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Research Paper

Genetic study of diversity and blast resistance in Ethiopian rice cultivars adapted to different ecosystems

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Rice (*Oryza sativa* L.) has been considered one of the most important crops in Ethiopia. Landraces and improved accessions in Ethiopia were characterized on the basis of polymorphism data for SSR markers, and classified into two groups: I and II. Cluster I was further divided into two sub-clusters, Ia and Ib. Cluster Ia corresponded to Japonica-like type, Cluster Ib to Japonica type, and Cluster II to Indica type with some Indica-like type. Many landraces and improved varieties belonged to Cluster Ia. Superior landraces were included in Cluster Ib. Further categorization based on blast resistance demonstrated three groups: Clusters A, B1, and B2. Cluster A comprised accessions with relatively high resistance, whereas Clusters B1 and B2 included susceptible accessions. Most of the improved varieties were found in Cluster A. Superior landraces, X-Jigna classified into Ib or DNA type tended to be susceptible in Cluster B2 for blast resistance. These results demonstrated that traditional landraces preferred by farmers should be improved for disease resistance using blast-resistant varieties. In order to avoid hybrid sterility occurring in cross-hybridizing breeding between Indica and Japonica types, desirable parental accessions can be chosen within the same DNA cluster. The clustering information among accessions may be useful in breeding schemes for selection of counterparts in cross-breeding programs.

Key Words: genetic variation, rice, DNA marker, blast, Ethiopia.

Introduction

Rice (*Oryza sativa* L.) is a crop of major economic and cultural importance in Asia, where over 90% of the world's rice is produced, feeding more than half of the world's population (Barker *et al.* 1985). Consumption of rice is growing faster than any other food crop in Africa and it is also a cash crop providing employment in African countries. Twenty-two of the 43 rice-producing countries in Africa are experiencing growing demand for rice, necessitating import of 10–90% of their needs, at an estimated cost of over US$5.5 billion per year (AfricaRice 2017). In Ethiopia, rice is an economically important and strategic food security grain crop and its production has doubled within a short time despite fluctuations over the years (EthioRice Project 2016: https://sites.google.com/site/ethiorice/). Currently, total annual paddy production and productivity have reached 136 thousand tons and 2.81 ton/ha, respectively, making it the second highest yielding cereal crop after maize. Despite an increase in domestic production, rice import has also increased drastically from ~43,000 tons (2010) to ~312,000 tons (2016) with values of ~US$26 million and ~US$ 171 million, respectively (Addis *et al.* 2018).

Although currently expanding, the exact timing of initial introduction of rice is unclear. According to Gebey *et al.* (2012) rice was introduced in the early 1970s, whereas EthioRice (2018) suggested that rice cultivation started in the early 1980s. Addis *et al.* (2018) also reported that the first rice introduction was started at Gambella and Pawe to address issues of food security and resettlement and at Fogera for food security, in the 1970s and 1980s, respectively.

Rice breeding research in Ethiopia has focused on the introduction of germplasms to evaluate for adaptation. Collaboration with external research institutions in this regard has led to the introduction of numerous rice germplasms, many of which were bred by AfricaRice and IRRI. NERICAs (New Rice for Africa) developed and released

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by the Africa Rice Center (then WARDA: West Africa Rice Development Association) are one of these introduced improved varieties, which were developed by inter-species hybridization between Asian rice, *O. sativa*, and stress resistant African rice, *O. glaberrima* (Jones *et al.* 1997).

Despite the release of new varieties for upland, lowland rainfed, and intermittently irrigated conditions including NERICAs, farmers in some localities tended to continue cultivating landraces introduced in the past. Including landraces, several cultivars exist in Ethiopia. X-Jigna is one of the landraces preferred by farmers because of its higher productivity and chilling tolerance adapted to highlands such as Fogera, which is the main rice-producing area. However, more than 20 years of cultivation has seen the emergence of various difficulties in cultivation, such as diseases. In order to improve rice, Ethiopian rice breeding requires not only introduced material but also a cross-hybridization program to fine-tune genetic resources depending upon regional demands. For this purpose, rice breeders must know the genetic background of potential materials. Rice genetic resources, however, have not been characterized genetically.

The genetic differentiation of rice accessions has been studied based on morphological and physiological characteristics (Matsuo 1952, Morishima and Oka 1981, Oka 1953). Other markers were developed, such as isozymes, SSR, and chloroplast markers, for evaluation of the diversity of genetic resources (Chen *et al.* 1993, Garris *et al.* 2005, Ishikawa *et al.* 1991, Oka 1988, Pai *et al.* 1975, Second 1982). These classifications always distinguished two main varietal groups, namely Indica and Japonica. Cross-hybridization between these remote groups always induces high sterility as a reproductive barrier (Oka 1988). In this report, we applied DNA markers to precisely classify Ethiopian materials.

Another limiting factor for the production and productivity is rice blast, which is becoming a major disease in Ethiopia, and it reduces productivity in various areas, including Fogera, Pawe, Assosa, Gambella, Guraferda, and Tepi. Survey reports by Mebratu *et al.* (2015) and Wasihun and Flagote (2016) indicated that leaf blast and panicle blast symptoms were observed in all assessed fields in Guraferda, Pawe, and Assosa. Similarly, the most popular and high-yielding landrace, X-Jigna, has been affected by blast in Fogera. Although some improved varieties including NERICAs showed resistance, but they are susceptible to cold stress in Fogera and similar areas. Thus, new varieties corresponding to local demands are required in Ethiopia.

Kawasaki-Tanaka and Fukuta (2014) investigated 324 Japanese rice accessions using 64 SSR markers and 16 blast isolates, and classified them into two DNA clusters and three resistance groups. They also postulated nine resistance genes among accessions including *Pik-s*, *Pish*, *Pia*, *Pii*, *Piz*, *Piz*(t), *Pik*, and *Pita* based on reaction patterns with different varieties. Odjo *et al.* (2017) studied genetic diversity and blast resistance using 61 SSR markers and 32 blast isolates among 195 rice accessions from West Africa, and found resistant and susceptible groups. This research exhibited compositions of resistant genes among local breeding materials. In this report, we also tried to understand Ethiopian rice accessions of both landraces and improved varieties. Both, genetic background examined with DNA markers, and blast resistance will accelerate rice breeding programs in Ethiopia.

