Genetic characterization of rainfed upland New Rice for Africa (NERICA) varieties based on multiple sites' evaluation

Summary

A total of 18 rainfed upland New Rice for Africa (NERICA) varieties were categorized as the heavy panicle and low tillering types and early heading, in compared with 32 different varieties based on several field evaluations in Temparate (Tsukuba, Japan).

The characteristics of early maturity and heavy panicle of upland NERICA varieties were succeeded from Asian rice varieties, and high dry matter production was introduced from CG 14 (*O. glaberrima* Steud.) or the other unknown donor varieties.

These chromosome components were clarified using 243 SSR markers which showed polymorphism among NERICA varieties and their parents, CG 14 and one of the recurrent parents, WAB-56-104 (*O. sativa* L.).

A total of 14 differential markers were also selected to classify NERICA varieties based on the polymorphism data. However, three groups: NERICAs, 3 and 4, NERICAs, 8, 9 and 11 and NERICAs, 15 and 16 were not distinguishable.

Four primary datasets of agronomic traits are released on JIRCAS website.

Introduction

Rice consumption in West Africa has been increasing due to population growth and change of food preferences among the people (Maclean et al., 2002). Meanwhile, rice production increase through field expansion and cropping system improvement has not kept pace with consumption (WARDA, 2005). Thus, improvement in yield potential is one of key points to increase rice production. Although upland rice fields make up 1.8 million ha of the 4.7 million ha of rice fields (Somado *et al*., 2008), farmers' average yield of upland rice is far below the yield potential of existing varieties. Thus, yield barriers such as tolerance to unfavorable conditions seem to be another critical wall to overcome. Past gains from conventional breeding to overcome yield-limiting factors in upland conditions, such as drought, pest, weeds and low inputs, have been limited, in part because the majority of Asian rice (*Oryza sativa* L.) varieties cultivated in West Africa carried limited resistance to many of the stresses that affected upland rice in the region (Jones *et al*., 1997).

Jones *et al*. (1997) started developing New Rice for Africa (NERICA) varieties for rainfed upland using interspecific hybridization between *Oryza sativa* L. and African rice (*Oryza glaberrima* Steud.) at the African Rice Center (AfricaRice, Former name: Western Africa Rice Development Association, WARDA). The aim was to combine the high yield potential of *O. sativa* based on high spikelet number and useful traits of *O. glaberrima*, such as rapid leaf canopy establishment and high nitrogen responsiveness. The BC_2F_1 progenies between some Asian Japonica-type rice (*O. sativa*) varieties, namely WAB 56-50, WAB

56-104 and WAB 181-18, which were used as the recurrent parents, and an African rice variety, CG 14 (*Oryza glaberrima* Steud.), were established and subjected to advance generation. Then, rainfed upland NERICA varieties were selected. The first seven varieties, NERICAs 1–7, were released by AfricaRice in 2000 (Kaneda 2007a, WARDA 2006) and an additional 11 varieties were released, NERICAs 8–18, in 2005 (Africa Rice Center 2008). The recurrent parents, WAB 56-104 and WAB 56-50, were bred in AfricaRice from a cross between a variety, IDSA6 which was bred at the Institute of Savanna of Ivory Coast and a Brazilian upland variety, IAC164.

