sativa* accessies (12 % van de totale onderzochte populatie) geselecteerd, die oorspronkelijke genetische variatie van het plantenmateriaal best weerspiegelt. Daarbij werden de geografische origine, onderlinge genetische afstand (de grootste afstand die kon worden gevonden met 77 AFLP-merkers) en de verschillende resistenties tegen pyriculariose als criteria gebruikt.

Om een beter beeld te krijgen van de populatiestructuur en de genetische diversiteit van de onderzochte rijstaccessies werd de bovenvermelde subset geanalyseerd aan de hand van 20 SSR (*simple sequence repeat*) en 77 AFLP merkers, en 17 morfologische kenmerken. De polymorfe informatie-inhoud (PIC) van de SSR-merkers varieerde van 0.58 tot 0.91 met een gemiddelde van 0.78, wat duidt op een hoog informatief karakter van deze merkers. Analyse van de genetische structuur van de onderzochte rijstaccessies, bracht drie verschillende rijstpopulaties aan het licht die overeen kwamen met de drie genetische groepen die werden geïdentificeerd aan de hand van fylogenetische analyse, maar zonder dat daarbij correlatie kon worden vastgesteld met de geografische oorsprong van de accessies. Moleculaire variantieanalyse (AMOVA) onthulde aanzienlijke variatie (80 %) tussen de verschillende individuen, wat aantoont dat ook binnen de drie genetische groepen er een grote genetische differentiatie variatie bestaat. Er werden op 18 loci van de 20 SSR-merkers geen significante verschillen gevonden in de allelen (Fst = 0.001, P = 0.48) tussen de 5 *O. glaberrima* en alle *O. sativa* accessies (populatie 1), wat wijst op een intense genetische migratie tussen *O. glaberrima* en *O. sativa*, mogelijks als gevolg van de dichte co-evolutie van beide rijstsoorten

in West-Afrika. We vonden ook belangrijke morfologische gelijkenissen tussen deze *O. glaberrima* accessies en *O. sativa*, vooral met betrekking tot de vorm van het tongetje en de pluim, en het uitbundig voorkomen van secondaire vertakkingen, wat de hypothese van genetische migratie ondersteunt. Die genetische migratie is een bron van genetische variabiliteit die kan gebruikt worden in veredelingsprogramma's met het oog op de ontwikkeling van nieuwe rijstvariëteiten die zijn aangepast aan de West-Afrikaanse omgeving. Met behulp van een Manteltestanalyse op basis van de AFLP-merkers, werd een sterke correlatie (R = 0.72) gevonden tussen de genetische afstand en de geografische origine. De genetische diversiteit die daarentegen kon worden aangetoond met de AFLP-merkers was relatief laag (PIC = 0.35). Met deze merkers kon dan ook geen populatiestructuur worden aangetoond in de rijstaccessies uit de bestudeerde subset.

De subset van 42 rijstaccessies werd verder beoordeeld op basis van 15 agronomische kenmerken om de mogelijkheden voor het combineren van hoge opbrengst en ziekteresistentie te onderzoeken. Deze studie werd uitgevoerd onder zowel geïrrigeerde hoogland- als laaglandomstandigheden in Benin, en maakte gebruik van 42 rijstaccessies, zeven hooglandvariëteiten (ARICA 4, ARICA 5, CG 14, NERICA 1, NERICA 2, NERICA 4 (*O. sativa* x *O. glaberrima*) en Moroberekan (*O. sativa*)) en zeven laaglandvariëteiten (ARICA 1, ARICA 2, ARICA 3, NERICAL 14, NERICAL 19 (*O. sativa* x *O. glaberrima*), TOG 5681 (*O. glaberrima*) en IR 841, (*O. sativa*)). De hoogland- en laaglandvariëteiten werden onderzocht als referentievariëteiten voor wetenschappelijk onderzoek op rijstveredeling in Afrika. De resultaten toonden aan dat er voor zeven agronomische kenmerken significante verschillen waren tussen de laagland- en hooglandveldproeven (percentage vruchtbare scheuten, totaal aantal aren, vruchtbaarheid van de aar, secundaire vertakking van de pluim, aantal dagen tot 80% bloeiwijzen waren gevormd, aantal dagen tot 80% van de aren was gerijpt, en opbrengst). In de hooglandomgeving was de opbrengst van de sterk pyriculariose-resistente *O. glaberrima* accessies (WAB0002143, WAB0029182, en WAB0029194) hoger dan elk van de zeven gebruikte referentievariëteiten met opbrengsten van respectievelijk 540, 573 en 603 g/m^3. In

de geïrrigeerde laagland groeicondities resulteerden de *O. glaberrima* rijstaccessies WAB0019882, WAB0008956 en WAB0015043 in een hogere opbrengst (567.78, 698.90 and 600.05 g/m^2, respectievelijk) in vergelijking met de zeven gebruikte laagland-referentievariëteiten.

Ook was de agronomische prestatie van drie *O. glaberrima* rijstaccessies (de sterk ziekteresistente WAB0008956 en WAB0015043, en de accessie WAB0029342, die zeer vatbaar is voor pyriculariose) in laagland condities beter dan die van alle *O. sativa* rijstaccessies afkomstig van Benin. De meest vroegrijpe accessie (minder dan 108 dagen) was de sterk vatbare *O. glaberrima* rijstaccessie WAB0030263. In de eerste veldexperimenten vertoonden de ziekteresistente accessies een relatief hogere secundaire vertakking van de pluim in vergelijking met de vatbare accessies, in zowel laagland (R=0.52) als hoogland (R=0.44) omgeving. Onze studie identificeerde dus een set *O. glaberrima* rijstaccessies, resistent tegen pyriculariose en met een goed opbrengstpotentieel. Deze resultaten kunnen direct door de Beninese nationale onderzoeksinstellingen gebruikt worden om de deze resistente rijst voor de teelt te ontwikkelen. Er moet wel verder onderzocht worden of de consument de graankwaliteit van deze variëteiten zal accepteren. Pyriculariose resistentie werd initieel aangetoond voor verschillende rijstaccessies aan het AfricaRice instituut in Cotonou. Het was echter niet duidelijk of de resistentie ook efficiënt zou blijken in de rest van Benin, daar de rijstaccessies enkel werden blootgesteld aan pyriculariose pathotypes die aanwezig waren aan het onderzoekscentrum van AfricaRice.

Het is moeilijk en duur om alle 350 rijstaccessies te screenen voor sterke resistentie tegen pyriculariose. Daarom werden een aantal rijstaccessies uit de subset van 42 gescreend onder gecontroleerde omstandigheden met isolaten geselecteerd op een mogelijke bron van sterke resistentie tegen pyriculariose. Een set van 9 isolaten uit Benin, die de Afrikaanse diversiteit van het pathotype van *M. oryzae* representeren, werd gebruikt voor de screening van de 21 geselecteerde rijstaccessies (18 *O. glaberrima* en 3 *O. sativa* rijstaccessies) die resistent waren op het veld enerzijds, en drie van *O. glaberrima* afgeleide F2 populaties anderzijds. Het

grootste deel (66.67%) van deze rijstaccessies bleek resistent te zijn tegen ten minste vijf isolaten. Het potentieel voor sterke resistentie werd bevestigd in zes *O. glaberrima* accessies (WAB0029182, WAB0029194, WAB0032298, WAB0032497, WAB0015703, WAB0002143 en WAB0008956) die resistent bleken tegen alle negen isolaten. Deze resistente accessies zouden geschikt zijn voor de rijstteelt in omgevingen met hoge pyriculariose-ziektedruk. Deze accessies hebben bovendien ook een heel aantal agronomisch belangrijke kenmerken die geschikt zijn voor de Beninese omstandigheden. Chi kwadraat analyses toonden aan dat de sterke resistentie tegen pyriculariose pathotypes van Benin het meest waarschijnlijk gecontroleerd wordt door één enkel of verschillende gelinkte genen (met een simpele mendeliaanse overerving).

Genetische analyses toonden ook aan dat de sterke resistentie tegen pyriculariose niet wordt aangestuurd door het Pi2 of Pi9 gen. Omdat de door ons gebruikte *M. oryzae* isolaten de meest gekende resistentiegenen bevatten (R-genen), is het mogelijk dat deze accessies nieuwe R-genen of een combinatie van gekende R-genen bevatten die potentieel hebben in de veredeling tot resistentie tegen pyriculariose. Bovendien werden in onze studie droogtetolerantiegenen (DTY3.1) en genen die aanleiding geven tot tolerantie voor waterzieke gronden geïdentificeerd in respectievelijk *O. glaberrima* rijstaccessies WAB0030263 en WAB0032298. Er moeten dringend genen gevonden worden die pyriculariosesistentie verhogen. De identificatie van kandidaat DNA merkers, gelinkt aan deze nieuw geïdentificeerde resistentiegenen, zal door selectie aan de hand van deze DNA merkers (MAS), een effectieve introgressie van de R-genen in de momenteel gangbare, vatbare rijstvariëteiten mogelijk maken. Deze kunnen gecombineerd worden met de introgressie van genen die zorgen voor een hoge opbrengst, tolerantie voor waterzieke gronden en droogtetolerantie om meer productieve en ziekteresistente variëteiten te ontwikkelen die veerkrachtiger zijn in omgevingsomstandigheden zoals die in Benin.

Dit doctoraatsonderzoek brengt nieuwe inzichten aan in de genetische diversiteit en het potentieel van *O. glaberrima*. Ze helpen om rijstvariëteiten te verbeteren die *O. glaberrima*

zullen helpen promoten bij boeren en consumenten.

Een breder gebruik van deze variëteiten zal de inspanningen voor hun instandhouding ondersteunen.

De resultaten van deze studie dragen bij tot een duurzame rijstproductie in de context van onstabiele omgevingsomstandigheden.

List of tables

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List of abbreviations

AFLP: Amplified Fragment Length Polymorphism

AfricaRice: Africa Rice Center

AMOVA: Analysis of MOlecular VAriance

ANOVA: ANalysis Of Variance

AVR: specific "AViRulence" effectors

BICs: Biotrophy-Interfacial Complexes

CF: Receptor-Like

BLB: Bacterial Leaf Blight

bp: base pair

CIAT: International Center for Tropical Agriculture

DI: Mean Disease Index

DNA: Deoxyribonucleic Acid

ETI: Effector-Triggered Immunity

FAO: Food and Agriculture Organization of the United Nations

FAOSTAT: Food and Agriculture Organization of the United Nations Statistics

Fst: Fixation index

GLM: General Linear Model

He: Expected heterozygosity

Ho: Observed heterozygosity

HR: Hypersensitive Response

I: Shannon's diversity index

InDel: small insertions/deletions

INRAB: Institut National des Recherches Agricoles du Bénin

K: The number of resumed populations

LDA: Linear Discriminant Analysis

LTH: International rice differential blast resistance gene (Lijiangxintuanheigu)

MAEP: *Ministère de L'Agriculture, de l'Elevage et de la Pêche*

MAMPs: Microorganism-Associated Molecular Patterns

MAS: Marker-Assisted Selection

NARS: National Agricultural Research System

NBS-LRR: Nucleotide-Binding Site Leucine-Rich Repeat

NERICA: NEw RIce for Africa

NJ: Neighbor-Joining

PAMPs: pathogen-associated molecular patterns

PCA: Principal Component Analysis

PCoA: Principal Coordinate Analysis

PCR: Polymerase Chain Reaction

PIC: Polymorphic Information Content

PRRs: Pattern Recognition Receptors

PTI: PAMP-Triggered Immunity

PVS: Participatory Variety Selection

QTL: Quantitative Trait Locus

RAPD: Random Amplified Polymorphic DNA

RFLP: Restriction Fragment Length Polymorphisms

RIL: Recombinant Inbred Lines

ROS: Reactive Oxygen Species

RYMV: Rice Yellow Motte Virus

SNP: Single-Nucleotide Polymorphisms

SSA: Sub-Saharan Africa

SSR: Simple Sequence Repeat

UPGMA: Unweighted Pair-Group Mean Arithmetic

UWNJ: UnWeighted Neighbour-Joining

ΔK: Log probability for the rate of change of the data

CHAPTER ONE

1. General introduction

1.1. Context and problem statement

Food and nutrition security is of the utmost importance for achieving sustainable development in Sub-Saharan Africa (SSA). SSA is the only region of the world in which chronic food insecurity and threats of famine remain endemic for most of the population, and the number of malnourished people is steadily increasing (Devereux and Maxwell 2001, Rukuni 2002). The prevalence of undernourishment appears to have risen from 20.8 to 22.7% between 2015 and 2016 (FAO 2017). Approximately 239 million people (23.2%) in SSA (FAO 2018) still suffer from hunger and malnutrition (Van Eeckhout 2010). Improving food security remains a central concern for SSA development and requires a concerted effort on the part of African governments and the international community (Boussard *et al.* 2006). Benin is an SSA country that borders Nigeria in the east and Niger in the north, Togo in the west and Burkina Faso in the northwest. Although Benin is one of the poorest countries of the world, considerable achievements have been made during the period 1999-2016 to reduce the prevalence of undernourishment from 22.6 to 10.3% (FAO 2017). Nevertheless, the Benin population continues to struggle with hunger and food shortages hunger index score in 2017 was 24.4%) (von Grebmer *et al.* 2017; Fry 2018). Despite years of progress, the food and nutrition security situation of Benin is still under threat (FAO 2019). Benin needs to develop strategies to achieve complete food security.

Humanity is dependent on crops for food, feed, biofuel, fiber, and other ecological services. Cereals are the most important staple crops regarding food and nutrition security of the global population (Poutanen 2009). About 50 % of all global calories are derived from cereals such as wheat, rice and maize (Gnanamanickam 2009). Rice is ranked as the third most important cereal crop worldwide in terms of production (770 million tons), with more than 167 million ha of the harvested area each year after maize (1,135 million tons; 197 million ha) and wheat (772 million tons; 219 million ha) (FAOSTAT 2019). Considering rice is a high-value crop for feeding

the world, the 57th session of the United Nations General Assembly decided to declare 2004 as the International Year of Rice reaffirming the need to focus world attention on the role that rice can play in providing food security and eradicating poverty around the world (FAO 2005; Norman and Kebe 2006). Rice is not only known to play a major role in diets, but it has also shaped the cultures and economies of thousands of millions of people (Gnanamanickam 2009). Rice production is indeed an important economic activity and a source of livelihood for millions of rice-farming households and poor rural working in this sector as hired labor forces (Samal *et al.* 2006; FAO 2014).

Over 20 million farmers grow rice in SSA while rice demand is rapidly increasing (Tollens 2006; Nwanze *et al.* 2006). However, rice production in SSA only covers 62% of the actual needs, leading to further food security challenges (Somado *et al.* 2008; Seck *et al.* 2013; OECD 2015). Therefore, SSA is compelled to import rice as production is insufficient, enlarging the trade deficit with risks of food shortages and consequences for its food security due to the disruption of markets. The major rice exporting countries in SSA include India, Thailand, Vietnam and Pakistan (Varma *et al.* 2017; FAO 2018). Many of these Asian countries provide countries in SSA with a variety of commercial rice food products such as Thai rice, Indian rice, Uncle Ben's rice (Nwanze *et al.* 2006). As a consequence, rice price and availability become significant determinants of the welfare of the poorest African consumers, whereas rice trade contributes to the economic growth of these countries from which it is exported to the world market. Olivier de Schutter, the special rapporteur of the United Nations on the right to food, has stated that: 'If most poor countries are still very vulnerable, it is because their food security still depends too much on food imports whose prices are increasingly high and volatile' (Sasson 2012).

World Population Prospect (2019) estimates the total population of Benin at 11,541,297 inhabitants in 2018. Food demand is continuously growing due to rapid population growth (growth rate of 2.75% in 2017) (World Bank 2019). There are up to 205,000 ha of lowlands and 110,000 ha of irrigable dry land that can be exploited to increase agricultural production in the country (MAEP 2011). However, the Food and Agriculture Organization of the United

Nations (FAO) estimates that the actual land area used for rice cultivation is only 82,351 ha (FAOSTAT 2018). Rice is produced throughout Benin with the highest occurrence in the Oueme/Plateau (southeast, bordering Nigeria), Borgou/Alibori (northeast), Zou/Collines (south-central), and Mono/Couffo (southwest, bordering Togo) departments (USDA 2013). In 2016, the country's rice production was estimated at 281,428 tons, whereas the population rice consumption is much higher. Moreover, the rice yield of 3.42 tons/ha in 2016 was still low (FAOSTAT 2018). As a consequence, Benin has to import about 400,000 tons of milled rice each year, corresponding to about 58.70% of actual needs, to compensate this rice deficit. On top of the general food crisis of 2008, the Benin government began to develop policies and programs to boost agricultural production through the intensification of farming systems with particular emphasis on rice sector development (MAEP 2010; Republic of Benin 2011). In 2010, with the support of the African Coalition for Rice Development (CARD), Benin implemented the National Rice Development Strategy (MAEP 2011). However, despite the efforts of the Benin government, food insecurity is still a major problem in this country.

The key cause of food insecurity in SSA is inadequate food production as a result of climate change, depleted soils and poor mechanization, lack of well-adapted varieties, limited access of farmers to agricultural credit, etc. (Sasson 2012). Addressing the problem of food insecurity in SSA will require further concerted efforts from African governments and international organizations to mobilize the necessary resources that aim to increase the current food production levels. Increasing production and satisfying rice demand can be done through either of the following strategies: (1) the production area can be increased, or (2) productivity can be improved on existing farmlands (Pender *et al.* 2004; Sakurai *et al.* 2006; Edgerton 2009). Of both options, increasing productivity on existing agricultural land is preferable as it avoids additional greenhouse gas emissions and the large-scale disruption of existing ecosystems associated with setting new land into agricultural production (Edgerton 2009). Arable land has become scarce, and land degradation is increasing because of reduced fallow periods (de Ridder *et al.* 2004; Franke *et al.* 2008; Giertz *et al.* 2012). Climate change and variability put

extra pressure on the agricultural production that is largely rainfall dependent. In this context, emphasis should be put on strategies leading to sustainable high-yield agriculture for the continuous production of more and better food in SSA.

As Benin rice production is still unacceptably low when compared to the actual needs, yields per production surface area could be better improved to solve our rice deficit (Edgerton 2009). Promoting more productive and sustainable agriculture would help to improve the local rice production level and strengthen food security in the country. Many (a)biotic constraints (e.g., drought, poor soils, pests, and diseases) impair rice yield potential in Benin (Vodouhe *et al.* 1981: Afouda *et al.* 2009; MAEP 2011; Odjo *et al.* 2017). Recent participatory field surveys indicate that the majority of the currently cultivated rice varieties are highly susceptible to blast disease. Rice blast, caused by the fungus *Magnaporthe oryzae* (anamorph *Pyricularia oryzae* Cav.) (Couch and Kohn 2002) is the major devastating biotic constraint to Benin rice production causing 30-100% yield reduction (Vodouhe *et al.* 1981; Afouda *et al.* 2007).

As a consequence, rice blast disease causes significant economic losses and instability in the food supply. In Benin, control of this disease is primarily based on the use of fungicides (Benomyl, edifenphos, and tricyclazole see Sere *et al.* 2013). However, fungicides are expensive, often ineffective under high disease pressure (high inoculum; in most cases, adversity of pathotypes). Fungicides work preventively and show only short-term efficacy resulting in the need for repeated treatments (Ivic 2010; Ricardo 2010). Moreover, fungicides with a bad ecotoxicological profile are often used in SSA, resulting in a threat to human health and the environment (water, groundwater, and air) (Kookana *et al.* 1998; Wightwick and Allinson 2007; Kibria *et al.* 2010; Komarek *et al.* 2010; Nicolopoulou-Stamati *et al.* 2016). The use of resistant genotypes is considered as the best way of managing blast in the field (Jensen 1952; Zhu *et al.* 2004; Roy-Chowdhury *et al.* 2012a).

Strong genetic resistance heavily relies on genetic diversity within a crop. The characterization of genetic diversity from plant genetic resources provides opportunity for plant breeders to develop new and improved cultivars with desirable characteristics, which include both farmer-

preferred traits (yield potential and grain quality, etc.) and breeders preferred traits (pest and disease resistance and photosensitivity, *etc.*) (Govindaraj *et al.* 2015; Reig-Valiente *et al.* 2016). One way to manage stress (blast) in rice production in Benin is the selection of high-performing genotypes with high adaptability to as many environmental conditions as possible (Tao *et al.* 2009). For example, major diseases greatly affected rice yields (blast, Bacterial Leaf Blight or BLB (*Xanthomonas oryzae* pv. *oryzae*), Rice Yellow Mottle Virus "RYMV") in SSA until breeders developed the "NEw RIce for Africa" (NERICA) varieties that tend to have better resistance to most African stress factors including diverse weeds and drought (Rodenburg *et al.* 2006). They have high-yielding potential and generally out-yield local varieties, under both low- and high-input conditions (Jones and Wopereis-Pura 2001).

There are only two species of cultivated rice in the world: *Oryza sativa* (L.) and *O. glaberrima* (Steud.) (Portères 1962; Crawford and Chen 1998; Zhijun 1998; Linares 2002). SSA holds an important potential of rice genetic diversity since Africa is the only continent where these two species are cultivated (Vaughan *et al.* 2003). Rice went through a relatively long domestication process in SSA (Viguier 1939; Portères 1976). Native to SSA, *O. glaberrima* was domesticated in West Africa, whereas *O. sativa* was introduced later from Asia into this region and adopted by farmers living in the Upper Guinea Coast (Crawford and Chen 1998; Zhijun 1998; Linares 2002; Wang *et al.* 2014). This attests to the existence of a high diversity within SSA. Thousands of cultivated African rice accessions (over 20,000) have been collected and are currently conserved in the genebank of AfricaRice, a member of the Consultative Group for International Agricultural Research, or CGIAR (Sie *et al.* 2012). These are a valuable resource for genetic rice improvement to successfully meet future food requirements in Benin and to reduce the country's dependence on Asian imports. Several African rice accessions, particularly those from *O. glaberrima*, exhibit high resistance to pests and diseases, tolerance to various abiotic stressors (drought, iron, acidity, salinity), as well as weed competitiveness (Pham 1992; Jones *et al.* 1997; Futakuchi and Sie 2009; Sie *et al.* 2010; Thiemele *et al.* 2010). NERICA varieties are the result of crosses between *O. glaberrima* and *O sativa*, which combine the desirable

traits of both species (the adaptability of *O. glaberrima* to several stress factors and high yield of *O. sativa*) (Dingkuhn *et al.* 1998; Sie *et al.* 2012). Some NERICA varieties with proven blast resistance have been well promoted by AfricaRice and the National Agricultural Research System (NARS) in Benin for cultivation.

Despite important rice breeding progress, new and more aggressive races of *M. oryzae* are emerging in SSA. This is particularly problematic because it leads to frequent blast outbreaks (Baboy *et al.* 1995; WARDA 1999; Odjo *et al.* 2011). Also, half of the farmers in Benin have recently ceased cultivation of NERICA varieties, especially in the center of the country. The reasons for abandonment were the combined effects of reduced seed demand and low yields, which were attributed to a lack of access to credit and training on NERICA cultivation (Yokouchi *et al.* 2017). They prefer traditional high-yielding varieties, of which many of them are also susceptible to blast disease.

There are still gaps in AfricaRice's interspecific breeding programs as only a limited number of African rice varieties have been characterized and utilized for improving rice productivity (Obilana and Okumu 2005; Mokuwa *et al.* 2013). It is therefore critical to draw more research attention to further rice-breeding programs in Africa. Nowadays, plant breeders are strongly oriented towards creating varieties that are suitable for local environmental conditions and production systems, with respect for preferences of the target communities (Brummer *et al.* 2011; Da Silva Dias 2015). In Benin, however, few studies exist in which both the communities and environments are considered in rice breeding. Therefore, we need to implement appropriately breeding programs with a focus on adaptability aspects to achieve higher rice yields under low-input conditions. In this regard, we started the characterization of a selected African rice germplasm collection for subsequent improvement of national breeding programs for increased rice productivity. Molecular and phenotypic investigations are conducted for efficient exploitation of genetic potentials of this germplasm. The results of this study will contribute in many aspects for breeding for blast-resistant varieties less dependent on fungicides with higher productivity for better food security in Benin.

1.2. Objectives

The overall goal of this study is to characterize the genetic diversity of cultivated rice in Africa to select varieties of high productivity and tolerance to blast for use in subsequent breeding programs. The characterization of this diversity by molecular and conventional field selection will provide breeders with traits of interest for rice improvement, especially blast resistance in Benin. The results of this approach should lead to improved nutrition and livelihoods of people living in Benin and beyond. Four specific objectives were defined:

1. Assess blast-resistance/susceptibility of 350 African cultivated rice accessions and their linkage with genetic structure/AFLP markers to make a selection of germplasm subset representative of total variation available within the whole germplasm collection.
2. Analyze population structure and genetic diversity within this subset by using Simple Sequence Repeat (SSR) and AFLP DNA markers combined with phenotypic rice descriptors;
3. Evaluate yield performance of this subset under both upland and lowland conditions, to identify blast-resistant accessions with high grain productivity.
4. Screen under laboratory conditions accessions of this subset and their Recombinant Inbred Lines (RIL) to select the ones that have strong resistance against *M. oryzae* isolates from Benin.

1.3. Research questions and hypotheses

Four hypotheses are put forward in our attempt to achieve the specified objectives. The first hypothesis to test is that a wide range of phenotypic reactions to blast is found to be correlated with AFLPs genetic variation within the 350 cultivated rice accessions. This hypothesis inspired the formulation of three research questions:

1. How do our rice accessions react to natural blast attacks in the field?
2. What is the most useful combination of AFLP markers for understanding the variability of blast resistance in the field?

3. Which accessions derived from the whole initial germplasm collection can furnish the richest source of genetic variability?

These questions are addressed in chapter 3 entitled "**Exploring genetic diversity and disease response of cultivated rice accessions against *Magnaporthe oryzae* under rainfed upland conditions in Benin".**

The second hypothesis postulates that higher genetic diversity exists in the selected subset of accessions that can provide useful genes for rice genetic improvement. There are three research questions to validate this hypothesis:

1. To what extent and in what ways are the selected rice accessions genetically different from one other?
2. Which population structure better represents the maximum possible genetic variation contained in our rice subset?
3. Is there a clear separation between *O. sativa* and *O. glaberrima* at the germplasm structure level and what can we say about gene flow?

Questions under the second hypothesis are addressed in chapter 4 entitled "**Analysis of population structure and genetic diversity reveals the existence of gene flow and geographic patterns in cultivated rice (*O. sativa* and *O. glaberrima*) in West Africa".**

The third hypothesis argues that many, possibly all blast-resistant accessions of our subset may have better grain yields compared with some currently cultivated varieties in Benin. To have this hypothesis tested, it is necessary to answer three research questions:

1. To what extent the grain performances of our accessions differ from those of varieties currently grown by farmers under lowland and upland conditions of Benin in absence of blast?
2. Which agronomic characteristics most contribute to the accessions' grain yields in both growing conditions?

3. Can accessions with proven blast resistance agronomically perform well? i.e. does high-yield of our accessions occur in combination with blast resistance?

These questions are addressed in chapter 5 entitled "**Combining High Yields and Blast Resistance in Rice (*Oryza* spp.): Results of Screening selected germplasm under Upland and Lowland Conditions in Benin".**

Finally, the fourth hypothesis postulates that it is possible that many accessions possess strong resistance to all blast pathotypes occurring in Benin and most likely have a great potential for blast resistance breeding. Three research questions are considered under this hypothesis:

1. How diverse are the accessions' responses to the Benin *M. oryzae* isolates, which represent part of African blast pathotypic diversity?
2. Does our subset contain accessions that resist all blast pathotypes?
3. What is the mode of inheritance of strongly blast-resistance in recombinant inbred lines?

Questions under this last hypothesis are addressed in chapter 6 entitled "**Screening of selected cultivated rice accessions (*Oryza* spp.) for strong resistance against *Magnaporthe oryzae*, the causal agent of rice blast disease.**

1.4. Outline of the thesis

The present dissertation is divided into seven chapters. Figure 1.1 illustrates the thesis framework.

Chapter 1 provides a general introduction presenting the context and problem statement of this research. Furthermore, this chapter summarizes the objectives, hypotheses and research questions.

Chapter 2 presents an overview of the current knowledge and challenges in rice breeding and cultivation. This chapter reviews the origin and distribution of the different rice species, their genetic diversity, ecological production conditions, and constraints. Then, the importance of

blast disease and management strategies are documented. Finally, knowledge of conventional and molecular blast resistance breeding strategies is discussed.

In Chapter 3, we present and discuss the results of our varietal screening for field disease resistance conducted in the field for 350 African rice accessions. This study also examined genetic variation within our germplasm collection by using fluorescent polymorphic AFLP markers. The possible linkage between the latter markers and accessions' reactions to the disease in the field was discussed. A number of the studied accessions were designed as a subset to represent the maximum diversity contained in the initial germplasm based on geographical origins, pairwise genetic distance (revealed by AFLPs) and accessions' different responses to blast disease.

Chapter 4 presents our studies on population structure and genetic differentiation of the accessions that belong to our new germplasm subset by using SSR and AFLP DNA markers. We assessed the extent of genetic diversity and its link with accessions' geographical origin. Finally, we examined genetic structuring and possible gene flow between *O. sativa* and *O. glaberrima*.

Chapter 5 evaluates the yield of our accessions under both upland and lowland conditions. The accessions' performance and their possible adaptability to the Beninese environment were assessed. The main goal of this study was to identify accessions with proven blast resistance that have also high-yield potential.

Chapter 6 identifies strongly blast-resistant genotypes that may be suitable for Benin. This study screen accessions for strong blast resistance by using *M. oryzae* isolates collected in Benin that take account of African blast pathotypic diversity.

In chapter 7, implications of the outcomes of this study for improved rice production, in particular in Benin are highlighted and discussed. We also presented research perspectives to use these accessions in future breeding programs on the basis of our results.

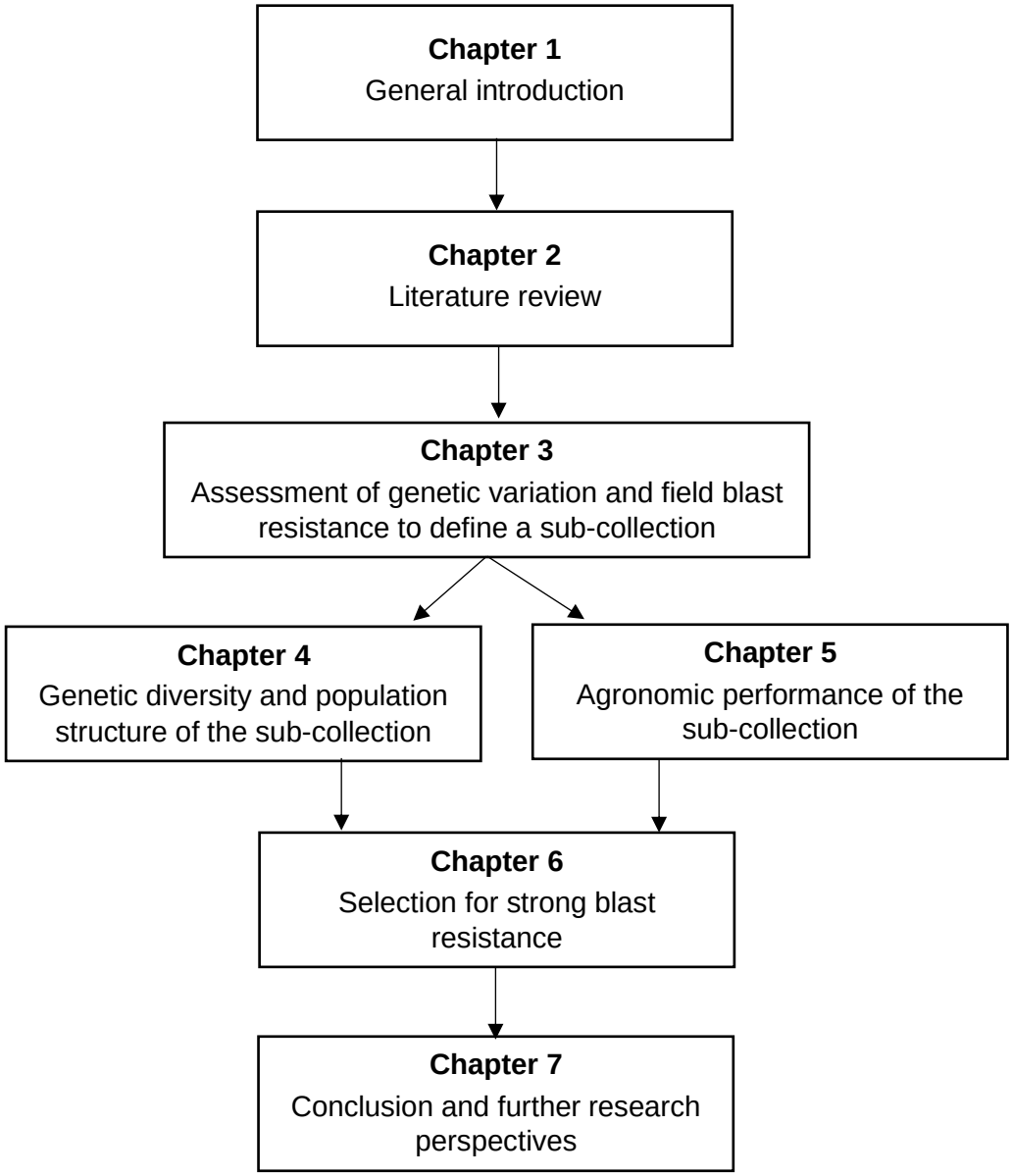


Figure 1.1. Thesis framework

CHAPTER TWO

2. Literature review

2.1. Origin of rice

Genus *Oryza* named by Linnaeus (1753) is classified under the tribe *Oryzeae*, subfamily *Oryzoideae*, of the grass family *Poaceae* (Gramineae). Genus *Oryza* is composed of 24 wild and two cultivated species, *O. sativa* and *O. glaberrima*. Both cultivated rice species are of particular interest for human and animal dietary energy worldwide (Vaughan *et al.* 1994; Ge *et al*. 2001; Ammiraju *et al*. 2010; USDA-ARS 2013). The origin of rice has long been a source of debate. The taxonomy and phylogenetic relationships of cultivated rice species and their relatives are discussed in this chapter.

According to historical and archaeological evidence, the 26-species in this genus originated about 130 million years ago from Gondwanaland, the ancient landmass from which Africa, South America, Australia, and Antarctica drifted apart since the tertiary era (Chang 1976a, 1976b; ORSTOM, 1987). Today's *Oryz*a spp. are distributed in all these continents except for Antarctica (Kole 2006). *O. sativa* occurs in all rice production areas throughout the world, whereas *O. glaberrima*, indigenous to Africa, is only cultivated in West and Central Africa.

The oldest archaeological evidence of rice used by humans has been found in the middle and lower Yangtze River Valley region in China (Wang 1986; Gross and Zhao 2014). Rice phytoliths and silicon microfossils of plant cell structures have been found at the Xianrendong and Diotonghuan sites and dated to 11,000–12,000 BC (Zhao 1998; Agnoun *et al.* 2012). Scientists have discovered other sites in this region, including Shangshan and Bashidang (Higham and Lu 1998; Pei 1998; Jiang and Liu 2006; Fuller 2007; Gross and Zhao 2014).

Parallel and independent evolution of rice gene pools have occurred in Africa and in Asia and many scientists have tried to elucidate the evolutionary relationships within and between the different species (Purugganan 2014; Wang *et al.* 2014; Veltman *et al.* 2019). *O. sativa* is comprised of two major varietal groups or subspecies, the *Indica* group, and the *Japonica*

group. Several studies have suggested that *Indica* cultivars are derived from the annual wild ancestor *O. nivara*, whereas *Japonica* cultivars are derived from the perennial wild ancestor *O. rufipogon* (Khush 1997; Cheng *et al.* 2003; Yamanaka *et al.* 2004; Xu *et al.* 2007) (Figure 2.1). A recent phylogenomic study of 446 geographically diverse accessions of the wild species *O. rufipogon* and 1,083 cultivated *Indica* and *Japonica* varieties supports that *O. Japonica* was first domesticated from a specific population of *O. rufipogon* around the middle area of the Pearl River in southern China, whereas *O. sativa Indica* was subsequently developed from crosses between *Japonica* rice and local wild rice as the initial cultivars spread into South East and South Asia (Huang *et al.* 2012).

O. nivara has a more restricted distribution than *O. rufipogon* (Vaughan *et al.* 2008). In fact, *O. nivara* is most common in South and Southeast Asia. *O.rufipogon* has many ecotypes and is widely distributed across Asia to Papua New Guinea and Australia. The *Indica* varietal group is grown in tropical and subtropical regions of low latitude and low altitude with warmer climatic conditions, whereas the *Japonica* varietal group is grown in high altitudes or temperate regions of high latitudes with cooler climatic conditions (Vaughan *et al.* 2008). *Indica* varieties are grown exclusively in tropical latitudes, whereas *Japonica* varieties can be found either in tropical or temperate climates (Mackill and Lei 1997; Reig-Valiente *et al.* 2016). In Yunnan province of China, the *Japonica* rice varieties were mostly concentrated in areas at altitudes between 1,400 and 2,320 m, whereas the majority of *Indica* varieties were found in areas below the altitudes of 1.400 (Xiong *et al.* 2010). *O. sativa* f. *spontanea* is a weed type of *Poaceae*, accompanying rice and is widely distributed in rice-planting areas all over the world. This species can be particularly found in south and south-east Asia, south and north America, and southern Europe (Mortimer *et al*. 2000).

Research findings supported by genetic data based on isozyme, simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) indicate that the direct progenitor of *O. glaberrima* is annual wild *O. barthii*. Some studies, however, argued that *O. glaberrima* might have evolved from *O. stapfii* (Suh 2008), which is found in Africa as wild form and distributed

in the same region as this *O. glaberrima* rice. It differs from *O. glaberrima* in the presence of awns, hairs on lemma and palea and shattering habit of the spikelets. The range of variation in this species is similar to that of *O. glaberrima* (Suh 2008).

O. longistaminata is the perennial rice from which the annual *O. barthii* wild species seem to be derived, and both occur in Africa (Second 1982; Zhu and Ge 2005). However, some phylogenetic studies support the annual *O. barthii* evolved from another ancestor of Asian wild rice, not *O. longistaminata* (Zhu and Ge 2005; Vaughan *et al.* 2005).

The domestication of rice (*O. glaberrima*) in Africa occurred later than in Asia but unequivocally prior to the introduction of *O. sativa* (Vaughan *et al.* 2004; Sweeney and McCouch 2007). The best evidence from archaeobotanical data for the domestication of *O. glaberrima* comes from the site of Dia, in the middle Niger Delta, and Mali (Portères 1956; Angladette 1966; Chang 1976a; Carney 1998; Murray 2004). Abundant grains were recovered at this site, of which the earliest occupation was dated at between 2800 and 2500 BC. Based on grain dimensions and their lack of change in size over time, they were all presumed to be from domesticated plants. The accelerator-based mass spectrometry C-14 dating of these rice grains suggests that the period previously proposed by scientists for the domestication of African rice of about 3500 BC might be close to the correct time (Portères 1976). Portères (1976) and Chang (1976a) also suggested two secondary centers of diversification dating back to 1,000 BC, with the first center on the coasts of The Gambia, Casamance in Senegal and Bissau Guinea, and the second center in the Guinea forest between Sierra Leone and the West Ivory Coast. Sweeney and McCouch (2007) reported that archaeologists found some specimens of rice grains dating back 1,800 BC to 800 BC in Ganjigana located in North-East Nigeria. However, despite these suggestions, the correct origin of *O. glaberrima* remains unknown as evidence is missing.

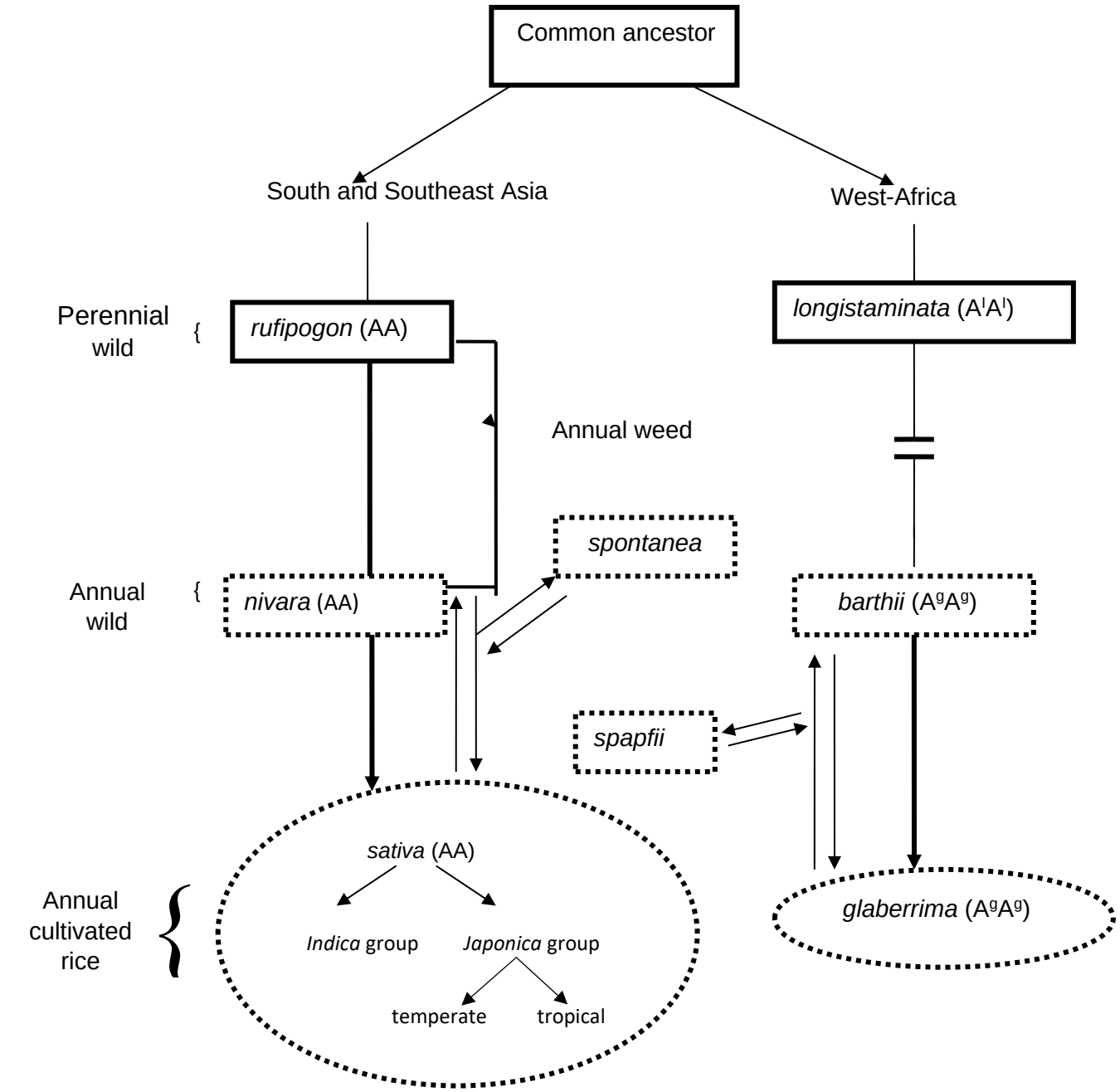


Figure 2.1. Evolutionary pathway of the two cultivated rice species, *O. sativa* and *O. glaberrima*

Taxa boxed by solid lines are wild perennials. Taxa boxed by broken lines are annuals. An arrow with a solid line indicates direct descents. An arrow with a broken line indicates indirect descent. Double arrows indicate introgressive hybridization (adapted from Chang (1976a) and Agnoun *et al.* (2012))

2.2. Diversity, potential, and spread of rice

Genus *Oryza* has two cultivated and 24 wild species. Both cultivated species, *O. sativa* and *O. glaberrima* are diploid (2n = 24). Nine of the 26 *Oryza* species are allotetraploid (Table 2.1). The wild species (2n = 24, 48 chromosomes) are grass-like plants that are weedy and inferior in morphological traits, having poor grain characteristics, low grain yield, and are shattering and lodging in nature, whereas domestication selection was mainly focused on plants that have less lodging and shattering (Callaway 2014). Since the 1960s, genus *Oryza* has been divided into several species' complexes. Taxonomists have divided this genus into four species complexes: (1) *sativa* complex, (2) *officinalis* complex, (3) *meyeriana* complex, and (4) *ridley* complex (Vaughan *et al.* 1994 Khush 2005 Sanchez *et al.* 2013). *Sativa* and *officinalis* complexes are the most studied ones, particularly for the genetic diversity, and species relationships. Genus *Oryza* has also been classified into ten genome groups (AA, BB, CC, BBCC, CCDD, EE, FF, GG, KKLL, and HHJJ) on the basis of genomic analysis using F_1 hybrid population (Ge *et al.* 2001). With 8 species of both cultivated and wild rice species, representing six out of the ten known genome types (Table 2.1), Africa appears to be the greatest source of rice genetic diversity.

Sativa is the only complex which comprises both cultivated *O. sativa* and *O. glaberrima*. This complex has a total of 8 diploids (2n = 24) species. The latter form the primary gene pool for rice improvement (Brar *et al.* 2018). The six other species in *sativa* complex are of wild types: *O. rufipogon* and *O. nivara* that are broadly distributed in Asia, whereas *O. longistaminata* and *O. barthii* in Africa; *O. meridionalis*, and *O. glumaepatula* occurs in Australia and South and Central America, respectively. The species *O. sativa* has been classified into two varietal groups of which the *Indica* group and the *Japonica* group share identical non-shatter mutations in gene sh4 on chromosome 4 (Sang and Ge 2013). It is suggested that this mutation occurred in an ancestor of *Japonica* rice first, and then found its way to *Indica* (Sang and Ge 2013). The most important domestication trait in rice is the loss of shattering because that results in rice being dependent on humans for survival. Traditional *Indica* varieties are usually characterized

by tall stature, weak stem, droopy leaves, high tillering capacity, long grains, and poor response to high nutrient input conditions. *Japonica* varieties have stiff, short stalk, and erect type with round grains and are highly responsive to nutrient inputs (Prasad *et al.* 2017).