Materials and Methods

Plant materials

A total of 79 rice accessions were subjected to DNA genotyping (**Table 1**). As landraces, 27 local accessions were used, collected from five areas in four regions: Fogera in Amhara, Pawe and Assosa in Benshangul Gumize, Guraferda in South Nations, Nationalities and Peoples Region (South NNPR), and Abobo in the Gambella region (**Fig. 1**). These regions are distinctly different in terms of temperature, rainfall pattern and intensity, relative humidity, cropping duration, and environmental stress types to which local cultivars have adapted. In order to compare genetic diversity and relationships, 33 improved accessions that had been released between 1998 and 2017 by different research centers in Ethiopia were included. The improved accessions comprised upland NERICAs, other upland rice, lowland rice, and intermitteńtly irrigated rice. As controls, seven Indica and 12 Japonica varieties comprising six Tropical-Japonica (Tr-J) and six Temperate-Japonica (Tm-J) types were used, as characterized Muto *et al.* (2016) (**Supplemental Table 1**). In addition, 42 Japanese Rice Core Collection (JRC; https://www.gene.affrc.go.jp/databases-core_collections_jr_en.php), and 63 World Rice Core Collection (WRC; https://www.gene.affrc.go.jp/databases-core_collections_wr_en.php) varieties were employed to clarify genetic similarity with Ethiopian Japonica- and Indica-type accessions, respectively. Most accessions we used in this experiment from the JRC accessions were Japonica type, whereas the WRC includes Indica and Japonica types (Ebana *et al.* 2010, Ichitani *et al.* 2016). The JRC and WRC were provided by the National Institute of Agrobiological Sciences (NIAS), Japan.

For blast resistance evaluation, among 79 accessions used in molecular analysis, only 62 accessions including; 27 landraces, 33 improved accessions, one Japonica accession (WAB56-104) and another Indica accession (IR64) were used. As controls, 25 standard differential varieties and one susceptible accession, the Chinese Japonica group accession, Lijiangxintuanheigu (LTH) were used as characterized by Kawasaki-Tanaka and Fukuta (2014). The differential varieties and LTH were kindly provided by JIRCAS TARF, at Ishigaki Island, Okinawa, Japan.

Molecular markers and DNA extraction

A total of 50 SSR markers showed polymorphic and

Table 1. Details of accessions and their Indica-Japonica classification as presumed by four INDEL markers

Accession	Cultivation type	Type	Origin	Location/ Country	DNA Cluster	INDEL[a]				Indica-Japonica[b] classification
						ORF100	Pgi 1	Cat 1	Acp2	
SGU01	Upland	Landrace	South NNPR	Ethiopia	Ia	ND	D	D	INS	Rec.
GAM02	Upland	Landrace	Gambella	Ethiopia	Ia	ND	D	ND	Non-INS	Rec.
AMF13	Lowland	Landrace	Amhara	Ethiopia	Ia	ND	D	D	INS	Rec.
GAM04	Upland	Landrace	Gambella	Ethiopia	Ia	ND	D	D	Non-INS	Rec.
BGP-01	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	ND	Non-INS	Rec.
BGP-03	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-04	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-05	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-06	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-07	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-09	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-10	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-11	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-12	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-13	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-14	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
BGP-15	Upland	Landrace	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
AMF14	Lowland	Landrace	Amhara	Ethiopia	Ia	ND	D	D	INS	Rec.
Fogera 1	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	D	INS	Rec.
Adet	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
NERICA-12	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
NERICA-13	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	ND	ND	Non-INS	Rec.
Chewaqa	Upland	Improved	China	China	Ia	ND	D	D	INS	Rec.
Hiddasse	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
NERICA-3	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	D	INS	Rec.
NERICA-4	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	D	INS	Rec.
SUPERICA-1	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	D	Non-INS	Rec.
Kokit	Upland	Improved	Amhara	Ethiopia	Ia	ND	D	D	Non-INS	Rec.
Pawe-1	Upland	Improved	Benshangulgumize	Ethiopia	Ia	ND	D	D	INS	Rec.
Hiber	Lowland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	D	Non-INS	Rec.
Ediget	Lowland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	D	INS	Rec.
NERICA-15	Intermittent irrigated	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
NERICA-6	Intermittent irrigated	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
NERICA-14	Intermittent irrigated	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
Kallafo-1	Intermittent irrigated	Improved	Madagascar	Madagascar	Ia	ND	D	D	INS	Rec.
NERICA-1	Intermittent irrigated	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
NERICA-2	Intermittent irrigated	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
NERICA-10	Intermittent irrigated	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
Abay	Upland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	D	INS	Rec.
Candidate 3	Lowland	Improved	International Rice Research Institute	Philippines	Ia	ND	D	ND	Non-INS	Rec.
Erib	Lowland	Improved	Africa Rice Center	Côte d'Ivoire	Ia	ND	D	ND	Non-INS	Rec.
Candidate 4	Lowland	Improved	Madagascar	Madagascar	Ia	ND	D	D	INS	Rec.
Wanzaye	Lowland	Improved	Madagascar	Madagascar	Ia	ND	D	ND	Non-INS	Rec.
Shaga	Lowland	Improved	Madagascar	Madagascar	Ia	ND	D	D	INS	Rec.
Demoze	Lowland	Landrace	Amhara	Ethiopia	Ib	ND	D	ND	INS	J
X-JIGNA	Lowland	Landrace	Amhara	Ethiopia	Ib	ND	D	ND	INS	J
GAM01	Upland	Landrace	Gambella	Ethiopia	Ib	ND	D	ND	INS	J
SGU09	Upland	Landrace	South NNPR	Ethiopia	II	D	ND	D	Non-INS	I
AMF06	Lowland	Landrace	Amhara	Ethiopia	II	ND	ND	ND	INS	Rec.
AMF12	Lowland	Landrace	Amhara	Ethiopia	II	ND	ND	ND	Non-INS	Rec.
GAM03	Upland	Landrace	Gambella	Ethiopia	II	ND	ND	D	Non-INS	Rec.
BGA01	Upland	Landrace	Benshangulgumize	Ethiopia	II	ND	ND	D	Non-INS	Rec.
BGP-02	Upland	Landrace	Benshangulgumize	Ethiopia	II	ND	ND	D	INS	Rec.
Getachew	Upland	Improved	Amhara	Ethiopia	II	ND	ND	D	Non-INS	Rec.
Andassa	Upland	Improved	Amhara	Ethiopia	II	ND	ND	D	Non-INS	Rec.
Tana	Upland	Improved	Amhara	Ethiopia	II	ND	ND	D	Non-INS	Rec.
Fogera2 (Komboka)	Lowland	Improved	International Rice Research Institute	Philippines	II	D	ND	D	Non-INS	I
Gumara	Lowland	Improved	Amhara	Ethiopia	II	ND	ND	D	Non-INS	Rec.
Candidate 1	Lowland	Improved	International Rice Research Institute	Philippines	II	ND	ND	D	Non-INS	Rec.
Candidate 2	Lowland	Improved	International Rice Research Institute	Philippines	II	ND	ND	D	Non-INS	Rec.