It was reported that seven NERICAs 1–7 were heavy panicle type which had big panicles and fewer numbers of tillers, based on the data of yield component investigated at three locations in Ghana in 2004 (Kaneda 2007b). Several lines among them were identified as tolerant to drought both at vegetative and reproductive stages. It was also indicated that NERICAs failed to succeed the target traits from *O. glaberrima,* such as effective nitrogen use, plant growth in the young vegetative stage or weed competitiveness, phosphoric acid (P) absorption ability and highly responsive to its application. Ekeleme *et al*. (2009) examined the ability of weed competition in NERICAs 1, 2 and 4 together with WAB 56-104, CG 14 and ITA 150 as check varieties, but significant differences were not detected among NERICAs and WAB 56-104. Oikeh *et al*. (2008) found the differences in responses to nitrogen and phosphorus. In their study, NERICAs, 3 and 6, were categorized as the suitable varieties at low-input condition, and NERICA 1 showed the highest response to nitrogen. Oikeh *et al*. (2009) evaluated three NERICAs, 1, 2 and 4, and WAB 56-104 under different nitrogen and planting space. Sanni *et al*. (2009a) evaluated stability among 18 NERICAs based on the genotype by environment interaction study and indicated that four NERICAs, 3, 10, 11 and 18, could be considered stable in comparison with the others. Sanni *et al*. (2009b) reported a difference of ratooning formation among NERICAs, WAB 56-104 and CG 14. Ishizaki and Kumashiro (2008) evaluated the abilities of shoot differentiation from calluses and transformation, and found a variation among 18 NERICAs. Semagn *et al*. (2006) tried to evaluate the relationships and genetic differences among 18 NERICAs using 102 SSR markers, which covered all rice chromosomes, and ten agronomic traits: days to heading, days to maturity, plant height, panicle length, number of primary branches, number of secondary branches, grain shattering, filled grain number, empty grain number and yield (kg/ha). Cluster analysis using the data of SSR markers indicated that these varieties were classified into two groups, NERICAs 1–7 and NERICAs 8–18. In their study, the evaluation of agronomic traits was carried out using only seven varieties, NERICAs 1–7, and the relationships between the genotypes of SSR markers and the phenotypes of ten traits in NERICAs was not clarified. These studies carried out with NERICAs were using only a small number of check varieties and the characterization data for various kinds of agronomic and physiological traits related to the yield performance were still limited. Thus, a systematic analysis of upland NERICAs had not yet been carried out for comparison with various types of rice varieties. In addition, these characterizations of agronomical traits in NERICAs were not clearly evaluated in comparison with genetic factor(s) introgressed from *O. glaberrima*.

Ikeda *et al*. (2007) reported that off types and sergegants were observed in the several varieties among NERICAs 1–7 for many morphological traits among the breeder and foundation seeds in AfricaRice. The homozygote selection and its seed propagation of all NERICAs were conducted under the collaboration project between the Japan International Cooperation Agency (JICA) and Africa Rice (Personal communication). Japan International Research Center for Agricultural Sciences (JIRCAS) introduced these seeds through the project and obtained the visually confirmed homozygotes of 18 NERICAs. By using these seeds, agronomic traits related with yield components and heading date were evaluated in comparison with various rice types to characterize NERICAs morphologically and physiologically. Chromosome components were also surveyed to identify the introgressions from *O. glaberrima* into NERICAs using SSR markers distributed in all genome chromosomes.

This database contains four primary datasets of the agronomic traits of upland NERICAs and figures, which were characterized in comparing with several Asian rice varieties based on the data collected from different environment conditions, temperate (Tsukuba city, Ibaraki, Japan). It also present differential SSR markers against NERICAs which are selected based on the polymorphism patterns of SSR markers to classify and distinguish each NERICA varieties.

Materials and Methods

Rice varieties

A total of 18 rainfed upland NERICA varieties were used (Table 1). NERICAs 1–11 were bred with WAB 56-104, three upland NERICAs, 12, 13 and 14, were bred with WAB56-50 and four NERICAs 15-18 were bred with WAB181-18 as the recurrent parents and adapted upland conditions (Jones *et al*. 1997, Kaneda. 2007a, WARDA 2006). The seeds of 18 NERICAs, which were selected and purified by JICA-AfricaRice project in Benin, were introduced to JIRCAS in 2005 and used for characterization. The agronomic traits of NERICAs were evaluated in comparison with 32 controls selected from several categories, Japonica- and Indica-types, landrace and improved types and lowland and upland varieties (Table. 2). These varieties were selected as

Table 1. Polymorphic SSR markers in upland NERICA varieties

NERICA variety	Cross combination [a]
NERICA 1	WAB 56-104 / CG 14//2*WAB 56-104
NERICA 2	WAB 56-104 / CG 14//2*WAB 56-104
NERICA 3	WAB 56-104 / CG 14//2*WAB 56-104
NERICA 4	WAB 56-104 / CG 14//2*WAB 56-104
NERICA 5	WAB 56-104 / CG 14//2*WAB 56-104
NERICA 6	WAB 56-104 / CG 14//2*WAB 56-104
NERICA 7	WAB 56-104 / CG 14//2*WAB 56-104
NERICA 8	WAB 56-104 / CG 14//2*WAB 56-104
NERICA 9	WAB 56-104 / CG 14//2*WAB 56-104
NERICA 10	WAB 56-104 / CG 14//2*WAB 56-104
NERICA 11	WAB 56-104 / CG 14//2*WAB 56-104
NERICA 12	WAB 56-50 / CG 14//2*WAB 56-50
NERICA 13	WAB 56-50 / CG 14//2*WAB 56-50
NERICA 14	WAB 56-50 / CG 14//2*WAB 56-50
NERICA 15	CG 14 / WAB 181-18//2*WAB 181-18
NERICA 16	CG 14 / WAB 181-18//2*WAB 181-18
NERICA 17	CG 14 / WAB 181-18//2*WAB 181-18
NERICA 18	CG 14 / WAB 181-18//2*WAB 181-18