Although *O. glaberrima* is endemic to West Africa, a recent study of 14 unlinked nuclear genes in 40 individuals found *O. glaberrima* to have 70% lower genetic diversity and hardly any population structure compared to its progenitors *O. barthii* and *O. nivara* (Li *et al.* 2011). During the last two decades, different molecular techniques have been used for analyzing genetic diversity and relationships within the *sativa* complex. Techniques such as Random Amplified Polymorphic DNA (Bautista *et al.* 2001; Ishii *et al.* 1996; Ren *et al.* 2003), Simple Sequence Repeat or SSR (Ishii *et al.* 1996; Ren *et al.* 2003), Restriction Fragment Length Polymorphisms or RFLP (Bautista *et al.* 2001; Lu *et al.* 2002; Wang *et al.* 1992), Amplified Fragment Length Polymorphism or AFLP (Park *et al.* 2003a,b), Inter Sequence Simple Sequence Repeat or ISSR (Joshi *et al.* 2000), Short Interspersed Elements (SINEs) and Miniature Inverted-Repeat Transposable Element (MITE) insertions (Cheng *et al.* 2002; Iwamoto *et al.* 1999; Mochizuki *et al.* 1993), single-nucleotide polymorphism (SNP) (Caicedo *et al.* 2007; Molina *et al.* 2011; Ndjiondjop *et al.* 2017; Veltman *et al.* 2019) have been used for a better understanding of phylogenetic relationships between and within these different species complexes.

More, in particular, the genetic diversity within the different cultivated African rice germplasms has been the subject of many genetic studies (Dramé *et al.* 2011; Orjuela *et al.* 2014; Wang *et al.* 2014; Chen *et al*. 2017; Ndjiondjop *et al.* 2017; Veltman *et al.* 2019). Using 93 SSR markers, Semon *et al.* (2005) assessed the population structure of 198 *O. glaberrima* accessions from 12 West African countries. They found that 67 % of *O. glaberrima* accessions show some genetic evidence for spontaneous hybridization with *O. sativa.* They argued that natural gene flow might have occurred between the two species as West African *O. glaberrima* is often grown close to *O. sativa*. Similar findings were reported by Barry *et al.* (2007) who characterized 26 *O. glaberrima* and 144 *O. sativa* accessions collected in maritime Guinea by means of 11 SSR markers. They detected the presence of gene flow between *O. glaberrima*

and *O. sativa*. Their study also revealed two ecotypes (floating and erect) among the *O. glaberrima* accessions. Sanni *et al.* (2008) analyzed the link between geographic origin and phenotypic diversity of 880 *O. sativa* upland rice landraces collected from the four main rice production regions of Ivory Coast; i.e. Gagnoa, Touba, Boundiali, and Danané. Significant variations were found across geographical zones and ecologies with respect to the 13 traits measured. Quantitative traits (plant height, leaf length, and width, days to 50% heading, maturity, *etc.*) and four qualitative traits (leaf blade pubescence, grain awn, panicle exertion, and tillering ability) were assessed. Results from morphological and molecular analysis confirmed the view that *O. sativa* has a higher genetic diversity compared to *O. glaberrima*.

With ten species representing five genome types, the *officinalis* complex is the largest species complex in the *Oryza* genus (Table 2.1). It is closely related to the *sativa* complex (Tateoka 1962; Vaughan 1989 Kumagai *et al.* 2010). This complex has related-species groups found in Asia, Africa, and Latin America. Using a data set of 68 single-copy genes, Zang *et al.* (2011) showed a close sister relationship between *officinalis* and *O. rhizomatis*, with *O. eichingeri* being much more diverged. *O. eichingeri* being the only wild *Oryza* species found in both Asia and Africa (Vaughan 1989, Sanchez *et al.* 2013). This distribution has drawn considerable research interest. Due to the significant differentiation between the *O. eichingeri* species from Africa and those from Sri Lanka, it has previously been suggested that they should be treated as distinct species (Federici *et al.* 2002). However, based on population genetic analysis, Zhang and Ge (2007) suggested that these geographically isolated populations should be treated as geographic races rather than distinct species.

The *meyeriana* complex has two diploid species, *O. granulate* and *O. meyeriana,* which are grown in South and Southeast Asia (Table 2.1). Phylogenetic analysis conducted using nuclear (Adh1 and Adh2) and chloroplast (MatK) genes indicated that both *O. meyeriana* and *O. granulate* are the earliest diverging *Oryza* species (Ge *et al.* 1999). Kumagai *et al.* (2010) using selected variable regions of the chloroplast genome showed a monophyletic relationship between both species of *Meyeriana* complex. No efforts seem to have been made to study the

evolutionary relationships between these species using current genomic approaches (Wambugu *et al.* 2018).

The complex *ridleyi* has two closely related tetraploid species, *O. ridleyi,* and *O. longiglumis.* The latter species is found along the Komba River, Irian Jaya, Indonesia, and Papua New Guinea. *O. ridleyi* grows across Southeast Asia.

Many *Oryza* spp. possess traits that are useful in rice breeding (Table 2.1). Scientists demonstrated that *O. glaberrima* possesses abilities of tolerance to several diseases and pests (Sarla and Swamy 2005; Cabasan *et al.* 2018; Agnoun *et al.* 2019). Many other important traits such as weed-competitiveness, drought tolerance and ability to respond to low input conditions, are uniquely linked with the *O. glaberrima* species (Sarla and Swamy 2005; Mondal *et al.* 2018; Sikirou *et al.* 2018). This species thus constitutes a potential source of genes for genetic improvement of *O. sativa* against these stress factors (diseases, insect pests, abiotic stress, and weeds) in Africa (Pham 1992; Fofana *et al.* 1995; Jones *et al*. 1997; Futakuchi and Sie, 2009; Sie *et al.* 2012; Dufey *et al.* 2015). *O. glaberrima* is, however, highly susceptible to lodging and grain shattering, whereas *O. sativa* usually has interesting characteristics associated with high grain yielding (National Research Council 1996; Jones *et al.* 1997; Linares 2002).

Table 2.1. Chromosome number, genomic composition, and distribution of the *Oryza* complex species and their useful traits

Species	Chromosome number (2n)	Genome group	Number of accessions	Distribution	Useful traits
***Sativa* complex**					
O. sativa L.	24	AA	96,564	Worldwide	High-yielding
O. glaberrima Steud.	24	A^gA^g	1,562	West Africa	Tolerance to drought, acidity, iron toxicity, P-deficiency; resistance to BB, blast, RYMV, African gall midge, nematodes; weed competitiveness
O. nivara Sharma et Shastry	24	AA	1,260	Tropical and subtropical Asia	Resistance to grassy stunt virus, BB
O. rufipogon Griff.	24	AA	858	Tropical and subtropical Asia, tropical Australia	Resistance to BB, blast, BPH, tungro virus; moderate tolerance to Shb; tolerance to aluminum and soil acidity; increased elongation under deep-water conditions and yield-enhancing loci
O. breviligulata A. Chev. Et Roehr. *O. barthii*	24	A^gA^g	218	Africa	Resistance to BB; tolerance to heat and drought
O. longistaminata A. Chev et Roehr	24	A^1A^1	203	Africa	Resistance to BB, GLH, nematodes, stemborer; drought tolerance
O. meridionalis Ng	24	A^mA^m	56	Tropical Australia	Elongation ability; drought and heat tolerance
O. glumaepatula Steud.	24	$A^{gp}A^{gp}$	54	South and Central America	Elongation ability; source of CMS; heat tolerance
***Officinalis* complex**					
O. punctata Kotschy ex Steud.	24, 48	BB, BBCC	71	Africa	Resistance to BB, BPH and zigzag leafhopper (*Cicindelidae*); drought and heat tolerance
O. minuta J.S. Presl. ex C.B. Presl.	48	BBCC	63	The Philippines and Papua New Guinea	Resistance to BB, blast, BPH, and GLH
O. officinalis Wall ex Watt	24	CC	265	Tropical and subtropical Asia, tropical Australia	Resistance to thrips, BPH, GLH, WPH, BB, stem rot; heat tolerance
O. rhizomatis Vaughan	24	CC	19	Sri Lanka	Drought and heat tolerance; blast resistance;
O. eichingeri A. Peter	24	CC	30	South Asia and East Africa	Resistance to BPH, WBPH, GLH
O. latifolia Desv.	48	CCDD	40	South and Central America	Resistance to BPH and BB; high biomass production

Species	Chromosome number (2n)	Genome group	Number of accessions	Distribution	Useful traits
O. alta Swallen	48	CCDD	6	South and Central America	Resistance to striped stemborer; high biomass production
O. grandiglumis (Doell) Prod.	48	CCDD	10	South and Central America	High biomass production
O. australiensis Domin.	24	EE	36	Tropical Australia	Resistance to BPH, BB, blast; drought and heat tolerance
***Meyeriana* complex**					
O. granulata Nees et Arn. ex Watt	24	GG	24	South and Southeast Asia	Shade tolerance; adaptation to aerobic soil
O. meyeriana (Zoll. et (Mor. Ex Steud.) Baill.)	24	GG	11	Southeast Asia	Shade tolerance; adaptation to aerobic soil
***Ridleyi* complex**					
O. longiglumis Jansen	48	HHJJ	6	Irian Jaya, Indonesia, and Papua New Guinea	Resistance to blast and BB
O. ridleyi Hook. F.	48	HHJJ	15	Southeast Asia	Resistance to blast, BB, tungro virus, stem borer, and whorl maggot
Unclassified					
O. brachyantha A . Chev. et Roehr	24	FF	19	Africa	Resistance to BB, yellow stemborer, leaf folder, whorl maggot; tolerance to laterite soil
O. schlechteri Pilger	48	KKLL	1	Papua New Guinea	Stoloniferous
O. coarctata Tateoka	48	KKLL	1	Asian Coastal Area	Salinity tolerance; stoloniferous;
Leersia perrieri A. Camus	24	UNKNOWN	1	Africa	Shade tolerance; stoloniferous

BPH brown planthopper, GLH green leafhopper, WBPH white backed plant hopper, BB bacterial blight, Shb sheath blight, CMS cytoplasmic male sterility, RYMV rice yellow mottle virus. Sources: Vaughan *et al.* (1994), Khush (2005), and Sanchez *et al.* (2013)

2.3. Major rice ecosystems and production constraints in Africa

Africa has enormous agricultural potential that ought to be exploited better than in the past to supply local communities with sufficient quantities of food. African ecosystems, in particular, the drylands, rainfed wetlands, deepwater and mangrove swamps, and irrigated wetlands are used for rice cultivation (Balasubramanian *et al.* 2007; Seck *et al.* 2012). Wetlands, such as inland valleys in the Equatorial Forest Zone deliver a range of associated ecosystem functions and deserve special attention due to their relatively high and secure water availability and soil fertility (Adams 1993; Turner *et al.* 2000, Dossou-Yovo *et al.* 2017). Global changes, such as population growth and climate change, provide new incentives for inland valley agricultural use

(Windmeijer and Andriesse 1993; Rodenburg 2013; Zorom *et al.* 2013). Inland valley ecosystems are estimated to cover about 3.6% of SSA, corresponding to approximately 85 million ha (Dossou-Yovo *et al.* 2017). In Benin, for example, the local population currently uses sixty-seven percent of the valleys, primarily for crop cultivation including rice. Other uses, such as pisciculture, animal husbandry and the collection of firewood, are of minor importance (Giertz *et al.* 2012). Figure 2.2 and Figure 2.3 presents the main agroecological zones and water management practices for rice production in West and Central Africa. The characteristics of the different rice growing ecosystems are summarized in Table 2.2.

Rainfed uplands mainly occur in the Guinea savannah and the humid forests where the landscape is characterized by plateaus and undulate slopes (Diagne *et al.* 2013). Although different soil types are present in the five major agro-ecological zones of Africa (Figure 2.6), some of them are ideally suitable for extensive rice production. Ferralsols and acrisols are dominant in the humid zones, whereas ferralsols and lixisols dominant in the sub-humid zone. Ferralsols are dominant in the humid southwestern and southeastern parts of Sierra Leone, Liberia, and Cameroon. However, their level of organic matter content is relatively low leading to low soil fertility. Acrisols occur in the transition area between the Equatorial Forest Zone and the Guinea Savanna Zone (eastern Guinea, Ivory Coast, southwestern Ghana, Togo, Benin, and southeastern Nigeria). They have few weatherable minerals, and their clay fraction consists mainly of kaolinite and some gibbsite. They are more susceptible to erosion than ferralsols, particularly if their organic matter content is low. Lixisols are the major soils in southern Senegal; Gambia; parts of Guinea Bissau; southern Mali; Burkina Faso; northern, central, and eastern Ghana; Togo; Benin; and western, central, and northern Nigeria. Lixisols most occur in the semi-arid zone (Deckers 1993) with a higher pH but lower structure stability than ferralsols and acrisols. Slaking and compaction of these soils is common under continuous cultivation and poor land management.

Upland rice is often grown on ferralsols, acrisols, and lixisols in SSA. Upland soils are generally poor in nitrogen, phosphorus, and sulfur, with severe iron deficiency. However, the majority of

African farmers have little resources and cannot afford additional agricultural inputs that would compensate for this soil nutrient deficiency. Water in upland ecosystems mainly comes from precipitation. In the context of erratic and poor rainfall associated with climate change, rice may be particularly affected by drought. High weed competition is one of the most important biophysical production constraints. The major rice diseases include blast, bacterial leaf blight (BLB, *Xanthomonas oryzae pv. oryzae*), rice yellow mottle virus (RYMV), African rice gall midge (AfRGM, *Orseolia oryzivora* Harris & Gagné) and stem borers (*Chilo suppressalis* Walker) (Abo and Sy 1997; Sere *et al.* 2013), which cause severe production losses.

The rainfed lowland ecosystem covers agro-ecological zones from Sudan savannah to the humid forest. Water sources in rainfed lowlands are direct rainfall, raised water tables and (unregulated) floods. In SSA, around 130 million ha (Diagne *et al.* 2013) include considered to be wetlands. Lowland soils (fluvisols, gleysols, and vertisols) are suitable for intensive rice cultivation within the humid zones to sub-humid wooded savannah zones in Africa. Planosols, as well as solonetz and solonchak soils that mainly occur in the semi-arid zone, may sometimes become flooded. Rainfed lowland rice predominates in west, central, east and southern Africa. Rice production in rainfed lowland ecosystems is constrained by (a)biotic stress conditions, including weeds, insect pests (such as stem borers, African Rice Gall Midge, and rice sucking bugs), and diseases (rice blast, brown spot of rice, and rice yellow mottle virus) and abiotic constraints such as cold, drought and salinity (Table 2.2). Due to poor drainage, rainfed lowland soils can contain high levels of iron and manganese, which often induce Fe (iron) toxicity. Fe toxicity is recognized as one of the most widespread soil problems in west African (Benin, Burkina Faso, Côte d'Ivoire, Liberia, Nigeria, Senegal, and Sierra Leone, *etc.*) wetlands and is considered as a major constraint to rice production in these environments (Virmani 1979; Gridley *et al.* 2006; Sikirou *et al.* 2015). Rice grown under rainfed lowland conditions also requires good water management practice (proper drainage and flood control measures).

Wetland ecosystems can be categorized into deep-water and mangrove swamp ecosystems. The deep-water ecosystem widely occurs in the low-lying wetlands of Madagascar and the poorly drained inland valleys of Chad, Guinea, Mali, Niger, and Nigeria. This ecosystem is currently shrinking due to the expansion of dam constructions that lead to reduced water movement. Due to the prevailing stress types in this ecosystem (including insect pests and diseases), their Africa-wide rice yield is only 2.4 ton/ha (FAOSTAT 2019).

Mangrove ecosystems mainly occur along the west African coast of Guinea Bissau, The Gambia, and Guinea Conakry, Sierra Leone, Liberia, Senegal and Nigeria (Defoer *et al.* 2007). Mangrove swamps are characterized by high levels of salinity as a result of seawater intrusion caused by ocean tides. As a result, rice yields in mangrove swamps are below 1 ton/ha especially due to salinity stress (Lancon and Erenstein 2002). Mangrove ecosystems are also constrained by insect pests, diseases and nutrient deficiencies (Table 2.2).

The irrigated lowland ecosystems provide the best conditions for rice cultivation as it offers better water control than the other production systems. The major water sources are diverted rivers and wells (Saito *et al.* 2013). In Africa, Madagascar has the largest irrigated area for rice production (782,487 ha) followed by Egypt (518,520 ha) and Mali (335,269 ha) (Diagne *et al.* 2013). The International Rice Research Institute (IRRI 2002) has classified this ecosystem as the irrigated wet season ecosystem often characterized by wet rice cultivation and irrigated ecosystem that uses irrigation water. The major constraints of rice production in irrigated lowlands include both nutrient deficiencies (e.g. N, P, S, and Zn) and toxicity (e.g. Fe, Mn, and Al and soil acidity), biotic stresses e.g. weeds such as *Sphenoclea zeylanica*, *Cyperus difformis, Echinochloa* spp.; diseases including RYMV, blast, glume discoloration, sheath rot, and bacterial blights; and insect pests such as stem borers (*Chilo suppressalis* Walker), African rice gall midge (AfRGM, *Orseolia oryzivora* Harris & Gagné).

Most Beninese rice production systems occur in the rainfed upland (Figure 2.4), rainfed lowland and irrigated lowland (Figure 2.5) ecosystems. According to Djagba *et al.* (2018), the majority (83.3%) of rice farmers in Benin uses rainfed lowland systems of the inland valley to

grow rice. Apart from the most common constraints, the rainfed upland ecosystems also face climate change and soil degradation that leads to low soil fertility. (MAEP 2011).

Table 2.2. Rice production ecosystem characteristics in Africa

Rice ecosystems	Actual/potential Yield (t/ha)	Abiotic factors	Biotic factors	Input use
Rainfed upland	1.2/2-5	P and N deficiency, acidity, Al toxicity, drought, erosion, poor soil fertility	Weeds, termites, stem borers, AfRGM Diseases (blast, BLB, RYMV), birds, nematodes, and rodents	Very low
Rainfed lowland	1.9/3-6	Water control, N and P deficiency, Fe toxicity	Weeds, termites, stem borers, AfRGM Diseases (blast, BLB, RYMV), birds	Low
Mangrove and deep water	<1/2-4	Acid sulfate, salinity, Fe toxicity, excess water	Salinity, diseases (blast, BLB, RYMV), insect pests, birds	Very low
Irrigated lowland	1.9-3.7/5-12	N deficiency, salinity and alkalinity, extreme temperatures	Weeds, stem borers, AfRGM Diseases (blast, BLB, RYMV), birds	High
High elevation (upland and lowland)	1.2/2-6	Cold, Fe toxicity, P and N deficiency, excess water	Weeds, stem borers Diseases (blast, BLB, RYMV), birds	Low

BB bacterial blight; RYMV rice yellow mottle virus; AfRGM African rice gall midge. Sources: Defoer *et al.* (2004): Wopereis *et al.* (2007).

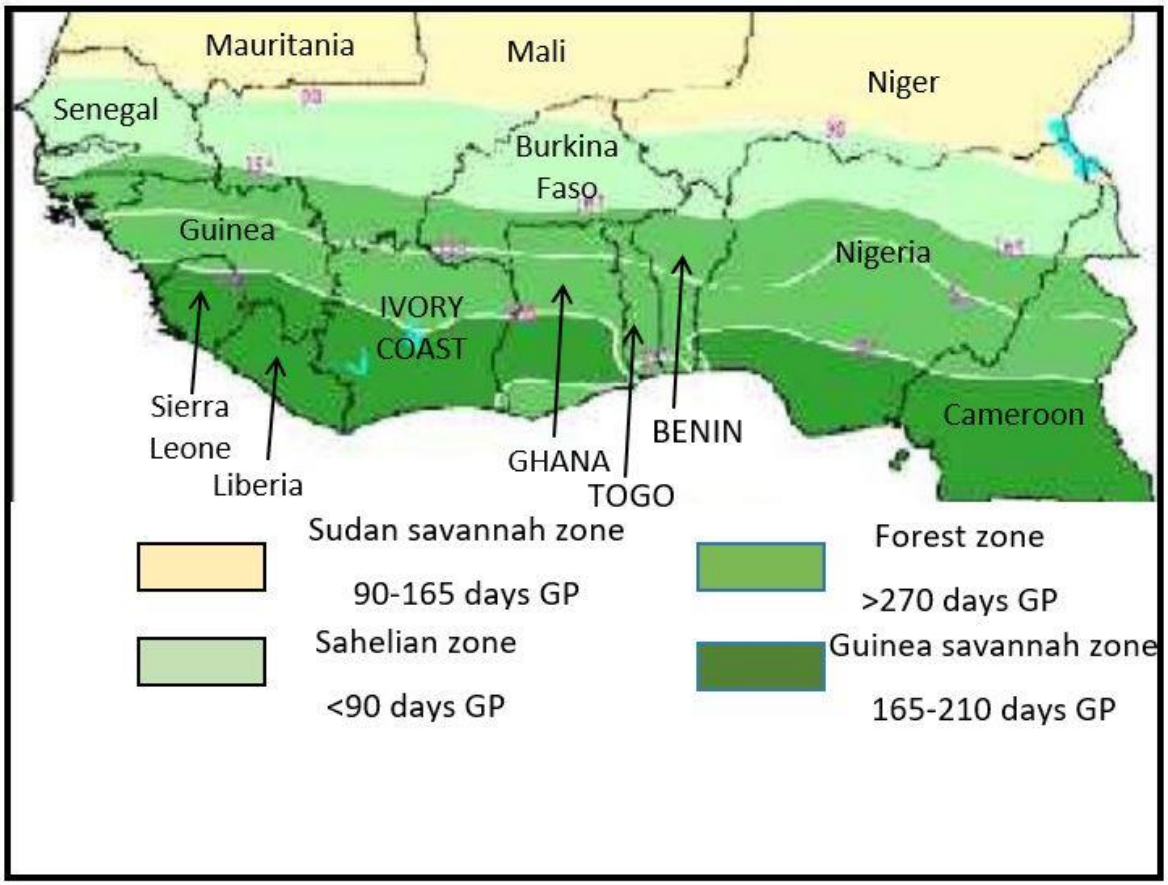
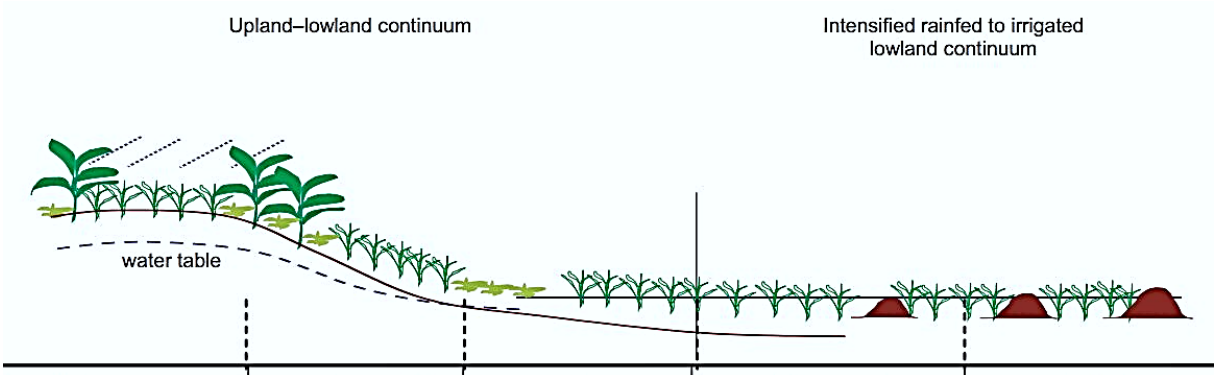


Figure 2.2. Agro-ecological zones for rice production in West and Central Africa; GP growing period. Source: Defoer *et al.* (2004)



Upland	Hydromorphic slopes	Lowland	Intensified lowland	Irrigated lowland
Main water supply:				
Rainfall	Rainfall + watertable	Rainfall + watertable + floodwater	Regulated floodwater	Irrigation
Agro-ecological zone:				
Guinea savannah to humid forest zone	Guinea savannah to humid forest zone	Sudan savannah to humid forest zone	Sudan savannah to humid forest zone	Sahel to humid forest zone

Figure 2.3. Water management for rice production in West and Central Africa modified from Defoer *et al.* (2004)

Figure 2.4. Rainfed upland rice field in Benin

Figure 2.5. Irrigated lowland rice field in Benin

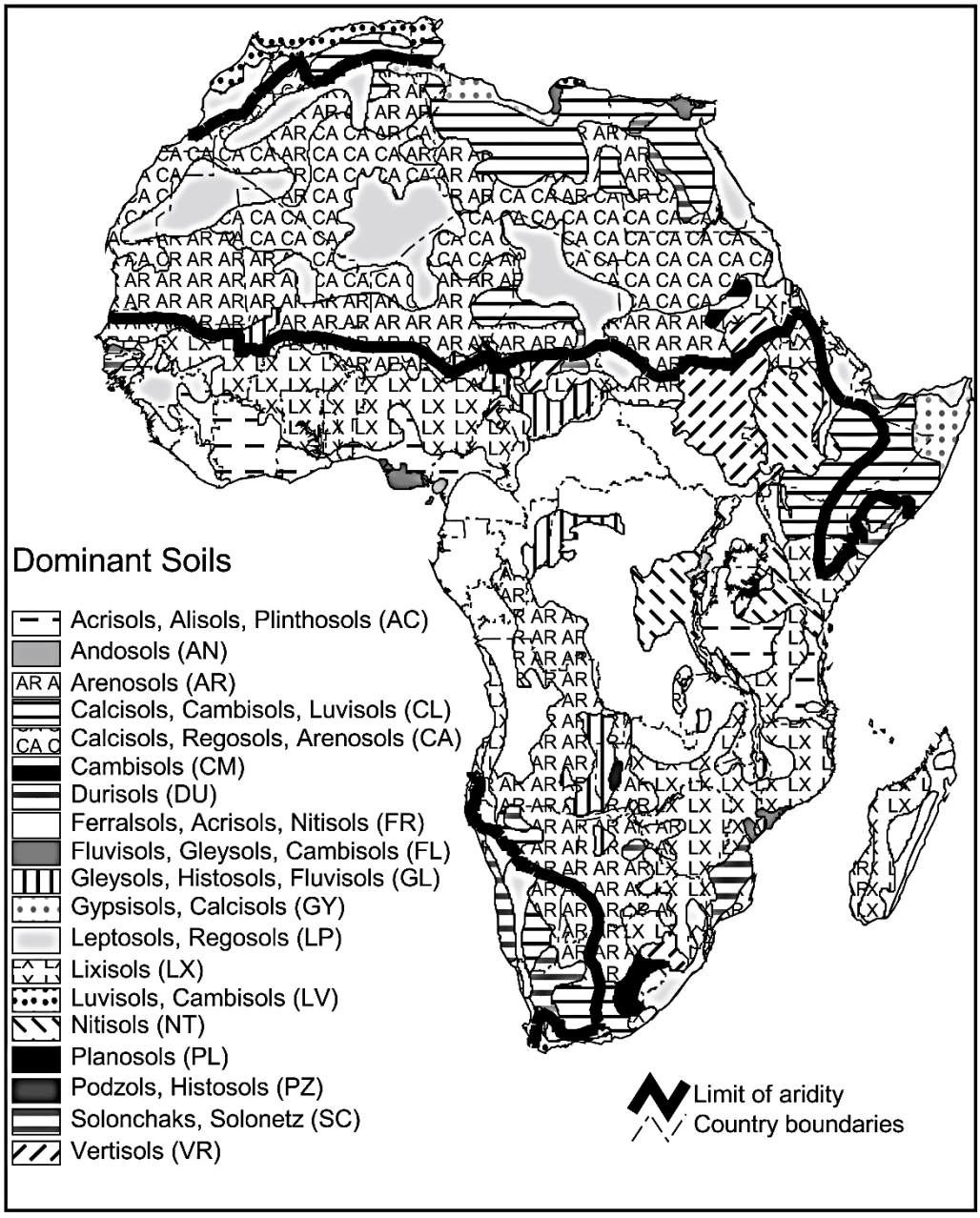


Figure 2.6. Soils diversity present in the African continent according to the FAO World Soil Resources map (2003)

2.4. Rice blast: impacts and management

2.4.1. Blast fungus-host interaction

The filamentous fungus *Magnaporthe oryzae* causes rice blast, the most serious disease of cultivated rice. Chumley and Valent (1990) began defining the fungus' utility as a model for genetics with their studies on the vegetative diploid phase, and

development of genetic mutants to examine specific traits, such as melanin production. Since then the rice–*M. oryzae* pathosystem has become a model in the study of plant-fungal interactions because of its scientific advancement and economic importance (Dodds *et al.* 2006; Thrall *et al.* 2012; Liu *et al.* 2013). Several studies and reviews reported the *M. oryzae* infective life cycle (reviewed in Li *et al.* 2012). The life cycle of *M. oryzae* is shown in Figure. 2.7 and described according to Wilson *and* Talbot (2009), and Donofrio *et al.* (2014).

The conidiophores, the asexual and the most important stage of *M. oryzae*, will germinate on a hydrophobic leaf surface. Given sufficient moisture levels, a single, polarized germ tube emerges from the spore, normally from its tapering end, and grows across the leaf surface, before differentiating into the dome-shaped appressorium (Struck 2006; Galhano and Talbot 2011), as illustrated in Figure 2.7.

This dome-shaped, melanized structure, utilizes mechanical pressure to breach the leaf surface, grows invasively into the first epidermal cells by means of invasive hyphae (Donofrio *et al.* 2014). This brief phase is rapidly followed by a lengthier biotrophic phase whereby bulbous hyphae grow within epidermal cells, producing ‘‘biotrophy-interfacial complexes’’ or ‘‘BICs’’ (Kankanala *et al.* 2007; Mosquera *et al.* 2009; Figure 2.7). The filamentous invasive hyphae enlarge into bulbous hyphae. Invaded cells are alive as the fungus enters them but later die as they become full of hyphae (Fernandez and Wilson 2014).

BICs are actually factories for the production and release of fungal effectors into plant cells, as reported by Khang *et al.* (2010). By 48 h post-inoculation, the fungus becomes necrotrophic, producing thin, invasive hyphae followed by eventual development of lesions and production of more conidiophores (Figure 2.7). The importance of

pathogen produced effectors during the infection process has been well studied in the infamous oomycete pathogen, *P. infestans* (reviewed in Kamoun 2006).

The first line of the plant immune system involves basal defense responses that are triggered by the detection of broadly conserved molecular features of pathogenic microorganisms called pathogen-associated molecular patterns (PAMPs) or microorganism-associated molecular patterns (MAMPs) (Giraldo and Valent 2013).

PAMP-triggered immunity (PTI) involves pattern recognition receptors (PRRs), which are transmembrane receptor kinases and transmembrane receptor-like proteins (Liu *et al*. 2010; Chen and Ronald 2011). PAMPs include pathogen cell wall components (chitin for fungi and glucans for fungi and oomycetes) and their detection occurs in the apoplast, which is the plant extracellular compartment (Giraldo and Valent 2013). Plant defenses include the production of reactive oxygen species (ROS), as well as the secretion of antimicrobial compounds, hydrolytic enzymes (proteinases, chitinases, and glucanases) that are damaging to the pathogen, and inhibitors of pathogen hydrolytic enzymes, which are damaging to plants. Pathogens (e.g. *M. oryzae*) secrete effectors, which are generally small unique proteins, many of which function to defeat PTI (Hogenhout *et al.* 2009; Bozkurt *et al.* 2012; Rafiqi *et al.* 2012).

In turn, plants have developed a second line of defense, effector-triggered immunity (ETI), which involves the detection of specific avirulence effectors (AVR effectors), either through direct ligand-receptor interactions or through indirect detection of effector action on host targets (Dodds and Rathjen 2010; Kanzaki *et al.* 2012; Liu *et al.* 2013). Apoplastic effectors of the extracellular fungus *Cladosporium fulvum* are detected by transmembrane receptor-like CF resistance proteins 21.

More commonly, pathogen effectors called cytoplasmic effectors are delivered to the host cytoplasm, and they are recognized by intracellular resistance (R) proteins of the cytoplasmic nucleotide-binding site leucine-rich repeat (NBS-LRR) class (Dodds *et al.* 2010). This recognition triggers the hypersensitive response (HR) and blocks pathogen growth. Mutation or complete loss of effector genes enables the pathogen to avoid R protein recognition, which leads to the boom-and-bust cycle that has often defeated efforts to control important plant diseases including blast (McDonald and Linde 2002; Singh *et al.* 2011).

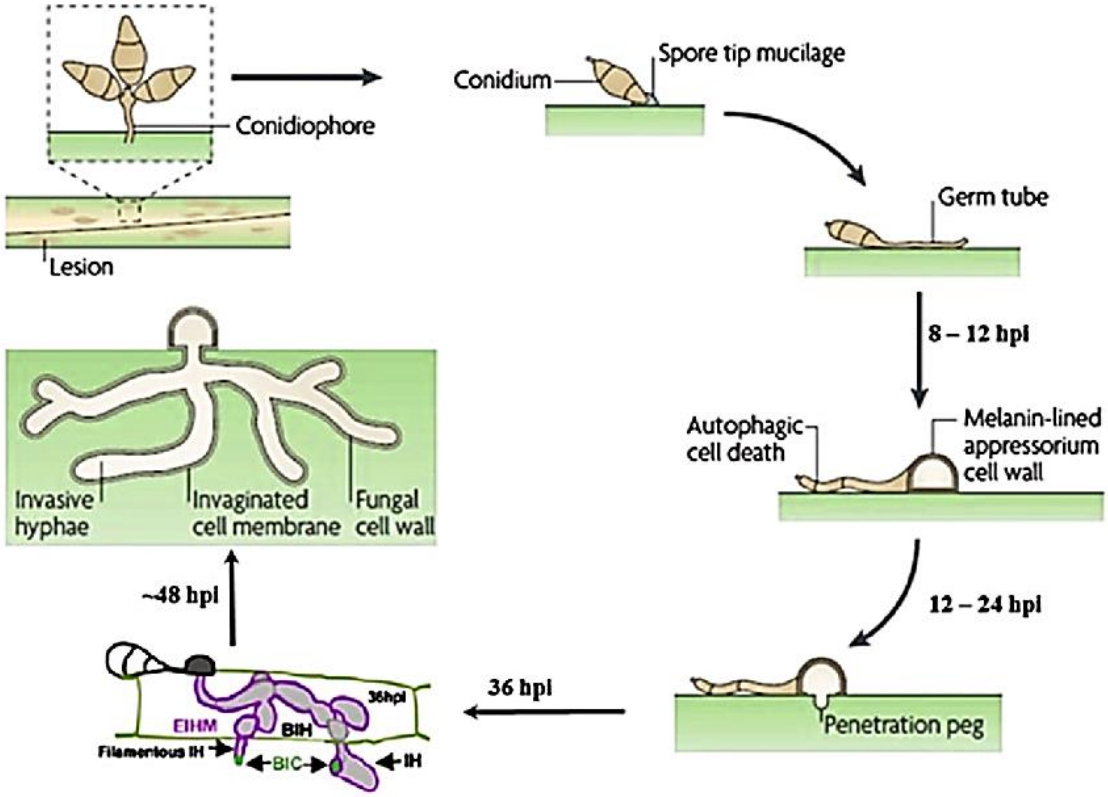


Figure 2.7. Life cycle of the rice blast fungus *Magnaporthe oryzae*. Sources: Wilson and Talbot (2009), Mosquera *et al.* (2009) and Donofrio *et al.* (2014). This figure has been adjusted to include the biotrophic portion of the life cycle, which includes the production of bulbous invasive hyphae (IH), biotrophic-interfacial complexes (BICs) and an extra-invasive hyphal membrane (EIHM)

2.4.2. Impacts of blast disease

Blast disease is one of the most devastating rice diseases causing production losses in over 85 countries (Ou 1985; Wang and Valent 2009, Séré et al, 2013; Boddy 2016; Bregaglio *et al*. 2016). It occurs in different ecological regions including at high altitudes (e.g. Madagascar, 1,400 m) (Raveloson *et al.* 2018). The fungus is capable of infecting rice at any developing stage. However, the crop is most vulnerable at seedling, tillering and flowering stages. The fungus usually attacks leaves, leaf collars, panicle nodes, panicle neck, branches, and spikelets. On the leaves, lesions first appear as minute brown specks, which then grow to become spindle-shaped, pointed at both ends. Under favourable conditions, these small necrotic lesions become larger and coalesce and eventually kill the leaves.

Panicle neck and panicle node blast are particularly devastating causing up to 80% yield losses in severe epidemics (Boddy 2016). Triangular, purple-coloured lesions form on the panicle neck and node, which elongate on both sides, seriously impairing rice crop development. Panicles become white when a young neck or node is invaded. Infection occurring later at a stage in plant growth may cause incomplete grain filling and poor grain quality. Blast lesions can also occur at the junction of the leaf blade and sheath causing collar rot and killing the flag or penultimate leaf. In this case, grain fertility is affected and this causes direct yield reductions. Furthermore, *M. oryzae* can infect and overwinter in alternative hosts, especially from the *Poaceae* family and then spread to the rice crop.

Crop losses attributed to rice blast vary according to regions, the susceptibility of varieties, and degree and moment of infection. When chemicals are used, the actual damage depends on the type of fungicides used, the dosage, the spraying technique and timing of application. The earlier blast infection starts during the growth cycle, the greater the production loss (Ou 1985). The blast can be more devastating in upland than in flooded conditions (Bonman 1992; Pooja and Katoch 2014; Miah *et al.* 2017). High fertilization, especially with nitrogen to increase rice grain yield under intensive system of rice cultivation (system of rice intensification, SRI), can also lead to severe blast epidemics (Mahesh *et al.* 2012; Poerwanto and Padmini 2017).

Currently, there is no literature data on estimations of the cost and benefit effects of blast outbreaks, even though blast is responsible for approximately 30% of global production loss, which is equivalent to feeding 60 million people (Nalley *et al.* 2016). Heavy yield losses of up to 100 % associated with blast have been reported in many African countries (Sere *et al.* 2013), which often results in costly fungicide applications. According to Peter *et al.* (2013), there is a continued threat to global food security due to blast. In fact, the cost of mitigating blast infections, especially via fungicides results in increased production costs and subsequently drives up food prices for consumers. This gives rise to food insecurity, in turn, especially in low-income countries.

2.4.3. Blast management strategies

Blast disease management has been extensively investigated, and many control procedures have been proposed. In the past, when no other methods were known, rice growers mainly used crop management practices (e.g. a variety of cultivated rice); nutrient and water control, planting time; and plant spacing to control blast.

Flooding rice fields is one of the crop management practices that help to reduce blast epidemics (Kahn and Libby 1958; Choong-hoe 1986; Bonman 1992; Miah *et al.* 2017). Blast is particularly devastating in upland and water stress conditions (drought) (Pooja and Katoch 2014). The flooding method represents helpful guidance for blast control in Africa.

Nutrient management practices are useful in reducing blast disease. Excessive uses of nitrogen (N) and silicon (Si) fertilization influence the efficacy of different blast control methods in opposite ways. High nitrogen fertilization has been shown to increase rice blast-susceptibility in many previous studies (Hori 1898; Ou 1985; Freitas *et al.* 2010; Siregar *et al*. 2016). Silicon (Si) has been shown to reduce plant disease impact, especially in rice blast (Ou 1985; Long *et al.* 2000). Silicon application to deficient soils has been shown to reduce blast severity (Wattanapayapkul *et al.* 2011; Ashtiani *et al.* 2012a, b). Silicon fertilization in rice has become a routine practice in the United States of America (USA), Florida with positive effects on

suppression of blast disease (Datnoff *et al.* 1997; Cai *et al.* 2008; Cacique *et al.* 2012). Nutrient management practices can also be useful in controlling rice blast fungus in Africa.

Wind can modulate the dynamics of *M. oryzae* populations in the field. Taguchi *et al.* (2014) investigated wind effects on blast incidence in Japan. They used different wind treatments on paddy fields in two successive seasons for controlling blast disease. Results showed a significant reduction in the incidence and severity of both leaf and panicle blast. Best results were recorded using wind treatments with a velocity of 3–6 m/s. Wind treatment was shown to be more effective than chemical fungicides in controlling leaf blast (Taguchi *et al.* 2014). This study shows the relative importance of wind as dispersal agents of *M. oryzae* and its effect on the transmission of rice blast. However, the mechanism of spores dispersal by wind remains unknown and the method difficult to apply on a large scale.

The variety mixture method (known as multi-lines) was also found to be efficient in China and South Asia (Boudreau 2013; Petrie and Bates 2017). This method combines different disease-resistant varieties that will actually constitute a heterogeneous environment for pathogens, such that the effect of plant varieties on pathogen performance, their frequencies, and their spatial arrangement would maintain the epidemic at a low level and hamper pathogen adaptation to varietal resistance. This strategy is easier than many others and can be introduced in Africa but would need to ensure their agronomic performance.

Chemical fungicides are widely used to combat blast disease in many parts of the world. Among the most currently used, one can cite Probenazole, Isoprothiolane, Carpropamid, Fenoxanil, Kresoxim methyl, *etc.* (Srivastava *et al.* 2017). The cost of their application in the field can reach $70 ha-1 (Nalley *et al.* 2016). Although fungicide application by foliar spraying, soil drenching, and seed treatment have been proven successful for controlling blast (Prabhu *et al.* 2003; Kunova *et al.* 2014; Chen *et al.* 2015), some active ingredients are no longer effective because of high pathogen diversity (Todorova and Kozhuharova, 2010; Ganesh *et al.* 2012; Hajano *et al.* 2012). Different fungicides with different modes of action are recommended to apply either in alternation or together in a mixture in order to delay the pathogen's resistance

development. However, these common practices are found to be environmentally harmful as fungicide residues can persist in the soil or migrate off-site and enter waterways (e.g. due to spray drift, run-off) (Wightwick *et al.* 2010). Also, many African farmers currently, live on small farms and have limited access to fungicides; Moreover, they lack the proper knowledge to effectively use them.

As opposed to synthetic fungicides, the use of organic compounds to control blast disease is an environmentally friendlier practice that represents fewer public health risks (Yokoyama 1981; Teng 1994). Some organic manure (Obilo *et al.* 2012) such as triterpenoid glycosides derived from alfalfa (*Medicago sativa*) (Abbruscato *et al.* 2014), neem (*Azadirachta indica*) seed extracts (Sireesha and Venkateswarlu 2013), and some essential oils or plant extracts with proven antifungal properties are used to control blast (Sun *et al.* 2014).

Antagonistic micro-organisms such as *Pseudomonas* spp., *Bacillus* spp. and *Streptomyces* spp. have been tested by spray drying as biological control agents of *M. oryzae* (Prabavathy *et al.* 2006; Tendulkar *et al.* 2007; Karthikeyan and Gnanamanickam 2008; Goud and Muralikrishnan 2009; Filippi *et al.* 2011; Khalil *et al.* 2014; Meng *et al.* 2015). They were shown to have the ability to synthesize antifungal compounds that inhibit mycelial growth of *M. oryzae*. A recent study demonstrated that microorganisms such as *Burkholderia* sp. isolated from the soil of Gotsu city in Japan could yield candidate control agents for plant diseases such as rice blast (Lemtukei *et al.* 2017). Antagonistic yeast isolates collected from fruit surfaces and rice leaves in Thailand have been shown to significantly reduce blast disease incidence in greenhouse and field conditions (Kunyosying *et al.* 2018). These biocontrol organisms occur naturally in the soil in the field environments. The non-native micro-organisms can adapt to new environments if applied as inoculants (Babu 2011; Raaijmakers and Mazzola 2012). But these practices are not used in Africa.

Blast control also depends to a great extent on the rice crop's genetic resistance. The use of resistant germplasm is an environmentally safe option for blast control. It is also much more effective and cheaper than using chemical fungicides (Tokunaga *et al.* 1965; Villareal *et al.*

1981; Koizumi and Kato 1987: Deepti *et al.* 2017). The blast control measure found effective and most utilized in Africa is rice genetic resistance. Deploying resistant varieties is especially advantageous in Africa because it requires no additional cost to farmers (does not require fungicide treatments), and is environmentally safe (Mew 1991). Furthermore, resistant varieties can be easily disseminated as seeds, leading to wide adoption (Bonman *et al.* 1992), whereas this deployment of different resistance genes is based on an understanding of the pathogen population structures (Leung *et al.* 1993; Leach *et al.* 2002; Leung *et al.* 2002).

Lots of science has been dedicated to the identification, cloning, and characterization of several resistance genes (R genes) for blast control. Considerable progress has been made to use R genes to develop blast-resistant rice hybrids adapted to different rice-growing regions worldwide (Suwarno *et al.* 2001; Narayanan *et al.* 2002; Abe 2004; Liu *et al.* 2008; Selvaraj *et al.* 2011). However, breeding for resistant rice takes a long time, and durability is short-lived (in general, two years) or not easily achieved (Suwarno *et al.* 2001; IRRI 2010).

Most improved varieties actually carry a few resistance genes in a uniform genetic background and are thus vulnerable to rapid adaptation of pathogens and pose uncertainty to farmers (Leung *et al.* 2003). Blast resistant varieties become susceptible over time as the fungus adapts its resistance mechanism. One of the strategies used to deal with a high diversity *of M. oryzae* populations is that If a variety becomes susceptible to blast, a new variety with different resistance genes is released to farmers (Leung *et al.* 2003). Continuous effort in the identification of new R genes is therefore critical for guaranteeing and bringing new effective genetic control against blast disease. Although the sequential release of varieties over time and space is one form of diversifying varieties or resistance genes used in the field, this strategy is valid only if the investment in developing a new variety is not out-balanced by the rapid loss of its usefulness. The system would also need to be supported by good race prediction and survey data (Leung *et al.* 2003).

All these methods, taken separately, are time-consuming and not sufficient to control blast disease completely. For example, a single change in crop management practices or in

resistance genes may result in significant blast epidemics, even after many years of successful disease control (TeBeest *et al.* 2007). This led to the development of Integrated Disease Management (IDM) strategies, which combine various methods of plant protection against blast. IDM became imperative for effective disease control of the entire complex of pests, diseases, and weeds encountered in vegetable crops. The development of IDM based control method of the blast is now viewed not only as an eco-friendly but also sustainable agriculture (Balgude and Gaikwad 2019).

Field trials were recently conducted by integration of cultural (e.g. soil application of rice husk ash (RHA) at sowing on raised beds (1 kg m^{-2}) + soil application of rice straw (RS) @ 2 tones ha^{-1} at transplanting, *etc.*), biological (*Pseudomonas fluorescens* 0.5%) and chemical (benomyl 0.3%) methods for rice blast management allowing disease reduction of 78.09% (leaf blast), 63.84% (neck blast) and 72.32% (node blast) with substantial increase in the grain yields (Balgude and Gaikwad 2019). In fact, rice straw used as compost replaces part of fertilizer used in the field, which reduces the susceptibility of the crop to blast and contributes to the plant growth.

