Developing Lowland Rice Germplasm with Resistance to Multiple Biotic Stresses through Anther Culture in Uganda

Article *in* Journal of the Korean Society of International Agriculture · November 2015

DOI: 10.12719/KSIA.2015.27.4.415

韓國際農誌(Korean J. Int. Agric.), 27(4): 415~420(2015)
http://dx.doi.org/10.12719/KSIA.2015.27.4.415
ISSN 1225-8504 (Print)
ISSN 2287-8165 (Online)

한국국제농업개발학회지
The Korean Society of International Agriculture

Developing Lowland Rice Germplasm with Resistance to Multiple Biotic Stresses through Anther Culture in Uganda

Lamo Jimmy*, Gyoung-rae Cho, Jane Ininda***, Dartey Paul Kofi Ayirebi****, Ekebu James*, Ekobu Moses*, Alibu Simon*, Okanya Stephen*, Oloka Bonny, Otim Michael*, and Asea Godfrey*, Kyung-Ho Kang*****†**

**National Crops Resources Research Institute, Namulonge Box 7084 Kampala Uganda*
***International Technology Cooperation Center, RDA (RDA), Jeonju 54875, Korea*
****Alliance for a Green Revolution in Africa P.O BOX 66773, Westlands, 00800, Nairobi*
*****CSIR-Crops Research Institute, P.O. Box 3785, Kumasi, Ghana*
******Crop Breeding Division, National Institute of Crop Science, RDA, Wanju 55365, Korea*

약배양에 의한 우간다의 복합내병성 벼 유전자원 개발

라모 지미* · 조경래 · 제인 이닌다*** · 다티 폴 코피 아이레비**** · 에크부 제임스* · 에코부 모세* · 알리부 시몬* · 아카냐 스테펜* · 오로카 보니* · 오팀 미카엘* · 아시 고프레이* · 강경호*****†**

*우간다 국작작물자원연구소, **,농촌진흥청 국제협력센타, ***아프리카녹색혁명연대,
****CSIR 작물연구소, *****농촌진흥청 국립식량과학원

ABSTRACT: The lowland rice genotypes grown in Uganda were introduced in the 1970s. These genotypes (now landraces) are threatened by multiple biotic stresses namely; Rice Yellow Mottle Virus (RYMV) disease, Bacterial Leaf Streak(BLS). Bacterial Leaf Blight (BLB), and Rice Blast (BL). There are currently no rice lines with multiple resistance to these stresses although attempts have been made to develop them through hybridization involving cultivated, local and introduced lines and four varieties with tolerance to RYMV have been released. The use of potential resistance donor such as the traditional African cultivated rice, *Oryza glaberrima*, could be an alternative approach to furnish multiple resistance to the cultivated rice. The rice germplasm developed from a cross of an *Oryza glaberrima* from Niger Delta and Milyang23, a high-yielding Korean rice variety were evaluated for multiple resistance in Uganda as a Korea-Africa Food & Agriculture Cooperation Initiative (KAFACI)-Alliance for a Green Revolution in Africa (AGRA) joint cooperative project, "Enhancement of High Yielding Rice Germplasm in African Countries through Anther Culture Breeding". Milyang23 was back crossed 4 times with *Oryza. glaberrima* and fixed through anther culture in Korea. An evaluation of 50 lines generated showed that up to 98%, 92%, 88% and 88% of the test plants showed resistance to the RYMV, BLS, BLB and BL diseases, respectively. There was no symptoms of the four diseases in 74% of the genotypes tested. The plants that showed symptoms of the three diseases had scores of not more than 3 on a 1 to 9 scale. This preliminary finding demonstrates that these generations of rice lines could help solving the current problem of susceptibility to multiple diseases.

Key words: germplasm, anther culture, hybridization, rice yellow mottle virus, bacterial leaf sreak, bacterial leaf blight, rice blast

†Corresponding author
(Phone) +82-63-238-5233
(E-mail) khkang@korea.kr
<Received Sep. 10, 2015/Revised Nov. 6, 2015/Accepted Nov. 6, 2015>

Rice has attained a status of a major food crop and an import commodity traded at various levels of its value chain in Uganda. However, self sufficiency in its production has not yet been achieved. The government of Uganda responded to this need gap by devoting funds to support upland rice research and production. These effort resulted in the release of 11 high yielding rain-fed upland varieties between year 2002 and 2014 (Lamo *et al*., 2010; Lamo *et al*., 2014). However, there was limited interventions in the development of lowland rice varieties during the same period. The lowland rice is ground in bunded flooded fields for some part of the growing season. These production areas are more fertile than the upland conditions and have opportunity to provide stable yields due to ease of irrigating the crop. In Uganda, rice is cultivated under three production systems: upland (71% of cultivated rice area), rain-fed lowland (25%) and irrigated wetland (4%), with each system contributing 40%, 42% and 18% of rice production in Uganda (MAFAP, 2013). The lowland and irrigated areas have the potential to bridge demand gap currently standing at 20% (240,000MT) of domestic rice demand of the country which is around 300,000MT. More so a cropping intensity of 1.2 have been demonstrated with landraces namely K85, K5 and K23 in the lowland and irrigated production areas.