[a] WARDA, 2006.

the representatives including almost all types of *O. sativa* and these made heading under the environmental conditions in the city of Tsukuba. Among the fourteen Indica-type varieties, four landrace varieties, Kasalath, Surjamkuhi, Tadukan and Tetep, are cultivated in tropical countries and the other ten improved varieties, IR 8, IR 24, IR 36, IR 64, IR 74, Hokuriku 143, Milyang 23, Taichung Native 1, Takanari and Mahsuri were bred from temperate and tropical areas. Nine landraces and nine improved varieties were included in the 18 Japonica-types. These nine landraces have been cultivated in lowland or upland areas as the leading varieties in several countries with tropical and temperate areas. Four varieties, Azucena and Davao from the Philippines, Trembese from Indonesia, and Moroberekan from Guinea, Africa, are landraces and cultivated in upland areas. The other four landraces, Basmati217 and Dular from India and Kamenoo and Kibi from Japan, are cultivated in irrigated areas. All nine improved Japonica varieties, Kotobukimochi, Owarihatamochi, Koshihikari, Reiho, Dontokoi, Aichiasahi, Nipponbare, Akihikari and Toride 1, were developed by crossbreeding in Japan. Kotobukimochi and Owarihatamochi were the upland varieties and the others are for irrigated lowlands.

Table 2 . Varieties used in the survey of agronomic traits

	Lowland	Upland
Indica improved	Hokuriku143, IR8, IR24, IR36, IR64, IR74, Mahsuri, Milyang23, Takanari, Taichung Native1	
Indica land race	Basmati217, Kasalath, Dular, Surjamkuhi, Tadukan, Tetep	
Tropical japonica land race		Azucena, Davao, Trembese, Moroberekan
Japonica improved	Aichi Asahi, Akihikari, Dontokoi, Koshihikari, Kotobuki mochi, Reiho, Toride1, Kamenoo, Nipponbare	Owarihatamochi
Japonica land race		Kibi, Oiran
Interspecific varieties		Nerica1, Nerica2, Nerica3, Nerica4, Nerica5, Nerica6, Nerica7, Nerica8, Nerica9, Nerica10, Nerica11, Nerica12, Nerica13, Nerica14, Nerica15, Nerica16. Nerica17, Nerica18

Survey of chromosomal components of NERICA

All 18 upland NERICAs, a donor variety, CG 14 (*O. glaberrima*), one of the recurrent parents, WAB 56-104 (*O. sativa*), and two controls of Asian rice varieties, Nipponbare and Kasalath, were used for genotyping with SSR markers. The recurrent parent, WAB 56-104, was selected as a control variety among three WAB lines, because it was used as the crossing parent in 11 NERICAs and the sister line, WAB 56-50, was also used the other three NERICAs. A total of 295 SSR markers distributing in whole genome chromosomes (McCouch *et al*. 2002, Temnykh *et al*. 2000), were used for genotyping these varieties.

Whole genomic DNA was extracted from the fresh leaves of each variety using a simple method and subjected to PCR (Fukuta *et al*. 2012). These polymorphic data of SSR markers among WAB 56-104, CG 14 and 18 NERICAs were used for the investigations of chromosome components of each NERICA variety. The chromosome regions for introgression from the donor variety, CG 14, were identified and then specific SSR markers were selected to classify each NERICA variety from the others.

Characterization of agronomic traits

A total of 50 varieties including 18 upland NERICAs and 32 control varieties were cultivated at following site(s) and season(s);

1. Irrigated paddy field at JIRCAS, May to October 2007.
2. Upland field at JIRCAS, June to October 2007.
3. Irrigated paddy field at JIRCAS, May to October 2008
4. Upland field at JIRCAS, June to October 2007

Eleven agricultural traits, days to heading, panicle weight , culm and leaf weight, total weight including panicle per plant, ratio of panicle against culm and leaf weight, culm length, panicle length and total length, panicle number per plant, spikelet number per plant and ratio of fertility seeds, were investigated in each variety. Ten plants were investigated with three duplications and the average of 30 plants was used as the representative value in each variety. Days to heading were defined as the time when half the plants showed exerted panicles in each variety.