2.5. Breeding strategy for blast resistance in rice

2.5.1. Traditional breeding approaches for blast resistance in rice

Breeding plants with resistance against a specific disease such as rice blast requires the identification of resistant plants, which are then crossed with high-yielding but usually susceptible plants. Blast disease has been under intensive field investigation and many rice varieties resistant against *M. oryzae* were identified from infested fields.

Plant disease defense involves various mechanisms triggered by both host plant and pathogen (Yang *et al.* 2013). Proper understanding of the relationship between pathogen virulence genes and plant resistance genes of the defense mechanisms would allow a more directed program for the development of durable resistance and for plant breeding. Many authors have reported that for each gene (resistance genes or R genes that condition resistance in the host

plant) there is a corresponding gene (avirulence genes or Avr-genes) in the pathogen that determines if the pathogen will be able to injure the plant (Flor 1956; Bonas and Lahaye 2002). In rice, scientists attempted to describe the specificity of the host-pathogen interactions between *Oryza sativa* and blast fungus, *M. oryzae* (Kiyosawa (1974; Silue *et al.* 1992). They found that the type of interaction between rice R genes and blast pathogen's avirulence genes (Avr gene) is the typical gene-for-gene system (Flor 1971; Silue *et al.* 1992) where a pathogen's Avr gene interacts with a corresponding rice R gene (Silue *et al.* 1992; Valent 1997; Zeigler *et al.* 1994) to confer resistance to the host. In fact, host resistance is triggered by the direct or indirect recognition of an avirulence gene product of the pathogen by an R gene product of the plant (the gene-for-gene concept) (Silue *et al.* 1992; Valent 1997). Several differential sets of cultivars known as differential varieties (DVs) with different single R genes (Odjo *et al.* 2016) have been selected for resistance genes characterization. DVs also can be used to screen *M. oryzae* isolates in view of differentiating pathogen races in terms of frequency of virulence and avirulence genes diversity (Flor 1945; Odjo *et al.* 2016). Through using DVs with known resistance, it is possible to study the reaction pattern of each variety (DVs) to blast isolates, which gives an indication of the presence or absence of the corresponding avirulence gene (Sere *et al.* 2004). Goto *et al.* (1961, 1964) first constructed a differential system and then cooperative research between the US and Japan developed an international differential system in 1967 (Goto 1967). However, these differential systems were not based on the gene for gene relationship between host and blast pathogen. Kiyosawa (1974) performed gene analyses of blast resistance and found many resistance genes in rice cultivars. Tsunematsu *et al.* (2000) and Telebanco-Yanoria *et al.* (2010) subsequently developed 23 monogenic lines and 2 near-isogenic lines (NILs), respectively as a new set of international DVs to contribute in understanding pathogenicity of *M. oryzae* and rice gene diversity. As these systems are based on the gene for gene concept and involve the majority of known R genes, they can be used for the monitoring of blast races on an international scale.

Pathogenicity is defined as the capacity of a microbe to produce a disease or to cause damage to a host (D'Arcy *et al.* 2011). Pathogenicity studies are crucial for developing strategies to ensure strong rice resistance (Xia *et al.* 2000). However, reliable pathogenicity tests are time-consuming (Kiyosawa 1980) since they require large population samples to ensure a complete array of pathotypes within the population. Pathotypes differ in their abilities to infect rice varieties, depending on what resistance gene is present in the host plant. Pathotype diversity tests of *M. oryzae* revealed the presence of variable numbers of four, 12, 20 pathotypes in Benin, Burkina Faso, and Ghana, respectively (Sere *et al.* 2007; Nutsugah *et al.* 2008; Koide *et al.* 2011a). However, it is difficult to compare these populations of pathotypes/races across countries since the authors used different sets of DVs harbouring different R genes.

Pathogenicity tests of a total of 152 blast isolates led to classify Benin blast pathogen into 134 races (Odjo *et al.* 2016). Amongst these, 26 blast races were selected to represent Benin differential system for blast pathogen races diversity. Mutiga *et al.* (2017) recently studied virulence diversity and virulence spectrum of a collection of 122 *M. oryzae* isolates from 9 sub-Saharan African (SSA) countries (Benin, Burkina Faso, Mali, Ghana, Kenya, Nigeria, Tanzania, Togo, and Uganda). The virulence spectrum was assessed by pathotype analysis with a panel of 43 rice genotypes consisting of differential lines carrying 24 blast resistance genes (R-genes), contemporary African rice cultivars, and susceptible controls. They found that 5 isolates were avirulent to all rice germplasm tested, whereas two isolates were virulent to 75% of the germplasm. Rice cultivar 75-1-127 (Pi9 donor) was resistant to all isolates (100%), followed by four African rice cultivars (AR105, NERICA 15, 96%; NERICA 4, 91%; and F6-36, 90%). An intense screening study for rice blast resistance was conducted in the Philippines using 4,246 rice accessions collected from diverse geographic origins worldwide. Screening germplasm for rice blast resistance was carried out under both fields and controlled conditions. The aim was to select candidate accessions that would be deployed as donors for blast resistance in breeding programs (Vasudevan *et al.* 2014). The study identified a set of

289 rice accessions with broad-spectrum blast resistance, some of which carried the Pi2 resistance gene.

More than 100 R genes (Wang *et al.* 2014) have been identified. Most have been utilized in rice blast resistance breeding programs (Causse *et al.* 1994; Wang *et al.* 1994; Nagato and Yoshimori 1998; Fukuoka and Okuna 2001; Yang *et al.* 2001; Berruyer *et al.* 2003; Pan *et al.* 2003; Fukuta *et al.* 2004; Liu *et al.* 2005; Gowda *et al.* 2006; Nguyen *et al.* 2006; Jueng *et al.* 2007; Liu *et al.* 2007). However, the majority of these R genes have been identified in *O. sativa* (Asian cultivated rice), more especially in rice cultivars from Japan and China, except for R genes Pi9, Pi54rh, Pi40(t), and Pirf2-1(t) identified in *O. minuta*, *O. rhizomatis*, *O. australiensis*, and *O. rufipogon*, respectively (Wang *et al.* 2014). In Benin, recent studies reported the emergence of new more aggressive *M. oryzae* populations capable to overcome blast resistance genes such as Pi1, Pi7, Pi5, Pikp, Pia, Pita2, Piks, Pi3 and Pik in northern (Kokey, Kandi and Bagou) and blast resistance genes such as Pi1, Pi7, Pi5, Pikp, Pia, Pita2, Piks, Pi3, Pik, Pita, Piz, Pikh and Pikm in southern part of this country (Odjo *et al.* 2011).

Conventional plant breeding has been going on for hundreds of years and is still commonly used for introducing new resistance gene variations via sexual hybridization of contrasting parental lines and mutations. Plants are generally bred for i) increased yield, (ii) increased tolerance to environmental stresses (salinity, extreme temperature, drought) combined with increased tolerance to pests and diseases and (iii) improved grain quality, such as increased nutritional values or better flavour. This is the case of Basmati-flavour varieties developed in India, a good example of plant breeding for Africa (Palanga *et al.* 2016). Over the last decades, scientists have tried to use conventional breeding methods such as the pedigree method, backcrossing, recurrent selection, and mutation breeding to generate hundreds of improved high-yielding rice varieties resistant to multiple diseases including blast (Khush 1989; Miah *et al.* 2013).

The vast majority of varieties of self-pollinating crop species have been developed through the pedigree method. Pedigree breeding consists of making a cross between two parents to

incorporate desirable traits into successive future generations by self-pollination until a set of lines that combines the specific desired characteristics of both parents is obtained. The latter method is suitable when resistance is linked with a single gene. It is mostly used for breeding for qualitative traits, such as disease resistance, or traits, such as plant architecture or the colour or shape of plant parts. However, the pedigree method requires land, much time, intense labour and is too often expensive to achieve results. Also, it cannot be used in environments where genetic variability for the trait is not expressed and may demand experienced persons for selecting inbred lines.

Backcrossing is the most common technique used in rice breeding for introgression or substitution of desired characters (genes) from a donor into a recipient parent. The high heritability of the characters being transferred is important. Then, the procedure would be to cross the progeny resulting from the first generation back to the same recipient parent. This process should be repeated generation after generation until the backcross progeny would be phenotypically similar to the recipient parent (Allard 1999; Xi *et al.* 2008). Backcrossing is the most indicated for both self- and cross-pollinated species. It can also be used to transfer recessive genes backcrossing but, the procedure is laborious, since it is necessary to self after the first backcross in order to obtain homozygotes in relation to desired genes for further backcrossing. Third and fifth backcross selections would similarly be selfed. An alternative method is to select several plants in each backcross progeny for further backcrossing and to obtain self-bred seed from each. However, backcross selection on a quantitative trait is less successful and requires to use molecular markers that facilitate to follow an efficient transfer of desired genes.

The recurrent selection has also been used as a method for breeding rice blast resistance (Fujimski 1979). Recurrent selection is defined as reselecting generation after generation, with intermating of the selected plant to produce the population for the next generation. This method consists of selecting any superior plants from base population and self-pollinating them to select superior plants at maturity and then, growing the progeny in the next season to self-

pollinate again and so on till the fourth or fifth generation. Recurrent selection is most practiced in both self- and cross-pollinated species. The method can be used to bring together desirable genes and improve the level of quantitative traits in a breeding population (Frey 1983). Recurrent selection is expected to ensure an increase of favorable alleles/genes frequency without reducing the population's genetic variability (Hallauer and Miranda Filho, 1981; Paterniani and Miranda Filho, 1987; Ribeiro *et al.* 2016). However, this method has not been extensively applied in rice breeding practices due to a lack of male-sterility genes and involves a lot of selection crossing and intense labour (Pang *et al.* 2017). Using this method, upland cultivar CG-91 was developed with resistance to rice blast (Courtois *et al.* 1997; Guimarães and Correa-Victoria 2000).

A gene mutation is a permanent, heritable alteration in the DNA sequence in a living cell. Mutation results in new traits that are passed on from parent to offspring and thereby, drives evolution (van Harten 1998; FAO/IAEA 2018). Mutation breeding can be described as the purposeful application of mutations in plant breeding. One of the problems often encountered in conventional crossbreeding is the poor combining the ability of some parental genotypes. Under such circumstances, mutation induction can be of advantage to produce cultivars with desired traits within defined germplasm pools (Pathirana 2011). For example, aromatic rice varieties have a poor combining ability, and cross-breeding with nonaromatic varieties will lead to a decrease in aroma and quality (Bourgis *et al.* 2008; Pathirana *et al.* 2009). In the latter case, mutation induction can be done to improve important traits, such as resistance to pests and diseases, improved physical grain characters and eating quality by using a variety of procedures. Of these, alkylating agents and ionizing radiation mutagenesis have been routinely used to develop lots of mutant rice lines with desirable traits (Pathirana 2011). Currently, scientific advances make it possible to detect mutated genes and pyramid them into a single breeding line and monitor them in subsequent breeding programs (Shu 2009). Many blast-resistant rice mutant lines have been developed in this way.

For example, a blast R gene allele was introduced into the high-yielding variety Ratna (IR8/TKm 6) using chemo-mutagenesis with 0.1% and 0.2% ethylmethane sulfonate (EMS) (Kaur *et al*. 1975). R917 is a *Japonica* rice mutant with a broad spectrum of resistance to blast and was selected after treatment of F1 seeds (by radiation with 10 Krad ^{60}Co γ-ray) from the cross between Chengte 232 and Xiushui 37 (Zhang *et al*. 2003). Mutant rice variety Zhefu 802 derived from the blast-resistant variety Simei 2 was also developed by gamma-ray irradiation (Ahloowalia *et al.* 2004). The disadvantage of mutation breeding is its limited power in generating dominant alleles that might be desired; it is also less effective than cross-breeding for a trait that needs a combination of multiple alleles, such as tolerance to abiotic stresses. The low mutation frequency requires growing and screening a large population for the selection of desired mutants at reasonable confidence. This becomes very expensive for traits that have to be evaluated through laborious phenotypic analysis (Shu 2009).

2.5.2. Molecular breeding approaches for blast resistance in rice

Despite the fact that conventional breeding has helped develop blast-resistant rice varieties, the process is laborious, time-consuming and highly dependent on environmental conditions as well (Werner *et al.* 2005; Zhang *et al*. 2006). Molecular breeding which is also an important component of any crop improvement program has led to the identification of a large number of blast resistance genes and their effective incorporation into germplasm. At least 347 quantitative trait loci (QTLs) and 102 single blast R genes (Table 2.3) have been identified and mapped in the rice genome (Khanna *et al.* 2015; Zheng *et al.* 2016; Khan *et al.* 2018). Blast R genes actually cover all 12 rice chromosomes. Out of these, chromosome 11 contains the largest number of known blast R genes, followed by chromosome 12, whereas only 1% located on chromosomes 3 and 7 (Ashkani *et al.* 2016). Moreover, a number of 27 blast R genes (Pita, Pib, Pb1, Pizt, Pid2, Pii, Pikm, Pit, Pid3, Pid3-A4, Pish, Pik, Pikp, Pia, PiCO39, Pi1, Pi2, Pi5, Pi9, Pi21, Pi25, Pi33, Pi36, Pi37, P50, Pi54, and Pi65(t)) have been successfully cloned and used in rice breeding programs.

Molecular markers are important developments in the field of plant breeding (Kebriyaee *et al.* 2012). A variety of molecular markers (Table 2.3) have been identified to be linked with rice blast resistance genes (Ashkani *et al.* 2014; Tanweer *et al.* 2015). The usefulness of a given molecular marker is dependent on its capability in revealing polymorphisms in the nucleotide sequence allowing discrimination between different molecular marker alleles. These polymorphisms are revealed by molecular techniques such as random amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), single-nucleotide polymorphism (SNP), small insertions/deletions (InDels), and cleaved amplified polymorphic sequences (CAPS) (Fukuta *et al.* 2009; Wang *et al.* 2014).

Recent advances in genomic sequencing also provided valuable information for the discovery, validation, and assessment of new molecular markers (Perez-de-Castro *et al.* 2012; Sahebi *et al.* 2015). A successful application of these molecular markers to assist breeding procedures for blast resistance has become possible through various techniques such as Marker-Assisted Selection (MAS), Quantitative Trait Locus (QTL) analysis, gene pyramiding, genetic transformation techniques, *etc.* However, despite the facility of breeding by molecular markers, a phenotypical validation is still necessary.

Table 2.3. Summary of blast R (major and minor, mapped and cloned) genes on rice chromosomes

Chr.	Name of R Gene	Name of germplasm	Map position (cM)	Marker name	Type of marker
1	Pit	Tjahaja , BL10, K59, Tongil	12.2	t311, t256, t8042	SNP
1	Pi27(t)	Q14	28.4-38.8	RM151, RN259	SSR
1	Pi24(t)	Azucena	64.4		
1	Pitp(t)	Tetep	114.1	RM246	SSR
1	Pi35(t)	Hokkai 188	132.0-136.6	RM1216	SSR
1	Pi37	St. No. 1	136.1	RM302,RM212	SSR

Chr.	Name of R Gene	Name of germplasm	Map position (cM)	Marker name	Type of marker
1	Pish	Shin 2, Nipponbare, Pi No. 4, Fukunishiki, Norin 22,, Kusabue, BL1, Akihikari	148.7-154.8		
2	PiDa(t)	Dacca6	10.8-14.4	RM211, RM5529	SSR
2	Pid1(t)	Digu	87.5-89.9	RM262	SSR
2	Pig(t)	Guangchangzhan	142.0-154.1	RM166, RM208	SSR
2	Pitq5	Teqing	150.5-157.9	RG520, RZ446b	
2	Piy1(t)	Yanxian No.1	153.2-154.1	RM3248, RM20, RM208	SSR
2	Piy2(t)	Yanxian No.1	153.2-154.1	RM3248, RM20, RM208	SSR
2	Pib	Tohoku IL9	154.1	RM138, RM166, RM208	SNP, SNP, SSR, SSR, SSR
2	Pi25(t)	IR64	157.9		
2	Pi14(t)	Maowang	0-197		
2	Pi16(t)	AUS373	0-197		
3					
4	pi21	Owarihatamochi *Japonica*	58.6	P702D03_#79	STS
4	Pikur1	Kuroka	86		
4	Pi39(t)	Chubu 111 (Haonaihuan)	107.4-108.2	RM3843, RM5473, RM3743, RM5473	SSR
4	Pi(t)		22-122		
5	Pi26(t)	Azucena	22.5-24.7		
5	Pi23(t)	Sweon 365	59.3-99.5		
5	Pi10	Tongil	88.5-102.8	OPF62700	InDel
6	Pi22(t)	Sweon 365	38.7-41.9		
6	Pi26(t)	Gumei 2	51.0-63.2		
6	Pi27(t)	IR64	51.9		
6	Pi40(t)	IR65482-4-136-2-2 (Acc100882)	54.1-61.6	RM3330, RM527, S2539	SSR, CAPS
6	Piz-5	Tadukan	58.7		
6	Piz	Zenith	58.7	z60510, z5765, z56592, z565962	SNP

Chr.	Name of R Gene	Name of germplasm	Map position (cM)	Marker name	Type of marker
6	Piz-t	Toride 1	58.7	z60510, z5765, zt56591, zt5659	SNP
6	Pi9	75-1-127 (101141)	58.7		
6	Pi25(t)	Gumei 2	63.2-64.6		
6	Pid2	Digu	65.8		
6	Pigm(t)	Gumei 4	65.8	C26348, S47656	CAPS, InDel
6	Pitq1	Teqing	103.0-124.4		
6	Pi8	Kasalath	98-98		
6	Pi13(t)	Maowang	63.2-69.6		
6	Pi13	Kasalath	16-90		
7	Pi17(t)	DJ 123	94.0-104.0		
8	Pi36	Q61	21.6-25.2	RM5647, CRG2, CRG3, CRG4	SSR, CAPS
8	Pi33	IR64, Bala	45.4	RM72, RM44	SSR
8	Pizh	Zhai-Ye-Quing	53.2-84.8		
8	Pi29(t)	IR64	69		
8	PiGD-1(t)	Sanhuangzhan 2			
9	Pii2(t)	Ishikari shiroke	0-72		
9	Pi5(t)	RIL125, RIL249, RIL260 (Moroberekan)	31.3-33.0	94A20r, 76B14f, 40N23r, JJ817 (SNP)	CAPS, SNP
9	Pi3(t)	Pai-Kan-Tao	31.3-33.0		
9	Pi15	GA25	31.3-34.9		
9	Pii	Ishikari shiroke			
10	Pi28(t)	Azucena	114.7		
10	PiGD-2(t)	Sanhuangzhan 2			
11	Pia	Aichi Asahi, CO39, Zenith	36	yca72	CAPS
11	PiCO39(t)	CO39	49.1	RGA8, RZ141, RGACO39	CAPS
11	Pilm2	Lemont	56.2-117.9		
11	Pi30(t)	IR64	59.4-60.4		
11	Pi7(t)	RIL29 (Moroberekan)	71.4-84.3		
11	Pi34	Chubu 32	79.1-91.4		
11	Pi38	Tadukan	79.1-88.7	RM206, RM21	SSR
11	PBR	St No.1	80.5-120.3		
11	Pb1	Modan	85.7-91.4		

Chr.	Name of R Gene	Name of germplasm	Map position (cM)	Marker name	Type of marker
11	Pi44(t)	RIL29 (Moroberekan)	91.4-117.9		
11	Pik-h	Tetep	101.9	RM206, RM144 , RM224, RM1233	SSR
11	Pi1	C101LAC (Lac23)	112.1-117.9		
11	Pik-m	Tsuyuake	115.1-117.0	k641, k6441, k4731, k7237	SNP
11	Pi18(t)	Sweon 365	117.9		
11	Pik	Kusabue	119.9-120.3	k6438, k6415, k8823, k8824	SNP
11	Pik-p	HR22	119.9-120.3	k641, k39575, k403, k3957	SNP
11	Pik-s	Shin 2	115.1-117.3	RM144 , RM224, RM1233	SSR
11	Pik-g	GA20			
11	Pise1	Sensho	39.8-64.8,62.5-81.5		
11	Pi f	Chugoku 31-1 (St. No.1)			
11	Mpiz	Zenith			
11	Pikur2	Kuroka	35.3-69.3,58-86		
11	Piisi	Imochi shirazu			
12	Pi24(t)	Zhong 156	10.3		
12	Pi62(t)	Yashiromochi	12.2-26.0		
12	Pitq6	Teqing	29.2-47.5		
12	Pi6(t)	Apura	32.6-63.2		
12	Pi12(t)	RIL10 (Moroberekan)	42.8-53		
12	Pi21(t)	Sweon 365	43.4-59.6		
12	Pi31(t)	IR64	44.3		
12	Pi32(t)	IR64	47.5		
12	Pi12(t)	K80 (Hong-jiao-zhan)	47.6-48.2		
12	Ipi(t)		47.6-58.3		
12	IPi3(t)		47.6-58.3		
12	Pi157	Moroberecan	49.5-62.2		
12	Pita	Taducan	50.4	ta642, ta801, ta3, ta577, ta5	SNP
12	Pita-2	Shimokita	50.4	ta642, ta801/RM155, RM7102	SNP, SSR
12	Pi19(t)	Aichi Asahi	32.6-63.2		

Chr.	Name of R Gene	Name of germplasm	Map position (cM)	Marker name	Type of marker
12	Pi39(t)	Q15	50.4	39M6, 39M7	CAPS
12	Pi20(t)	IR24	51.5-51.8	RM1337, RM5364 , RM7102	SSR
12	PiGD-3(t)	Sanhuangzhan 2	55.8		

Chr.= chromosome. Sources: Fukuta *et al.* (2009) and Wang *et al.* (2014)

2.5.2.1. Marker-assisted selection

Marker-assisted selection (MAS) is a marker-based breeding strategy used to select plant progenies that contain the wanted gene to be transferred from a donor variety by using molecular markers closely linked to this gene. MAS is an approach that has been developed to avoid the problems connected with conventional plant breeding changing the selection criteria from a selection of phenotypes towards the selection of genes, either directly or indirectly (Francia *et al.* 2005). Traditionally, plant breeders select the desired progeny based on plant phenotypes. With the availability of an array of molecular markers and genetic maps, MAS offers better selection strategies both for traits governed by major genes as well as for quantitative trait loci (QTLs) than conventional phenotypic selection in term of precision, efficiency and time saving (Young 1996; Koide *et al.* 2010; Zhu *et al.* 2012). Different methods that use polymerase chain reaction (PCR)-based DNA markers have been developed for MAS (Hayashi *et al.* 2006; Ashkani *et al.* 2011). MAS has shown to be effective for the development of several improved rice varieties (Wang *et al*. 2007; Ashkani *et al*. 2012) with resistance genes such as Pita (Rybka *et al.* 1997), Piz (Conaway-Bormans *et al.* 2003), Pi37 (Chen *et al.* 2005), Pi35 (Nguyen *et al.* 2006) and Pi1, Pi9 (Du *et al.* 2007).

Marker-Assisted Back-crossing (MAB) is a traditional breeding method commonly employed to transfer resistance genes from an agronomically inferior source (the donor parent) into an elite variety (the recurrent parent) by monitoring the genes with markers in the breeding process (Allard 1960; Reyes-Valdés 2000). The progeny resulting from the first generation is crossed back to the same recipient parent so that to recover the recurrent parent genotype

using only two or three backcrosses. MAB has been employed to improve elite rice varieties such as KDML105, Basmati and Manawthukha for their resistances to blast in the South and Southeast Asia (Joseph *et al.* 2004; Toojinda *et al.* 2005; Sreewongchai *et al.* 2010; Hasan *et al.* 2015). Improvement of blast resistance of rice variety named Feng39S was also found to be successful through molecular marker-assisted backcrossing. Feng39S possesses high yield potential with good grain quality but was susceptible to blast. It was thus used as the recurrent parent in this study. With this marker-assisted backcrossing method, the blast resistance gene Pi2 from rice variety Hua1201S (donor parent) was transferred into Feng39S to improve its resistance (Yang *et al.* 2019).

2.5.2.2. Quantitative trait locus (QTL) analysis

Researchers have long observed that Instead of the qualitative resistance characterized by the complete inability of a pathogen to infect plants, plants can carry resistance that is phenotypically incomplete varying their susceptibility, which is known as partial or quantitative resistance (Ou 1985; Wang *et al.* 1994; Niks *et al.* 2015).

More and more breeders are recognizing the use of this quantitative resistance (QR) as a valuable approach to protect crops (Niks *et al.* 2015). Often, QR remains durably effective, which is the primary driver behind the interest in it. In fact, QR is regulated by several genes known as QTLs, whereas qualitative resistance is regulated by one or two genes, i.e. major gene(s), (R genes) (McCouch *et al.* 1994; Young 1996; Niks *et al.* 2015). QTL genes, act against a broad range of pathogen races, each gene contributing a small proportion of the resistance level (Song and Goodman 2001; Wisser *et al.* 2005; Roy-Chowdhury *et al.* 2012b; Wang *et al.* 2013). Thus, a pathogen able to suppress a certain defense gene may not defeat all QR (Niks *et al.* 2015).

By opposite, resistance genes (R gene) such as Pia, Pib, Pii, Pi-km, Pi-t, Pi12(t) and Pi19(t) confer pathogen race-specific resistance (Ashikawa *et al.* 2008; Yang *et al.* 2008; Hayashi and Yoshida 2009). We point out that a number of blast R genes in rice such as Pi-1(t), Pi2, Pi9, Pi20(t) Pi27(t), Pi39(t), Pi40(t) and Pikh may also confer resistance to multiple

pathogen races (Mackill and Bonman 1992; Chen *et al.* 1996; Liu *et al.* 2002; Zhu *et al.* 2004; Jeung *et al.* 2007; Liu *et al.* 2007; Li *et al.* 2008; Yang *et al.* 2008; Tacconi *et al.* 2010). Therefore, monogenic resistance (one gene) is in itself not sufficient to qualify a resistance as qualitative. Moreover, QR would not rule out that some plant accessions may have only one significant minor-effect gene contributing to the resistance (Talukder *et al.* 2004; Lopez-Gerena 2006). Four QR genes have been identified and described as specific: Pif (Yunoki *et al.* 1970), Pi21 (Fukuoka *et al.* 1997), Pb1 (Fujii *et al*. 1995) and Pi34 (Zenbayashi-Sawata *et al*. 2005).

Many genes for QR to rice blast have been mapped (Wang *et al.* 1994; Fukuoka and Okuno 2001; Miyamoto *et al*. 2001; Tabien *et al.* 2002; Zenbayashi *et al.* 2002; Chen *et al.* 2003; Sallaud *et al.* 2003; Talukder *et al.* 2004; Wu *et al.* 2005; Ashkani *et al.* 2013a,b).

Mapping genes that confer QR enabled marker-assisted selection (MAS) to introduce certain quantitative genes into a large number of cultivars (Ashkani *et al.* 2015). There are two types of genome maps: genetic maps that provide landmark locations or markers and physical maps that provide both markers and genetic sequences between these landmarks.

One type of quantitative resistance against blast was found in the durably resistant cultivar IR64, which confers protection against many *M. oryzae* races found in Asia, Latin America and Africa (Sharma *et al.* 2012; Waiyalert *et al.* 2015). This IR64 has recently been used as a gene donor to confer a strong resistance in RD15, the most cultivated rice variety used in Thailand and in many other countries (NBACFS 2003; Wangsawang *et al.* 2018). Pi-b and Pi-kh were pyramided into Malaysian MR219 rice variety to make resistance durably effective against blast (Tanweer *et al.* 2015).

2.5.2.3. Gene-pyramiding

Gene-pyramiding is a breeding method aimed at combining multiple desirable genes from multiple parents into a single genotype (Joshi and Nayak 2010). Pyramiding different sources of resistance has become an important strategy to achieve broad-spectrum and durable

resistance in rice, and thus giving crop protection against many diseases including blast (Fu *et al.* 2012; Ashkani *et al.* 2015). Based on an evolutionary risk assessment model, it has been argued that rice blast and bacterial blight (*Xanthomonas oryzae*) can be managed by gene pyramiding, because it is unlikely that a sequence of multiple virulence mutations will occur in the same clonal lineage of the pathogen (McDonald and Linde 2002). Past experience, however, suggests that gene pyramids are most likely to be effective for controlling bacterial blight but less so for blast due to the high level of pathogenic variation exhibited by *M. oryzae* (Leung *et al.* 2003).

Owing to the dominance and epistasis effects (masking effect of one gene over the others), of resistance genes, it is difficult to pyramid genes using conventional breeding methods (Suh *et al.* 2015). With a marker-based selection, multiple genes can be identified in a single genotype (Nelson 1996). Molecular markers can be successfully used to identify and pyramid desirable and multiple alleles for blast resistance.

The gene-pyramiding process is more efficient when combined with the use of linked DNA markers. MAS breeding provides a new opportunity for the selective transfer of desirable genes that confer tolerance to multiple stress factors (Jairin *et al.* 2009). Molecular markers precisely estimate the introgression of chromosome segments from donor parents and can speed up the recipient genome recovery via backcrossing (Jairin *et al.* 2009; Suh *et al.* 2009).

Three R genes (Pi1, Piz-5, and Pita-2) were pyramided into rice varieties using MAS for broad-spectrum resistance against different *M. oryzae* races (Hittalmani *et al*. 2000). Many other successful cases of gene pyramiding in rice using MAS have been reported (Chen *et al.* 2004; Pinta *et al.* 2013; Fukuoka *et al.* 2015; Suwannual *et al.* 2017). The effectiveness of MAS depends on the availability of closely linked DNA markers for the target locus, the size of the population, the number of backcrosses, and the position and number of markers for background selection (Frisch and Melchinger 2001; Suh *et al.* 2015).

A review of the literature indicates that better blast disease management can be achieved by using multi-lines/cultivar mixtures than with pure stands (Mundt 1994; Koizumi 2001). Rice varietal mixtures are commonly used in traditional rice culture in Asia and Africa (Bonman *et al.* 1986). Winterer *et al.* (1994) argued that a varietal mixture is the best strategy for rice blast as compared with gene pyramids and gene rotation. Chin and Husin (1982) showed that a mixture containing 66% resistance component lines was adequate to control blast, whereas Koizumi (2001) found that 75% resistance in a multiline was required to reduce severity to the level of control achieved by fungicide treatments.

Cultivars mixture, multi-lines, near-isogenic lines or resistance inbred lines (NILs, pure lines) are reported to be effective approaches for disease management (Joshi *et al.* 2014). The adoption of these multiline varieties led to durable control of blast resistance in Japan (Ashizawa *et al.* 2007; Fukuoka and Okuno 2019). According to Mackill and Bonman (1992), allelism tests of blast resistance genes would be much easier in NILs than in donor cultivars where resistance is often conferred by two or more major genes, thus allowing more accurate determination of the number and distribution of these resistance genes. Nils could be also used as an improved differential set for describing *M. oryzae* isolates Mackill and Bonman (1992).

A number of nine near-isogenic lines (NILs) for blast resistance genes has been developed by using *an Indica*-type elite rice variety, IR49830-7-1-2-2, suitable for the rainfed lowland conditions in the tropics, as a genetic background through recurrent backcrossing. These NILs carry eight resistance genes (Pik, Pi7(t), Pi3, Pi5, Pita-2, Piz-5, Pish, and Pi9). The introgression of each resistance gene in the NILs was confirmed by reaction patterns to specific blast isolates, allelism tests, and DNA marker analysis (Koide *et al.* 2011b). Besides, multiline varieties of “Koshihikari”, the leading varieties of Japan, were effectively used in Niigata and Toyama prefectures, with different gene components to control rice blast (Kojima *et al.* 2004; Ishizaki *et al.* 2005).

2.5.2.4. Genome editing and conventional transgenic approaches for rice blast resistance

In recent years, sequence-specific genome editing technologies were found to be useful tools for crop improvement (Georges and Ray 2017). Different from human cells, genome editing in plants has typically made use of transgenes that specify the expression of the genome-editing machinery such as TALENs or Cas9 and its guide RNAs. In particular, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein9 (Cas9) genome editing technology (CRISPR/Cas9) has so far shown the greatest promise for addressing emerging challenges in agriculture. This technology can be used to modify almost any genomic sequence to achieve desired traits in organisms including plants, with the only known limitation being the availability of the protospacer adjacent motif (PAM) sequence. Compared to other genome editing tools such as zinc-finger nucleases (ZFNs) and transcriptional activator-like effector nucleases (TALENs), CRISPR/Cas9 is easier, more cost-effective, precise and is highly efficient even at multiplex genome editing (Wang *et al.* 2018).

Using this CRISPR/Cas9-based technology, Wang et al. (2016) developed mutagenized rice lines possessing enhanced blast resistance (Wang *et al.* 2016). They engineered the CRISPR/Cas9 vector (pCERF922) to target the rice OsERF922 gene, which negatively regulates rice blast resistance (Liu *et al.* 2012). In this process, pC-ERF922 was delivered into calli derived from seeds of blast susceptible rice variety Kuiku131 by Agrobacterium-mediated transformation with a mutagenic frequency of 42.0% in T_0 transgenic plants. Edited plants that harbored the desired modification in the OsERF922 were identified in the T_1 and T_2 segregating populations. The edited lines showed significantly enhanced blast resistance compared with wild-type. This study provided a successful example of improving PTI to enhance rice blast resistance using CRISPR/Cas9 (Haque *et al.* 2018).

Recently, Ma *et al.* (2017) used CRISPR/Cas9 to knock out the function of OsSEC3A gene in rice and demonstrated that rice plant that lacks a functional copy of OsSEC3A provide better defense response as well as enhanced resistance to the fungal pathogen *M. oryzae*, which causes rice blast disease (Haque *et al.* 2018).

In 2018, Foster *et al.* demonstrated a co-editing strategy for the creation of single nucleotide changes at specific loci in *M. oryzae*. Their study showed that the stable expression of Cas9 is highly toxic to *M. oryzae*. Yamato *et al.* (2019) developed a novel genome editing strategy by using Single crossover-mediated targeted nucleotide substitution and knock-in strategies with CRISPR/Cas9 system in the model filamentous fungus *M. oryzae*. Using this strategy, these authors demonstrated highly efficient and freely programmable base substitutions within the desired genomic locus, and target gene disrupted mutants were also obtained via a shortened (100–1000 bp) single homology arm. Furthermore, this method allowed a one-step GFP gene knock-in at the C-terminus of the targeted gene.

In conventional transgenic methods, genes that encode desired traits are inserted into the genome at random locations through plant transformation (Lorence and Verpoorte 2004). These methods typically result in varieties containing foreign DNA. In contrast, genome editing allows changes to the endogenous plant DNA, such as deletions, insertions, and replacements of DNA of various lengths at designated targets (Barrangou and Doudna, 2016). Depending on the type of edits introduced, the product may or may not contain foreign DNA.

Researchers have long observed that transgenic plants expressing genes derived from pathogens often display immunity to the pathogen and its related strains (Lomonossoff 1995). Trangenic expression of antifungal protein from *Aspergillus giganteus* conferred robust resistance to rice blast disease (Coca *et al.* 2004). The developed transgenic plants showed stable integration as well as inheritance of the transgene. In another study, the development of improved Basmati rice against fungal infection through gene transfer technology has been reported. Introduced RCC2 gene (for blast fungus resistance gene) by Agrobacterium-mediated transformation showed significant levels of resistance in Basmati rice under the laboratory and field conditions (Asghar *et al.* 2007). Studies by Bundo and Coca (2015) evidenced an over-expression of calcium-dependent protein kinase (OsCPK4) in transgenic rice, which led to resistance against blast. In 2017, transgenic rice plants having two elicitor

genes (MoHrip1 and MoHrip2) from *M. oryzae* showed elevated resistance against rice blast and higher tolerance to drought stress (Wang *et al.* 2017).

CHAPTER THREE

3. Exploring genetic diversity and disease response of cultivated rice (*Oryza* spp.) accessions against *Magnaporthe oryzae* under rainfed upland conditions in Benin

Based on a research article published as:

> Exploring genetic diversity and disease response of cultivated rice accessions against *Pyricularia oryzae* under rainfed upland conditions in Benin. Yelome OI, Audenaert K, Landschoot S, Dansi A, Vanhove W, Silue D, Van Damme P, Haesaert G. Genet. Resour. Crop Evol. (2018) 65:1615-1624.

Abstract

The main goal of this study is to gain insight into the relationship between the genetic profile of cultivated rice (*Oryza* ssp.) accessions and their resistance to rice blast. We, therefore, examined the genetic and phenotypic variability of a set of 350 African cultivated rice accessions. Most of them were collected from Benin, Burkina Faso, Guinea Conakry, Democratic Republic of Congo, Ivory Coast, Mali, and Nigeria. We assessed genetic variation to classify our germplasm collection by using seventy-seven fluorescent amplified fragment polymorphism (AFLP) markers. In addition, we evaluated the germplasm for its resistance to blast disease caused by *Magnaporthe oryzae* in upland field conditions. We observed huge differences in responses of our rice accessions to blast in the field. They ranged from highly susceptible to highly resistant. Twelve percent of all accessions were highly resistant to *M. oryzae*. Based on their AFLP marker profile, these highly resistant accessions could be separated from the other ones. Stepwise regression revealed that the best prediction of blast resistance level was achieved with a maximum number of 13 AFLP markers. Marker CTA22 was the most accurately predicted blast resistance and was present in all highly resistant accessions. It can be concluded that AFLP markers are a valuable tool to screen rice accessions for resistance to blast disease and that, based on a subset of markers, it is possible to predict the resistance to rice blast.

Keywords: Fluorescent-AFLP; genetic diversity; rice; blast

3.1. Introduction

Blast disease, caused by the filamentous ascomycete fungus *Magnaporte oryzae*, anamorph *Pyricularia oryzae* Cav. (Couch 2002) is one of the most important biotic constraints to rice production in Benin (Vodouhe *et al.* 1981; Afouda *et al.* 2007). It is the most widespread disease in African rice production causing yield losses of up to 100 % (Séré *et al.* 2013). At present, blast disease poses a significant threat to rice food security in Benin (Odjo *et al.* 2011). In Benin, blast disease hotspots have recently been mapped by the Africa Rice Center (AfricaRice) and partners, revealing the presence of isolates that belong to different pathotypes (Odjo *et al.* 2011, 2016). Extensive and uncontrolled use of fungicides is injurious to human health and poses environmental safety concerns. Host resistance in rice cultivation has been recognized to be the most economical and effective way of controlling blast and reducing the need for chemical applications. More than 100 single resistance genes (R genes) and around 347 quantitative trait loci (QTL) have been identified till now, whereas a number of them were mapped on rice chromosomes (Ballini *et al.* 2008; Sharma *et al.* 2012; Khan *et al.* 2018). Unfortunately, country-specific field evaluations of a set of differential lines that carry most of these identified genes demonstrated the capacity of unidentified virulent *M. oryzae* races to overcome a number of rice resistance genes (Pi1, Pi7, Pi5, Pikp, Pia, Pita2, Piks, Pi3, Pik, Pita, Piz, Pikh and Pikm) in Africa (Baboy *et al.* 1995; WARDA 1999; Odjo *et al.* 2011).

M. oryzae is highly variable, and loss of resistance in varieties is quite common (Zeigler *et al.* 1994; Jia *et al.* 2003). The latter often appears when resistance is based on a single R gene (Mackill and Bonman 1992; Dai *et al.* 2010). Scientists argued that each of the R genes known so far only acts against a subset of *M. oryzae* isolates. Over time, varieties may thus become susceptible when new, more virulent isolates will occur. As a result, continuous search for new R genes and their pyramiding into genotypes is necessary for efficient disease management (Wang *et al.* 2010; Roy-Chowdhury *et al.* 2012b). The approach of gene pyramiding will confer overlapping resistance spectra to multiple isolates/races of *M. oryzae* (Hittalmani *et al.* 2000). Also, it was shown that quantitative resistance that confers broad-spectrum resistance is

controlled by several minor or major genes, durably protects the host plant against *M. oryzae* (Wisser *et al.* 2005; Roy-Chowdhury *et al.* 2012b; Wang *et al.* 2013).

There are only two types of cultivated rice species in the world: *Oryza sativa* L. and *O. glaberrima* Steud. Both are commonly grown in Africa. *O. glaberrima* was domesticated in West Africa approximately 3,500 years ago (Jones et *al.* 1997). This indigenous rice possesses useful traits such as weed competitiveness, tolerance to various abiotic stressors (drought, iron, acidity, salinity) as well as pest and disease resistance (Pham 1992; Jones *et al.* 1997; Futakuchi & Sie 2009; Sié *et al.* 2010; Thiemele *et al.* 2010). However, *O. glaberrima* is becoming rare in African cropping systems where it has mostly been replaced by *O. sativa* cultivars due to the former's negative traits such as poor yield, grain shattering and poor lodging resistance (Linares 2002; National Research Council 1996). *O. sativa* rice comprises two major groups: i) the *Japonica* group with sticky, short-grained genotypes that are mainly cultivated in upland conditions; and ii) the *Indica* group with non-sticky, long-grained genotypes that are mostly grown in lowlands. Asian rice was introduced into West Africa some 500 years ago by Portuguese sailors. Today, most varieties grown in Africa belong to the latter species.

Successful artificial interspecific hybridizations of both *O. glaberrima* and *O. sativa* have led to a series of popular hybrids known as NERICA ("New Rice for Africa"), which combine the resilience of the African species with the productivity of the Asian ones (Pham 1992; Jones *et al.* 1997; Futakuchi & Sie 2009; Sié *et al.* 2010; Thiemele *et al.* 2010).

To date, only limited information about agricultural characteristics such as pest and disease resistance of the African rice gene pool is available. Germplasm without consistent data on disease and pest tolerance is of little value for breeding programs and sustainable paddy production.

The present work studies genetic variation of a set of cultivated rice accessions (*O. sativa* and *O. glaberrima*) of diverse African provenances (Benin, Mali, Nigeria, Ivory Coast, *etc*.) and examines their reactions to blast disease infestation. The linkage between genetic

structure/markers and disease resistance is also investigated. Finally, the germplasm subset was proposed for a thorough evaluation of their agronomic traits.

3.2. Material and methods

3.2.1. Source of the germplasm collection

The 350 rice (5 *O. sativa* and 345 *O. glaberrima*) accessions used in this study (Table A.1, appendix), were obtained from the germplasm collection of AfricaRice, a Consultative Group for International Agricultural Research (CGIAR) center located in Cotonou, Benin. Three reference rice accessions (WAB0006871 and WAB0007880 from *O. glaberrima* species and WAB0006881 from *O. sativa* species) were also included in the study as taxonomic and blast-resistant references. WAB0007142 (CO 39) and GSOR301253 (Maratelli) were included as blast susceptible references. All these accessions originated from Benin, Burkina Faso, Guinea Conakry, Democratic Republic of Congo, Ivory Coast Mali and Nigeria (Table A.1, appendix)

3.2.2. DNA extraction and AFLP analysis of rice accessions

Genomic DNA extraction from each accession was done according to the CetylTrimethyl Ammonium Bromide (CTAB) method described by Saghai-Maroof *et al.* (1984). Five seeds from each of the 353 rice samples were grown in a greenhouse at 27 °C. Approximately 200 mg of leaf samples were harvested on 21 days-old seedlings and ground in liquid nitrogen in 2 mL of CTAB using a pestle and mortar. Then, 500 µL of each of the obtained suspensions were transferred to centrifuge tubes and incubated in a water bath for 30 min at 60 °C. An equal volume of chloroform:isoamyl alcohol (24:1, v/v) was added to each tube. The suspension was gently mixed by inverting the tubes. Tubes were centrifuged at 12,000 rpm for 10 min and the supernatant recovered and mixed with an equal volume of ice-cold isopropanol. DNA was recovered as a pellet by centrifugation at 12,000 rpm for 5 min, washed with 100 µL of 70 % ethanol, dried under vacuum and dissolved in 30 µL of TE buffer. Two µL of RNase

were added to the DNA and incubated for 1 h. DNA concentrations were measured using the Quantus™ Fluorometer (Promega) and adjusted to approximatively 250 ng/µL.

AFLP polymorphic markers were used to analyze genomic DNA according to the modified protocol described by Vos *et al.* (1995). To this end, DNA (250 ng/µL) was completely digested with 5 U of both MseI and EcoRI restriction enzymes and ligated to 5 pmol EcoRI and 25 pmol MseI adapters. Pre-amplification of ligation products was done with specific EcoRI and MseI primers without additive nucleotides, and over 20 cycles of 94 °C during 30 s, 56 °C during 60 s, and 72 °C during 60 s were run in a thermal cycler. The concentration of the amplified DNA was checked on a 1.5 % agarose gel. Next, DNA was diluted 25 times in TE buffer. For selective amplification, three primer combinations with three selective bases at the end (EcoRI + ACC/MseI + CTC, EcoRI + ACC/MseI + CTA, EcoRI + ACC/MseI + CAT) were used to reveal a maximum number of polymorphic markers in the rice samples. EcoRI primer was end-labeled with NED-fluorescent dye. Amplified products were mixed with GeneScan-500 ROX size standard (Applied Biosystems Inc., USA) and deionized formamide. Finally, they were loaded on an Applied Biosystems electrophoresis instrument 3130 for electrophoretic size separation with a performance-optimized polymer (POP 4).

3.2.3. Field screening for resistance to blast in Benin

Benin has two rainy seasons during which blast disease can naturally occur. The AfricaRice Cotonou experimental site was identified as a disease hotspot (Odjo *et al.* 2011). Two field experiments were successively conducted under upland rainfed conditions in 2015 during the two rainy seasons, i.e. from April to July and from August to October, respectively. The first experiment was implemented using an augmented randomized complete block design with 17 control varieties replicated 10 times in the design, whereas an Alpha Lattice design with four replicates was used during the second experiment. For each of the 350 test rice accessions, 18 seeds were sown in a plot of three, 50 cm long rows with a spacing of 20 cm between rows and 10 cm between plants. Fourteen days prior to sowing of the latter accession seeds, three infesting rows, consisting of three blast-susceptible reference varieties (Maratelli, CO39 and

IRBLTA2-PI) were sown perpendicularly to test plots in order to spread the disease. Thinning of rice plants was done 14 days after sowing to maintain only one seedling per hill. A pre-drilling base application of 300 kg/ha of NPK fertilizer (15-15-15) was applied at the sowing of the test accessions. Next, 300 kg/ha of urea was applied in two equal splits at 21 and 42 days after sowing. Hand-weeding and watering were done as per need. No treatment against diseases or pests was done. Disease symptoms were scored on the 12 inner plants of each row of each plot leaving aside the first and last plant in each row. Scoring was done weekly starting from the appearance of the first symptoms until harvest. The IRRI (2013) 1-9 scale was used and four to five scoring events were performed during each of the two experiments. Mean Disease Index (DI) was obtained by calculating the average disease scores for each accession. Four disease resistance classes were considered: resistant (R): DI<3, moderately resistant (MR): 3≤DI<4, moderately susceptible (MS): 4≤DI<5 and susceptible (S): DI≥5. A subset representing the genetic diversity of the total germplasm was then selected according to their blast response and variability for AFLP markers for efficient use in crop improvement programs. Actions were taken to avoid duplicating the most similar rice accessions in order to constitute a subset with maximum genetic diversity with minimum repetitiveness.