[a] Genotypes of ORF100, -Pgi 1-INDEL-Cat 1-INDEL-Acp2-INDEL: ND-D-ND-INS for Japonica, D-ND-D-Non-INS for Indica type.
[b] Classification into Indica (I) or Japonica (J) was based on Muto *et al.* (2016); Rec. refers to recombinant.

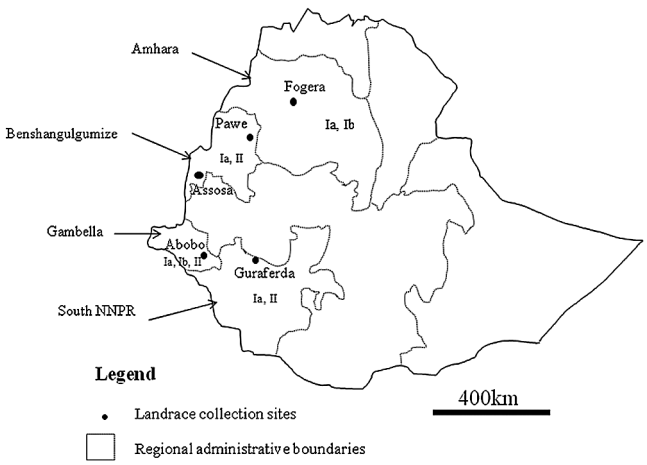

Fig. 1. Rice accession collection sites in Ethiopia. Landrace accessions were collected from Fogera in the Amhara region, Pawe and Assosa in Benshagul Gumize, Abobo in Gambella, and Guraferda in the South NNPR. DNA genotype cluster groups (Ia, Ib and II) to which landraces belonged are indicated for each region.

reproducible amplifications (McCouch *et al.* 2002, Temnykh *et al.* 2000) (**Supplemental Table 2**). These polymorphic markers were applied to all 79 accessions. Ten primer pairs listed in **Supplemental Table 2** were also applied to detect similarity between Ethiopian Japonica accessions and the JRC, and between Indica accessions and the WRC. Four INDEL markers; ORF100 (Chen *et al.* 1993), Cat1-INDEL, Pgi1-INDEL, and Acp2-INDEL (Muto *et al.* 2016) were applied to classify each accession as Indica or Japonica by genotype to confirm the Indica-Japonica classification based on SSRs. In order to detect either type of Acp2 alleles, alternative reverse primers were adopted because the difference is due to CACTA transposon insertion in the Indica allele and its whole insertion is hard to amplify. Genomic DNA was extracted from fresh leaves of 2-week-old seedlings for each individual using the urea method as described by Chen and Dellaporta (1993) with minor modifications.

PCR was carried out in a total volume of 20 µL per reaction containing 1.5 µL of template DNA, 2 µL 10× PCR buffer, 2 µL dNTPs (2 mM), 1 µL of forward and reverse primers, 0.1 µL *Taq* DNA polymerase (Takara Bio Inc., Shiga, Japan), and 12.4 µL sterile water. PCR amplification was performed using a thermal cycler (Bio-Rad Laboratories, Inc., California, USA) in the following PCR conditions: pre-heating at 94°C for 3 min, 30 cycles of 95°C for 10 s, 55°C for 30 s, and 72°C for 30 s, and a final extension of 72°C for 1 min. The amplification products were separated on 6% polyacrylamide gels at 1500 V for 1: 30 to 2 h in 0.5× TBE and detected by silver staining, as described by Creste *et al.* (2001). ORF100, Cat1-INDEL, Pgi1-INDEL, and Acp2-INDEL were amplified with supplier-recommended reaction buffer with 0.25 U rTaq (NEB Inc., Tokyo, Japan). The PCR conditions were pre-heating at 94°C for 3 min, followed by 35 cycles of 95°C for 10 s, 55°C for 30 s, 72°C for 1 min, and post-heating at 72°C for 5 min. The amplified DNA fragments were electrophoresed on 1.5% agarose gels at 100 V for 1 h in 1× TAE to allow genotyping by their relative migration distances.

