THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE L'UNIVERSITÉ DE MONTPELLIER

En mécanismes des interactions parasitaires pathogènes et symbiotiques
École doctorale [GAIA – Biodiversité, Agriculture, Alimentation, Environnement, Terre, Eau]

Unité de recherche [Unité Mixte de Recherche Biologie et Génétique des Interactions Plante-Parasite]

Biologie et génétique des populations de *Pyricularia oryzae* pour le contrôle de la pyriculariose du riz au Mali

Présentée par Diariatou DIAGNE

Le 18 mai 2021

Sous la direction de Didier THARREAU
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UNIVERSITÉ DE MONTPELLIER

Je dédie cette thèse à mon défunt père Seydou DIAGNE, qui de son vivant n'a jamais cessé de m'encourager et de m'exhorter à continuer mes études au plus haut niveau possible. « Que ton âme repose en paix papa ».

Je rends un vibrant hommage à mon regretté superviseur Pr. Lassana B TRAORE pour m'avoir accueilli à bras ouverts au sein de la Faculté des Sciences et Techniques de Bamako dans le cadre de mes premiers pas dans l'assistanat. « Que son âme repose en paix ».

REMERCIEMENTS

Mes vifs remerciements à l'endroit de mon jury de thèse d'avoir accepté d'évaluer mes travaux de thèse, mes rapporteurs Dr. Christel LEYRONAS et Dr. Christophe LE MAY aussi mon examinatrice Dr. Anne-Sophie WALKER.

J'adresse également mes remerciements :

- aux membres de mon comité de thèse pour leur suivi tout au long de mes travaux de thèse, Pr. Claire NEEMA, Dr. Christophe BRUGIDOU, Dr. Marc-Henri LEBRUN, Dr. Boris SZUREK, pour leurs conseils, leurs suggestions et encouragements ;

- à l'Ambassade de France au Mali à travers Mme Fanta TOURE, Responsable de la Bourse SCAC, pour leur assistance qui n'a pas fait défaut en prenant en charge le financement de ma thèse ;
- au fond d'appui pour les prospections de la Jeune Equipe Associée à l'IRD (JEAI CoANA) qui m'a permis de mener les missions d'échantillonnage ;
- au Programme de Formation des Formateurs de l'Université des Sciences Juridiques et Politiques de Bamako (USJPB-R/SG.PFF) à travers le Pr. Sinaly DEMBELE, Coordinateur du dit programme pour leur soutien ;
- au corps professoral de la Faculté des Sciences et Techniques (F.S.T) de l'Université des Sciences des Techniques et des Technologies de Bamako (USTTB) pour leur encouragement ;

- à mon Directeur de thèse, Dr. Didier THARREAU pour son encadrement indéfectible, son soutien sans relâche pour m'avoir ouvert toutes les portes afin d'aboutir à tous ces résultats. Sa disponibilité et son management ont fait de cette thèse une réalité ;

- à mon Co-Directeur de thèse, Pr. Ousmane KOITA, qui grâce à son coaching a enclenché ce prestigieux projet de thèse et qui continue de près ou de loin à mettre toutes les cartes pour la réussite efficiente de cette thèse ;

- au Dr. Valérie VERDIER qui est à la base et constitue pour moi la lanterne qui illumine ce parcours de thèse, son apport est d'une importance capitale ;

Henri, je tiens vivement à te remercier pour ta disponibilité, ton accompagnement et ton encadrement sans faille qui m'ont permis d'effectuer mes travaux de thèse. Grace à ton hospitalité, j'ai passé un séjour agréable avec ta famille qui était si joviale et accueillante. Je suis également très touchée par ta générosité qui n'a jamais fait défaut ;

Joëlle, mes vifs remerciements à ton égard pour n'avoir ménagé aucun effort pour ton soutien et ton encadrement pour la réussite de cette thèse. Nos échanges amicaux me remontaient le moral et me permettaient d'évacuer le stress, ce qui me rendait plus efficace ;

Géneviève, merci pour ton assistance, ton appui et tes orientations dans toutes mes démarches administratives qui m'ont facilité beaucoup de choses sur le plan technique et logistique. Merci également à Florence pour son soutien dans certaines de mes démarches administratives ;

Mes remerciements sont adressés à Elisabeth, Sébastien, Pierre, Florian et Lucas pour leur aide dans mes analyses de données génomiques ;

Mes remerciements à toute l'équipe 5 de BGPI, pour l'aide technique apportée lors de mes activités de thèse, pour la compréhension et les encouragements quotidiens et l'excellente atmosphère de travail, pour l'organisation qu'ils ont mis en place au sein de l'équipe pour faciliter mon insertion ;

Durant mon séjour doctoral à BGPI, j'ai croisé des merveilleuses personnes avec qui j'ai partagé des moments agréables, il s'agit notamment d'Edwige, Mireille, Thomas, Maud, Valentin, Faihad, Brigitte, Romain, Adel, Mahsa, Lieu, Emiline, Flavia, Gislène, Faustine, Alexandre, Gloria Mosquera, Katia, Cica, Laurence, Véronique, à toutes les deux Sandrine, Kristell, Sonia, Michel, Loic, un grand merci à tous ;

Hilaire, un grand merci à toi pour m'avoir aidé à un moment crucial de mes travaux de thèse et aussi des échanges fructueux qui ont suscité pas mal d'intérêt en termes de partage d'expériences ;

Mohamed DIABY, merci infiniment pour avoir confectionner mes données cartographiques ;

A toute mon équipe de collecte particulièrement M. Mamadou DEMBELE (qui m'a montré pour la première fois au champ les symptômes de la pyriculariose du riz) et M. Dotian DIALLO de l'IER Niono à l'Office du Niger ; l'équipe JEAI CoANA au LBMA notamment Dr. Hinda DOUCOURE, Ibrahim KEITA, Dr. Moussa KANTE, Lazeni KONATE, Jean Marie SANOU et Dr. Boubacar MACALOU ; aux différentes équipes des stations rizicoles notamment M. Mamadou TOGOLA (Directeur Général), et M. Yaya COULIBALY (guide) de l'Office du Périmètre Irrigué de Baguineda (OPIB), M. Souleymane DEMBELE (responsable du périmètre irrigué de Manikoura), M. Mahamadou MAIGA (guide), M. N'do COULIBALY et M. Ousmane DIABATE (guide) de l'Office de Développement Rural de Sélingué (ODRS) ; à toute l'équipe des stations de Sikasso à travers M. Seydou FOMBA et Mme Mariam BERTHE ; à l'équipe rizicole de Mopti à travers M. Ousmane CAMARA. Merci infiniment à tous pour votre disponibilité pour la réussite des différentes prospections ;

Je voudrais exprimer ma reconnaissance aux Dr. Moussa KANTE, Dr. Youssouf DIARRA et Dr. Lassina DOUMBIA pour leur disponibilité, leur aide précieuse pour l'aboutissement de la rédaction de ce manuscrit. Merci pour votre franche collaboration ;
Pr. Lansana SANGARE, Mamadou Coulibaly, Dr. Abdrahamane TOUNKARA, Dr. Ousmane KODIO et Dr. Abdoulaye BORE, merci pour votre soutien ;
Mes remerciements sont adressés à tout le personnel du Laboratoire de Biologie Moléculaire Appliquée (LBMA) ;

A mes familles d'accueil, mes amis en France, en Suisse et en Espagne notamment les familles SIDIBE, CAMARA, REMUND, SCHMUTZ, HOFER et DIALLO, je vous réitère mes sincères remerciements pour votre hospitalité légendaire ;

Abou, je tiens à te remercier infiniment pour ta disponibilité ;

A ma famille, maman, mes sœurs et frères, mes oncles particulièrement tonton Mamadou DIAKITE et tonton Salif SAMAKE et mes tantes pour leur accompagnement et leurs bénédictions ;

Enfin, je remercie également tous ceux et celles qui de près ou de loin ont contribués au bon déroulement de ma thèse, puisse Dieu vous récompenser de vos efforts, Amen.

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Introduction Générale

INTRODUCTION GENERALE

I- Généralité sur le riz

1-Taxonomie du riz

Le riz est une céréale monocotylédone de la famille des Poacées (ou Graminées) (Cronquist, 1988), cultivée dans les régions tropicales, subtropicales et tempérées chaudes Subudhi et al., 2006). Le genre *Oryza* comprend 24 espèces parmi lesquelles deux sont cultivées : *Oryza sativa* L. et *O. glaberrima* Steud (Vaughan et Chang, 1992). Après de nombreuses recherches, tant morphologiques que moléculaires, ces différentes espèces ont été regroupées en complexes selon diverses modalités. Ge et al. (2001) suggèrent sur une base moléculaire, une division en quatre complexes (*sativa, officinalis, ridleyi* et *meyeriana*), similaire à la division en sections et en séries suggérée par le Centre de Ressources Génétiques de l'International Rice Research Institute (sur des bases morphologiques ; Tableau I). Le complexe *sativa* regroupe les deux espèces de riz cultivées *O. sativa* et *O. glaberrima,* ainsi que six espèces sauvages apparentées : *O. barthii* A. Chev., *O. glumaepatula* Steud, *O. longistaminata* A. Chev. et Roehr., *O. meridionalis* Ng., *O. nivara* Sharma et Shastry, et *O. rufipogon* Griff. Toutes ces espèces sont diploïdes, de génome AA, avec 2n = 24 (Ge et al., 2001).

2-Les riz sauvages

Il existe plusieurs espèces sauvages du riz dans le monde parmi lesquelles nous retrouvons l'espèce *O. barthii* et *O. longistaminata* en Afrique (Portères, 1959). Ces espèces sauvages sont pérennes ou annuelles, allogames ou autogames (Arraudeau M., 1998).

O. longistaminata peut être définie par les caractéristiques suivantes :

- Présence des rhizomes remarquables qui lui confèrent une forte pérennité ;
- Une reproduction allogame régit par un système d'auto-incompatibilité faisant subsister cependant certaines formes auto-compatibles ;
- La thermosensibilité des graines d'*0. longistaminata* au cours de la germination leur confère une variabilité intrapopulation permettant à certains individus de se développer à des températures extrêmes moins bien supportées par d'autres espèces (Second et al., 1977 ; Bezançon et al., 1989).

O. barthii (anciennement appelée *O. breviligulata)* (Portères, 1966 ; Second et al., 1977) est une espèce pérenne et rhizomateuse (Nayar, 1967). Ses chaumes sont de type flottant avec la crue pour maintenir ses extrémités foliaires et ses inflorescences au-dessus de l'eau. Dès avant

maturité apparente, *O. barthii* égrène abondamment. La maturité physiologique étant plus tardive, les graines détachées de très bonne heure ne sont pas capables de germer tout de suite. Elles n'acquièrent leur maturité qu'à la saison suivante (dormance). Cette espèce a une capacité d'adaptation étonnante aux inondations périodiques afin de se maintenir en peuplements denses par ses graines et ses rhizomes.

3-Les riz cultivés et leur domestication :

Les espèces de riz cultivées seraient issues d'un ancêtre commun, *O. perennis*, qui aurait existé dans le Gondwana à l'époque où les continents étaient unis (Chang, 1976 ; Khush, 1997).

Oryza sativa est l'espèce la plus cultivée dans le monde. D'origine asiatique, elle a été domestiquée à partir *d'O. rufipogon*, une espèce complexe qui comprend une forme pérenne et une forme annuelle (aussi décrite sous le nom d'*O. nivara* ; Second, 1985 ; Kovach et al., 2007 ; Tang et Shi, 2007). Selon les découvertes archéologiques, la domestication du riz asiatique aurait commencé dans la zone qui s'étend à l'est de la Chine, à travers le piémont de l'Himalaya, au Vietnam, en Thaïlande, au Myanmar, et à l'est de l'Inde. Le plus ancien type de riz cultivé a été trouvé dans la vallée du fleuve Yang-Tse en Chine, datant de près de 8000 ans avant notre ère (Normile, 1997 ; Itzstein-Davey et al., 2007). *O. sativa* est l'espèce la plus cultivée dans le monde et comporte des groupes génétiques: indica, japonica, aus, basmati et aromatiques (Wang et al., 2014 ; Fig.1). Le groupe indica serait originaire de l'Asie tropicale (Inde) et se caractérise en général par un fort tallage et des grains longs et fins, contrairement au groupe japonica qui serait originaire de la zone tempérée et subtropicale d'Asie (Chine), qui se caractérise souvent par un tallage moyen et des grains courts et ronds (Garris et al., 2005). L'origine des groupes indica et japonica n'est pas clairement établie, en particulier l'existence d'hybridations secondaires entre les deux groupes fait l'objet de discussion (Ghesquiere, 1988).

O. glaberrima est cultivée uniquement dans la zone subsaharienne. Son rendement est faible par rapport à *O. sativa*. (Linares, 2002 ; Orjuela et al., 2014). *O. glaberrima* aurait été domestiqué dans la boucle du Niger au Sud de Tombouctou (Mali) avant de s'étendre à l'Ouest vers les fleuves Sénégal et Gambie, et à l'Est vers le lac Tchad (Cubry et al., 2018 ; Lachowsky, 2019). Cette espèce aurait été domestiquée il y a 3000 ans dans le delta intérieur du fleuve Niger (Portères, 1966 ; Veltman et al., 2019) à partir d'une espèce sauvage annuelle *O. barthii* (Monat, 2016 ; Pidon, 2016 ; Gnacadja et al., 2018), elle-même issue de l'espèce pérenne *O. longistaminata*, *O. glaberrima* possèderait certains caractères d'adaptation aux contraintes biotiques et abiotiques rencontrées dans sa zone de culture. Ses ravageurs ou agents pathogènes sont entre autres, la panachure jaune du riz (RYMV), la pyriculariose (*Pyricularia oryzae*), la

bactériose vasculaire (*Xanthomonas oryzae*) et des nématodes. La sécheresse et à la toxicité ferreuse sont des contraintes abiotiques fréquentes. Les caractéristiques morphologiques les plus importantes qui permettent de différencier *O. glaberrima d'O. sativa* sont la ligule plus courte et tronquée (Arraudeau, 1998), le péricarpe souvent rouge, l'absence de pilosité des glumelles, une ramification secondaire des panicules plus faible, les grains plus petits et ayant une plus forte déhiscence, et un port plus dressé de la panicule (Sweeney et McCouch, 2007 ; Pidon, 2016).

Tableau I : Composition génomique et répartition géographique des espèces *Oryza*
(Ge et al., 2001 ; Khush et al., 2001)

Complexe d'espèces	**Génome**	**Distribution**
Complexe *Oryza sativa*	AA	
O. sativa	AA	Mondiale
O. nivara	AA	Asie tropicale et subtropicale
O. rufipogon	AA	Asie tropicale et subtropicale, Australie tropicale
O. glaberrima	AA	Afrique de l'ouest
O. barthii	AA	Afrique
O. glumaepatula	AA	Amérique central et du sud
O. longistaminata	AA	Afrique
O. meridionalis	AA	Australie tropicale
Complexe *Oryza officinalis*		
O. punctata	BB, BBCC	Afrique
O. minuta	BBCC	Philippines et Papouasie-Nouvelle Guinée
O. officinalis	CC	Asie tropicale et subtropicale, Australie tropicale
O. rhizomatis	CC	Sri Lanka
O. eichingeri	CC	Asie du Sud et Afrique de l'Est
O. latifolia	CCDD	Amérique du sud et centrale
O. alta	CCDD	Amérique du sud et centrale
O. grandiglumis	CCDD	Amérique du sud et centrale
O. australiensis	EE	Australie tropicale
Complexe *Oryza meyeriana*		
O. granulata	GG	Asie du sud et du sud-est
O. meyeriana	GG	Asie du sud-est
Complexe *Oryza ridleyi*		
O. ongiglumis	HHJJ	Irian Jaya (Indonésie) et Papouasie-Nouvelle-Guinée
O. ridleyi	HHJJ	Asie du sud
Espèces n'appartenant à aucun complexe		
O. brachyantha	FF	Afrique
O. schlechteri	HHKK	Papouasie Nouvelle Guinée

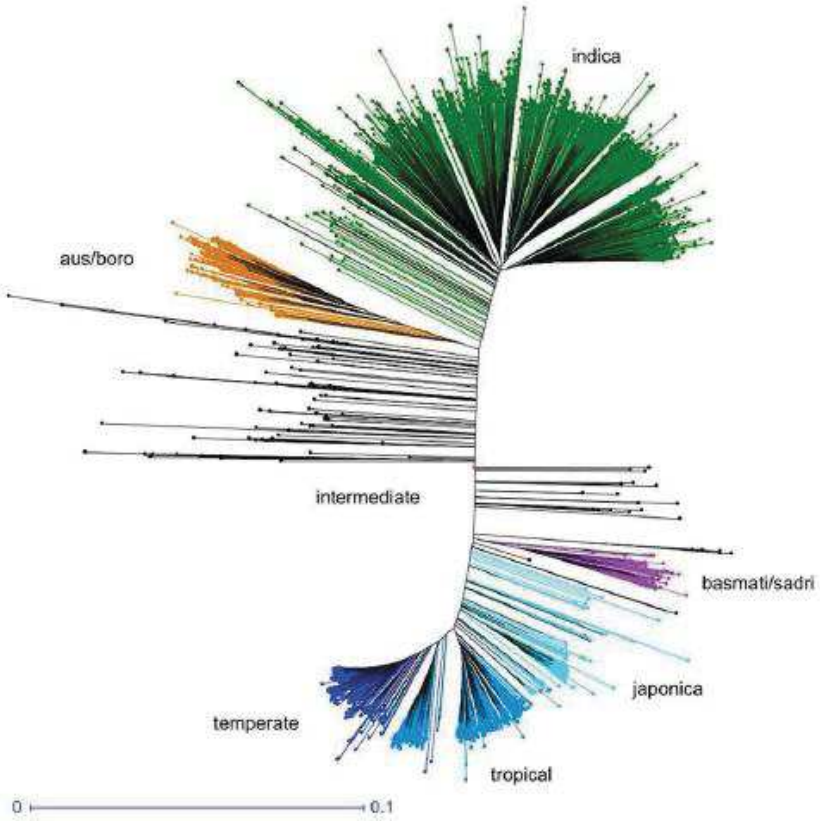

Figure 1 : Arbre de diversité génétique d'*O. sativa* (Wang et al., 2018)

4-Production et importance du riz dans le monde

Le riz est une plante cultivée adaptée à une gamme variée de conditions agroclimatiques. Le riz se cultive sur tous les continents. Il constitue la base alimentaire de près de 40 % de la population mondiale après le blé selon un rapport de la Conférence des Nations Unies sur le commerce et le développement (CNUCED). Selon les prévisions de la FAO, la production mondiale de riz est de 515 millions de tonnes (FAO, 2020). L'Asie occupe la première place dans la production de riz suivie par l'Afrique, l'Amérique, l'Europe et l'Océanie (FAO, 2018). Près de 85 % de la superficie totale cultivée du riz se trouve dans cinq pays d'Asie, à savoir la Chine, l'Inde, l'Indonésie, le Bangladesh et les Philippines. La Chine occupe la première place de ce classement avec un volume de production supérieur à 200 M de tonnes par an (FAOSTAT, 2019).

L'Afrique représente 8 % de la production rizicole mondial (FAOSTAT, 2016). Le riz est cultivé sur tout le continent africain, cependant l'Afrique de l'Ouest représente 55% de la

production de riz dans le continent (FAO, 2018). Le Nigeria est le plus gros producteur du riz du continent. Il était le 13ème producteur mondial de riz avec 8 M de tonnes par an (FAOSTAT, 2019) et 1er producteur de riz en Afrique de l'Ouest (Boutsen et Aertsen, 2013). L'Egypte était le 15ème producteur mondial de riz avec 6 M de tonnes par an (FAOSTAT, 2019). Madagascar était le 19ème producteur mondial de riz avec 4 M de tonnes par an (FAOSTAT, 2019). Toutefois, la production locale de riz ne satisfait pas la demande croissante dans de nombreux pays africains (Hossain, 2006) où le riz demeure un aliment de base. Ainsi, l'Afrique de l'Ouest a besoin d'importer 40% de sa consommation de riz. Le Mali par exemple parvient à assurer pratiquement la totalité de ses besoins en riz, tandis que des pays comme le Sénégal, le Ghana et le Bénin assurent à peine 40% de leur consommation (Boutsen et Aertsen, 2013). Des efforts visant à réduire la dépendance aux importations sont également en cours au Sénégal. En Côte d'Ivoire, le riz représente 65% de la production de céréales, le déficit de production est comblé par des importations à environ 40% (Bouet et Tahouo, 2015).

5-Etat des lieux de la riziculture au Mali

5.1- Production du riz au Mali

La filière rizicole représente l'une des réussites du Mali , 22ème producteur mondial de riz avec 3,2 M de tonnes par an (FAOSTAT, 2019) ; 2ème producteur du riz en Afrique de l'Ouest derrière le Nigéria (AMASSA / Afrique Verte Mali et al., 2018 ; FAO, 2018). Dans les zones rurales au Mali, la production locale de riz est autoconsommée majoritairement et constitue la 3ème céréale la plus consommée dans ses zones après le mil/sorgho et le maïs. (Balié et al., 2013). La culture du riz couvre plus de 0,5 million d'hectares (Mendez del Villar et al., 2011 ; Boutsen et Aertsen, 2013). La production annuelle du riz est passée d'un million de tonnes au début des années 2000 à plus de 2,5 millions de tonnes à partir de 2010 pour atteindre environ 3 millions de tonnes en 2018 avec un excédent de production de 584 000 tonnes (Commodafrica, 2018 ; FAO, 2018). Cette augmentation de production pourrait être due à l'implication de l'État qui a favorisé l'accès aux intrants, l'expansion des infrastructures d'irrigation et la mécanisation de l'agriculture par la mise en place d'un programme d'appui à la culture du riz en 2008 nommé « Initiative riz ». Le Mali est l'un des pays d'Afrique subsaharienne où les importations sont les plus faibles ; il a presque atteint l'autosuffisance alimentaire (Balié et al., 2013 ; Fall, 2019). Cependant, les importations restent nécessaires pour satisfaire une demande en croissance, en particulier en milieu urbain. De nos jours, les Maliens

consomment plus de 80 kg de riz par habitant et par an. La part du riz dans la consommation totale de céréales du pays augmente et serait à 35 % (Baudouin et al., 2017). Cela serait dû à sa simplicité de préparation par rapport à d'autres céréales, à l'urbanisation et à diversification des modes de consommation dans le pays en général et dans les villes en particulier (Baudouin et al., 2017).

5.2 -Importance de la riziculture dans l'économie du Mali

L'agriculture est le socle de l'économie malienne qui occupe 75% de la population active. Elle contribue à plus de 40% à la formation du Produit Intérieur Brut (PIB). La contribution de la filière riz au PIB est significative (5% en valeur du PIB national et environ 10 % en valeur du PIB agricole), et avec une tendance à la hausse (FAO, 2010 ; FMI, 2011 ; Coulibaly et Ouologuem, 2014 ; Baudouin, 2018).

Les principales régions de production sont : Ségou (zone de l'Office du Niger), Mopti, Sikasso et Tombouctou (Fig. 2). La zone de l'Office du Niger contribue à 45% de la production nationale de riz (Maïga, 2019).

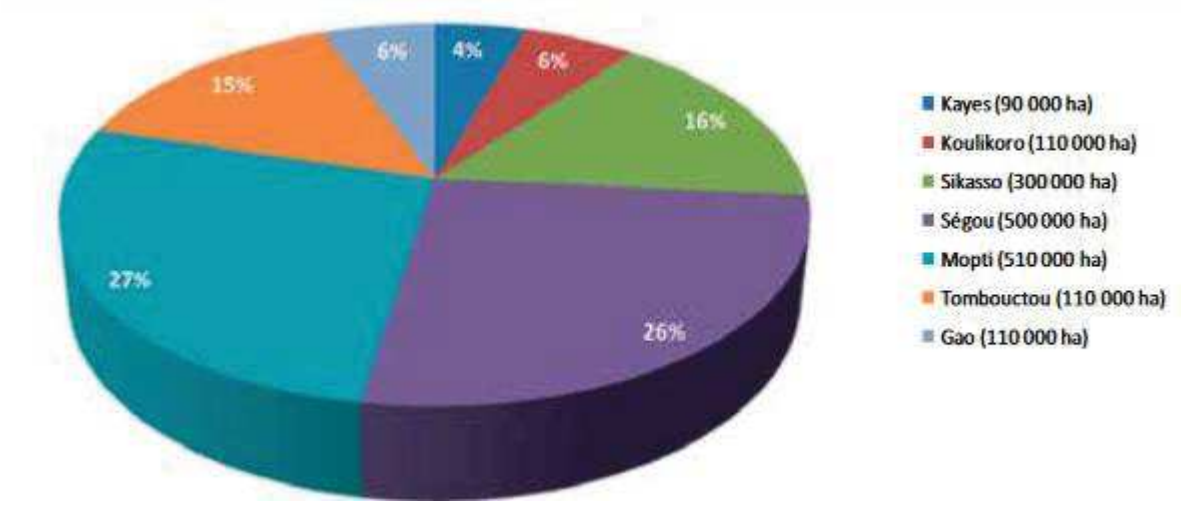

Figure 2: Répartition régionale des zones de production du Mali (DNA, 2009)

5.3- Les différents agrosystèmes du riz au Mali

Le riz est cultivé dans toutes les régions du Mali actuellement, dans des agrosystèmes diversifiés (Fig. 3) en fonction de la spécificité des zones. Cette spécificité concerne la disponibilité en eau de surface, le climat, les conditions topographiques…. (CORAF/WECARD, 2009). On distingue cinq grands écosystèmes de riziculture :

5.3.1-La riziculture avec maîtrise totale de l'eau

Dans cet agrosystème, les aménagements sont réalisés dans l'objectif d'un contrôle total de l'eau dans les parcelles (CORAF/WECARD, 2009). La zone de l'Office du Niger constitue la plus grande zone suivie des grands périmètres aménagés de Sélingué et de Baguinéda. A cela s'ajoute la plaine de San-Ouest où les rendements varient entre 4 à 8 t/ha, les périmètres irrigués par pompage (plaines d'Amadia et de Korioumé), les petits périmètres villageois le long du fleuve Niger (Mopti et Tombouctou) et le fleuve Sénégal (Bafoulabé, Kita, Kayes et Manantali) (FAO, 2010 ; Camara et al., 2014; Fig 4).

5.3.2- La riziculture pluviale

Cette culture est pratiquée sur de petites superficies dans la zone sud du pays où la pluviométrie est supérieure ou égale à 800 mm par an. Les rendements sont généralement faibles à raison de 800 kg/ha mais en amélioration constante avec l'avènement du projet « Riz NERICA » et la diffusion des nouvelles variétés pluviales mises au point par l'IER (Institut d'Economie Rurale). Ces nouvelles variétés ont contribué à une prise d'envergure de la riziculture pluviale dans les régions de Sikasso, Kayes, Koulikoro et une partie de la région de Ségou avec des rendements nettement améliorés avec 3 à 3,5 tonnes/ha (CORAF/WECARD, 2009 ; FAO, 2010 ; Camara et al., 2014).

5.3.3- La riziculture de submersion contrôlée

Dans cet agrosystème, l'aménagement est constitué d'un chenal d'amenée d'eau à partir du fleuve, d'un ouvrage de régulation (vanne) et d'une digue qui entoure partiellement ou totalement le périmètre cultivé (CORAF/WECARD, 2009 ; FAO, 2010). Elle est essentiellement pratiquée dans les plaines inondables du Niger et du Bani (régions de Ségou et Mopti en majorité) sur de vastes superficies aménagées au début des années 70. Elle représente 74 000 ha de périmètres aménagés et des rendements de l'ordre de 0.8 à 2.5 t/ha (Camara et al., 2014).

5.3.4- La riziculture de bas – fonds

Au Mali, cet agrosystème est caractérisé par une grande diversité et représente 12% des emblavures. Elle est pratiquée en général par les femmes dans les régions de Ségou, Sikasso et Kayes avec en moyenne 0,8 à 2 t/ha. Il peut s'agir d'une submersion temporaire ou permanente

due à la remontée de la nappe phréatique, à l'accumulation de l'eau de ruissellement et à la crue de la rivière ou du marigot (Camara et al., 2014 ; Fall, 2019).

5.3.5- La riziculture traditionnelle de submersion libre

Cet agrosystème est pratiqué surtout dans le delta intérieur du Niger et le long du fleuve dans la boucle du Niger. Le rendement maximal est de 1 t/ha. L'espèce cultivée est *O. glaberrima* car elle a la particularité de croître très rapidement et de s'adapter à la crue du fleuve qui peut monter à un rythme de 5 cm par jour. Le semis a lieu dès les premières pluies, puis la crue survient et inonde les rizières durant l'hivernage (CORAF/WECARD, 2009 ; Camara et al., 2014).

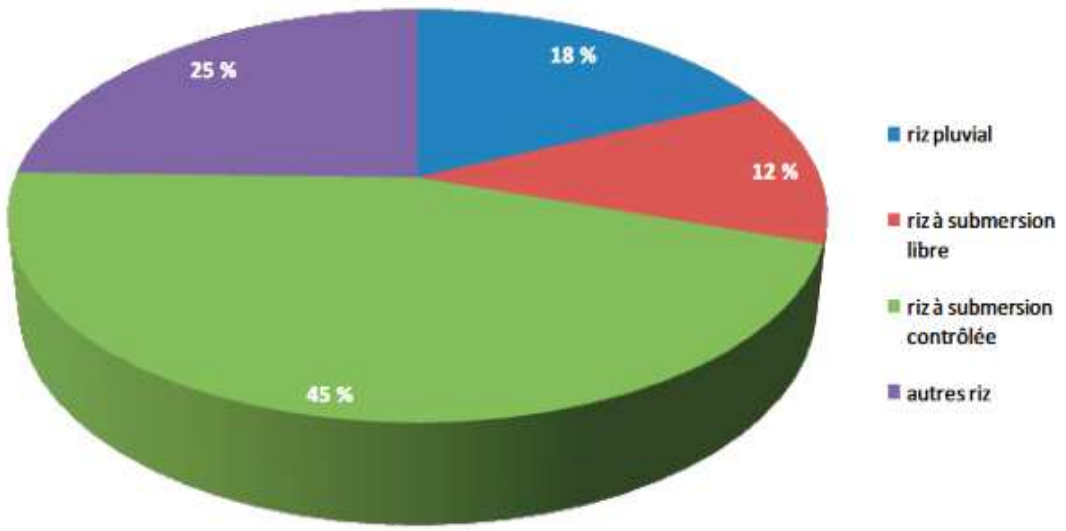

Figure 3 : Allocation des terres rizicoles en fonction des modes de production

Source : (DNA, 2009)

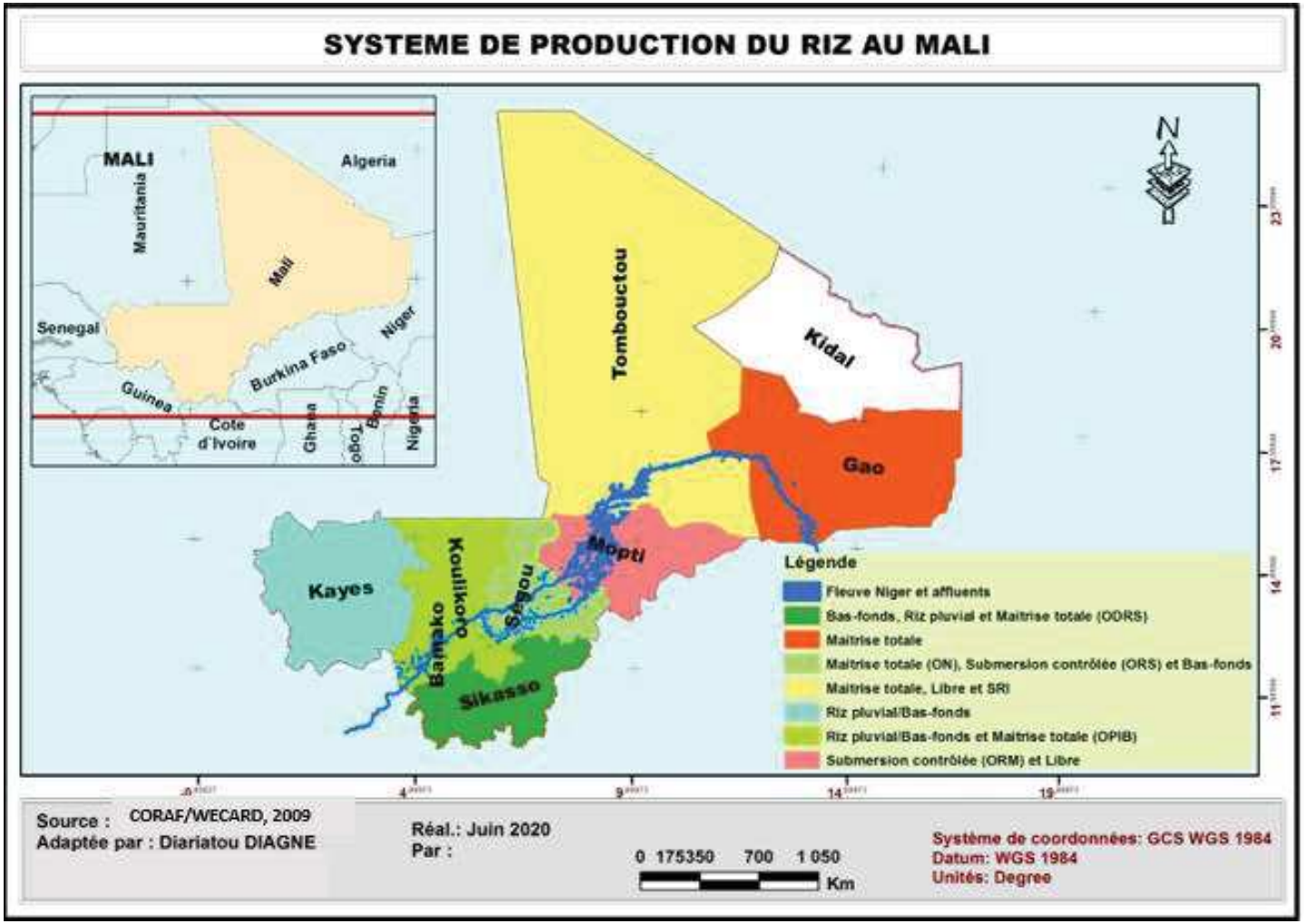

Figure 4 :Cartographie des zones rizicoles par région au Mali (adapté de CORAF/WECARD, 2009)

Au Mali, la date optimale de semis est établie en fonction de la saison de culture et du système de riziculture (Tableau II).

Tableau II : Calendrier de la riziculture au Mali

Type de saison	Riziculture irriguée		Riziculture pluviale et de bas-fond
	Semis en pépinière	Repiquage	Semis direct
Hivernage	20 mai au 20 juin	15 juin au15 juillet	-
Pluviale	-	-	1er juin au 15 juillet
Contre saison	1er au 28 février	21 février au 30 mars	-

Source : Ministère de l'Agriculture, 2012

5.4- Contraintes de la riziculture au Mali

Parmi les contraintes au développement de la riziculture au Mali, nous avons le faible niveau d'utilisation de variétés et de pratiques culturales améliorées et rentables, l'encadrement insuffisant des producteurs, des difficultés d'accès aux équipements, aux infrastructures de stockage et au crédit, des difficultés d'accès à de meilleures semences et à des engrais minéraux, des réseaux de distribution et de commercialisation peu performants (FAO et APRAO, 2013). En post-récolte, la qualité des produits est affectée par les techniques de conservation et de traitement du riz (Doumbia, et al., 2002 ; Traoré et Boly, 2004). A ces contraintes structurelles s'ajoutent les contraintes abiotiques que sont les conditions climatiques et les carences et toxicités de certains éléments minéraux. Les principales contraintes biotiques de la production rizicole au Mali demeurent la panachure jaune du riz (RYMV), les attaques des ravageurs (cécidomyie africaine oiseaux granivores, poissons rhizophages) et les adventices (AFAR et ODI, 2000 ; Hamadoun et al., 1998). La culture du riz est aussi affectée par des maladies causées par des bactéries, d'autres virus, des champignons et des mycoplasmes entraînant des pertes de rendement considérables (Wopereis et al., 2008)

Les principales maladies observées au Mali sont présentées plus en détail ci-après.

- **Les maladies virales**

Le virus de la panachure jaune du riz (Rice yellow mottle virus ou RYMV) n'est connu qu'en Afrique (y compris Madagascar ; SY, 2014) et est spécifiquement inféodé aux écosystèmes rizicoles irrigués et de bas-fonds non aménagés. Cette maladie identifiée en 1966 au Kenya (Bakker, 1970), est la principale contrainte virale à la production de riz en Afrique (AFAR et ODI, 2000 ; Sarra et al., 2004). Les pertes de rendement varient de 25 à 100% suivant la variété cultivée,

le stade phénologique d'infection (précocité de l'infection) et les conditions environnementales biotiques et abiotiques (Sy, 2014). Des études sur la dissémination ont montré que, parmi les vecteurs du RYMV, les insectes et en particulier les mangeurs de feuilles trouvés dans les rizières (coléoptères, sauterelles et cicadelles) sont la principale source de transmission du virus (Bakker, 1970 ; Bakker, 1974). Le virus de la nécrose à rayure du riz (RSNV pour Rice stripe necrosis virus) a été identifié en Côte d'Ivoire dans les années 1977 et progressivement dans les pays d'Afrique de l'Ouest. Il est transmis par un protiste du sol endoparasite obligatoire des racines de plantes appelé *Polymyxa graminis* (Fauquet, 1983 ; Decroës et al., 2017 ; Maurino et al., 2018).