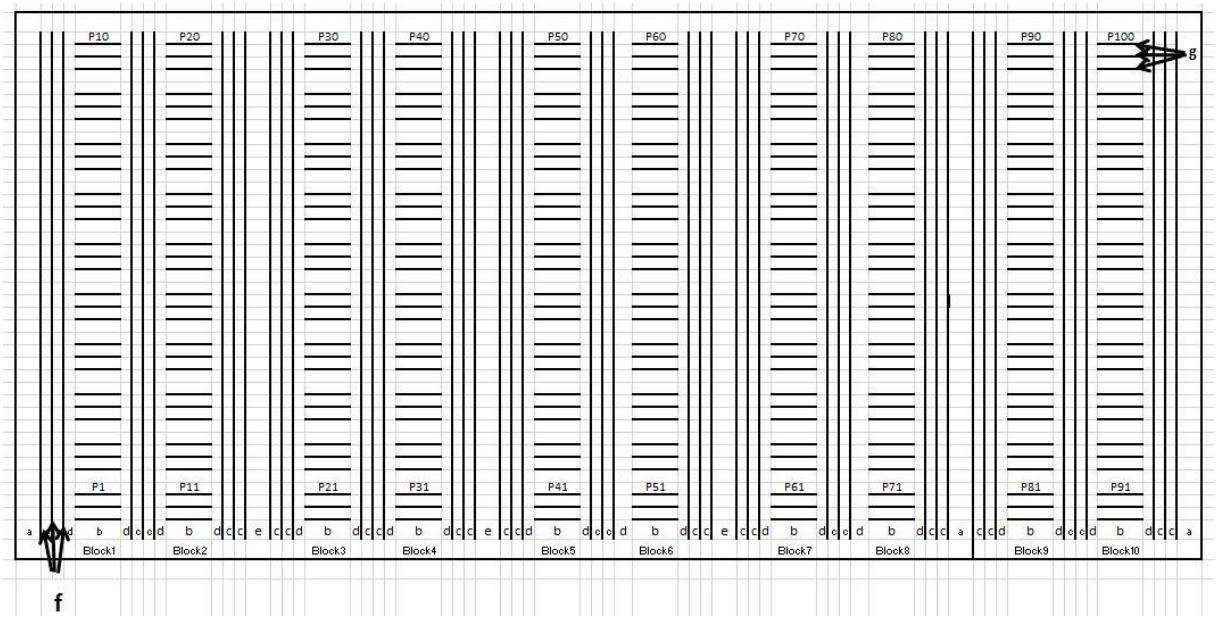


Figure 3.1. General view of the experimental layout

a: 1 m; b: 50 cm; c: 10 cm; d (distance between infesting band and entries): 20 cm; e: 1 m;

a: 1m path;

b: Length of a plot = 50 cm;

P: Plot;

c: Distance between rows and infesting band;

d: Distance between entries and infesting band;

e: 1m path;

f: Infesting band (3 different accessions are sown on each of the 3 rows);

g: Number of plants per row (6 plants per row x 3 rows = 18 plants per plot)

3.2.4. Statistical data analysis

AFLP electropherograms were analyzed by GeneMapper Software Version 4.0 using the GeneScan -500 ROX marker in the 30-500 size range. Genotyping results were automatically compiled in a standard binary Excel format (1; present, 0: absent). Polymorphism Information Content (PIC) values were calculated according to Botstein *et al.* (1980). Genetic dissimilarity coefficients using Jaccard distance were calculated to determine the genetic relationship between accessions. The phylogenetic clustering tree was constructed using Unweighted Neighbor-Joining (UWNJ) and the clusters determined according to the axis origin, the main node that directly links them. Analysis of molecular variance (AMOVA) was carried out to estimate molecular diversity at each hierarchical level among and within groups identified and

genetic diversity parameters calculated using GenAlEx 6.502. DI values were subjected to Linear Discriminant Analysis (LDA) with identified polymorphic AFLP markers based on continuous and categorical approaches using the R software (R Core Team, 2017) to define the linear combination of AFLP markers which best separate accessions according to blast resistance. In addition, stepwise regression models were constructed for selecting an appropriate subset of markers to predict rice blast resistance. The germplasm subset was constituted based on geographical origins, pairwise genetic distances analysis (as revealed by 77 AFLP markers) and differential reactions to blast disease. This subset was created stepwise, by selecting from each genetic group formed by UWNJ method, the most genetically distant accessions, as possible. The selection was done so that it included blast-resistant accessions more than susceptible ones in order to maintain a high diversity of blast resistance genes in this subset. We also selected the entries of this subset according to their geographic provenance so that the origin diversity is represented as well as possible. For the choice of these entries at least 30 accessions were considered so that we can create genetic populations within the selection set.

3.3. Results

3.3.1. Genetic diversity detected by AFLP markers among assembled rice germplasm

The three primer combinations generated a total of 14,506 bands, out of which 72 % were polymorphic at 77 different loci (putative loci/genome landmarks). Average Polymorphic Information Content (PIC) detected was 24 ± 4 % (standard deviation (SD)). Primer combination EcorI-ACC:Mse-CAT appeared to be the most-informative with a PIC of 29 % (Table 3.1). Jaccard's genetic dissimilarity coefficients ranged from 0.03 to 0.98 across our germplasm collection.

UWNJ tree based on Jaccard's dissimilarity coefficients derived from the 77 polymorphic AFLP markers evidenced three main genetic clusters: one with 184 accessions including *O.*

glaberrima control WAB0006871; a second with 83 accessions including the only *O. sativa* controls (WAB0006881, Moroberekan), and a third with 86 accessions including *O. glaberrima* control WAB0007880 (Figure 3.2). Dissimilarity coefficients varied from 0.05 to 0.84 in cluster 1 and from 0.03 to 0.59 in cluster 3, whereas a larger range was observed in cluster 2 (0.03 to 0.98). The highest Shannon's diversity index (*I*) was found within cluster 2 with *I* = 0.4 followed by cluster 1 with 0.32, whereas a much smaller *I*-value was observed in cluster 3 (0.26). Expected heterozygosity averaged 0.26, 0.21 and 0.17 in clusters 2, 1 and 3, respectively (Table 3.2). A high proportion of genetic diversity was most apparent in cluster 2, which comprised all *O. sativa* accessions, including control variety WAB0006881. Cluster 1 and cluster 3 contained exclusively *O. glaberrima* accessions. AMOVA used to determine the percentage of genetic variation within and among the three identified clusters (Table 3.3), revealed that within-cluster diversity explained most of the genetic variation (77 %), whereas only 23 % of the total variance was due to differences between the three clusters ($P < 0.001$).

Table 3.1. Results of polymorphism detection for each primer combination

Primer combinations	Number of loci detected	Number of polymorphic loci	Number of total bands	Number of polymorphic bands	Percentage of Polymorphism (PP, %)	Polymorphic Information Content (PIC, %)
ACC-CAT	34	34	4918	3900	79	29
ACC+CTC	28	24	4475	3092	69	20
ACC+CTA	27	22	5113	3392	66	23
Total	89	77	14506	10384	71	73

Table 3.2. Level of genetic variability estimated among accessions of the three clusters identified

Cluster	Number of accessions	Dissimilarity range	I	He
1	184	0.05-0.84	0.32	0.21
2	83	0.03-0.98	0.40	0.26
3	86	0.03-0.59	0.26	0.17

I = Shannon's diversity Index; He = Expected Heterozygosity

Table 3.3. AMOVA analysis for cluster differentiation by AFLP markers

Source of variation	df	SS	MS	Estimated variance	Percentage of total variance	Probability
Among clusters	2	535.68	267.84	2.4	23%	
Within clusters	350	2820.29	8.06	8.06	77%	P<0.001
Total	352	3355.98		10.46	100%	

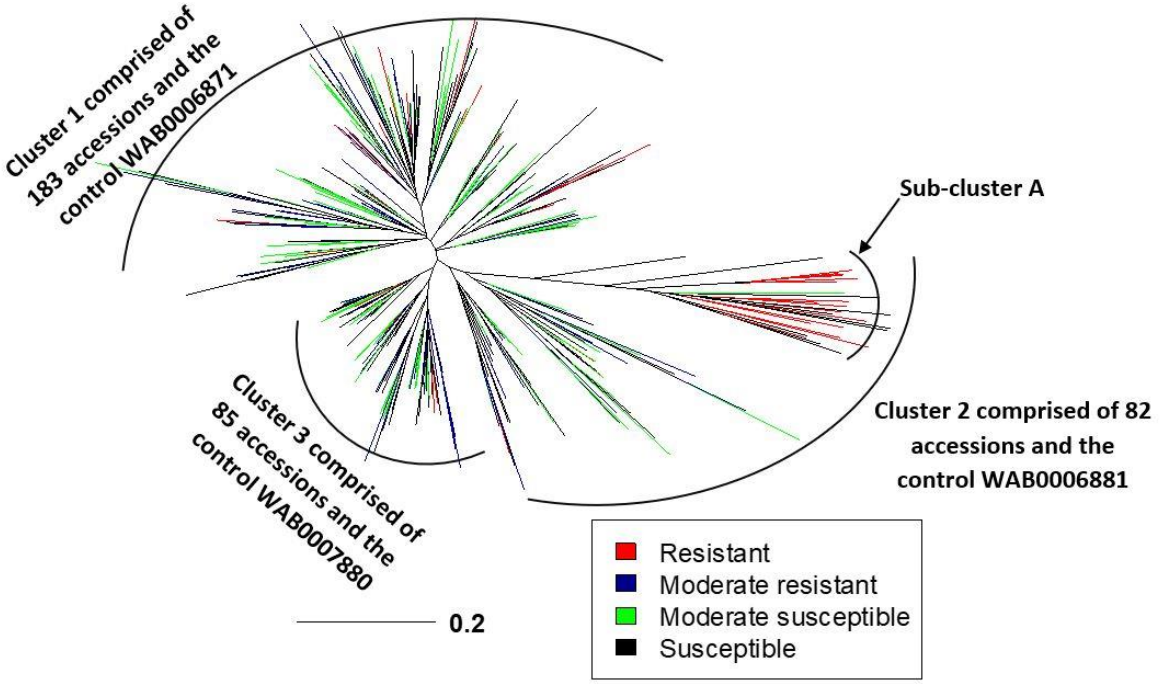


Figure 3.2. UWNJ tree cluster analysis showing accession reaction to blast disease. Colours indicate disease status: Resistant accessions (Severity<3) are presented in red; Moderate resistant (3≤Severity<4) in blue; moderate susceptible (4≤Severity<5) in green and Susceptible (Severity≥5) are indicated in black

3.3.2. Frequency of blast disease among 350 cultivated rice accessions

DI ranged from 0 to 9 in the first experiment, whereas for the second experiment, DI ranged from 0.33 to 7 (Figure 3.3). A significant positive association ($R^2 = 0.64$, $P < 0.001$) was found for blast disease indices between the two successive field experiments. In other words, blast disease score distributions were similar in the two experiments. However, we observed that overall disease pressure was lower during the second experiment. Nevertheless, the resistance/susceptibility status of our accessions did not change. Average disease scores recorded on the susceptible control varieties WAB0007142 (CO 39) and GSOR301253 (Maratelli) were 8 and 7.6, respectively. Out of the total 350 test rice accessions, 12 % were highly resistant, 24 % moderately resistant, 29% moderately susceptible and 35% susceptible. The highest number of resistant accessions was found in cluster 2 (20), whereas cluster 1 contained the highest number of moderately resistant rice accessions (50).

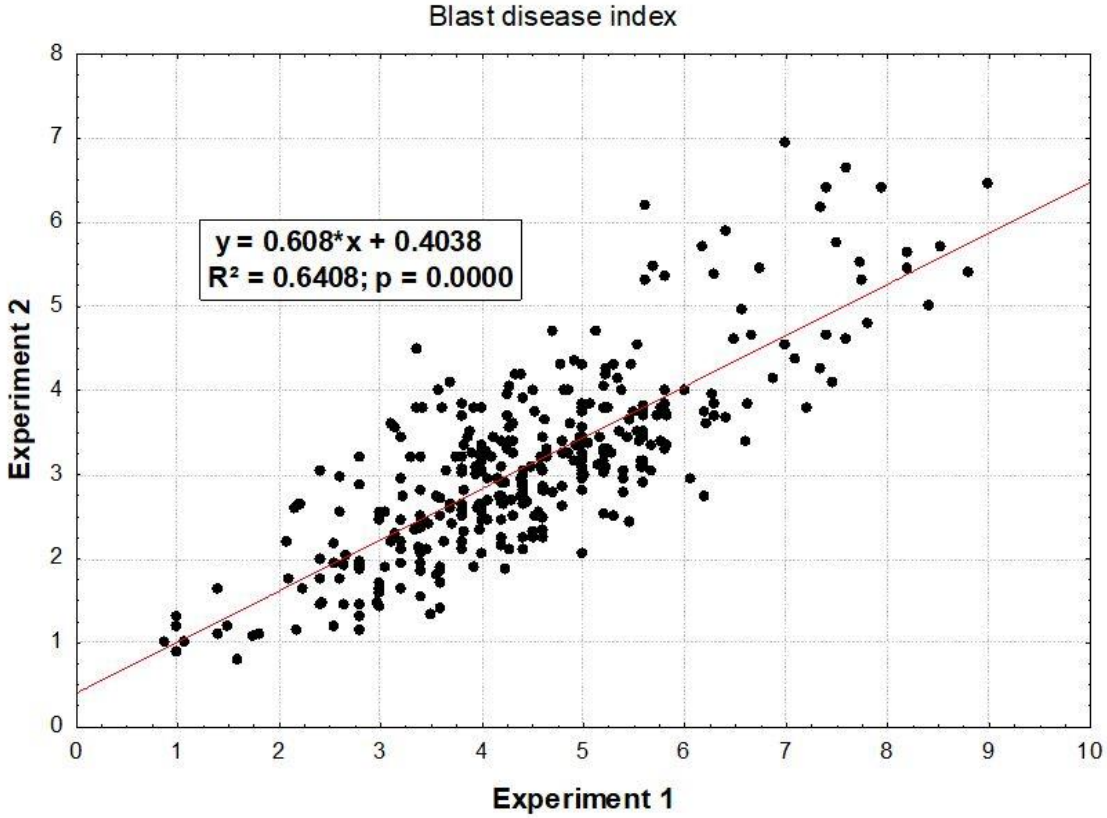


Figure 3.3. Correlation of disease severity means of two field experiments for blast resistance screenings carried out in Benin from April to October 2015

3.3.3. Relationship between AFLP markers and blast disease resistance/susceptibility

Among the major genetic clusters identified based on 77 polymorphic AFLP markers, we evidenced a sub-cluster A within cluster 2 that contained the highest number of resistant accessions compared to clusters 1 and 3. We concluded the existence of strong genetic affinity among these rice accessions that show the same disease reaction, which gives an indication of the fact that AFLP markers may help to predict blast (Figure 3.2). Linear Discriminant Analysis (LDA) using all 77 AFLP markers was performed (Figure 3.4) revealing a significant association of AFLPs to accessions' reactions in the field ($R^2 = 0.37$). A series of models was performed to better examine genetic diversity relationship with blast disease. Stepwise regression analysis revealed that only 13 out of 77 polymorphic markers were necessary to accurately explain accessions' reactions patterns (Table 3.4). Based on these 13 most-significant AFLP markers, we attempted to predict three disease classes (Resistant: DI<3, moderate resistant: 3≤DI<4 and susceptible with DI≥4) using a General Linear Model (GLM). This GLM showed significant coefficient values ($P < 0.05$) for a total of seven markers linked with disease scores (Table 3.5). The probability of accession assignment to the "right" disease class was 68 %. Better results were obtained when we considered only two disease classes (DI < 3 and DI ≥ 3). In case this binary classification was used, the algorithm was able to explain 90 % of the responses with a number of eight significant markers associated (Table 3.6).

Among the significant markers, CTA22-$_{366}$ with a size of 366 base pairs was exclusively found in highly resistant rice accessions. Marker utility was such that at the molecular level when CTA22-$_{366}$ was present, the observed status of the rice accessions was always scored as resistant. But when this marker was absent, we found that rice accessions were either resistant or susceptible. A representative sub-set of 42 rice accessions (12 % of total germplasm) of the total of 350 rice accessions studied was proposed (based on their molecular variation and

diversified blast resistance responsiveness) for further evaluation purposes. This selected rice sub-set consisted of 26 resistant, 9 moderately resistant and 7 susceptible accessions.

Table 3.4. Stepwise regression revealed 13 AFLP markers associated with blast disease

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	4.4103	0.1636	26.956	< 2e-16 ***
CAT7	0.7149	0.1772	4.034	6.77e-05 ***
CAT10	-0.6234	0.1981	-3.147	0.00179 **
CAT19	-3.4332	0.679	-5.056	7.02e-07 ***
CAT20	1.403	0.5495	2.553	0.01111 *
CAT21	-0.4396	0.1924	-2.285	0.02294 *
CAT29	4.4162	0.7253	6.089	3.08e-09 ***
CAT32	-0.8009	0.18	-4.449	1.17e-05 ***
CAT34	-0.4286	0.1402	-3.056	0.00242 **
CTC12	0.3687	0.1429	2.58	0.01030 *
CTC15	-1.4344	0.5746	-2.496	0.01303 *
CTA20	0.472	0.159	2.968	0.00321 **
CTA22	-2.2543	0.4733	-4.762	2.84e-06 ***
CTA27	1.7764	0.5479	3.242	0.00130 **

Table 3.5. Association (R^2) of AFLP loci with blast severity split into three main disease clusters: resistant (R), moderate resistant (MR) and susceptible (S)

AFLP loci	Multiple R^2	dl Modèle	F	P
CTA22	0.190130	2	41.08409	0.000000
CAT19	0.132719	2	26.77993	0.000000
CTA27	0.090294	2	17.36995	0.000000
CTC15	0.072827	2	13.74579	0.000002
CAT20	0.048344	2	8.89004	0.000171
CAT32	0.035157	2	6.37667	0.001905
CAT29	0.031221	2	5.63982	0.003884
CTC12	0.015694	2	2.79024	0.062773
CTA20	0.013683	2	2.42774	0.089720
CAT10	0.013050	2	2.31387	0.100389
CAT21	0.009757	2	1.72437	0.179797
CAT34	0.009265	2	1.63662	0.196122
CAT7	0.001562	2	0.27381	0.760639

Table 3.6. Association (R^2) of AFLP loci with blast severity split into two main disease clusters: resistant (R), and susceptible (S)

AFLP loci	Multiple R^2	df	F	P
CTA22	0.187690	1	81.10116	0.000000
CAT19	0.128381	1	51.69885	0.000000
CTA27	0.086193	1	33.10718	0.000000
CTC15	0.070069	1	26.44748	0.000000
CAT20	0.046012	1	16.92900	0.000048
CAT29	0.024686	1	8.88392	0.003078
CTC12	0.014893	1	5.30636	0.021832
CTA20	0.013635	1	4.85198	0.028264
CAT10	0.008857	1	3.13671	0.077416
CAT21	0.008448	1	2.99064	0.084626
CAT34	0.004771	1	1.68261	0.195430
CAT32	0.004114	1	1.44982	0.229368
CAT7	0.001260	1	0.44268	0.506267

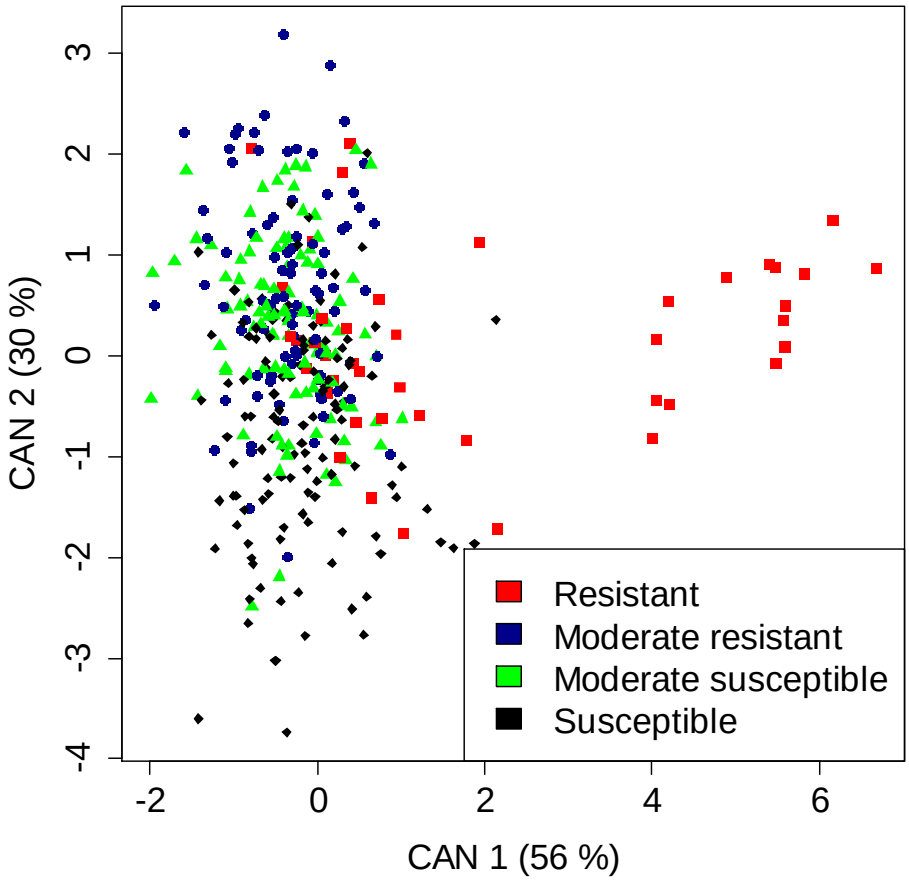


Figure 3.4. Genetic differentiation for the response of 350 rice accessions to blast disease by Linear Discriminant Analysis using AFLP markers. Colours indicate disease status: Resistant accessions (Severity<3) are presented in red; Moderate resistant (3≤Severity<4) in blue; moderate susceptible (4≤Severity<5) in green and Susceptible (Severity≥5) are indicated in black.

3.4. Discussion

The PIC value obtained for the studied germplasm collection (mean = 24 %) was lower than those reported by Semon *et al.* (2005) and Dramé *et al.* (2011) who assessed the genetic diversity of 198 and 82 African rice accessions, respectively, using Simple Sequence Repeat (SSR) markers. They reported PIC values of 34 % and 52 %, respectively. According to Vieira *et al.* (2016), there are some advantages of using SSR markers to detect polymorphisms due to their multi-allelic and codominant nature. Furthermore, we examined here a higher rice accession number (350) than in studies by Semon *et al.* (2005) and Dramé *et al.* (2011). Our germplasm collection might have contained more genetically similar samples.

Partitioning the molecular variance present in our germplasm collection indicated significant genetic variation both among (23 %) and within (77 %) clusters. Genetic diversity was found to be higher within than between clusters. Earlier studies have also reported in African cultivated rice, the existence of high amount for within-group variance, which ranged from 71 % to 95 % (Ming *et al.* 2010; Salem and Sallam 2016; Ndjiondjop *et al.* 2017).

In our study, all *O. sativa* rice accessions including the *O. sativa* reference variety WAB0006881 were classified into cluster 2. The highest level of genetic diversity was detected in cluster two. This *O. sativa* reference variety could have contributed largely to genetic diversity observed in cluster 2. Many previous studies have noted higher levels of genetic diversity in *O. sativa* than in *O. glaberrima* (Li *et al.* 2011; Wang *et al.* 2014). We also found that within cluster 2, sub-cluster A, which discriminated 20 *O. glaberrima* rice accessions together with all six *O. sativa* rice accessions. As it sometimes happens that farmers grow both species in the same field, this study supports the hypothesis that a gene flow might have occurred between *O. sativa* and *O. glaberrima*. Spontaneous interspecific hybridization is not uncommon in rice fields (Second *et al.* 1982; Barry *et al.* 2007; Nuijten *et al.* 2009). When it occurs, pollen flow is usually from *O. sativa* to *O. glaberrima* (Sano *et* al; 1989). According to Jusu (1999) and Semon et *al.* (2005), interspecific hybridizations may result in new varieties.

Among all screened rice accessions, 12 % were highly resistant and 24 % were moderately resistant to blast disease. The other accessions were moderately susceptible or susceptible (29 % and 35 %, respectively). Results suggest a large genetic variability in blast resistance in our germplasm collection. Accessions showing high blast resistance might possess a single resistance gene. According to Vasudevan *et al.* (2014) quantitative blast resistance, which is controlled by multiple genes should be looked for the category of moderately resistant accessions. Out of 353 rice accessions tested (including the three controls), 50 were moderately resistant in cluster 1, whereas only 15 and 23 moderately resistant were found in clusters 2 and 3, respectively. Results from this study suggest a higher level of quantitative resistance within cluster 1 than within the other clusters. But we need first to confirm the results

by artificial inoculation using specific Beninese blast isolates for comparing with present levels of field resistance. Also, there is a need to quantify the yield potential of these resistant accessions for breeding purposes; genebanks and breeders will more likely use *O. glaberrima* germplasms when they would have a high-yield (Wambugu *et al.* 2013). The remaining, lower-yielding resistant germplasm would then be used by breeders as resistance gene donors.

Diversity in cultivated rice accessions was found to correlate well with varying phenotypic blast disease reactions. Based on their AFLP pattern, susceptible and resistant accessions could be distinguished. A number of AFLP markers were significantly associated with blast resistance. These associations were strongest with the CTA22-$_{366}$ marker. The use of these markers in screening tests for blast resistance will be examined through future genetic studies by developing hybrid individuals and backcross populations.

This study suggested a total of 13 AFLP markers that have the potential to select a highly variable rice germplasm subset that is small enough to evaluate and would facilitate the conservation and use in future breeding programs. Particularly, subset selection will help formulate future strategies for improving rice blast resistance. Plant genetic diversity information is important pre-breeding information for selecting parents that can be used in intra- and inter-crossing to broaden the genetic base of modern rice cultivars (Thakur *et al.* 2015).

3.5. Conclusions

The present study identified three different genetic groups among the 350 rice accessions studied based on AFLP-marker analysis. A large proportion of our total rice germplasm (36%) was resistant to blast and this was accurately discriminated by AFLPs. Results are relevant for effective germplasm management and use and may also contribute to better blast disease management in Benin. AFLP appears to be a very useful molecular tool for exploring rice genetic diversity and patterns with blast resistance. Information can be integrated into breeding for new resistant rice varieties: the choice of donor parents could be based on the knowledge of these accessions' genetic groups. However, further studies are needed to clarify which

tightly linked AFLP markers can reliably predict blast phenotypes and might be used for MAS. A subset of 42 accessions, with variable phenotypes, was designed as a core collection for further evaluation and for future use in breeding programs. This new germplasm collection is an added value for broadening horizons towards the identification of new blast resistance genes.

CHAPTER FOUR

4. Analysis of population structure and genetic diversity reveals the existence of gene flow and geographic patterns in cultivated rice (*O. sativa* and *O. glaberrima*) in West Africa

Based on a research article published as:

Analysis of population structure and genetic diversity reveals gene flow and geographic patterns in cultivated rice (*O. sativa* and *O. glaberrima*) in West Africa. Yelome OI, Audenaert K, Landschoot S, Dansi A, Vanhove W, Silue D, Van Damme P, Haesaert G. Euphytica(2018) 214:215.

Abstract

In order to fully exploit the diversity of the African rice germplasm, it is necessary to generate reliable information on population genetic diversity and phenotypic characteristics of the available gene pool. In this chapter, we investigated the population structure and genetic diversity of 42 cultivated African rice (*Oryza* spp.) accessions originating from West Africa (Benin, Mali, Nigeria, Ivory Coast, Liberia, and Guinea) using 20 Simple Sequence Repeat (SSR) and 77 Amplified Fragment Length Polymorphism (AFLP) markers. Additionally, field trials were set up to gain insight into phenotypic characteristics that differentiate the genetic populations these rice accessions belong to. The analysis revealed considerably high polymorphisms for SSR markers (Polymorphism Information Content mean = 0.78) in our germplasm collection. A significant association was found between AFLP markers and the materials' geographic respective origin (R = 0.72). Germplasm structure analysis showed that *O. sativa* accessions were not totally isolated from *O. glaberrima* accessions. Our results allowed to identify five *O. glaberrima* accessions which grouped together with a number of *O. sativa* acquisitions, sharing common alleles on 18 loci out of the 20 SSR markers analyzed. Population structure analysis further revealed the existence of gene flow between *O. sativa* and *O. glaberrima* accessions, whereby these progenies combined several traits of interest for breeding programs. Further studies are needed to characterize the valuable traits derived from this gene flow. They could concern resistance against abiotic and/or biotic factors including disease resistance.

Keywords: amplified fragment length polymorphism; genetic diversity; gene flow; rice; simple sequence repeat.

4.1. Introduction

Rice (*Oryza* spp.) is one of the world's most important self-pollinating diploid food crops that provides a significant part of staple food for billions of people (Chang 1976a; Kubo and Purevdoj 2004). Genus *Oryza* is composed of two domesticated (*Oryza sativa* L. and *O. glaberrima* Steud.) and 22 wild species representing ten rice genome types (Vaughan *et al*. 1994; Ge *et al*. 2001; Ammiraju *et al*. 2010; USDA-ARS 2013). Both domesticated species are grown in West Africa (Nuijten *et al*. 2009) with *O. glaberrima* being indigenous to the continent (Portères 1956; Angladette 1966). Also, six out of the ten known genome types are present on the African continent and show that Africa has a very rich gene pool that would be adapted to its growing conditions (Sanchez *et al*. 2013; Wambugu *et al*. 2013).

Many *O. glaberrima* and *O. sativa* cultivars have been collected in the past along with wild species (*O. barthii*, *O. longistaminata*, *O. punctata, O. brachyantha and O. eichingeri*) (over 20,000 rice accessions in total). They have been conserved in the AfricaRice genebank, a Consultative Group for International Agricultural Research (CGIAR) (Sié *et al.* 2012). However, because of the lack of knowledge of their genetic characteristics, they are still only limitedly used in breeding for sustainable production (Roy-Choudhury *et al*. 2014). Today, most *O. glaberrima* cultivars have been replaced with *O. sativa* ones because of the latter's higher productivity and grain yield (Linares 2002; National Research Council 1996). Teeken *et al*. (2012) reported that only limited research has been conducted to genetically improve *O. glaberrima*, whereas more attention was paid to *O. sativa* general improvement. Given the known *O. glaberrima* potential to adapt to a wide range of adverse environments (Pham 1992; Jones *et al*. 1997a; Futakuchi and Sie, 2009; Sié *et al*. 2010; Mokuwa *et al*. 2013), strategies for a better valorization of its cultivars must be encouraged.

Recently, we initiated screenings of selected rice accessions (345 *O. glaberrima* and five *O. sativa* accessions) for their resistance to blast disease in upland conditions in Benin (See chapter 3). We evidenced a close relationship between genetic diversity based on AFLP markers and blast resistance. We, therefore, undertook the present work for further examining

the genetic structure and diversity, and relationships among this same set of African rice germplasm. The characterization of genetic diversity can serve as a basis for formulating strategies to expand this diversity and to broaden the gene pool through diverse hybridization schemes (Nisar *et al*. 2008, Salem and Sallam 2016). Furthermore, both the relationship between genetic structure and geographic distribution and the level of the gene flow among rice accessions will be examined in our work to constitute a solid knowledge base for genebank management. Knowledge of this genetic structure is very important for rice breeding and conservation. It can guide the selection of genetically distant genotypes to incorporate a higher variation into segregating populations (Sié *et al*. 2012). Investigations to evidence gene flow may also serve for introducing potentially adaptive alleles into populations, and thus increasing genetic diversity. Contrary to natural selection, gene flow distributes, homogenizes, and maintains genetic variation (North *et al*. 2011; Sexton *et al*. 2011; Sork 2016). Molecular SSR and AFLP markers (Vos *et al*. 1995; Vieira *et al*. 2016) have been widely used for screening, characterizing and evaluating genetic diversity in rice. In this chapter, we report the use of both molecular markers along with a number of morphological traits to investigate the genetic diversity of our germplasm collection, which will inform further germplasm management activities.

The objectives of this part of our study are to (i) assess genetic diversity and phylogenetic relationship of an African rice accession selection collected from Benin, Mali, Nigeria, Ivory Coast, Liberia, and Guinea; (ii) examine the relationship between genetic diversity and geographic origin of these rice accessions, and (iii) determine the genetic structure and level of gene flow in the germplasm. This information will be important for the development of strategies aiming, among others, at selecting high-yielding accessions with strong blast disease resistance.

4.2. Material and methods

4.2.1. Plant material

The germplasm of *Oryza* spp. included in this work is a subset of 42 rice accessions (five *O. sativa* and 37 *O. glaberrima* accessions) collected from six West African countries (Table 4.1). This subset of rice accessions is derived from a wider collection of 350 African rice accessions that was constituted based on geographical origins, pairwise genetic distances analysis (revealed by 77 AFLP markers) and differential reactions to blast disease. The accessions' resistance levels can be found in the appendix, Table 4.1. For the 350 accessions collection, the average Polymorphic Information Content (PIC) was 24 ± 4 %, whereas Jaccard's genetic dissimilarity coefficients ranged from 0.03 to 0.98 (See chapter 3). Among the selected germplasm, we identified several accessions with different field blast resistance: 26 were highly resistant, 9 moderately resistant, 3 moderately susceptible and 4 susceptible (Table 4.1).

Table 4.1. The subset of 42 African rice accessions and reference controls used.

S/N	Accession number	Species name	Country of origin	Reaction to field blast
1	WAB0015772	*O. s*	Benin	R
2	WAB0006684	*O. s*	Benin	R
3	WAB0035038	*O. s*	Benin	R
4	WAB0035055	*O. s*	Benin	R
5	WAB0035059	*O. s*	Benin	R
6	WAB0008937	*O. g*	Guinea	R
7	WAB0015043	*O. g*	Ivory Coast	R
8	WAB0032497	*O. g*	Liberia	R
9	WAB0032495	*O. g*	Liberia	R
10	WAB0008956	*O. g*	Liberia	R
11	WAB0029182	*O. g*	Mali	R
12	WAB0023837	*O. g*	Mali	R
13	WAB0024105	*O. g*	Mali	R
14	WAB0024116	*O. g*	Mali	R
15	WAB0029194	*O. g*	Mali	R
16	WAB0032487	*O. g*	Mali	MR
17	WAB0032298	*O. g*	Mali	R
18	WAB0032848	*O. g*	Mali	R
19	WAB0026783	*O. g*	Mali	R
20	WAB0002093	*O. g*	Mali	MR

S/N	Accession number	Species name	Country of origin	Reaction to field blast
21	WAB0002136	*O. g*	Mali	R
22	WAB0002143	*O. g*	Mali	R
23	WAB0002145	*O. g*	Mali	MS
24	WAB0032345	*O. g*	Mali	MR
25	WAB0026176	*O. g*	Mali	R
26	WAB0029335	*O. g*	Nigeria	MR
27	WAB0030263	*O. g*	Nigeria	S
28	WAB0008589	*O. g*	Nigeria	MR
29	WAB0020477	*O. g*	Nigeria	MR
30	WAB0032397	*O. g*	Nigeria	R
31	WAB0029323	*O. g*	Nigeria	MR
32	WAB0029333	*O. g*	Nigeria	MR
33	WAB0019882	*O. g*	Nigeria	R
34	WAB0029315	*O. g*	Nigeria	MR
35	WAB0020505	*O. g*	Nigeria	MS
36	WAB0015703	*O. g*	Nigeria	R
37	WAB0001360	*O. g*	Nigeria	S
38	WAB0029342	*O. g*	Nigeria	S
39	WAB0032230	*O. g*	Nigeria	S
40	WAB0032550	*O. g*	Nigeria	R
41	WAB0009280	*O. g*	Nigeria	MS
42	WAB0032394	*O. g*	Nigeria	R
43	WAB0006871	*O. g*	Control	R
44	WAB0006881	*O. s*	Control	R
45	WAB0007880	*O. g*	Control	R

O. g = *Oryza glaberrima* ; *O. s* = *Oryza sativa;* R = resistant, MR = moderately resistant; MS = moderately susceptible; S = susceptible

4.2.2. DNA extraction

Genomic DNA extraction from each rice accession was done according to the CetylTrimethyl Ammonium Bromide (CTAB) method described by Saghai-Maroof *et al*. (1984). Five seeds from each of the 42 rice accessions were grown in a growth chamber at 27 °C. Leaf samples of approximately 200 mg were harvested for each accession on 21 days-old seedlings and ground in liquid nitrogen in 2 mL of CTAB using a pestle and mortar. Then, 500 µL of each of the obtained suspensions were transferred to centrifuge tubes and incubated for 30 min in a water bath kept at 60 °C. An equal volume of chloroform:isoamyl alcohol (24:1, v/v) was added to each tube. Suspensions were then gently mixed by inverting the tubes. Tubes were

centrifuged at 12,000 rpm for 10 min and the supernatant recovered and mixed with an equal volume of ice-cold isopropanol. DNA was then recovered as a pellet by centrifugation at 12,000 rpm for 5 min, washed with 100 µL of 70 % ethanol, dried under vacuum and dissolved in 30 µL of TE buffer. Two µL of RNase (10 g/mL) was added to the DNA and the mixture was incubated for 1 h. DNA concentrations were measured using the QuantusTM Fluorometer (Promega).

4.2.3. AFLP analysis

AFLP polymorphic markers used in this study were selected on the basis of their polymorphisms we evidenced in our previous work on the 350 rice accessions (See chapter 3). Genomic DNA was completely digested with 5 U of each of both MseI and EcoRI restriction enzymes and ligated to 5 pmol EcoRI and 25 pmol MseI adapters. Pre-amplification of ligation products was done with specific EcoRI and MseI primers without additive nucleotides, and over 20 cycles of 94 °C during 30 s, 56 °C during 60 s, and 72 °C during 60 s were run in a thermal cycler. The concentration of the amplified DNA was checked on a 1.5 % agarose gel. Next, DNA was diluted 25 times in TE buffer. For selective amplification, three primer combinations with three selective bases at the end (EcoRI + ACC/MseI + CTC, EcoRI + ACC/MseI + CTA, EcoRI + ACC/MseI + CAT) were used to reveal a maximum number of polymorphic markers in the tested rice samples. Based on the analysis of the results obtained with the entire collection of 350 rice accessions, the primer combination EcorI + ACC/Mse + CAT appeared to be most informative with 79 % polymorphic bands, followed by EcorI+ ACC/Mse+ CTC and Ecor I+ ACC/Mse + CTA, with 69 % and 66 % polymorphic bands, respectively (See chapter 3). The EcoRI primer was end-labeled with NED-fluorescent dye. Amplified products were mixed with GeneScan-500 ROX size standard (Applied Biosystems Inc., USA) and deionized formamide. Finally, they were loaded on an Applied Biosystems electrophoresis instrument 3130 for electrophoretic size separation with performance-optimized polymer (POP 4).

4.2.4. SSR analysis method

A total of 20 polymorphic SSR markers distributed across the 12 rice chromosomes were used to conduct genotyping of our germplasm collection (Table 4.2). These markers were selected from a panel of 30 standard SSR markers developed by the Generation Challenge Program for rice diversity analysis (http://gramene.org/markers/microsat/50_ssr.html) and from other scientific projects/programs (Temnykh *et al*. 2000; McCouch *et al*. 2002; Dramé *et al*. 2011) based on their ability to reveal polymorphisms in relation to genetic diversity. Forward primers were labeled at the 5' end of the oligonucleotide either with the fluorescent 6-FAM™ (Blue) or 5-TET™ (Green) dyes and supplied by Applied Biosystems Inc. (USA). These primers were first tested for amplification and polymorphism on a subset of 11 DNA samples. Then, individual polymerase chain reaction (PCR) amplifications were carried out in a total volume of 10 µL containing 2 µL of genomic DNA (5 ng), 0.125 µL of each of the forward and reverse primers (diluted to a 5 µM concentration), 0.4 µL of the dNTPs mix (100 mM), 2 µL PCR buffer (Promega), 0.6 µL of $Mgcl_2$ and 0.0625 µL of Taq DNA polymerase (5u/µL, Promega).

PCR runs were performed according to the following program: initial denaturation at 95 °C during 2 minutes, followed by 30 cycles of 30 s at 95 °C, the annealing temperature during 1 min; elongation at 72 °C during 1 min; and a final elongation step at 72 °C during 5 min. After the PCR reaction, PCR products were transferred on 2 % agarose gel to check the amplification. Of the PCR product, a master mix of 10 µL was prepared into optical 96-well MicroAmp plates using non-overlapping markers of two different dyes (FAM and TET) with reference to the allele sizes published on the Gramene website. The master mix was composed of 1 µL of each of the fluorescent dyes (FAM: Blue and TET: Green), 7.7 µL of deionized formamide and 0.3 µL GeneScan-500 ROX size standard (Applied Biosystems Inc., USA). Products were denatured by heating them for 3 min at 90 ° C. They were then loaded on an Applied Biosystems electrophoresis instrument 3130 for electrophoretic size separation with performance-optimized polymer (POP 4).

Table 4.2. Primer sequences and PCR conditions used for the amplification of the microsatellites in our rice accessions

Panel	SSR marker	Primer sequences (5' - 3')		*Ta* (°C)	PCR cycle	Dye
		Forward	Reverse			
1	RM234	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	55	30	FAM
1	RM287	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC	55	30	TET
2	RM125	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC	63	30	FAM
2	RM223	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG	55	30	TET
3	RM1236	AGAAAAGTTAATTCCAAAGG	CAAGGAATTCTAGAGGAGTG	48	30	TET
3	RM162	GCCAGCAAAACCAGGGATCCGG	CAAGGTCTTGTGCGGCTTGCGG	61	30	FAM
4	RM237	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC	55	30	FAM
4	RM280	ACACGATCCACTTTGCGC	TGTGTCTTGAGCAGCCAGG	55	30	TET
5	RM55	CCGTCGCCGTAGTAGAGAAG	TCCCGGTTATTTTAAGGCG	55	30	FAM
5	RM1235	GAAAACTAAAAAGCAGAGGA	AAGCTATCCATTTTGGATTA	48	30	TET
6	RM171	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG	55	30	TET
6	RM219	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG	55	30	FAM
7	RM19	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA	55	30	TET
7	RM3483	CCTAGCTTTCAGGAGCAAG	CCCACAATGAGAAACAGTTG	55	30	FAM
8	RM3740	ATCCCAACTCTAAGCCACCC	CTACCCGTCACCAACTCACC	50	30	FAM
8	RM1075	CCAGTTCAGTAGTTCACACACC	GTTGGGTTGCTGTGTTGTTC	50	30	TET
9	RM5851	GCTGTCGGGGATGTAATACG	GCTTTGCGGCTGGTTAATTG	50	30	FAM
9	RM3317	CCTGACAGAAGAATGGTACACC	TGTGGCTTCTCGTTGAGTTG	50	30	TET
10	RM316	CTAGTTGGGCATACGATGGC	ACGCTTATATGTTACGTCAAC	55	30	FAM
10	RM133	TTGGATTGTTTTGCTGGCTCGC	GGAACACGGGGTCGGAAGCGAC	63	30	TET

4.2.5. Phenotypic description

Morphological characterization (phenotypic description of our rice accessions) was conducted at AfricaRice's experimental site in Cotonou, Benin in upland conditions. The experimental layout was an augmented randomized complete block design with three reference controls (WAB0006881 (*O. sativa*), WAB0006871 (*O. glaberrima*) and WAB0007880 (*O. glaberrima*)) (Table 4.1). Such a design does not require replicated experiments (replication of accessions) and is convenient for trials with limited accession seeds as was the case in our work (Venugopalan *et al.* 2008). A total of 7 blocks with 9 plots was used including all of our 42 rice accessions that appear exactly once in each block. Our reference controls were replicated 9 times over the 9 blocks. Each single rice accession was sown in a plot of 5 m^2 (5x1 m^2) with 20 cm spacing within rows and lines (Figure A.1, appendix). Thinning of seedlings was done

at 14 days after sowing. We evaluated 17 morphological traits using the Standard Evaluation System for Rice (http://www.knowledgebank.irri.org/ses/SES.htm) (Table A.5, appendix). NPK (15-15-15) was applied during sowing (200 kg/ha), whereas two applications of urea were performed at the top-dress stage (35 kg/ha) and panicle initiation stage (65 kg/ha), respectively.

4.2.6. Statistical data analysis

AFLP/SSR electropherograms were analyzed by GeneMapper Software Version 4.0. Genotype marker results were automatically compiled in a standard Excel format. Statistical parameters in relation to genetic diversity, such as polymorphism information content (PIC), (Botstein *et al*. 1980), Shannon's diversity index, allele number, frequency of major alleles, and heterozygosis were determined using GenAlEx V6.502. We investigated population structure using the Bayesian model-based software program in STRUCTURE V2.3.4 (Pritchard *et al*. 2000).

Optimum population number (K) was set from 1 to 10 with the 50, 000 lengths of burn-in period and 200,000 Markov Chain Monte Carlo (MCMC) repeats after a burn-in period of 50,000 steps and 10 independent runs for each K number. Python Script Structure Harvester.py v0.6.92 (Earl and vonHoldt, 2012) was used to summarize the STRUCTURE output. This script generates ΔK values using the method described by Evanno *et al.* (2005). The most probable number of populations was determined by plotting the K number against the ΔK (log probability for the rate of change of data) calculated. The run with maximum likelihood was then used to assign each accession into a population. Accessions with affiliation probabilities (inferred ancestry) ≥80 % were assigned to a distinct population, whereas those <80 % were treated as admixture, which means that these accessions have mixed ancestry from the identified parents that belong to different gene pools or geographical origins. We calculated genetic dissimilarity coefficients (Jaccard's distance) by using DARwin V6.0.8 software, in order to examine the genetic relationship between accessions. We then constructed dendrograms according to the unweighted pair group mean arithmetic method (UPGMA). An Analysis of MOlecular VAriance

(AMOVA) was used to estimate Fst (fixation) values and determine the partitioning of the molecular variance of our rice accessions. Mantel test was used to investigate the correlation between genetic distances and geographical, and phenotypical distances (Mantel 1967). We performed Principal Coordinate Analysis (PCoA) to show the links between genetic populations we detected.