Blast resistance evaluation

A total of 20 standard differential blast isolates originating from Japan (JPF 494, JPF 500, JPF 506, JPF 507, JPF 509, JPF 510, JPF 513, JPF 514, and JPF 517), the Philippines (PHL 2, PHL 4, PHL 8, PHL 14, PHL 15 and PHL 16), China (CHN 125), Laos (LAO 12), Benin (BEN 43), Nigeria (NIG 1), and Kenya (KNY 135) were used to evaluate blast resistance among 60 accessions from Ethiopia genotyped for SSRs in this study, 25 differential varieties, and other three control accessions (LTH as the most susceptible accession, IR64, and WAB56-104). Three seeds of each of 88 accessions were planted in plastic cell trays with two replications in a greenhouse at about 25–28°C. The universally susceptible control accession, LTH, was sown at each replication. At the 4th–5th leaf stage, seedlings were sprayed with freshly prepared conidial suspensions of each standard differential blast isolate standardized to 10×10^4 spores/ml at a volume of 80 ml per tray. After inoculation, the seedlings were kept in an incubator at 25°C and more than 90% relative humidity for 24 hours and then transferred to a greenhouse. The disease reaction score for each cultivar was assessed 7 days after inoculation. The reaction scoring was based on the 0–5 scale as described by Hayashi and Fukuta (2009). Scores of 0–2 and 3–5 corresponded to resistant (R) and susceptible (S) reactions, respectively. The resistance frequency (RF) of accessions in relation to blast isolates was calculated as RF = (no. of incompatible isolates/total no. of isolates used) × 100% (Wu *et al.* 2015). In order to postulate resistance genes in Ethiopian accessions, their patterns of reaction to differential blast isolates were compared with those of differential varieties. These differential varieties comprised 23 different specific *R* genes, namely IRBLsh-B for *Pish*, IRBLb-B for *Pib*, IRBLt-K59 for *Pit*, IRBLa-A for *Pia*, IRBLi-F5 for *Pii*, IRBL3-CP4 for *Pi3*, IRBL5-M[LT] for *Pi5*(t), IRBLks-F5 for *Pik-s*, IRBLkm-Ts for *Pik-m*, IRBL1-CL[LT] for *Pi1*, IRBLkh-K3[LT] for *Pik-h*, IRBLk-Ka[LT] for *Pik*, IRBLkp-K60 for *Pik-p*, IRBL7-M for *Pi7*(t), IRBL9-W for *Pi9*(t), IRBLz-Fu for *Piz*, IRBLz5-CA-1 for *Piz-5*, IRBLzt-T for *Piz-t*, IRBLta2-Pi[LT] and IRBLta2-Re for *Pita-2*, IRBL12-M for *Pi12*(t), IRBLta-K1[LT] and IRBLta-CP1 for *Pita*, IRBL19-A for *Pi19*(t), and IRBL20-IR24 for *Pi20*(t) (Kobayashi *et al.* 2007, Telebanco-Yanoria *et al.* 2010, Tsunematsu *et al.* 2000).

Data analysis

Molecular data based on SSR loci and INDEL markers were subjected to analysis using GenAlEX6.5 software (http://www.anu.edu.au/BoZo/GenAlEx/) to determine molecular variance, the number of alleles, expected heterozygosity (He), polymorphic information content (PIC),

and major allele frequency (MAF). Cluster analysis of DNA polymorphism data and the blast reaction mean score was carried out to classify accessions using Ward's hierarchical method (Ward 1963) using JMP 14.0 software (JMP version 14.0 for Windows, 2018; SAS Institute, Inc., Cary, NC, USA). Genetic diversity in terms of expected heterozygosity (Nei 1973) was calculated by following formula, $He = 1 - \sum_{i=1}^{n} xi^2$ where, n is the number of distinct alleles at a locus, and x_i ($i = 1, 2 \ldots n$) is the frequency of allele i in the population.

Results

Genetic characterization of rice cultivars in Ethiopia

Analysis of DNA polymorphism based on 50 SSR markers among 79 accessions showed multiple alleles ranging from 2 to 13, with an average of 7.02 alleles per locus, and a total of 351 alleles were found across the SSR markers and rice accessions used (**Supplemental Table 3**). Expected heterozygosity among 79 accessions varied from 0.23 to 0.88, with an average of 0.65. PIC among the markers ranged from 0.12 (RM6313) to 0.68 (RM8137) and the MAF varied from 0.51 to 0.90. The results were similar to those found by Aljumaili *et al.* (2018), who obtained 4.09 alleles per locus, expected heterozygosity of 0.60 and PIC equal to 0.63 when they evaluated 50 aromatic rice accessions using 32 SSR markers. Landraces were originated from Amhara, South NNPR, Benshangul Gumize, and Gambella regions. In addition, expected heterozygosity of landraces for Amhara (n = 6), Gambella (n = 4), Benshangul Gumize (n = 15), and South NNPR (n = 2) were 0.62, 0.52, 0.35, and 0.42, respectively (**Table 2**).

Cluster analysis demonstrated two major groups, Clusters I and II. Cluster I included control Japonica accessions and NERICA accessions and Cluster II included the control Indica accessions. Therefore, the two clusters were considered to correspond to both Japonica with Japonica-like types and Indica with Indica-like types, respectively (**Fig. 2**). Cluster I was divided into two sub-clusters, Clusters Ia and Ib. Cluster Ia consisted of 45 accessions, including 18 landraces and 26 improved varieties including upland NERICAs, and one Japonica control, WAB56-104 (**Table 1**). Although landraces were mainly upland rice, improved accessions in Cluster Ia originated from diverse production systems; upland NERICAs (n = 4), lowland rice (n = 7), and other upland rice (n = 9), including varieties of intermittently irrigated rice (n = 6) released from various research centers. Although they were from different ecosystems, the relatively lower genetic diversity of Cluster Ia (expected heterozygosity = 0.40) suggested their genetic similarity, whereas Clusters Ib and II showed higher expected heterozygosity values of 0.57 and 0.62, respectively (**Table 2**). Landraces from Amhara (33.3%), Gambella (50%), Benshangul Gumize (86.7%), South NNPR (50%), and the improved varieties (78.8%) belonged to Cluster Ia (**Table 2**). Based on chloroplast INDEL (ORF100), 44 Ethiopian accessions in Cluster Ia showed non-deletion type suggesting that the maternal donor of all accessions in this cluster was Japonica type whereas the other three nuclear INDELs (Cat 1-INDEL, Pgi 1-INDEL, and Acp2-INDEL) showed inconsistency, which carry alternative alleles specific to Indica or Japonica (**Table 1**). All the Ethiopian accessions in Cluster Ia belonged to Japonica-like type. Because the alternative alleles were mixed but maternal donor is Japonica. Eleven Ethiopian accessions of Cluster II belonged to Indica-like type because they carried Indica type deletion type ORF100 but nuclear INDELs were in mixture. These recombinant types between Indica and Japonica types may be attributed to their complex breeding history. The upland NERICA varieties in Cluster Ia, crossbreeds of *O. sativa* (Japonica) and *O. glaberrima* were also shown as Japonica-like type because of different alleles were introduced from *O. glaberrima* (**Table 1**). Cluster Ib consisted of 14 accessions, including 11 control Japonica and three landrace accessions (X-Jigna, GAM01 and Demoze). ORF100 and three nuclear INDELs also confirmed that these three landraces belonged to Japonica type (**Table 1**).