- **Les maladies bactériennes**

Nous rencontrons au Mali deux bactérioses du riz qui sont dues à *Xanthomonas oryzae* (*Xo*), une espèce de protéobactéries de la famille des *Xanthomonadaceae*. Ces espèces se différencient par leur mode d'infection de l'hôte et leur spécificité tissulaire. *X. oryzae* pv. *oryzae (Xoo)* est un agent pathogène vasculaire causant la bactériose vasculaire du riz, encore appelée flétrissement bactérien ou *Bacterial leaf blight* (BLB). *X. oryzae* pv. *oryzicola (Xoc)* est l'agent causal de la strie foliaire, aussi appelée *Bacterial leaf streak* (BLS). Les symptômes des deux maladies peuvent être facilement distingués au début de l'infection, et devenir plus difficiles à distinguer à des stades avancés. (Doucoure, 2017 ; Yasmin et al., 2017). L'incidence de la strie foliaire au Mali varie d'une zone à une autre et peut atteindre 45% avec une perte atteignant 80% dans certains cas (Sarra et al., 2010).

- **Les maladies fongiques**

La culture du riz est affectée par plusieurs maladies fongiques telles que le gigantisme (*Fusarium* moniliforme), l'helminthosporiose, la pyriculariose, la décoloration des grains (complexe d'espèces) et les maladies des chaumes (complexe d'espèces). Parmi ces maladies fongiques, les plus observées au Mali sont décrites ci-dessous.

- **Helminthosporiose**

Parmi les diverses maladies fongiques du riz, nous rencontrons l'helminthosporiose, provoquée le plus souvent par *Bipolaris oryzae*. En plus de *B. oryzae*, d'autres espèces fongiques sont responsables de l'helminthosporiose du riz : *B. victoriae, B. indica, B. bicolor, Exserohilum rostratum* (Motlagh et Kaviani, 2008 ; Sunder et al., 2014). A l'échelle mondiale, l'helminthosporiose représente la 3ème cause de maladie fongique par sa distribution et l'importance des dégâts causés après la pyriculariose (CAB IMI, 1984 ; Savary et al., 2019). Les symptômes de la maladie sur les feuilles sont caractérisés par des lésions ovales dont la taille peut atteindre 10 mm de longueur. Des tâches nécrotiques sont également observées sur les glumes. Ils peuvent aussi apparaître sur la coléoptile, les gaines foliaires, le rachis

paniculaire et, plus rarement, sur les racines de jeunes plantes et sur les tiges (CILSS et UCTR/PV, 1991 . ; Ouedraogo, 2008).

- **Curvulariose**

La curvulariose est causée par *Curvularia* spp (Hassikou et al., 1997, 2001). La curvulariose se présente sous la forme de taches allongées ou ovales, brunes à noires, sur les feuilles et les tiges des plantes de certaines variétés de riz, qui s'élargissent par la suite et deviennent coalescentes. L'infection des grains de riz par *Curvularia* entraine une coloration brune à noire, une perte de poids et une diminution du pouvoir germinatif (Hassikou et al., 2003).

La pyriculariose du riz est la principale maladie fongique au Mali et fait l'objet de cette thèse. La maladie et l'agent pathogène responsable sont présentés dans les paragraphes suivants.

II- Pyriculariose du riz

1-Importance et symptômes de la pyriculariose du riz :

La pyriculariose, causée par *Pyricularia oryzae* (synonyme *Magnaporthe oryzae*) est la plus importante maladie fongique du riz avec estimée à 4% par an à l'échelle mondiale (Savary et al., 2019). Dans les cas sévères (variétés très sensibles), la perte de rendement causée par *P. oryzae* peut atteindre 80 à 100 % (Skamnioti et Gurr, 2009 ; Jahaar Singh et al., 2019). La maladie est décrite dans tous les pays rizicoles (Ou, 1985).

La pyriculariose affecte chaque partie de la plante : feuilles (Fig. 5A), nœuds paniculaires (cous), ligules, grains et plus rarement nœuds de la tige (Nottéghem et Baudin, 1981). Des infections racinaires ont été provoquées par inoculation artificielle et Marcel et al. (2010) ont même suggéré une éventuelle infection des racines in natura, sans le démontrer. La pyriculariose du cou (Fig. 5B) et la pyriculariose des panicules (Fig. 6A) sont les phases les plus dommageables de la maladie et il a été démontré qu'elles réduisent considérablement le rendement, le poids et la qualité des grains (Fig. 6B). Une lésion typique de la pyriculariose sur une feuille de riz (Fig. 5A) est grise au centre, a un bord sombre et est en forme de fuseau (Manjunatha et Krishnappa, 2019). Toutefois, la forme et la couleur des lésions varient suivant les conditions du milieu, l'âge des lésions et le degré de sensibilité du cultivar (Ou, 1985 ; Abadassi, 1989).

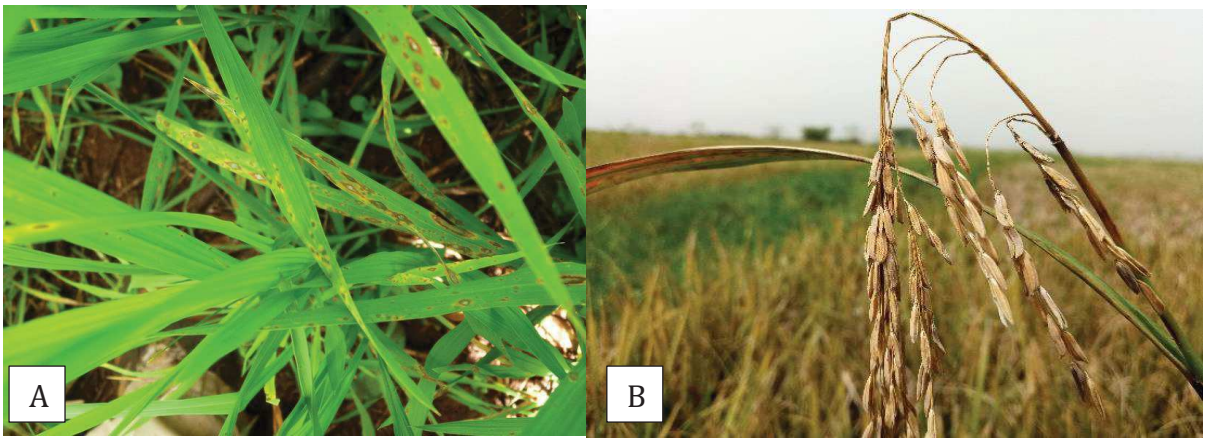

Figure 5 : A) Pyriculariose foliaire sur le riz pluvial. Source : (M. Sester/Cirad) ; B) Pyriculariose du cou (Niono/Mali), 2018 /Photo : D. DIAGNE

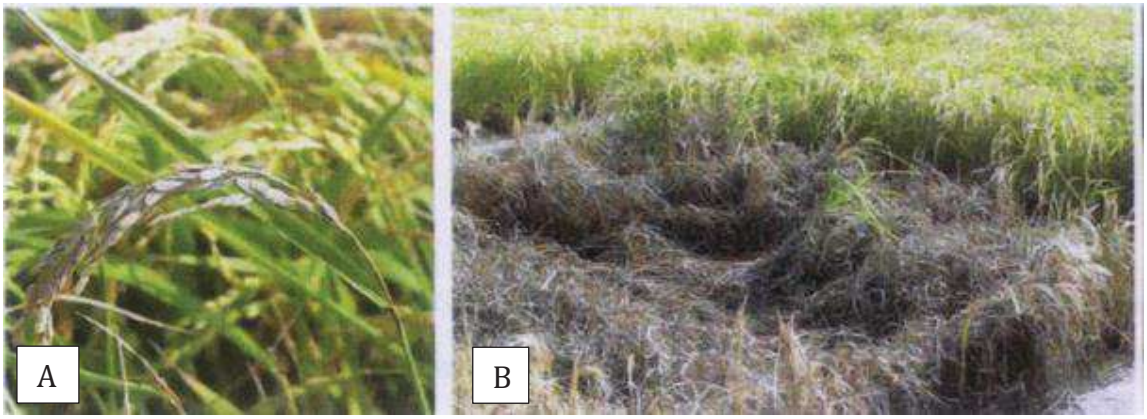

Figure 6 : Symptômes et dégâts provoqués par *P. oryzae* sur la culture de riz. A) Symptômes sur panicules ; B) Dégâts au champ en Camargue. Photos : JB Morel, INRAE

2-Biologie et épidémiologie de la pyriculariose du riz

2.1-Morphologie de *P. oryzae*

P. oryzae possède des conidiophores groupés à partir d'un stroma ; la partie basale du conidiophore est gonflée, sombre et la région apicale s'amincit progressivement. La conidie (spore asexuée) initiale est formée à l'apex du conidiophore, les suivantes étant formées sur les extrémités de nouvelles ramifications émergeant juste au-dessous du point d'émission de la conidie initiale ; plusieurs conidies (de 2 à 5) peuvent naitre d'un même conidiophore. Les conidies sont pyriformes (en forme de poire ; Fig. 7B) biseptées, le plus souvent hyalines à olive pâle. Leur taille est comprise entre 19 et 23 µm x 7 à 9 microns µm. Elles portent généralement un petit appendice basal mesurant 1,6 à 2,4 microns. La germination de la conidie (Fig. 7A) se fait le plus souvent au niveau de la cellule apicale ou de la cellule basale, parfois d'une cellule médiane (Sy, 1976).

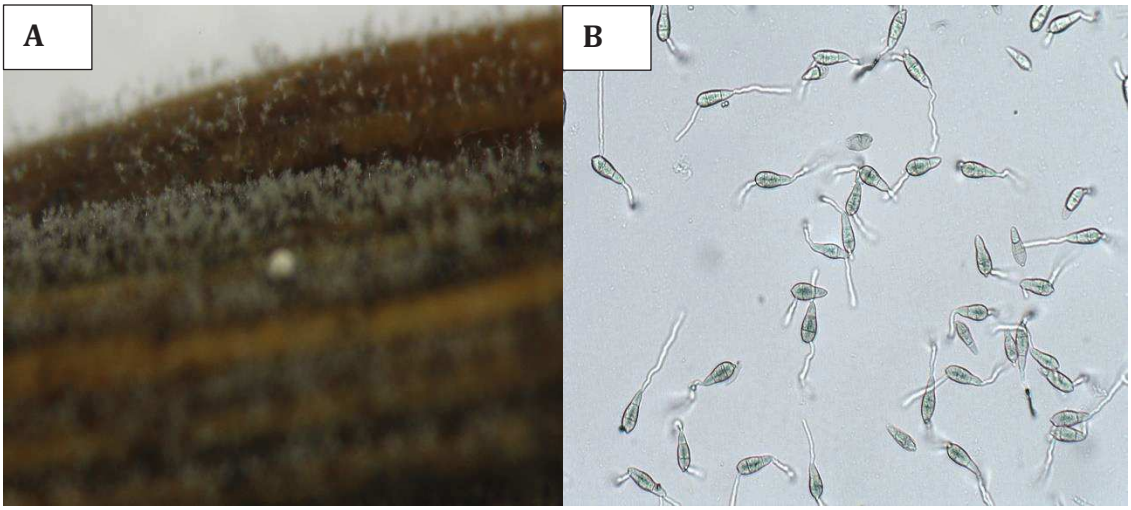

Figure 7: A) Sporulation de *P. oryzae* au miscroscope (LEICA MA205 C)
Photo : D. Diagne / BGPI CIRAD, Montpellier, 2020 ; B) Spores germées de *P. oryzae* au miscroscope (LEICA MA205 C) Photo : D. Diagne / BGPI CIRAD, Montpellier, 2020

2.2-Cycle de vie de *P. oryzae*

Le cycle asexué est le seul mode de reproduction observé dans la nature (Zeigler, 1998). Lorsque les conditions sont favorables, à partir du mycélium, des conidies sont produites (Saleh, 2011). Il existe également un cycle sexué (Fig. 8), qui n'a jamais a été directement observé dans la nature mais qui est produit *in vitro.* Le cycle sexué de *P. oryzae* est suspecté avoir existé au sein des populations attaquant le riz dans des zones très localisées d'Asie (Zeigler 1998 ; Tharreau et al 2009 ; Saleh et al., 2012 ; Gladieux et al., 2018 ; Thierry et al, 2020).

La pyriculariose du riz est une maladie polycyclique. Cinq étapes de base (Fig. 9) peuvent résumer le processus d'infection par *P. oryzae* : (1) la formation et dispersion des conidies, (2) la fixation des conidies sur une surface hôte, (3) la formation d'appressorium, (4) la pénétration de la première cellule hôte et (5) la croissance des hyphes invasifs (Hamer et al., 1988). Pendant les périodes de forte humidité relative (c'est-à-dire 90 % ou plus), le champignon produit et libère des conidies (Kato, 2001 ; Miah et al., 2017). Le champignon commence son processus d'infection lorsqu'une conidie atterrit à la surface de la feuille de riz (Ou, 1985 ; Wilson et Talbot, 2009). Les conidies germent lorsque de l'eau libre est présente et le tube germinatif forme un appressorium par lequel le champignon pénètre les parois végétales (Wilson et Talbot, 2009 ; Miah et al., 2017). Les symptômes apparaissent dans les 4 à 5 jours suivant l'infection (Kato, 2001). Ces lésions sporulent et libèrent des conidies qui seront dispersées par le vent. Une seule lésion peut générer 20 000 conidies et un épillet de riz infecté peut produire jusqu'à 6 000 conidies en une nuit (Ou, 1981).

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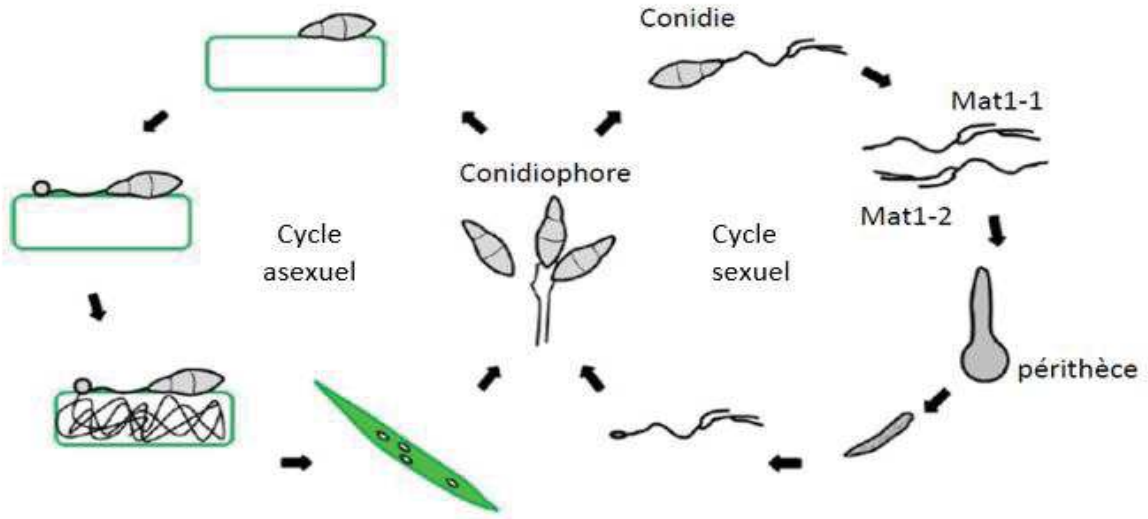

Figure 8: Cycle de reproduction sexué et asexuée de *P. oryzae* (Saleh, 2011 ; Thierry, 2019)

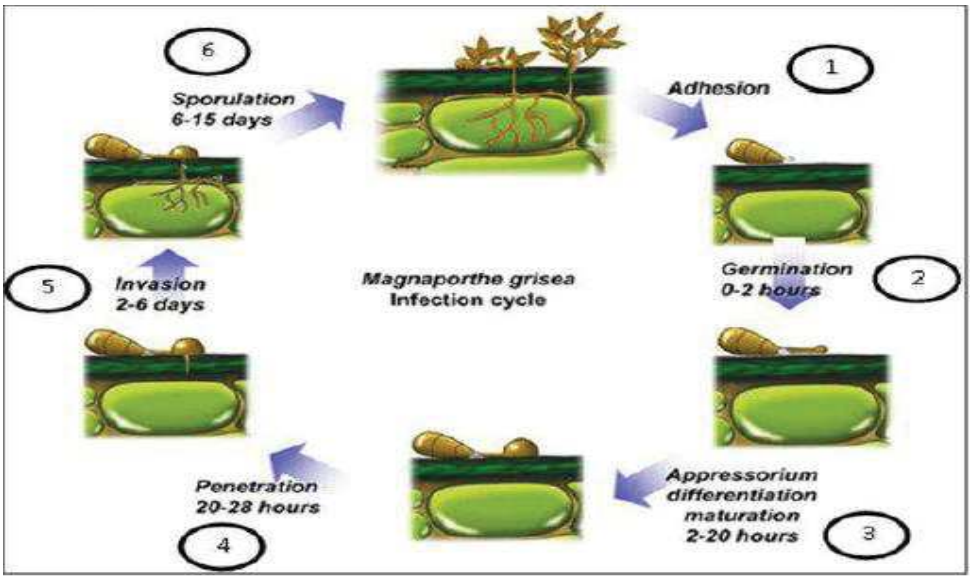

Figure 9**:** Processus d'infection de *P. oryzae* (Ribot et al., 2008)

2.3-Conditions de développement de la maladie

L'expression de la pyriculariose du riz varie considérablement en fonction des facteurs de l'environnement, ou de facteurs spécifiques à la plante elle-même. Les principaux facteurs sont l'humidité, la température, la fertilisation et la lumière. L'humidité est indispensable au développement de *P. oryzae* notamment pour la germination et la formation des conidies. En outre, de fortes concentrations d'azote favorisent l'infection. La température optimale de croissance pour *P. oryzae* se situe entre 24 et 28°C. A ces températures, la pénétration du

champignon dans la plante de riz s'opère rapidement en 6 ou 8 heures sous réserve d'une humidité suffisante, alors qu'elle semble impossible à 34°C (Traoré, 2000).

2.4-Structure génétique des populations de *P. oryzae* dans le monde

La structure génétique des populations de *P. oryzae* infectant le riz a été largement étudiée pour comprendre l'évolution de l'agent pathogène et pour adapter les techniques de lutte et les programmes d'amélioration variétale. Ces études ont porté sur la caractérisation des populations mondiales de *P. oryzae* avec différents types de marqueurs moléculaires : simple sequence repeats (SSR), sequence-characterised amplified region (SCAR), single nucleotide polymorphism (SNP), etc. (Adreit et al., 2007 ; Didier Tharreau, 2008 ; Gladieux et al., 2018 ; Zhong et al., 2018 ; Thierry, 2019 ; Thierry et al., 2020). Zeigler (1998) a conclu que la diversité génétique de *P. oryzae* était plus importante dans la zone englobant l'Asie du Sud, de l'Est et du Sud-Est que dans d'autres régions du monde, en comparant le nombre de lignées clonales trouvées dans différentes études. Les études les plus détaillées sur la structure de la population de *P. oryzae* à l'échelle mondiale ont révélé une structure génétique de trois ou quatre groupes principaux. Ces études ont montré que l'Asie serait le centre de diversité et l'origine des populations pathogènes du riz de toutes les populations mondiales (Tharreau et al., 2009 ; Saleh et al., 2014 ; Gladieux et al., 2018 ; Zhong et al., 2018 ; Thierry et al., 2020). Cependant, il existe peu de données sur la diversité et la structure des populations de cet agent pathogène en Afrique. Mutiga et al. (2017) ont étudié la diversité génétique des populations de riz *P. oryzae* et ont évalué le spectre de virulence de cet agent pathogène en Afrique de l'Ouest et en Afrique de l'Est. Ces auteurs ont identifié 7 groupes génétiques et ont montré une différenciation entre les populations d'Afrique de l'Ouest et de l'Est. Kassankogno et al., (2017) ont montré que les populations des deux pays voisins, le Burkina Faso et le Togo, sont distinctes, mais que certains génotypes multilocus sont partagés, ce qui suggère certains flux migratoires entre les pays. L'étude de Nutsugah et al. (2008) a montré la présence de quatre lignées distinctes reparties en différentes régions du Ghana. Parmi ces lignées, la lignée 1 a été identifiée dans presque toutes les régions du Ghana, tandis que les lignées 2 et 3 semblait être plus spécifiques de certains sites. Ces auteurs ont suggéré que la structure de la population de l'agent pathogène pouvait être influencée par les conditions agro-environnementales, en particulier les génotypes d'hôtes présents. A l'échelle mondiale, Thierry et al. (2020) ont confirmé cette hypothèse en montrant que la structure des populations de *P. oryzae* est déterminée par des facteurs climatiques, par une spécialisation partielle à l'hôte et par des barrières génétiques aux flux de gènes.

2.5-Sources d'inoculum

L'identification des sources d'inoculum peut contribuer efficacement à réduire l'incidence et la sévérité de la maladie (Raveloson et Sester, 2011). Les principales sources d'inoculum primaire pour la pyriculariose sont les résidus de riz infecté et de semences infectées par la maladie (Long, et al., 2001; Guerber et TeBeest, 2006; Raveloson et al., 2013). Des informations complémentaires sont fournies dans les introductions des chapitres 1 et 3.

2.6-Moyen de lutte contre la pyriculariose du riz

Différentes méthodes sont utilisées pour lutter contre la pyriculariose du riz et parfois combinées (Ghazanfar et al., 2009) : pratiques culturales (lutte agronomique), lutte chimique, utilisation de variétés résistantes (lutte génétique). A notre connaissance, la lutte biologique n'est pas utilisée au champ.

- **Lutte agronomique**

Elle est basée sur l'adaptation des pratiques culturales. Le développement de la pyriculariose du riz dépend de la date de semis, de la fertilisation (éviter une fertilisation excessive à l'azote), de la maîtrise de l'irrigation (maintenir une inondation continue et éviter l'assèchement de la rizière), de la profondeur et de la densité de plantation (faire des semis non denses ; Ou, 1985). La pyriculariose peut se conserver et se transmettre par les semences et les pailles (Raveloson et al., 2013) donc l'utilisation des semences saines ou certifiées et la destruction de tous les résidus végétaux infectés (destruction de la paille par enfouissement ou exportation des pailles) sont nécessaire pour l'introduction et le maintien du champignon dans le champ.

- Lutte chimique

De nombreux fongicides de synthèse ont été utilisés contre la pyriculariose du riz. Actuellement, les fongicides contenant de l'azoxystrobine ou des ingrédients actifs de la famille des triazoles (Leroux, 1996 ; Bouet et al., 2012) ou des strobilurines sont utilisés en pépinière au stade du tallage et de l'émergence des panicules. Le tricyclazole est un fongicide systémique très utilisé pour la lutte contre la pyriculariose du riz. Il bloque spécifiquement la pénétration de *P. oryzae* dans les tissus du riz en inhibant la biosynthèse de la mélanine et, par conséquent, la formation d'un appressorium fonctionnel (Yamaguchi, 2004).

- Lutte génétique

L'utilisation de variétés résistantes reste le moyen de lutte le plus pratique et le plus économique contre la pyriculariose du riz (Dembele, 2001 ; Agrios, 2005 ; Suh et al., 2009). Pour cela, une caractérisation de la résistance à la pyriculariose des ressources génétiques est

nécessaire pour optimiser les combinaisons de géniteurs utilisés dans les croisements contrôlés ou pour la création de nouvelles populations récurrentes (Raboin, et al., 2010). Deux formes de résistance sont connues : la résistance partielle et la résistance complète. La résistance complète a été très étudiée dans l'interaction entre le riz et *P. oryzae*. Elle est le plus souvent contrôlée par un gène dominant, spécifique (c'est-à-dire efficace vis-à-vis d'une partie des souches seulement), et peu durable. Une quinzaine de gènes de résistance complète (gènes R) ont été clonés (Liu et al., 2013). Ils codent quasiment tous pour des récepteurs reconnaissant des petites molécules sécrétées par le champignon (effecteurs ou facteurs d'avirulence - avr). L'interaction entre gènes R et avr correspond au modèle gène pour gène. La résistance partielle du riz à la pyriculariose a été moins étudiée. Les variétés durablement résistantes combineraient résistance complète et résistance partielle (Ballini et al., 2008).

- **Lutte biologique**

Le contrôle biologique contre la pyriculariose est actuellement un enjeu majeur en agriculture, dans la perspective de réduire, voire supprimer les pesticides de synthèse. Des essais d'efficacité ont été conduits, le plus souvent en conditions contrôlées mais n'ont pas abouti à une utilisation au champ. Les souches bactériennes comprenant *Bacillus circulans*, *B. subtilis, B. megaterium et Pseudomonas* fluorescens réduisent de manière significative la sévérité de la pyriculariose du riz par rapport au témoin, mais leur efficacité reste inférieure à celle obtenue avec un traitement fongicide (Padasht et al., 2004). Wei et al. (2020) ont montré que les souches bactériennes telles que *Bacillus. safensis, Pseudomonas. koreensis, Pseudomonas. saponiphila, Stenotrophomonas. rhizophila, et Bacillus. tequilensis* ont présenté de fortes inhibitions sur la germination des conidies et la pathogénicité du champignon de la pyriculariose du riz, tant à titre préventif que curatif. Les résultats de l'étude de (Chaiharn et al., 2020) montrent le potentiel de *Streptomyces palmae* comme biocontrôle contre le champignon de la pyriculariose du riz. Le traitement des semences et la pulvérisation foliaire par *Trichoderma harzianum* ont démontré une réduction significative de la sévérité de la pyriculariose du riz (Khalili et al., 2012).

III- Objectifs scientifiques

Au Mali le riz occupe une place centrale dans les habitudes alimentaires de la population, principalement en milieu urbain (Coulibaly, 2014). La productivité du riz est affectée par plusieurs agents pathogènes, notamment des champignons, et, en particulier, *Pyricularia oryzae*, agent de la pyriculariose. Pour réduire les pertes, des études sont nécessaires pour générer des connaissances pour améliorer la lutte contre la maladie. La connaissance des facteurs pouvant influer sur le développement des épidémies est un enjeu capital pour améliorer la lutte intégrée contre la pyriculariose par des mesures préventives. Par ailleurs, la diversité et la structure des populations de l'agent pathogène sont des données à prendre en compte pour adapter les méthodes de lutte basées sur la résistance variétale. Or, les connaissances sur l'épidémiologie et la structure des populations sont limitées en Afrique en général et au Mali en particulier.

Cette thèse a donc pour objectif général de contribuer à une meilleure connaissance de la pyriculariose du riz au Mali et pour objectifs spécifiques (i) d'évaluer la prévalence de la maladie et (ii) d'identifier les sources d'inoculum au Mali, et (iii) de caractériser la structure des populations de l'agent pathogène en Afrique.

Au cours de cette thèse, j'ai produit et analysé des données pour répondre aux questions suivantes :

- Quelles sont l'incidence et la sévérité de la pyriculariose dans les différentes zones rizicoles au Mali ?
- Est-ce que les semences, les pailles et/ou le riz sauvage sont sources d'inoculum pour le riz cultivé ?
- Quelle est la structure des populations de *P. oryzae* en Afrique ?

Nous avons utilisé différentes méthodes pour répondre à ces questions : évaluation de la maladie sur le terrain, analyses microbiologiques d'organes infectés, génétique des populations, tests de pouvoir pathogène.

Les résultats produits au cours de cette thèse sont présentés et discutés sous forme d'articles. : (i) Le premier est un article en préparation pour Phytopathology (« Prevalence of the rice blast disease and potential sources of inoculum in Mali ») et présente les résultats de prévalence de la maladie au Mali et de recherche de l'agent pathogène sur les semences et pailles de riz. Le deuxième article publié dans Phytopathology (« Structure of African populations of *Pyricularia oryzae* from rice ») décrit la structure des populations de *P. oryzae* en Afrique. Pour cet article, en plus de la production de donnés de génotypage de souches

maliennes que j'ai collectées, j'ai contribué à compiler deux jeux de données de génotypage de plusieurs milliers de souches et j'ai analysé la diversité et structure des populations. Le troisième article, soumis à Phytopathology, teste l'hypothèse du riz sauvage comme source d'inoculum pour le riz cultivé (« Wild and cultivated rice host different populations of the blast fungus, *Pyricularia oryzae*, in Mali »). Un dernier chapitre de conclusion générale et perspectives dresse le bilan des travaux de cette thèse et propose des perspectives pour poursuivre ces études. Un article de signalement (« First report of rice leaf brown spot caused by *Exserohilum rostratum* in Mali »), auquel j'ai participé, soumis à Plant Disease est également fourni en annexe.

Chapitre 1

Prevalence of the Rice Blast Disease and Potential Sources of Inoculum in Mali

CHAPITRE 1 : article 1 « Prevalence of the rice blast disease and potential sources of inoculum in Mali »

Ce chapitre comprend également 3 annexes :

Annexe 1 : Fiche de prospection (pyriculariose) ;

Annexe 2 : Fiche d'évaluation de l'incidence et de la sévérité foliaire et paniculaire ;

Annexe 3: Article « First report of rice leaf brown spot caused by *Exserohilum rostratum* in Mali ».

Prevalence of the rice blast disease and potential sources of inoculum in Mali

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ABSTRACT

In Mali, rice production is affected by several diseases that represent a major constraint and lead to yield losses. Among them, blast disease, caused by the fungus *Pyricularia oryzae*, alone accounts for losses estimated between 1 and 4% at the world scale, despite the implementation of control methods. Both the prevalence and the sources of inoculum for this disease are poorly documented in Mali. The aim of the study was therefore to assess the incidence and severity of rice blast and to diagnose *P. oryzae* on seeds and straw in order to determine the potential sources of inoculum in Mali. Leaf and panicle incidence and severity were assessed in the localities of Baguineda, Manikoura (Koulikoro region), Sélingué, Niéna, M'pegnesso, Loulouni Faraka Banankoro and Loulouni Plaine (Sikasso region) and in Niono Nango Sahel, Niono N3-bis, Niono N5 (Ségou region). Surveys conducted in farmers' and experimental plots in 2018, allowed to observe leaf and panicle blast in 10 out of 17 sites surveyed. In symptomatic fields, leaf incidence ranged from 24 to 72% and leaf severity from

2 to 15%. The highest values of panicle incidence and severity reached 94% and 52%, respectively. In 2019, leaf blast was observed on 3 out of 4 sites surveyed, and incidence and severity were much lower (maximum of 7% and 1%, respectively). Incidence and severity were not significantly different between irrigated and rainfed-lowland agrosystems. The highest values for panicle blast incidence and severity confirm that blast is a potential cause of high yield losses. In addition, the presence of *P. oryzae* was tested on 20 seed lots collected in different localities between 2016 and 2018. Visual inspection showed the presence of genera responsible for brown spot disease (*Bipolaris* and *Exserohilum*) and *Curvularia* in 17 of the 20 lots with an average of 4.6 and 4.3% of contaminated seeds, respectively. The third pathogen observed was a fungus of the genus *Fusarium. P. oryzae* was not detected on the observed seeds. In addition to seeds, seven lots of straws were collected and analyzed during the 2018 campaign in the irrigated perimeters of Sélingué, Niono, Baguineda, and Manikoura. We observed the presence of *P. oryzae* in the straw lots of Niono (3%) and Sélingué (6%). Based on these results, seeds are not expected to be a major source of inoculum for blast epidemics in Mali. The role of infected straws requires further investigation.

Keywords: Blast, *Pyricularia oryzae*, rice, prevalence, inoculum sources.

INTRODUCTION

Rice (*Oryza sativa* L.) is the main food for more than half of the world's population. It thus occupies an important position in agriculture and food security. Although more than 90% of the world's rice is grown and consumed in Asia (Kole 2006; Khush 1997), it is also a major food crop in Africa. In Mali, after millet/sorghum and maize, rice is the third most important cereal crop. Rice cultivation is considered as one of the priorities of the agricultural sector for the country's development. The contribution of the rice sector to gross domestic product (GDP) is significant (5% in terms of national and about 10% in terms of agricultural GDPs), and with an upward trend (Coulibaly and Ouologuem 2014; Baudouin 2018). Rice productivity is affected by the occurrence of several fungal diseases, in particular rice blast caused by the pathogenic fungus *Pyricularia oryzae* (Pennisi 2010). *Pyricularia oryzae* is presently considered as the 2nd main fungal disease of rice (Savary et al. 2019). Although currently available blast management methods can significantly reduce the disease, outbreaks of blast are still frequent, resulting in disastrous yield losses. In a recent study, Savary et al. (2019) estimated that *P. oryzae* alone accounts for 14% of the annual yield losses of rice caused by all pests and diseases. Other estimates have shown that yield losses due to blast are equivalent to

a rice grain quantity that could feed 60 million people (Zeigler et al. 1994; Pennisi 2010). In addition, farmers frequently experience heavy yield losses of up to 100%, although disease incidence and yield losses vary greatly between cropping systems and practices, varieties, countries and years (Dar et al. 2010; Hossain et al. 2017; Yashaswini et al. 2017; Raveloson 2018).

Primary sources of inoculum lead to early onset of disease and have a direct impact on the severity of an outbreak and associated yield loss. Therefore, it is of critical importance to identify primary sources of infection such as seeds, straw and alternative host/reservoir plants. In addition to its ability to survive on infected seeds, *P. oryzae* is also known to survive on residues such as rice straw. These two sources are probably the main sources of primary inoculum (Long et al. 2001; Guerber and TeBeest, 2006; Te Beest et al. 2007; Raveloson, et al. 2013 Hubert et al. 2015). The role of alternative hosts in blast epidemics is controversial. In a recent study on the possible role of wild rice as inoculum source for cultivated rice in Mali, *P. oryzae* populations on cultivated and on wild rice were different, suggesting that wild rice is not a source of inoculum (Diagne and Tharreau, unpublished data).

The blast epidemic situation and the sources of primary inoculum in farmer's fields are poorly documented in Africa in general (Barro M. and Tollenaere C., personal communication) and in Mali in particular (Hamadoun et al. 1998). The first objective of this study was to assess the rice blast prevalence and severity in the different rice-growing areas in Mali. The second objective was to evaluate whether seeds and/or straws could be potential sources of inoculum for cultivated rice by studying the presence/absence of fungal species on seeds and straws.

MATERIAL AND METHODS

Evaluation of the disease. The assessment of the incidence and severity of rice blast were carried out in the localities of Baguineda and Manikoura in Koulikoro region, Sélingué, Niena, M'pegnesso, Loulouni Faraka Banankoro and Plaine in Sikasso region, and in Niono Nango Sahel, Niono N3-bis, Niono N7 in Segou region (Fig. 1). It was also carried out to allow the comparison between plots assessed in the same site. The disease assessment took place in September and November 2018, and October 2019. It was carried out at the same stage of rice development, i.e., at the heading/flowering stage, to allow comparisons between sites.

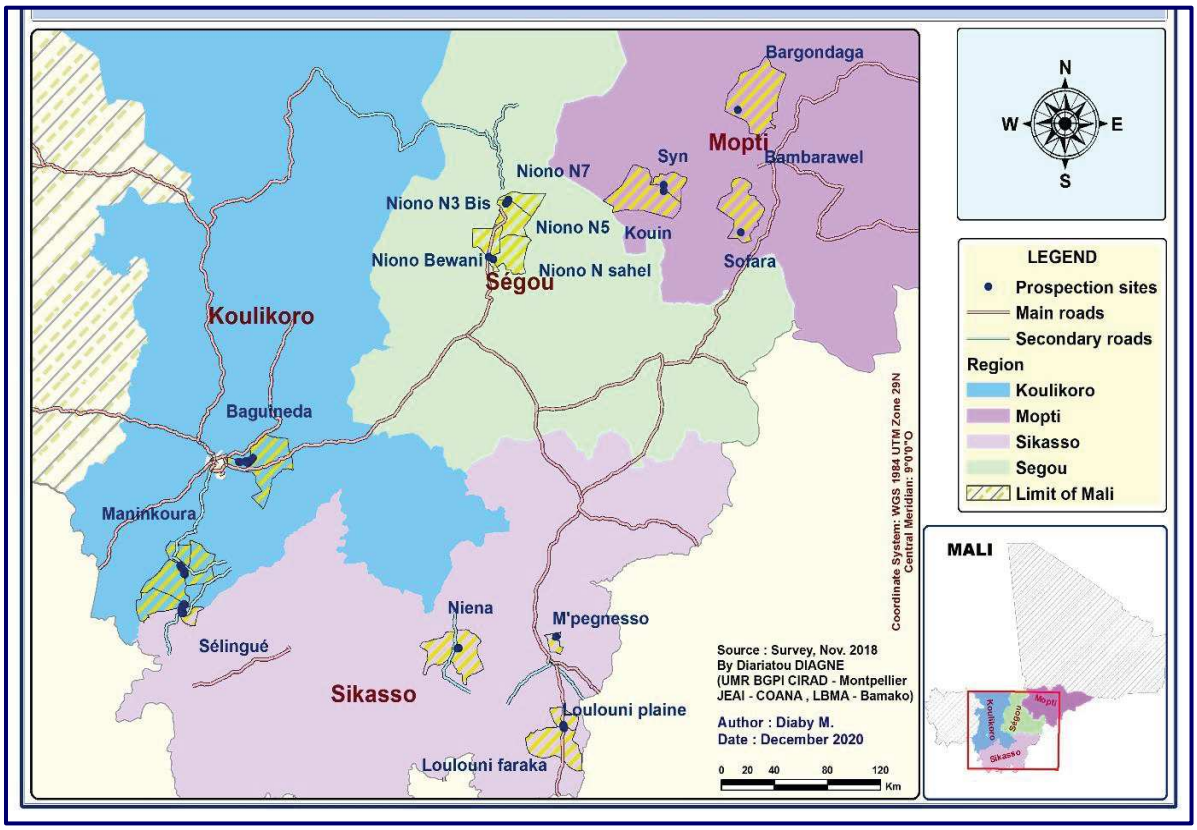


Figure 1: Location of sampling sites

All sites prospected in 2018 were not prospected in 2019. Seventeen and four sites were prospected in 2018 and in 2019, respectively. Plots where blast was observed were evaluated for leaf and panicle incidence and severity. The evaluation team was guided only to plots where the disease was suspected. So, the proportion of diseased plots measured in our sample may be overestimated compared to the actual proportion.