4.3. Results

4.3.1. Genetic variability as evidenced by AFLP and SSR markers among a subset of 42 West African rice accessions

A total of 1232 fragments were identified in our subset of 42 rice accessions by 77 polymorphic AFLP markers with an average of 25.67 loci for each of the three primer combinations tested (Table 4.3). Shannon's information index (*I*) and Polymorphism Information Content (PIC) averaged 0.46 and 0.35, ranging 0.42-0.49 and 0.33-0.37, respectively. Jaccard's distance coefficients varied from 0.15 to 0.96 across all samples tested.

Twenty SSR markers were used to identify 223 different alleles in the tested germplasm, with an average of 11.15 alleles per locus (Table 4.4). Nearly half (49.32 %) of the detected alleles were rare with a frequency lower than 0.05. Major allele (the most common alleles) frequency ranged from 0.16 to 0.56, averaging 0.35. Three loci (RM162, RM287, RM316) revealed abundant alleles (frequency ≥ 50 %) across all tested samples. The highest number of these different alleles was observed with marker RM5851 (19 alleles) followed by RM219 (18 alleles) and RM1236 (17 alleles), whereas a lower number (5 alleles) was found at RM125, RM162, and RM171 loci. Shannon's information index (*I*) varied from 1.01 (RM162) to 2.68 (RM5851) with an average value of 1.88. PIC values ranged from 0.58 (RM162) to 0.91 (RM5851 and RM219), with an average of 0.78. Markers RM55, RM162, and RM1075 revealed more than 40 % heterozygous individuals, whereas RM125 was homozygous. Jaccard's distance coefficients varied from 0.1 to 1 among our germplasm subset.

Table 4.3. Polymorphism levels detected by the number of loci and bands per primer combination, Shannon's information index (I) and Polymorphism Information Content (PIC) for AFLP markers.

Primer combinations	Number of loci detected	Total number of bands	I	PIC
ACC+CAT	31.00	478.00	0.46	0.37
ACC+CTC	24.00	341.00	0.42	0.33
ACC+CTA	22.00	413.00	0.49	0.35
Mean	25.67	410.67	0.46	0.35
Total	77.00	1232.00		

Table 4.4. Polymorphism levels detected by major allele frequency, number of different alleles (Na), number of effective alleles (Ne), Shannon's information index (I), observed heterozygosity (Ho) and Polymorphism Information Content (PIC) for 20 SSR markers on 11 chromosomes (Chr.)

Locus	Chr.	Frequency	Missing (%)	Na	Ne	I	Ho	PIC	Allele size (bp)
RM19	12	0.40	0.00	10.00	4.48	1.84	0.10	0.78	204-246
RM55	3	0.17	2.38	15.00	9.01	2.37	0.46	0.89	217-238
RM162	6	0.50	2.38	5.00	2.36	1.01	0.59	0.58	191-209
RM171	10	0.41	2.38	5.00	3.70	1.45	0.05	0.73	327-344
RM219	9	0.16	2.38	18.00	10.92	2.59	0.29	0.91	190-230
RM234	7	0.21	0.00	10.00	7.19	2.10	0.07	0.88	130-159
RM237	1	0.31	4.76	8.00	5.46	1.87	0.10	0.82	122-131
RM287	11	0.50	0.00	9.00	3.25	1.57	0.10	0.69	93-111
RM316	9	0.56	2.38	6.00	2.65	1.23	0.02	0.63	174-206
RM1075	2	0.23	0.00	13.00	7.52	2.23	0.45	0.87	181-210
RM1235	8	0.43	2.38	11.00	3.36	1.66	0.05	0.70	100-119
RM1236	10	0.43	2.38	17.00	4.41	2.01	0.29	0.77	119-198
RM3317	4	0.42	0.00	10.00	4.33	1.81	0.17	0.79	126-145
RM3483	12	0.48	0.00	11.00	3.51	1.64	0.12	0.72	144-207
RM3740	1	0.33	0.00	12.00	5.34	2.00	0.02	0.81	114-146
RM5851	12	0.17	0.00	19.00	11.68	2.68	0.05	0.91	204-270
RM125	7	0.34	2.38	5.00	3.81	1.42	0.00	0.74	118-122
RM133	6	0.37	0.00	11.00	5.04	1.94	0.36	0.81	216-226
RM223	8	0.21	0.00	15.00	7.98	2.35	0.26	0.88	137-160
RM280	4	0.43	0.00	13.00	4.27	1.91	0.02	0.77	147-180
Mean		0.35	1.19	11.15	5.51	1.88	0.18	0.78	
Min		0.16	0.00	5.00	2.36	1.01	0.00	0.58	
Max		0.56	4.76	19.00	11.68	2.68	0.59	0.91	

4.3.2. Population structure and genetic variation among a subset of 42 rice accessions

The Bayesian-based analysis of population structure using SSR markers showed that the log-likelihood at K = 3 was optimal to group the subset of 42 rice accessions into three genetically distinct populations (Figure 4.1). A number of 9, 6 and 26 rice accessions were classified with more than 96 % ancestry into population 1 (red colour), population 2 (green colour) and population 3 (blue colour), respectively (Figure 4.2). Accession WAB0006684 (*O. sativa*) from Benin shared ancestry with rice accessions in population 1 (77 %) and population 2 (23 %). Population 1 contained all remaining *O. sativa* accessions we used in this work.

Cluster Analysis based on the UPGMA method using the 20 SSR markers confirms that germplasm can be divided into three genetic groups (Figure 4.3a). Accessions in each group corresponded to the three populations identified by STRUCTURE except for accession WAB0006684 (*O. sativa*) that shared ancestry with accessions of both populations 1 and 2.

AMOVA results (Table 4.5) reveal that genetic variation was higher between rice accessions (80 %) than within accessions (18 %), whereas only 2 % of this variation was due to differences between the three populations. Overall Fixation (Fst) value was equal to 0.018 across populations. Fst-values were low between all pairwise populations, but significant ($P < 0.05$) for defining population genetic structure of our germplasm. A comparable level of population genetic differentiation (Fst) was observed between population 1 and population 2 (0.032), and between population 2 and population 3 (0.032), whereas a much lower Fst (0.016) was found between population 1 and population 3. The average number of different and private alleles (Alleles found only in a single population) per locus was higher in population 3 (6.60 and 3.95) than in population 1 (5.65 and 3.25) and population 2 (2.50 and 0.80). The number of major alleles for 50 % of the accessions was zero across all three populations. Shannon's Information Index (*I*) and expected heterozygosity (*He*) were lower in population 2 (0.58 and 0.32) and population 3 (1.34 and 0.63) than in population 1 (1.43 and 0.68) (Table 4.6). Minimum Nei

genetic distance of 1.5 was found between populations 1 and 3, whereas populations 2 and 3 were the most divergent ones (2.12).

Using AFLP-data, STRUCTURE analysis showed that no definite population could be identified in the subset of 42 rice accessions. On the other hand, UPGMA analysis showed two genetic clusters, cluster 1 (blue colour) and cluster 2 (black colour) that comprised 26 and 16 rice accessions, respectively (Figure 4.3b). Cluster 1 corresponds to population 3, whereas cluster 2 corresponds to both populations 1 and 2. Mantel-test results (Figure 4.4) show a significant correlation between genetic distances based on AFLP markers and genetic distances based on SSR markers (R = 0.76, P = 0.01).

AMOVA results (Table 4.5) point out significant variations between clusters (52 %) and within clusters (48 %). Nei genetic distance between cluster 1 and cluster 2 was estimated to be 0.29. Both clusters showed a similar value of Shannon's Information Index (*I*) (0.33) and expected heterozygosity (*He*) (0.22), whereas the number of total bands detected in cluster 1 (772) was much higher than in cluster 2 (460) (Table 4.6).

Table 4.5. AMOVA analysis for genetic groups differentiation by AFLP and SSR markers in the subset of 42 rice accessions.

Source of variation	df	SS	MS	Estimated variance	Percentage of the total variance	Probability
AFLP markers						
Among clusters	1	203.48	203.48	9.81	52%	
Within clusters	40	366.38	9.159	9.16	48%	P<0.001
Total	41	569.86		18.97	100%	
SSR marker						
Among Pops	2	43.13	21.56	0.179	2%	
Among Ind.	38	672.21	17.69	7.96	80%	P<0.001
Within Ind.	41	73.00	1.78	1.78	18%	
Total	81	788.34		9.91	100%	

Table 4.6. Polymorphism levels estimated among accession groups identified by AFLP and SSR markers

Genetic groups	Number of samples	Total number of alleles	I	He
AFLP markers				
Cluster 1	26 (*glaberrima*)	772	0.33	0.22
Cluster 2	16 (5 *sativa*, 11 *glaberrima*)	460	0.33	0.22
SSR markers				
Pop 1	9 (5 *glaberrima*, 4 *sativa*)	5.65	1.43	0.68
Pop 2	6 (*glaberrima*)	2.50	0.58	0.32
Pop 3	26 (*glaberrima*)	6.60	1.34	0.63

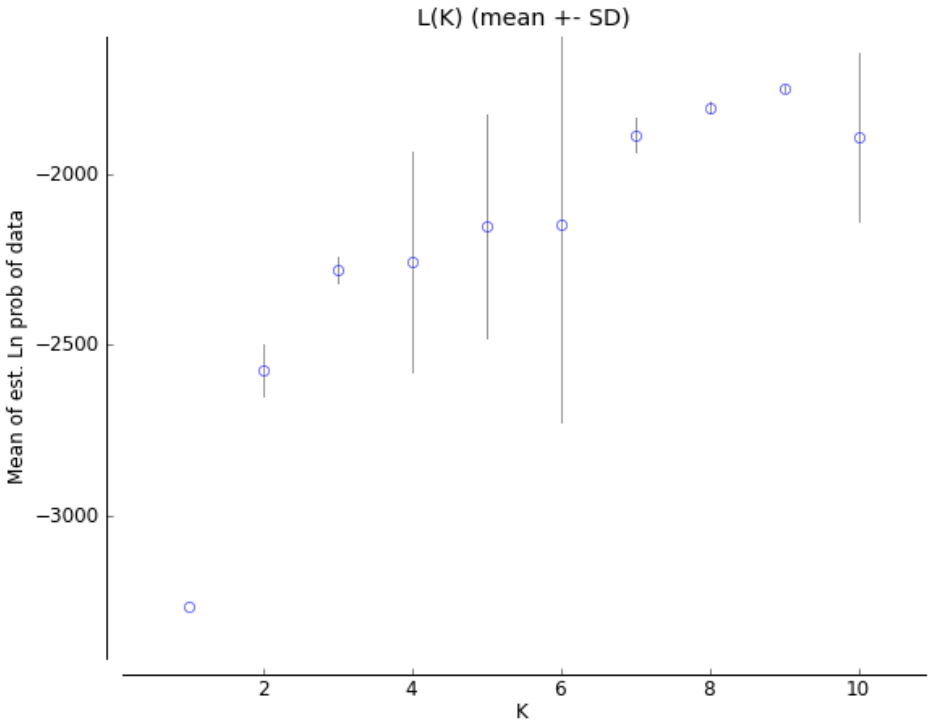


Figure 4.1. Population structure analysis of 42 rice accessions using 20 SSR markers based on the log-likelihood (posterior probability) values for differing numbers of populations (k)

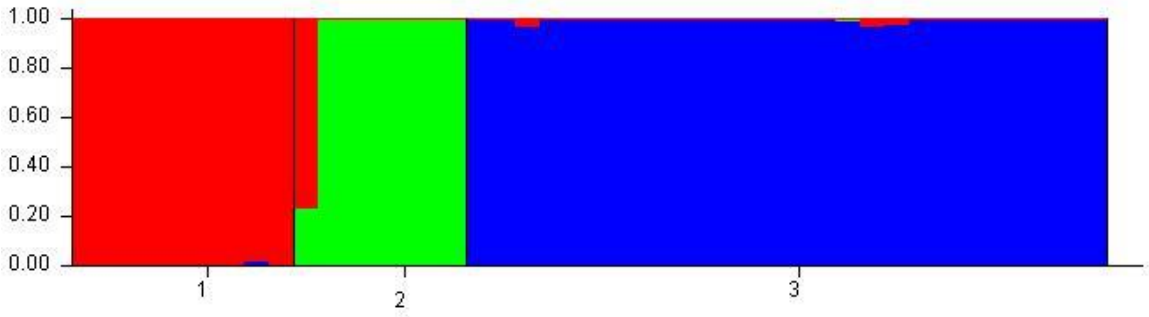


Figure 4.2. Results of the population structure analysis conducted with the 42 rice accessions at k=3 using 20 SSR markers

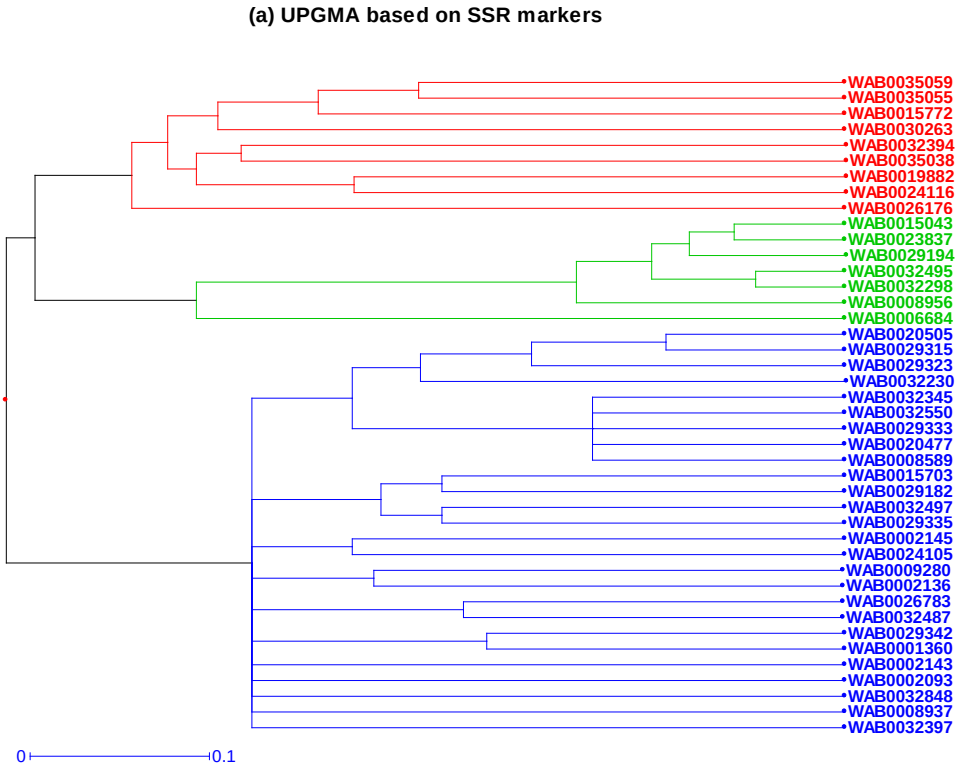


Figure 4.3a. UPGMA tree base on SSR markers

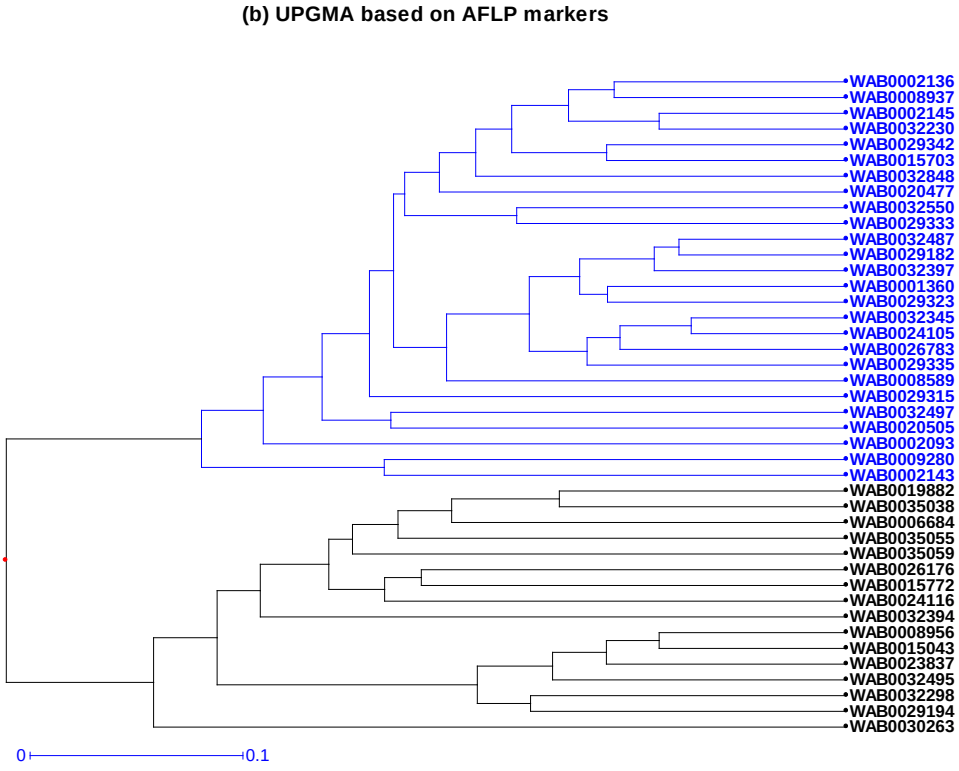


Figure 4.3b. UPGMA tree base on AFLP markers

Figure 4.3. UPGMA dendrogram of the subset of 42 rice accessions based on Jaccard's distance from (a) SSR markers and (b) AFLP markers; colours indicate clusters

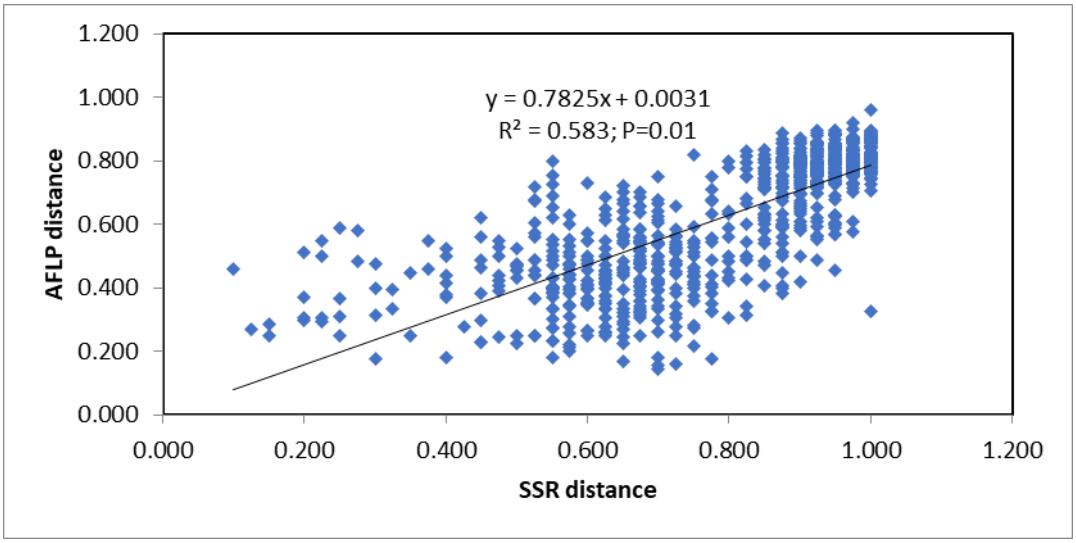


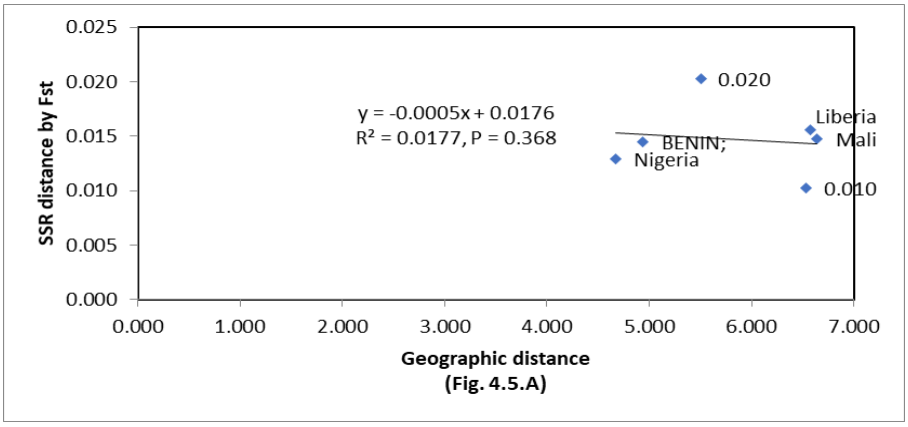
Figure 4.4. Genetic distance correlation test using AFLP and SSR markers

4.3.3. Relationships between geographic provenances and genetic structure of a subset of 42 rice accessions subset

Mantel-test revealed no correlation between genetic distances estimated by pairwise Fst using SSR markers and geographic provenances (Benin, Liberia, Mali, and Nigeria) of our germplasm collection (R = 0.13, P = 0.37). Samples from Guinea and Ivory Coast, containing only one sample each, were not included in the analysis (Figure 4.5). Jaccard's genetic dissimilarity coefficients ranged from 0.48 to 9 in population 1. The highest similarity was found between Beninese accessions WAB0035055 and WAB0035059, whereas a large divergence was found between WAB0026176 (Mali) and WAB0035038 (Benin). In population 2, accessions WAB0032495 (Liberia) and WAB0032298 (Mali) shared 10 % of genetic similarity, whereas WAB0023837 (Mali) and WAB0008956 (Liberia) were more divergent (40 % of dissimilarity). In population 3 the highest genetic similarity (20 %) was found between Nigerian accessions WAB0029315 and WAB0020505, whereas WAB0020477 (Nigeria) and WAB0032848 (Mali) were the most divergent (88 % of dissimilarity) rice accessions.

Results from AFLP markers analysis reveal a significant negative association between geographic and genetic distances (R = -0.72, P = 0.02) within our germplasm collection. The graphics show a continuous and linear decrease of genetic similarity when geographic

distance increases (Figure 4.5). High genetic distance was found between accessions from Benin and Nigeria (0.41), whereas those from Mali were genetically closer to the accessions from Benin (0.01). Cluster 1 was dominated by accessions from Mali and Nigeria, whereas all accessions from Benin were classified in cluster 2 together with 2 accessions (out of the three) from Liberia. High genetic similarity of 15 % was found between Malian accessions (WAB0032345 and WAB0024105) in cluster 1, whereas Nigerian accessions WAB0029333 and WAB0009280 were divergent at 80 % (20% of genetic similarity). The most similar (82 %) accessions in cluster 2 were WAB0015043 (Ivory Coast) and WAB0008956 (Liberia), whereas WAB0030263 (Nigeria) and WAB0023837 (Mali) were most divergent (80 % dissimilarity).



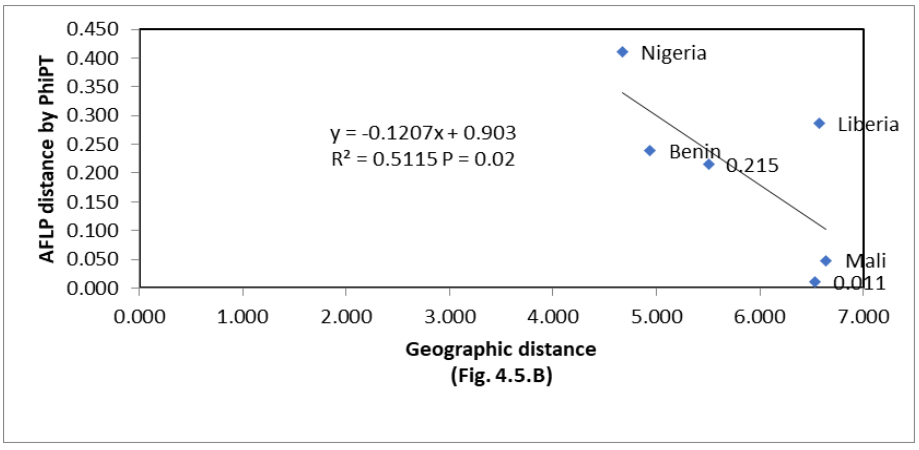


Figure 4.5. Relationship between geographic distance and genetic distance as measured by SSR (Fig. 4.5.A) and AFLP (Fig. 4.5.B) markers

4.3.4. Phenotypic discrimination and gene flow between *O. sativa* and *O. glaberrima* rice accessions

Mantel's statistical test revealed a significant association between AFLP markers and the 17 morphological traits evaluated (R = 0.60, P = 0.01). Several morphological characteristics differentiated cluster 1 from cluster 2. Accessions in cluster 1 were discriminated by the presence of anthocyanin coloration on basal leaf sheath, open culm habit and spreading type of panicle branches, whereas accessions in cluster 2 showed relatively opened culm habit and semi-compact panicles. Secondary branching was particularly sparse in cluster 1, whereas cluster 2 had dense panicle type. Cluster 1 showed truncate ligule shape and erect panicle form, whereas cluster 2 had cleft ligule shape and drooping panicles. Based on these morphological distinctive traits, accessions in cluster 1 were very similar to the two *O. glaberrima* reference controls (WAB0006871 and WAB0007880), whereas cluster 2 resembled the *O. sativa* (WAB0006881) control. UPGMA tree constructed using 77 AFLP markers classified WAB0006881 (*O. sativa* control) in cluster 2, whereas the two *O. glaberrima* (WAB0006871 and WAB0007880) controls were grouped together in cluster 1 (Figure 4.6).

Based on SSR markers, we investigated allelic distribution patterns between the five *O. sativa* and all *O. glaberrima* accessions that belong to the three identified populations. On average, in both populations 1 and 2, *O. glaberrima* accessions showed a similar pattern of genetic diversity indexes as *O. sativa* accessions, whereas *O. glaberrima* accessions in population 3 displayed a specific different pattern (Figure 4.7).

Principal coordinates analysis (PCoA) based on Nei's genetic distance (Figure 4.8) showed that *O. glaberrima* accessions in population 1 were more closely related to *O. sativa*, whereas *O. sativa* was itself more isolated from the other accessions. The lowest fixation index (Fst = 0.001, P-value = 0.48), was observed between *O. glaberrima* accessions in population 1 and *O. sativa* accessions. Fst-values between *O. glaberrima* (population 1) and *O. sativa* accessions were shown to be non-significant for alleles of 18 loci out of 20 SSR markers studied (P > 0.05). The intensity of geneflow Nm observed was 217.08 (Nm>1).

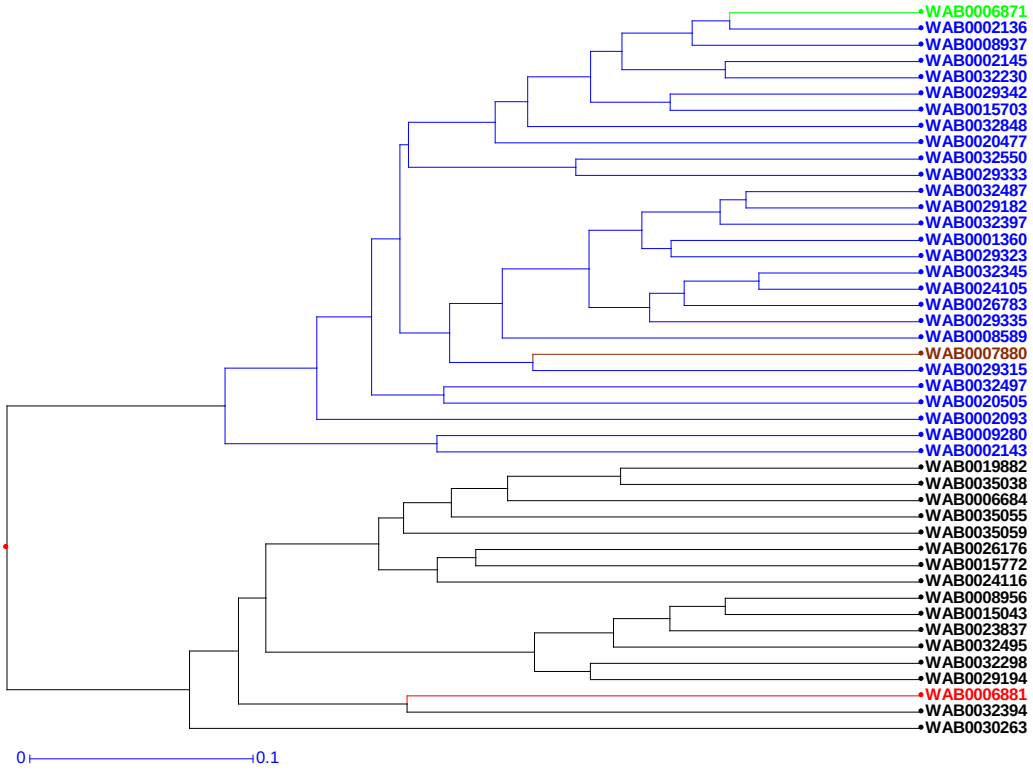


Figure 4.6. UPGMA dendrogram of 42 rice accessions and three reference controls: WAB0006871 (*O. glaberrima*, presented in green), WAB0007880 (*O. glaberrima*, presented in brown), and WAB0006881 (*O. sativa* presented in red) based on AFLP markers

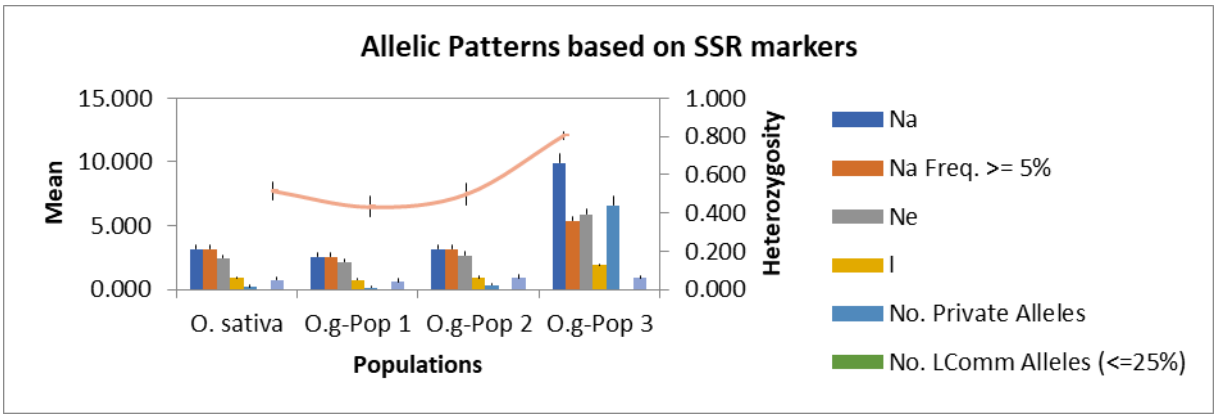


Figure 4.7. Comparison of allelic distribution patterns between *O. sativa* and *O. glaberrima* accession groups for the number of different alleles (Na), the number of different alleles (Na) with a Frequency >= 5%, number of effective alleles (Ne), number of alleles unique to a single population, number of locally common alleles (Freq. >= 5%) found in 25% or fewer populations and Shannon's Information Index (I)

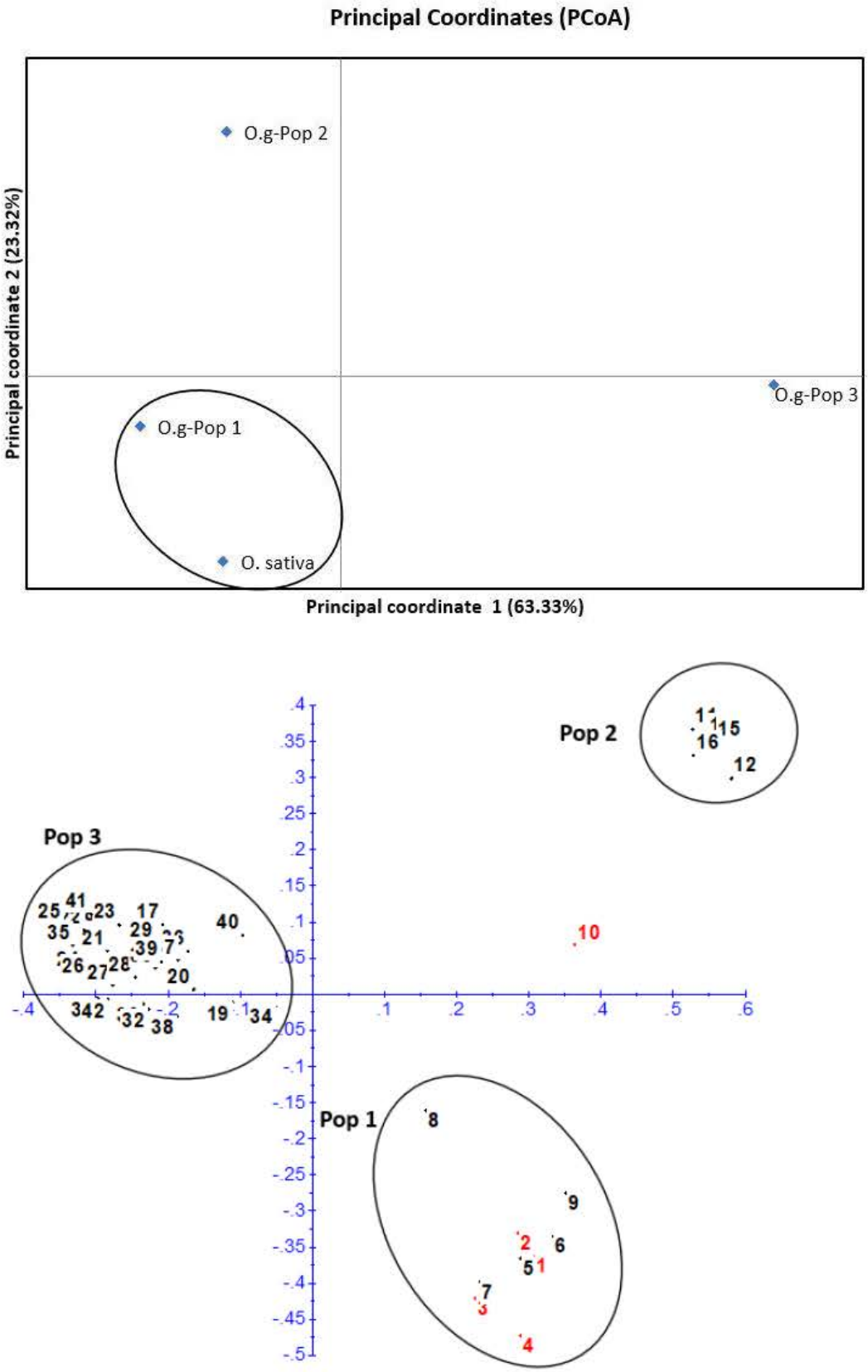


Figure 4.8. Principal coordinates analysis based on genetic distances between all *O. sativa* accessions presented in red and the three populations of *O. glaberrima* accessions presented in black

4.4. Discussion

Mean PIC value (0.35) revealed by AFLPs in our germplasm collection is higher (31%) than that observed (0.24) previously for a set of 350 accessions (See chapter 3). Genetic diversity usually increases in rice with the elimination of genetically similar accessions during core collection establishment (Agrama *et al*. 2009; Roy-Choudhury *et al*. 2014). UPGMA cluster analysis using AFLPs identified two genetically different clusters, which seem to reflect geographic provenances of the assembled accessions. The highest genetic similarity was recorded between the Malian accessions (cluster 1) and those collected from Ivory Coast and Liberia (cluster 2). It has been demonstrated that historically, *O. glaberrima*, which is derived from a wild ancestor *O. barthii* A. Chev. (syn. *O. breviligulata* A. Chev. & Roehr.), was first domesticated in the Niger river delta (Mali), and subsequently migrated to two secondary centers: (1) through the Guinea highlands to Niger, crossing along the border of Benin and diffusing into Nigeria; and (2) through the Guinea forest (between Sierra Leone and western Ivory Coast) to Liberia (Portères 1962; Second 1982; Sarla and Swamy 2005; Wang *et al*. 2014). *O. sativa* was domesticated from the wild progenitor *O. rufipogon* in the Yangtze basin of China and introduced later to West Africa (Choi and Purugganan 2018). Both species were domesticated independently (Wang *et al*. 2014). Our results show that genetic similarity decreased when geographic distance increased, thus showing a gradual variation of AFLP markers over the geographical origin (Diniz-Filho *et al*. 2013). This suggests that samples collected in the same country are not always genetically the most closely related (Semon *et al.* 2005).

Although AFLPs generated the largest number of total alleles (1232 bands), higher ranges of Shannon's diversity index (1.01-2.68) and PIC (0.58-0.91) were obtained by SSR markers. We conclude that SSR markers were more effective in uncovering at least part of genetic variation. Comparative studies with AFLP and SSR markers support that microsatellites are able to reveal most of the polymorphism in soybean (Powell *et al*. 1996), lentil (Idrissi *et al*. 2015) and also rice germplasm (Tarang and Gashti 2016). Vieira *et al*. (2016) explained that there are

some advantages of using SSR markers to evidence polymorphisms because they are multi-allelic and codominant markers. SSR markers have the potential to detect high degrees of polymorphism compared to other marker systems (Wu and Tanksley, 1993; Saghai-Maroof *et al.* 1996; Powell *et al*. 1996 and Morgante *et al*. 2002). AFLP is a straightforward technique, but potential individual bands may actually be composed of multiple fragments (Shan *et al.* 1999). Other than AFLPs, SSR markers make a clear differentiation between homozygous and heterozygous genotypes. Overestimations of the "true" genetic diversity levels can be observed with the use of molecular markers such as AFLPs, whereas the use of whole-genome sequencing would have been a better choice in the current study. However, the evaluation of genetic diversity with the whole-genome sequencing technique is very time-consuming and more expensive than the application of molecular markers.

In the present study, the mean PIC value of 0.78 obtained using SSR markers is comparable to that reported by Ming *et al*. (2010) who studied the genetic diversity of 32 *O. sativa* and 4 *O. glaberrima* accessions. They reported a PIC average of 0.716 using 54 SSR markers. Higher polymorphism levels were revealed in the present work compared to the results of previous studies on African rice (Semon *et al*. 2005; Dramé *et al*. 2011; Chen *et al*. 2017) where a range of 0.34 to 0.64 was observed. Possible reasons could be because molecular markers used by these authors are less informative, whereas accessions studied are not the same. The major reason, however, could be attributed to a large number of different alleles and the heterozygosity we detected (Kalinowski 2002). The most informative SSR markers of this study were RM219 and RM5851 (PIC value of 0.91), which produced 18 and 19 alleles, respectively. These results are partially supported by Dramé *et al*. (2011), who observed a maximum PIC value of 0.90 at RM219 locus with 17 alleles detected within a set of 82 African rice accessions (including 72 *O. glaberrima* accessions). However, the average number of different alleles per locus (11.15) detected in our study was higher than the numbers reported by Dramé *et al*. (2011) (8.4) and Chen *et al.* (2017) (8.4 and 6, respectively). Out of the 20 SSR markers tested, only RM125 was found to be homozygous, whereas Dramé *et al*. (2011)

recorded the greatest proportion of heterozygous individuals (0.86) at the same locus, RM125. We can assume that marker RM125 is specific to the accessions studied in the present work.

The presence of three different populations was revealed by SSR markers, whereas there was no clear geographic structure in the three populations. Average Fixation (Fst) value (0.018) was less than 0.05, but significant, suggesting little genetic differentiation between these populations (Balloux and Moulin 2002). Our germplasm structure shows that *O. sativa* accessions were not totally isolated from *O. glaberrima* accessions. Four out of the five *O. sativa* accessions were grouped together with five *O. glaberrimas* in population 1. *O. sativa* accession WAB0006684 shared ancestry with populations 1 and 2. No morphological difference in ligule shape, panicle form or secondary branching was found between *O. glaberrima* accessions in population 1 and all *O. sativa* accessions (five *O. sativa* accessions from Benin and the control WAB0006881 of the same species) studied. These morphological traits are among the criterion taken into consideration for arguing whether or not a particular variety belongs to *O. sativa* species (Linares 2002; Bezançon and Diallo 2006). The narrow range of fixation indices (Fst) across the three populations taken together with accession similarities at morphological level attests intense gene flow between both species. Fst value that we recorded between *O. glaberrima* and all *O. sativa* accessions in population 1 (0.001) was significantly lower than those in populations 2 and 3. According to Slatkin (1985) and Ingvarsson and Giles (1999), gene flow strongly influences the spatial scale over which genetic differentiation will be observed. Because Fst and gene flow are inversely related (Wright 1951) a higher amount of gene flow occurred between *O. glabberrima* accessions in population 1 and the *O. sativa* material. The movement of pollen is usually from *O. sativa* to *O. glaberrima* (Sano *et al.* 1989). The occurrence of gene flow in the germplasm studied could be attributed to (1) evolutionary history of these populations; (2) out-crossing between rice accessions from different species: (Ko *et al*. 1994; Jusu 1999 and Nuijten *et al*. 2009). Spontaneous interspecific hybridization is not uncommon in the rice gene pool (Second *et al*. 1982; National Research Council 1996; Barry *et al*. 2007; Nuijten *et al*. 2009). The observed gene flow may have strong

implications for the conservation of genetic diversity and plant breeding. Because genetic diversity is often much higher in *O. sativa* than in *O. glaberrima*, overwhelming gene flow from *O. sativa* may increase genetic diversity in *O. glaberrima* (Vecchi-Staraz *et al.* 2009; North *et al*. 2011; Sexton *et al*. 2011;). Thus, for strategies for the conservation of both species, it is important to acquire knowledge about the genetic structure of the populations and the extent of gene flow (Ellstrand 2003). This flow over many generations might have an effect on the evolution of *O. glaberrima* in the future. Gene flow from cultivated *O. sativa* to *O. glaberrima* accessions revealed in this study also raises the question of the substantial reality whether current *O. glaberrima* samples still belong to the latter species group. It is also important to note that pollen flow arising from *O. sativa* may have improved the yield potential of this small set of *O. glaberrima* accessions discovered in population 1 because *O. sativa* is often of high grain performance. Because also of the known wide adaptability of *O. glaberrima,* these accessions discovered in population 1 are promising for different biotic or abiotic resistance traits. Given the importance of our findings, additional analysis of the traits of interest for production should e conducted. Our germplasm collection could thereafter be used for the development of further improved varieties.

4.5. Conclusions

Results of this study clearly show that using AFLP markers, the 42 *O. glaberrima* and *O. sativa* accessions of the studied subset could be classified according to their country of origin. However, the highest polymorphism levels were detected only with SSR markers. In addition, Fst values for genetic differentiation of populations showed low genetic variation between the three populations identified with SSR markers. At the morphological level, a number of *O. glaberrima* accessions were very similar to *O. sativa*. With both AFLP and SSR markers, our work also demonstrated that gene flow occurred between *O. glaberrima* and *O. sativa* rice. Gene flow can be used to introduce new genetic variation and expand genetic diversity for resistance improvement against pathogens including blast fungus.

CHAPTER FIVE

5. Combining High Yields and Blast Resistance in Rice (*Oryza* spp.): Results of Screening Selected Germplasm under Upland and Lowland Conditions in Benin

Based on a research article published as:

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Abstract

The future rice supply in Africa heavily depends on improving the level of local production to achieve self-sufficiency. In order to cope with the current gap between production and actual demand, combining a high level of rice blast resistance and high yield potential is necessary. The current work was conducted under upland and lowland conditions in Benin to gain insight into the performance of selected, blast-resistant accessions along with some currently grown varieties. This study revealed high phenotypic variability among these accessions. Furthermore, we evidenced differences in the performance of these accessions under lowland and upland conditions. The principal component analysis showed their grouping in three clusters. The analysis also demonstrated a high yield potential among the blast-resistant rice accessions whether they were *Oryza sativa* or *O. glaberrima*. Furthermore, there was a significant correlation between yield, and both spikelet fertility and growth cycle duration. In conclusion, the present study identified promising rice accessions for future breeding projects/programs. High phenotypic variability combined with interesting traits can help to develop new resilient varieties. Finally, when these traits correlate with high yield, they can be used as markers for an early screening method for identifying promising accessions at an early stage.

Keywords: rice, breeding, blast, food security, high-yield potential

5.1. Introduction

Rice (*Oryza* spp.) is the third most widely grown cereal crop in the world after maize and wheat, and a staple food consumed by billions of people worldwide (Nguyen and Ferrero 2006; Sarkar *et al.* 2013; FAOSTAT 2019). Rice provides consumers with the bulk of their essential calories but also with a number of micronutrients (iron, zinc, and ß-carotene) (Dipti *et al.* 2012). Africa has an abundant supply of natural resources (e.g. water, land, biodiversity, *etc*.) that can support a huge expansion of food supply, specifically rice production (Balasubramanian *et al.* 2007; Seck *et al.* 2012). Rice can be grown under diverse environments, e.g., rainfed dryland, wetland, deepwater and mangrove swamps, and irrigated wetland (Balasubramanian *et al.* 2007; Seck *et al.* 2012). Africa harvests annually more than 12,5 million ha of rice to feed many low-income households with limited access to food (FAOSTAT 2018). However, annual African rice production only covers 62% of actual needs, whereas demand is growing faster than for any other staple food on the continent (Seck *et al.* 2008; OECD and FAO 2015). To meet future rice demands, the yield increase is seen as a key component to achieve self-sufficiency. Improvements in resource management (e.g. water, land, biodiversity, *etc*.) and farm mechanization are also essential for achieving this goal. Breeding for better rice varieties with resistance to stress factors is also an option for increasing production. In addition, the introduction of biotechnology using DNA-based markers in the development of new breeding methodologies could facilitate further yield improvements (Edgerton 2009).

In Africa, there are two major cultivated rice species, *Oryza sativa* (L.) and *O. glaberrima* (Steud.). Better knowledge of the phenotypic variability of these two rice species has the potential for improving germplasm management, conservation, and use. An important step in rice breeding was the creation of improved hybrids, using the most-promising germplasm. A prime example of such a hybrid is “NEw RIce for AfriCA” (NERICA), which was derived from crosses between *O. sativa* and *O. glaberrima*, both well-adapted to African, rice-growing environments (Pharm 1992; Jones *et al.* 1997; Futakuchi *et al.* 2009; Sie *et al.* 2010; Thiémélé *et al.* 2010). Their rapid adoption by small-scale farmers has significantly contributed to

strengthening food security and improving livelihoods in most Sub-Saharan African countries (Adegbola *et al.* 2006; Rodenburg *et al.* 2006; Kijima *et al.* 2008; Nguezet *et al.* 2011; Dibba *et al.* 2017).