Table 2. Regional composition of DNA clusters

Variety types	Region	No. of accessions in each DNA cluster				Total (%)	Genetic diversity[a]
		I			II		
		Ia (%)	Ib (%)	Sum (%)	II (%)		
Landrace	Amhara	2 (33.3)	2 (33.3)	4 (66.7)	2 (33.3)	6 (100.0)	0.62
	Gambella	2 (50.0)	1 (25.0)	3 (75.0)	1 (25.0)	4 (100.0)	0.52
	Benshangulgumize	13 (86.7)	0 (0.0)	13 (86.7)	2 (13.3)	15 (100.0)	0.35
	South NNPR	1 (50.0)	0 (0.0)	1 (50.0)	1 (50.0)	2 (100.0)	0.42
	Sum	18 (66.7)	3 (11.1)	21 (77.8)	6 (22.2)	27 (100.0)	0.48
Improved varieties	—	26 (78.8)	0 (0.0)	26 (78.8)	7 (21.2)	33 (100.0)	0.55
Control	—	1 (5.3)	11 (57.9)	12 (63.2)	7 (36.8)	19 (100.0)	0.68
Total		45 (57.0)	14 (17.7)	59 (74.7)	20 (25.3)	79 (100.0)	0.65
Genetic diversity		0.40	0.57	0.54	0.62	0.65	—

[a] Genetic diversity was estimated in terms of expected heterozygosity (Nei 1973).

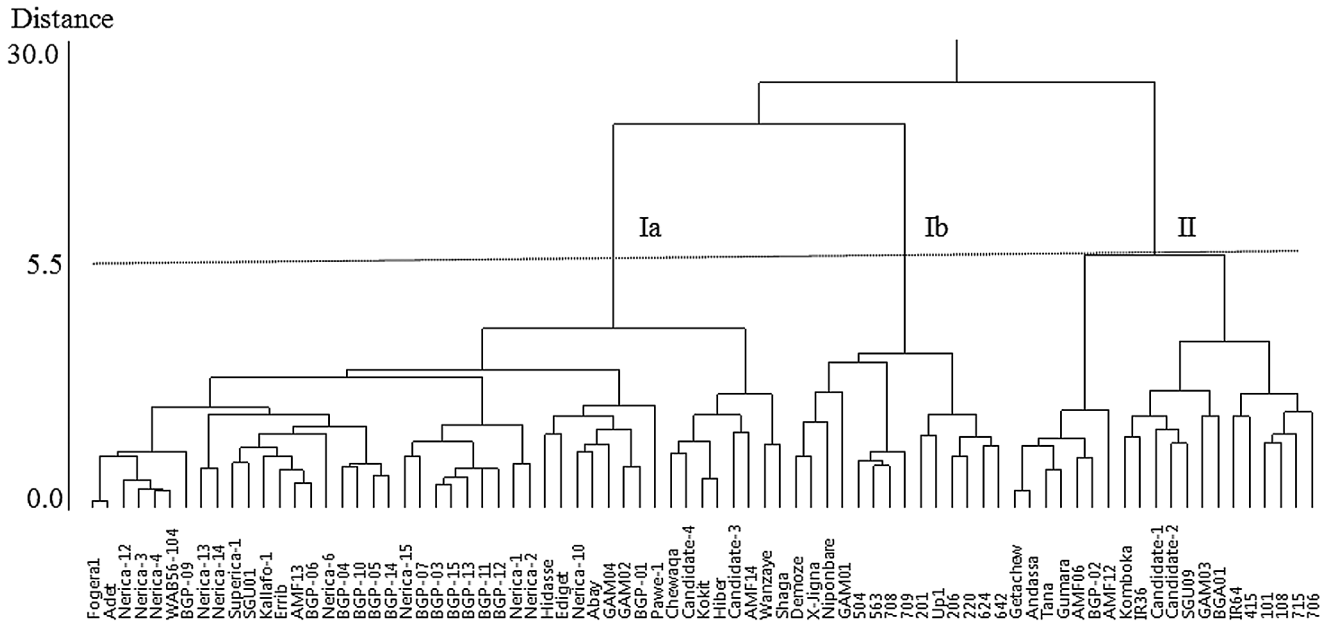

Fig. 2. Classification of rice accessions in Ethiopia based on polymorphism data for 50 SSR markers. Cluster analysis was carried out using Ward's hierarchical method (Ward 1963) with JMP14.0 software (SAS Institute Inc., Cary, NC, USA) for Windows. A total of 79 accessions including 27 landraces, 33 improved cultivars and 19 controls (12 Japonica and 7 Indica group control cultivars) were classified into two major clusters, I and II. Cluster I was further divided into Ia and Ib. Ia included entirely Japonica-like recombinant type while Ib comprised Japonica type. Cluster II corresponded to Indica and Indica-like types.

Accessions in Cluster II comprised six landraces (AMF06, AMF12, BGA01, BGP-02, GAM03, and SGU09) and seven improved varieties including Fogera2 (Komboka) along with seven control Indica accessions. SGU09 and Komboka carried deletion type ORF00 as Indica type cytoplasm and all Indica type alleles for the three INDELs. Remaining five landraces and six improved varieties in Cluster II carried various mixtures of genotypes for the three INDELs. Their cytoplasm donor was Japonica type. Thus, the two of the 13 Ethiopian accessions were identified as Indica type and the eleven out of them as Indica-like type (**Table 1**).