The incidence of blast for each plot was defined as the proportion of leaves/panicles with at least one blast symptom to the total number of leaves/panicles in a defined area. Observations were made at four points in each plot and on five hills per plot (about 1m²). For leaf blast, we counted the number of tillers with symptomatic leaves and the total number of tillers. For panicle blast, we counted the number of diseased panicles and the total number of panicles. The incidence was calculated as the ratio of tillers or panicles with symptoms over the total number of tillers or panicles (Fig. 2). Severity was also assessed on leaves and panicles. For leaf blast, we estimated the percentage of leaf area affected by the disease on four upper leaves of three tillers (Sester et al. 2014). For panicle blast, we evaluated the percentage of seeds affected by the disease on three panicles (Sester et al. 2014; Fig. 2).

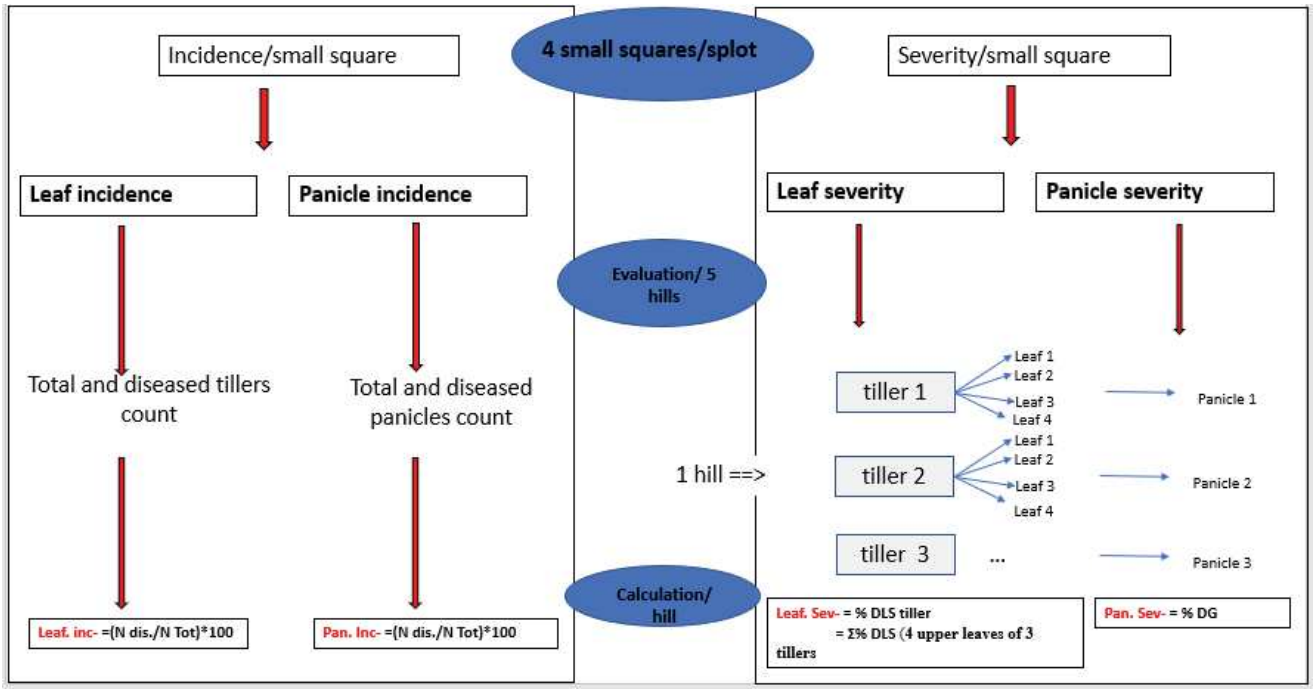


Figure 2: Procedure for assessing the incidence and severity of rice blast disease

DLS: Diseased leaf surface; DG: Diseased grains; Pan. Inc-: Panicle incidence; Leaf. Sev-: leaf severity; Leaf. Inc.: leaf incidence; Pan. Sev-: Panicle severity.

Mycoflora of seeds. A total of 20 seed lots were collected from different varieties (ARICA 10, ARICA 9, Nenekala, Kogoni, BG 90-2, IR32310, ADNY-11, Wapmo (WAS 197), WAS 49, 08Fan2, Swarna 2, Bw 348-1, Sika, Shwetasoke, NERICA) and sites: 2 lots in Baguinéda, 1 lot in Loulouni, 2 lots in M'Pegnesso, 3 lots in Niena, 2 lots in Sélingué and finally 10 lots in Niono. Seventeen certified seed lots were collected in 2016, 2017 and 2018. Three seed lots were collected directly from the fields in 2017 and 2018 **(**Supplementary TABLE 1).

Seed mycoflora was determined following the protocol established by the International Seed Testing Association (ISTA, 2020). Twenty-five seeds were deposited on a filter paper moistened with distilled water (4 mL) in a Petri dish. For each seed lot from the field, 400 grains were distributed in 16 dishes. The seeds were incubated at 20°C for one week with a 12 h day/night alternation. Then, they were observed with a binocular microscope (Olympus SZ-PT). Fungal species were identified based on spore morphology. The number of infected seeds was counted for each fungal species.

Search for *P. oryzae* on straws. In October 2018, after harvest, seven lots of straw left in the field after threshing were collected in irrigated perimeters in Baguinéda (1 lot), Manikoura (2 lots), Sélingué (2 lots), and Niono (2 lots). The necrotic parts of the straws were cut with scissors, placed in sterile Petri dishes containing a filter paper disc moistened with distilled water, and incubated at 25°C for 24 h. The fragments were then observed with a binocular microscope (Olympus SZ-PT) to assess the presence of conidia of *P. oryzae*. The presence of other phytopathogenic fungal species was also recorded.

The analysis of variance was done to compare the mean incidence and severity within study site. STATA 16 was used for statistical analyses. A p-value of less than 0.05 was deemed to be statistically significant at a confidence level of 95%.

RESULTS

Incidence and severity of leaf and panicle blast. In 2018, 7 sites (5 sites in the Mopti region and 2 sites in the Segou region; Fig.1) were free of blast disease, and 10 sites (22 plots) were evaluated for leaf and panicle blast incidence and severity. In 2019, out of 4 prospected sites, 1 site was free of blast disease, and 3 sites (5 plots) were evaluated for leaf incidence and severity. Panicle blast was only observed at 2 sites (Niono and Niena) in 2018. There were no cases of panicle blast at the other sites, nor in 2019.

In 2018, leaf blast incidence was of 54% on average. The highest leaf blast incidence (73%) was observed in M'pegnesso (Sikasso region) and the lowest (24%) in Baguineda (Koulikoro region). Leaf blast severity was of 5% on average. The highest leaf blast severity (15%) was observed in Niena and the lowest (2%) in M'pegnesso (Sikasso region), (Fig. 3; Fig.4; Table 1**).** Incidence and severity of panicle blast were relatively high and both were higher in Niena than in Niono: 94% of blasted panicle (incidence) with 52% of diseased grains (severity) in Niena versus 68% and 49% in Niono (Fig. 5).

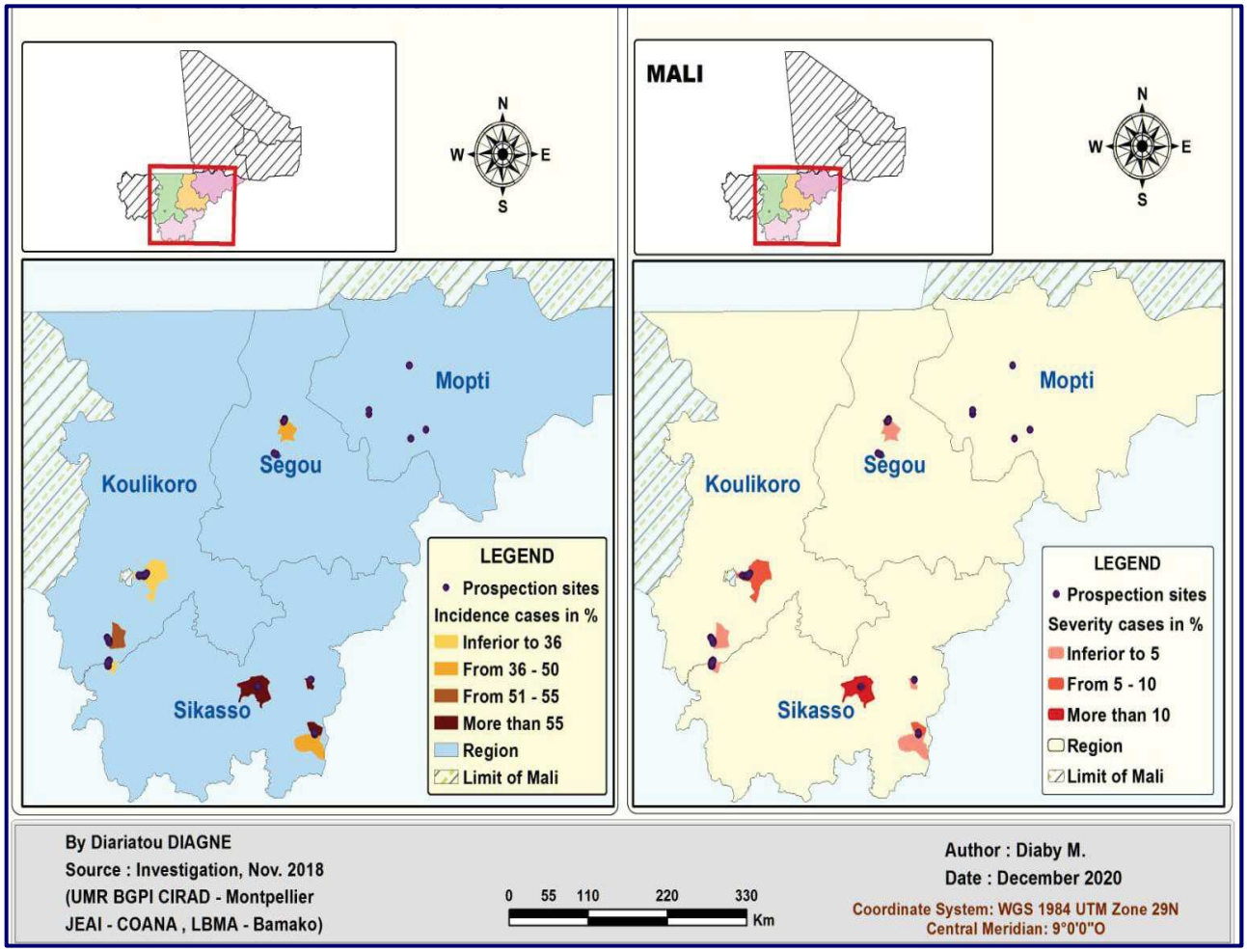


Figure 3: Mapping of rice leaf blast incidence (left panel) and severity (right panel) assessment in Mali (2018)



Figure 4: Incidence and leaf severity of blast disease in Mali per site and region (2018-2019). Blue, incidence in 2018 - 2019, orange, severity in 2018 - 2019

TABLE 1: Incidence and severity of leaf blast disease in different locations in Mali (2018-2019)

Type of ecosystem	Region	Location	Incidence 2018	Severity 2018	Incidence 2019	Severity 2019
Lowland	Sikasso	Niena	71	15	3	1
		M'pegnesso	73	2	ND	ND
		Loulouni P	67	6	ND	ND
		Loulouni B	48	2	ND	ND
		Sélingué	37	4	ND	ND
Irrigated	Ségou	Niono*	52	2	ND	ND
Irrigated	Koulikoro	Baguineda	24	6	7	0.4
		Manikoura	57	2	5	0.2
		Average	54	5	5	0.5

* Niono, indicates results of three sites (Niono Nango Sahel, Niono N7 et Niono N3-bis)
ND: Not determined

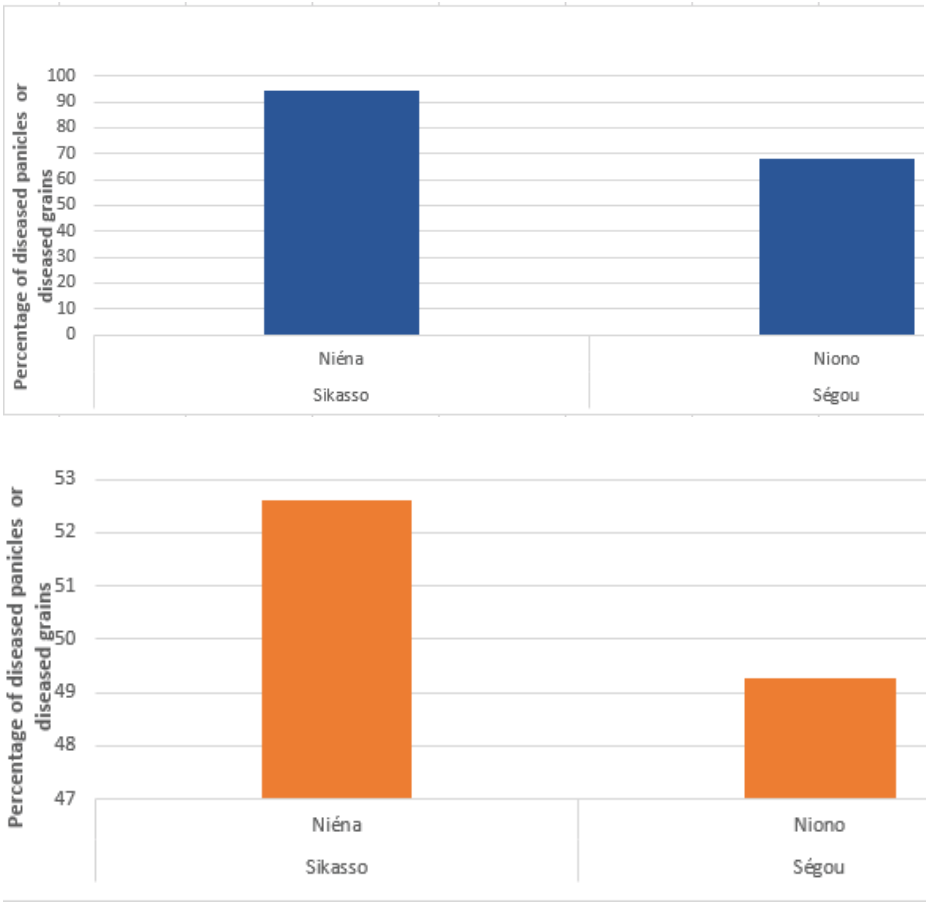


Figure 5: Assessment of panicle blast incidence and severity in 2018 in two sites of Mali. Blue, incidence; orange, severity.

In 2019, leaf blast incidence was low since the disease was not observed in all sites evaluated in 2018. Among the sites not evaluated in 2019 there is the Niono site in the Office du Niger in the Segou region, which has is irrigated agrosystem and accounts for half of the country's rice production. There are also three sites in the Sikasso region in the lowlands (M'pegnesso, Loulouni Plaine and Loulouni Faraka Banakoro) that were not included in the 2019 evaluation. In sites where leaf blast was observed highest incidence value was 7% (in Baguineda, Koulikoro region). Severity was also very low, the highest value being 1% (in Niena, Sikasso region) (Fig. 4; TABLE 1).

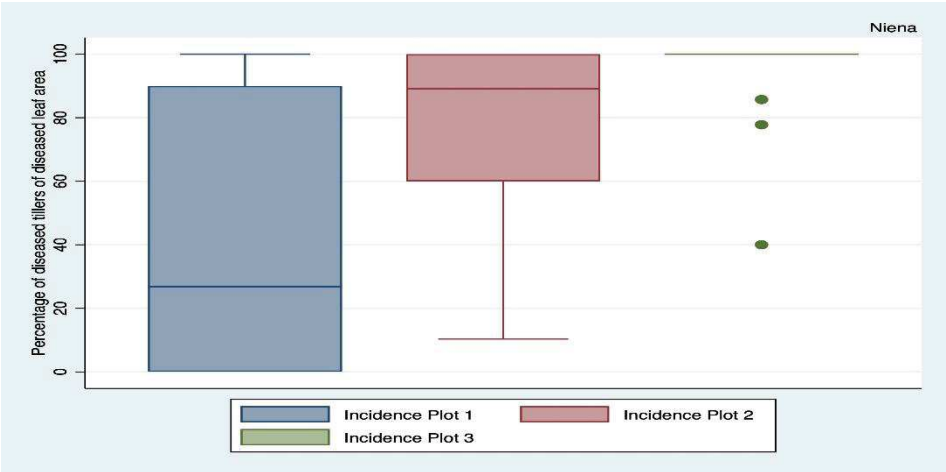
In 2018, the average incidence of leaf blast was higher in lowland ecosystem (65%) than in the irrigated areas (42%). However, this difference was not statistically significant ($t=2.02$, $p=0.14$). The average severity (6%) of leaf blast in the lowland areas was almost double that of the irrigated ecosystem (3%), but the difference was also not significant statistically ($t=1.04$, $p=0.37$. In 2019, leaf blast incidence was slightly higher in irrigated (6%) than in lowland (3%), and leaf severity was also slightly higher in lowland (1%) than in irrigated (0.2%).

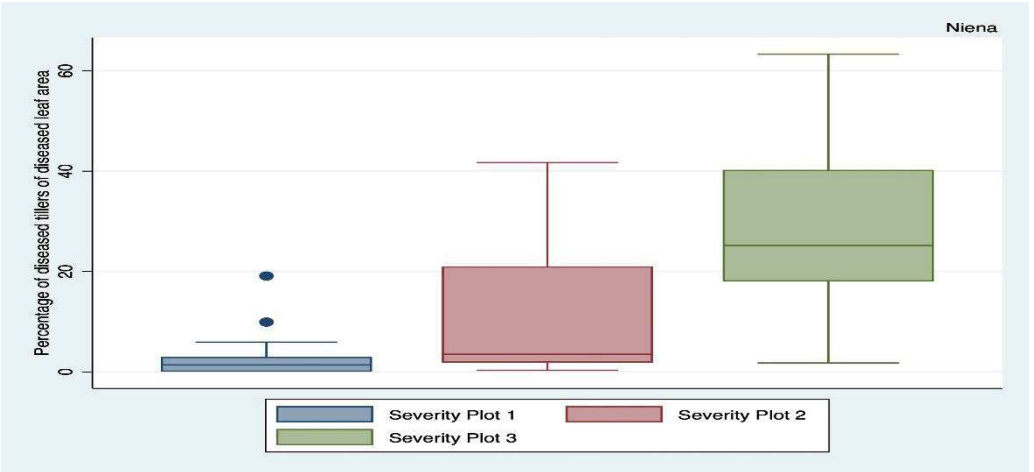
For the leaf incidence and severity in Niena (lowland), there was a significant difference when comparing the severity of plot 3 to the other two plots (plot 3 vs plot 2, $p<0.001$; plot 1 vs plot 3, $p<0.0001$). However, there was no significant difference between the severity of plots 1 & 2 ($p=0.083$). For the case of M'pegnesso, there was no significant difference between the incidences in plots 1 & 2 ($p=0.5638$). The severity was not significantly different between plots 1 & 2 ($p=0.7778$) (Fig. 6 A).

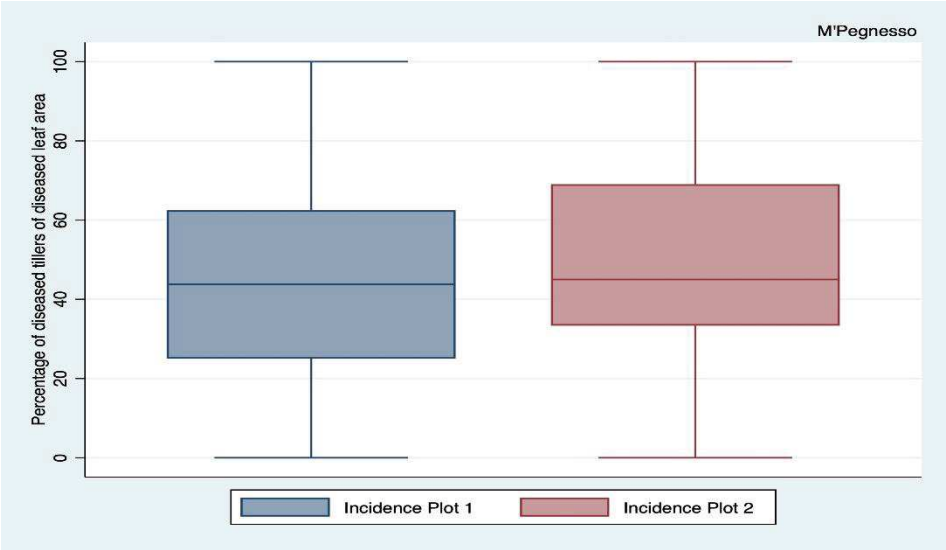
For Loulouni P (lowland), overall, the incidence was not significantly different at the plot level ($p=0.235$). Nonetheless, there was a significant difference between the severities at the plot level ($p<0.0001$). However, no significant difference was observed for the following plot pairs: plot 1 vs. plot 3, $p=0.440$; plot 3 vs. plot 4, $p>0.999$; plot 1 vs. plot 4, $p=0.271$; plot 1 vs. plot 5, $p=0.119$; plot 3 vs. plot 5, $p>0.999$; plot 4 vs. plot 5, $p>0.999$. For Loulouni B, the incidence was not significantly different between plots 1 & 2 ($p=0.7905$).

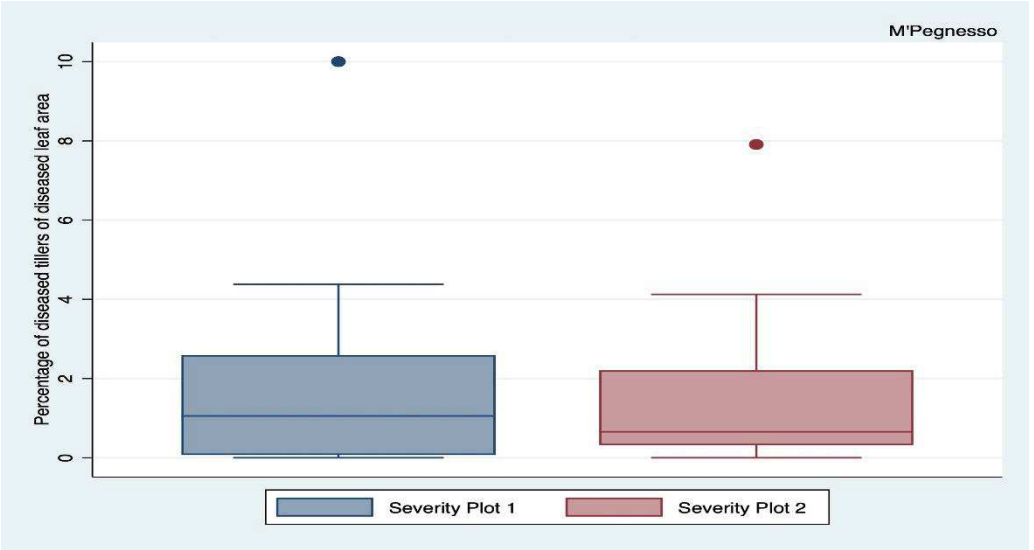
For the Manikoura (irrigated) site, both the incidence and severity were significantly different between plots ($p<0.0001$). For the Niono site, the incidence was not significantly different between plots 1 & 2 ($p=0.1506$), but the severity was significantly different between plots 1 & 2 ($p=0.0008$). (Fig. 6C).

A)

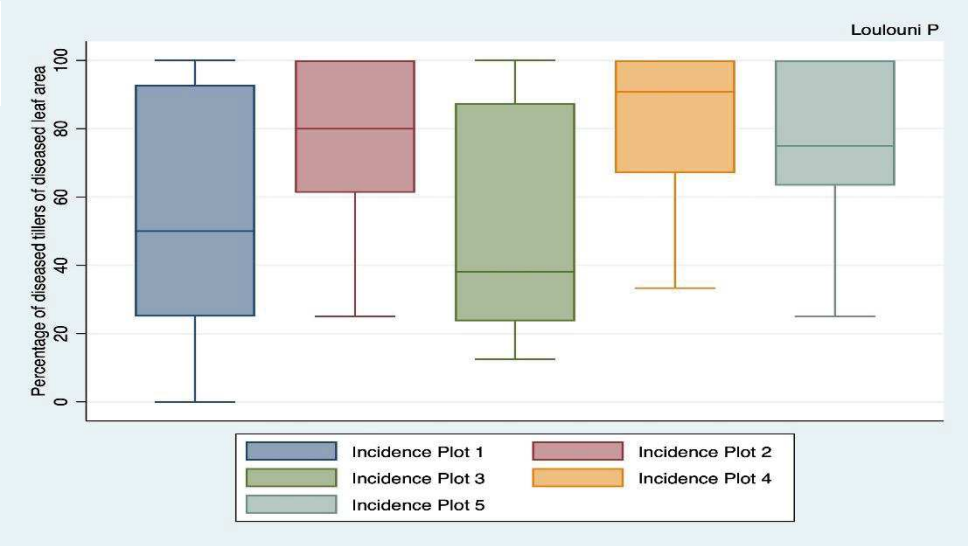

Niena
Percentage of diseased tillers of diseased leaf area
0
20
40
60
80
100
Incidence Plot 1
Incidence Plot 2
Incidence Plot 3

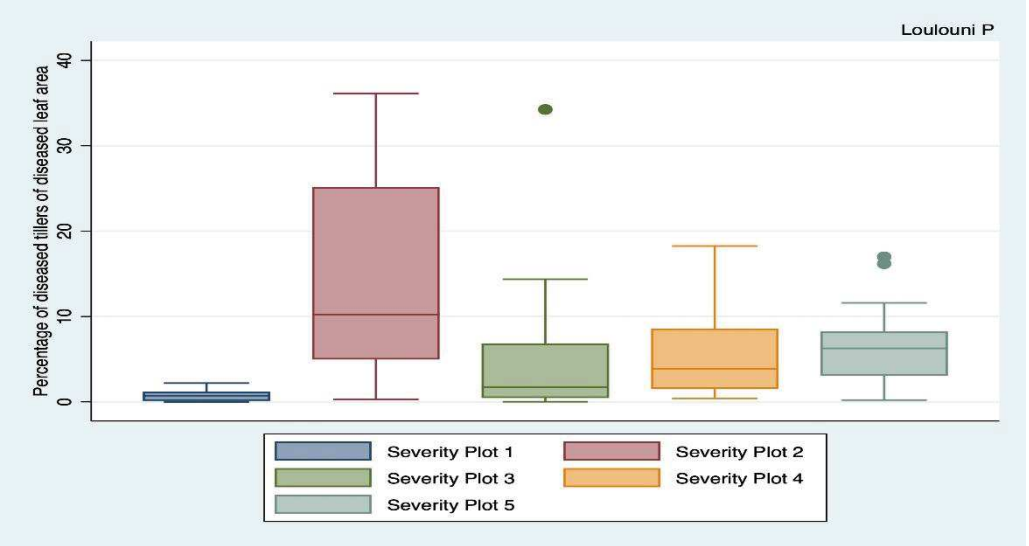

Niena
Percentage of diseased tillers of diseased leaf area
0
20
40
60
Severity Plot 1
Severity Plot 2
Severity Plot 3

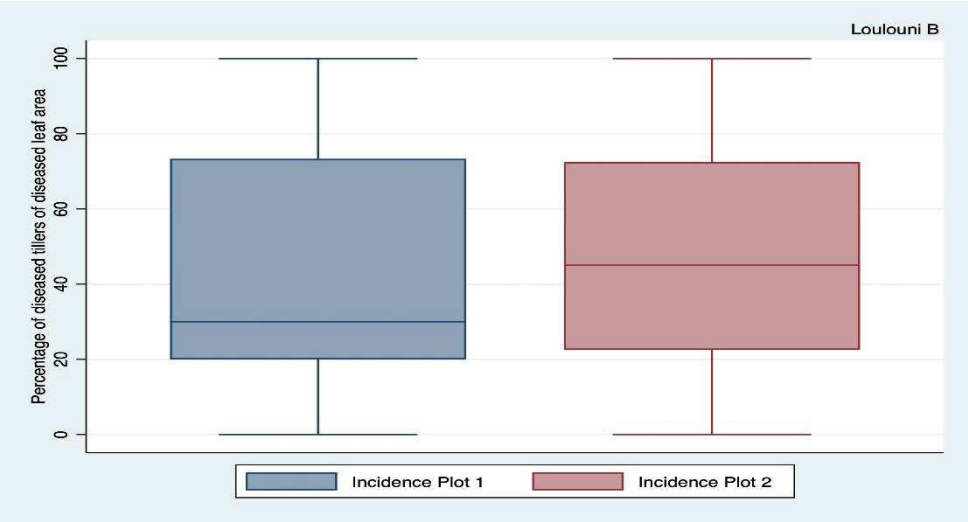

M'Pegnesso
Percentage of diseased tillers of diseased leaf area
0
20
40
60
80
100
Incidence Plot 1
Incidence Plot 2

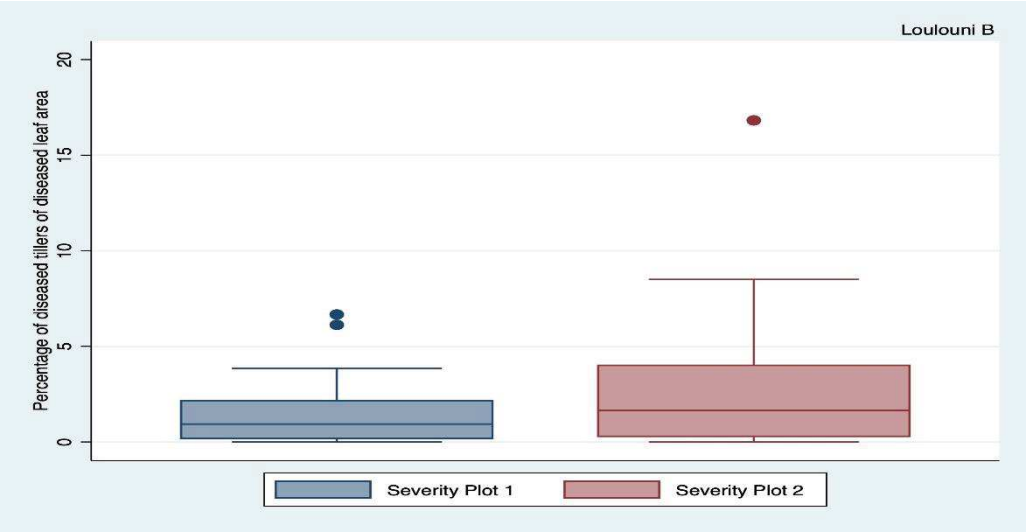

M'Pegnesso
Percentage of diseased tillers of diseased leaf area
0
2
4
6
8
10
Severity Plot 1
Severity Plot 2

B)


Loulouni P
Percentage of diseased tillers of diseased leaf area
0
20
40
60
80
100
Incidence Plot 1
Incidence Plot 2
Incidence Plot 3
Incidence Plot 4
Incidence Plot 5


Loulouni P
Percentage of diseased tillers of diseased leaf area
0
10
20
30
40
Severity Plot 1
Severity Plot 2
Severity Plot 3
Severity Plot 4
Severity Plot 5


Loulouni B
Percentage of diseased tillers of diseased leaf area
0
20
40
60
80
100
Incidence Plot 1
Incidence Plot 2


Loulouni B
Percentage of diseased tillers of diseased leaf area
0
5
10
15
20
Severity Plot 1
Severity Plot 2

C)

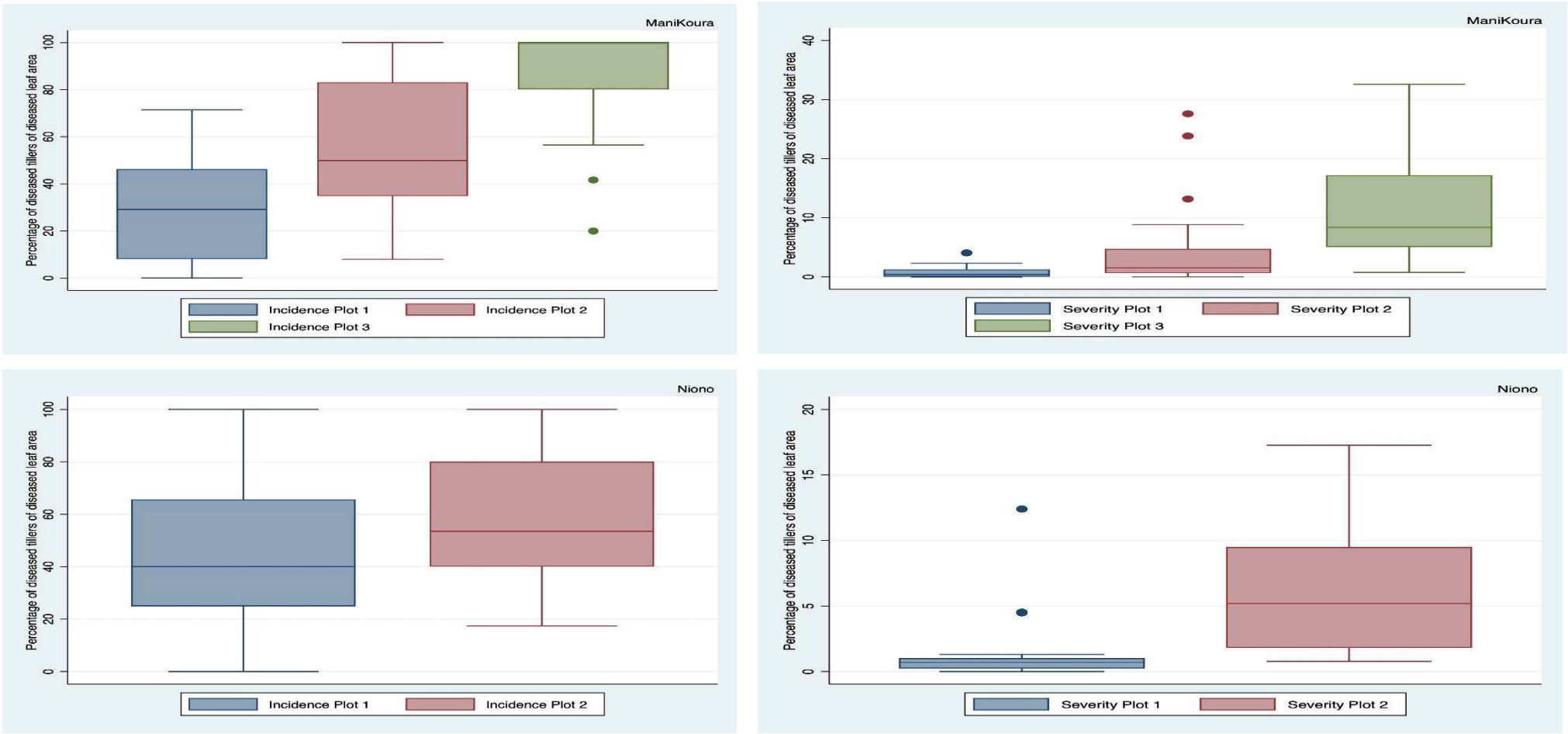


Figure 6: Incidence and leaf severity of blast disease in Mali per site and plots (2018). A) Incidence and leaf severity of blast disease in Niena and M'pegnesso per plot; B) Incidence and leaf severity of blast disease in Loulouni P and Loulouni B per plot; C) Incidence and leaf severity of blast disease in Manikoura and Niono per plot.

For panicle incidence at Niena, there was no significant difference between plots ($p=0.5629$). There was a statistically significant difference between severity at plot level 1 & 2 ($p< 0.0001$).