Classification of NERICA and control varieties based on agricultural traits and polymorphic data of SSR markers.

Data analysis for classifying 50 varieties was conducted using phenotypic values of five traits, culm length, panicle length, number of panicles, clum and leaf weight and panicle weight, which were related with plant type, performing Ward's hierarchical analysis (Ward 1963) using a computer program, JMP6.0 (SAS Institute Inc., Cary, NC, USA) for Windows. The variety groups classified by cluster analysis were characterized in more detail based on the 12 agronomic traits: days to heading, panicle weight, leaf and culm weight, total weight per plant, ratio of panicle against culm and leaf weight, culm length, panicle length, total length, panicle number per plant, number of fertile seeds and number of sterile seeds per plant and ratio of fertility seeds. Cluster analysis by using polymorphism data of 127 SSR markers, which showed polymorphism among 18 NERICAs and four varieties, WAB 56- 104, CG-14, Nipponbare and Kasalath, was also conducted to investigate the differences and relationships among 18 NERICAs.

Results

Chromosomal components of NERICAs

The results of surveys, chromosomal components of NERICAs and their classification have been published in Fukuta *et al*. (2012). It reported that the frequencies of SSR markers with the different types of polymorphic patterns from WAB 56-104 were varied from 7.0 to 34.2% among 18 NERICAs. The introgression included some polymorphism types, CG 14 and different types from CG 14 and WAB 56-104. The frequencies of CG 14 and other type SSR marker introgression varied in the ranges from 1.2 to 10.7% and from 5.8 to 23.5%, respectively. NERICA 6 showed that a total of 34.2% SSR markers were not WAB 56-104 types and was the highest introgression value among NERICAs. The average introgression frequencies in each chromosome also varied among NERICAs. Chromosomes 4, 6, 7, 10, 11 and 12 were higher frequencies than 12.5%, and chromosome 9 was lower. The distributions of introgression frequencies in 18 NERICAs were also different in 12 chromosomes. Almost all chromosomes showed variations in the ranges from 0 to approximately 40%, but three chromosomes, 10, 11 and 12, varied the introgressions with the widest ranges from 0 to approximately 60%. These results indicated that the introgressions varied among NERICAs and also in each chromosome.

Differential DNA maker among upland NERICA s

A set of SSR markers was suggested as differential tools for 18 upland NERICAs (JIRCAS 2012). Seven SSR makers showed the specific polymorphic patterns in each variety. The NERICA varieties were classified by those markers as follows: upland NERICA1, 5, 6, 7, 10, 14, and 17 were classified by their respective single markers; upland NERICA2, 12, 13, and 18 were classified by combining more than one marker. On the other hand, the following varieties did not show any polymorphisms: upland NERICA3 and 4, upland NERICA8, 9 and 11, upland NERICA15 and 16. Thus, they were identified as three separate groups.

Classification of upland NERICAs based on the genotypes of DNA markers

A total 22 varieties, 18 upland NERICAs, and four controls, CG 14, Kasalath, Nipponbare and WAB 56-104, were classified into six groups by cluster analysis using polymorphism data of 127 SSR markers, which consisted of the complete matrix and no missing data between markers and varieties. There were three groups which NERICAs were classified; eleven NERICAs, 1, 2, 3, 4, 5, 7, 10, 12, 13 and 17 together with WAB56-104, three NERICAs, 15, 16 and18, and five NERICAs 6, 8, 9, 11 and 14. Among them, variety groups for NERICAs were closer to the group in which Nipponbare is included, in comparison with the other groups, one with CG 14 and another with Kasalath.

Agronomical characterization of NERICA varieties

A total of 50 varieties including 18 upland NERICAs varied in the wide ranges in each trait. NERICAs showed several unique characters in comparison with the other 32 varieties among nine agronomic traits: panicle weight (g), leaf and culm weight (g), ratio panicle weight against leaf and culm weight (%), panicle number, panicle length (cm), culm length (cm), days to heading, number of seeds per panicle and setting ratio of fertile seeds (%).The average of NERICAs for weight of panicle, panicle length and culm length showed similar to those of the other 32 varieties but had narrower range of distribution. The values of three traits, days to heading, number of panicles and leaf and culm weight, of NERICAs were smaller than those of the other varieties. In contrast, number of seeds per panicle, ratio of panicle weight against leaf and culm weight and ratio of fertility seed per panicle, were higher. The results indicated that NERICAs had the characteristics for heavy panicle-type rice as compared with the other varieties used in this study. Thus, these dry matter productions of NERICAs were not high, but they had heavy panicles in compare with these stems.