Relationships of Ethiopian Japonica and Indica types to the JRC and WRC

In order to clarify trends, some Ethiopian landraces were compared with core collections developed by NARO, known as the JRC and WRC (**Supplemental Figs. 1**, **2**). Japonica accessions from Ethiopia (X-Jigna, Demoze, and GAM01) were genotyped with the JRC, and Indica accessions (Komboka and SGU09) with the WRC. We found that two accessions, X-Jigna and Demoze, showed a close genetic association with Dango (JRC 25), Rikutourikuu (JRC 49), Aikoku (JRC 26), and Ginbouzu (JRC 27), whereas GAM01 was closely associated with Wataribune (JRC 19) and Himenomochi (JRC 50), although not identical (**Supplemental Fig. 1**). This result demonstrated that the two landraces, X-Jigna and Demoze, had a closer genetic relationship to each other than was the case for GAM01. This may have been due to the uncertain origin of these landraces. GAM01 was collected from another area of Ethiopia with different environmental conditions. With regard to the Indica type, Komboka showed genetic similarity to Jena 035 (WRC 04), Beikhe (WRC 03), and Puluikarang (WRC 06), whereas SGU09 showed similarity to Naba (WRC 05), Tadukan (WRC 20), Kemasin (WRC 62), and Bingala (WRC 66) (**Supplemental Fig. 2**). These WRC accessions were classified as Indica type but originated from different countries, indicating that Indica accessions from Ethiopia may have been introduced from diverse origins.

Genetic variation in blast resistance

Ethiopian blast races have not been established for genetic analysis. Thus, we applied known blast races from other sources and differential varieties carrying known resistance genes. The majority of accessions from Ethiopia exhibited resistance to most blast isolates from Japan, but some showed susceptibility to blast isolates from the Philippines, Laos, Benin, China, and Kenya. A total of 57 accessions (32 improved and 25 landraces) from Ethiopia showed resistance or moderate resistance to all of the blast isolates tested. Cluster analysis based on the pattern of reaction classified accessions into two major clusters, A and B, and Cluster B was further divided into two subclusters, B1 and B2 (**Fig. 3**). Accessions in Cluster A (n = 66, 75%) represented a resistance group with a RF of 75.8% (**Table 3**). In addition to two resistant controls, Japonica accession (WAB56-104) and Indica accession (IR64) and the majority of landraces, most improved accessions including upland, lowland rainfed, intermittently

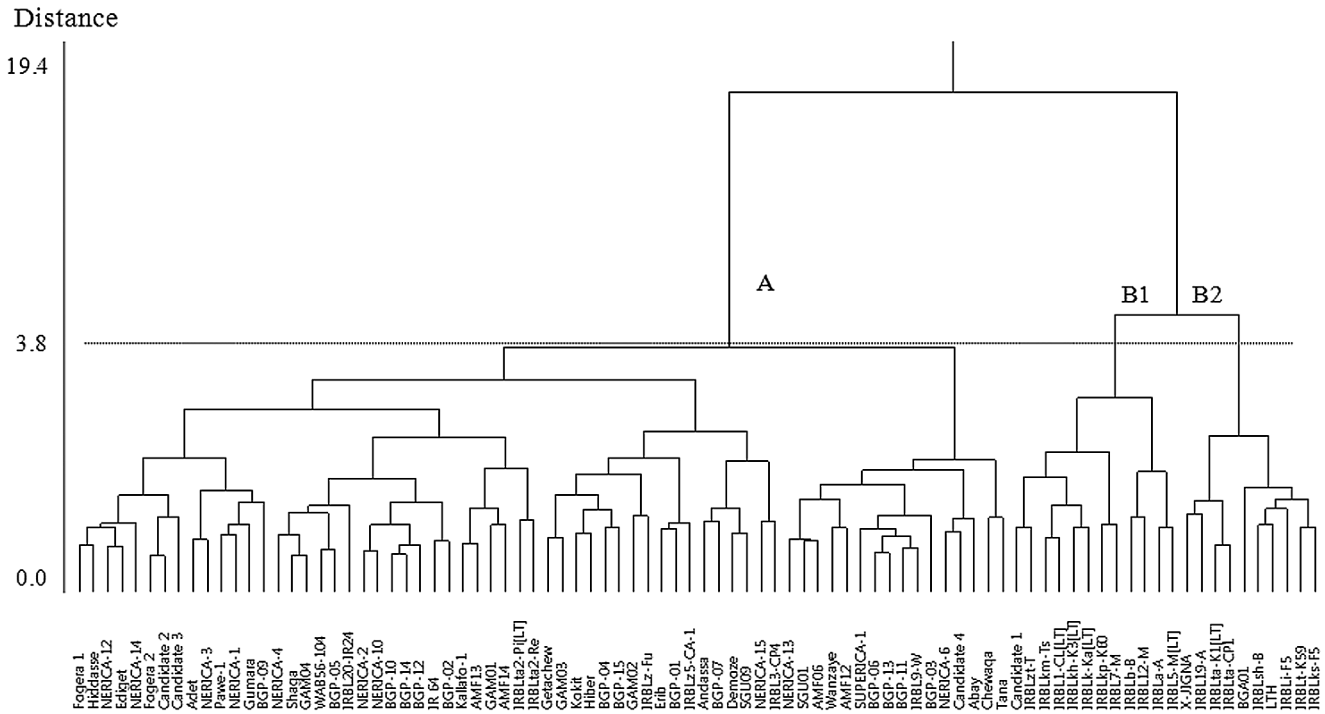

Fig. 3. Classification of rice accessions in Ethiopia based on reactions to standard differential blast isolates. Cluster analysis was carried out using Ward's hierarchical method (Ward 1963) with JMP14.0 software (SAS Institute Inc., Cary, NC, USA) for Windows. A total of 88 accessions including 27 landraces, 33 improved accessions, 25 differential accessions, 1 susceptible accession, LTH and WAB56-104 as Japonica cultivars and IR64 as an Indica cultivar were used.