For panicle incidence and severity in Niono, the incidence was significantly different between plots 1 & 2 ($p<0.0001$). There was a significant difference between the incidence and severity in plots 1 & 2 ($p<0.0001$) (Fig. 7)

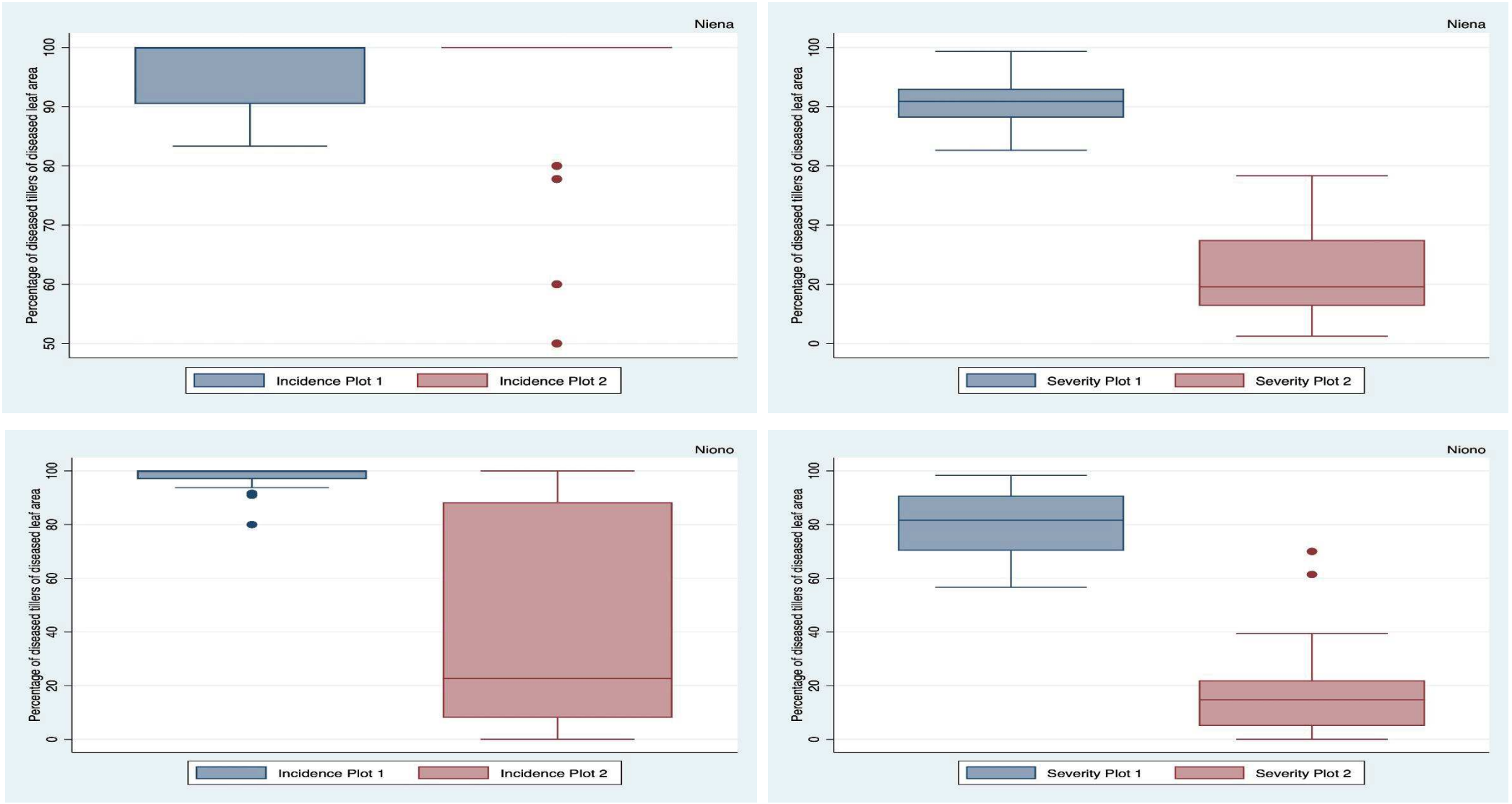


Figure7: Incidence and panicle severity of blast disease in Niena and Nion per plot (2018).

Search for *P. oryzae* and other fungal pathogens on seeds. After incubation on wet filter paper, there was no *P. oryzae* conidia observed on seeds. But we encountered different genera of fungi known to include phytopathogenic species such as *Curvularia* and *Fusarium*. We also identified fungal species causing brown spot disease. From a small sample of these strains, *Bipolaris* and *Exserohilum* were distinguished by sequencing reference genes (data not shown) but could not be distinguished visually. They are hereafter named collectively "brown spot species". The "brown spot species" and *Curvularia* were the most frequently observed species. Co-infection of the same lot by different species was frequently observed. Seventeen lots out of 20 were co-infected, each of them being contaminated by two or three pathogens. Hence, 12 seed lots (1 from Baguineda, 2 from M'pegnesso, 1 from Loulouni 3, 2 from Niena, 1 from Selingué, and 5 from Niono) were contaminated by two pathogens (“brown spot species” and *Curvularia*). In addition, 5 seed lots (1 from Baguineda, and 4 from Niono) were contaminated with three pathogens ("brown spot species", *Curvularia* spp and *Fusarium* spp; Fig. 8). For infected lots, the average percentage and range (in parenthesis) of infected seeds were 4.6% (0.3-31.8%), 4.3% (0.5-15.8%), and 1.2% (0.3-4.8%) respectively for “brown spot species”, *Curvularia* spp, and *Fusarium* spp (TABLE 2).

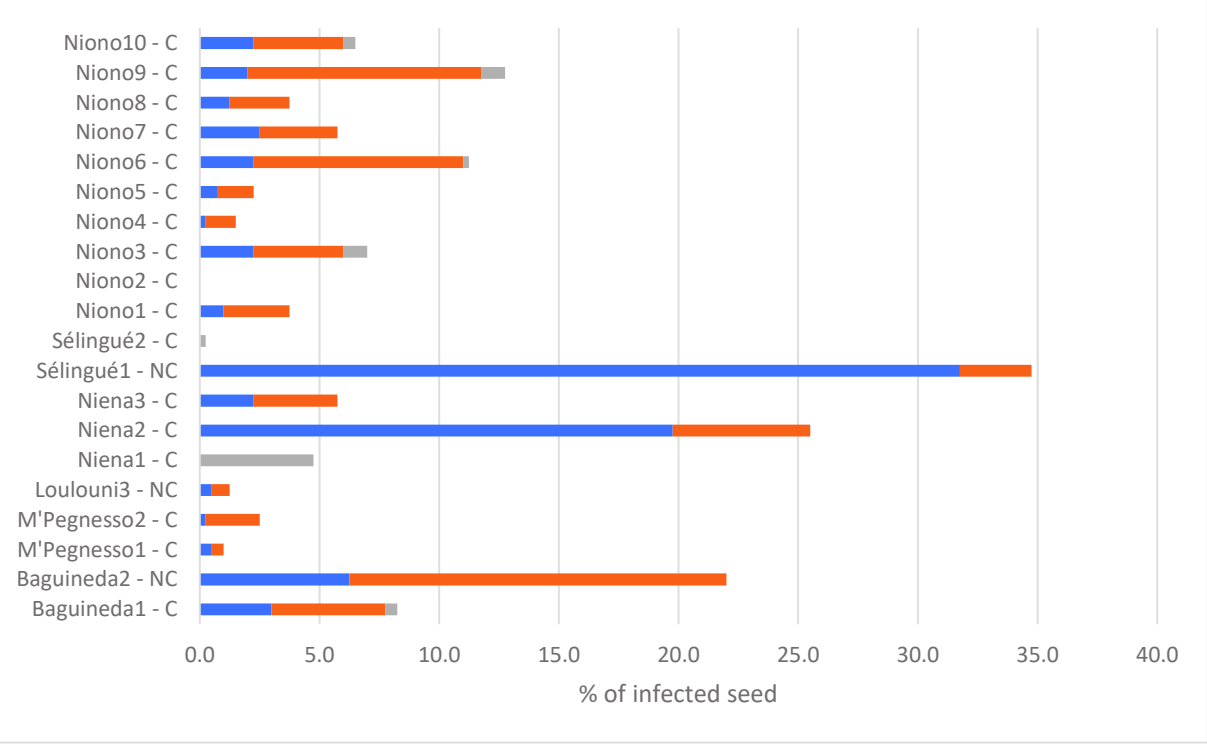


Figure 8: Percentage of infected seeds per pathogenic fungi for seed lots from different sites in Mali. Blue: brown spot species (*Bipolaris* spp and *Exserohilum* spp); orange: *Curvularia* spp; grey: *Fusarium* spp. C: Certified; NC: Not certifiedTABLE 2**:** Percentage of infected seeds per fungal pathogen species for seed lots collected in different locations in Mali

N° lot	Location	% of infected seed			
		Brown spot species	*Curvularia* spp	*Fusarium* spp	*Pyricularia oryzae*
1	Baguineda1	3.0	4.8	0.5	0.0
2	Baguineda2	6.3	15.8	0.0	0.0
3	M'Pegnesso1	0.5	0.5	0.0	0.0
4	M'Pegnesso2	0.3	2.3	0.0	0.0
5	Loulouni3	0.5	0.8	0.0	0.0
6	Niena1	0.0	0.0	4.8	0.0
7	Niena2	19.8	5.8	0.0	0.0
8	Niena3	2.3	3.5	0.0	0.0
9	Sélingué1	31.8	3.0	0.0	0.0
10	Sélingué2	0.0	0.0	0.3	0.0
11	Niono1	1.0	2.8	0.0	0.0
12	Niono2	0.0	0.0	0.0	0.0
13	Niono3	2.3	3.8	1.0	0.0
14	Niono4	0.3	1.3	0.0	0.0
15	Niono5	0.8	1.5	0.0	0.0
16	Niono6	2.3	8.8	0.3	0.0
17	Niono7	2.5	3.3	0.0	0.0
18	Niono8	1.3	2.5	0.0	0.0
19	Niono9	2.0	9.8	1.0	0.0
20	Niono10	2.3	3.8	0.5	0.0
	Average	3.9	3.7	0.4	0.0
	Average of infected lots	4.6	4.3	1.2	0.0

As expected, fungal saprophytes were also observed on seeds. In Niono, all lots were carriers of fungi belonging to the genera *Alternaria, Rhizopus, Aspergillus, Epicoccum* and *Penicillium*. Out of 10 lots, these species were observed in 1, 6, 4, 3 and 4 lots, respectively. Their presence was relatively low with contamination intensities not exceeding 30%. Nine lots were contaminated with a sixth genus that infected 18% of the seeds on average. (Supplementary Fig. 1-A). However, it was not possible to identify this genus due to the lack of spore production. In other sites, the saprophytic species present were the same as in Niono but with a notable difference in the over-representation of the genera *Aspergillus*, *Penicillium*, and *Rhizopus*. *Penicillium* and *Rhizopus* were found in 8 and 7 out of 10 lots, respectively, while *Aspergillus* was found in 5 lots. In general, all seed lots showed a high percentage of infected seeds: 43.8% for *Rhizopus*, 99.5% for *Aspergillus*, and 99% for *Penicillium* (Supplementary Fig. 1-B).

Identification of *P. oryzae* and other pathogens on straws. All seven straw lots examined were contaminated with two to four different pathogens. *Fusarium* spp and *Curvularia* spp were the most frequently observed genera. Hence, 3 straw lots (1 from Baguineda, and 2 from Manikoura) were contaminated with two pathogens (*Curvularia* spp and *Fusarium* spp). Two lots (from Selingué and Niono) were contaminated with three pathogens (*Curvularia* spp, "brown spot species" and *Fusarium* spp). Finally, 4 lots of straw (Selingué 1 and Niono) were contaminated with the four pathogens (*P. oryzae*, *Curvularia* spp, "brown spot species" and *Fusarium* spp; Fig. 9). For infected lots, the average percentage and range (in parenthesis) of infected straws were 4.5% (3-6%), 41% (6-100%), 19% (10-30%) and, 62% (30-96%) for *P. oryzae*, *Curvularia* spp, "brown spot species", and *Fusarium* spp, respectively (TABLE 3).

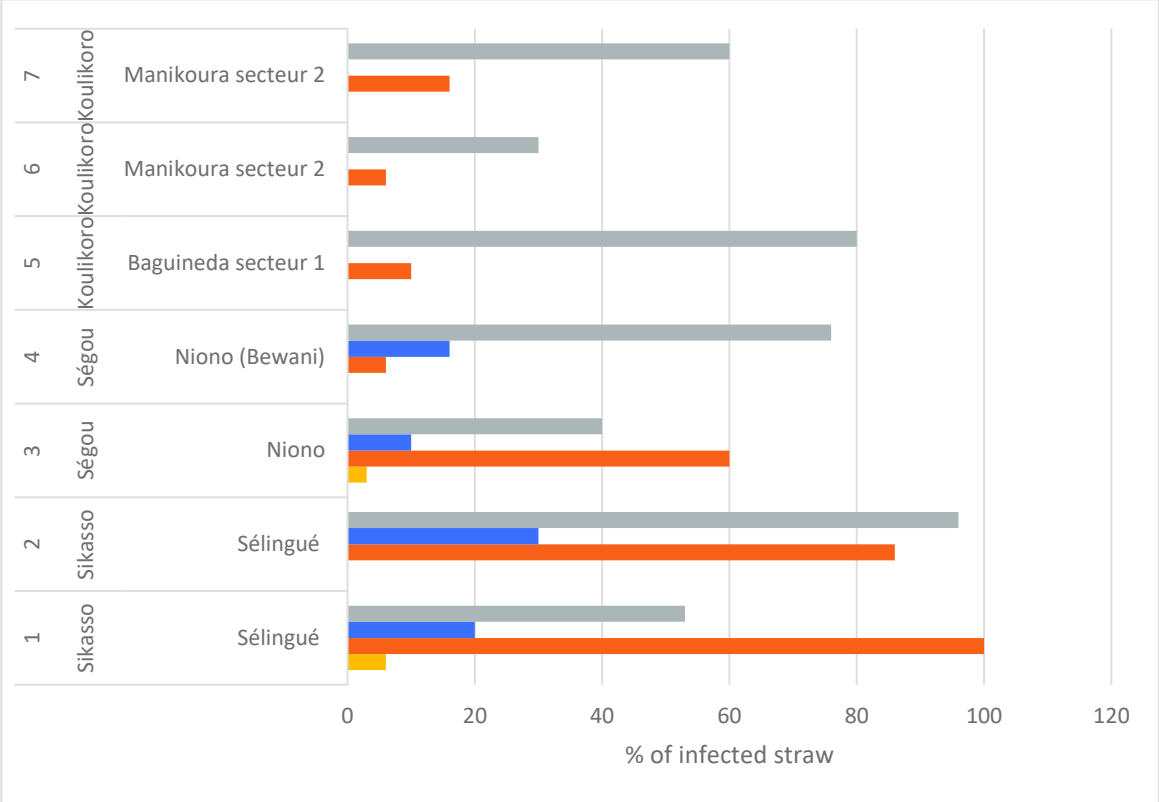


Figure 9**:** Identification of pathogenic fungi on straws by site. Blue: brown spot species (*Bipolaris* spp and *Exserohilum* spp); orange: *Curvularia* spp; grey: *Fusarium* spp, Yellow*: Pyricularia oryzae*.

TABLE 3: Percentage of infected straws per fungal pathogen species in samples collected from different locations in Mali

Type of ecosystem	N° lot	Localité	% of infected straw			
			Pyricularia oryzae	*Curvularia sp*	*Brown spot species*	*Fusarium sp*
Irrigated	1	Sélingué	6	100	20	53
	2	Sélingué	0	86	30	96
	3	Niono	3	60	10	40
	4	Niono (Bewani)	0	6	16	76
	5	Baguineda secteur 1	0	10	0	80
	6	Manikoura secteur 2	0	6	0	30
	7	Manikoura secteur 2	0	16	0	60
		Average	1.2	41	11	62
		Average of infected lots	4.5	41	19	62

DISCUSSION

Although rice blast is considered as a major disease in Sub-Saharan Africa, recent evaluations of its incidence and severity in farmer fields are rare. Surveys on large areas were conducted in upland and lowland rainfed agrosystems in the central part of Madagascar (Ratsimba 2005; Rakotonindraina et al. 2010; Raveloson 2018), in rainfed and irrigated agrosystems in Uganda (Amayo et al. 2020) and in Ghana (Nutsugah et al., 2005), and in lowland rainfed and irrigated agrosystems in Burkina Faso (Barro M. and Tollenaere C., personal communication). To our knowledge, our study is the first to conduct a large-scale survey of blast disease in Mali, the second rice producer in West Africa (FAO 2018). Leaf blast was encountered in most of the farmer fields prospected in the Koulikoro, Segou and Sikasso regions of Mali and its incidence was relatively high. On the contrary, leaf severity was low and panicle blast was not frequently encountered. However, panicle incidence and severity were high in the few fields were it was observed. Since yield losses caused by the blast disease are mainly due to attacks on the panicles, the impact on rice yield of the blast disease is probably low at the country scale. However, the devastating potential and the large distribution of the disease in Mali require maintaining surveillance and using preventive control measures.

It is commonly admitted among rice blast specialists that the incidence and severity of blast disease is lower in irrigated agrosystems compared to upland rainfed agrosystems. This is

usually explained by the fact that droughts at the end of wintering are favorable to attacks of *P. oryzae* especially on panicles (Mbod 1989). But few surveys were conducted to measure the impact of agrosystems on blast disease. In Uganda, the highest blast incidence was recorded in the rainfed lowland rice (72%), followed by irrigated lowland (60%) and rainfed upland rice (47%; Amayo et al. 2020). A study conducted in Bangladesh in 10 agro-ecological areas (Hossain et al. 2017) reported higher incidence and severity in the irrigated ecosystem compared to the rainfed ecosystem. In Mali in 2018, although the average leaf incidence of rice blast was higher in the lowland agrosystem (65%) than the irrigated agrosystem (42%), it was not significantly different between agrosystems. Despite low disease pressure, this result was confirmed in 2019. All together, these results suggest that other factors than the water management system are influencing the development of blast epidemics. Agronomic practices such as fertilization and choice of varieties are known to have a major impact on blast epidemics. The management of inoculum sources such as straw and infected seeds (Long, et al., 2001; Te Beest et al., 2007; Raveloson et al., 2018) is also expected to have an impact on the incidence of blast.

Limiting primary sources of inoculum is an important preventive control measure against plant diseases in general and blast in particular. Many species of pathogenic fungi and saprophytes have been observed on rice seeds (Ouedraogo et al. 2016; Sharma and Kapoor 2016). For several fungal diseases of rice, seeds were demonstrated to be inoculum sources. Infected seeds also increase the risk of dissemination between different areas through the exchange of materials. Species causing brown spot disease (*Bipolaris* spp and *Exserohilum* spp) and the genera *Curvularia* and *Fusarium* were commonly found. Most of the seed lots examined were infected with one or more of these fungal species pathogenic to rice. In general, we observed rice organs with brown spot symptoms in the surveyed fields in many rice-growing areas of Mali. In some countries, the percentage of seeds infected with *Bipolaris oryzae* and *Curvularia sp* can be high (Sadia 2012; Ouedraogo et al. 2016; Dossou and Silue 2018). In addition to *Curvularia sp*., Dossou and Silue, (2018) also diagnosed other pathogens of medium importance such as *Alternaria oryzae* and saprophytes affecting seed germination such as *Penicillium sp*. and *Aspergillus,* which were also found in the seed lots we examined. Although the percentage of infected seeds in our study was relatively low (between 1 and 5 %), the high frequency of infected seed lots observed for 'brown spot species' and *Curvularia* should raise questions about the role of seeds as a source of inoculum for these pathogens in Mali.

Despite the presence of pathogenic fungi on rice seeds, we did not observe *P. oryzae* on seeds in Mali. Similar results have been obtained in different African countries: Ghana (Sadia 2012), Chad (Lamrani et al. 2013; Serferbe et al. 2016), and Nigeria (Aluko 1969). In contrast, in Burkina Faso, Ouedraogo et al (2016) found *P. oryzae* in 13.5% of seed samples using the same method as described here. The infected seed samples came from humid localities (more than 800 mm of rainfall per year) with conditions close to those of the Sikasso region (where a significant part of the seeds in our study originated from). The use of certified seeds could explain the absence of *P. oryzae* on seeds from Mali but is not consistent with the infection frequency of seed lots by other fungi that we observed. However, seed certification was based on specific purity, germinability and varietal purity, but the phytosanitary quality of the seed was not assessed, which could exlain the presence of fungal pathogens. Alternatively, the low panicle blast incidence and severity observed in the field is likely to result in low or absence of seed infection by the *P. oryzae*. Our results thus support the hypothesis that seeds are not a major source of primary inoculum for blast epidemics in Mali. It has been shown by Raveloson et al. (2013) that alternative to infected seeds, infected straws or residues left on the soil are major sources of inoculum in rainfed upland rice in Madagascar. Monitoring the viability of the inoculum on the infected residues showed that infected straws could be sources of primary inoculum for nearly two seasons. In our study, *P. oryzae* was observed on straw samples, particularly in irrigated areas. The presence of other pathogenic fungi on straw was also observed. This could be evidence that straws are a source of inoculum for several fungal diseases of cultivated rice in Mali.

ACKNOWLEDGEMENTS

We thank Lassina DOUMBIA, Youssouf DIARRA, Kouka Hilaire KABORE and the team that collected the samples, JEAI CoANA team at LBMA, in particular Moussa KANTE, Hinda DOUCOURE, Ibrahim KEITA, Lazeni KONATE, Jean Marie SANOU and Aboubacar MACALOU; the teams of the stations of Baguineda, Manikoura, Sélingué, Niena, M'pegnesso and Loulouni for their contribution. This work was supported by grants of the RICE CRP, a grant from the French Embassy in Mali to Diariatou Diagne, IRD support to Ousmane Koita group (JEAI CoANA) and the support of Institutes of the coauthors (CIRAD and USTT-B).

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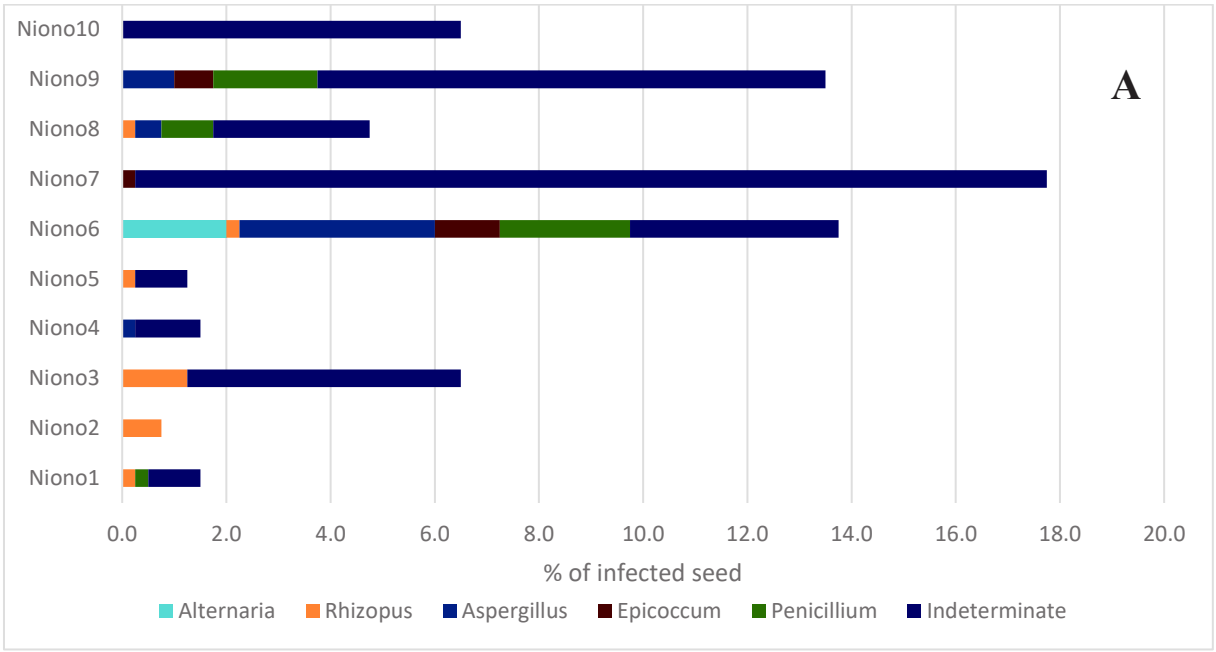
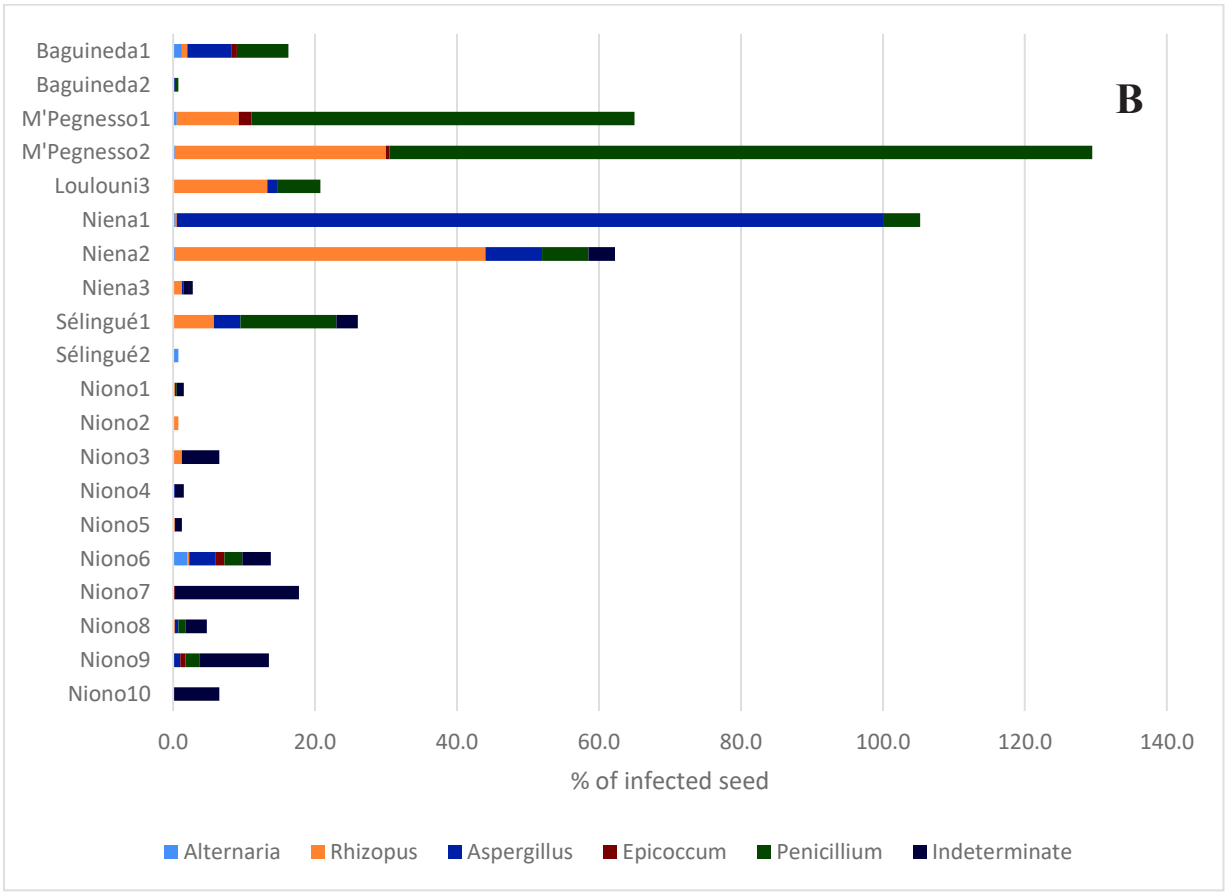
Supplementary TABLE 1: Information on seed lots collected

Seed date	Type seeds	Region	Location	Genus	Species	Variety	Type of ecosystem
2017-2018	Certified	Koulikoro	Baguineda	*Oryza*	*sativa*	ADNY 11	Irrigated
2017-2018	Uncertified (farmers)	Koulikoro	Baguineda	*Oryza*	*sativa*	ADNY 11	Irrigated
2017	Uncertified (farmers)	Sikasso	Sélingué	*Oryza*	*sativa*	ADNY 11	Irrigated
2018	Certified	Sikasso	Sélingué	*Oryza*	*sativa*	SWARNA 2	Irrigated
2015-2016	Certified	Sikasso	Niena	*Oryza*	*sativa*	SIKA	Rainfed lowland
2017	Certified	Sikasso	Niena	*Oryza*	*sativa*	IR32000	Rainfed lowland
2017-2018	Certified	Sikasso	Niena	*Oryza*	*sativa*	BW 348-1	Rainfed lowland
2017	Certified	Sikasso	M'pegnesso	*Oryza*	*sativa*	BW 348-1	Rainfed lowland
2017	Certified	Sikasso	M'pegnesso	*Oryza*	*sativa*	NERICA	Rainfed lowland
2017	Uncertified (farmers)	Sikasso	Loulouni	*Oryza*	*sativa*	SHWETASOKE	Rainfed lowland
2017	Certified	Ségou	Niono	*Oryza*	*sativa*	08FAN 2	Irrigated
2017	Certified	Ségou	Niono	*Oryza*	*sativa*	WAS 49	Irrigated
2017	Certified	Ségou	Niono	*Oryza*	*sativa*	WAPMO (WAS 197)	Irrigated
2017	Certified	Ségou	Niono	*Oryza*	*sativa*	ADNY 11	Irrigated
2017	Certified	Ségou	Niono	*Oryza*	*sativa*	IR32000	Irrigated
2017	Certified	Ségou	Niono	*Oryza*	*sativa*	BG 90 -2	Irrigated
2017	Certified	Ségou	Niono	*Oryza*	*sativa*	KOGONI	Irrigated
2017	Certified	Ségou	Niono	*Oryza*	*sativa*	AFRICA 9	Irrigated
2017	Certified	Ségou	Niono	*Oryza*	*sativa*	NENEKALA	Irrigated
2017	Certified	Ségou	Niono	*Oryza*	*sativa*	AFRICA 10	Irrigated





Supplementary Figure 1: Identification of saprophytes by site on seeds A) in Niono, B) in other sites.

Chapitre 2

Structure of African Populations of *Pyricularia oryzae* from Rice

CHAPITRE 2 : article 2 « Structure of African populations of *Pyricularia oryzae* from rice »

Structure of African populations of *Pyricularia oryzae* from rice

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ABSTRACT

Rice blast, caused by the filamentous ascomycete *Pyricularia oryzae*, is one of the most devastating diseases of rice. Four genetic clusters were previously identified and three have a large geographic distribution. Asia is the center of diversity and the origin of most migrations to other continents and sexual reproduction persisted only in the South China–Laos–North Thailand region, which was identified as the putative center of origin of all *P. oryzae* populations on rice. Despite the importance of rice blast disease, little is known about the diversity and the population structure of the pathogen in Africa (including Madagascar). The present study was aimed at describing the structure of African populations of *P. oryzae* and

identifying the relationship between African and worldwide genetic clusters. A set of 2057 strains (including 937 African and 1120 Madagascan strains) were genotyped using 12 SSR markers to assess the diversity and the population structure of *P. oryzae*. Four genetic clusters were identified in Africa and Madagascar. All the four clusters previously identified are present in Africa. Populations from West Africa, East Africa and Madagascar are highly differentiated. The geographic structure is consistent with limited dispersion and with some migration events between neighboring countries. The two mating types are present in Africa with a dominance of Mat1.2 but no female fertile strain was detected supporting the absence of sexual reproduction on this continent. This study showed an unsuspected high level of genetic diversity of *P. oryzae* in Africa and suggested several independent introductions.

INTRODUCTION

The ascomycete fungus *Pyricularia oryzae* (syn. *Magnaporthe oryzae*) is a haploid fungus, responsible for the most damaging disease of rice worldwide: blast. This model species for the study of host–pathogen interactions (Dean et al. 2012) is a major threat to food security (Pennisi 2010). The first written record about rice blast was probably made in China and dates back to 1637 (Ou 1985). Since then, this disease has been reported in all rice producing areas.

The genetic diversity of *P. oryzae* was characterized in many studies using molecular markers, mainly based on repetitive DNA sequences (Hamer et al. 1989) and clonal lineages were described in several countries or continents: India (Kumar et al. 1999), China (Chen et al. 2006), Thailand (Zeigler 1998), Japan (Don et al. 1999a), Korea (Park et al. 2003, 2008), Philippines (Chen et al. 1995; Zeigler et al. 1995), Vietnam (Don et al. 1999b), Europe (Roumen et al. 1997; Piotti et al. 2005), Iran (Javan-Nikkah et al. 2004), USA (Levy et al. 1991; Xia et al. 1993, 2000; Correll et al. 2009; Wang et al. 2017), Argentina (Consolo et al. 2008), Colombia (Levy et al. 1993; Zeigler, 1998) and Cuba (Fuentes et al. 2003). In West and East Africa, the diversity studies of *P. oryzae* focused mainly on trap analysis in rice resistance screening sites (so called blast nurseries; Séré et al. 2004; Nutsugah et al. 2008; Odjo et al. 2011; Takan et al. 2012). However, little is known about the diversity and population structure of the pathogen in Africa. Onaga et al. (2015) investigated the population structure, pathogenicity, and mating type distribution of 65 strains of *P. oryzae* from East Africa, eight strains from West Africa, 14 from the Philippines and one from Japan, using AFLP markers and mating-type-specific primer sets. From this study, five genetic groups were resolved, and

the results showed a clear separation of the Asian strains from the African groups. No evidence of population structure was found among the 65 strains from East Africa (14 strains from Tanzania, 20 from Rwanda and 31 strains from Uganda), which was interpreted as high gene flow among East Africa populations. The number of West Africa strains was too small to draw any conclusions. Mutiga et al. (2017) also studied the genetic diversity of *P. oryzae* populations from rice and evaluated the virulence spectrum of this pathogen in West Africa (Benin, Burkina Faso, Mali, Ghana, Nigeria, and Togo) and East Africa (Kenya, Tanzania, and Uganda). Diversity analysis was based on SNP markers using GBS from the 86 isolates. The study identified seven genetic groups and showed a differentiation between West and East African isolates.

By comparing the number of clonal lineages identified in different studies Zeigler (1998) concluded that the genetic diversity of *P. oryzae* was higher in the area encompassing South, East and Southeast Asia than in other regions. The most exhaustive studies of the population's structure of *P. oryzae* at the worldwide scale depicted a genetic structure of three or four main clusters distributed widely, Asian strains being scattered in four clusters (Tharreau et al. 2009; Saleh et al. 2014; Gladieux et al. 2018; Zhong et al. 2018; Thierry et al. 2020). These studies concluded that Asia was the center of diversity and the origin of most migrations to other continents. It was also shown that the South China–Laos–North Thailand region is the unique area were sexual reproduction might persist, and that it is the putative center of origin of all *P. oryzae* populations on rice (Saleh et al. 2014). In other parts of the world, strains of different genetic clusters can coexist without exchanging genes (Thierry et al. 2020). Unfortunately, African strains were scarcely represented in these studies (Tharreau et al. 2009, Saleh et al. 2014, Gladieux et al. 2018; Zhong et al. 2018; Thierry et al. 2020).

Crop domestication, diversity and structure are known to influence the emergence and evolution of plant pathogens (Gladieux et al. 2010; Saleh et al. 2014; Stükenbrock and McDonald 2008; Stükenbrock et al. 2011). In Africa, two rice species are cultivated. *O. sativa* was introduced from Asia by the Portuguese as early as the middle of the 16th century (Portéres 1962). Like Asia, Africa experienced the domestication of a rice species, *O. glaberrima*. Indeed, native to sub-Saharan Africa, *O. glaberrima* is thought to have been domesticated from the wild ancestor *O. barthii* (formerly known as *O. brevilugata*) by people living in the floodplains at the bend of the Niger river some 2,000–3,000 years ago (Portéres 1962 and 1976). Population genomics analyses of 20 *O. glaberrima* and 94 *O. barthii* accessions, demonstrated that *O. glaberrima* was domesticated in a single region along the Niger river (Wang et al. 2014). Today,

the species *O. sativa* is dominant in West Africa, while *O. glaberrima* and *O. barthii* are barely found in the region. However, the existence of two potential host species in Africa may have influenced the population structure of the rice blast fungus. There have been no blast population studies conducted on African cultivated or wild species of rice to date.

To investigate the population structure and diversity of *P. oryzae* in Africa (including Madagascar), 2057 strains were collected from West Africa, East Africa and Madagascar; mainly from 2009 to 2018. They were analyzed with 12 SSR markers and the genotypes obtained were compared to the strains of the worldwide sample of Saleh et al. (2014). These SSR markers were previously used successfully to characterize the genetic structure of the blast fungus populations (Saleh et al., 2014). We used the same markers to allow comparison with previous published results. We aimed at answering the following questions: (i) What is the population structure of *P. oryzae* in Africa? (ii) Do African strains originate from Asia? (iii) Are there specific genetic clusters of *P. oryzae* in Africa? and (iv) Is there sexual reproduction of *P. oryzae* in Africa?