Wang *et al.* (2015) recently investigated the relationship between rice blast resistance, and plant height, heading date and seed weight. For this purpose, they evaluated blast disease reactions of rice plants from a core collection for their yield-related components under both greenhouse and field conditions. Results showed that shorter plants were more resistant to blast disease. It was also found that blast resistance gene *Pi-ta* was associated with lighter seed weight, whereas susceptible alleles of RM171 and RM6544 were associated with heavier seed weight (Wang *et al.* 2015).

Benin is one of Africa's countries where rice demand is constantly growing. In the country, approximately 48% of the active population earn their income from farming, notably from rice cultivation (MAEP 2010). The land area for rice cultivation is estimated at about 82,351 ha with an overall production of 281,428 tons (FAOSTAT 2018) whereby farmers mainly practice rainfed and irrigated lowland production systems (Adégbola and Singbo 2005; Diagne *et al.* 2013). However, rice production is not increasing fast enough to keep up with the rising demands of the rapidly growing population. This obliges Benin to annually import almost 400,000 (42% of production) tons of milled rice to fulfill rice consumption in the country (FAOSTAT 2018). Benin is thus far from self-sufficient for rice.

A number of constraints affect Benin rice production, e.g., climate change, depleted soils, and poor mechanization. Also, the lack of well-adapted varieties is a major constraint especially because of abiotic and biotic stress factors that affect production. Participatory field evaluation was recently conducted by Odjo *et al.* (2017) who reported that there are very few effective rice varieties that can cope with biotic and abiotic stress conditions Beninese farmland. About half of the farmers have stopped the cultivation of NERICA varieties to grow other high-yielding rice varieties (Yokouchi and Saito 2017) which are highly vulnerable to damage caused by blast disease caused by the fungus *Magnaporthe oryzae* (Couch *et al.* 2002). The variation in the motivations of farmers to continue or stop NERICA cultivation could be attributed to various

factors. These include (1) the way in which the NERICA varieties were promoted at the farmers level (offering a higher price for rice seeds); (2) characteristics of NERICA varieties and their adaptation and (3) farmers' socio-demographic characteristics.

Blast disease is one of the most widespread and devastating biotic stresses in Benin leading to rice yield reductions of more than 30% (Vodouhe *et al.* 1981; Afouda *et al.* 2007). Rice production is hampered dramatically by this disease. It is thus important to continue the search for well-adapted and high-yielding varieties to achieve rice self-sufficiency for this country.

The 2008 African food crisis led to a high priority being placed upon food security in the continent (AfricaRice 2011) and highlighted the fact that Africa needs to increase production capacity and reduce rice importation that hampers these efforts. This goal can be achieved if each African country taps into the continent's high rice genetic diversity to introduce a series of high-performing rice varieties suitable for its different ecologies (Norman 2006).

Regarding the limitation in natural resources (e.g. land, water, labour force, and energy), providing sufficient rice to the growing Beninese population will require the use of locally adapted, high-performing varieties. Such varieties with good resistance to blast disease would be profitable and marketable worldwide (Wang *et al.* 2015). In order to select new blast-resistant genotypes, field resistance of a set of 350 cultivated rice (*Oryza sativa* L. and *O. glaberrima* Steud.) accessions was evaluated (See chapter 3). This work revealed the existence of large variability in blast resistance (See chapter 3). Additionally, a subset of 42 accessions, with variable phenotypes, was selected and considered as a core collection for further evaluation and future use in breeding programs.

Realizing the importance of rice production for Benin, the present work was undertaken to evaluate the grain production potential of this selected subset under irrigated upland and lowland field ecologies. This will allow determination of whether these rice accessions have better grain yields than currently cultivated varieties. The study can also provide a wide range of accessions with interesting agronomic characteristics that would form the basis of future

breeding programs in Benin. Furthermore, scientific knowledge generated from this study would help ultimately improve rice production in Africa.

5.2. Material and methods

5.2.1. Plant material

Oryza spp. germplasm included in our study is a subset of 42 rice accessions (5 *O. sativa* accessions and 37 *O. glaberrima*) originating from six West African countries (Table A.2, appendix). This subset of rice accessions is derived from a wider collection of 350 African rice accessions that was constituted based on geographical origins, pairwise genetic distances analysis (revealed by 77 AFLP markers) and differential reactions to blast disease (See chapter 3). Several field blast resistance patterns were observed in the core germplasm collection: 26 highly resistant accessions; 9 moderately resistant, 3 moderately susceptible and 4 susceptible.

Seven upland (ARICA 4, ARICA 5, CG 14, NERICA 1, NERICA 2, NERICA 4 and Moroberekan) and seven lowland rice (ARICA 1, ARICA 2, ARICA 3, IR 841, NERICAL 14, NERICAL 19 and TOG 5681) accessions which are cultivated by farmers in Benin were included as reference varieties.

5.2.2. Field trials for agronomic evaluation

Two field experiments were conducted concurrently under protective measures against diseases and pests in upland (from June 14th to December 15th) and lowland conditions (from June 18th to December 5th) in Cotonou (Benin) at AfricaRice's experimental site (2°21′20 E, 6°26′54 N). In that area, rainfall usually starts in mid-March and ends (with a total of 1100 to 1200 mm) in early November, with a mid-season dry period from mid-July to mid-August.

The experimental set up was a randomized complete block design (RCBD) with three replications. Forty-two rice accessions (5 *O. sativa* and 37 *O. glaberrima*) and seven reference controls (ARICA 4, ARICA 5, CG 14, NERICA 1, NERICA 2, NERICA 4 and Moroberekan) of

upland rice, were sown with 121 seeds per accession in the plots of 2 × 2 m^2. The inter-row spacing of plants was 20 cm, whereas intra-row spacing was 20 cm.

The lowland field experiment was also performed in an RCBD with three replications. Here, however, only 37 of 42 rice accessions of the subset were tested (because of insufficient seed) along with the seven lowland reference controls (ARICA 1, ARICA 2, ARICA 3, IR 841, NERICAL 14, NERICAL 19 and TOG 5681). Pre-germinated seeds of each accession were transplanted 21 days after sowing into small plots of 1.60 × 1.60 m^2 at a spacing of 20 × 20 cm^2 between and within rows.

Chemical treatments with mancozeb (80 g/15 L) and deltamethrin (Decis®, 40 mL/15 L) were performed bi-weekly to protect plants against diseases and pests. Plots were weeded regularly to minimize weed infestation. A pre-planting base application of 200 kg ha^{-1} of NPK (15-15-15) was done followed by a total of 100 kg ha^{-1} of urea at panicle initiation (35 kg ha^{-1}) and booting stages (65 kg ha^{-1}), respectively.

5.2.3. Data collection

Fifteen agronomic traits were evaluated in both field experiments (lowland and upland): total number of tillers (Tillers_Total), number of fertile tillers (Tillers_Fertile), percentage of fertile tillers (%Fertile_Tillers), panicle length (Length_Pan), plant height at maturity (Plant_height), total number of spikelets (Spikelets_TotalNum), number of filled spikelets (Filled_Spikelets), percentage of filled spikelets (%Fertile_Spikelets) number of primary branching (Ram_Iaire), number of secondary branching (Ram_IIaire), ratio secondary branching/primary branching (Ratio_RamIIRamIaire), total number of panicles per square meter (Panicules_Num), number of days to 80% flowering (CSE), number of days to 80% maturity (CSM) and grain yield (Yield). A number of plants in the middle of the inner two rows of each elementary plot were considered for data collection using the Standard Evaluation System for Rice (IRRI, 2007). The list of data collected and the methodology used are presented in Table 5.1. At 80% crop maturity stage, a quadrat of 1 × 1 m^2 size was measured and all plants in each quadrate of each plot were harvested to estimate grain production of each rice accession.

Table 5.1. List of the 15 parameters assessed in the study

Traits evaluated on five representative plants	Codes used	Description
Total number of tillers/plant	Tillers_Total	Number of tillers at the maturity stage
Number of fertile tillers/plant	Tillers_Fertile	Fertile tillers at the maturity stage
Percentage of fertile tillers/plant	% Fertile_Tillers	Calculated in Excel
Panicle length	Length_Pan	Measured from the base of the lowest spikelet to the tip of spikelets on the panicle, excluding awn
Plant height at maturity (cm)	Plant_height	Measured height from the base of the plant to the tip of spikelets on the panicle, excluding awn
Total number of spikelets/panicle	Spikelets_TotalNum	Total number of grains in sampled panicles
Number of filled spikelets/panicle	Filled_Spikelets	Number of filled grains in sampled panicles
Percentage of filled spikelets	% Fertile_Spikelets	Calculated in Excel
Number of primary branching	Ram_Iaire	Number of primary branches of the panicle
Number of secondary branching	Ram_IIaire	Number of secondary branches of the panicle
Ratio secondary branching/primary branching	Ratio_RamIIRamIaire	Computed in Excel
Total number of panicles/m^2	Panicules_Num	Number of panicles per square meter
Number of days to 80% flowering	Number of days to 80% flowering (FLW)	Number of days from effective seeding to 80% heading
Number of days to 80% maturity	Number of days to 80% maturity (MAT)	Number of days from effective seeding to 80% maturity
Grain yield (g/m^2)	Yield	Weight of a square meter of 36 plants (1 m × 1 m) of each plot

5.2.4. Statistical analysis

An ANalysis Of VAriance (ANOVA) was conducted to gain insight into the effect of genotypes on phenotypic traits. Correlation analyses were performed to assess the relationships between variables. To reduce the data dimension for a better description of the relationships between accessions, Principal Component Analysis (PCA) was used to identify phenotypic traits and use them to identify superior accessions and similarities between accessions. This PCA was performed using identified traits via ANOVA which contributed most to phenotypic variation. (Ivosev *et al.* 2008).

Additionally, principal component regression was used to predict yield based on linear combinations of the phenotypic traits. A *t*-test was performed to compare lowland and upland grain yield characteristics of all rice accessions.

5.3. Results

5.3.1. Phenotypic variability for 15 agronomic traits measured

ANOVA of the 15 agronomic traits measured in the respective lowland and upland ecologies reveals significant variations for some characteristics, namely the total number of tillers, number of fertile tillers, plant height at maturity and spikelet fertility, were observed between replications in lowland conditions, and for spikelet fertility in upland conditions (Table 5.2). Accessions' performance in the lowland was significantly different from that in upland for seven traits (Table 5.3). The data for the percentage of fertile tillers, total number of spikelets, spikelet fertility, panicle secondary branching, days to 80% heading, days to 80% maturity, and grain yield were the major discriminant characters between lowland and upland ecological conditions.

A correlation matrix (Table 5.4) was constructed with lowland data, showing that the number of days to 80% flowering was positively and significantly associated with the number of days to 80% maturity (R = 0.94, P = 0.0001), but negatively associated with spikelet fertility. Total number of spikelets was positively correlated with secondary branching (R = 0.61, P = 0.004). In upland conditions, a positive association was found between grain yield and spikelet fertility (R = 0.57, P = 0.0001) and both were significantly and negatively correlated with the number of days to 80% flowering and maturity. Moreover, secondary branching had a positive correlation with total number of spikelets (R = 0.69, P = 0.0001) (Table 5.5).

Table 5.2. Mean sum of squares for the effect of “Accession (Access.)”, “Replication (Rep.) and residuals (Error) for the 15 agronomic traits measured for the experiments under lowland and upland conditions. ***, ** or * indicate a significant effect of accession and/or replication on a certain trait at a significance level of α = 0.001, α = 0.01 and α = 0.05, respectively.

Trait	Lowland			Upland		
	Access. (*df* = 48)	Rep. (*df* = 2)	Error (*df* = 96)	Access (*df* = 44)	Rep. (*df* = 2)	Error (*df* = 88)
Tillers_Total	19 ***	9.99 *	2.84	35 ***	4.92	1.85
Tillers_Fertile	19 ***	9.65 *	2.98	34 ***	3.59	2.29
%Fertile_Tillers	36.45 ***	33.91	17.04	46.19	4.93	33.20
Length_Pan	37 ***	31.31	16.53	17 ***	1.48	1.22
Plant_Height	444 ***	419.70 ***	44.39	238 ***	5.14	21.20
Spikelets_TotalNum	2180 ***	313.1	233.8	2368 ***	17.43	85.67
Filled_Spikelets	1804 ***	93.39	248.77	2110 ***	262.20	143.5
%Fertile_Spikelets	49.96 ***	77.71 *	16.69	355.37 ***	246.43 **	37.45
Ram_Iaire	18 ***	8.72	4.85	8 ***	0.15	0.70
Ram_IIaire	197 ***	4.88	16.38	184 ***	9.64	8.15
Ratio_RamIIRamIaire	1.88 ***	0.14	0.19	1.69 ***	0.05	0.05
Panicules_Num	34,223 ***	8434	3348	31,117 ***	503.1	2141.7
FLW	440 ***	36.33	12.16	1150 ***	9.64	9.77
MAT	475 ***	36.18	12.20	961 ***	3.11	13.48
Yield	42,975 ***	16,730	11,962	62,184 ***	8141	5963

Table 5.3. Descriptive statistics (minimum value (Min.), maximum (Max.) values and standard deviation (Std.) of rice traits measured under lowland and upland conditions, together with the *p*-values indicating significance of the differences between upland and lowland conditions for a certain trait; *p*-values marked with ***, **, and * indicate significant differences between upland and lowland rice at α = 0.001, α = 0.01 and α = 0.05, respectively

Trait	Lowland			Upland			*p*-value
	Min.	Max.	Std.	Min.	Max.	SD	
Tillers_Total	5	20	2.89	3	21	3.56	0.4125
Tillers_Fertile	5	20	2.88	3	21	3.56	0.8075
%Fertile_Tillers	61.45	100.00	4.86	60.98	100.00	5.97	0.001 **
Long_Pan	15	69	4.85	18	30	2.52	0.8641
Plant_Height	76	134	13.46	82	135	9.60	0.5462
Spikelets_TotalNumber	54	185	29.57	62	193	28.90	0.0001 ***
Filled_Spikelets	51	172	27.51	35	163	28.14	0.1483
%Fertile_Spikelets	72.21	98.65	5.34	29.42	98.82	12.03	0.0000 ***
Ram_Iaire	4	29	3.02	6	16	1.75	0.1866
Ram_IIaire	1	39	8.70	2	40	8.13	0.0039 **
Ratio_RamIIRamIaire	0.02	3.35	0.86	0.16	3.20	0.77	0.0518
Panicules_Num	105	714	117.16	114	590	107.91	0.9754
FLW	75	136	12.63	64	156	19.74	0.0071 **
MAT	94	155	13.10	86	173	18.03	0.0208 *
Yield	96	783	158.01	31	869	159.92	0.0002 ***

Table 5.4. Associations of different yield-contributing traits and their direct effect on yield

Traits	%Fertile_ Tillers	Spikelets_T otalNumber	%Fertile Spikelets	Ram_Ilaire	FLW	MAT	Yield
%Fertile_Tillers		0.01	−0.17	−0.04	0.21	0.19	−0.27
Spikelets_Total Number	0.03		−0.09	0.69*	0.01	−0.01	0.09
%Fertile Spikelets	0.12	−0.12		−0.03	−0.70*	−0.69	0.57*
Ram_Ilaire	−0.16	0.61*	−0.17		−0.15	−0.10	0.27
FLW	0.16	0.17	−0.41*	−0.23		0.96*	−0.52*
MAT	0.1	0.12	−0.44*	−0.22	0.94*		−0.49*
Yield	0.17	0.08	0.06	0.24	−0.05	−0.03	

* = Significant at 0.001; correlation estimates in the upland appear above the diagonal and correlation estimates in the lowland appear below the diagonal

5.3.2. Performance of rice accessions for yield and yield components in upland conditions

PCA was performed using seven prior identified traits via ANOVA which contributed most to phenotypic variation. The first two principal components explained 68.82% of phenotypic variability within the whole group of accessions tested (42 rice accessions and 7 reference varieties). The trait contribution revealed by both principal components is presented in Table 5.5.

PC 1 showed a positive association with yield (0.42) and spikelet fertility (0.47) whereas the number of days to 80% flowering (−0.53) and the number of days to 80% maturity (−0.52) were negatively linked with PC 1.

Panicle secondary branching (0.69) and the total number of spikelets (0.69) were positively correlated with PC 2. A two-dimensional scatter plot involving all 42 accessions and the seven controls are presented in Figure 5.1. Three accessions groups were clearly separated with reference to PCs 1 and 2. Cluster 1 included 13 accessions (4 *O. sativa* and 9 *O. glaberrima*) and all seven reference controls, except CG 14. Cluster 2 included only 8 rice accessions: WAB0015772 (*O. sativa*), and 7 *O. glaberrima* accessions (WAB0024116, WAB0002145, WAB0032394, WAB0002093, WAB0024105, WAB0032487 and WAB0032230). Cluster 3 was composed of 21 *O. glaberrima* accessions and reference control CG 14. The majority of the accessions in cluster 1 were characterized by a short growth cycle and had high grain yields. Furthermore, accessions

in this cluster were characterized by relatively higher spikelet fertility, total number of spikelets and secondary panicle branching compared to accessions in cluster 2. By contrast, accessions in cluster 2 were low-yielding and had a long cycle duration. Accessions in this cluster, namely WAB0032230, WAB0002093, WAB0032394, and WAB0015772 had a very long growth cycle (146, 151, 170 and 172 days to 80% maturity) and low grain yield (50.62, 98.18, 51.06 and 257.30 g/m^2), respectively. Accessions in cluster 3 showed intermediate agronomic performance as evidenced by trait correlation analysis with both PCs 1 and 2. Three highly resistant *O. glaberrima* accessions, namely WAB0002143 and WAB0029182 from cluster 1 and WAB0029194 (cluster 3), out-yielded all seven reference controls with yields of 540, 573, and 603 g/m^2, respectively. Two highly resistant *O. sativa* accessions, namely WAB0035059 and WAB0035038 from cluster 1, out-yielded all seven reference controls used in the upland trials with yields of 669 and 717 g/m^2, respectively.

Table 5.5. Results of the percent of the variance of traits explained by the principal components in both upland and lowland growing conditions

Ecology	Upland		Lowland		
Traits	**PC 1**	**PC 2**	**PC 1**	**PC 2**	**PC 3**
%Fertile_Tillers	−0.20			−0.11	−0.74 *
Spikelets_TotalNumber		0.69 *	0.11	0.62 *	−0.07
%Fertile Spikelets	0.47 *	0.17	−0.40 *	−0.22	−0.32
Ram_IIaire	0.13	0.69 *	−0.13	0.70 *	0.06
FLW	−0.53 *		0.63 *		−0.08
MAT	−0.52 *		0.63 *		−0.04
Yield	0.42 *	0.11		0.26	−0.58 *

* = Significant correlation at α = 0.05

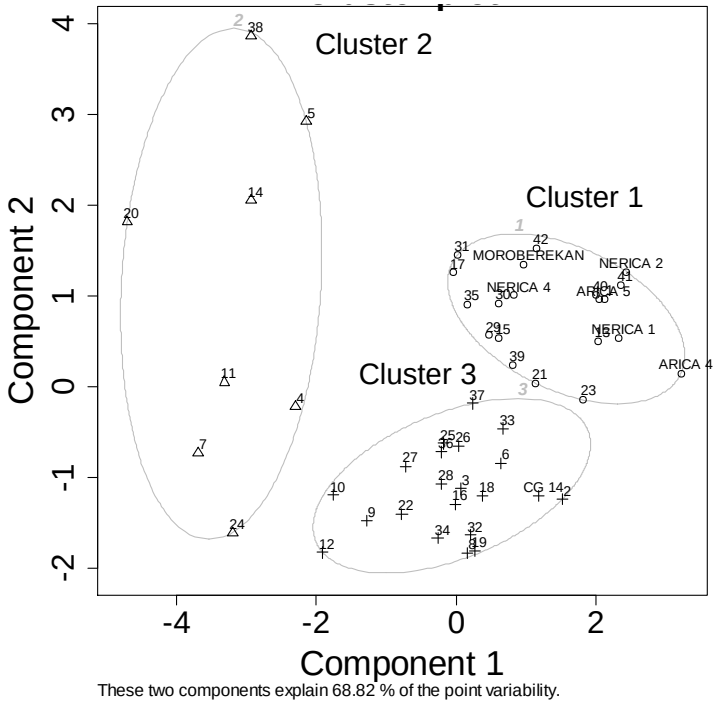

Figure 5.1. Scatter plot of rice accessions for two principal components based on seven agronomic traits in upland conditions

5.3.3. Performance of rice accessions for yield and yield components in lowland conditions

A similar PCA analysis was conducted to examine the relationships between accessions in lowland ecology test conditions. The number of days to 80% heading, and days to 80% maturity were positively correlated with PC 1 (0.63), whereas a significant negative correlation was found with spikelet fertility (−0.40). The total number of spikelets (0.62) and secondary panicle branching (0.70) were positively correlated with PC 2, whereas grain yield (−0.58) and percentage of fertile tillers (−0.74) were negatively correlated with PC 3 (Table 5.5).

All 37 rice accessions and the seven reference controls were split into three clusters according to the two first principal components (Figure 5.2). The two principal components (PCs 1 and 2) accounted for 57.64% of total variation among the studied germplasm. The first group, cluster 1, included seven accessions (6 *O. glaberrima* and 1 *O. sativa*) and 4 reference controls (ARICA 2, ARICA 3, NERICAL 19 and IR 841). Most rice accessions in cluster 1 produced the highest total number of spikelets and secondary panicle branching, whereas higher spikelet fertility was found in cluster 2. Cluster 2 included 21 rice accessions (three *O. sativa* and 18 *O.*

glaberrima) and two reference controls (NERICAL 14 and TOG 5681), whereas cluster 3 was composed of 9 *O. glaberrima* rice accessions and reference control ARICA 1. The majority of rice accessions in cluster 3 had a long duration for the number of days to 80% flowering and 80% maturity. Four rice accessions highly resistant to blast, namely WAB0035055 (*O. sativa*), WAB0019882, WAB0008956, and WAB0015043 (*O. glaberrima*) out-yielded all the reference controls (569.18, 567.78, 698.90 and 600.05 g/m^2, respectively). Among these, the three *O. glaberrima* rice accessions tested (WAB0008956, WAB0029342, and WAB0015043) performed better than all the *O. sativa*. One highly susceptible *O. glaberrima* accession WAB0029342 out-yielded all reference controls (ARICA 1, ARIC 2, ARICA 3, NERICAL 14, NERICAL 19, the *O. sativa* IR841 and the *O. glaberrima* TOG 5681) with a grain yield performance of 644.95 g/m^2. Eight rice accessions, including WAB0029182 (highly resistant) and WAB0030263 (highly susceptible), matured earlier than all controls used (less than 108 days). Accession WAB0030263 was found to be the earliest maturating one within all these tested (94 days).

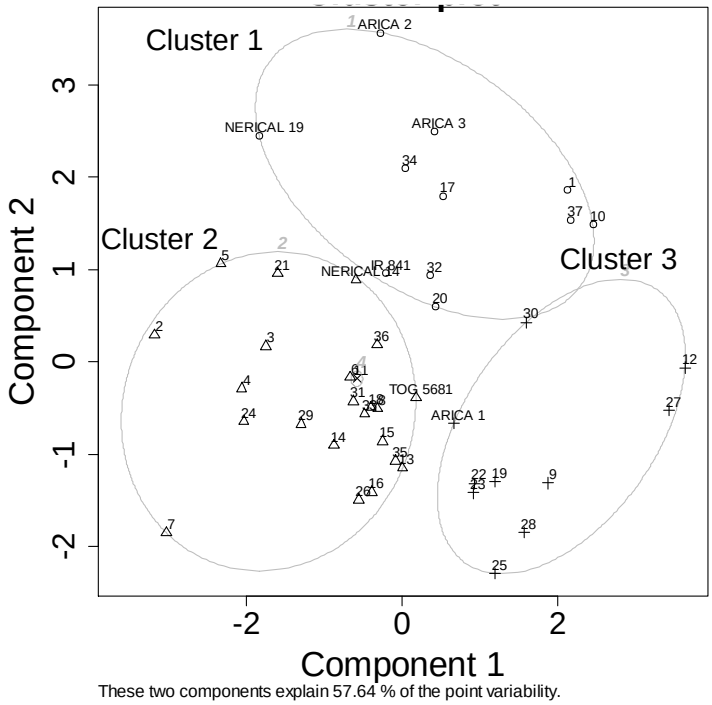

Figure 5.2. Scatter plot of rice accessions for two principal components based on seven agronomic traits in lowland conditions

5.3.4. Pairwise comparison of grain yield performance of accessions in lowland and upland agroecology

Grain yield scores were used as a selection index to rank accessions from the most- to the least-yielding. In upland ecology, WAB0035059 showed the best yield score followed by WAB0029194 and WAB0029182, whereas lower yields were observed for WAB0032394, WAB0002093, and WAB0024105. In lowland conditions, WAB0008956 and WAB0029342 achieved the best yield performance, whereas the lowest performance was recorded in WAB0002136 and WAB0030263 (Table 5.6). Blast-resistant accession WAB0035055 was ranked at positions 4 and 5 for yield performance in both lowland and upland conditions, respectively, and might be suitable for cultivation in both ecologies. Blast-susceptible accession WAB0008937 achieved similar and good yield performance in both lowland (498.34 g/m^2) and upland (669.49 g/m^2) conditions.

Accessions, WAB0030263 versus WAB0006684, WAB0015772 versus WAB0029315 and WAB0029323 versus WAB0026176 showed similar performance in both environments (in circles showed in Figure 5.3). Principal components analysis enabled the identification of the accession group named cluster 1 whether in lowland or upland conditions, which contained the majority of the reference controls used. Most rice accessions of this cluster 1 achieved yield performance superior to that of these lowland and upland reference controls. These accessions were found to be well-performing and might be suitable for either of the two environments. Most rice accessions of cluster 2 (upland) were classified with no upland reference controls. Based on these results accessions of cluster 2 might be unsuitable for upland.

Tukey's honest significance test was performed to compare the accessions' performance between lowland and upland test for grain yield and number of days to maturity. The results are presented in Table 5.6. On the basis of the ranking scores and the significant differences detected in grain yield and cycle duration, we identified 13 and 6 rice accessions that might be recommended to grow in lowland and upland conditions in Benin, respectively. Nineteen rice

accessions were relatively well-performing in both ecologies and might be proposed for cultivation in Benin.

Table 5.6. Results of the Tukey's honest significance test between lowland and upland test for grain yield (g/m^2) and number of days to 80% maturity (MAT) to determine their possible ecology; Accessions with the same letter in the same row are not significantly at α = 0.05

		Upland			Lowland			
Accessions	**Species**	**Grain Yield**	**MAT**	**Rank**	**Grain Yield***	**MAT**	**Rank**	**Possible Ecology**
WAB0015772	SATIVA	257[a]	172[a]	30	489[b]	140[b]	14	Lowland
WAB0006684	SATIVA	319[a]	95[a]	25	168[a]	99[b]	35	Upland
WAB0035055	SATIVA	494[a]	126[a]	5	569[a]	107[b]	4	BOTH
WAB0035059	SATIVA	669[a]	124[a]	1	498[b]	107[b]	12	Upland
WAB0029182	GLA	573[a]	117[a]	3	510[a]	108[b]	11	BOTH
WAB0029335	GLA	338[a]	132[a]	21	474[a]	118[b]	16	BOTH
WAB0030263	GLA	327[a]	103[a]	23	168[a]	94[b]	36	Upland
WAB0023837	GLA	395[a]	134[a]	13	555[a]	125[b]	6	BOTH
WAB0024105	GLA	100[a]	140[a]	35	358[b]	145[a]	27	Lowland
WAB0024116	GLA	322[a]	157[a]	24	421[a]	135[b]	25	Lowland
WAB0029194	GLA	603[a]	129[a]	2	476[a]	128[a]	15	BOTH
WAB0032487	GLA	190[a]	168[a]	33	258[a]	151[b]	34	Lowland
WAB0032298	GLA	371[a]	132[a]	17	343[a]	126[a]	29	BOTH
WAB0008589	GLA	409[a]	123[a]	12	471[a]	117[b]	18	BOTH
WAB0020477	GLA	425[a]	127[a]	9	295[a]	120[b]	32	Upland
WAB0029323	GLA	363[a]	137[a]	19	427[a]	127[a]	24	BOTH
WAB0019882	GLA	212[a]	135[a]	32	567[b]	127[b]	5	Lowland
WAB0029315	GLA	259[a]	131[a]	29	493[b]	122[a]	13	Lowland
WAB0020505	GLA	269[a]	133[a]	26	430[a]	135[a]	21	BOTH
WAB0032497	GLA	469[a]	129[a]	8	446[a]	129[a]	20	BOTH
WAB0015703	GLA	374[a]	115[a]	16	526[b]	112[a]	8	Lowland
WAB0001360	GLA	411[a]	131[a]	10	427[a]	124[b]	23	BOTH
WAB0029342	GLA	365[a]	132[a]	18	644[b]	135[a]	2	Lowland
WAB0008937	GLA	491[a]	123[a]	6	536[a]	107[b]	7	BOTH
WAB0032848	GLA	268[a]	138[a]	27	260[a]	136[a]	33	BOTH
WAB0032550	GLA	220[a]	125[a]	31	318[a]	120[a]	31	BOTH
WAB0002093	GLA	98[a]	150[a]	36	333[b]	150[a]	30	Lowland
WAB0002136	GLA	103[a]	147[a]	34	155[a]	143[b]	37	BOTH
WAB0002143	GLA	540[a]	112[a]	4	358[b]	111[a]	28	Upland
WAB0002145	GLA	263[a]	147[a]	28	454[a]	140[b]	19	Lowland
WAB0032345	GLA	333[a]	125[a]	22	512[a]	117[b]	9	BOTH
WAB0009280	GLA	348[a]	122[a]	20	510[b]	126[a]	10	Lowland
WAB0015043	GLA	410[a]	126[a]	11	600[a]	127[a]	3	BOTH
WAB0026176	GLA	375[a]	125[a]	15	429[a]	120[a]	22	BOTH
WAB0032495	GLA	389[a]	127[a]	14	413[a]	125[a]	26	BOTH
WAB0008956	GLA	469[a]	132[a]	7	698[b]	124[b]	1	Lowland
WAB0032394	GLA	51[a]	169[a]	37	471[b]	143[b]	17	Lowland
WAB0035038	SATIVA	717	134	-	-	-	-	Upland
WAB0029333	GLA	238	125	-	-	-	-	-
WAB0032230	GLA	50	146	-	-	-	-	-
WAB0032397	GLA	346	129	-	-	-	-	-
WAB0026783	GLA	270	152	-	-	-	-	-

GLA = *Oryza glaberrima*; SATIVA = *O. sativa*; BOTH = can be grown in both upland and lowland conditions *: g/m^2

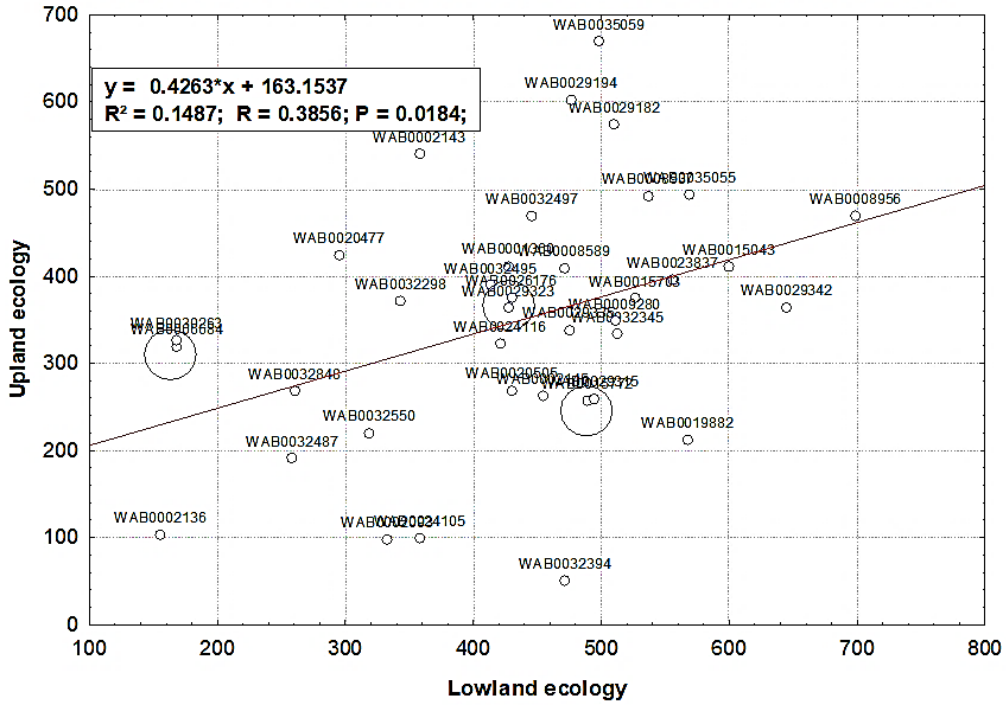

Figure 5.3. Grain yield/plot *(*g/m^2) of 37 rice accessions evaluated under upland and lowland conditions in Benin

5.3.5. Relationship analysis of yield components with blast resistance and genetic population diversity

The results of the correlation test (between blast severity (resistance/susceptibility) and agronomic traits) reveal that resistant accessions tend to produce a relatively higher number of secondary branching compared to susceptible provenances in both lowland (R = 0.52) and upland (R = 0.44) growing conditions. However, there was no direct association between blast resistance and grain yield in lowland (R = 0.08) nor upland (R = 0.26) environments, respectively.

The presence of three genetically distinct populations (populations 1, 2 and 3) was revealed in the current germplasm subset using 20 SSR polymorphic markers (See chapter 4). Population genetic structure was found to be significantly correlated with secondary panicle branching (R = 0.67) and primary panicle branching (R = 0.41) of our rice accessions in upland growing conditions. Accessions in population 1 produced a significantly higher number of secondary

panicle branches than those in populations 2 and 3. Also, accessions in population 3 produced a significantly higher number of primary panicle branches than those in populations 1 and 2.

In the lowland trial, we found significant correlations between population genetic structure and the following agronomic traits: total number of tillers (R = 0.41), percentage of fertile tillers (R = 0.45), secondary panicle branching (R = 0.70) and ratio of secondary branching to primary branching (R = 0.73). The majority of accessions in populations 2 and 3 tended to develop a better tiller ability, whereas accessions in population 1 showed a higher number of secondary panicles branching and a higher ratio of secondary branching to primary branching.

5.4. Discussion

Significant differences were observed between our rice accessions for the 15 agronomic traits measured under both lowland and upland ecologies, respectively. This confirms the existence of high genetic variability in the studied rice germplasm (Ogunbayo *et al.* 2014). This variability will help rice breeders properly select parental lines for further yield improvements (Acquaah 2007).

Furthermore, it was seen that our rice accessions responded differently across the three repetitions in upland conditions for spikelet fertility, and in lowland conditions for the total number of tillers, number of fertile tillers, plant height, and spikelet fertility. Plant height and number of tillers are in general sensitive to environmental conditions (high water level), especially when there is long-standing water. We observed there was no significant difference in grain yield between the number of repetitions. Differences between our accessions for spikelet fertility in both ecologies were possibly due to bird damage. Many studies show that rice grains are often damaged by birds, especially during grain maturation process (milk to hard-dough stages) (Ruelle and Bruggers 1982; Tréca 1987).

The performances of our accessions differed significantly for seven agronomic traits between upland and lowland rice. Both experiments were simultaneously conducted on AfricaRice's site in similar physicochemical soil conditions. These significant differences observed between the lowland and the upland tests might be most likely attributed to the hydrological conditions

(duration and intensity of flooding) (Montcho *et al.* 2013). We suggest the subset of seven traits to be given a greater priority for the selection of suitable lowland and upland rice accessions in Benin.

Concerning the relationship between yield and other traits, it was concluded that secondary panicle branching was strongly correlated with the total number of spikelets and could have contributed to grain yield performance of our accessions under lowland conditions. Zhao *et* al. (2016) recently identified a Single-Nucleotide Polymorphism locus (G/C) at the -1253 bp of promoter region, which substantially affects both the total number of spikelets per panicle and number of primary and secondary branches in some high-yielding *Japonica* rice varieties. According to Ashikari *et* al. (2005), grain yield is mostly determined by the total number of spikelets per panicle.

The upland experiment revealed that spikelet fertility and growth cycle duration (the number of days to 80% flowering and 80% maturity) were the most-important grain yield contributors. The shorter the cycle duration, the more spikelets were fertile and the higher grain yield under upland conditions. Mokuwa *et* al. (2013) also pointed out a negative relationship between grain yield and the number of days to 50% flowering in *O. glaberrima* and *O. sativa* accessions.

Under stress conditions, it can be observed that early-maturing varieties can produce higher grain yield than late-cycle varieties; therefore, they would be harvested before the end of the growth cycle thus reducing the risk of damage (Lafitte *et al.* 2003). Several authors (Mokuwa *et al.* 2013; Laborte *et al.* 2015; Richards 1986) suggested that grain yield and maturity duration are the most important characteristics used by farmers to select varieties. A recent participatory, ethnobotanical survey indicated that Beninese farmers put particular emphasis on high-productive and early-maturing varieties for selecting varieties (Odjo *et al.* 2017).

Three *O. glaberrima* rice accessions (i.e. WAB0008956, WAB0029342, and WAB0015043) showed higher grain productivity than *O. sativa* rice accessions in the lowland. Previous work demonstrated that *O. glaberrima* is the species better-adapted to Africa's adverse environmental conditions compared to *O. sativa* (Jones *et al.* 1997; Linares 2002; Sarla and Swamy 2005; Ndjiondjop *et al.* 2012; Mokuwa *et al.* 2013). However, a significant reduction in

grain yield is often observed in this species due to the grain shattering and susceptibility to lodging (Linares 2002; Mokuwa *et al.* 2013). Also, it is often seen that *O. glaberrima* possesses lower secondary panicle branching compared to *O. sativa* (Sarla and Swamy 2005). In our collection, most *O. glaberrima* accessions in cluster 1 (lowland and upland) achieved better performance for the total number of spikelets and panicle secondary branching than *O. sativa*, which contributed to their grain yield potential. Based on the results of our study, we need to reconsider some *O. glaberrima* as of agronomic importance for a better valorization of this species.

A better agronomic performance was particularly observed in the earlier identified blast-resistant rice accessions (See chapter 3) when comparing to the reference controls used. This means in case severe blast attacks would occur in the field, these blast-resistant accessions would yield better than most blast-susceptible varieties currently grown by farmers in Benin. It is more interesting for a variety not only to achieve good yield performance but also to have resistance to diseases. In fact, our findings in chapter 3 are among the first ones giving any insight on the resistance/susceptibility of each rice accession across two different environments in Benin. The study clearly demonstrated that rice germplasm exhibiting high blast disease resistance is potentially resistant to all isolates/races of the pathogen that prevail in those two environments (See chapter 3). Studies by Odjo *et al.* (2011) clearly highlighted the risk that blast disease represents to these environments (hotspots) where we also set up these agronomic evaluation trials. Planting our accessions in these environments carries with it a further advantage in that the resistant rice reduces the probability of blast invading. These results could enhance the potential acceptability of our accessions by farmers in Benin.

Higher secondary panicle branching was positively correlated with blast resistance in both lowland (R = 0.52) and upland (R = 0.44) conditions. Identification of blast resistant accessions, along with high-secondary branching would be important to employ them in future breeding programs. Since blast disease is the most harmful biotic threat to Beninese rice production, the combination of high-yield potential of rice accessions with their resistance to the disease

can help minimize yield losses and thus reduce chemical pesticide applications (Zhu *et al.* 2004; Roy-Chowdhury *et al.* 2012a).

Genetic structure analysis of our germplasm collection revealed the presence of three genetically distinct populations where an intense gene flow has occurred between *O. sativa* and *O. glaberrima* accessions across population 1 (See chapter 4). Valuable information on the relationships between genetic population structure and agronomic characteristics was highlighted in the present chapter. Thus, information can be used in breeding for attaining higher yields (Sie *et al.* 2012). Also, we showed in this chapter that nearly all *O. glaberrima* accessions in population 1 yielded at least 4 tons ha^{-1} in lowland ecology except for WAB0030263. However, this WAB0030263 was the most-early maturing accession out of the total number of accessions tested and this is one of the characteristics preferred by farmers for growing rice.

5.5. Conclusions

This study revealed high phenotypic variability among the screened rice accessions, which is of high value for breeding programs. Differential performance in upland and lowland conditions for several traits indicates that these traits are substantially influenced by environmental factors. In addition, we observed significant correlations between yield and several phenotypic traits, which are important and can be used as markers for early screening for identifying promising, high-yielding varieties. The results of the present study highlight the potential of the core selection of African rice germplasm for developing new blast-resilient rice varieties in general and more especially for the Beninese environment. The agronomic traits associated with blast resistance and genetic structure will both globally and locally help breeders improve rice production. However, multi-year trials at multiple locations are still required to check for stability of these agronomic traits to validate the performance of these promising accessions.

CHAPTER SIX

6. Screening of a set of rice accessions: (*Oryza* spp.) for strong resistance against *Magnaporthe oryzae*, the causal agent of rice blast disease

Abstract

Blast disease caused by *Magnaporthe oryzae* is worldwide a major constraint to rice (*Oryza* spp.) cultivation. Screening rice varieties with strong resistance to the disease is known to provide farmers with efficient and low-cost blast management alternatives. The objective of the present study was to identify rice accessions with strong resistance to nine blast isolates that represent a diversity of blast pathotypes occurring in Africa. For this purpose, twenty-one rice accessions originating from West Africa were screened using a set of *M. oryzae* isolates collected in Benin. All experiments were done in a greenhouse. High differences in our germplasm responses were observed. Overall, 66.67% were resistant to at least five isolates, whereas 19% were susceptible to all isolates tested. Six rice accessions (WAB0029182, WAB0029194, WAB0032298, WAB0032497, WAB0015703, WAB0002143, and WAB0008956) were found to be particularly resistant to all Beninese blast pathotypes tested. Analyses of resistance inheritance to pathotype BN0252 in the best-resistant accessions WAB0029194, WAB0008956 and WAB0032298 crossed with the susceptible accession WAB0030263, revealed a Mendelian segregation ration 3:1 (Resistant:Susceptible). The chi-squared values (0.14, 1.04 and 3.07) indicated that resistance is most likely controlled by (one) single dominant gene. We suspect that the observed resistance may be controlled by genes other than the resistance genes known so far in rice. Accession WAB0030263, which was shown to be highly susceptible to all 9 Beninese blast isolates was found to carry the gene DTY3.1 for drought tolerance. Also, we found that accession WAB0032298 which has a strong resistance to all blast pathotypes tested possesses a gene responsible for submergence tolerance as well. This study revealed new information that supports the existence of valuable sources for breeding rice varieties for tolerance/resistance to multiple stress factors, including blast resistance.

Keywords: Benin, blast isolates, drought and submergence tolerance, rice, intraspecific lines

6.1. Introduction

Rice (*Oryza* spp.) blast caused by *Magnaporthe oryzae* (Couch and Kohn 2002) is the most widespread and harmful fungal rice disease worldwide (Kwon and Lee 2002; Li *et al.* 2007). Blast pathogen is a serious threat to rice production because of the wide distribution and ability to survive in diverse environmental conditions. Yield reductions up to 100% have been reported. This severely impairs rice food security and economy on the continent (Sere *et al.* 2013). Extensive and uncontrolled use of fungicides for the management of blast is hazardous to human health and poses serious environmental safety concerns as well (Nicolopoulou-Stamati *et al.* 2016). A number of *M. oryzae* strains have become resistant to fungicides (DHN-melanin biosynthesis inhibitors (MBIs) and quinone outside inhibitors (QoIs)), in a relatively short time (about three years) after their introduction (Yamaguchi *et al.* 2002; Kaku *et al.* 2003; Takagaki *et al.* 2004). These uses of fungicides are not enough to protect against this disease. In most cases, it is expensive and difficult to protect rice crops from blast by chemical fungicides. The use of resistance in rice crop varieties is the most-effective and most-sustainable way of managing blast epidemics in the field (Zhu *et al*. 2004; Roy-Chowdhury *et al.* 2012). However, blast resistance is sometimes known to be also unstable and lasts for only a short period of time in the field because of the rapid evolution of *M. oryzae* populations (Dean *et al.* 2005; Valent and Khang 2010; Fukuoka *et al.* 2015) to these new varieties.

Two major categories of rice blast resistance have been reported, i.e. qualitative and quantitative resistance (Ou *et al.* 1975). Qualitative resistance, controlled by major resistance genes (R genes) is specific to pathogen pathotypes. It is generally of a limited lifetime (Mackill and Bonman 1992; McDonald and Linde 2002; Dai *et al.* 2010). By contrast, quantitative resistance that is non-pathotype-specific and controlled either by several minor or major genes (QTLs), confers durable resistance to disease (Wisser *et al.* 2005; Roy-Chowdhury *et al.* 2012; Wang *et al.* 2013). To date, almost 347 quantitative trait loci (QTLs) and 102 single blast R genes have been identified of which 27 genes (Pita, Pib, Pb1, Pizt, Pid2, Pii, Pikm, Pit, Pid3, Pid3-A4, Pish, Pik, Pikp, Pia, PiCO39, Pi1, Pi2, Pi5, Pi9, Pi21, Pi25, Pi33, Pi36, Pi37, P50,

Pi54, and Pi65(t)) have been cloned using specific molecular markers (Khanna *et al.* 2015; Zheng *et al.* 2016; Khan *et al.* 2018).

Blast resistance genes have been successfully transferred from a donor variety into susceptible recipients by using marker-assisted selection (MAS) (Wang *et al*. 2007; Ashkani *et al*. 2012). Genetic improvements of *O. sativa* L. with *O. glaberrima* Steud. genes have been carried out in Africa to develop varieties with stable protection against multiple stress factors, including blast. A series of popular hybrids known as "New Rice for Africa" or NERICA have been developed and were proven to be well-adapted to African rice-growing environments (Pham 1992; Jones *et al.* 1997; Futakuchi and Sié 2009; Sié *et al.* 2010; Thiémélé *et al.* 2010). However, new, more aggressive *M. oryzae* races were able to overcome resistance in these varieties controlled by a single gene such as Pi1, Pi7, Pi5, Pikp, Pia, Pita2, Piks, Pi3, Pik, Pita, Piz, Pikh and Pikm (Baboy *et al.* 1995; WARDA 1999; Odjo *et al.* 2011).