Table 3. Regional composition of blast reaction clusters (A, B1, and B2) categorized by the phenotype of the blast reaction pattern

Varietal type	Region	No. of rice accessions in each blast reaction cluster				Total (%)	RF (%)[a]
		A	B				
		A (%)	B1 (%)	B2 (%)	Sum (%)		
Landrace	Amhara	5 (83.3)	0 (0.0)	1 (16.7)	1 (16.7)	6 (100.0)	67.5
	Gambella	4 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (100.0)	66.3
	Benishangulgumize	14 (93.3)	0 (0.0)	1 (6.7)	1 (6.7)	15 (100.0)	74.0
	South NNPR	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	77.5
	Sum	25 (92.6)	0 (0.0)	2 (7.4)	2 (7.4)	27 (100.0)	71.3
Improved varieties	—	32 (97.0)	1 (3.0)	0 (0.0)	1 (3.0)	33 (100.0)	77.6
Control	—	9 (32.1)	11 (39.3)	8 (28.6)	19 (67.9)	28 (100.0)	50.0
Total		66 (75.0)	12 (13.6)	10 (11.4)	22 (25.0)	88 (100.0)	67.0
RF (%)		75.8	51.7	27.0	40.5	67.0	—

[a] Resistance frequency (RF) = (No. of incompatible isolates / total no. of isolates used) × 100% (Wu *et al.* 2015).

irrigated, and all NERICA varieties belonged to this group (**Supplemental Table 4**). Accessions in this cluster showed stronger resistance to blast isolates originating from Japan than to those from the Philippines, Laos, China, and Africa. Cluster A also included seven differential varieties with specific resistance genes, namely IRBL3-CP4 for *Pi3*, IRBL9-W for *Pi9*(t), IRBLz-Fu for *Piz*, IRBLz5-CA-1 for *Piz-5*, IRBLta2-Pi [LT] and IRBLta2-Re for *Pita-2*, and IRBL20-IR24 for *Pi20*(t). Thus, accessions clustered together with these control accessions may carry the same genes as *Pi3*, *Pi9*(t), *Piz*, *Piz-5*, *Pita-2*, and *Pi20*(t) along with *Pish*, *Pib*, *Pit*, and *Pia*, in addition to other unknown resistance genes (**Supplemental Table 4**). The overall average of reaction score for accessions in Cluster A was 1.5 (data not shown).

Cluster B1 included one improved accession and 11 differential varieties, many of which showed relatively intermediate to high susceptibility. The reaction score for accessions in this group ranged from 1.2 to 3.9 with an overall mean of 2.5. Each of these 11 differential varieties possessed specific resistance genes such as IRBLb-B for *Pib*, IRBLa-A for *Pia*, IRBL5-M[LT] for *Pi5*(t), IRBLkm-Ts for *Pik-m*, IRBL1-CL[LT] for *Pi1*, IRBLkh-K3[LT] for *Pik-h*, IRBLk-Ka[LT] for *Pik*, IRBLkp-K60 for *Pik-p*,

IRBL7-M for *Pi7*(t), IRBLzt-T for *Piz-t*, and IRBL12-M for *Pi12*(t). Therefore the improved accession, Candidate 1, which belonged to this cluster may have one or more of these resistance genes in its genetic background. A further gene postulation revealed the presence of three genes—*Pi7*(t), *Pik-m*, and *Pik-p* in Candidate 1 (**Supplemental Table 4**).

Cluster B2, on the other hand, comprised 10 accessions including two landraces (X-Jigna and BGA01), a susceptible accession (LTH), and seven differential varieties with an overall average reaction score of 3.0. Most accessions in this cluster were highly susceptible to blast isolates from the Philippines, Benin, Nigeria, China, Laos, and Kenya. These differential varieties possessed specific, predetermined resistance genes, including IRBLsh-B for *Pish*, IRBLt-K59 for *Pit*, IRBLi-F5 for *Pii*, IRBLks-F5 for *Pik-s*, IRBLta-K1[LT] and IRBLta-CP1 for *Pita*, and IRBL19-A for *Pi19*(t). The two landraces, X-Jigna and BGA01, probably contain one or more of these resistance genes in their genetic background. Thus, based on the patterns of blast reactions to differential varieties, gene postulation showed that X-Jigna harbored *Pit* and other unknown resistance genes, whereas the genetic background of BGA01 was found to include an unknown type of resistance gene (**Supplemental Table 4**).

Relationships of blast phenotype clusters to regional compositions

Relationships between blast phenotype clusters and regional compositions of rice accessions were compared (**Table 3**). Except for Candidate 1, which was in cluster B1, all improved accessions (97% of the total) were grouped in Cluster A. Similarly, the majority of landraces from four regions; Amhara (83.3%), Gambella (100%), Benshangul Gumize (93.3%), and South NNPR (100%) belonged to Cluster A (**Table 3**). One landrace from Amhara (X-Jigna) and another from Benshangul Gumize (BGA01) belonged to the most susceptible group, Cluster B2 (**Fig. 3**, **Table 3**). RF of the accessions in Clusters A, B1, and B2 were 75.8%, 51.7%, and 27.0%, respectively. On the other hand, landraces from Amhara, Gambella, Benshangul Gumize, and South NNPR showed RF of 67.5%, 66.3%, 74.0%, and 77.5%, respectively. Improved varieties also showed RF of 77.6%, whereas the control accessions showed RF of 50.0% (**Table 3**). This result demonstrated that a couple of landraces from the Amhara and Benshagulgumize regions showed susceptibility to the blast isolates considered, while those from Gambella and South NNPR exhibited resistance. Moreover, improved accessions tended to be resistant to blast isolates (**Table 3**).

Relationships between blast phenotype and DNA clusters

Blast phenotypes clustered as A, B1, and B2, and DNA clusters Ia, Ib, and II were compared (**Table 4**). In this comparison, accessions that appeared only in both the DNA polymorphism and blast inoculation test were considered.

Table 4. Relationship between DNA and blast reaction clusters

DNA cluster	Blast reaction cluster[a]			
	A (%)	B1 (%)	B2 (%)	Total (%)
Ia	45 (100.0)	0 (0.0)	0 (0.0)	45 (100.0)
Ib	2 (66.7)	0 (0.0)	1 (33.3)	3 (100.0)
Sum	47 (97.9)	0 (0.0)	1 (2.1)	48 (100.0)
II	12 (85.7)	1 (7.1)	1 (7.1)	14 (100.0)
Total	59 (95.2)	1 (1.6)	2 (3.2)	62 (100.0)

[a] Accessions used in both DNA polymorphism and blast inoculation experiments were considered here, i.e. all Ethiopian accessions (33 improved and 27 landraces) and two controls: IR64 and WAB56-104.