MATERIAL AND METHODS

Strains collection, isolation and storage. The collection was composed of strains isolated from cultivated rice mainly between 2009 and 2018, from farmers' or experimental fields (Supplementary Table 1). Rice diseased samples were collected in farmer fields from four different ecologies - irrigated, rainfed lowland, upland, and flooded upland - in nine countries of West Africa (Benin, Burkina-Faso, Ghana, Ivory Coast, Mali, Niger, Nigeria, Senegal and Togo), five countries in East and Central Africa (Burundi, Cameroon, Kenya, Mozambique, Tanzania and Uganda), and in Madagascar. Some samples were collected in field experiments on differential varieties (near isogenic lines) with known resistance genes to blast and commonly used to characterize the virulence spectrum of blast isolates. A total of 2057 strains - Benin (165), Burkina Faso (210), Ivory Coast (51), Ghana (4), Kenya (5), Mali (105), Niger (14), Nigeria (245), Tanzania (94), Mozambique (1), Togo (34), Senegal (1) Uganda (5), Burundi (2), Cameroon (1) and Madagascar (1120) - were used to investigate population structure of *P. oryzae* in Africa. Most of the strains from countries with small sample sizes (1 to 5) were included in the analyses because they represent historic reference strains in our collection. To compare our results to the worldwide survey of Saleh et al. (2014) we included in relevant analyses the genotyping data of 804 strains from Asia, Europe, and Americas. The

genotypic data of 354 Madagascan strains from the study of Saleh et al. (2014) were also part of the 1120 Madagascan genotypic data set used in this study. Fungal strains were isolated from infected plant material placed in humid chamber at 25˚C for 1–2 days, and genetically pure fungal strains were obtained by monospore isolation. Fungal strains were grown on a 7 cm diameter paper disk (Whatman® N°5) placed on autoclaved rice flour medium (20 g of rice flour, 15 g of agar, 2.5 g of yeast extract, 500,000 units of penicillin, 1 L of water), as previously described (Silué and Nottéghem 1990). After colonization of the paper by the mycelium, i.e. about seven days in a climatic chamber (25°C, fluorescent lighting, 12 h photoperiod), the paper was removed, dried in an oven at 37°C for 5 days, cut in pieces for collection in a sterile paper bag, numbered, bagged in a vacuum sealed plastic bag and were stored at -20˚C, as described by Valent et al. (1986).

DNA extraction and SSR amplification. DNA extraction was performed following the procedure described by Adreit et al. (2007). Paper stocks were deposited on rice flour medium maintained in a climatic chamber for 4 - 5 days in order to obtain actively growing mycelium. This mycelium was used to inoculate 6 mL of 2YEG liquid medium (2 g yeast extract, 10 g glucose, 3 g KNO_3, 2 g KH_2PO_4, 500,000 units of penicillin, and 1 L water). Cultures were maintained 4 days in the dark at 25°C. The mycelium from this culture was collected for the extraction of genomic DNA following the Adreit et al. (2007) protocol.

Twelve simple sequence repeat (SSR) markers (Supplementary Table 2), previously developed for population genetics studies of *P. oryzae* (Kaye et al., 2003; Adreit et al. 2007, Saleh et al. 2014) were used in this study. These markers were amplified by PCR (QIAGEN Multiplex PCR kit). PCR assays were run with a total volume of 5 µL, including 2.5 µL of Master Mix, 0.5 µL of 10 x Mix primers, 0.5 µL of 5x Q solution and 1.5 µL of genomic DNA (10n g/µL). The PCR program was performed as follows: i) predenaturation at 95°C for 15 min, ii) denaturation at 94°C for 30 s, iii) hybridization at 57 to 63°C for 90 s, iv) extension at 72°C for 60 s, v) repeat steps ii - iv for 40 cycles, and vi) final extension at 72°C for 30 min. The products obtained were separated and analyzed on a 16-capillary ABI Prism 3130XL machine (Applied Biosystems, Foster City, CA, USA) and the size of the amplicons was assessed by fluorescence measurement. For this analysis, 1.5 µL of amplified products (1/70 dilution) were mixed with 15 µL Formamide HiDi and GeneScan-500LIZ size marker (Applied Biosystems). The raw data collected were then analyzed and converted to allele size with GENEMAPPER 4 software (Applied Biosystems).

Determination of mating type and fertility. In order to make assumptions about the occurrence of sexual reproduction of *P. oryzae* in Africa, the mating type of 435 strains and the female-fertility of 245 of them were determined. These 435 strains were chosen after preliminary analysis of genotyping data to be representative of the diversity of *P. oryzae* populations in Africa. The mating type and female-fertility of 245 African strains were determined by *in vitro* crosses as described by Nottéghem and Silué (1992). Mating of *P. oryzae* requires strains of opposite mating types and at least one of the strains must be female-fertile (i.e., able to produce perithecia). Crosses were performed by confronting the tested strain to hermaphroditic strains for which the mating type is known (reference strains). Mat1.1 reference strains used were the Chinese strains CH999 and CH1003, and Mat1.2 reference strains were the Chinese strains CH997 and CH1019 (Saleh et al. 2012). Tested strains were classified as Mat1.1 when inducing or forming perithecia with a Mat1.2 reference strain (and conversely). Tested strains were classified as female-fertile when forming perithecia with reference strains. The mating type of 190 additional strains was determined by PCR amplification with the primers specific for Mat1.1 (Primers A1 and A5) and Mat1.2 (Primers B15 and B16; Xu and Hamer, 1995). PCR assays were run with a total volume of 19 µL (for each mating type of a fungal strain) including 11 µL of sterile MiliQ H_2O, 2 µL of 10 x PCR buffer, 2 µL of dNTPs (10 mM each), 0.8 µL of MgCl2 (15 mM), 1 µL of specific primer, 0.2 µL of Taq polymerase and 2 µL of DNA solution. The PCR program was: i) pre-denaturation at 95°C for 5 min, ii) denaturation at 95°C for 1 min, iii) hybridization at 60°C for 2 min (55°C for Mat1.2), iv) amplification at 72°C for 2 min, v) repeat steps ii - iv for 29 cycles, vi) elongation at 72°C for 5 min. The product of the PCR amplification was then deposited on a 1% agar gel in TAE buffer and separated electrophoretically for 90 min at 130 V. Visual scoring was made under UV light after staining for 15 min in ethidium bromide (0.5 µg/mL). For the strains assessed only by PCR amplification, female fertility was not assessed.

Multilocus genotypes, clustering and assignment analyses. All the 2862 strains analyzed had no more than three missing loci per strain and, overall, 7% of the genotypic data were missing. Since *P. oryzae* is haploid, each missing data corresponds to a missing locus. Polymorphism information content (PIC) was used to evaluate the diversity of SSR markers. PIC value for each SSR was calculated as $PIC = 1-\Sigma(Pi)^2$, where Pi stands for the ith allele frequency of one SSR locus (Anderson et al. 1993; Botstein et al. 1980).

In order to clarify the genetic structure of *P. oryzae* populations in Africa and compare

with populations from other continents, we first studied the overall genetic structure. Because, *P. oryzae* populations are clonal in most part of the World (Saleh et al. 2014, Thierry et al. 2020), we first identified and analyzed the distributions of multi-locus genotypes (MLGs) present in the African population. Then, we studied their grouping into genetic clusters. Strains were assigned to a particular multi locus genotypes (MLGs) when they share the same alleles at all loci. This was done by using a script developed in house by Sebastien Ravel (https://pypi.org/project/MLG-assign/). This script compares genotypes and deals with missing data. If because of missing data a strain is potentially assigned to two MLGs, the script does not assign the strain to any MLG.

To determine the putative origin of African populations of *P. oryzae*, we compared these populations with populations from other continents. Since four genetic clusters were previously described to have migrated worldwide from Asia (Gladieux et al. 2018; Thierry et al. 2020), we considered that analyzing the population structure at the genetic cluster level was needed. Thus, we assigned MLGs to genetic clusters determined *de novo* in this study. We used the discriminant analysis of principal components (DAPC; Jombart et al. 2010) that does not require any assumption on the biology of the organism, especially regarding panmixia. The DAPC was conducted using the "adegenet" package of R software (v3.2.2) (Jombart and Ahmed 2011, R Development Core Team 2017, Jombart and Ahmed 2011, Jombart 2008). The find clusters function was used to try to determine the optimal number of genetic clusters (K) based on the Bayesian information criterion (BIC).

Indices of genetic diversity in genetic groups. To characterize the diversity, for each genetic group identified after DAPC analysis and for the six geographic regions sampled (East Africa, West Africa, Madagascar, Americas, Asia, and Europe), the mean number of alleles per locus Na, and the unbiased gene diversity $H_{n.b.}$ (Nei, 1987) were calculated using GENETIX v4.05 (Belkhir et al. 2004). We calculated the mean number of private alleles (N_p) as the number of alleles that were present only in one genetic group, averaged over all markers.

Genetic differentiation and genetic distances. Pairwise F_{ST} (Weir & Cockerham, 1984) was calculated between genetic clusters. It was also calculated between populations from geographic sub-regions in Africa and from different continents. The null hypothesis $F_{ST}=0$ was tested using exact tests implemented in GENEPOP v4 (Raymond and Rousset 1995). The

proportion of the shared alleles between genetic clusters was calculated using POPULATIONS software version 1.2.32.

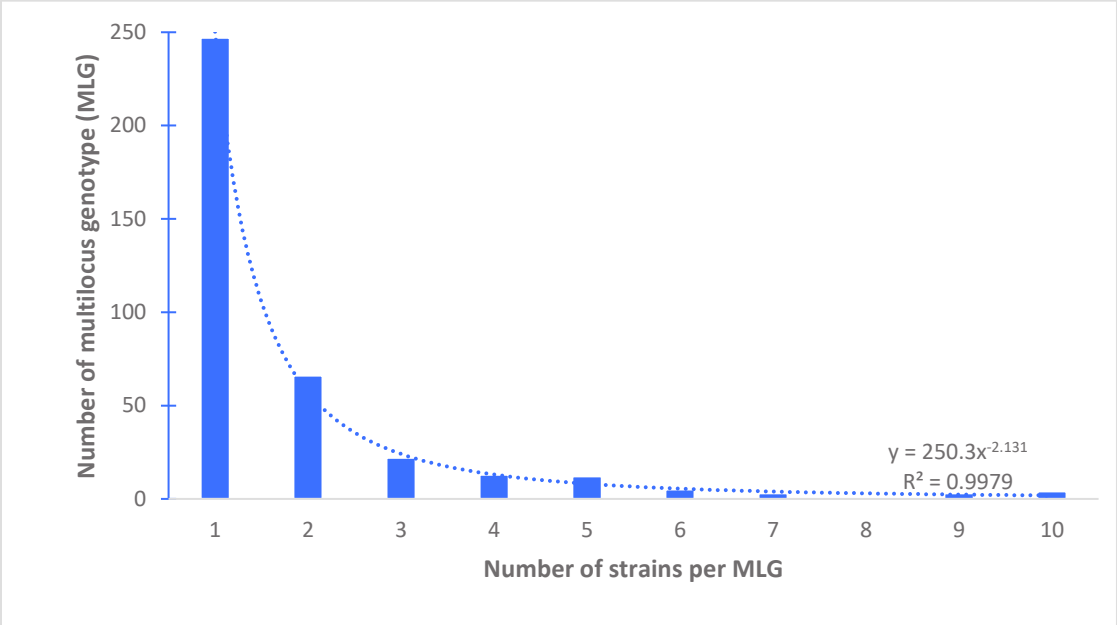
Linkage disequilibrium. Pairwise linkage disequilibrium was tested for all 66 pairs of markers and for populations from West Africa, East Africa and Madagascar. Significance at the 0.05 level was tested by an exact test based on 10000 permutations implemented in Arlequin version 3.5.1.2 (Excoffier and Lischer 2010).

RESULTS

Genetic structure. Hereafter, "African population" of *P. oryzae* refers to strains collected in continental Africa and in Madagascar. All the twelve microsatellite markers were polymorphic in the African population of *P. oryzae*. The number of alleles per locus ranged from 3 to 12 and averaged 6.3 over all loci (Supplementary Table 2). PIC per locus loci varied from 0.21 to 0.68 and averaged 0.42 over all loci (Supplementary Table 2).

Among the 2057 strains genotyped from African populations, 1222 could be unambiguously assigned to one of the 393 MLGs. Figure 1 shows the distribution of the number of MLGs according to the number of strains they represent in *P. oryzae* African populations. One-hundred forty- eight MLGs were represented by at least two strains and 245 MLGs were represented by a unique strain (Fig. 1). The most common MLG was represented by 82 strains. The distribution of the number of strains per MLG followed a Pareto distribution and is typical of clonal organisms (Arnaud-Haond et al. 2007).

a)



b)

Number of trains/MLG	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18	20	22	25	29	42	55	61	82
Number of MLGs	245	65	22	13	12	5	3	1	3	4	2	3	1	1	1	1	1	1	2	1	1	2	1	1	1

Fig. 1. Distribution of the number of multi-locus genotypes (MLGs) according to the number of strains they represent in *Pyricularia oryzae* African populations. a) Only MLG encompassing 10 strains or more are presented in the graph. b) The full distribution is provided in the table below the graph.

To further characterize the genetic structure of African populations of *P. oryzae* and compare it to populations from elsewhere, genotyping data of 804 strains from Asia, Europe, and Americas from the study of Saleh et al. (2014), were added to African and Madagascan strain samples. In total, the genotypic data of 2861 strains from West and East Africa (936 strains), Madagascar (1120 strains), Asia (322 strains), Europe (395 strains), and North and South America (87 strains) were analyzed. In a DAPC (Jombart et al. 2010), the 30 principal components retained explained more than 90% of the observed variance. The BIC value regularly decreased with the number of genetic clusters (K). The curve did not show a marked inflection (Supplementary Fig. 1) and we could not determine an optimal K value based on this method. Alternatively, we applied the recommendation of the DAPC tutorial (Jombart and

Collins, 2015) and tried to find the value of K that "better, more efficient[ly] summaries of the data than others". DAPC was run for K from 2 to 10. The analysis of DAPC results graphs (Fig. 2) showed that clustering in more than 5 groups seemed to create unnecessary and unreliable level of structure. Hence, four clusters (K=4; numbered from 1 to 4; Fig. 2) seemed to best describe the genetic structure of *P. oryzae* populations. In continental Africa and Madagascar, 1917 strains out of 2057 could be assigned to these clusters (Supplementary Table 3). The 140 remaining strains had admix genotypes and were not assigned to a single cluster.

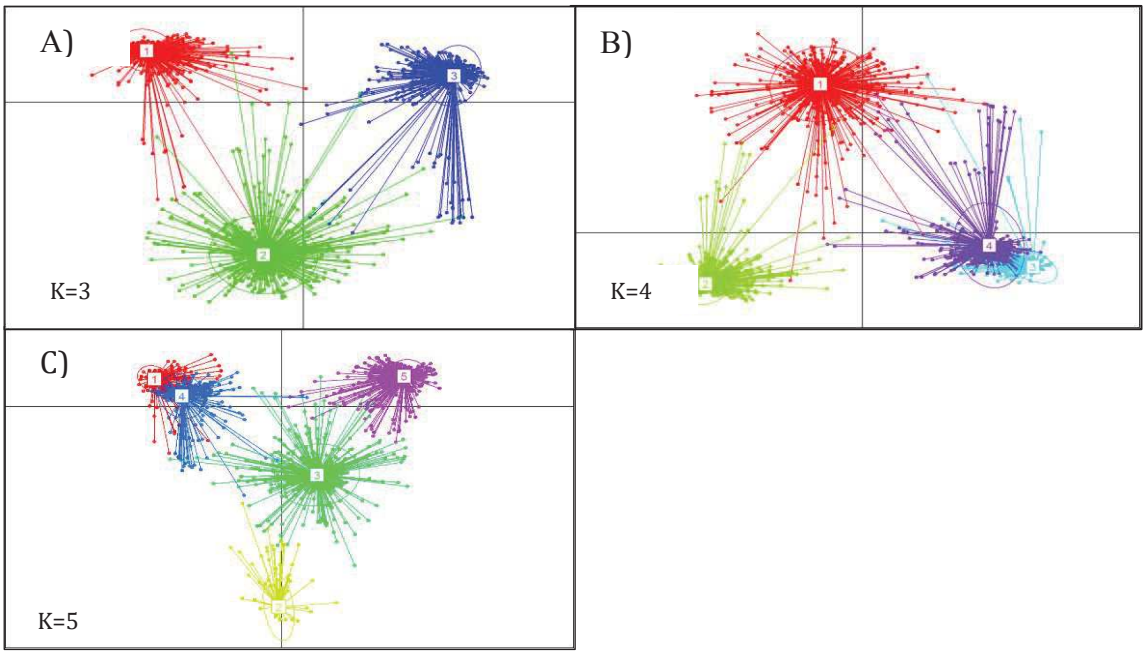


Fig. 2. Clustering of genotypes by DAPC method with number of clusters (K) varying between 3 (A), 4 (B) and 5 (C). Each color represents a different cluster.

For African and Madagascan strains, the genetic differentiation between genetic clusters measured by the F_{ST} index (Supplementary Table 4 showed high values for all pairwise comparisons. Each cluster was significantly differentiated from the others. The pairwise F_{ST} value varied from 0.37 between genetic clusters (GC) 1 and 2, to 0.77, between GC1 and 3. On average GC2 was the most differentiated cluster (F_{ST} = 0.60) and GC1 was the least differentiated (F_{ST} = 0.41). The mean number of private alleles (N_p) varied between 0.25 for GC1 to 3.83 for GC4 (Table 1).

We also evaluated the genetic diversity within genetic clusters for African and Madagascan strains. The genetic diversity ($H_{n.b.}$) calculated over all individuals within clusters,

ranged from 0.18 for GC1 to 0.61 for GC4. The mean number of alleles per locus (N_a) varied between 5.1 for GC1 and GC3 to 10 for GC4. So, GC1 was the least diverse genetic cluster whereas GC4 was the most diverse. GC2 and 3 showed intermediate levels of diversity (Table 1).

TABLE 1. Genetic diversity of genetic clusters of *Pyricularia oryzae* in West Africa, East Africa and Madagascar

Genetic clusters	**N**	**Diversity parameters**		
		H_{nb}	N_a	N_p
1	1154	0.18	5.1	0.25
2	430	0.32	8.3	0.67
3	120	0.31	5.1	0.50
4	213	0.61	10	3.8
Total	1917	-	-	-

Total number of individuals (N), unbiased gene diversity ($H_{n.b.}$), mean number of alleles per locus (N_a), mean number of private alleles per locus (N_p).

Geographic structure in Africa. The differentiation between geographic sub-regions in Africa was quantified by calculating the differentiation index F*st* (Fig. 3). Populations from Madagascar and East Africa were highly differentiated (F*st* = 0.800), whereas populations from Madagascar and West Africa on one side and populations from East Africa and West Africa on the other side were moderately differentiated (F*st* = 0.380 and 0.384, respectively).

To further characterize the geographic structure of *P. oryzae*, we studied the distribution of genotypes (MLGs) and of GCsbetween countries and between sub-regions (West Africa, East Africa and Madagascar). Most MLGs were country specific since 139 out of 148 MLGs represented by more than one strain were detected only in one country. Ten MLGs were shared between West African countries (Table 2, Supplementary Table 5). Nine of them were shared between two countries and one between three countries. Most of the time these, countries are neighbors (only one exception). In East Africa, no MLGs were shared between countries. Despite the relative proximity between East Africa and Madagascar, no MLGs were shared between the two regions.

TABLE 2. Geographic distribution of multi-locus genotypes shared between different countries

MLGs	Genetic clusters	Benin	Burkina Faso	Ghana	Côte d'Ivoire	Mali	Nigeria	Togo
118	2		1		2			
119	1		5		4			
124	2	6	2				1	
138	2		1					1
198	2	3	2					
200	2	11	2					
238	2			1			54	
243	2					3	3	
244	4					4	1	
474	2	1					1	

In West Africa, all four genetic clusters were observed in five out of seven countries (excluding countries with small sample size i.e., below six strains). Despite shared clusters, each country showed some specificity. GC2 was highly represented (56.2% and 42% respectively) in two countries (Burkina Faso and Nigeria; Table 3; Fig. 4). GC1 dominated in Côte d'Ivoire (80.4% strains) and GC4 dominated in Mali (61% strains) and Niger (100%). In Benin GC3 (45.4% strains) and GC2 (43% strains) co-dominated. In East Africa, all genetic clusters were detected but limited sample size per country only allowed to describe the population structure in Tanzania. In this country, only two GCs were detected: GC3 (24.5% of the strains) and GC4 (75.5%). In Madagascar, all four GCs were detected and GC1 was the most represented (92% strains).

Overall, the three sub-regions showed very different distribution of clusters (Fig 4, Table 3). GC2, GC4 and GC1 were dominant in West Africa, East Africa and Madagascar, respectively.

TABLE 3. Geographic structure of *Pyricularia oryzae*: number of strains per genetic cluster and country

	Genetic clusters					
Countries	**1**	**2**	**3**	**4**	**Adm**	**Total**
West Africa						**829**
Nigeria	28	103	3	1	110	245
Burkina Faso	38	118	5	37	12	210
Benin	8	71	75	10	1	165
Mali	8	30	1	64	2	105
Côte d'Ivoire	41	4	1	4	1	51
Togo		26		8		34
Niger				14		14
Ghana		4				4
Senegal					1	1
East and Central Africa						**108**
Tanzania			23	71		94
Kenya			4	1		5
Uganda		1	4			5
Burundi	1			1		2
Mozambique		1				1
Cameroon	1					1
Madagascar	1029	72	4	2	13	1120
Total	**1154**	**431**	**120**	**213**	**140**	**2057**

Adm: Admix strains; unassigned to a single genetic cluster by DAPC.

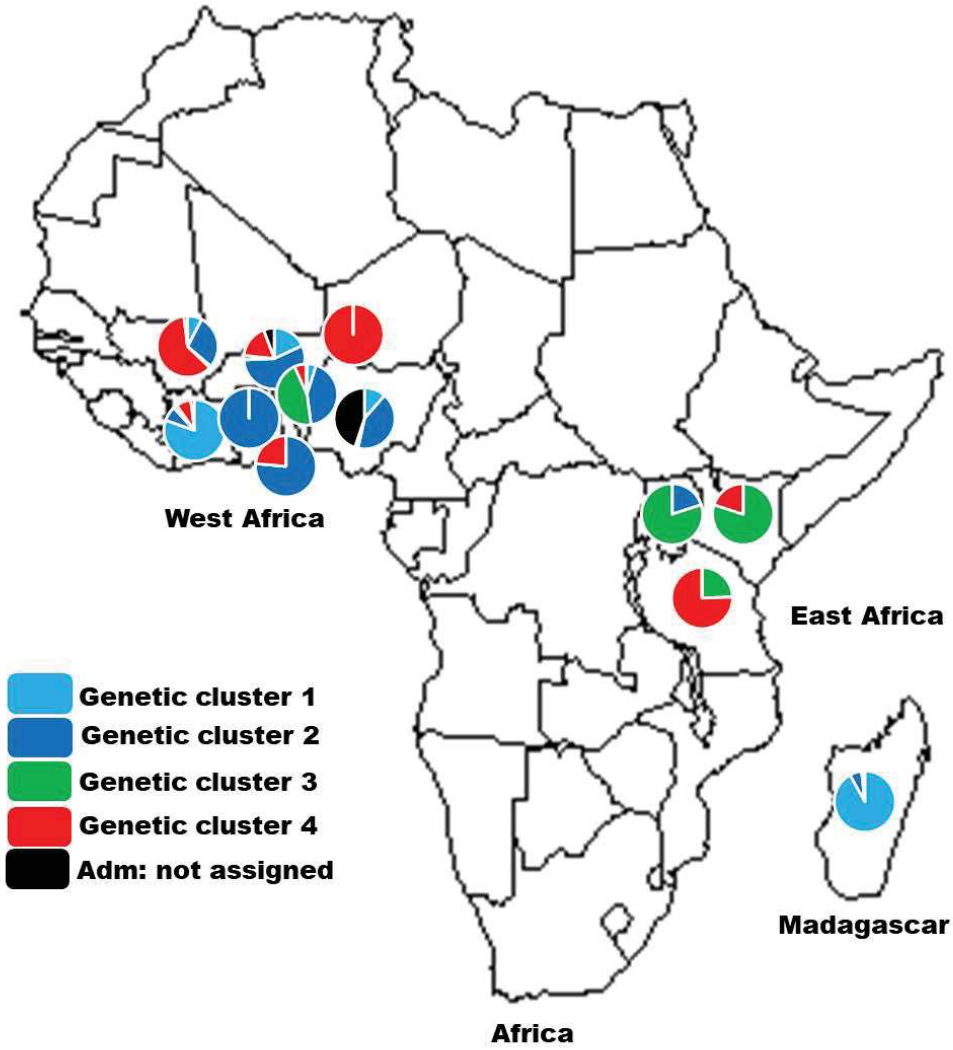
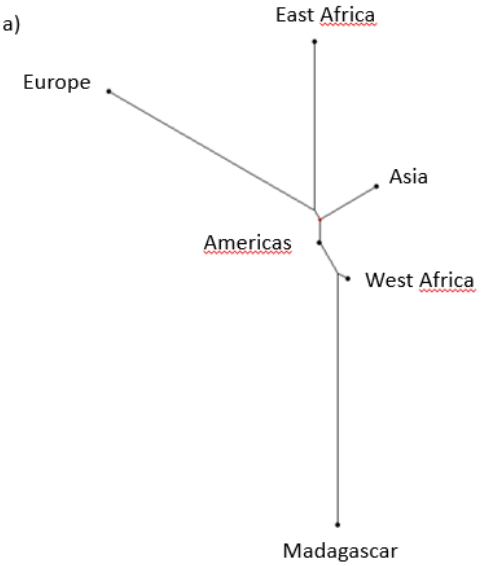


Fig. 4. Geographic distribution of genetic clusters of *Pyricularia oryzae.* Discriminant analysis of principal components method was used to classify 937 strains from West and East Africa, and 1120 strains from Madagascar. Cluster 1, light blue; cluster 2, blue; cluster 3, green; cluster 4, red; Admix strains (unassigned to a single genetic cluster), black.

Relationship between African strains and worldwide samples of *P. oryzae*. In order to make hypotheses about the origin of African populations of *P. oryzae*, we measured the differentiation between populations from geographic sub-regions in Africa and populations from other continents (Fig 3). In all pairwise comparisons with other continents, the population from West Africa was the least differentiated and the population from Madagascar was the most differentiated. Populations from West Africa, East Africa, and Madagascar were highly differentiated from the European population (F*st* values of 0.501, 0.587 and 0.768, respectively). On the contrary, the American population showed the lowest differentiation with African populations (F*st* values of 0.118, 0.253 and 0. 365, respectively for populations from West Africa, East Africa, and Madagascar). Compared to the American population, the Asian population was slightly more differentiated from the West and East African populations and much more differentiated from the population from Madagascar (F*st* values of 0.190, 0.344 and 0.651, respectively).

a)



b)

	East Africa	Madagascar	West Africa	Asia	Americas
Madagascar	0.800				
West Africa	0.384	0.381			
Asia	0.344	0.651	0.190		
Americas	0.253	0.365	0.118	0.076	
Europe	0.587	0.768	0.501	0.461	0.369

Fig. 3 Relatedness between *P. oryzae* populations from African subregions and other continents. Neighbor joining tree (a) based on F*st* values given in the table (b).

We also compared the genetic clusters found in Africa in this study to those identified on a worldwide sample by Saleh et al. (2014; Table 4). Saleh et al. identified three worldwide genetic clusters (A, B and C) and four Asian genetic clusters (1 to 4). In our study, 208 out of 210 strains previously assigned to worldwide cluster A were assigned to GC4. Asian strains assigned to GC4 in our study, were clustered mainly in Asian clusters 1 and 4 (39.9% and 43.3% of strains, respectively). A large majority (492 out of 499) of the strains assigned by Saleh et al. to cluster B were assigned to GC3. All 63 Asian strains assigned to GC3, had been assigned to Asian cluster 2 by Saleh et al (2014). Most the strains (327 out of 449) assigned by Saleh et al. to cluster C were assigned to GC1; 108 were assigned to GC2 (24.1%) and 14 could not be assigned (3.1%). The majority of Asian strains (72 out of 78) assigned to GC2 had been assigned to Asian cluster 3 by Saleh et al. (2014) (the remaining strains were not assigned to a unique GC in our study). Altogether, these results show that GC4, GC3 and GC1 identified in this study correspond respectively to Cluster A, B and C of Saleh et al. GC2 also corresponds to Cluster C. GC2 and 3 correspond to Asian clusters 3 and 2 respectively, and GC4 gathers strains from Asian clusters 1 and 4.

TABLE 4. Distribution of genetic clusters defined by Saleh et al. (2014) and genetic clusters identified in this study

Genetic groups (Saleh et al 2014)		Genetic Clusters (this study)					
World	Asia	1	2	3	4	Adm	Total
A	1			2	83		85
	2				4		4
	3				2		2
	4				90		90
	NA				29		29
B	2			63			63
	NA			429	7		436
C	3		72			6	78
	NA	327	36			8	371
Total World (Saleh et al. 2014)		327	108	494	215	14	1158
Africa and Madagascar (this study)		1154	430	120	213	140	2057

A/B/C and 1 to 4 correspond to groups defined by Saleh et al. (2014) for worldwide and Asian samples respectively; NA: Non-Asian strains; Adm: admix strains (strains unassigned to a single genetic cluster by DAPC).

These results show that the genetic clusters to which African strains belong are also found in other continents. In particular, all the genetic clusters identified in Asia by Saleh et al. (2014) are detected in Africa.

MLGs shared between different continents were searched for. We identified no MLGs shared between African and Madagascan strains and other continents. The single shared MLG identified by Saleh et al. (2014) between one strain of Madagascar and one strain of Indonesia was not found because this Indonesian strain was missing in the present data set. Thus, this strain was excluded from our analyses because of uncertainty on the host of origin, although the identity of genotypes was confirmed by reexamination of the data.

The genotypic diversity in West Africa and Madagascar (Shannon and Genotypic diversity indexes in Supplementary Table 3) was higher than in Americas and Europe. It was lower than in Asia, which is considered as the center of diversity (and include the putative center of origin) of the blast fungus. The population from East Africa was the least diverse.

Mating type, fertility of strains and linkage disequilibrium. The results of mating type showed contrasted distributions between West Africa, East Africa and Madagascar with Mat1.1/Mat1.2 ratios of 20/72, 20/1, and 1/320 respectively (Supplementary Table 6). These ratios were significantly different from 1:1 (Chi^2 = 29.4, 17.2, and 317.0 respectively; P value < 0.001 for all three tests). Both mating types were found in all GCs (Table 5) but one mating type was largely dominant for each GC: Mat1.1 for GC3 and GC4 (89.5% and 95.2% of strains respectively), Mat1.2 for GC1 and GC2 (99.7% and 94.7% respectively). All these frequencies were significantly different from 50% (Chi^2 = 297, 60.8, 11.8, 17.2; P value ≤ 0.001 for all four tests). None of the 245 strains tested produced perithecia in crossing with reference strains. Thus, no female-fertile strain was identified in this representative sample.

Linkage disequilibrium analysis demonstrated a general trend towards non-random association of alleles at different loci for the three geographic populations in Africa. Significant (5% level) pairwise linkage disequilibrium was detected for 86%, 95% and 100% of the 66 pairs of loci for the populations from West Africa, Madagascar and East Africa, respectively.

TABLE 5. Distribution of the mating type of 435 selected strains and of the number of strains analyzed for female fertility in the different genetic clusters found in Africa and Madagascar

Genetic clusters	Mating type				[z]strains tested for female-fertility (n)	N
	1.1	1.2	Total	1:1 ratio Chi² (p value)		
1	1	300	301	297 (<0.001)	141	1154
2	4	72	76	60.8 (<0.001)	54	430
3	17	2	19	11.8 (0.001)	18	120
4	20	1	21	17.2 (0.001)	21	213
Adm	0	18	18	18.0 (<0.001)	11	140
Total	42	393	435	283 (<0.001)	245	2057

Adm: admix strains unassigned to a single genetic cluster. N is the total number of strains per genetic clusters. [z] Strains were tested *in vitro* for their female-fertility. All tested strains were female-sterile.

DISCUSSION

The presence of repeated Multi-locus genotypes (MLGs), the distribution of strains per MLG, and the non-random association of alleles at different loci were indicative of a clonal structure of the African populations (including Madagascar). Clonal reproduction of *P. oryzae* rice populations is the rule (Saleh et al. 2012b). We here confirmed that both mating types are present in Africa (Nottéghem and Silué 1992; Takan et al. 2012; Onaga et al. 2015) and sometimes in the same country. But the coexistence of strains of both mating types is not sufficient for sexual reproduction to take place. The presence of female fertile strains is also required (Saleh et al. 2012a; Saleh et al. 2012b; Saleh et al. 2014). We did not find any female-fertile strains in Africa. Mating types also deviated very significantly from the 1:1 ratio expected for random mating. These results are consistent with the clonal population structure observed and also supports the hypothesis that sexual reproduction is absent in Africa. As shown at a broader scale (Thierry et al. 2020), *P. oryzae* genetic clusters on rice coexist and have no or very limited gene exchanges out of Asia.

Genetic differentiation between East and West African populations of the rice blast fungus was previously observed by Mutiga et al. (2017) on a limited number of samples. With a larger sample and on a broader geographic area, we revealed that differences between populations from the subcontinental regions of West Africa, East Africa and Madagascar were marked: mid to high differentiation index values, no MLGs were shared between them and frequencies of genetic clusters were different between these sub-regions. Although each

country showed specificity, MLGs were shared between neighboring countries in West Africa. This pattern is consistent with a combination of frequent short distance and rare events of long-distance migrations due to natural dispersion of *P. oryzae* spores and transportation of infected seeds respectively (Tharreau et al. 2009), exchanges of infected seeds within a subcontinental region likely being more frequent than between subcontinental regions.

We showed the presence in Africa of the three worldwide clusters and the four Asian clusters previously described (Saleh et al. 2014). To date, Asia was the only continent in which the three worldwide clusters were represented within a given local geographic area. In addition to belonging to all genetic clusters, we identified African populations (genetic cluster 4) related to the Asian clusters 1 and 4, which encompass the most genetically diverse populations (Saleh et al. 2014). This places Africa in second position after Asia in terms of genetic diversity of *P. oryzae* at the continental level. High genotypic diversity could be explained by two mechanisms. Sexual reproduction generates genotypic diversity by recombination. But, based on our results and similarly to other non-Asian populations, sexual reproduction seems unlikely in African *P. oryzae* populations. Genotypic diversity may also result from multiple introductions. This hypothesis is supported by the fact that all genetic clusters found in Africa are also found in Asia. According to the literature, the Asian rice (*O. sativa*) was introduced to East Africa (by 10th century AD) by Polynesians using the southern Indian Ocean route from Indonesia to Madagascar and East Coast of Africa (Carpenter 1978; Crowther et al. 2016). It was subsequently introduced to West Africa, by the Portuguese in about 1,500 AD through the Atlantic Ocean coast (Portéres 1950). Introductions of new varieties for the local development of new rice cropping systems and of accessions for breeding purposes have also taken place during the last century with limited attention to phytosanitary quality of seeds. The high genetic diversity of *P. oryzae* observed in Africa is likely to have resulted from multiple introductions of rice infected seeds.

In summary, this work sheds new light on the genetic structure of *P. oryzae* strains in Africa. Unexpectedly, the genetic diversity of the fungus in Africa is high, placing the continent in second position after Asia. In addition, clusters identified in Africa were shared with other continents and no genetic clusters specific to Africa was detected. Such diversity increases the risk of emergence of new pathotypes in Africa. So, it is urgent to monitor the evolution of the pathogen populations in this area and to propose new and durable strategies for the deployment of resistance genes, exploiting knowledge of the genetic structure of the pathogen.

ACKNOWLEDGEMENTS

We thank Christophe Tertois and Youssouf Diarra for their contribution. This work was supported by grants of the RICE CRP and the Grisp (Menergep project), a financial support from Nick Talbot lab to Théophile Odjo, a grant from the French Embassy in Mali to Diariatou Diagne, IRD support to Ousmane Koita group (JEAI CoANA) and the support of Institutes of the coauthors (CIRAD, INERA, USTT-B). Genotyping Data used in this work were produced through the GenSeq technical facilities of the « Institut des Sciences de l'Evolution de Montpellier » with the support of LabEx CeMEB, an ANR "Investissements d'avenir" program (ANR-10-LABX-04-01). We are grateful to the numerous colleagues who contributed to the collection of samples or provided strains.