Currently the huge potential for producing rice under lowland conditions is being undermined by several biotic stresses namely rice yellow mottle virus (RYMV), African rice gall midge(ARGM), Bacterial leaf streak (BLS), Bacterial leaf blight (BLB) and rice blast (BL). For instance, the RYMV occurs both in lowland and upland conditions but affects mainly lowland indica species of rice (Traore *et al*., 2009) because of lack of resistance (Ochola and Tussime, 2011). This problem is threatening the lowland rice industry considering that lowland rice genotypes grown in Uganda were introduced as early as the 1970s and yields from these traditional rice varieties in the lowland ecology are declining further. There is an urgent need to replace these varieties with better adapted genotypes. Currently there are no rice lines with multiple resistance to these stresses at the moment although attempts have been made to develop them through hybridization involving cultivated, local and introduced lines. Recently, however, a few lowland varieties that are tolerant to rice were released. These varieties are tolerant to RYMV. The use of traditional landraces including *Oryza glaberrima* is an alternative approach to furnish multiple resistance to the cultivated rices in Africa. A breeding population from a cross of an African cultivated rice (*Oryza glaberrima* from Niger Delta) and Milyang 23, a Tongil-type Korean rice variety was developed for combining high-yielding and resistance to multiple biotic stresses in Korea. The African landraces are known to harbor genes for resistance to various biotic and abiotic stresses.

Diverse sources of resistance to BLB, BLS and LB have been identified and reported by a number of researchers in Uganda (Habarurema, *et al*., 2013, Lussewa *et al*., 2012, Lado *et al*., 2011). However, majority of these studies have been conducted using the germplasm of Philippines, West Africa, Colombia, and China. Considering that limited sources of resistance that have been identified in African germplasm in light of the importance of rice and its diverse biotic constraints, exploring new sources of resistance would be essential. The purpose of this study was therefore to screen wide-cross progenies derived from a cross of *Oryza glaberrima* and a Korean tongil-type variety for resistance to RYMV, BLB, BLS and LB for use in breeding program of Uganda.

MATERIALS AND METHODS

Plant material

The germplasm screened were the double-haploid lines of an advanced backcross population derived from a cross of an African cultivated rice (*Oryza glaberrima* from Niger Delta) and Milyang23. *Oryza glaberrima* was the resistant donor and was used as the male parent in the cross. Milyang23 was the recurrent parent for the backcrosses. Anther culture was conducted in the BC_4F_1 and fifty double-haploid lines were generated through anther culture in Korea.

Experimental site and design

The screening was carried out at the National Crops Resources Research Institute (NaCRRI) Namulonge in Uganda (032.62661^0, 00.52924^0, 1,122 meters above sea level) during 2013-2014 season. Namulonge has a bimodal type of rainfall with an annual mean of 1,300mm, where the first rain season starts from April and ends in July and the second season from September to December. The site also has a tropical wet and a mild dry climate with slightly humid conditions of average 65%. Temperatures rarely rise beyond 28?, while the minimum is about 15?. It experiences a less than 70% relative humidity.

A total of 50 lines including one parental line was used in the evaluation. The lines were planted in 5 by 10 alpha lattice design. The trial was planted on the 12 February 2014. The plot size was two rows, 3m long with 0.2m inter-row spacing and 0.2m intra-row spacing. K85, a rice variety susceptible to all major rice diseases in the country was grown all around the trial plot two months before the planting date. Fertilizer was applied at rate of 30N (DAP)

Table 1. Scale used for RYMV severity in the rice field.

Scale	Description and level of resistance
1	No visible symptoms, Resistant (R)
3	Green leaves with sparse dots of streaks and < 5% of height reduction, Medium resistant (MR)
5	Green leaves or pale green leaf with mottling and 6-25% height reduction; flowering slightly delayed, Medium susceptible (MS)
7	Leaves pale yellow to yellow, with 26 - 75% height reduction; flowering delayed, Susceptible (S)
9	Leaves turn yellow or orange, > 75% height reduction; no flowering or some plants dead. Highly susceptible (HS)

Source: Standard Evaluation System of Rice (IRRI, 1988)
A modified scale developed by Zouzou et al. (2008) was used to classify the mean scores for disease severity. Values between 1 and 1.5 were given a score of 1 = highly resistant, 1.6-4.5 were assigned a score of 3 = resistant, 4.6-6.5 were rated as 5 = moderately resistant, 6.6-8.5 as 7 = susceptible, and 8.6-9 as 9 = highly susceptible.

at the time of transplanting and 30N (Urea) at 40 days after transplanting. Standard weeding methods was followed. Flood irrigation was applied until maximum tillering (35d after sowing), three times a week, to maintain field capacity till the grain filling stage.