Classification of NERICA based on agronomical traits using data of JIRCAS, Tsukuba, Japan in 2007

Fifty varieties were classified into five cluster groups based on the characterizations of five agronomic traits, culm length, panicle length, number of panicles, clum and leaf weight and panicle weight, which were related with the plant types of rice (Table 3). Cluster A included a Japanese upland variety, Owarihatamochi and all NERICAs except for NERICA 6 and these were all upland Japonica varieties. This variety group was characterized as low dry culm weight and matter production (Total weight), high ratio of panicle against culm and leaf (P/CL) weight, small panicle number, and short days for heading, in comparison with the other groups. Thus, this group was categorized as a heavy panicle type variety which showed low tiller, many seeds (spikelets) per panicle, high harvest index, low dry matter production and early heading. Cluster B had five improved Japanese Japonica-type lowland varieties: Aichiasahi, Koshihikari, Reiho, Toride 1 and Nipponbare. These varieties were characterized by low panicle weight, low ratio of P/CL weight, low number of total seeds (spikelets) per plant, low number of seeds per plant and low number and ratio of fertile seeds per plant, short panicle length and high panicle number. This variety group showed the most panicle number types among the five.Cluster C included 11 improved lowland varieties. All varieties were

Table 3. Classification of NERICA based on agronomical traits using data of JIRCAS, Tsukuba, Japan in 2007

Cluster goup	
B	Aichi Asahi, Koshihikari, Reiho, Toride1, Nipponbare, Owarihatamochi
A	Nerica11, Nerica3, Nerica4, Nerica14, Nerica17, Nerica1, Nerica2, Nerica5, Nerica8, Nerica10, Nerica9, Nerica7, Nerica15, Nerica16, Nerica18, Nerica12, Nerica13

Cluster goup	
E	Basmati217, Mahsuri
D	Kasalath, Oiran, Trembese, Tadukan, Tetep, Azucena, Surjamkuhi, Nerica6, Kamenoo, Kibi, Davao, Dular, Moroberekan, Kotobuki mochi
C	Hokuriku143, IR64, IR8, IR74, IR24, Milyang23, IR36, Taichung Native1, Takanari

Modified from Fukuta *et al*. (2012)

semidwarf type except for Akihikari. Fivevarieties, IR 64, IR 8, IR 74, IR 24 and IR 36 were bred through introducing a semidwarf gene, sd-1, from a Chinese variety, Dee-geo-woo-gen, at the International Rice Research Institute (IRRI). Three varieties, Milyang 23, Tanaknari (Imbe *et al*. 2004) and Dontkoi (Tabuchi *et al*. 2000, Uehara 2001) also harbor the sd-1 from IR24 (Deegeo- woo-gen). Hokuriku 143 is a non-shattering mutant line from Nan-jing 11 (Fukuta 1995, Fukuta *et al*. 1994) and Nan-jing 11 introduced a semidwarf gene from Ai-Jio-Nan- Te (Lin and Min 1991). Oba *et al*. (1990) confirmed that Ai- Jio-Nan-Te harbored the same allele of sd-1. These varieties harbor the common semidarf gene, sd-1, and were characterized as the other heavy panicle type, which had the higher values in these traits, weights of panicle, culm and total, panicle length and number of panicles and lower ratio of P/CL weight and shorter culm length, than those in group A. Cluster D included a total of 14 landraces, four Indicatype varieties, Kasalath, Tadukan, Tetep and Surjamkuhi and eight Japonica-type upland varieties, Oiran, Trembese, Azusena, Kamenoo, Kibi, Davao, Kotobukimochi and Moroberekan and one Japonica-type lowland variety, Dular and NERICA 6. These varieties showed the intermediate values in all traits among the five groups. Cluster E had two lowland varieties, Basmati 217 and Mahsuri, and showed the higher weights of culm and total of panicle, culm and leaf and longer lengths of panicle, culm and total of panicle and culm, longer days to heading, and lower sterile and high ratio of fertile seeds and lower ratio of P/CL weight than those in the other groups. This group was characterized by the long vegetative stage and low harvest index. These results indicated that almost all NERICAs were heavy panicle type, but the type was different from that of the improved lowland varieties.

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