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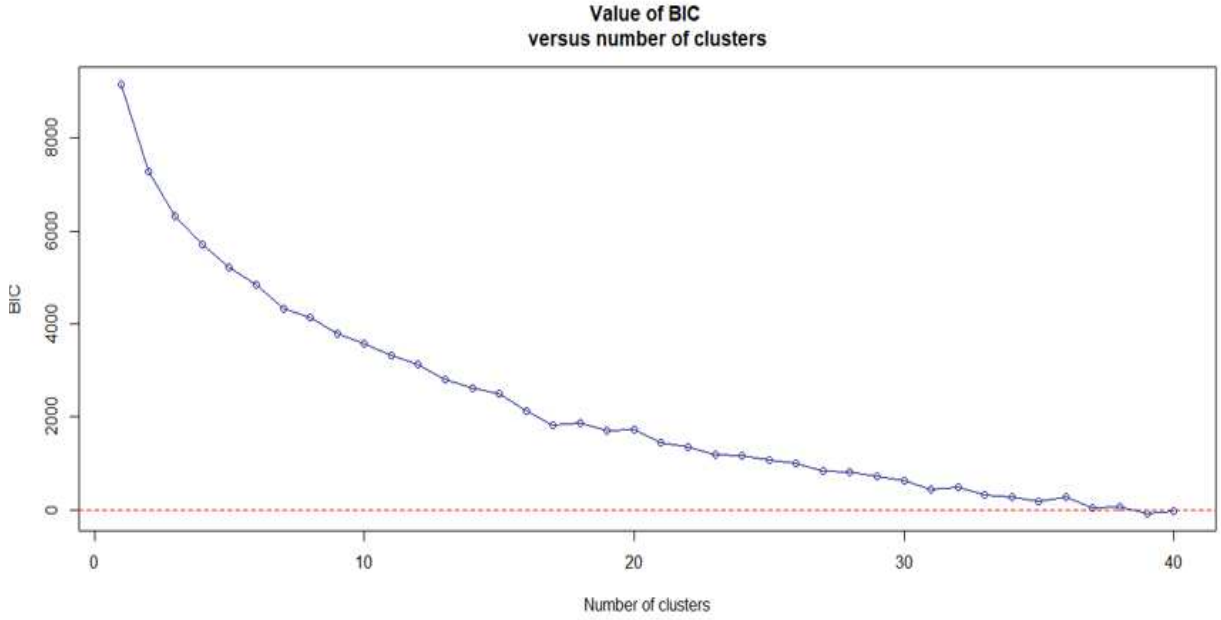
Supplementary FIGURE 1: Variation of Bayesian Information Criteria value with the number of genetic clusters (from 1 to 40).



Supplementary TABLE 1. Geographic origin of samples of *Pyricularia* oryzae collected in West and East Africa

Country	Site	Type of the site	Year of the	Variety	Ecosystem	Host	Strain
Benin							165
	Agoua	unknown	2013	1	Rainfed lowland	*Oryza*	4
	Bétérou	Farmer field	2012	1	Rainfed lowland	*Oryza*	4
	Calavi	Experimental	2010	2	Rainfed upland	*Oryza*	3
	Calavi	Experimental	2011	1	Rainfed upland	*Oryza*	3
	Djougou	Farmer field	2013	2	Rainfed lowland	*Oryza*	5
	Gogounou	unknown	2013	1	Rainfed lowland	*Oryza*	1
	Kalalé	Farmer field	2013	1	Rainfed lowland	*Oryza*	13
	Kérou	Farmer field	2012	3	Rainfed upland	*Oryza*	33
	Kokey (Banikoara)	Farmer field	2012	1	Rainfed lowland	*Oryza*	4
	Kommon (Banikoara)	Farmer field	2012	1	Rainfed lowland	*Oryza*	3
	Lokossa	Farmer field	2010	42	Irrigated	*Oryza*	65
	Lokossa	unknown	2011	4	Irrigated	*Oryza*	4
	Malanville	unknown	2011	1	Rainfed lowland	*Oryza*	2
	Natitingou	Farmer field	2012	1	Rainfed lowland	*Oryza*	5
	N'Dali	unknown	2013	1	Rainfed lowland	*Oryza*	3
	Papane	unknown	2013	1	Rainfed lowland	*Oryza*	3
	Sinendé	Farmer field	2013	1	Rainfed lowland	*Oryza*	3
	Suru	unknown	2013	1	Rainfed lowland	*Oryza*	1
	Tanguiéta	Farmer field	2013	1	Rainfed upland	*Oryza*	6
Burkina Faso							210
	Bagré	Farmer field	2009	1	Irrigated	*Oryza*	1
	Bagré	Farmer field	2013	1	Irrigated	*Oryza*	1
	Bagré	Farmer field	2014	1	Irrigated	*Oryza*	1
	Bagré	Farmer field	2015	1	Irrigated	*Oryza*	2
	Banfora	Experimental	2013	7	Rainfed lowland	*Oryza*	33
	Banfora	Experimental	2014	10	Rainfed lowland	*Oryza*	13
	Banfora	Experimental	2015	6	Rainfed lowland	*Oryza*	13
	Banzon	Farmer field	2013	4	Rainfed lowland	*Oryza*	26

	Banzon	Farmer field	2014	1	Rainfed lowland	*Oryza*	1
	Fada	Farmer field	2015	1	Rainfed lowland	*Oryza*	1
	Farako-bâ	Experimental field	2013	3	Rainfed upland	*Oryza*	3
	Farako-bâ	Experimental field	2014	23	Rainfed upland	*Oryza*	43
	Farako-bâ	Experimental field	2015	6	Rainfed upland	*Oryza*	7
	Karfiguela	unknown	2013	1	Rainfed lowland	*Oryza*	3
	Karfiguela	unknown	2014	1	Rainfed upland	*Oryza*	1
	Loto	unknown	2009	1	Rainfed upland	*Oryza*	4
	Loto	unknown	2013	1	Rainfed upland	*Oryza*	1
	Mongtedo	Farmer field	2015	1	Rainfed lowland	*Oryza*	1
	Nare yô	Farmer field	2015	1	Rainfed upland	*Oryza*	3
	Navielgane	Farmer field	2013	1	Rainfed upland	*Oryza*	4
	Ndorola	Farmer field	2013	2	Rainfed lowland	*Oryza*	12
	Niéna Dionkéle	Experimental field	2009	9	Rainfed lowland	*Oryza*	11
	Ouezindougou	Farmer field	2013	1	Rainfed upland	*Oryza*	3
	Pankatiora	Farmer field	2013	1	Rainfed lowland	*Oryza*	1
	Pankatioro	Farmer field	2013	unknown	Rainfed lowland	*Oryza*	3
	Sagoume	Farmer field	2015	1	Rainfed upland	*Oryza*	1
	Uezindougou	Farmer field	2013	1	Rainfed upland	*Oryza*	1
	Vallée du kou	unknown	2013	2	Irrigated	*Oryza*	15
	Vallée du kou	unknown	2014	1	Irrigated	*Oryza*	1
Ivory Coast							51
	Agboville	Farmer field	2014	1	Rainfed lowland	*Oryza*	19
	Bouaké	unknown	1981	1	unknown	*Oryza*	2
	Man	Farmer field	1984	1	unknown	*Oryza*	1
	Odienne	Experimental field	1984	2	unknown	*Oryza*	2
	Odienne	Experimental field	1989	2	unknown	*Oryza*	2
	Tiassalé	Farmer field	2014	1	Rainfed lowland	*Oryza*	23
	San Pedro	Farmer field	1983	1	unknown	*Oryza*	1
	Waninou	Farmer field	1984	1	unknown	*Oryza*	1
Ghana							4

	Akuse AGRIC farms	Farmer field	2009	1	Irrigated	*Oryza*	2
	unknown	unknown	2010	1	Irrigated	*Oryza*	1
	Akuse AGRIC farms	Farmer field	2012	1	Irrigated	*Oryza*	1
Kenya							5
	Ahero	Farmer field	2014	1	unknown	*Oryza*	2
	Mwea	Farmer field	2013	1	unknown	*Oryza*	2
	Mwea	Farmer field	2014	1	unknown	*Oryza*	1
Mali							105
	Loulouni Faraka	Farmer field	2018	1	Rainfed lowland	*Oryza*	1
	Manikoura	Experimental	2018	1	Irrigated	*Oryza*	14
	M'pegnesso	Farmer field	2017	1	Rainfed lowland	*Oryza*	1
	M'pegnesso	Farmer field	2018	2	Rainfed lowland	*Oryza*	9
	Niena	Farmer field	1986	1	Rainfed lowland	*Oryza*	1
	Niena	Farmer field	2018	1	Rainfed lowland	*Oryza*	7
	Niono N7	Farmer field	2018	1	Irrigated	*Oryza*	6
	Niono Nango N3-Bis	Farmer field	2018	1	Irrigated	*Oryza*	25
	Niono Nango Sahel	Farmer field	2018	1	Irrigated	*Oryza*	8
	Sélingué	Farmer field	2009	1	Irrigated	*Oryza*	1
	Sélingué	Farmer field	2017	2	Irrigated	*Oryza*	10
	Sélingué	Farmer field	2018	1	Irrigated	*Oryza*	13
	Sikasso	Farmer field	2009	4	Irrigated	*Oryza*	4
	Sikasso	Farmer field	2011	1	Irrigated	*Oryza*	5
Niger							14
	unknown	Farmer field	2013	1	Rainfed upland & lowland	*Oryza*	14
Nigeria							245
	Abéocuta	Farmer field	2010	1	Irrigated	*Oryza*	12
	Edozhigi	Farmer field	2009	2	Irrigated	*Oryza*	6
	Edozhigi	Farmer field	2010	2	Irrigated	*Oryza*	6
	Ibadan	Experimental	2009	20	Rainfed upland	*Oryza*	131
	Ibadan	Experimental	2010	16	Rainfed upland	*Oryza*	42
	Ikenne	Experimental	2010	15	Rainfed upland	*Oryza*	46

	Lagos	Farmer field	2010	1	Irrigated	*Oryza*	2
Tanzania							94
	Ifakara	Farmer field	2013	1	Irrigated	*Oryza*	16
	Ifakara	Farmer field	2013	2	Rainfed upland	*Oryza*	47
	Kyela	Farmer field	2013	1	Rainfed upland	*Oryza*	12
	Kyela	Farmer field	2013	2	Rainfed upland	*Oryza*	19
Togo							34
	Assomè	Farmer field	2012	1	Irrigated	*Oryza*	25
	Elavayo	Farmer field	2012	2	Rainfed upland	*Oryza*	5
	Kovié	Farmer field	2014	1	Irrigated	*Oryza*	1
	Sankargo	Farmer field	2014	1	Rainfed lowland	*Oryza*	1
	Toaga	Farmer field	2014	1	Rainfed lowland	*Oryza*	1
	Yelivo	Farmer field	2014	1	Rainfed lowland	*Oryza*	1
Uganda							5
	Bulega	Farmer field	unknown	1	unknown	*Oryza*	1
	Butaleja	Farmer field	unknown	2	unknown	*Oryza*	3
	Namulonge	Farmer field	unknown	1	unknown	*Oryza*	1
Burundi							2
	Noebe	Farmer field	1989	1	unknown	*Oryza*	1
	Ntega	Farmer field	1988	1	unknown	*Oryza*	1
Cameroon	M'Bos	unknown	1987	1	unknown	*Oryza*	1
Mozambique	unknown	unknown	1999	1	unknown	*Oryza*	1
Senegal	Djibelor Plateau	unknown	1986	1	unknown	*Oryza*	1
Madagascar							1120
	unknown	Farmer field	2003	1	Rainfed upland	*Oryza*	1
	Ambaiboho	Farmer field	2005	1	Rainfed lowland	*Oryza*	2
	Ambatolampy	Farmer field	2005	2	Irrigated	*Oryza*	6
	Ambatomainty	Farmer field	2015	1	Rainfed upland	*Oryza*	17
	Ambohitsilaozana	Farmer field	2001	1	Rainfed lowland	*Oryza*	4
	Amparafaravola	Farmer field	2005	1	Rainfed lowland	*Oryza*	1
	Andraikiba Mangalaza	Farmer field	2003	1	Rainfed upland	*Oryza*	2

Andranomanelatra	Experimental	2003	3	Rainfed upland	*Oryza*	6
Andranomanelatra	Experimental	2004	6	Rainfed upland	*Oryza*	17
Andranomanelatra	Experimental	2005	3	Rainfed upland	*Oryza*	138
Andranomanelatra	Experimental	2006	2	Rainfed upland	*Oryza*	112
Andranomanelatra	Farmer field	2015	2	Rainfed upland	*Oryza*	66
Andranomanelatra	Farmer field	2016	2	Rainfed upland	*Oryza*	40
Antananarivo	Farmer field	2003	undetermined	Irrigated	*Oryza*	2
Antananarivo	Farmer field	2005	1	Irrigated	*Oryza*	1
Antanifotsy	Farmer field	2005	undetermined	Rainfed lowland	*Oryza*	2
Antanivao	Farmer field	2001	2	Rainfed lowland	*Oryza*	2
Antsapanimahazo	Farmer field	2005	1	Rainfed lowland	*Oryza*	3
Antsapanimahazo	Farmer field	2016	2	Rainfed upland & lowland	*Oryza*	61
Antsirabe	Farmer field	2003	1	Rainfed lowland	*Oryza*	7
Antsirabe	Farmer field	2004	4	Rainfed lowland	*Oryza*	9
Antsirabe	Farmer field	2005	2	Rainfed upland & lowland	*Oryza*	7
Belazao	Farmer field	2003	1	Rainfed lowland	*Oryza*	2
Belazao	Farmer field	2004	7	Rainfed upland & lowland	*Oryza*	14
Belazao	Farmer field	2005	2	Rainfed lowland	*Oryza*	5
Cala	Experimental	2001	1	Rainfed lowland	*Oryza*	3
Fiantsonana	Farmer field	2015	1	Rainfed upland	*Oryza*	19
Fiantsonana	Farmer field	2016	2	Rainfed upland	*Oryza*	96
Fifamanor	Experimental	2004	22	Rainfed upland	*Oryza*	41
Ibity	Experimental	2001	2	Rainfed upland	*Oryza*	2
Ibity	Farmer field	2003	4	Rainfed upland	*Oryza*	6
Ibity	Farmer field	2004	1	Rainfed upland	*Oryza*	2
Ibity	Farmer field	2005	1	Rainfed upland & lowland	*Oryza*	1
Ivory	Farmer field	2004	2	Rainfed upland	*Oryza*	5
Manakara	Experimental	2006	2	Rainfed upland	*Oryza*	13
Manandona	Farmer field	2002	1	Irrigated	*Oryza*	3
Manandona	Farmer field	2003	1	Irrigated	*Oryza*	2
Manandona	Farmer field	2004	3	Irrigated	*Oryza*	7

Manandona	Farmer field	2005	1	Irrigated	*Oryza*	9
Mananetivohitra	Experimental	2015	5	Rainfed upland & lowland	*Oryza*	105
Mananetivohitra	Experimental	2016	2	Rainfed upland & lowland	*Oryza*	97
Mangalaza	Farmer field	2003	1	Rainfed upland	*Oryza*	1
Mangalaza	Farmer field	2004	2	Rainfed upland	*Oryza*	5
Mangalaza	Farmer field	2005	5	Rainfed upland & lowland	*Oryza*	31
Morarano	Experimental	2015	3	Rainfed upland & lowland	*Oryza*	34
Morarano	Experimental	2016	3	Rainfed upland & lowland	*Oryza*	58
Moratsiazo	Farmer field	2004	undetermined	Rainfed upland	*Oryza*	3
PK47 to Mandoto	Farmer field	2003	1	unknown	*Oryza*	1
Sambaina	Farmer field	1992	1	Irrigated	*Oryza*	1
Soanindrariny	Farmer field	2005	1	Rainfed upland	*Oryza*	5
Talata	Experimental	2001	12	Rainfed upland	*Oryza*	19
Tamatave	unknown	1977	1	unknown	*Oryza*	1
Tongarivo	Farmer field	2016	1	Rainfed upland	*Oryza*	17
Tsaramody	Farmer field	2003	1	Rainfed lowland	*Oryza*	2
Tsi-kamo	Farmer field	2001	1	Rainfed lowland	*Oryza*	1
Vinaninkarena	Farmer field	2005	2	Rainfed lowland	*Oryza*	3
Total						**2057**

Supplementary TABLE 2. Primers and polymorphism of the 12 Simple Sequence Repeat (SSR) markers used for genotyping (Adreit, et al., 2007)

[a] Number and size of alleles in the African population. [b] PIC: Polymorphic Information content.

Name	Chromo-some	Primers	Number of alleles[a]	PIC[b]	Size (pb)[a]
Pyrms63	1	F:TTGGGATCTTCGGTAAGACG R: GCCGACAAGACACTGAATGA	3	0.22	149-161
Pyrms37	4	F:ACCCTACCCCCACTCATTTC R: AGGATCAGCCAATGCCAAGT	3	0.21	197-209
Pyrms319	2	F:TAAGACCACTGGCGGAATCT R: GGCTTTGTCTGGTTGTACGG	3	0.24	281-287
Pyrms657	6	F:ATCAGTCGAACCCACAAAGC R: ATGTGTGGACGAACCAGTCC	4	0.30	164-170
Pyrms427	5	F:CTGTCACCACAACCAAGACG R: TTGCCCTGATTTGTCAGTCA	9	0.61	199-221
Pyrms607	3	F:CCCAAGCTCCATAATACGCTAC R:TCCGAGACTCTTTGGATAGCAC	4	0.35	272-287
Pyrms233	5	F:TGAGATGGACCGCATGATTA R: TTGATGGCAGAGACATGAGC	6	0.33	241-268
Pyrms47	4	F:TCACATTTGCTTGCTGGAGT R: AGACAGGGTTGACGGCTAAA	7	0.49	157-169
Pyrms77B	3	F:AGGCTCTCTGCCTACGAAGT R: GCTTTCGGCAAGCCTAATC	7	0.44	194-226
Pyrms83B	2	F:GTCTGCCTCGACTCCTTCAC R: GCAAAGTTGTTTGAGCAAGG	4	0.62	103-115
Pyrms99B	5	F:CACCACTTTATGGCGCAGT R: ACCTAGGTAGGTATACATGTTGTT	11	0.53	226-313
Pyrms409	4	F: TCCCAGTACTTGCCCATCTC R: ATCTCATATCCGTCGGTCGT	15	0.68	299-343

Supplementary Table 3: *Pyricularia oryzae* diversity indices by geographic area and per Genetic Cluster

Geographic origin	Genetic Cluster	Number of isolates	Number of MLGs	Number of isolates assigned to MLGs[1]	Clonal fraction[2]	Shannon index[3]	Eveness[4]	Genotypic diversity[5]	Hnb[6]	Na[7]	Np[8]
East Africa	1	1	1	0	-[9]	-	-	-	-	-	-
	2	2	2	2	-	-	-	-	-	-	0
	3	31	15	22	0.32	2.4	0.89	0.86	0.24	2.58	0
	4	73	17	72	0.76	1.7	0.61	0.64	0.11	3.17	0
	All isolates	107	34	96	0.65	2.5	0.71	0.79	0.47	5.25	0
West Africa	1	124	31	115	0.73	2.9	0.86	0.93	0.23	3.00	0
	2	356	120	336	0.64	4.1	0.86	0.96	0.28	7.17	0.08
	3	85	19	84	0.77	1.3	0.46	0.47	0.16	3.92	0.42
	4	138	68	125	0.46	3.7	0.88	0.94	0.63	9.25	0.50
	nd[10]	127	12	123	0.90	1.2	0.49	0.53	0.14	2.75	0
	All isolates	830	250	783	0.68	4.6	0.83	0.97	0.52	13.08	4.00
Madagascar	1	1029	66	263	0.75	3.4	0.81	0.94	0.09	4.00	0.17
	2	72	27	61	0.56	2.6	0.80	0.86	0.31	3.67	0.17
	3	4	4	4	-	-	-	-	-	-	-
	4	2	2	2	-	-	-	-	-	-	-
	nd	13	10	13	0.23	2.2	0.98	0.89	0.28	2.00	0
	All isolates	1120	109	343	0.68	3.9	0.83	0.96	0.11	6.50	1.67
Americas	1	4	2	3	-	-	-	-	-	-	-
	2	34	14	33	0.58	2.2	0.84	0.84	0.25	2.08	0
	3	48	16	47	0.66	1.9	0.68	0.70	0.20	2.33	0
	4	37	14	24	0.42	2.3	0.88	0.85	0.44	3.42	0
	nd	9	2	2	-	-	-	-	-	-	-
	All isolates	132	48	109	0.56	3.3	0.84	0.92	0.56	5.67	0.00
Asia	1	17	9	17	0.47	1.9	0.88	0.81	0.23	1.92	0

	2	396	162	310	0.48	4.7	0.93	0.99	0.36	6.92	0.17
	3	172	74	163	0.55	3.7	0.86	0.95	0.36	6.83	0.25
	4	740	301	637	0.53	4.7	0.82	0.95	0.67	13.75	1.67
	nd	41	16	37	0.57	2.4	0.86	0.87	0.32	3.25	0
	All isolates	1366	562	1164	0.52	5.6	0.88	0.98	0.68	15.33	3.27
Europe	2	1	1	1	-	-	-	-	-	-	-
	3	1530	163	1260	0.87	3.3	0.65	0.88	0.25	4.92	0.17
	4	29	10	29	0.66	1.6	0.68	0.63	0.19	2.50	0
	All isolates	1560	174	1290	0.87	3.4	0.66	0.89	0.25	5.67	1.00
All		5115	1177	3787	0.69	5.8	0.82	0.98		18.40	-

1: Due to missing data, some genotypes could be assigned to a single MLG. Only isolates assigned unambiguously to a single MLG are counted in this column. 2: Clonal fraction was calculated as 1- (nb of MLGs/nb of isolates assigned to MLGs).

3: Shannon index was calculated folowing the formula: -Sum(pi*Ln(pi) where pi is the frequency of the ith MLG. 4: Evenness was calculated following the formula: Shannon index/Ln (Nb of MLGs).

5: Genotypic diversity was calculted following the formula: 1-Sum(pi^2) where pi is the frequency of the ith MLG.

6: Unbiased gene diversity (Nei, 1987).

7: Average number of alleles per locus.

8: Average number of private alleles per locus.

9: When population size was below 10 isolates, indices were not calculated.

10: Some admix genotypes could not assigned to a single Genetic Cluster (GC) and the GC was thus considered as not determined (nd).

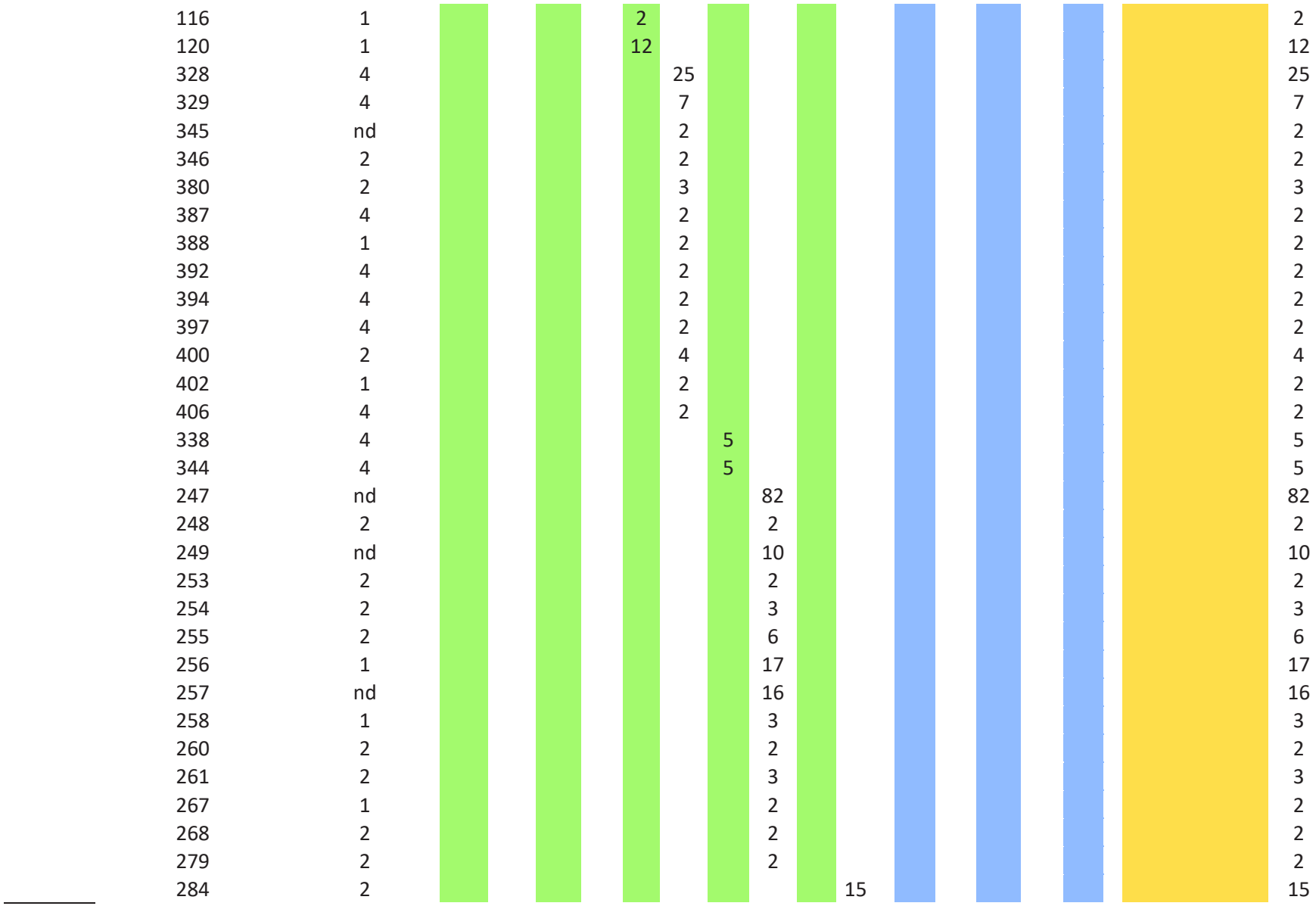
Supplementary Table 4. Differentiation between genetic clusters of *Pyricularia oryzae* in Africa. F*st* values between pairs of populations defined by clustering.

Genetic clusters	1	2	3
2	0.37	-	-
3	0.77	0.58	-
4	0.65	0.46	0.41

Supplementary TABLE 5: Geographic distribution of Multi-Locus Genotypes between countries

	MLGs	Genetic clusters	N° of strains															
			West Africa									East Africa					Madagascar	Total
			BN	BF	CM	GH	CI	ML	NR	NG	SN	TG	BD	KN	MZ	OG	TN	MD	
MLGs sampled in more than one African country	118	2		1			2												3
	119	1		5			4												9
	124	2	6	2						1									9
	138	2		1								1							2
	198	2	3	2															5
	200	2	11	2															13
	238	2				1				54									55
	243	2						3		3									6
	244	4						4		1									5
	474	2	1							1									2
MLGs specific of one African country	206	2	5																5
	207	2	6																6
	209	3	4																4
	210	2	5																5
	211	1	8																8
	223	3	61																61
	226	3	3																3
	228	4	2																2
	229	2	3																3
	231	2	3																3
	232	2	5																5
	233	2	4																4
	234	2	3																3
	235	2	2																2
	480	4	3																3
	123	1		3															3
	125	2		2															2
	126	2		10															10

127	1		10										10
130	2		3										3
131	2		2										2
133	2		22										22
134	2		2										2
135	1		4										4
136	2		2										2
142	2		5										5
144	2		4										4
152	4		2										2
153	2		7										7
157	2		5										5
159	2		2										2
163	1		4										4
167	2		2										2
168	4		2										2
170	2		2										2
173	2		2										2
174	2		3										3
175	2		2										2
178	2		2										2
180	1		3										3
183	nd		6										6
185	1		5										5
186	4		2										2
187	4		3										3
191	2		2										2
201	2		2										2
202	1		2										2
239	2				1								2
240	2				2								2
114	1					13							13



116	1			2									2
120	1			12									12
328	4				25								25
329	4				7								7
345	nd				2								2
346	2				2								2
380	2				3								3
387	4				2								2
388	1				2								2
392	4				2								2
394	4				2								2
397	4				2								2
400	2				4								4
402	1				2								2
406	4				2								2
338	4					5							5
344	4					5							5
247	nd						82						82
248	2						2						2
249	nd						10						10
253	2						2						2
254	2						3						3
255	2						6						6
256	1						17						17
257	nd						16						16
258	1						3						3
260	2						2						2
261	2						3						3
267	1						2						2
268	2						2						2
279	2						2						2
284	2							15					15

285	4						3					3
287	2						2					2
288	2						2					2
293	2						3					3
296	4									42		42
297	4									2		2
298	3									7		7
301	4									4		4
303	4									4		4
307	4									2		2
309	4									6		6
311	4									2		2
312	3									2		2
489	1										4	4
490	1										3	3
491	1										29	29
492	2										2	2
493	1										14	14
496	2										2	2
497	1										42	42
498	1										12	12
499	1										2	2
502	1										2	2
504	1										22	22
507	2										3	3
510	1										5	5
511	nd										2	2
512	1										3	3
515	1										3	3
516	1										13	13
518	2										2	2
520	1										2	2

521	1																2	2
522	1																4	4
523	2																2	2
525	1																2	2
532	1																4	4
544	2																2	2
545	nd																2	2
546	nd																2	2
553	1																2	2
555	1																2	2
558	1																2	2
560	1																18	18
564	1																2	2
566	1																5	5
567	1																10	10
573	1																2	2
575	1																2	2
577	1																3	3
584	1																4	4
585	2																20	20
587	2																9	9
589	1																2	2
591	1																2	2
592	1																2	2
593	1																4	4
N° of unique MLGs		21	53	1	1	9	35	4	24	0	7	1	3	1	3	17	65	245
N° of ND strains		6	15			9	6		9	1	1	1	2		2	6	777	835
Total N° of strains		165	210	1	4	51	105	14	245	1	34	2	5	1	5	94	1120	2057

Supplementary Table 6: Geographic distribution of mating types

		Mating type	
Region	**Country**	**Mat1.1**	**Mat1.2**
West Africa	Benin	7	12
	Burkina Faso		1
	Cameroun		1
	Ghana		4
	Ivory Coast	2	5
	Mali	4	4
	Nigeria	1	39
	Senegal		1
	Togo	6	5
East Africa	Burundi	1	
	Mozambique		1
	Tanzania	19	
Madagascar		1	320
	Total	**41**	**393**

Chapitre 3

Wild and Cultivated Rice Host Different Populations of the Blast Fungus, *Pyricularia oryzae*, in Mali

CHAPITRE 3 : article 3 « Wild and cultivated rice host different populations of the blast fungus, *Pyricularia oryzae*, in Mali »

Wild and cultivated rice host different populations of the blast fungus, *Pyricularia oryzae*, in Mali

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ABSTRACT

Blast is a devastating disease of rice caused by the fungus *Pyricularia oryzae*. The role of infected seeds and infected straws as primary inoculum sources is well known. The role of alternative hosts is yet to be confirmed. The current study sought to assess if wild rice is a major source of inoculum for cultivated rice by comparing the genetic structure of *P. oryzae* populations from both hosts. Cross infectivity of *P. oryzae* isolates was also assessed using pathogenicity tests. Samples were collected from cultivated and wild rice organs with blast symptoms in irrigated and lowland areas of Mali in Koulikoro, Sikasso, and Ségou regions. Under controlled conditions, *P. oryzae* isolates from wild rice were pathogenic to cultivated rice but, on average, appeared to have a narrower range of cultivar compatibility. Results of pathogenicity tests suggest that *P. oryzae* isolates from wild rice have the potential to attack cultivated rice in the field. However, populations of *P. oryzae* on cultivated and wild rice were genetically differentiated. Thus, although physically close, each host plant is hosting a different population of the pathogen and our results support the hypothesis that wild rice is not a source of blast inoculum for cultivated rice.

INTRODUCTION

Blast is recognized as one of the most devastating diseases of rice in the world (Savary et al. 2000; Gurr et al. 2011). The disease is caused by the fungus *Pyricularia oryzae* (synonym *Magnaporthe oryzae*). This model pathosystem for research on host-pathogen interactions (Dean et al. 2012) is also a major challenge for food security (Pennisi, 2010). Rice blast was reported for the first time in China in 1637 (Ou, 1985). It is distributed in all continents and in every area where rice is grown (Ou, 1985). Globally, despite the use of control methods, losses are estimated at 4% of the annual world rice harvest (Savary et al. 2019) and represent a lack of staple food for millions of persons.

The genetic structure of *P. oryzae* populations infecting rice has been extensively studied on a global scale to understand the evolution of the pathogen and to adapt control techniques, in particular the deployment of varietal resistance management strategies. These studies on the characterization of *P. oryzae* populations have been carried out at different geographic scales with different molecular markers and with genome sequences (Adreit et al. 2007; Tharreau et al. 2009; Gladieux et al. 2018a; Zhong et al. 2018; Thierry et al. 2020). By genotyping samples from more than 185 locations in 51 countries with SNP markers, Thierry et al. (2020) identified four genetic groups structured into one recombinant lineage and three clonal lineages, each with a wide geographical distribution. Based on 52 complete genome sequences, Gladieux et al. (2018a) also found four major groups and two additional groups, which were represented by one and two individuals. Other studies on the population structure of *P. oryzae* have shown a genetic structure of at least three major genetic groups, with Asian isolates represented in all three groups (Tharreau et al. 2009; Saleh et al. 2014; Zhong et al. 2018). These studies also showed that Asia is the center of diversity and origin of the pathogen populations (Saleh et al. 2014). However, little data exist on the diversity and population structure of the pathogen in Africa. Mutiga et al. (2017) studied the genetic diversity of *P. oryzae* rice populations and evaluated the virulence spectrum of this pathogen in West Africa and East Africa. These authors identified seven genetic groups and showed differentiation between West and East African populations. Kassankogno et al. (2017) showed that populations from the two neighboring countries Burkina Faso and Togo are differentiated but that some multi-locus genotypes are shared, suggesting some migrations between these countries. A recent study (Odjo et al. 2021) confirmed this hypothesis and showed the presence in Africa and Madagascar of the four genetic groups previously described in Asia

(Gladieux et al. 2018a; Thierry et al. 2020). Populations in West Africa, East Africa, and Madagascar were highly differentiated (Odjo et al. 2021).

Like for any disease, knowing inoculum sources is necessary to develop sustainable control methods. For the blast disease, some primary inoculum sources are well known, such as infected seeds and infected straws (Long, et al. 2001; Guerber and TeBeest, 2006; Raveloson et al. 2013, 2018; Hubert et al. 2015). On the other hand, the role of weeds as inoculum sources is controversial. Based on pathogenicity test in control conditions, some weeds have been presented as sources of inoculum for blast epidemics on rice, including *Digitaria sanguinalis* (Choi et al. 2013), *Festuca arundinacea*, *Lolium multiflorum*, *Anthoxanthum odoratum*, *Phalaris arundinacea* (Kato et al. 2000), *Rottboellia exaltata, Echinochloa colona*, and *Leersia hexandra* (Mackill and Bonman 1986). But, to date, this hypothesis was not confirmed by observations in the field. On the contrary, genetic (Borromeo et al. 1993) and phylogenetic studies (Couch et al. 2005; Hirata et al. 2007; Gladieux et al. 2018b) show that strains causing epidemics on rice belong to a single clade, which is not sampled on other hosts (with the exception of a barley sample from Thailand). Similarly to weeds, certain species of wild rice were suspected of serving as alternative hosts. Pathogenicity tests of *P. oryzae* isolates from wild rice (*O. meridionalis*) in North Australia showed that many local rice varieties of *O. sativa* are susceptible. On the contrary, although on a limited number of samples (10 isolates), *P. oryzae* population from wild rice (*O. rufipogon*) in Cambodia showed a different pathogenicity spectrum than populations from cultivated rice (Fukuta et al. 2014). This result suggests that both pathogen populations are differentiated. So, to date, there is no evidence that wild species of rice are inoculum sources for blast epidemics on cultivated rice.

The specific situation in Mali offers an opportunity to address this question. *O. sativa*, the Asian rice (cultivated worldwide), coexists with the African rice *O. glaberrima* (cultivated on a limited scale in West Africa) and with wild species of rice (*O. longistaminata, O. barthii*). In that context, wild species of rice could be sources of inoculum for cultivated Asian rice. We tried to answer this question by carrying out population surveys on *O. sativa* and *O. longistaminata* in Mali. Our working hypothesis was that, if wild rice is a major source of inoculum for cultivated rice, then *P. oryzae* populations on both hosts should be genetically identical. We also performed pathogenicity tests in controlled conditions to evaluate the infectivity on *O. sativa* of *P. oryzae* isolates collected on *O. longistaminata*.

MATERIAL AND METHODS

Sampling and conservation of samples. Sampling was carried out on organs of cultivated and wild rice showing blast symptoms from 2017 to 2019, from irrigated and lowland areas, at different stages of rice development in four Regions in Mali (Koulikoro, Sikasso, Ségou; Fig. 1; Supplementary Table 1). Diseased organs were collected on different plants distributed in the plots.