Thus, most accessions in Clusters I and II belonged to Cluster A. Although only one accession from Cluster Ib belonged to Cluster B2, all other accessions from Clusters Ia and Ib were grouped into Cluster A. Out of 14 accessions in Cluster II, 12 accessions belonged to Cluster A and only two belonged to Cluster B1 or B2. This result demonstrated that both of the majority of Cluster I except for X-Jigna, and the majority of Cluster II except for BGA01 tended to belong to Cluster A. The result also showed that all accessions as recombinant types in Clusters I and II corresponded to Cluster A. Overall, 92.6% of landraces and 97% of improved rice accessions collected from Ethiopia, the majority of which belonged to Custer A, showed high resistance to the blast races considered here. These results suggested that the accessions had a narrow variation of blast resistance because most of them skewed to the resistant group. This was in contrast to Kawasaki-Tanaka and Fukuta (2014), who evaluated 324 accessions with 16 blast isolates and 64 SSR markers, and clustered them into two clusters, I and II, in which accessions in Cluster I showed a wider variation of resistance from susceptible to highly resistant.

Discussion

Rice cultivation is new to Ethiopia and its actual timing of introduction is uncertain, with various dates provided in previous reports. Gebey *et al.* (2012) reported that rice was introduced there in the early 1970s, whereas EthioRice (2018) suggested the early 1980s. On the other hand, Addis *et al.* (2018) reported that the first rice introduction was started at three locations; Gambella, Pawe, and Fogera during the same period to address challenges of food security and resettlement at Gambella and Pawe, and food security at Fogera in the 1970s and 1980s, respectively. Unlike the others, the Fogera initiative technically supported by North Korean experts was successful and currently impacted the livelihood of farmers. X-Jigna, a high-yielding lowland and chilling tolerant landrace, was one of the first introductions that was adapted and has been widely cultivated to date. X-Jigna and recently introduced varieties have become susceptible to blast, which makes it necessary to search for

new varieties that are resistant to stresses and meet local demands. Thus, there is urgent demand to start cross-hybridization. In cluster analysis, we classified these materials into two groups Cluster I mainly corresponded to Japonica with Japonica-like type and Cluster II to Indica with Indica-like type. This classification was again separated by chloroplast and nuclear INDEL markers. In all the eight gene combinations for three nuclear INDELs, such as Pgi1, Cat1, and Acp2, the haplotype, D-D-INS is predominant (41.7%), followed by D-ND-(Non-INS) (25%) and ND-D-(Non-INS) (15%). It is due to the fact that improved varieties have been established with various parental combinations including NERICA. Most improved varieties and landraces including X-Jigna tended to cluster with control Japonica type. These materials are predominantly distributed in northwestern Ethiopia where low temperature frequently induces chilling stress because of high altitude. Because of hybrid sterility in the progeny of Indica-Japonica crosses, understanding of the genetic background is necessary to choose ideal counterparts to improve rice varieties further. We will procced our rice breeding program by crossing with these genetic background data.

Blast is another major biotic stress challenging rice in Ethiopia. Use of blast resistant varieties is the most economical and feasible approach for growing rice, especially in developing countries like Ethiopia where most growers are subsistence farmers who cannot afford other blast control options. We examined landraces and improved accessions for blast resistance using 20 blast isolates. Accessions were compared with one susceptible control (LTH), and 25 differential varieties carrying 23 different blast resistance genes. This is the first report of its kind to characterize Ethiopian rice accessions for blast resistance using standard blast isolates. Two landraces, X-Jigna related to Japonica type, and BGA01 related to Indica type, revealed high susceptibility to blast isolates considered. The majority of improved accessions and landraces showed high resistance to 20 blast isolates. Vasudevan *et al.* (2014) screened 4246 IRRI accessions for blast resistance including six monogenic lines and two susceptible controls (IR72 and CO39) against five blast isolates through artificial infection and found that 289 accessions exhibited broad-spectrum resistance to all five blast isolates. In this study, the blast resistance of Ethiopian accessions was higher to blast isolates from Japan than those from the Philippines, China, Laos, and Africa. Khan *et al.* (2017) also evaluated 334 rice accessions from Bangladesh in comparison with 25 differential varieties, and LTH using 20 blast isolates obtained from Bangladesh, Kenya, and Japan and reported that the resistance potential of Bangladesh rice accessions was comparably higher against blast isolates from Japan than against those from Bangladesh and Kenya. Similarly, Kawasaki-Tanaka and Fukuta (2014) investigated 324 Japanese rice accessions and 26 controls (23 monogenic lines and three others) using 16 blast isolates from Japan and the Philippines, and indicated that blast isolates from tropical countries such as the Philippines were significantly important for discriminating Japanese accessions as resistant or susceptible.

Resistance genes in Ethiopian accessions were postulated by comparing reaction patterns in differential varieties (Kawasaki-Tanaka and Fukuta 2014, Khan *et al.* 2017). In this study, postulated resistance genes varied markedly among accessions. Out of 60 accessions from Ethiopia, 50 carried more than one resistance gene. Resistant accessions were grouped in Cluster A. Overall, 92.6% of landraces and 97% of improved rice accessions collected from Ethiopia belonged to Cluster A. These accessions are adapted to different ecologies, comprising upland, lowland rainfed, and irrigated conditions. However, the most chilling tolerant accession, X-Jigna, was not in this cluster. The present results of genetic diversity based on molecular markers and blast resistance phenotyping suggested that Ethiopian rice accessions are good reservoirs of useful genetic resources to be used in rice breeding. Good counterparts with superior landraces such as X-Jigna would be chosen with the resistant group but belonged to the same DNA cluster. However, further investigation among Ethiopian accessions for blast resistance using local blast races may reveal some specific blast resistance genes, which can be useful to mitigate blast incidence in rice growing localities in Ethiopia.

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