About 100 rice varieties were recently documented from 55 different villages of Benin, many of them also are no longer efficient against blast disease (Odjo *et al.* 2017). As new *M. oryzae* strains may lead to resistance breakdown as well as to increased blast disease epidemics, there is needed to evaluate and identify additional sources of blast resistance to be used in subsequent breeding programs.

We performed a screening of a set of 350 cultivated rice (*Oryza sativa* & *O. glaberrima*) accessions for field resistance to blast in upland conditions in Benin. We noted the existence of a broad source of variability for blast resistance (See chapter 3). Further, a phenotypically variable subset of 42 accessions was selected to represent the diversity of the initial germplasm collection for further evaluation and use in breeding programs. However, this study needs to be followed by inoculating rice seedlings in laboratory conditions with pathogenic blast isolates in order to ascertain the effectiveness and spectrum of field resistance. In addition, we need to determine whether the observed resistance is controlled by combinations of the classical-known genes or rather by new unidentified R genes. There is thus necessary

to perform further inoculation experiments with specific *M. oryzae* isolates under controlled conditions.

We selected in our previous work, different isolates that represent blast pathogen diversity occurring in Africa (Odjo *et al.* unpublished). These isolates were tested on 54 differential accessions and 10 known resistant varieties, which carry the majority of the Blast R genes known till now (Awande *et al.* unpublished). This set of blast isolates was then used in the present study to investigate whether the observed field-resistant rice accessions have strong (i.e. effective against all blast pathotypes) resistance against the disease. Our hypothesis was rice accessions that would resist all these Beninese isolates would most likely resist anywhere in Africa. We also evaluated resistance in F_2 segregating rice populations and backcross to examine the Mendelian inheritance of blast resistance. The hypothesis was considered true if the associated probability was higher than 5%. We also used sets of Single Nucleotide Polymorphism (SNP) markers for exploring the presence or absence of genes for blast resistance, Bacterial Leaf Bight (BLB) resistance, drought and submergence tolerance. The results of these investigations will together help better valorize the resistant accessions possessing desired characteristics in the breeding process (Hittalmani *et al.*, 1995; Naqvi and Chattoo, 1996).

6.2. Materials and methods

6.2.1. Plant material

Three *Oryza sativa* L. and 18 *O. glaberrima* Steud rice accessions collected from Benin, Guinea, Liberia, Nigeria, and Mali were used in this study (Table A.3, appendix). This germplasm is part of a wider African collection of 350 rice accessions (acquired from AfricaRice) which was previously evaluated for field resistance to blast disease in Benin (See chapter 3). Several blast reaction patterns were observed: 14 accessions were shown to be highly resistant, 2 moderately resistant, 1 moderately susceptible and 4 susceptible. Accessions Tetep and CO39 (acquired from AfricaRice) were included as respectively resistant and susceptible controls. To study the genetic inheritance of blast resistance, we

choose the best-resistant rice accessions (3 male parents) that we crossed with one highly blast-susceptible accession (female parent) to develop F_2 segregating and backcross populations. We performed this experiment to verify whether the observed segregation matches the expected Mendelian inheritance, considering the hypothesis that resistance is controlled by a single gene.

6.2.2. *Magnaporthe oryzae* isolates

Nine *M. oryzae* isolates collected in diverse rice-growing areas of Benin (Malanville, Bétérou, Djougou, Kérou, Lokossa, *etc.*) were used against our subset of 21 rice accessions for evaluating the latter's resistance behavior. These pathogenic blast isolates were provided by Dr. Drissa Silué head of AfricaRice pathology laboratory. The selected *M. oryzae* strains were tested on 64 differential blast-resistant varieties (harboring specific known blast resistance genes) that carry the majority of the known R genes (Table A.4, appendix). These isolates all together match the resistance of these R genes and thus represent the genetic diversity of the majority of blast pathotypes occurring in Africa including Benin (Awande *et al.* unpublished).

6.2.3. Inoculation and disease evaluation

Stock blast isolates used for inoculation were re-cultured on rice polish agar plates consisting of 10 g of rice polish powder, 2 g of yeast extract, and 15 g of agar powder per liter of tap water) (Table A.4, appendix). To induce conidia formation, plates were incubated at 25°C under 12 hours of fluorescent light and 12 hours of darkness for 12 days (Bhaskar *et al.* 2018).

Plates were washed with distilled water by using a paintbrush to make the spore suspension. Spore suspension of *M. oryzae* isolates was adjusted to 5×10^4 conidia/mL. One hundred and twenty rice seedlings/tray at 4- to 5-leaves stage were spray-inoculated per in an inoculation chamber by spraying with about 50-100 mL conidial suspension (5×10^4 conidia/mL) according to the method described by Roumen *et al.* (1997). Inoculated plants were kept in darkness in a confined chamber for 24 h where the temperature was at 26-28°C and with a relative humidity

of 95%. Plants were then transferred to the greenhouse (28°-30°C) for six days before disease evaluation.

A total of 23 rice accessions (including Tetep and CO39 used as controls) were grown in plastic trays of 12 rows (10 seeds of each accession were sown per row) containing standard greenhouse potting mixture (perlite and vermiculite). Three trays replicates were inoculated at the same time with each blast isolate. We repeated this experiment (inoculation) two times for each blast isolate tested. A third experiment was done when the results of the first two experiments were inconsistent.

Disease evaluation was performed 1 week after inoculation for each experiment. Blast disease scores (ranging from 0 to 5) were based on lesion type and diseased area as described by Shi *et al.* (2010). Mean Disease Index (DI) was calculated by averaging disease scores obtained for each rice accession. Disease reactions were classified into two groups based on DI: accessions with DI < 3 were considered as resistant, while those with DI ≥ 3 were considered as susceptible. The percentage of resistance reactions to the set of nine blast isolates was calculated as a ratio of the total resistance reactions for a specific accession/isolate interaction over a total of nine isolates tested. This may give some insight into the effectiveness of blast resistance.

6.2.4. Investigating blast resistance inheritance and presence/absence of resistance genes

Three accessions, namely WAB0008956, WAB0029194, and WAB0032298, which showed strong resistance to all tested blast isolates based on disease reactions, were chosen and crossed with susceptible accession (WAB0030263) to develop segregating F_2 populations for inheritance analysis of blast resistance. Genotyping by gene-specific DNA markers was also conducted according to the method used by Kitazawa *et al.* (2019). We inoculated a number of 230, 233 and 352 rice plants from F_2 of the three populations, respectively with the most pathogenic blast isolate BN0252. A backcross population from $F_{2\ (WAB0032298\ X\ WAB0030263)}$ X

WAB0030263 was also tested to confirm blast resistance inheritance in WAB0032298. Additionally, individual plant leaves were randomly harvested and DNA extraction was done according to the CetylTrimethyl Ammonium Bromide (CTAB) method described by Saghai-Maroof *et al.* (1984). DNA samples were genotyped using a set of 34 trait-based Single Nucleotide Polymorphism (SNP) markers provided by an external KASP™ service provider in the USA. The SNPs are associated with different genes responsible for biotic and abiotic stresses tolerance in rice. Among the 34 SNP markers used, 3 markers were associated with bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*, BLB) resistance, 2 with blast resistance, 2 with drought tolerance, three with submergence tolerance and grain chalkiness and fragrance, while 24 other SNPs were used for progeny verification. Details of the 10 gene-linked SNP markers are given in Table 6.1.

Table 6.1. Characteristics of the 10 gene-linked SNP markers tested

SNP marker code	Gene/QTL	Chromosome	Physical position (Mb)	Trait	Favorable Allele
snpOS0089	DTY3.1	3	31.3	Drought tolerance	C
snpOS0024	Chalk5	5	3.3	Grain chalkiness	G
snpOS0040	Submergence	9	6.4	Submergence	T
snpOS0002	xa13	8	27.5	Bacterial leaf blight resistance	G
snpOS0061	Xa21	11	21.3	Bacterial leaf blight resistance	C
snpOS0054	xa5	5	0.4	Bacterial leaf blight resistance	AG
snpOS0031	BADH2	8	20.38	Fragrance	-
snpOS0096	DTY12.1	12	17.6	Drought tolerance	A
snpOS0009	Pi2	6	10.38	Blast resistance	-
snpOS0007a	Pi9	6	10.38	Blast resistance	-

Source: IRRI website at http://gsl.irri.org/genotyping/trait-based-genotyping/10-snp-panel

6.2.5. Statistical analysis

Mean Disease Index (DI) was calculated in Excel by averaging disease scores obtained for each rice accession. The chi-square analysis for the genotypic and phenotypic ratio was calculated by using the formula, $\chi^2 = (O - E)^2 / E$, where O is the observed value, and E is the expected value of DI. By the hypothesis of one dominant gene, the expected ratio of Mendelian segregation was 3:1 in F_2, and 1:1 in BC_1F_1 populations. This hypothesis was considered true if the associated probability calculated in Excel was higher than 5%. In other words, the chi-square value was not higher than 3.84 (Miah *et al.* 2015).

6.3. Results

6.3.1. Resistance spectrum of 21 cultivated rice accessions to 9 isolates

We observed a wide range of disease reactions among the rice germplasm examined. Accessions Tetep and CO39, which were used as controls, were consistently found resistant

and susceptible, respectively. A high proportion of rice accessions (33%) showed resistance reactions to all 9 blast isolates, whereas 19% were found susceptible to all isolates tested (Table 6.2). In previous pathogenicity tests conducted by Odjo *et al.* (unpublished), only two differential rice lines (Oryzica_llanos5 and Pin4) resisted all *M. oryzae* isolates out of the 64 differential varieties tested for blast resistance (Table 6.2). Figure 6.1 shows the relationship among the 21 rice accessions and groups them into two main clusters. Cluster 1 (in black) and cluster 2 (in red) are composed of 13 and 8 rice accessions, respectively. Accessions in cluster 1 were shown to have resistance to at least 5 isolates, whereas those in cluster 2 were susceptible to the majority of tested blast isolates (at least 5 isolates).

Accessions WAB0008956, WAB0002143, WAB0015703, WAB0032497, WAB0032298, WAB0029194, and WAB0029182 resisted all isolates tested and were grouped in cluster 1. WAB0029335 and WAB0008937 were susceptible to only one isolate (BN0119 and BN0050, respectively). WAB0020477 and WAB0032550 were found resistant to 6 blast isolates. Further rice accessions in cluster 1, WAB0006684 and WAB0019882 showed resistance against 5 blast isolates.

In cluster 2, accessions WAB0030263, WAB0001360, WAB0029342, and WAB0032230 were susceptible to all blast isolates tested. WAB0035059, WAB0009280, and WAB0035055 were susceptible to at least 6 out of the 9 isolates used. Only WAB0026176 in cluster 2 showed susceptibility to 4 blast isolates.

The most aggressive isolates were BN0119, BN0202 and BN0252 with broad pathogenicity (pathogenic on 12, 11 and 11 rice accessions, respectively), whereas isolates BN0082 and BN0094 had the lowest pathogenicity (pathogenic on 6 rice accessions only).

Table 6.2. Reaction pattern of 21 rice accessions against 9 Beninese blast isolates tested

Accession number	BN0013	BN0040	BN0050	BN0066	BN0082	BN0094	BN0119	BN0252	BN0202	Resistance rate (%)
WAB0006684	R	R	R	S	S	R	R	S	S	55.56
WAB0029182	R	R	R	R	R	R	R	R	R	100
WAB0029335	R	R	R	R	R	R	S	R	R	88.89
WAB0029194	R	R	R	R	R	R	R	R	R	100
WAB0032298	R	R	R	R	R	R	R	R	R	100
WAB0020477	R	R	S	R	R	R	S	S	R	66.67
WAB0019882	S	R	R	R	S	R	S	R	S	55.56
WAB0032497	R	R	R	R	R	R	R	R	R	100
WAB0015703	R	R	R	R	R	R	R	R	R	100
WAB0008937	R	R	S	R	R	R	R	R	R	88.89
WAB0032550	S	R	R	R	R	R	S	S	R	66.67
WAB0002143	R	R	R	R	R	R	R	R	R	100
WAB0008956	R	R	R	R	R	R	R	R	R	100
WAB0035055	S	S	R	S	R	S	S	S	S	22.22
WAB0035059	R	S	R	S	R	S	S	S	S	33.33
WAB0030263	S	S	S	S	S	S	S	S	S	0
WAB0001360	S	S	S	S	S	S	S	S	S	0
WAB0029342	S	S	S	S	S	S	S	S	S	0
WAB0032230	S	S	S	S	S	S	S	S	S	0
WAB0009280	S	S	S	S	R	R	S	R	S	33.33
WAB0026176	R	S	R	R	R	R	S	S	S	55.56
									Average	60.32
Tetep	R	R	R	R	R	R	R	R	R	100
CO39	S	S	S	S	S	S	S	S	S	0

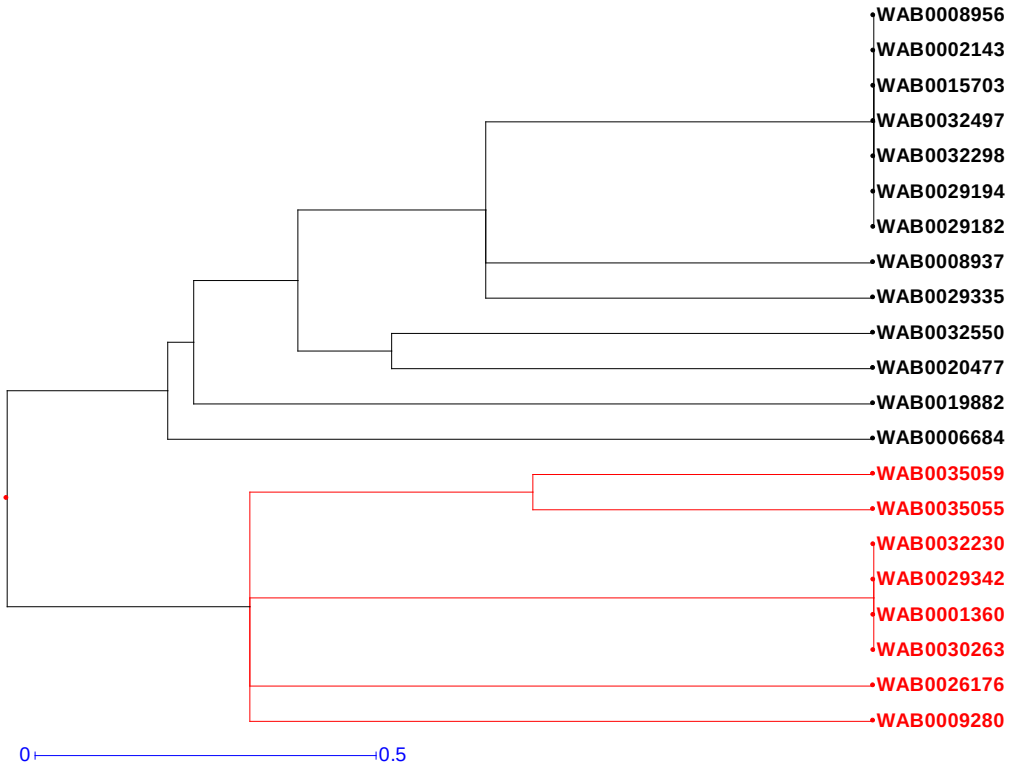

Figure 6.1. Dendrogram of the 21 rice accessions based on their reactions to a diverse set of isolates from Benin. Colours indicate the nature of clusters: accessions in cluster 1 are written in black and resisted at least five isolates, while those in cluster 2 (in red) are susceptible to at least five isolates.

6.3.2. Inheritance of Blast resistance in our rice accessions

Inheritance of blast resistance from the crosses between resistant rice accessions WAB0029194, WAB0032298, and WAB0008956 (male parent) and susceptible accession WAB0030263 (female parent) to isolate BN0252 is presented in Table 6.3. The F_2-population of the WAB0008956 x WAB0030263 cross segregated in 267 resistant and 85 susceptible plants. The chi-square value of 0.14 with P-value = 71% (P > 5%) corresponded well to the expected ratio 3:1 of Mendelian segregation for a dominant gene. The F_2-population of the WAB0029194 x WAB0030263 cross segregated in 168 resistant and 65 susceptible plants. The chi-square value was 1.04 with P-value = 31% showing that resistance also segregated into a 3:1 ratio for a dominant gene. In the F_2-population of the WAB0032298 x WAB0030263 cross, there were 161 resistant and 69 susceptible plants. The value of the chi-square $\chi 2$ =

3.07 with P-value = 8% (P > 5%) was high but non-significant. The maximum expected value for this test was less than 3.841. This also indicates a segregation ratio of 3:1 for a dominant gene. We found that backcross family BC_1F_1 of the WAB0032298 x WAB0030263 cross achieved 67 resistant vs 77 susceptible plants with a chi-square value of $\chi 2 = 0.69$ (P-value = 40%), which corresponds well to the expected segregation ratio of 1:1 for a dominant gene. The results of all crosses were similar to the expected Mendelian ratios 3:1 in F_2 and 1:1 in BC_1F_1 progenies for a dominant gene. Our results thus confirm the hypothesis of one dominant resistance gene in accessions WAB0029194, WAB0032298, and WAB0008956 against isolate BN0252.

Table 6.5. The response of F2 and backcross populations to blast isolate BN0252

Cross	Generation	Nbr. of plants	Nbr. of resistant	Nbr. of susceptible	Expected ratio R:S	χ2 test	P-value
P_1 x P_2	F2	352	267	85	3:1	0.14	0.71
P_3 x P_2	F2	233	168	65	3:1	1.04	0.31
P_4 x P_2	F2	230	161	69	3:1	3.07	0.08
(P_4 x P_2) x P_2	BC1F1	144	67	77	1:1	0.69	0.40

Nbr.= Number ; P_1 = WAB0008956 (resistant) ; P_2 = WAB0030263 (susceptible); P_3 = WAB0029194 (resistant); P_4 = WAB0032298 (resistant); P-value = probability value significant at $\alpha = 0.05$

6.3.3. SNP genotyping of strongly resistant rice accessions and intraspecific F_2 populations

SNP analysis did not show the presence of resistance genes either for blast resistance (Pi9 and Pi2) or bacterial leaf blight (Xa5, Xa13, and Xa21) among the F_2-populations and the three parental, resistant rice accessions (i.e. WAB0029194, WAB0032298, and WAB0008956). Nevertheless, an allele of the genes responsible for submergence tolerance was identified in parental blast-resistant accession WAB0032298. Additionally, susceptible accession WAB0030263 was found to carry a favorable allele of the gene DTY3.1 for drought tolerance. Gene DTY3.1 was present in several F2 progenies as well.

6.4. Discussion

Many blast-resistant rice varieties have been selected in the past for breeding purposes (Sere *et al*. 2004; Biotica *et al.* 2014; Vasudevan *et al.* 2014; Rama Devi *et al.* 2015). However, *M. oryzae*, the causal agent of rice blast, possesses a high genetic changing ability to challenge resistant lines/varieties. For this reason, rice yield losses due to blast remain usually much higher than those for any other fungal disease (Monsur *et al.* 2016). Despite the fact that rice breeding has been the focus of extensive research, new blast pathotypes were seen to occur in Africa, overcoming varietal resistance and leading to severe epidemics in the field (Sere *et al.* 2007; Odjo *et al.* 2011).

The present study thus concentrated on further screening, potentially unexploited germplasm, to broaden the set of currently known resistance genes. In the breeding work, this screening of resistance is indispensable. It is necessary not only for finding resistant materials but also for selection in progenies. The 21 rice accessions used were derived from a wider germplasm collection of 350 rice accessions that had shown a large variability for field blast resistance in Benin. Similar to the case of the latter field experiment, we also observed here a high variation for blast severity among our 21 rice accessions studied. The majority (66.67%) of these rice accessions resisted at least to 5 out of 9 blast isolates used that are potentially virulent to nearly all R genes known so far in rice blast (Odjo *et al*. unpublished). The isolates we used, represent the Africa-wide (including Benin) blast diversity for *M. oryzae* pathogenicity. We can thus assume that any accession in this work that resists all nine isolates should possess either R gene combinations (pyramids) or new resistance genes or QTL genes. A rice plant cannot be resistant to an isolate of *M. oryzae* unless the pathogen has the genes that make it avirulent on that rice plant. An isolate of *M. oryzae* cannot be avirulent on a rice plant unless the rice plant has genes that make it resistant to that isolate (Ellingboe and Chao 1994; Wang *et al.* 2014).

About 19% of our germplasm collection were shown to be highly susceptible to all blast isolates tested. Our previous results of chapter 3 also showed that these accessions were highly

susceptible to blast disease under field conditions in Benin. We performed a dendrogram which separated the most blast-resistant accessions in cluster 1, whereas cluster 2 was dominated by the most susceptible accessions. Therefore, breeders and researchers can use accessions in this cluster 1 to explore new genes for blast resistance and incorporate them in breeding programs (Zang *et al.* 2015).

The chi-square values of the crosses between resistant accessions WAB0029194, WAB0032298 and WAB0008956 (cluster 1) and susceptible WAB0030263 were consistent with the Mendelian segregation (P-value > 5%). Our study supports the hypothesis that heredity of blast resistance is controlled by a single dominant R gene. However, these results obtained here do not allow us to conclude whether the same gene is present in the three blast-resistant accessions WAB0029194, WAB0032298, and WAB0008956, or whether each accession has a different gene. Several studies also reported a similar inheritance pattern and concluded that blast resistance was mostly controlled by one or two dominant genes (Mackill *et al.* 1985; Yu *et al.* 1987; Pan *et al.* 1999; Sharma *et al.* 2007; Tanweer *et al.* 2015). We need to conduct additional research based on polymorphic molecular markers analysis like the set of 20 SSR markers used in chapter 4. Further research could allow us to confirm this segregation pattern of blast resistance from the crosses between accessions WAB0029194, WAB0032298 and WAB0008956 used as donor parents with the blast susceptible accession WAB0030263. The use of polymorphic molecular markers for genotyping these F_2 populations is required and could help to check for a possible additive effect of minor QTL genes present in accessions WAB0029194, WAB0032298, and WAB0008956.

Genotyping results using SNPs did not reveal the presence of blast resistance genes, Pi2 or Pi9, in parental accessions WAB0029194, WAB0032298 and WAB0008956 and or in the F_2 populations. These R genes, Pi2 and Pi9, were reported to confer strong resistance against blast disease (Qu *et al.* 2006; Zhou *et al.* 2006). Blast resistance we evidenced in these three accessions could not be linked to Pi2 or Pi9; We assume that alternative known genes or new genes control resistance to all Beninese blast pathotypes.

An allele of the gene responsible for submergence tolerance was present in the parental, blast-resistant accession WAB0032298. QTL for submergence was mapped on rice chromosome 9 (Xu and Mackill 1996; Septiningsih *et al.* 2012). Waterlogging and floods cause substantial rice yield losses worldwide, especially in the tropics and subtropics, including Africa (Das *et al.* 2009). Accession WAB0032298 can thus be used for breeding submergence tolerant rice.

Parental, blast susceptible accession WAB0030263 along with some progenies were found to carry an allele of the gene DTY3.1 responsible for drought tolerance in rice (Venuprasad *et al.* 2009; Dixit *et al.* 2014). WAB0030263 used as a recipient of blast resistance will offer an additional advantage for its adaptation to water deficit environments. Farmers highly value rice accessions that are tolerant to droughts and floods. Consequently, we found convenient to suggest to use both WAB0032298 and WAB0030263 to sustain a variety of breeding programs to improve rice tolerance to multiple stress factors, simultaneously.

6.5. Conclusion

We assessed a diverse set of rice accessions for resistance to *M. oryzae* and identified a cluster that contained 13 rice accessions with a wide blast resistance spectrum. Six of these accessions were resistant to all Benin blast isolates. Results further confirm that our rice collection consists of interesting sources for blast resistance genes that can be used in breeding programs.

We found that resistance to the very virulent isolate BN0252 was controlled by a single dominant gene. It will be valuable to further characterize and map these genes resistant to all blast pathotypes in order to use for breeding new cultivars with resistance to blast through genetic engineering or molecular assistance selection approaches.

Furthermore, we detected two alleles of the genes associated with drought and submergence tolerance, respectively. Benin rice production can be potentially improved when successfully combined blast resistance with other characters such as drought and submergence tolerance.

CHAPTER SEVEN

7. Conclusion and further research perspectives

7.1. Exploring rice genetic diversity and blast resistance under field condition (Chapter 3)

We characterized the genetic diversity of cultivated rice in Africa to select accessions of high productivity and resistant to blast for use in subsequent breeding programs. The study started with field evaluations of blast resistance of 350 rice accessions in upland conditions in Benin. Genetic diversity of these rice accessions assessed by AFLP markers was combined with accessions' response to blast in the field to select a sub-collection, which comprises most of the genetic diversity within our initial germplasm collection. This subset selection would be easier to evaluate as it contains a much smaller number of samples than the whole germplasm collection. It would also facilitate their conservation and use in further studies and breeding programs. Furthermore, high genetic diversity is expected to be found in this germplasm subset collection. This work was set up to answer three main research questions.

Research question 1: How do our rice accessions react to natural blast attacks in the field?

Our work reported high genetic variability for blast resistance within the collection of 350 rice accessions studied. Many accessions (24%) exhibited partial resistance with scores between 3 and 4, whereas a few accessions (12%) showed complete resistance when compared to the standard susceptible control varieties (CO39 and Maratelli). Among these two categories, we can select a high level of blast resistance variability since Awande (2015) had demonstrated that three blast pathogen races (ID, IE, and IF) often occur in the studied rice production environment. Understanding the genetic basis of this partial resistance (shown by 24% of the accessions studied) may require a broader search of QTL resistance genes than just characterization of R-genes (Vasudevan *et al.* 2014). In-plant breeding the screening for resistance is indispensable for finding resistant germplasm. This resistant germplasm is seen to be an essential consideration because, for resource-poor farmers (especially in developing

countries like Benin), the options for managing diseases are limited (Leung *et al.* 2003). Screening for blast resistant genotypes will enable farmers to select their preferred varieties to use to reduce grain losses caused by rice blast. This could lead to the widespread adoption of our accessions (Bonman *et al.* 1992). Also, our germplasm will allow breeding programs to develop rice varieties with resistance to specific and multiple races of rice blast. However, the observed resistance is not so reliable to be deployed on a large scale, since the results of our screening tests only rely on the diversity of *M. oryzae* populations found in the study environment. This work also stimulates considerable interest in extending our experiments to other locations of Benin so that to check for stability of blast resistance in our accessions.

Research question 2: What is the most useful combination of AFLP markers for understanding the variability of blast resistance in the field?

We demonstrated that the AFLP-based method has the potential to predict blast resistance. This chapter evidenced significant associations between AFLP markers and field blast resistance, with clear boundaries between ‘‘resistant’’ and ‘‘susceptible’’ accession groups. However, our findings were not conclusive enough to suggest the use of these AFLP markers for marker-assisted selection. Using marker-assisted selection could help to develop multi-line varieties in which different resistance genes are introduced for efficient blast management (Boudreau 2013; Petrie and Bates 2017).

Research question 3: Which accessions derived from the whole initial germplasm collection can furnish the richest source of genetic variability?

The ultimate question of this work was about the development of a germplasm subset collection. The methodology used allowed the constitution of 42 rice accessions in this subset, which represents the maximum of genetic diversity within our initial germplasm collection (Table 7.1). However, this subset can only represent a small part of the gene pool of known-cultivated rice species since we gave higher priority to the selection of *O. glaberrima* blast-resistant accessions.

The size of germplasm collections often limits their accessibility, and thus their utilization in plant breeding and research. The current subset selection would thus offer a good starting point when searching for new traits (Vaughan 1991). It could be used for in-depth evaluation, to broaden our knowledge of *O. glaberrima* potential (Knüpffer and van Hintum 1995). Further investigation on aspects such as agronomic traits is required to reveal the rice potential as added-values for a better valorization of these accessions.

Table 7.1. Overview potential and characteristics of the selected subset of 42 rice accessions

S/N	Accession number	Species name	Country of origin	Genetic group (population)	Yield (g/m^2)	Field resistance	Resistance rate (%) with infection by blast strains
1	WAB0015772	*O. s*	Benin	Pop 1	Upland: 257; Lowland: 489	R	-
2	WAB0035038	*O. s*	Benin	Pop 1	Upland: 717; Lowland: -	R	-
3	WAB0035055	*O. s*	Benin	Pop 1	Upland: 494; Lowland: 569	R	22.22
4	WAB0035059	*O. s*	Benin	Pop 1	Upland: 669; Lowland: 498	R	33.33
5	WAB0030263	*O. g*	Nigeria	Pop 1	Upland: 327; Lowland: 168	S & drought tolerant	0
6	WAB0024116	*O. g*	Mali	Pop 1	Upland: 322; Lowland: 421	R	-
7	WAB0019882	*O. g*	Nigeria	Pop 1	Upland: 212; Lowland: 567	R	55.57
8	WAB0026176	*O. g*	Mali	Pop 1	Upland: 375; Lowland: 429	R	55.56
9	WAB0032394	*O. g*	Nigeria	Pop 1	Upland: 51; Lowland: 471	R	-
10	WAB0006684	*O. s*	Benin	Mixed Pop1(77%) and Pop2(23%)	Upland: 319; Lowland: 168	R	55.56
11	WAB0023837	*O. g*	Mali	Pop 2	Upland: 395; Lowland: 555	R	-
12	WAB0029194	*O. g*	Mali	Pop2	Upland: 603; Lowland: 476	R	100
13	WAB0032298	*O. g*	Mali	Pop 2	Upland: 371; Lowland: 343	R & submergence tolerant	100
14	WAB0015043	*O. g*	Ivory Coast	Pop 2	Upland: 410; Lowland: 600	R	-
15	WAB0032495	*O. g*	Liberia	Pop 2	Upland: 389; Lowland: 413	R	-
16	WAB0008956	*O. g*	Liberia	Pop 2	Upland: 469; Lowland: 698	R	100
17	WAB0029182	*O. g*	Mali	Pop 3	Upland: 573; Lowland: 510	R	100

S/N	Accession number	Species name	Country of origin	Genetic group (population)	Yield (g/m^2)	Field resistance	Resistance rate (%) with infection by blast strains
18	WAB0029335	*O. g*	Nigeria	Pop 3	Upland: 338; Lowland: 474	MR	88.89
19	WAB0024105	*O. g*	Mali	Pop 3	Upland: 100; Lowland: 358	R	-
20	WAB0032487	*O. g*	Mali	Pop 3	Upland: 190; Lowland: 258	MR	-
21	WAB0008589	*O. g*	Nigeria	Pop 3	Upland: 409; Lowland: 471	MR	-
22	WAB0020477	*O. g*	Nigeria	Pop 3	Upland: 425; Lowland: 295	MR	66.67
23	WAB0032397	*O. g*	Nigeria	Pop 3	Upland: 346; Lowland: -	R	-
24	WAB0029323	*O. g*	Nigeria	Pop 3	Upland: 363; Lowland: 427	MR	-
25	WAB0029333	*O. g*	Nigeria	Pop 3	Upland: 238; Lowland: -	MR	-
26	WAB0029315	*O. g*	Nigeria	Pop 3	Upland: 259; Lowland: 493	MR	-
27	WAB0020505	*O. g*	Nigeria	Pop 3	Upland: 269; Lowland: 430	MS	-
28	WAB0032497	*O. g*	Liberia	Pop 3	Upland: 469; Lowland: 446	R	100
29	WAB0015703	*O. g*	Nigeria	Pop 3	Upland: 374; Lowland: 526	R	100
30	WAB0001360	*O. g*	Nigeria	Pop 3	Upland: 411; Lowland: 427	S	0
31	WAB0029342	*O. g*	Nigeria	Pop 3	Upland: 365; Lowland: 644	S	0
32	WAB0032230	*O. g*	Nigeria	Pop 3	Upland: 50; Lowland: -	S	0
33	WAB0008937	*O. g*	Guinea	Pop 3	Upland: 491; Lowland: 536	R	88.89
34	WAB0032848	*O. g*	Mali	Pop 3	Upland: 268; Lowland: 260	R	-
35	WAB0032550	*O. g*	Nigeria	Pop 3	Upland: 220; Lowland: 318	R	66.67
36	WAB0026783	*O. g*	Mali	Pop 3	Upland: 270; Lowland: -	R	-
37	WAB0002093	*O. g*	Mali	Pop 3	Upland: 98; Lowland: 333	MR	-
38	WAB0002136	*O. g*	Mali	Pop 3	Upland: 103; Lowland: 155	R	-
39	WAB0002143	*O. g*	Mali	Pop 3	Upland: 540; Lowland: 358	R	100
40	WAB0002145	*O. g*	Mali	Pop 3	Upland: 263; Lowland: 454	MS	-
41	WAB0032345	*O. g*	Mali	Pop 3	Upland: 333; Lowland: 512	MR	-
42	WAB0009280	*O. g*	Nigeria	Pop 3	Upland: 348; Lowland: 510	MS	33.33

O. s = O. sativa ; O. g = O. glaberrima; R = resistant; MR = moderately resistant; Ms = moderately susceptible; S = susceptible; Pop = genetic population

7.2. Population structure analysis revealed gene flow between *O. glaberrima* and *O. sativa* (Chapter 4)

This work addressed three relevant aspects of rice genetic resources management and use, which correspond to the research questions driving our Ph.D. research project. The work was focused on the assessment of genetic diversity and population structure of our new rice subset using both SSR and AFLP markers along with several morphological traits. This allowed us to study genetic relationships between *O. glaberrima* and *O. sativa* accessions. The concept of a genetic barrier between these two species is well accepted. However, gene flow occurrence, which can be defined as spontaneous inter-specific hybridizations, is not uncommon. We thus investigated this gene flow occurrence between *O. glaberrima* and *O. sativa.*

Research question 1: To what extent and in what ways are the selected rice accessions genetically different from one other?

Our research provided evidence for higher genetic diversity (as revealed by SSR markers) among accessions of the studied subset when compared to several previous studies in rice (Semon *et al.* 2005; Dramé *et al.* 2011; Chen *et al.* 2017; see chapter 3). Genetic diversity is of fundamental importance in the continuity of a species. It provides the necessary adaptation to the prevailing environmental factors (biotic and abiotic factors) and enables variation in the genetic composition to cope with changes in the environment (Caliskan 2012; Liu *et al.* 2015).

Nowadays, most farmers practice a monoculture system, which causes a gradual decline in the diversity of their cultivated rice varieties. As modern high-yielding varieties grown by farmers expand to millions of hectares, they also replace old traditional varieties. Although useful genes from these traditional varieties are being used in breeding for modern varieties, many unique attributes and gene combinations resulting from years of selection are difficult to reconstitute (Leung *et al.* 2003). To achieve the productivity needed for Benin, it is not possible to revert to planting diverse traditional varieties that are poor yielding. However, it is within our capacity to work for the conservation of this genetic diversity and for improving blast

management strategies that sustain productivity yet maintain adequate diversity and resilience in the production systems (Leung *et al.* 2003).

Based on SSRs, the amount of genetic diversity was higher than that revealed by AFLPs. Our results demonstrated that SSRs have the potential of revealing a high amount of polymorphism between the rice accessions studied. It may be attributed to their multiallelic and codominant nature (Vieira *et al.* 2016). The AFLP bands being dominant, homozygous genotypes were confused with the heterozygous ones. The reveal of high genetic diversity is critical for breeding programs since it can provide new sources of resistance genes to various stress factors, including blast. With recent advances in genomics, new opportunities exist to associate the genes present in our accessions with phenotypes at a genome-wide scale.

Genetic diversity by AFLPs differed across the country of origin of our accessions. The analysis indicates a high resemblance between the Malian accessions and between those originating from Ivory Coast and Liberia. This variation may be attributed to rice migration process by humans during rice domestication knowing that *O. glaberrima* was first domesticated in the Niger river delta (Mali), which was subsequently migrated to two secondary centers: (1) The Guinea highlands to Niger, crossing along the border of Benin and diffusing to Nigeria; and (2) through the Guinea forest (between Sierra Leone and the western Ivory Coast) to Liberia (Portéres 1962; Second 1982; Sarla and Swamy 2005; Wang *et al.* 2014).

Research question 2: Which population structure better represents the maximum possible genetic variation contained in our rice subset?

A second related aspect to our study was about the organization of genetic variation in this rice subset. As demonstrated, the studied subset collection represents three genetically different populations, of which some *O. glaberrima* accessions were classified with those of *O. sativa* (Table 7.1). Genetic diversity also differs somewhat within individuals of the three populations. This variation may result from human and natural selection effects and evolutionary processes such as recombination, mutation, and genetic drift over the years (Yan

et al. 2019). Eighty percent of genetic variation found among individuals of these populations attests that these accessions can be exploited for rice breeding.

Research question 3: Is there a clear separation between *O. sativa* and *O. glaberrima* at the germplasm structure level and what can we say about gene flow?

The third research question was to find out whether there is a clear separation between *O. sativa* and *O. glaberrima* at the germplasm structure level. As we mentioned above, *O. sativa* accessions were not totally isolated from *O. glaberrima* accessions. The extent of genetic variation observed between *O. sativa,* and *O. glaberrima* accessions were very low, which suggests that both species are intermix within the same genetic population. This could be explained by the phenomenon of gene flow. Gene flow is the movement of genes within and between populations. The direction of pollen flow may be explained by the higher efficiency of pollen capture in *O. glaberrima*. In fact, *O. glaberrima* tends to have a higher percentage of exserted stigma than *O. sativa* (Sano 1989). Gene flow has been widely reported from both cultivated rice species (Second 1982; Barry *et al.* 2007; Nuijten *et al.* 2009). Sano (1989) reported that the frequency of this gene flow ranged from 0.8 to 4.5%. According to Yan *et al.* (2019), this gene flow may have been caused by genetic drift. The intensity of gene flow (Nm = 217,08 in our study) prevented a clear genetic differentiation between *O. sativa* and *O. glaberrima* (Grant 1991; Vekemans and Hardy 2004). Maintained this gene flow may lead to a combination of *O. sativa* gene pool with that of *O. glaberrima.* As a consequence, *O. glaberrima*, which is becoming rare in Africa could be threatened by extinction risks.

7.3. Screening for combining high yields with blast resistance in (Chapter 5)

The agronomic performance of rice accessions in our subset was compared with that of currently rice varieties used in Benin. The obtained information can be combined with high blast resistance levels to achieve step by step sustainable rice production in this country. Genetic resources of *O. glaberrima* are still underutilized. Our study thus offers an opportunity

to use our accessions in subsequent breeding programs for a better valorization of their genetic potential.

Research question 1: To what extent the grain performances of our accessions differ from those of varieties currently grown by farmers under lowland and upland conditions of Benin?

This work identified several accessions as having higher yields than the reference controls used in both lowland and upland conditions. In 2009, in Benin, Sanni *et al.* reported the yield potential of 18 upland interspecific NERICA varieties (NERICA 1-18) that we used as reference controls in our work. They recorded a maximum total grain yield of 614 g/m^2 obtained by NERICA 2. Accessions under the current study showed grain yields ranging from 50.62 (WAB0032230) to 717.17 (WAB0035038) g/m2. These findings confirm the great potential for selection for yield improvement. Grain yield is the priority in rice improvement programs. The performance of rice varieties currently grown by Beninese farmers was estimated at 342 g/m^2 (FAOSTAT 2018). Our germplasm collection is thus offering a lot of possibilities for breeding superior cultivars. However, it should be pointed out that our study is not exhaustive. Further research is needed to better examine environmental adaptation and stability of the yields of these accessions throughout Benin over a minimum of three years.

Research question 2: Which agronomic characteristics most contribute to the accessions' grain yields in both growing conditions?

As we reported in this study, the best performing rice accessions had higher values of yield-related traits, particularly spikelet fertility (upland) and tiller fertility (lowland), which directly influence grain yield. This shows that the high potential of these rice accessions for the spikelet and tiller fertility under different water regimes are controlled by different QTLs and physiological mechanisms. Although the two characters are seen to contribute to yields in different ways, spikelet fertility was found to be most determined by cycle duration. This result was valid whether in upland or lowland conditions. We conclude that the rice accessions found capable of completing their life cycle within a short duration can produce better yields than

those with more extended life cycle periods. As a consequence, they could escape the effects of climate change and could perform better than late accessions. Scientists should devote special attention to these yield-related traits to select the most promising germplasm candidates for breeding programs.

However, despite our interesting findings, there is no evidence for the stability of these traits if the environmental conditions changed. In fact, these agronomic traits do not only depend on the genetic background but also on environmental and genotype by environment interaction effects (Singh 2002). It is, therefore, necessary to study yield stability and adaptability of the accessions across multiple environments either in different locations or seasons or both (Keneni *et al.* 2012). This information could contribute to make recommendations for better use of the *O. glaberrima* accessions and update breeding objectives and methods (Idrissi *et al.* 2019).

Research question 3: Can accessions with proven blast resistance agronomically perform well? i.e. does high-yield occur in combination with blast tolerance of accessions of our subset?

Higher yield performance was found in many blast-resistant accessions than in reference controls. This performance was observed whether in upland (WAB0002143, WAB0029182, WAB0029194, WAB0035059, and WAB0035038) or lowland conditions (WAB0035055, WAB0019882, WAB0008956, and WAB0015043) (Table 7.1). We conclude that our blast-resistant accessions could be great promising lines for cultivation, especially under environments of blast hotspot. They can maintain the high yield potential when blast pressure remains high. As we seek to improve Benin rice production, these accessions can also provide breeders with great opportunities for developing high-yield, blast-resistant rice cultivars to enhance the thirst for food security in this country.

High secondary panicle branching was associated with blast resistance. Some scientists argue that panicle secondary branching is one of the traits that most contribute to panicle size and weight (Ashikari *et al.* 2005; Zhao *et al.* 2016). This may explain why our blast-resistant

accessions had good yield performance in both upland and lowland conditions. Breeders can thus use this trait as markers for early screening for identifying high performing accessions with excellent resistance to blast.

7.4. Screening of some cultivated rice accessions (*Oryza* spp.) for robust resistance against blast disease (Chapter 6)

To sustain the development of new varieties for improved Benin rice production, it was necessary to confirm, under laboratory conditions, the prior blast-resistant accessions. This research complements the work done in chapter 3 through screening some accessions for strong blast resistance. A set of *M. oryzae* isolates that represent the Benin blast pathotypes diversity was used to screen these rice accessions. The purpose was to search for accessions having strong resistance to all Benin blast pathotypes, which could be of great interest for use in Africa as well. Also, we investigated the presence/absence of some rice blast, Bacterial Leaf Blight (BLB), drought, and submergence resistance genes that are indispensable for enhancing breeding programs.

Research question 1: How diverse are the accessions' responses to the Benin *M. oryzae* isolates that take account of African blast pathotypic diversity?

The results of our screen tests provide evidence for the presence of a broad diversity of responses, and critical levels of resistance to blast. The results also confirm that our accessions are interesting sources of blast resistance as found in the previous work (chapter 3). This differential reactions to *M. oryzae* isolates could be ascribed to different virulence spectra, which allowed to classify the germplasm collection into two categories: the most-resistant group and the most-susceptible group. The latter resistant group indicates the presence of race-specific resistance. The best-resistant accession group can be used by Benin rice researchers as a preliminary base material for breeding purposes. This germplasm is available for research purposes on request. The release of these resistant accessions in Benin can contribute to increasing the rice yield in two ways: (1) It can reduce yield loss due to blast,

and (2) it can expand the adaptability of rice varieties to production areas previously limited by high incidence of diseases (Leung *et al.* 2003).

Research question 2: Does our subset contain accessions that resist all blast pathotypes?

We found that some accessions possess a robust resistance against all isolates. Benin rice production can be managed by introducing these accessions possessing strong resistance against blast. On the other hand, broad-spectrum blast resistance critical for developing better germplasm and varieties. This is the first step towards sustainable strategy for efficient blast control in Benin (Fu *et al.* 2012; Ashkani *et al.* 2015). Most farmers' varieties are only resistant to a few blast rice pathotypes, whereas Benin production environments are seen to be habitats of different *M. oryzae* races, which cause high levels of damage (Odjo *et al.* 2011; Awande 2015). These accessions that we found resistant to all blast pathotypes, are of high value to explore genes and mechanisms that govern their resistance and durability.

Research question 3: What is the mode of inheritance of strongly blast-resistance in recombinant inbred lines?

Our work reported the mode of inheritance of this robust blast-resistance. As demonstrated, the robust resistance could involve either a resistance conferred by the accumulation of several race-specific genes or new dominant genes, given the blast isolates used were virulent on most race-specific genes known so far. Another hypothesis could be that the resistance is quantitative and controlled by a set of minor genes. This work thus provides the basis for further studies to understand the mechanisms and molecular basis of the resistance in our accessions.

Recent surveys by Odjo *et al.* (2017) indicated that few rice varieties resist biotic and abiotic stress factors in Benin, which suggests the need to check for new varieties adapted to as many environments as possible. The resistance found to be effective just against blast disease would offer only a partial solution for Benin's low rice production. In this line of thought, we identified the presence of alleles for submergence and drought tolerance in the blast-resistant accession

WAB0032298 and in the accession WAB0030263 (susceptible to blast), respectively (Table 7.1). These findings could facilitate the transfer of resistance genes in breeding programs, as a single cross may serve several purposes.

We thus answered many of the research questions brought up by this thesis. Our study is found to be relevant for future research. Finally, we will conclude this thesis by highlighting some key findings that may influence breeding and conservation programs on the African cultivated rice species. Also, perspectives for future researches will be proposed.

7.5. Summarized outcomes and practical significance

AFLP markers have contributed immensely to evidence the link between genetic diversity and blast resistance and thereby to select a subset collection that represents the genetic diversity of our initial germplasm collection. This subset has a small sample size, broad representation, low redundancy, and rich diversity (Song *et al.* 2010).

Breeders usually cross varieties for expanding genetic diversity, discovering genes of interest, and improving rice varieties with desirable traits. The greatest obstacle to accomplishing those traits is the lack of detailed evaluation and genetic characterization of large amounts of rice germplasm resources (Gai *et al.* 1999). Our subset collection is a valuable germplasm resource that can provide ways of better management and utilization of the accessions included (Guo *et al.* 2014).

In this study, we provided a detailed analysis of the genetic diversity and population structure of our subset. The obtained results are crucial for managing and conserving the accessions of this subset (Zaya *et al.* 2017). For example, breeders can use the information to select accessions within populations for crossings (Sié *et al.* 2012). Analysis of population genetic structure shows that breeders should consider three main genetic groups when making management decisions. The extent of genetic variation between and within populations suggests possibilities for a different level of improvement through selection.