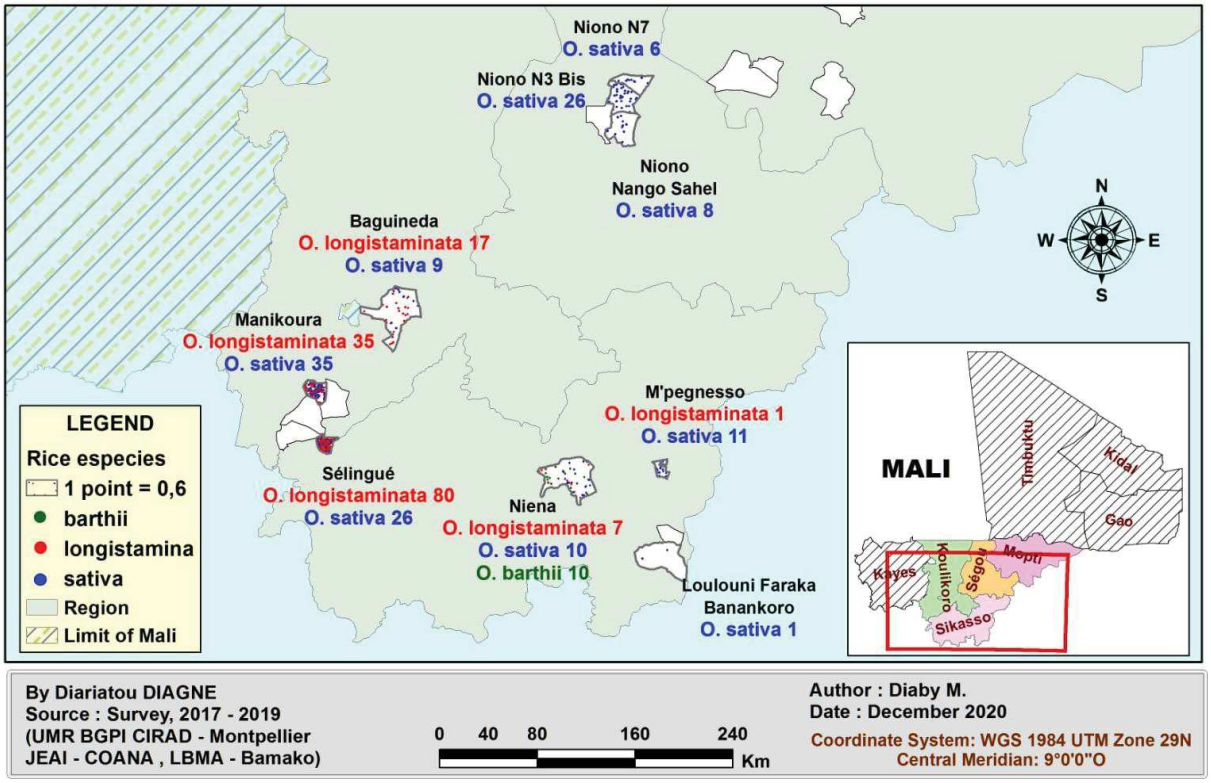

Fig. 1: Mapping of *P. oryzae* strains isolated from wild and cultivated rice by site and species from 2017 to 2019

A sample is here defined as the diseased organs collected in a single plot (and on a single variety, for cultivated rice). The samples were bagged in a paper envelope and labeled (date, species, variety, locality, organ, development stage, and GPS data), stored in the oven (BINDER, Tuttlingen, Germany) at 37°C at the Bamako Laboratory of Applied Molecular Biology (LBMA) before being sent to the UMR PHIM, CIRAD, in Montpellier, France.

Isolation and conservation of isolates of *P. oryzae*. Diseased organs were placed on a moistened filter paper disc in sterile Petri dishes and incubated at 25°C for 24 h. The

fragments were then observed under a binocular microscope (Olympus SZ-PT) to assess the presence or absence of *Pyricularia* conidia. The conidia were collected with a glass needle and deposited on the surface of bacto-agar medium (45 g of bacto-agar in 1 L water). The Petri dish was sealed with tape (Tesa® masking tape 50 m x 19 mm) and incubated at 25°C for 24 h for germination. A germinated conidium was placed on rice flour medium (agar 15 g, rice flour 20 g, yeast extract 2 g, water 1 L, and 500,000 units of Penicillin G added after autoclaving) and incubated at 25°C. A single strain was isolated per plant sampled. For storage, an actively growing mycelium plug was deposited on a sterilized filter paper (Whatman N°5) placed on rice flour agar medium (Silué and Nottéghem 1990). Five to seven days later, the mycelium-covered filter paper was removed and placed in a sterile empty Petri dish with a lid and incubated at 35°C for four days for drying. It was then cut into small fragments and placed in sterile paper bags, placed in a plastic sheath, and sealed under vacuum for conservation at -20°C (Valent et al. 1986).

A total of 282 strains were isolated from 2017 to 2019 from 9 locations (Table 1). In addition, 11 Malian reference strains collected between 1986 and 2011 were included in the study.

TABLE 1**:** Distribution of isolates by sites and hosts and number of multi-locus genotypes per site

Location	N° MLGs	N° of strains/host species			Total
		O. sativa	*O. longistaminata*	*O. barthii*	
Sélingué	58	25	79	-	104
Manikoura	27	32	35	-	67
Niéna	17	10	7	10	27
Baguineda	13	7	16	-	23
M'pegnesso	8	11	1	-	12
Niono Nango Sahel	5	8	-	-	8
Niono Nango N3-Bis	8	25	-	-	25
Niono N7	6	6	-	-	6
Loulouni Faraka Banankoro	1	1	-	-	1
ND	5	9	0	0	9
Total		134	138	10	282

DNA extraction and microsatellite amplification. After four days of culture in rice flour liquid medium (rice flour 20 g, yeast extract 2 g, water 1 L), the mycelium was recovered in Eppendorf tubes, and genomic DNA was extracted according to the protocol described in Gladieux et al. (2018a).

A total of 282 isolates (271 from the 2017-2019 sampling and the 11 reference strains) were genotyped with 12 Simple Sequence Repeats (SSR) markers routinely used (Adreit et al. 2007; Saleh 2011; Saleh et al. 2014; Odjo et al. 2021). These markers were amplified by PCR (QIAGEN Multiplex PCR kit) as previously described (Saleh et al. 2014) with a total volume of 5 µL, including 2.5 µL of Master Mix, 0.5 µL of 10x Mix primers, 0.5 µL of 5x Q solution, and 1.5 µL of genomic DNA (10 ng/µL). The PCR program was as follows: i) predenaturation at 95°C for 15 min, ii) denaturation at 94°C for 30 min, iii) hybridization at 57 to 63°C for 90 min, iv) extension at 72°C for 60 min, v) repetition of steps 2 to 4 for 40 cycles, and vi) final extension at 72°C for 30 min. The resulting products were separated and analyzed on a 16-capillary ABI Prism 3130XL machine (Applied Biosystems, Foster City, USA), and the amplicons were evaluated for size by fluorescence measurement. For this analysis, 1.5 µL of amplified products (1/70 dilution) were mixed with 15 µL Formamide HiDi and GeneScan-500LIZ size marker (Applied Biosystems).

Analysis of genotyping data. The raw data collected were analyzed and transcribed into allele sizes using the GeneMapper V4.1 (Applied Biosystems) tool. Multi-Locus Genotypes (MLGs) were identified using a script developed by Sébastien Ravel (Odjo et al. 2021; https://pypi.org/project/MLG-assign/). A discriminant analysis of principal components (DAPC) was performed using the "adegenet" package of the R software (v3.2.2) (Jombart 2008; Jombart and Ahmed 2011; R Core Team 2017). The mean number of alleles per locus (*Na*) and the unbiased genetic diversity ($H_{n.b.}$; Nei 1987) were calculated using Genetix v4.05 (Belkhir et al. 2004). The mean number of private alleles (N_p) was estimated as the number of alleles present in a single genetic group, combining all markers. Genetic differentiation between populations was estimated with the F_{ST} index (Weir and Cockerham 1984) calculated with Genepop version 4.2 (Raymond and Rousset 1995; Rousset 2008).

Pathogenicity testing of wild rice isolates on cultivated rice. The pathogenicity of 20 Malian isolates of *P. oryzae* collected from 2017 to 2019 was assessed. Among them, 12 isolates were collected from wild rice *O. longistaminata*, four isolates from wild rice *O. barthii,* and four isolates from cultivated rice *O. sativa*. Isolates were selected to represent the genetic diversity observed (Table 2).

TABLE 2: Pathogenicity test on cultivated varieties. Black cell: compatible interaction; white cell: incompatible interaction; gray cell: missing data

Original host	Isolates	MLGs	Genetic cluster	*O. sativa* varieties											
				Kogoni	Nenekala	ADNY 11	Swarna 2	ARICA 10	ARICA 9	Wapmo (Was 197)	Was 49	08Fan2	Bw 348-1	Shwetasoke	Maratelli
O. barthii	ML0312	94	2												
O. barthii	ML0321	97	4												
O. barthii	ML0320	98	4												
O. barthii	ML0322	99	4												
O. longistaminata	ML0097	9	1												
O. longistaminata	ML0134	26	2												
O. longistaminata	ML0160	40	2												
O. longistaminata	ML0176	48	2												
O. longistaminata	ML0296	92	2												
O. longistaminata	ML0093	8	3												
O. longistaminata	ML0156	18	3												
O. longistaminata	ML0138	28	3												
O. longistaminata	ML0145	31	3												
O. longistaminata	ML0089	111	3												
O. longistaminata	ML0103	13	4												
O. longistaminata	ML0328	101	4												
O. sativa	ML0223	121	1												
O. sativa	ML0276	124	2												
O. sativa	ML0339	103	3												
O. sativa	ML0130	116	4												
Compatible interactions per cultivar (%)	*O. barthii* isolates			0%	33%	25%	25%	25%	100%	100%	100%	100%	100%	100%	100%
	O. longistaminata isolates			0%	0%	0%	8%	8%	8%	8%	8%	66%	66%	75%	25%
	O. sativa isolates			25%	0%	0%	25%	25%	25%	25%	25%	75%	66%	75%	50%

Isolates were inoculated on 11 certified varieties of *O. sativa* cultivated in Mali [08Fan2, ADNY 11, ARICA 10, ARICA 9, Bw 348-1, Kogoni, Nenekala, Shwetasoke, Swarna 2, Wapmo (Was 197), Was 49], and *O. sativa* susceptible check variety Maratelli. The 12 accessions were sown in peat soil (Jiffy compost, reference 284795) in a 33 x 45 cm plastic tray and cultivated in the greenhouse (temperature set points of 26°C during the day and of 21°C at night; humidity between 50 and 70%). Inoculations were carried out 28 days after sowing (4- to 5-leaf stage) by spraying 30 mL per tray of a spore suspension (Gallet et al. 2016) and were repeated three times. *P. oryzae* isolates were cultured one week on rice flour agar medium following the conditions described above for isolate conservation. Spores were collected by flooding the plate with 5 mL of water and scrapping the medium. The spore suspension was adjusted to 25,000 spores/mL and 0.5% gelatin. The inoculated plants were incubated at 25°C and 100% relative humidity for one day and then transferred for seven days to a growth chamber (12 h photoperiod; 26°C during the day, 21°C at night). Disease symptoms were assessed seven days after inoculation. The scoring was done according to the 1 to 6 scale described in Gallet et al. (2016). Scores 1 to 3 were considered as incompatible reactions (resistant variety), whereas scores 4 to 6 were considered as compatible reactions (susceptible variety). Inconsistent data from the three replicates were considered as missing data (Table 2).

RESULTS

O. longistaminata was commonly found in the localities of Sélingué (Sikasso region), Manikoura, and Baguineda (Koulikoro region), whereas *O. barthii* was found only in Niéna (Sikasso region). Blast was observed and sampled in all these sites and on all species present, except on wild rice in Niono locality in Ségou region (Fig. 1). To compare *P. oryzae* populations on wild and cultivated rice in Mali, we carried out analyzes at two geographic scales (whole country/localities) and at two genetic levels of the pathogen population (genotype/genetic clusters). Because *P. oryzae* is clonal most population genetic analyses we conducted were based on multilocus genotypes rather than on allelic frequencies.

Population diversity and structure at the whole country scale. The genetic diversity was identical for *P. oryzae* populations sampled on wild rice (*O. longistaminata*) and on cultivated rice (*O. sativa*) at the whole country scale. The unbiased genetic diversity (H_{nb}) was 0.62 and 0.58, and the mean number of alleles per locus (N_a) was 9 and 8, respectively (Table 3). The two populations were moderately differentiated, as indicated by a significant but

relatively low F_{ST} value (0.16) for this species (Saleh et al. 2014). The average number of private alleles (N_p) were 3.1 and 2.2, respectively (Table 3). These relatively high numbers also supported differentiation between the two populations. The sample size (n=10) of the population sampled on *O. barthii* was considered too small to calculate parameters of genetic diversity and differentiation.

TABLE 3: Genetic diversity of *P. oryzae* populations on wild and cultivated rice in Mali

Host	N	Diversity parameters		
		H_{nb}	N_a	N_p
O. longistaminata	138	0.62	9	3.1
O. sativa	134	0.58	8	2.2
Total	272			

N: total number of individuals, $H_{n.b.}$: unbiased gene diversity; N_a: mean number of alleles per locus, $N_{p:}$ mean number of private alleles per locus

Two-hundred-fifty-four out of 282 isolates genotyped were unambiguously assigned to 125 multi-locus genotypes (MLGs). Eighty-three MLGs were represented by a single isolate (hereafter named single MLGs), and 42 MLGs were represented by two or more isolates (hereafter named multi MLGs; Table 4). Among these latter, 35 were sampled only on a single host: 18, 14, and 3 on Asian cultivated rice (*O. sativa*) and on wild rice species *O. longistaminata* and *O. barthii*, respectively. There was a very limited number of shared MLGs between hosts since only six were sampled on both *O. sativa* and *O. longistaminata* and one on both *O. longistaminata* and *O. barthii* (Table 4).

TABLE 4: Distribution of multi-locus genotypes by species

MLGs	Species-specific			Shared		
	O. sativa	*O. longistaminata*	*O. barthii*	*O. sativa/O. longistaminata*	*O. sativa/O. barthii*	*O. longistaminata/O. barthii*
*Single MLGs	36	47	0	-	-	-
Multi- MLGSs	18	14	3	6	0	1

*Single MLGs: MLGs represented by one isolate; Multi MLGs: MLGs represented by two or more isolates.

DAPC analysis was carried out to cluster MLGs in genetic groups without a priori. To determine the number of cluster (K), variation of the BIC value was calculated for varying values of K. BIC value regularly decreased with increasing K values (Supplementary Fig. 1). Because the curve did not show a marked inflection, we could not determine an optimal K value based on this approach. Instead, we applied the recommendation of the DAPC tutorial described by Jombart and Collins (2015) and tried to find the value of K that "better summaries the data than others". The analysis of DAPC results (Fig. 2; Supplementary Fig. 2) showed that clustering in more than five groups seemed to create an unnecessary and unreliable level of clustering. Hence four genetic clusters (named G1, G2, G3, and G4) were defined.

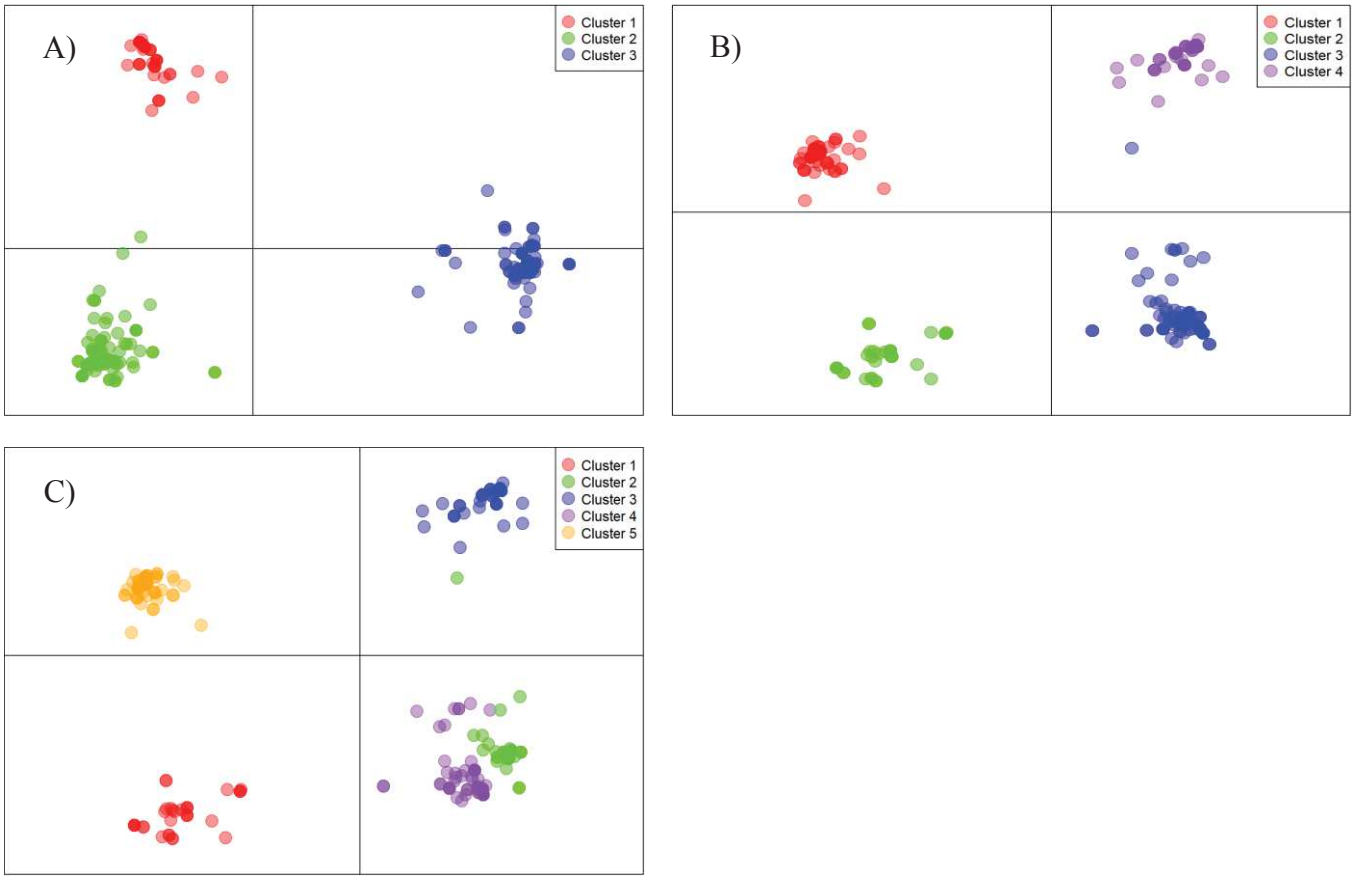

Fig. 2. Clustering of genotypes by the DAPC method with the number of cluster (K) varying between three (A), four (B) and five (C). Each color represents a different cluster.

As expected, and because genotypes were clustered based on genetic similarity, F_{ST} values were high between different genetic groups (Table 5). We then analyzed the genetic differentiation between *P. oryzae* populations on wild *O. longistaminata* and on cultivated *O. sativa* rice within each of the four genetic clusters. We observed that the populations on *O. longistaminata* and *O. sativa* were significantly differentiated. F_{ST} indices between the two populations ranged between relatively low to average values (0.41, 0.16 and 0.31 for G1, G2 and G3, respectively, Table 5). On the contrary, for the G4 cluster, populations from *O. longistaminata* and *O. sativa* were poorly differentiated (F_{ST} = 0.02; Table 5). A bias due to the small sample size could explain this exception.

TABLE 5: F_{ST} statistics between genetic clusters of *P. oryzae* populations on wild and cultivated rice in Mali. Values for populations from the same genetic cluster but from different hosts are highlighted in grey

Host	Host	*O. longistaminata*				O. *sativa*		
	Genetic cluster	1	2	3	4	1	2	3
O. longistaminata	2	0.65	-	-	-	-	-	-
	3	0.64	0.66	-	-	-	-	-
	4	0.46	0.59	0.51	-	-	-	-
O. sativa	1	0.41	0.72	0.73	0.66		-	-
	2	0.64	0.16	0.63	0.47	0.76	-	-
	3	0.65	0.65	0.31	0.50	0.73	0.62	-
	4	0.44	0.54	0.49	0.02	0.71	0.43	0.49

Population diversity and structure at the local scale. To compare populations at the local scale, we focused on Sélingué, Manikoura, Niéna, and Baguineda localities, where the sample size was sufficient for comparisons between populations isolated from wild rice and cultivated rice (Table 1).

In Sélingué, 10 single MLGs and 2 multi MLGs (representing a total of 9 isolates) were specific of cultivated rice *O. sativa*. Similarly, we identified 30 single MLGs and 11

multi MLGs (30 isolates) specific to wild rice *O. longistaminata* (Figure 3A, Supplementary Table 3)**.** Four MLGs were shared between both species. All four GCs were identified on *O. sativa* and *O. longistaminata.* Clusters G1 was dominant on *O. sativa* (12/25 isolates), whereas G2 (37/79 isolates) and G3 (27/79 isolates) were dominant on *O. longistaminata* (Table 6, Supplementary Table 3).

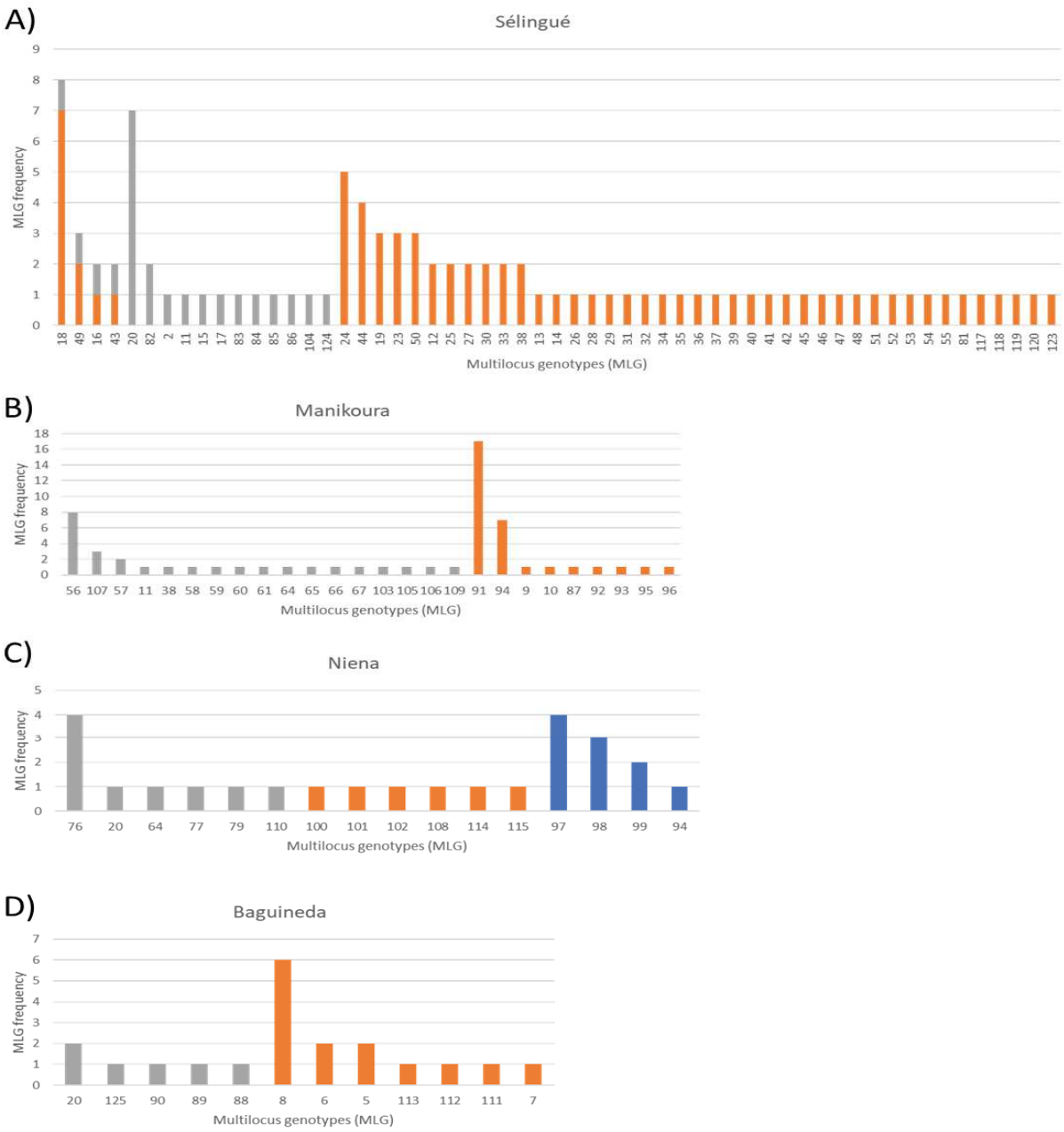

Fig. 3: Distribution of MLGs by site and species. Number of isolates per multi-locus genotype in four localities: A) Sélingué, B) Manikoura, C) Niena, and D) Baguineda. Orange: *O. longistaminata*; grey: *O. sativa*; blue: *O. barthii*.

TABLE 6: Distribution of genetic clusters by species and sites. Number of *P. oryzae* isolates per site, host species and genetic clusters.

Host species	Genetic clusters	Regions	Koulikoro		Ségou			Sikasso					Total
		Sites	Baguineda	Manikoura	Niono N7	Niono N-N3-Bis	Niono N Sahel	Loulouni FB	M'pegnesso	Niena	Sélingué	ND	
O. barthii	2									1			1
	4									9			9
O. longistaminata	1			3					1		3		7
	2		3	26							37		66
	3		13	3						1	27		44
	4			3						6	12		21
O. sativa	1		7	2	6	25	8			2	12	3	65
	2								2		6		8
	3			30					9	8	5	6	58
	4							1			2		3
Total			**23**	**67**	**6**	**25**	**8**	**1**	**12**	**27**	**104**	**9**	**282**

In Manikoura, 14 single and 3 multi MLGs (13 isolates) were specific to *O. sativa*, and 7 single and 2 multi MLGs (24 isolates) were specific to *O. longistaminata* (Figure 3B, Supplementary Table 3). There was no genotype shared between wild and cultivated rice in Manikoura. Genetic clusters G1 and G3 were sampled on *O. sativa*, G3 being more represented (30/32 isolates), whereas the four genetic clusters were identified on *O. longistaminata* with G2 as the dominant one (26/35 isolates; Table 6).

In Niéna, 5 single and 1 multi MLGs (4 isolates) were specific of *O. sativa*, 1 single and 3 multi MLGs (9 isolates) were specific to the wild rice *O. barthii*, and 6 single MLGs were specific of *O. longistaminata* (Figure 3C, Supplementary Table 2). There was no genotype shared between wild and cultivated rice. A dominance of cluster G4 on the two wild species in Niéna was observed**.** G2 (1/10 isolates) and G4 (9/10 isolates) were found on *O. barthii,* while G3 (1/7 isolates) and G4 (6/7 isolates) were found on *O. longistaminata* (Table 6). G1 (2/10 isolates) and G3 (8/10 isolates) were sampled on *O. sativa*.

In Baguineda, we identified 4 single and 1 multi MLGs (2 isolates) specific of *O. sativa* and 4 single and 3 multi MLGs (10 isolates) specific of *O. longistaminata* (Figure 3D, Supplementary Table 3). There was no genotype shared between wild and cultivated rice in Baguineda. G1 (7 isolates) was the only genetic cluster sampled on *O. sativa*, whereas G2 (3/16 isolates) and G3 (13/16 isolates) were identified on *O. longistaminata* (Table 6).

Pathogenicity of isolates from *O. longistaminata* on cultivated rice. A necessary condition for wild rice to serve as an inoculum source for blast epidemics on *O. sativa* is that blast isolates from wild rice should be pathogenic on *O. sativa*. Thus, we evaluated in controlled conditions the pathogenicity on *O. sativa* of *P. oryzae* strains isolated from *O. barthii* and *O. longistaminata*. Strains isolated from *O. sativa* in Mali were used as positive controls. Most of the varieties (including the susceptible check Maratelli) were incompatible with the majority of isolates, except three varieties (namely 08Fan2, Bw 348-1, and Shwetasoke; Table 2). The average ratio of compatible interactions between *P. oryzae* isolates and *O. sativa* varieties was similar for isolates from *O. longistaminata* and *O. sativa*: 0.24 (range: 0 to 0.75) and 0.34 (range: 0 to 0.75), respectively (Table 2). *O. barthii* appeared to have higher frequencies of compatible interactions than isolates from *O. sativa*: 0.69 (range: 0.66 to 0.75). This broad spectrum may be explained by a bias due to the small number of

isolates from *O. barthii* tested and probably a narrower genetic diversity because they came from the same place and have closely related genotypes.

DISCUSSION

In Africa, the role of wild rice as an inoculum source for Asian cultivated rice was demonstrated for two important diseases, Bacterial leaf streak (Wonni et al. 2014) and RYMV (Traoré et al. 2009). On the contrary, to date, there is no convincing evidence that weeds, in general, and wild rice species, in particular, are reservoirs for blast epidemics on cultivated rice. Isolating *P. oryzae* from wild rice and showing that these isolates are pathogenic on cultivated rice are not sufficient to demonstrate a role as inoculum source. Both hosts could act as separated compartments and epidemics could be independent, i.e., without demographic and genetic mutual impact on blast populations. Such an example of "parallel" epidemics was observed for blast populations on indica and japonica varieties in the Yuanyang terraces of Yunnan Province in China (Liao et al. 2016). *P. oryzae* isolates from indica varieties were genetically different from isolates from japonica varieties, although both types of varieties were cultivated very closely and although isolates from indica varieties had the potential to attack the cultivated japonica varieties. We took advantage of the specific context of Mali where wild rice (mainly *O. longistaminata)* is growing in the vicinity of cultivated rice (*O. sativa*) to test if the *P. oryzae* populations on both hosts were genetically similar.

The present genetic study shows that *P. oryzae* populations on cultivated and wild rice in Mali are significantly but barely differentiated. This result suggests that, at the country scale, the gene pool shared between both populations is broad. However, comparisons of the clones and genetic clusters composing pathogen populations from each host show a clear differentiation. Each population is mainly composed of specific MLGs. Few MLGs were sampled on both hosts, and an even smaller number were sampled in the same area on both hosts. Although the same four genetic groups are sampled on both hosts, their frequencies are different between hosts. These results show that wild rice (*O. longistaminata*) and cultivated rice (*O. sativa*) in Mali are hosting different populations of the blast fungus. These results do not support the hypothesis that wild rice could be a main source of inoculum for cultivated rice.

Maratelli is considered as a susceptible check because of its broad range of compatibility to *P. oryzae* isolates from rice. Results from different published studies with a very diverse collection of *P. oryzae* isolates from *O. sativa* showed that 94% of the 172 isolates tested were compatible (Gallet et al. 2016; Thierry et al. 2020; Odjo 2019). In this study, Maratelli was incompatible to an unexpectedly high ratio of isolates (50%). Most of these incompatible isolates (9/10) were isolated on *O. longistaminata*. Malian isolates from wild rice may carry an avirulence factors that impede them from attacking Maratelli. Two avirulence genes to Maratelli were already identified and mapped (Valent and Chumley 1991; Mandel et al. 1997). Alleles of these genes triggering an incompatible reaction in Maratelli originated from non-rice *P. oryzae* isolates.

Pathogenicity tests on cultivated rice also revealed that isolates from wild rice are pathogenic to cultivated rice under controlled conditions. In addition, isolates from wild rice did not appear to be less pathogenic on *O. sativa* varieties cultivated in Mali than *O. sativa* isolates. So, the isolates from *O. longistaminata* have the potential to infect *O. sativa* varieties cultivated in Mali. Similar results were observed with *P. oryzae* isolates from wild rice (*O. meridionalis*) in Australia. These isolates were pathogenic to local rice varieties after artificial inoculation (Khemmuk et al. 2016). Despite this potential, and based on population genetic structure, *P. oryzae* isolates generating epidemics on *O. longistaminata* do not seem to cause epidemics on *O. sativa* in the field. Two non-exclusive causes can be provided to explain this observation. First, our evaluation of compatibility did not take into consideration other pathogenicity components, which may be important in field epidemics (e.g., production of spores by lesion area unit). Second, isolates from *O. longistaminata* are less fit on *O. sativa* than isolates from *O. sativa* and are thus excluded when competing in the field. In *P. oryzae*, fitness differences were observed between populations from two rice subspecies and were hypothesized to contribute to the pathogen population structure (Liao et al. 2016). Additional experiments measuring quantitative differences in pathogenicity and fitness would be needed to test these hypotheses for *P. oryzae* populations of wild and cultivated rice.

ACKNOWLEDGEMENTS

We thank Youssouf Diarra and the team that collected the samples, specially Mamadou Dembelé of IER Niono at the Office du Niger, the JEAI CoANA team in LBMA, the teams of the Baguineda, Manikoura, Sélingué, Niena, M'pegnesso and Loulouni stations for their contribution. This work was supported by grants of the RICE CRP, a grant from the French Embassy in Mali to Diariatou Diagne, IRD support to Ousmane Koita group (JEAI CoANA), and the support of Institutes of the coauthors (CIRAD, USTT-B). Genotyping data used in this work were produced through the GenSeq technical facilities of the "Institut des Sciences de l'Evolution de Montpellier" with the support of LabEx CeMEB, an ANR "Investissements d'avenir" program (ANR-10-LABX-04-01).

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Supplementary TABLE 1: Samples collected per sites from 2017 to 2019

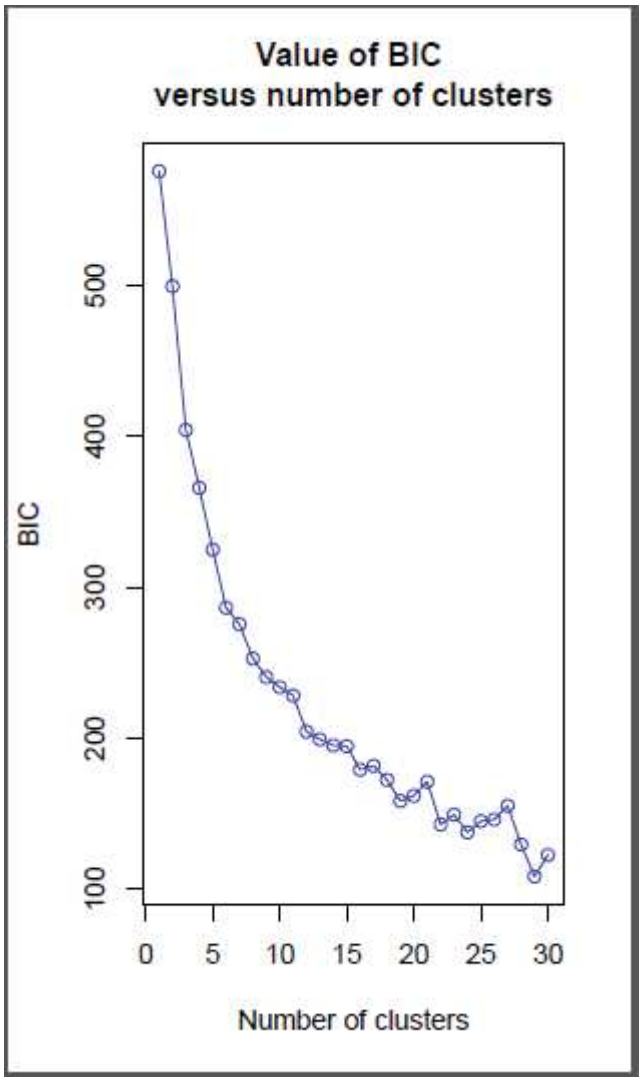
Region	Locality	Collection dates	Total number of plots	Total number of samples	Number of samples processed	Total number of strains collected
Koulikoro	Baguineda	2017	6	11	11	17
		2018	8	14	12	0
		2019	3	4	1	9
	Manikoura	2017	13	15	15	4
		2018	7	13	4	20
		2019	2	4	4	46
Sikasso	Selingué	2017	14	17	17	22
		2018	13	25	23	82
		2019	4	4	1	2
	Niena	2018	7	7	6	10
		2019	5	7	3	17
	M'pegnesso	2018	3	5	5	12
	Loulouni FB	2018	4	6	3	1
Ségou	Niono N sahel	2018	2	3	2	8
	Niono N3-bis	2018	1	2	1	26
	Niono N7	2018	3	3	2	6
Total			**95**	**140**	**110**	**282**

Supplementary TABLE 2**:** Distribution of *P. oryzae* isolates by site and host

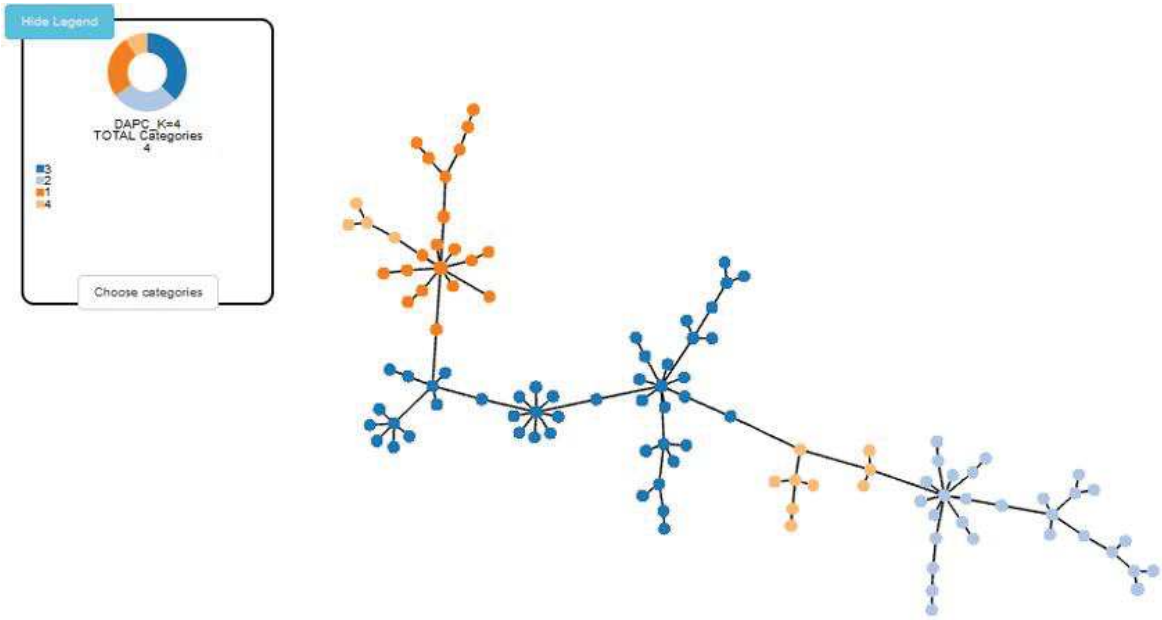
Varieties/species	Region/Location									Total
	Koulikoro		Ségou			Sikasso				
	Baguineda	**Manikoura**	**Niono N7**	**Niono N-N3-Bis**	**Niono N Sahel**	**Loulouni**	**M'pegnesso**	**Niena**	**Sélingué**	
BW 348-1 (*O. sativa*)							7	2		9
IR32310 (*O. sativa*)		33					4		19	56
Kogoni 91-1 (*O. sativa*)	9		6	26	8				7	56
Locale (*O. sativa*)		2								2
Shwetasoké (*O. sativa*)						1		8		9
O. barthii								10		10
O. longistaminata	17	35					1	7	80	140
Total	**26**	**70**	**6**	**26**	**8**	**1**	**12**	**27**	**106**	**282**

Supplementary TABLE 3**:** Distribution of MLGs by site and host species

MLGs	Especies	N° of strains										Total
		Koulikoro		Ségou			Sikasso					
		Baguineda	Manikoura	Niono N7	Niono N- N3-Bis	Niono N Sahel	0	Loulouni FB	M'pegnesso	Niena	Sélingué	
15	*longistaminata*								1			1
15	*sativa*										1	1
16	*longistaminata*										1	1
16	*sativa*										1	1
18	*longistaminata*										7	7
18	*sativa*										1	1
38	*longistaminata*										2	2
38	*sativa*		1									1
43	*longistaminata*										1	1
43	*sativa*										1	1
49	*longistaminata*										2	2
49	*sativa*										1	1
94	*barthii*									1		1
94	*longistaminata*		7									7
1	*sativa*						3					3
107	*sativa*		3									3
11	*sativa*		1	1	4	1					1	8
2	*sativa*						3				1	4
20	*sativa*	2		1	13	3				1	7	27
21	*sativa*								2			2
22	*sativa*								2			2
56	*sativa*		8									8
57	*sativa*		2									2
63	*sativa*								2			2
64	*sativa*		1							1		2
68	*sativa*					2						2
70	*sativa*				1	1						2
73	*sativa*				2							2
76	*sativa*									4		4
78	*sativa*								2			2
79	*sativa*								1	1		2
82	*sativa*										2	2
12	*longistaminata*										2	2
19	*longistaminata*										3	3
23	*longistaminata*										3	3
24	*longistaminata*										5	5
25	*longistaminata*										2	2
27	*longistaminata*										2	2
30	*longistaminata*										2	2
33	*longistaminata*										2	2
44	*longistaminata*										4	4
5	*longistaminata*	2										2
50	*longistaminata*										3	3
6	*longistaminata*	2										2
8	*longistaminata*	6										6
91	*longistaminata*		17									17
97	*barthii*									4		4
98	*barthii*									3		3
99	*barthii*									2		2
Unique MLGs		8	18	3	3	1	2	1	2	8	37	83
nd	*longistaminata*	2	4							1	8	15
nd	*sativa*	1	5	1	2		1			1	2	13
Total		**23**	**67**	**6**	**25**	**8**	**9**	**1**	**12**	**27**	**104**	**282**



Supplementary Fig. 1: Variation of bayesian information criterium value with the number of genetic clusters.



Supplementary Fig. 2: MLG haplotype network. MLGs clustered in genetic groups 1, 2, 3 and 4 are colored in orange, light blue, blue, light orange, respectively.

Conclusions et Perspectives

CONCLUSIONS ET PERSPECTIVES

Le riz est une production agricole de première importance dans le monde et l'une des céréales les plus consommées. Il est connu pour être cultivé sur tous les continents, du fait de son adaptation à une gamme variée de conditions agroclimatiques. En Afrique, comme ailleurs dans le monde, le riz est une plante vivrière qu'il faut produire en plus grande quantité mais avec de plus en plus de contraintes : s'adapter au changement climatique, réduire l'utilisation de fertilisants chimiques, réduire l'emploi de pesticides, réduire la consommation d'eau, émettre moins de gaz à effet de serre. L'augmentation de la productivité du riz en Afrique soulève donc des questions de recherche et de développement d'intérêt plus global.

Le Mali en sa qualité de deuxième pays producteur en Afrique de l'Ouest, se doit d'accorder beaucoup d'intérêt aux problèmes lié à la riziculture. Cependant, des problèmes d'ordre structurel, organisationnel et agro-parasitaires impactent sérieusement l'atteinte d'une production optimale. Au nombre des contraintes parasitaires, la pyriculariose, maladie de première importance économique sur le riz, reste à ce jour peu étudiée dans ce pays. Notre présente étude envisageait donc d'apporter des connaissances sur : (i) l'état sanitaire du riz au Mali par la détermination de la prévalence de *P. oryzae* dans les zones rizicoles, (ii) les potentielles sources de contamination (semences, pailles, espèces de riz réservoirs) ; (iii) la structure des populations de *P. oryzae* en Afrique.

Prévalence de la pyriculariose au Mali

Cette étude a montré la présence de la pyriculariose du riz dans certaines régions rizicoles du Mali notamment dans les régions de Koulikoro, Ségou et Sikasso. Globalement, en se fondant sur les valeurs de l'incidence et la sévérité moyenne de la pyriculariose foliaire et paniculaire, nous pourrions émettre l'hypothèse que l'agrosystème de bas-fond était plus favorable à la pyriculariose que celui de l'agrosystème irrigué. Nous avons constaté que les zones rizicoles de bas-fond de la région de Sikasso étaient en moyenne plus impactées par la pyriculariose du riz, toutefois sans différence statistique. Nos données d'incidence et de la sévérité foliaire et paniculaire de la pyriculariose du riz complètent les données antérieures notamment en Afrique et, plus particulièrement, en Afrique de l'Ouest où il y a peu de données. Des études antérieures en Afrique mettent en avant l'influence de l'agrosystème sur la prévalence de la pyriculariose. Cependant, le résultat de ces études d'incidence varie

considérablement d'une zone géographique à une autre. Par exemple, à Madagascar, l'incidence de la maladie est plus élevée dans l'agrosystème pluvial strict qu'en irrigué et qu'en bas-fond (Raveloson 2018), alors qu'en Ouganda, l'incidence la plus élevée a été enregistrée sur le riz pluvial de bas-fond, suivi du riz irrigué de bas-fond et du riz pluvial d'altitude (Amayo et al. 2020). Au Bangladesh, il a été signalé une incidence et une sévérité plus élevées dans l'agrosystème irrigué par rapport à l'agrosystème pluvial (Hossain et al. 2017). Il semble donc que les différentes pratiques de gestion de l'eau ne suffisent pas à expliquer l'incidence de la pyriculariose. D'autres pratiques, plus ou moins liées à l'agrosystème, peuvent également expliquer les différences observées. Les apports d'engrais azoté augmentent l'incidence de la pyriculariose. La sensibilité des variétés est également un facteur déterminant. Nos résultats actuels ne permettent pas de conclure pour l'instant sur la situation au Mali. Pour tester l'importance de la gestion de l'eau sur la pyriculariose, il faudrait concevoir des études spécifiques réduisant le nombre de facteurs étudiés.

L'équipe d'évaluation de la maladie était guidée uniquement vers des parcelles où la maladie sévissait donc la proportion de parcelles malades était probablement surestimée par rapport à la réalité du terrain. Il serait donc utile de mener des études complémentaires (prospections, suivi-épidémiologique, enquêtes, échantillonnage) pour compléter les données sur l'évaluation de la pyriculariose. De façon pratique il s'agira : (i) en terme de prospections, d'élargir les zones d'études, en évaluant l'incidence et la sévérité dans les zones les moins prospectées jusqu'à maintenant, (ii) pour le suivi-épidémiologique, de mettre en place un réseau dynamique des acteurs de la production (producteurs, vulgarisateurs/agents techniques d'agriculture, ….) pour la surveillance et le lancement d'alertes précoces en cas d'observation de la maladie, (iii) d'exploiter les enquêtes pour connaître les facteurs favorisant la maladie. Pour cela, il faudrait collecter toute information en lien avec la sociologie des exploitations agricoles, les systèmes de production, et les pratiques de protection phytosanitaire déployées.

Au terme de nos travaux sur l'évaluation de la maladie, nous faisons le constat que pour la détection de la pyriculariose du riz, il est nécessaire de développer des techniques de diagnostic au champ. Cette perspective ressort du fait que lors de nos travaux sur le terrain, contrairement aux symptômes typiques sur feuilles et tiges paniculaires, la pyriculariose sur les grains est difficile à distinguer d'autres maladies et que cela a parfois conduit à ne pas collecter des échantillons et peut être à sous-estimer l'incidence de la maladie. D'une manière générale, il serait utile de développer ou d'améliorer des outils de diagnostic et d'identification des agents

pathogènes fongiques. La détection simultanée de plusieurs espèces serait appropriée puisque nous avons observé fréquemment des cas de co-infections. Ceci suggère qu'il faut développer des tests de diagnostic multi-pathogènes portatif, d'application pratique sur le terrain.

Structure des populations de *P. oryzae* en Afrique

Nos collectes d'échantillons d'organes de riz portant des symptômes de la pyriculariose et l'isolement de souches de *P. oryzae* ont permis d'enrichir la collection de *P. oryzae* du Mali. La caractérisation moléculaire (génotypage) avec 12 marqueurs microsatellites (SSR) d'une large collection d'Afrique a permis de compléter les études précédentes conduites à l'échelle mondiale. En effet, les souches d'Afrique étaient peu représentées dans ces études. La caractérisation de la diversité et de la structure des populations de *P. oryzae* d'Afrique a nécessité l'intégration du jeu de données de génotypes produit avec la collection malienne, l'analyse sans a priori de la structure des populations (DAPC), l'identification de Génotypes Multi Locus, le calcul d'indices de diversité et de différenciation. Ce travail a abouti à un article intitulé « Structure of African populations of *Pyricularia oryzae* from rice », publié en janvier 2021 dans le journal « Phytopathology ».

Pour l'analyse du mode de reproduction des populations africaines, nous avons procédé à la détermination du signe de compatibilité sexuelle et de la fertilité par des croisements in vitro. Nous avons constaté que les deux signes de compatibilité sexuelle étaient présents en Afrique avec une dominance de Mat1.2 mais aucune souche femelle-fertile n'a été détectée. Ce résultat confirme les observations de terrain où seul le cycle asexué est observé. Il confirme également les résultats antérieurs de biologie et génétique des populations qui montrent que la reproduction sexuée de *P. oryzae* n'existe ou n'a existé que dans une zone limitée d'Asie (Zeigler, 1998 ; Saleh et al., 2012 ; Thierry, 2019). La caractérisation génétique des populations de *P. oryzae* a permis de montrer que la diversité en Afrique était plus élevée comparativement aux autres continents, à l'exception de l'Asie. Les quatre groupes génétiques présents en Afrique sont également présents sur d'autres continents. L'analyse de la structure des populations a mis en évidence une différenciation entre les populations d'Afrique de l'Ouest, d'Afrique de l'Est et de Madagascar. Par ailleurs, tous les groupes génétiques identifiés en Asie ont été détectés en Afrique. Ces résultats supportent deux hypothèses. La première est que les populations de *P. oryzae* en Afrique sont originaire d'Asie. La seconde est que la structure observée en Afrique résulte probablement d'introductions multiples.

Dans notre étude, l'échantillon d'Afrique de l'Est était de petite taille et couvrait peu de pays. Les données disponibles sur cette zone sont donc limitées et mériteraient d'être élargies à d'autres pays de la zone.

La forte diversité observée en Afrique peut favoriser l'émergence de nouveaux pathotypes qui, à leur tour, peuvent contourner les résistances variétales. Il apparait donc important de se doter de moyens de suivre et de comprendre l'évolution des populations de *P. oryzae* sur le continent Africain.

Enfin, pour conforter l'hypothèse d'introductions multiples en Afrique, une des perspectives de notre travail serait de retracer plus précisément les introductions (origine et date). Cela pourrait se faire par des analyses de séquences de génomes complets, les données de génotypage avec des marqueurs microsatellites dont nous disposons étant trop limitées pour tester cette hypothèse. L'utilisation de données de génotypage plus précises permettraient également de mieux retracer les migrations à l'échelle du continent.

Sources d'inoculum au Mali

L'importance relative des sources d'inoculum est encore mal connue pour la pyriculariose. Le rôle des semences infectées dans les épidémies de pyriculariose a été mesuré en riziculture irriguée et pluviale stricte. Les résidus infectés (dont les pailles) laissés sur le sol après récolte ont un rôle majeur pour le démarrage des épidémies en riziculture pluviale stricte mais n'est pas connu en riziculture inondée (irriguée ou bas fond). Enfin, le rôle d'hôtes secondaires est sujet à controverse. Notre travail a permis de produire des données qui contribuent à déterminer si le riz sauvage, les semences et les pailles pourraient être des sources d'inoculum au Mali.

La caractérisation de la mycoflore fongique d'échantillons de semences et de pailles prélevées dans des périmètres irrigués a été effectuée en évaluant la présence de conidies de *P. oryzae* et d'autres espèces fongiques phytopathogènes. Nous n'avons pas rencontré de conidies de *P. oryzae* dans les lots de semences. Par contre, les espèces responsables de l'helminthosporiose et des *Curvularia* ont été les plus fréquemment observées, ce qui conduit à s'interroger sur le rôle des semences comme source d'inoculum pour ces agents pathogènes. Des saprophytes fongiques ont également été observés sur les graines, leur présence pourrait être due aux conditions de conservation qui n'étaient probablement pas bonnes. Nos résultats ne soutiennent pas l'hypothèse que les semences sont une source majeure d'inoculum pour les

épidémies de la pyriculariose du riz au Mali. Toutefois, le nombre de lots de semences sur certains sites était limité. Il serait utile de collecter plus de semences dans ces zones pour conforter nos résultats. Par ailleurs, *P. oryzae*, et d'autres champignons pathogènes du riz, ont été observé sur des échantillons de pailles. Ce résultat suggère que les pailles pourraient être une source d'inoculum pour plusieurs maladies fongiques du riz cultivé au Mali. Pour confirmer cette hypothèse, des observations de terrain et des enquêtes plus détaillées (sur un nombre de parcelles plus restreintes) seront nécessaires. Des suivis chez les riziculteurs et des expérimentations de terrain pourraient être conduits pour comparer les niveaux de maladie sur des parcelles avec et sans pailles infectées sur le sol.

Des tests de pathogénicité ont révélé que les souches de *P. oryzae* isolées de riz sauvage étaient pathogènes pour le riz cultivé dans des conditions contrôlées, ce qui suggère qu'elles ont le potentiel pour créer des épidémies sur le riz cultivé. Toutefois, notre étude de génétique des populations de l'agent pathogène a montré que les populations sur les deux hôtes étaient différentes. Cette étude pourrait être confortée par un plus grand nombre de comparaisons et par des échantillons les plus équilibrés possibles entre riz sauvage et riz cultivé. Mais nos résultats tendent à montrer que le riz sauvage n'est pas une source d'inoculum pour le riz cultivé. Cette hypothèse pourrait aussi être testée par l'observation du démarrage des épidémies dans quelques sites où coexistent riz cultivé et riz sauvage. Des essais pourraient également être conduits au champ pour mesurer l'impact de la présence de riz sauvage infecté sur les épidémies sur le riz cultivé.

D'un point de vue pratique, la préconisation de traitements de semences ne semble pas utile pour lutter contre la pyriculariose du riz au Mali. De même, l'élimination du riz sauvage ne devrait pas avoir d'impact majeur sur les épidémies. Par contre, l'élimination des pailles infectées pourrait se révéler importante pour limiter le démarrage des épidémies.

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Annexes

ANNEXES

Annexe 1 : Fiche de prospection (pyriculariose)

DATE……..

Coordonnées GPS

- Latitude :……..
- Longitude :…….
- Altitude :……
- Région :……Localité :……

I INFORMATIONS RELATIVES A L'ESPECE DE RIZ CULTIVEE

I-1-Espèce (s) cultivées (s) :……
I-2- Variété (s) cultivées (s) :……
I-3- Superficie cultivée :…….....…… I-
4- Age de la parcelle :…… I-
5- Culture précédente :……... I-
6- Type d'écosystème :
Pluvial, Irrigué, Irrigué/bas-fond

I-7- Stade phénologique de la culture
Avant tallage, Tallage, Epiaison, Floraison/Montaison, Remplissage, Fructification/Maturité

I-8- Type d'organe
Feuille, Tige, Feuille paniculaire, Tige paniculaire, Graine

II QUESTIONS RELATIVES AU SYSTEME DE CULTURE

II-1- Type de culture : monoculture…Association de cultures… II-2- Cultures associées…….
II-3- Densité de culture……..
II-4- Variété (s) cultivée (s) : 1)……2)……3)……4)……
II-5- Origine de la semence :
II-5-1- Locale
Propre champ, Champ voisin, Autre localité, Autre pays
II-5-1- Améliorée
Certifiée, Non certifiée
II-6- Enherbement : Fort, Moyen, Faible
II-7- Utilisation de pesticides:

- Non/Oui
- Si oui :
- Type de pesticide :…….....
- Doses : 1 bouchon/15l 2 bouchons/15L.>2
- Fréquence d'apport : 1 fois/semaine fois/semaine fois /semaine

III QUESTIONS RELATIVES A LA CONNAISSANCE DU PAYSAN SUR LES MALADIES FONGIQUES DU RIZ

III-1- Attaques fongiques

III-1-1- Connaissez –vous les symptômes de la pyriculariose sur la plante infectée ?
Oui/ Non

III-1-2- Connaissez –vous les vecteurs ?
Oui/ Non

IV QUESTIONS RELATIVES A LA PRATIQUE AGRICOLE POUR GESTION DE LA MALADIE

IV-1- Quelles sont les moyens de gestion pour réduire les risques d'infestation ?

- Arrachage de plants infectés : Oui/Non
- Insecticides
- Herbicides
- Rotation de cultures
- Autres :

V IDENTIFICATION DU PAYSAN

VI-1- Place de la riziculture et autres
Activité professionnelle, Activité secondaire

VI-2- Nombre d'années d'expérience :……..

VI-3- Nom et Prénoms :……

VI-4- Contact :……..

NOM ET PRENOM DU PROSPECTEUR :

Annexe 2 : Fiche d'évaluation de l'incidence et de la sévérité foliaire et paniculaire

Date :…../……./……. | |Parcelle :…… | |

Localité :……

P…		Incidence						Sévérité					
		Talles			Panicules			Org		Org		Org	
	Poquet	N total	N Mal	Inc. Fol-	N total	N Mal	Inc. Pan-		% SFM Tige	F	% SFM-F	P	% GM
D1	Pq1							T1		F1		P1	
										F2			
										F3			
										F4			
								T2		F1		P2	
										F2			
										F3			
										F4			
								T3		F1		P3	
										F2			
										F3			
										F4			
	Pq2							T1		F1		P1	
										F2			
										F3			
										F4			
								T2		F1		P2	
										F2			
										F3			
										F4			
								T3		F1		P3	
										F2			
										F3			
										F4			
	Pq3							T1		F1		P1	
										F2			
										F3			
										F4			
								T2		F1		P2	
										F2			
										F3			
										F4			
								T3		F1		P3	
										F2			
										F3			
										F4			
	Pq4							T1		F1		P1	
										F2			
										F3			
										F4			
								T2		F1		P2	
										F2			
										F3			
										F4			
								T3		F1		P3	
										F2			
										F3			
										F4			
	Pq5							T1		F1		P1	
										F2			
										F3			
										F4			
								T2		F1		P2	
										F2			
										F3			
										F4			
								T3		F1		P3	
										F2			
										F3			
										F4			

NB : Pour chaque parcelle, usage de 4 fiches correspondant aux 4 carrés d'observation

Annexe 3 : Article "First report of rice leaf brown spot caused by *Exserohilum rostratum* in Mali"

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Rice brown spot is an emerging disease of concern in many rice-growing countries. Different fungal species of the genera *Bipolaris* and *Exserohilum* were reported as causal agents of this disease. These fungal pathogens cause similar necrotic lesions on leaves, and infect grains with a significant effect on seed germination. In 2018, samples of rice seeds and leaves with typical brown spot symptoms were collected from irrigated (Manikoura and Niono) and lowlands (M'pegnesso and Loulouni) rice fields in Mali and incubated for 5 to 7 days on wet filter paper (25°C; 12 h photoperiod). Conidia observed under a binocular were straight or slightly curved and light-brown or dark. They were also rostrate or obclavate and measured 31.4-275.6 µm x 7.3-18 µm (Supplementary Figure S1. **A**). These morphological characteristics are identical to those of *Exserohilum rostratum* (Hernández-Restrepo et al., 2018). This species identification was confirmed by sequencing three genes from eight monospores isolates. DNA from these strains was extracted by a CTAB-based protocol (Doyle and Doyle, 1987). ITS1-5.8S-ITS2, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and translation elongation factor 1 alpha (TEF1 alpha) genomic regions were amplified by polymerase chain reaction with the primers ITS5/ITS4 (White et al., 1990), GPD1/GPD2 (Berbee et al., 1999) and EF1 983/EF1 2218, respectively. Sequence similarity between these 8 isolates from Mali were 100% for ITS and GAPDH, and 99.8-100% for TEF1. Sequence similarity between Malian strains and reference *E. rostratum* sequences BRIP 11417 (GenBank accession No. LT837836, LT882553, LT896656) and CBS 128061 (GenBank accession No. KT265240, LT715900, LT896658) were 99.6-100%. The sequences were concatenated and aligned with sequences of reference strains of different species of *Exserohilum* (Hernández-Restrepo et al., 2018) (Supplementary Table

S1). The phylogenetic tree generated with MEGA-X 10.1.7 using the maximum likelihood method grouped all eight strains from Mali in the *E. rostratum* clade with a bootstrap value of 100% (Supplementary Figure S1. **C**).

To confirm the pathogenicity on rice of the *E. rostratum* strains isolated in Mali, spore suspensions were inoculated on rice plants. Four strains (ML9012, ML9026, ML9040 and ML9044) were grown onto rabbit food agar (50 $g.L^{-1}$ steeped filtrate of rabbit food pellets plus 15 g agar) during 14 days at 25°C and with a 12 h photoperiod (Hau and Rush, 1980). Spores were collected and spore suspensions adjusted to 1.5 x 10^5 conidia/ml with 0.5% gelatin final. The cultivated rice varieties ADNY 11, ARICA 9 and Shwetasoké were grown during 21 days in pots containing peat soil and NPK 13-5-18 at 3.5 g. L^{-1} of soil. Four pots of each variety (5 seedlings/pot) were placed in a tray (60 plants per tray) and the leaves were sprayed with 30 ml of the conidial suspensions or water at 0.5% gelatin (negative control). Plants were kept at maximum humidity (100%) at 21°C for one night and then transferred to a phytotron at 27°C. Seven days after inoculation, circular or oval foliar lesions of less than 5 mm long, either brown or dark, sometimes whitish in their center were observed (Supplementary Figure S1. **B**). These lesions were identical to those observed in rice fields infected by *E. rostratum* affects a wide range of plant species, particularly grasses. It has been observed on rice in many countries (Cardona and Gonzàlez, 2007; Silva et al., 2016; Mahmad Toher et al., 2016; Majeed et al., 2016). However, to our knowledge this is the first report of *E. rostratum* on rice in Mali. Additional monitoring is needed to determine its relative prevalence compared to *B.* oryzae.

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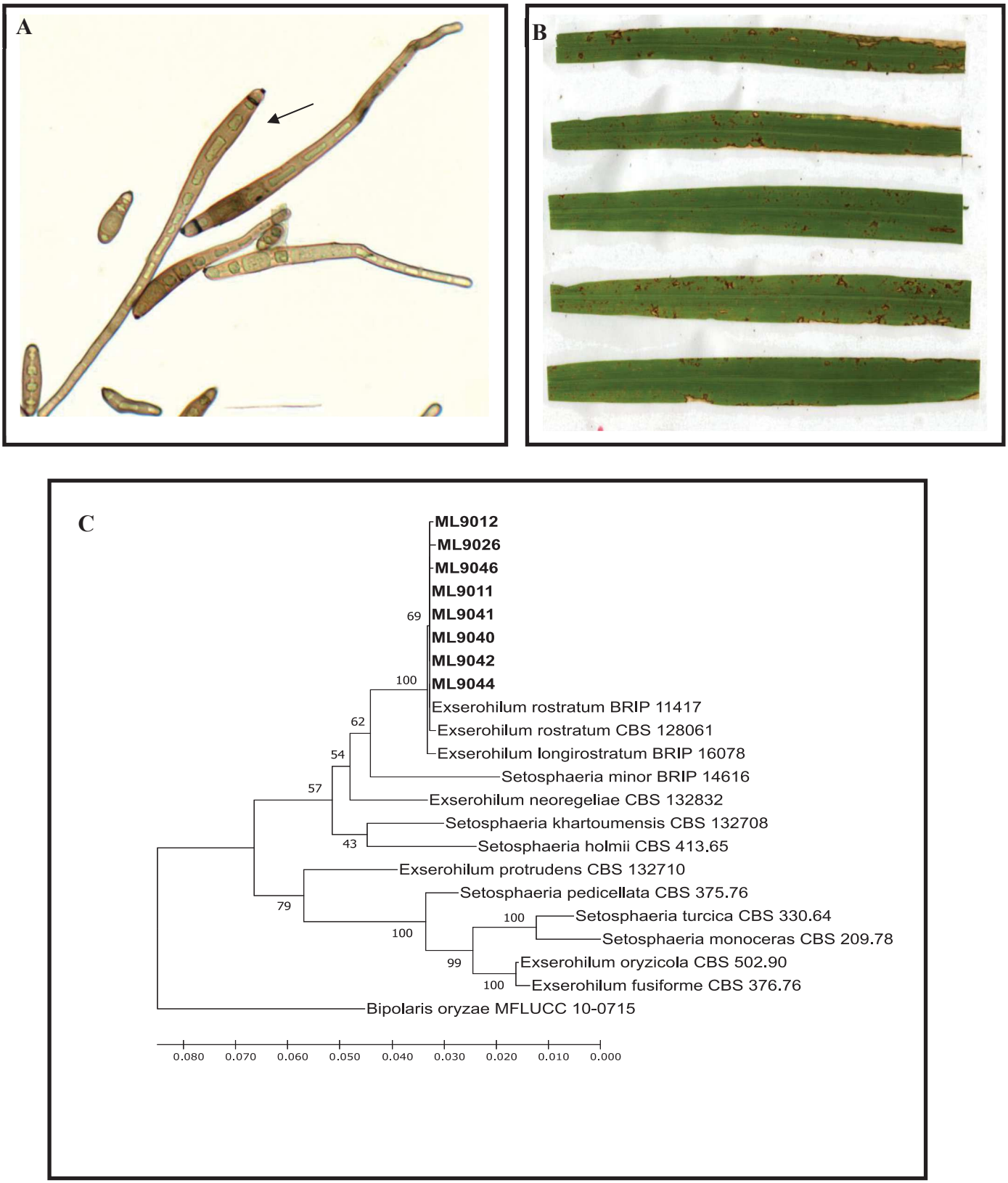

Supplementary Figure S1. **A**) Different sizes of conidia with protruding hilum observed under the microscope (400x). **B**) Brown spots on rice leaves 7 days after artificial inoculation at 1.5 x 10^5 spores/ml. **C**) Phylogenetic tree realized by the maximum likelihood method showing the relationship between isolates sampled in Mali (bold) and a selection of reference isolates and based on the comparison of concatenated sequences of ITS, GAPDH and TEF1 genomic regions.

Supplementary Table S1: Accessions from Genbank used to create the phylogenetic tree

Species	Strains numbers	GenBank accession number		
		ITS	GAPDH	TEF1
Exserohilum rostratum	BRIP 11417	LT837836	LT882553	LT896656
Exserohilum rostratum	CBS 128061	KT265240	LT715900	LT896658
Setosphaeria minor	BRIP 14616	LT837470	LT883545	LT883580
Exserohilum protrudens	CBS 132710	LT631308	LT715880	LT896651
Setosphaeria pedicellata	CBS 375.76	LT631330	LT715879	LT896631
Exserohilum fusiforme	CBS 376.76	LT837456	LT883535	LT883562
Exserohilum oryzicola	CBS 502.90	LT631331	LT715878	LT896629
Setosphaeria monoceras	CBS 209.78	LT837471	LT715875	LT883581
Setosphaeria turcica	CBS 330.64	HF934950	HG779153	LT896615
Exserohilum neoregeliae	CBS 132832	LT837476	LT715886	LT896607
Setosphaeria khartoumensis	CBS 132708	LT837461	LT715888	LT883569
Setosphaeria holmii	CBS 413.65	LT837459	LT715890	LT883567
Exserohilum longirostratum	BRIP 16078	LT837826	LT882563	LT896640
Bipolaris oryzae	MFLUCC 10-0715	JX256416	JX276430	JX266585

RESUME

Biology and population genetics of *Pyricularia oryzae* for rice blast control in Mali

Abstract

Rice production is affected by several plant pathogens, including the fungus *Pyricularia oryzae*, which causes blast disease. This disease is a major constraint to the intensification of rice production in the world. To improve integrated pest management, it is necessary to know the sources of inoculum and useful to have information on the diversity of the pathogen. However, presently, the disease prevalence and the relative importance of inoculum sources have not been assessed in West Africa and, more specifically, in Mali. Similarly, the genetic diversity and population structure of *P. oryzae* are poorly documented in Africa.

The general objective of this thesis is, therefore, to contribute to filling this knowledge gap, and the specific objectives are 1) to assess the incidence and severity of rice blast in different rice-growing areas in Mali, 2) to determine whether seeds, straw, and/or wild rice are sources of inoculum for cultivated rice, and 3) to determine the population structure of *P. oryzae* in Africa.

Surveys conducted in farmers' and experimental plots in 2018 allowed to observe leaf and panicle blast in 10 out of 17 sites surveyed. In symptomatic fields, leaf incidence ranged from 24 to 72% and leaf severity from 2 to 15%. The highest values of panicle incidence and severity reached 94% and 52%, respectively. In 2019, leaf blast was observed on 3 out of 4 sites surveyed, and incidence and severity were much lower (maximum of 7% and 1%, respectively). Incidence and severity were not significantly different between irrigated and rainfed-lowland agrosystems. The highest values for panicle blast incidence and severity confirm that blast is a potential cause of high yield losses.

The analysis of seed mycoflora showed the presence of the genera *Bipolaris* and *Curvularia* in 17 of the 20 plots with averages of 4.6 and 4.3% of contaminated seeds, respectively. The third pathogen observed was a fungus of the genus *Fusarium*. *P. oryzae* was not detected on the seeds. In contrast, *P. oryzae* was observed in straw lots from Niono (3%) and Sélingué (6%). The hypothesis that wild rice (*Oryza longistaminata*) is a source of inoculum for Asian cultivated rice (*O. sativa*) was also tested. Under controlled conditions, wild rice isolates are pathogenic to cultivated rice. But, on average, these isolates have a narrower range of compatibility than isolates collected from cultivated rice. Results of pathogenicity tests suggest that *P. oryzae* isolates from wild rice have the potential to attack cultivated rice in the field. However, the populations of *P. oryzae* on cultivated and wild rice are genetically differentiated. Thus, although physically close, each host plant harbors a different population of the pathogen, and our results suggest that wild rice is not a source of blast inoculum for cultivated rice. Seeds do not appear to either be a source of inoculum for blast epidemics in Mali. The role of infected straws needs to be further studied.

The analysis of the population structure of *P. oryzae* with microsatellite markers revealed four genetic groups in Africa and Madagascar. These groups correspond to the four groups identified at the worldwide level. The populations of West Africa, East Africa, and

Madagascar are highly differentiated. The geographic structure is consistent with limited dispersal and with some migratory events between neighboring countries. Both sexual compatibility groups (Mat1.1 and Mat1.2) are present in Africa with a dominance of Mat1.2, but no fertile female strains were detected, confirming the absence of sexual reproduction on this continent. This study showed an unsuspected high level of genetic diversity of *P. oryzae* in Africa and suggests several independent introductions.
Keywords : rice, blast disease, *P. oryzae*, inoculum source, population structure

Biologie et génétique des populations de *Pyricularia oryzae* pour le contrôle de la pyriculariose du riz au Mali

Résumé

La production du riz est affectée par plusieurs agents phytopathogènes dont le champignon *Pyricularia oryzae*, agent de la pyriculariose. Cette maladie est une contrainte majeure à l'intensification de la production du riz dans le monde. Pour améliorer la lutte intégrée, il est nécessaire de connaître les sources d'inoculum et utile de disposer d'informations sur la diversité de l'agent pathogène. Toutefois, actuellement la prévalence de la maladie et l'importance relative des sources d'inoculum n'ont pas été évaluées en Afrique de l'Ouest, et plus spécifiquement au Mali. De même, la diversité et la structure des populations de *P. oryzae* sont peu documentées en Afrique.

Cette thèse a donc pour objectif général de contribuer à combler ce déficit de connaissances et pour objectifs spécifiques 1) d'évaluer l'incidence et la sévérité de la pyriculariose du riz dans les différentes zones rizicoles au Mali, 2) de déterminer si les semences, les pailles et/ou le riz sauvage sont sources d'inoculum pour le riz cultivé et 3) d'établir la structure des populations de *P. oryzae* en Afrique.

Les prospections conduites sur des parcelles paysannes et expérimentales en 2018, ont permis d'observer la pyriculariose foliaire et paniculaire dans 10 des 17 sites prospectés. Dans les champs symptomatiques, l'incidence foliaire allait de 24 à 72 % et la sévérité foliaire de 2 à 15 %. Les valeurs les plus élevées d'incidence et de sévérité paniculaires atteignaient respectivement 94 % et 52 %. En 2019, la pyriculariose a été observée sur 3 des 4 sites prospectés et l'incidence et la sévérité étaient beaucoup plus faibles (respectivement 7 % et 1% maximum). L'incidence de la maladie n'était pas significativement différente entre culture irriguée et bas-fond. Les valeurs maximales d'incidence et de sévérité paniculaire confirment que la pyriculariose est un risque potentiel de pertes de rendement élevées.

L'analyse de la mycoflore des semences a montré la présence des genres *Bipolaris* et *Curvularia* dans 17 des 20 lots examinés avec des moyennes de 4,6 et 4,3% de semences contaminées respectivement. Le troisième agent pathogène observé était un champignon du genre *Fusarium*. *P. oryzae* n'a pas été détecté sur les semences. Par contre, nous avons observé la présence de *P. oryzae* dans les lots de paille de Niono (3%) et de Sélingué (6%). L'hypothèse que le riz sauvage (*Oryza longistaminata*) soit une source d'inoculum pour le riz cultivé asiatique (*O. sativa*) a aussi été testée. Dans des conditions contrôlées, les isolats de riz sauvage sont pathogènes pour le riz cultivé. Mais, en moyenne, ces isolats ont une gamme plus étroite de compatibilité que les isolats collectés sur le riz cultivé. Les résultats des tests de pathogénicité suggèrent donc que les isolats de *P. oryzae* provenant du riz sauvage ont le potentiel d'attaquer le riz cultivé dans les champs. Cependant, les populations de *P. oryzae* sur le riz cultivé et le riz sauvage sont génétiquement différenciées. Ainsi, bien que physiquement proches, chaque plante hôte abrite une population différente de l'agent pathogène et nos travaux suggèrent que le riz sauvage n'est pas une source d'inoculum de pyriculariose pour le riz cultivé. Les semences ne semblent pas non plus être une source d'inoculum pour les épidémies de pyriculariose au Mali. Le rôle des pailles infectées doit faire l'objet d'une étude plus approfondie.

L’analyse de la structure des populations de *P. oryzae* avec des marqueurs microsatellite a mis en évidence quatre groupes génétiques en Afrique et à Madagascar. Ces groupes correspondent aux quatre groupes identifiés à l’échelle mondiale. Les populations d'Afrique de l'Ouest, d'Afrique de l'Est et de Madagascar sont très différenciées. La structure géographique est cohérente avec une dispersion limitée et avec quelques événements migratoires entre pays voisins. Les deux signes de compatibilité sexuelle (Mat1.1 et Mat1.2) sont présents en Afrique avec une dominance de Mat1.2 mais aucune souche fertile femelle n'a été détectée, ce qui confirme l'absence de reproduction sexuée sur ce continent. Cette étude montre un niveau élevé insoupçonné de diversité génétique de *P. oryzae* en Afrique et suggère plusieurs introductions indépendantes.
Mots clés : riz, pyriculariose, *P. oryzae*, source d’inoculum, structure des populations