We found that significant amounts of gene flow occurred between *O. glaberrima* and *O. sativa* accessions, which shapes the genetic structure of these populations. The potential for gene flow depends on habitats or geographic distribution of the crops and occasionally has significant applied consequences, such as increasing the extinction risk for rare species like *O. glaberrima* (Peakall *et al.* 2003; Moran and Clark 2011). Nonetheless, this gene flow offers the potential benefit of interbreeding between populations of *O. sativa* and *O. glaberrima*, which is different from the traditional rice breeding approaches. It can lead to inbred and hybrid lines that possess a variety of useful traits, and the high genetic gain would be expected.

Our rice subset includes accessions with high levels of genetic diversity, blast resistance, drought and submergence tolerance, and with different desirable agronomic traits (Table 7.1). High genetic diversity constitutes the basis of evolution and essential resources for adaptation to environmental condition change, whereas narrow genetic diversity results in uniformity and reduces adaptability (Luan *et al.* 2006). The primary constraint to the genetic improvement is the narrow genetic diversity of the available genetic resources (Holbrook and Stalker 2003; Ortiz *et al.* 2013). The amount of genetic diversity available in our collection can be exploited to obtain desirable traits for rice improvement (Alonso-Blanco and Koornneef 2000).

Studies on agronomic performance of our subset were conducted under both upland and lowland conditions in Benin. This selected subset is dominated by *O. glaberrima* accessions (88%), whereas the latter are the most underutilized and being replaced by higher productive *O. sativa* accessions. Today, *O. glaberrima* is limited to only certain areas in West Africa, whereas *O. sativa* is distributed worldwide. Our results evidenced many high performing *O. glaberrima* accessions with blast resistance compared to those of *O. sativa*. Consequently, these accessions can be proposed for cultivation either in uplands or lowlands.

We also found high variability for agronomically new traits such as cycle duration, secondary and primary panicle ramifications, spikelet fertility, the total number of spikelets, *etc*. Increasing crop yield is a significant challenge, whereas the observed genetic variability for these agronomic traits is the critical component to improved grain yields.

Secondary and primary branching were shown to be associated with blast resistance. In many breeding programs, as far as rice is concerned, it is mainly the genetic variability available in *O. sativa* germplasm, which has exploited. To our knowledge, there are very few improvements of *O. glaberrima*. Therefore, our results could help to draw more attention to *O. glaberrima* and define efficient strategies for better utilization and development of intra-specific hybrid varieties.

Finally, our study revealed accessions with strong resistance to blast isolates that are virulent on most R-genes known so far. These accessions are interesting to be used for blast management in Africa but need to be further tested vis-à-vis pathotypes that occur in other African countries. Accessions were also shown to be a reservoir of useful alleles for tolerance to drought and flood. This information will allow for pyramiding these alleles together with blast resistance and high-yields by using MAS for broader adaptability.

7.6. Future perspectives

In this study, we selected a subset of promising rice accessions whose potential for resistance to multiple stress factors and excellent agronomic performance has been demonstrated. This subset is small enough and can thus be assessed across Benin in the field in terms of effectiveness and durability of blast resistance over two to three years.

The discovery of accessions with strong resistance could provide useful genes for blast disease management in Africa. However, the issue of the application of our research findings beyond the particular environments of Benin would require further assessments at multiple locations over two to three years in order to ascertain the durability and broad-spectrum of blast resistance. It also implies the need to extend our yield evaluation to other African countries for enhancing the utilization of these accessions.

Because of the future use of these accessions, our subset should be properly conserved in genebanks (seed storage rooms) to ensure the conservation and longevity of the seeds. The government of Benin has built at *Institut National des Recherches Agricoles du Bénin* (INRAB),

a seed storage unit (national genebank). This is viewed as an active collection of plant genetic resources. We thus suggest creating a backup of this subset, which is already in our local collection in order to ensure longevity of the seeds and their easy access for local research activities.

The current study indicated the inheritance of blast resistance. However, it is essential to characterize genes and mechanisms that make this resistance to be most active, given exploring the functionality of these genes. The dissection of mechanisms underlying the strong resistance of our rice accessions against Beninese blast isolates is vital for their future utilization in varietal improvement via marker aided selection approach (Chaipanya *et al.* 2017). The majority of plant R genes encode proteins that have putative central nucleotide-binding-site (NBS) and carboxy-terminal leucine-rich repeats (LRRs). There are many scenarios that describe the specificity of recognition between these resistance proteins and avirulence proteins of the pathogen. For example, one R gene can recognize more than one Avr gene, an example of which is Pia that can recognize both AvrPia and Avr1-CO39 (Cesari *et al.* 2013), or it could also be that many R genes can recognize the same Avr gene, as in the case of AvrPikD that can be recognized by Pik, Pikm, and Pikp (Yoshida *et al.* 2009). *O. glaberrima* possesses thousands of valuable genes that remain unrevealed and thus underutilized till now. Revealing the resistance genes of current *O. glaberrima* accessions and their mechanisms would allow the development of further strategies for resistance and durability to rice blast fungus populations in Benin and worldwide.

Candidate genes conferring strong resistance to Benin blast pathotypes are necessary for breeding programs. They can be pyramided into susceptible varieties using MAS. We need to develop molecular markers associated with these genes in order for MAS to be feasible.

To achieve sustainability of rice production in Benin, we need a rice production system built upon effective resistant varieties with broad resilience to a range of diseases. Apart from the blast pathogen (*M. oryzae*), many other rice pathogens such as *Xanthomonas oryzae* pv. *oryzae* (BLB), rice yellow mottle virus (RYMV), African rice gall midge (AfRGM, *Orseolia*

oryzivora Harris & Gagné) and stem borers (*Chilo suppressalis* Walker) (Abo and Sy 1997; Sere *et al.* 2013), often occur in Benin rice production environments. The damage caused by these diseases is a function of the prevalence of the pathogen, epidemic potential of the disease, and available control measures. These diseases collectively could pose a significant threat to rice production (Mew *et al.* 1992). Potential sources of resistance to these diseases might be preserved in the current accessions studied, given during their domestication process, they may have been subjected to distinct selective pressures for prevalent diseases. It would, therefore, be interesting to investigate their potential as a source of genetic resistance to the most relevant rice diseases.

Farmers, especially in Africa, have in the past adopted and continue to show interest in high performing and suitable new rice varieties to their environmental conditions. It has been an important goal to complement existing cultivars and to enrich the local rice gene pool. Our study has opened many research perspectives on varietal development. It will be necessary to promote these new varieties developed at the farmers' level. We can use Participatory Variety Selection (PVS) method or demonstration plots in order to involve farmers directly in the selection process with a focus on their preferences, and socioeconomic needs. This PVS method will help for widespread disseminating our accessions and lines and whereby field genebanks will be feasible.

In this study, our rice accessions were shown to be interesting to improve African rice production. However, yield and stress tolerance are not the only factors to be considered when developing new rice varieties. Also, quality (e.g., grain physical appearance, cooking and eating characteristics, milling quality, and nutritional value) has a significant impact on adoption by farmers and acceptance by consumers of any variety. To our knowledge, there has not been any research on the improvement of grain quality of our rice accession collection till now. Breeding for consumer-preferred grain qualities is an essential goal for rice breeding programs. Fofana *et al.* (2011) assessed the physicochemical and cooking characteristics of some rice varieties consumed in Benin. It was suggested that more considerable attention be paid to

grain quality characteristics in African rice breeding programs in order to meet consumer preferences on the continent. It is, therefore, necessary that we also investigate grain and nutritional quality of our accessions to be able to breed for specific consumer preferences. This information will help to identify strategies for future grain quality improvements in rice in Benin and beyond.

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Appendix

Table A.1. Characteristics of the 353 cultivated rice as referenced in AfricaRice genebank database

S/N	Accession number	Species name	Country of origin
1	WAB0032183	*O. glaberrima*	-
2	WAB0030260	*O. glaberrima*	Africa
3	WAB0030441	*O. glaberrima*	Africa
4	WAB0015772	*O. sativa*	Benin
5	WAB0035055	*O. sativa*	Benin
6	WAB0035059	*O. sativa*	Benin
7	WAB0006684	*O. sativa*	Benin
8	WAB0035038	*O. sativa*	Benin
9	WAB0029010	*O. glaberrima*	Burkina Faso
10	WAB0007981	*O. glaberrima*	Burkina Faso
11	WAB0006871	*O. glaberrima*	Control
12	WAB0006881	*O. glaberrima*	Control
13	WAB0007880	*O. glaberrima*	Control
14	WAB0008937	*O. glaberrima*	Guinea
15	WAB0030338	*O. glaberrima*	Guinea
16	WAB0029289	*O. glaberrima*	Ivory Coast
17	WAB0029199	*O. glaberrima*	Ivory Coast
18	WAB0015000	*O. glaberrima*	Ivory Coast
19	WAB0029225	*O. glaberrima*	Ivory Coast
20	WAB0029273	*O. glaberrima*	Ivory Coast
21	WAB0015043	*O. glaberrima*	Ivory Coast
22	WAB0014999	*O. glaberrima*	Ivory Coast
23	WAB0032495	*O. glaberrima*	Liberia
24	WAB0008956	*O. glaberrima*	Liberia
25	WAB0032315	*O. glaberrima*	Liberia
26	WAB0032497	*O. glaberrima*	Liberia
27	WAB0002034	*O. glaberrima*	Mali
28	WAB0009198	*O. glaberrima*	Mali
29	WAB0002020	*O. glaberrima*	Mali
30	WAB0029293	*O. glaberrima*	Mali
31	WAB0029178	*O. glaberrima*	Mali
32	WAB0032965	*O. glaberrima*	Mali
33	WAB0009234	*O. glaberrima*	Mali
34	WAB0032568	*O. glaberrima*	Mali
35	WAB0029183	*O. glaberrima*	Mali
36	WAB0029234	*O. glaberrima*	Mali
37	WAB0002137	*O. glaberrima*	Mali
38	WAB0029222	*O. glaberrima*	Mali
39	WAB0029202	*O. glaberrima*	Mali
40	WAB0002093	*O. glaberrima*	Mali

S/N	Accession number	Species name	Country of origin
41	WAB0002116	*O. glaberrima*	Mali
42	WAB0032835	*O. glaberrima*	Mali
43	WAB0002120	*O. glaberrima*	Mali
44	WAB0032789	*O. glaberrima*	Mali
45	WAB0002122	*O. glaberrima*	Mali
46	WAB0007994	*O. glaberrima*	Mali
47	WAB0002125	*O. glaberrima*	Mali
48	WAB0002130	*O. glaberrima*	Mali
49	WAB0032860	*O. glaberrima*	Mali
50	WAB0002136	*O. glaberrima*	Mali
51	WAB0002140	*O. glaberrima*	Mali
52	WAB0002142	*O. glaberrima*	Mali
53	WAB0002143	*O. glaberrima*	Mali
54	WAB0002145	*O. glaberrima*	Mali
55	WAB0015488	*O. glaberrima*	Mali
56	WAB0032383	*O. glaberrima*	Mali
57	WAB0032341	*O. glaberrima*	Mali
58	WAB0002153	*O. glaberrima*	Mali
59	WAB0032368	*O. glaberrima*	Mali
60	WAB0009245	*O. glaberrima*	Mali
61	WAB0009286	*O. glaberrima*	Mali
62	WAB0029207	*O. glaberrima*	Mali
63	WAB0032756	*O. glaberrima*	Mali
64	WAB0024096	*O. glaberrima*	Mali
65	WAB0024098	*O. glaberrima*	Mali
66	WAB0024104	*O. glaberrima*	Mali
67	WAB0032759	*O. glaberrima*	Mali
68	WAB0024051	*O. glaberrima*	Mali
69	WAB0024040	*O. glaberrima*	Mali
70	WAB0024054	*O. glaberrima*	Mali
71	WAB0024066	*O. glaberrima*	Mali
72	WAB0024081	*O. glaberrima*	Mali
73	WAB0024083	*O. glaberrima*	Mali
74	WAB0029226	*O. glaberrima*	Mali
75	WAB0032771	*O. glaberrima*	Mali
76	WAB0030311	*O. glaberrima*	Mali
77	WAB0029282	*O. glaberrima*	Mali
78	WAB0032848	*O. glaberrima*	Mali
79	WAB0032215	*O. glaberrima*	Mali
80	WAB0020178	*O. glaberrima*	Mali
81	WAB0032481	*O. glaberrima*	Mali
82	WAB0029186	*O. glaberrima*	Mali
83	WAB0029291	*O. glaberrima*	Mali
84	WAB0032297	*O. glaberrima*	Mali

S/N	Accession number	Species name	Country of origin
85	WAB0029271	*O. glaberrima*	Mali
86	WAB0030222	*O. glaberrima*	Mali
87	WAB0032387	*O. glaberrima*	Mali
88	WAB0029184	*O. glaberrima*	Mali
89	WAB0029261	*O. glaberrima*	Mali
90	WAB0032967	*O. glaberrima*	Mali
91	WAB0029253	*O. glaberrima*	Mali
92	WAB0029239	*O. glaberrima*	Mali
93	WAB0029212	*O. glaberrima*	Mali
94	WAB0032916	*O. glaberrima*	Mali
95	WAB0024099	*O. glaberrima*	Mali
96	WAB0024100	*O. glaberrima*	Mali
97	WAB0024111	*O. glaberrima*	Mali
98	WAB0024116	*O. glaberrima*	Mali
99	WAB0024121	*O. glaberrima*	Mali
100	WAB0024082	*O. glaberrima*	Mali
101	WAB0024086	*O. glaberrima*	Mali
102	WAB0029194	*O. glaberrima*	Mali
103	WAB0032298	*O. glaberrima*	Mali
104	WAB0029270	*O. glaberrima*	Mali
105	WAB0032223	*O. glaberrima*	Mali
106	WAB0030223	*O. glaberrima*	Mali
107	WAB0029278	*O. glaberrima*	Mali
108	WAB0029284	*O. glaberrima*	Mali
109	WAB0032300	*O. glaberrima*	Mali
110	WAB0030258	*O. glaberrima*	Mali
111	WAB0029214	*O. glaberrima*	Mali
112	WAB0032622	*O. glaberrima*	Mali
113	WAB0029189	*O. glaberrima*	Mali
114	WAB0029193	*O. glaberrima*	Mali
115	WAB0002019	*O. glaberrima*	Mali
116	WAB0030261	*O. glaberrima*	Mali
117	WAB0023837	*O. glaberrima*	Mali
118	WAB0002023	*O. glaberrima*	Mali
119	WAB0002031	*O. glaberrima*	Mali
120	WAB0007932	*O. glaberrima*	Mali
121	WAB0002126	*O. glaberrima*	Mali
122	WAB0032746	*O. glaberrima*	Mali
123	WAB0029210	*O. glaberrima*	Mali
124	WAB0026176	*O. glaberrima*	Mali
125	WAB0029179	*O. glaberrima*	Mali
126	WAB0029266	*O. glaberrima*	Mali
127	WAB0032307	*O. glaberrima*	Mali
128	WAB0002023	*O. glaberrima*	Mali
129	WAB0029272	*O. glaberrima*	Mali

S/N	Accession number	Species name	Country of origin
130	WAB0029191	*O. glaberrima*	Mali
131	WAB0024106	*O. glaberrima*	Mali
132	WAB0029258	*O. glaberrima*	Mali
133	WAB0029213	*O. glaberrima*	Mali
134	WAB0029201	*O. glaberrima*	Mali
135	WAB0029182	*O. glaberrima*	Mali
136	WAB0024042	*O. glaberrima*	Mali
137	WAB0002030	*O. glaberrima*	Mali
138	WAB0032893	*O. glaberrima*	Mali
139	WAB0032523	*O. glaberrima*	Mali
140	WAB0024067	*O. glaberrima*	Mali
141	WAB0002030	*O. glaberrima*	Mali
142	WAB0002034	*O. glaberrima*	Mali
143	WAB0032745	*O. glaberrima*	Mali
144	WAB0032925	*O. glaberrima*	Mali
145	WAB0029198	*O. glaberrima*	Mali
146	WAB0002039	*O. glaberrima*	Mali
147	WAB0029197	*O. glaberrima*	Mali
148	WAB0029221	*O. glaberrima*	Mali
149	WAB0029292	*O. glaberrima*	Mali
150	WAB0032389	*O. glaberrima*	Mali
151	WAB0009194	*O. glaberrima*	Mali
152	WAB0032446	*O. glaberrima*	Mali
153	WAB0002146	*O. glaberrima*	Mali
154	WAB0024105	*O. glaberrima*	Mali
155	WAB0023838	*O. glaberrima*	Mali
156	WAB0029208	*O. glaberrima*	Mali
157	WAB0029268	*O. glaberrima*	Mali
158	WAB0032927	*O. glaberrima*	Mali
159	WAB0032345	*O. glaberrima*	Mali
160	WAB0026783	*O. glaberrima*	Mali
161	WAB0029200	*O. glaberrima*	Mali
162	WAB0024119	*O. glaberrima*	Mali
163	WAB0002137	*O. glaberrima*	Mali
164	WAB0032225	*O. glaberrima*	Mali
165	WAB0024059	*O. glaberrima*	Mali
166	WAB0029233	*O. glaberrima*	Mali
167	WAB0024115	*O. glaberrima*	Mali
168	WAB0032207	*O. glaberrima*	Mali
169	WAB0032487	*O. glaberrima*	Mali
170	WAB0002148	*O. glaberrima*	Mali
171	WAB0008951	*O. glaberrima*	Mali
172	WAB0002126	*O. glaberrima*	Mali
173	WAB0029294	*O. glaberrima*	Mali
174	WAB0030262	*O. glaberrima*	Nigeria

S/N	Accession number	Species name	Country of origin
175	WAB0030263	*O. glaberrima*	Nigeria
176	WAB0032394	*O. glaberrima*	Nigeria
177	WAB0020682	*O. glaberrima*	Nigeria
178	WAB0026773	*O. glaberrima*	Nigeria
179	WAB0020592	*O. glaberrima*	Nigeria
180	WAB0010659	*O. glaberrima*	Nigeria
181	WAB0010459	*O. glaberrima*	Nigeria
182	WAB0007948	*O. glaberrima*	Nigeria
183	WAB0020501	*O. glaberrima*	Nigeria
184	WAB0020704	*O. glaberrima*	Nigeria
185	WAB0020144	*O. glaberrima*	Nigeria
186	WAB0010665	*O. glaberrima*	Nigeria
187	WAB0032171	*O. glaberrima*	Nigeria
188	WAB0020716	*O. glaberrima*	Nigeria
189	WAB0029331	*O. glaberrima*	Nigeria
190	WAB0032180	*O. glaberrima*	Nigeria
191	WAB0032182	*O. glaberrima*	Nigeria
192	WAB0010476	*O. glaberrima*	Nigeria
193	WAB0029337	*O. glaberrima*	Nigeria
194	WAB0032184	*O. glaberrima*	Nigeria
195	WAB0029338	*O. glaberrima*	Nigeria
196	WAB0015703	*O. glaberrima*	Nigeria
197	WAB0032187	*O. glaberrima*	Nigeria
198	WAB0020491	*O. glaberrima*	Nigeria
199	WAB0032192	*O. glaberrima*	Nigeria
200	WAB0032198	*O. glaberrima*	Nigeria
201	WAB0032201	*O. glaberrima*	Nigeria
202	WAB0032212	*O. glaberrima*	Nigeria
203	WAB0032216	*O. glaberrima*	Nigeria
204	WAB0032221	*O. glaberrima*	Nigeria
205	WAB0008980	*O. glaberrima*	Nigeria
206	WAB0029858	*O. glaberrima*	Nigeria
207	WAB0032227	*O. glaberrima*	Nigeria
208	WAB0032230	*O. glaberrima*	Nigeria
209	WAB0029344	*O. glaberrima*	Nigeria
210	WAB0032232	*O. glaberrima*	Nigeria
211	WAB0032236	*O. glaberrima*	Nigeria
212	WAB0029346	*O. glaberrima*	Nigeria
213	WAB0032247	*O. glaberrima*	Nigeria
214	WAB0008677	*O. glaberrima*	Nigeria
215	WAB0032262	*O. glaberrima*	Nigeria
216	WAB0032261	*O. glaberrima*	Nigeria
217	WAB0020063	*O. glaberrima*	Nigeria
218	WAB0020527	*O. glaberrima*	Nigeria
219	WAB0009280	*O. glaberrima*	Nigeria

S/N	Accession number	Species name	Country of origin
220	WAB0020094	*O. glaberrima*	Nigeria
221	WAB0032272	*O. glaberrima*	Nigeria
222	WAB0032293	*O. glaberrima*	Nigeria
223	WAB0020474	*O. glaberrima*	Nigeria
224	WAB0029306	*O. glaberrima*	Nigeria
225	WAB0032319	*O. glaberrima*	Nigeria
226	WAB0032322	*O. glaberrima*	Nigeria
227	WAB0009707	*O. glaberrima*	Nigeria
228	WAB0029313	*O. glaberrima*	Nigeria
229	WAB0032332	*O. glaberrima*	Nigeria
230	WAB0032339	*O. glaberrima*	Nigeria
231	WAB0029315	*O. glaberrima*	Nigeria
232	WAB0032352	*O. glaberrima*	Nigeria
233	WAB0032357	*O. glaberrima*	Nigeria
234	WAB0020487	*O. glaberrima*	Nigeria
235	WAB0009458	*O. glaberrima*	Nigeria
236	WAB0032365	*O. glaberrima*	Nigeria
237	WAB0032381	*O. glaberrima*	Nigeria
238	WAB0032382	*O. glaberrima*	Nigeria
239	WAB0009275	*O. glaberrima*	Nigeria
240	WAB0029320	*O. glaberrima*	Nigeria
241	WAB0025035	*O. glaberrima*	Nigeria
242	WAB0032400	*O. glaberrima*	Nigeria
243	WAB0032412	*O. glaberrima*	Nigeria
244	WAB0001875	*O. glaberrima*	Nigeria
245	WAB0030341	*O. glaberrima*	Nigeria
246	WAB0032511	*O. glaberrima*	Nigeria
247	WAB0001430	*O. glaberrima*	Nigeria
248	WAB0032550	*O. glaberrima*	Nigeria
249	WAB0032558	*O. glaberrima*	Nigeria
250	WAB0020477	*O. glaberrima*	Nigeria
251	WAB0032565	*O. glaberrima*	Nigeria
252	WAB0032575	*O. glaberrima*	Nigeria
253	WAB0029342	*O. glaberrima*	Nigeria
254	WAB0032592	*O. glaberrima*	Nigeria
255	WAB0032656	*O. glaberrima*	Nigeria
256	WAB0032678	*O. glaberrima*	Nigeria
257	WAB0032716	*O. glaberrima*	Nigeria
258	WAB0029329	*O. glaberrima*	Nigeria
259	WAB0029333	*O. glaberrima*	Nigeria
260	WAB0025343	*O. glaberrima*	Nigeria
261	WAB0032736	*O. glaberrima*	Nigeria
262	WAB0032742	*O. glaberrima*	Nigeria
263	WAB0029339	*O. glaberrima*	Nigeria
264	WAB0009480	*O. glaberrima*	Nigeria

S/N	Accession number	Species name	Country of origin
265	WAB0032765	*O. glaberrima*	Nigeria
266	WAB0020566	*O. glaberrima*	Nigeria
267	WAB0009292	*O. glaberrima*	Nigeria
268	WAB0032776	*O. glaberrima*	Nigeria
269	WAB0007985	*O. glaberrima*	Nigeria
270	WAB0020675	*O. glaberrima*	Nigeria
271	WAB0032788	*O. glaberrima*	Nigeria
272	WAB0020143	*O. glaberrima*	Nigeria
273	WAB0032840	*O. glaberrima*	Nigeria
274	WAB0020138	*O. glaberrima*	Nigeria
275	WAB003287	*O. glaberrima*	Nigeria
276	WAB0032880	*O. glaberrima*	Nigeria
277	WAB0032884	*O. glaberrima*	Nigeria
278	WAB0020067	*O. glaberrima*	Nigeria
279	WAB0008592	*O. glaberrima*	Nigeria
280	WAB0008985	*O. glaberrima*	Nigeria
281	WAB0023217	*O. glaberrima*	Nigeria
282	WAB0020073	*O. glaberrima*	Nigeria
283	WAB0007979	*O. glaberrima*	Nigeria
284	WAB0024973	*O. glaberrima*	Nigeria
285	WAB0020505	*O. glaberrima*	Nigeria
286	WAB0032185	*O. glaberrima*	Nigeria
287	WAB0010660	*O. glaberrima*	Nigeria
288	WAB0009273	*O. glaberrima*	Nigeria
289	WAB0020136	*O. glaberrima*	Nigeria
290	WAB0032330	*O. glaberrima*	Nigeria
291	WAB0032347	*O. glaberrima*	Nigeria
292	WAB0032344	*O. glaberrima*	Nigeria
293	WAB0032362	*O. glaberrima*	Nigeria
294	WAB0032363	*O. glaberrima*	Nigeria
295	WAB0032372	*O. glaberrima*	Nigeria
296	WAB0032371	*O. glaberrima*	Nigeria
297	WAB0020523	*O. glaberrima*	Nigeria
298	WAB0001255	*O. glaberrima*	Nigeria
299	WAB0032393	*O. glaberrima*	Nigeria
300	WAB0009472	*O. glaberrima*	Nigeria
301	WAB0029324	*O. glaberrima*	Nigeria
302	WAB0008673	*O. glaberrima*	Nigeria
303	WAB0032486	*O. glaberrima*	Nigeria
304	WAB0007918	*O. glaberrima*	Nigeria
305	WAB0032499	*O. glaberrima*	Nigeria
306	WAB0032502	*O. glaberrima*	Nigeria
307	WAB0019882	*O. glaberrima*	Nigeria
308	WAB0001424	*O. glaberrima*	Nigeria
309	WAB0032541	*O. glaberrima*	Nigeria

S/N	Accession number	Species name	Country of origin
310	WAB0032556	*O. glaberrima*	Nigeria
311	WAB0026777	*O. glaberrima*	Nigeria
312	WAB0029384	*O. glaberrima*	Nigeria
313	WAB0032793	*O. glaberrima*	Nigeria
314	WAB0032858	*O. glaberrima*	Nigeria
315	WAB0008982	*O. glaberrima*	Nigeria
316	WAB0020093	*O. glaberrima*	Nigeria
317	WAB0032397	*O. glaberrima*	Nigeria
318	WAB0032395	*O. glaberrima*	Nigeria
319	WAB0032545	*O. glaberrima*	Nigeria
320	WAB0001360	*O. glaberrima*	Nigeria
321	WAB0029407	*O. glaberrima*	Nigeria
322	WAB0032794	*O. glaberrima*	Nigeria
323	WAB0032755	*O. glaberrima*	Nigeria
324	WAB0029310	*O. glaberrima*	Nigeria
325	WAB0001254	*O. glaberrima*	Nigeria
326	WAB0029403	*O. glaberrima*	Nigeria
327	WAB0032543	*O. glaberrima*	Nigeria
328	WAB0032734	*O. glaberrima*	Nigeria
329	WAB0020089	*O. glaberrima*	Nigeria
330	WAB0007945	*O. glaberrima*	Nigeria
331	WAB0010505	*O. glaberrima*	Nigeria
332	WAB0008589	*O. glaberrima*	Nigeria
333	WAB0011753	*O. glaberrima*	Nigeria
334	WAB0032559	*O. glaberrima*	Nigeria
335	WAB0032503	*O. glaberrima*	Nigeria
336	WAB0022404	*O. glaberrima*	Nigeria
337	WAB0007970	*O. glaberrima*	Nigeria
338	WAB0007926	*O. glaberrima*	Nigeria
339	WAB0032203	*O. glaberrima*	Nigeria
340	WAB0008877	*O. glaberrima*	Nigeria
341	WAB0029319	*O. glaberrima*	Nigeria
342	WAB0032883	*O. glaberrima*	Nigeria
343	WAB0008908	*O. glaberrima*	Nigeria
344	WAB0029335	*O. glaberrima*	Nigeria
345	WAB0020707	*O. glaberrima*	Nigeria
346	WAB0020703	*O. glaberrima*	Nigeria
347	WAB0032229	*O. glaberrima*	Nigeria
348	WAB0007973	*O. glaberrima*	Nigeria
349	WAB0029343	*O. glaberrima*	Nigeria
350	WAB0022405	*O. glaberrima*	Nigeria
351	WAB0029323	*O. glaberrima*	Nigeria
352	WAB0032273	*O. glaberrima*	Philippines
353	WAB0001417	*O. glaberrima*	Democratic Republic of Congo

Table A.2. Information on the subset of 42 African rice accessions tested

S/N	Accessions Number	Species Name	Country of Origin	Blast Resistance Pattern*
1	WAB0015772	*O. sativa*	Benin	Resistant
2	WAB0006684	*O. sativa*	Benin	Resistant
3	WAB0035038	*O. sativa*	Benin	Resistant
4	WAB0035055	*O. sativa*	Benin	Resistant
5	WAB0035059	*O. sativa*	Benin	Resistant
6	WAB0029182	*O. glaberrima*	Mali	Resistant
7	WAB0029335	*O. glaberrima*	Nigeria	Moderately resistant
8	WAB0030263	*O. glaberrima*	Nigeria	Susceptible
9	WAB0023837	*O. glaberrima*	Mali	Resistant
10	WAB0024105	*O. glaberrima*	Mali	Resistant
11	WAB0024116	*O. glaberrima*	Mali	Resistant
12	WAB0029194	*O. glaberrima*	Mali	Resistant
13	WAB0032487	*O. glaberrima*	Mali	Moderately resistant
14	WAB0032298	*O. glaberrima*	Mali	Resistant
15	WAB0008589	*O. glaberrima*	Nigeria	Moderately resistant
16	WAB0020477	*O. glaberrima*	Nigeria	Moderately resistant
17	WAB0032397	*O. glaberrima*	Nigeria	Resistant
18	WAB0029323	*O. glaberrima*	Nigeria	Moderately resistant
19	WAB0029333	*O. glaberrima*	Nigeria	Moderately resistant
20	WAB0019882	*O. glaberrima*	Nigeria	Resistant
21	WAB0029315	*O. glaberrima*	Nigeria	Moderately resistant
22	WAB0020505	*O. glaberrima*	Nigeria	Moderately susceptible
23	WAB0032497	*O. glaberrima*	Liberia	Resistant
24	WAB0015703	*O. glaberrima*	Nigeria	Resistant
25	WAB0001360	*O. glaberrima*	Nigeria	Susceptible
26	WAB0029342	*O. glaberrima*	Nigeria	Susceptible
27	WAB0032230	*O. glaberrima*	Nigeria	Susceptible
28	WAB0008937	*O. glaberrima*	Guinea	Resistant
29	WAB0032848	*O. glaberrima*	Mali	Resistant
30	WAB0032550	*O. glaberrima*	Nigeria	Resistant
31	WAB0026783	*O. glaberrima*	Mali	Resistant
32	WAB0002093	*O. glaberrima*	Mali	Moderately resistant
33	WAB0002136	*O. glaberrima*	Mali	Resistant
34	WAB0002143	*O. glaberrima*	Mali	Resistant
35	WAB0002145	*O. glaberrima*	Mali	Moderately susceptible
36	WAB0032345	*O. glaberrima*	Mali	Moderately resistant
37	WAB0009280	*O. glaberrima*	Nigeria	Moderately susceptible
38	WAB0015043	*O. glaberrima*	Ivory Coast	Resistant
39	WAB0026176	*O. glaberrima*	Mali	Resistant
40	WAB0032495	*O. glaberrima*	Liberia	Resistant
41	WAB0008956	*O. glaberrima*	Liberia	Resistant
42	WAB0032394	*O. glaberrima*	Nigeria	Resistant

*: According to results in chapter 3: accessions' reaction to natural field infection by blast

Table A.3. Rice accessions and controls used

S/N	Accession number	Species name	Country of Origin	Blast Resistance Pattern *
1	WAB0006684	*O. sativa*	Benin	Resistant
2	WAB0035055	*O. sativa*	Benin	Resistant
3	WAB0035059	*O. sativa*	Benin	Resistant
4	WAB0029182	*O. glaberrima*	Mali	Resistant
5	WAB0029335	*O. glaberrima*	Nigeria	Moderately resistant
6	WAB0030263	*O. glaberrima*	Nigeria	Susceptible
7	WAB0029194	*O. glaberrima*	Mali	Resistant
8	WAB0032298	*O. glaberrima*	Mali	Resistant
9	WAB0020477	*O. glaberrima*	Nigeria	Moderately resistant
10	WAB0019882	*O. glaberrima*	Nigeria	Resistant
11	WAB0032497	*O. glaberrima*	Liberia	Resistant
12	WAB0015703	*O. glaberrima*	Nigeria	Resistant
13	WAB0001360	*O. glaberrima*	Nigeria	Susceptible
14	WAB0029342	*O. glaberrima*	Nigeria	Susceptible
15	WAB0032230	*O. glaberrima*	Nigeria	Susceptible
16	WAB0008937	*O. glaberrima*	Guinea	Resistant
17	WAB0032550	*O. glaberrima*	Nigeria	Resistant
18	WAB0002143	*O. glaberrima*	Mali	Resistant
19	WAB0009280	*O. glaberrima*	Nigeria	Moderately susceptible
20	WAB0026176	*O. glaberrima*	Mali	Resistant
21	WAB0008956	*O. glaberrima*	Liberia	Resistant

*: According to the results of chapter 3

Table A.4. Phenotypic variability of the 8 blast isolates tested on 54 differential blast accessions and 10 traditionally resistant varieties (Odjo *et al.* unpublished)

Accession number	Resistance Genes*	BN0013	BN0040	BN0050	BN0066	BN0082	BN0094	BN0119	BN0252	Incompatibility (%)
IRBL11-ZH	Pi11	S	S	R	S	S	S	S	R	25
IRBL5-M	Pi5	R	S	S	S	S	S	S	R	25
IRBL7-M	Pi7	S	S	S	S	S	S	S	R	12.5
IRBL12-M	Pi12	S	S	S	S	S	S	S	R	12.5
IRBL19-A	Pi19	S	S	R	S	S	S	S	R	25
GOTAK_GATIK	nd	S	S	S	S	R	R	S	R	37.5
IRBL20-IR24	Pi20	R	S	R	S	S	S	S	S	25
IRBL3-CP4	Pi3	S	S	S	S	S	S	S	R	12.5
IRBL9-W	Pi9	S	S	R	S	R	S	R	R	50
DA11	nd	S	S	R	R	S	R	R	R	62.5
IRBLB-B	Pib	S	S	S	S	S	S	S	R	12.5
IRBLI-F5	Pii	S	S	S	S	S	R	S	S	12.5
IRBLKH-K3	Pik-h	S	S	S	S	S	S	S	S	0
IRBLK-KA	Pik	R	S	S	S	S	S	S	R	25

Accession number	Resistance Genes*	BN0013	BN0040	BN0050	BN0066	BN0082	BN0094	BN0119	BN0252	Incompatibility (%)
IRBLKM-TS	Pik-m	R	R	S	R	R	R	S	R	75
IRBLKP-K60	Pik-p	S	S	S	S	S	S	S	R	12.5
IRBLKS-S	Pik-s	S	S	S	S	S	S	S	R	12.5
IRBLSH-S	Pish	S	S	S	S	S	R	S	R	25
IRBLTA-CP1	Pita	S	S	R	S	S	S	S	R	25
IRBLTA2-Pi	Pita-2	S	S	S	S	S	S	S	R	12.5
IRBLT-K59	Pit	S	S	S	S	S	S	S	R	12.5
IRBLZ5-CA	Piz-5	S	S	R	S	S	S	R	R	37.5
IRBLZ-FU	Piz	S	S	R	S	R	S	R	R	50
IRBLZT-T	Piz-t	S	S	S	S	S	S	S	S	0
MODAN	Pb-1	S	S	S	S	S	R	S	R	25
Owari hata mochi	pi-21	S	S	S	S	R	R	S	R	37.5
RIL249Moro	Pi5(t)	S	S	R	R	R	R	R	R	75
Aichi-Asahi	a + 19	S	S	S	S	S	S	S	S	0
75-1-127	9	R	R	R	R	R	R	R	S	87.5
Bala	33	S	S	R	S	R	S	R	R	50
BL1	b	S	S	R	S	R	S	S	R	37.5
C101A51	2 = z5	R	R	S	R	R	R	R	S	75
C101LAC	1 + 1b + 33	R	S	S	S	R	R	S	R	50
C104LAC	1	S	S	S	S	R	R	S	R	37.5
C104PKT	3	S	S	S	S	S	S	S	S	0
Co39	R0CO39	S	S	S	S	S	S	S	S	0
FujisakaNo5	i + ks	S	S	S	S	S	R	S	R	25
Fukunishiki	z + sh	S	R	R	R	R	R	R	R	87.5
IR1529	33	R	S	R	S	S	S	S	R	37.5
IR64	33	R	R	R	R	S	R	R	R	87.5
K1	ta	S	S	R	S	S	S	R	R	37.5
K2	kp + a	S	S	S	S	S	S	S	R	12.5
K3	kh	R	R	S	R	R	R	S	R	75
K59	t	S	S	S	S	S	S	S	R	12.5
K60	kp	S	S	S	S	S	S	S	R	12.5
Kanto51	k	R	S	S	S	S	R	S	R	37.5
Kusabue	k	R	R	S	R	R	R	S	R	75
Maratelli	none	S	S	S	S	S	S	S	S	0
Moroberekan	Pi12(t), Pi157(t), Pi44(t), Pi7(t)	R	R	R	R	R	R	S	R	87.5
Nato	i	S	S	S	S	S	S	S	R	12.5
Oryzica_llanos5	nd	R	R	R	R	R	R	R	R	100
Pin4	ta²	R	R	R	R	R	R	R	R	100
Rico1	ks	S	S	S	S	R	R	S	R	37.5
Shin2	ks + sh	S	S	S	S	S	R	S	R	25
St1	f	S	R	R	S	R	R	S	R	62.5
Toride1	zt	R	R	R	R	R	R	S	R	87.5
Tsuyuake	km	R	R	S	R	R	R	S	R	75

Accession number	Resistance Genes*	BN0013	BN0040	BN0050	BN0066	BN0082	BN0094	BN0119	BN0252	Incompatibility (%)
Zenith	z	S	R	R	R	R	R	R	R	87.5
Chiem-Chanh	nd	R	S	R	S	S	R	R	R	62.5
RTS14	nd	S	S	R	S	S	S	S	R	25
DHOLI-BORO	nd	R	R	R	S	S	R	R	R	75
GOGO-LEMPUK	nd	S	S	S	S	S	R	S	R	25
Nipponbare	nd	S	S	S	S	S	R	S	R	25
Som-Cau-70A	nd	S	S	S	S	S	S	S	S	0
Average										38.48

*: BN0202, the 9th blast isolate is compatible with resistance gene Pi- ta^{2}; nd = non-identified gene

Table A.5. List of 17 morphological characters used to differentiate *O. glaberrima* and *O. sativa*

S/N	Parameter	Description
1	Basal leaf sheath: colour	Colour of the outer surface of the leaf sheath. Stage: late vegetative
2	Leaf-blade: pubescence	Assess both visually and by touch, rubbing fingers over the leaf surface from the tip downwards. Stage: late vegetative
3	Leaf margin: pubescence	Assess pubescence of leaf margins. Stage: late vegetative
4	Ligule shape	Stage: late vegetative
5	Ligule colour	Stage: late vegetative
6	Flag leaf: attitude (late observation)	Observed near the collar. The angle of attachment between the flag leaf blade and the main panicle axis
7	Culm: habit	The estimated average angle of inclination of the base of the main culm from vertical. Stage: after flowering
8	Lemma and palea: colour (early observation	Stage: after anthesis to hard dough stage (pre-ripening stage)
9	Awns: distribution (cultivated species)	The presence and distribution of awns along the panicle. Stage: flowering to maturity
10	Awns: colour (early observation)	Stage: after anthesis
11	Panicle: exsertion	The extent to which the panicle is exserted above the flag leaf sheath. Stage: near maturity
12	Panicle: the attitude of branches	The compactness of the panicle, classified according to its mode of branching, angle of primary branches, and spikelet density
13	Panicle: secondary branching	The abundance and distribution of spikelets borne on secondary branches of the panicle. Stage: near maturity
14	Lemma: colour of apiculus (early observation)	Stage: cultivated species after anthesis to hard dough stage (pre-ripening stage
15	Lemma: colour of apiculus (late observation)	Stage: After harvest
16	Lemma and palea colour (late observation)	Stage: After harvest
17	Panicle form	Stage: at harvest

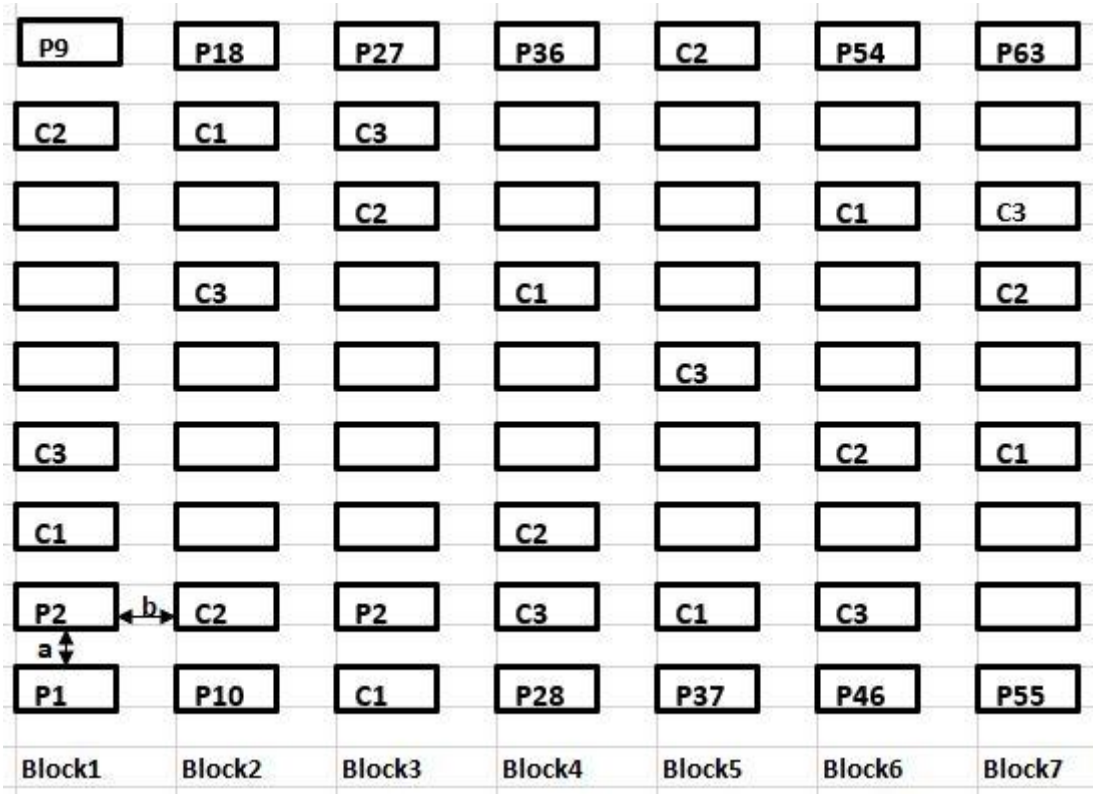

Figure A.1. General view of the Augmented experimental design

a: 2 m; b: 2 m; c: 10 cm; d (distance between infesting band and entries): 20 cm; e: 1 m;

P1 : experimental unit of 5x1 m^2

P: Plot;

C1, C2, and C3: rice reference controls

a: 2m path between plots;

Curriculum Vitae

Professional information

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Education

2003-2004 Secondary school of Abomey-Calavi, Benin

Baccalaureate of Biological Sciences

2007-2008 University of Abomey-calavi, Benin

Bachelor of Science (Biochemistry)

2008-2009 University of Abomey-calavi, Benin

Maîtrise of Science (Biochemistry)

2010-2011 University of Abomey-calavi, Benin

Master of Science (Plant Genetic Resources and Breeding)

Professional experience

2011-2014 Africa Rice Center, Cotonou, Benin

Postmaster position

2011-present University of Abomey, Faculty of Sciences and Technics of Dassa Benin

Assistant Lecturer (Biological Sciences)

Language skills

- French: Fluency in speaking, reading, and writing
- English; satisfactory in speaking, reading, and writing

Publications

1. **Yelome OI**, Audenaert K, Landschoot S, Dansi A, Vanhove W, Silue D, Van Damme P, Haesaert G (2018a) Exploring genetic diversity and disease response of cultivated rice accessions against *Pyricularia oryzae* under rainfed upland conditions in Benin. Genet. Resour. Crop Evol. 65:1615-1624.
2. **Yelome OI**, Audenaert K, Landschoot S, Dansi A, Vanhove W, Silue D, Van Damme P, Haesaert G (2018b) Analysis of population structure and genetic diversity reveals gene flow and geographic patterns in cultivated rice (*O. sativa* and *O. glaberrima*) in West Africa. Euphytica 214:215.
3. **Yelome OI**, Audenaert K, Landschoot S, Dansi A, Vanhove W, Silue D, Van Damme P, Haesaert G (2018c) Combining High Yields and Blast Resistance in Rice (*Oryza*

spp.): Screening under Upland and Lowland Conditions in Benin. Sustainability (Switzerland). 10:1-16.

4. Agnoun Y, **Yelome I**, Sie M, Albar L, Ghesquière A, Silué D (2019) Resistance of selected *Oryza glaberrima* landraces and their intra-specific breeding lines to Beninese Rice yellow mottle virus isolates. Crop Protection 119:172-176.

Scientific conferences

1. IBPO conference (2018) on Scientific innovation for sustainable development of African agriculture, Belgium; Exploring genetic diversity and disease response of cultivated rice accessions against *Pyricularia oryzae* under rainfed upland conditions in Benin

   Yelome OI, Audenaert K, Landschoot S, Dansi A, Vanhove W, Silue D, Van Damme P, Haesaert G

2. IX International Agriculture Symposium “AGROSYM” (2018), Bosnia and Herzegovina; Grain yield performance of selected blast-resistant rice (*Oryza* spp.) under lowland and upland growing conditions in Benin

   Yelome OI, Audenaert K, Landschoot S, Dansi A, Vanhove W, Silue D, Van Damme P, Haesaert G

Short term training

1- Leadership and Management skills course for Monsanto's Beachell-Borlaug International Scholars organized by Tero International, Iowa, USA, 14-16 October 2017

2- Leadership and Management skills course for Monsanto's Beachell-Borlaug International Scholars organized by Tero International, Iowa, USA, 13-16 October 2015

Scholarships and Awards

1. Belgium BOF sandwich Ph.D. scholarship 2014
2. Monsanto's Beachell-Borlaug International Scholars Program 2015