Inoculation with RYMV isolates

Inoculum of RYMV was collected from Namulonge in Uganda that represent “hot-spots” for RYMV disease based on earlier work (Munganyinka et al., 2012) The virulence of these isolates was confirmed by mechanical inoculation of the susceptible genotypes, SUPA and K85 with these two isolates. In order to prepare the inoculum, one gram of infected leaf tissue was first crushed in a drop of double distilled water using sterile mortars and pestles until 80% of the leaf tissue were macerated. The resultant leaf extract was diluted 10 times by addition of 10 ml of double distilled water. Thereafter, inoculation was achieved by rubbing test plants at 14 days post-emergence (when most plants had achieved a 3-leaf stage) from the leaf-base to the tip with fingers moistened with prepared inoculum. Non-inoculated plants were used as the control. The inoculation was repeated after a week to avoid any escapes.

Augmentation of BLB, BL and BLS infection.

The experimental layouts, treatments and fertilizer applications used for blast and BLB trapping nurseries were according to Sere *et al*. (2007). The standard fertilizer rate of 30N (DAP) at the time of transplanting and 30N (Urea) at 40 days was considered adequate to render the plant easily attacked by the BLB and BLS diseases.

Data Collection

Reaction of individual plants to RYMV was scored at one, two, three and four weeks after the initial inoculation, based on the intensity of visual foliar symptoms. The mean disease severity scores in plant populations were obtained using the IRRI standard evaluation system for rice (IRRI, 2002) as detailed in Table 1.

Table 2. Scale used for scoring bacterial leaf blight disease severity in the rice field.

Scale	Percentage of Diseased Leaf Area	Description
1	1-5	Resistant (R)
3	6-12	Medium resistant (MR)
5	13-25	Medium susceptible (MS)
7	26-50	Susceptible (S)
9	> 50	Highly susceptible (HS)

Source: Standard Evaluation System of Rice (IRRI, 1988)

Data on BLB and blast was collected from 10 plants per line by taking disease scores at 42 days after transplanting using scores scale based percentage blast disease incidence (BDI) and % BLB disease lesion (BDL) following Standard Evaluation System of Rice (IRRI, 1988) with modification by Onasannya et al (2007). These scoring scales for rice blast and BLB are detailed in Table 2 and Table 3, respectively. The disease scoring scale for BLS was similar to that of BL.

Statistical analysis

Using the % BDI and % BDL, The analysis of variance for all data was performed using GLM in Genstat following the linear model.

RESULTS AND DISCUSSIONS

The resistance reactions of the 50 double-haploid lines which were produced by anther culture in the BC4F1 plants from a combination of Milyang23 x *O.glaberrima* to RYMV, BLB, BLS, and Bl revealed that RYMV and Blast scores ranged from 1 to 5 while BLB ranged from 1 to 3 and BLS varied from 0 to 1. However, the distribution

Table 3. Scale used for scoring rice blast disease severity in the rice field.

Scale	Description
1	No lesion and small brown specks of pinpoint size or larger brown specks without sporulating center
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. Lesions are mostly found on the lower leaves
3	Lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves
4	Typical susceptible blast lesions, 3 mm or longer, infecting less than 2% of the leaf area
5	Typical blast lesions infecting 2-10% of the leaf area
6	Typical blast lesions infecting 11-25% of the leaf area
7	Typical blast lesions infecting 26-50% of the leaf area
8	Typical blast lesions infecting 51-75% of the leaf area and many leaves dead
9	More than 75% leaf area affected

Source: Modified Standard Evaluation System of Rice (IRRI, 1988)

Table 4. Resistance reaction of the fifty double-haploid lines derived from a BC_4F_1(Milyang23*5 /*O. glaberrima*) to major diseases in Africa.

Diseases	Lines tested	Resistance reactions to diseases			
		RYMV [1/]	BLS[2/]	BLB[3/]	BL[4/]
No. of Resistant lines (%)	50 50	49 (98)	46 (92)	44 (88)	44 (88)

[1/]RYMV: Rice Yellow Mottle Virus disease, [2/] BLS: Bacterial Leaf Streak, [3/]BLB: Bacterial Leaf Blight, [4/]BL: Rice Blast

of the scores were all skewed to the complete resistance, showing that up to 98%, 92%, 88% and 88% of the 50 lines tested did not show any of the RYMV, BLS, BLB and BL diseases symptoms, respectively (Table 4).

Further analysis involving mean severity revealed that all the genotypes were highly resistant to BLS, while 41 plants (82%) were highly resistant falling to a scale of 1.0–1.5 and 9 plants (18%) resistant to BLB (Figure 1). Results of the reaction of the genotypes to RYMV revealed that 45 plants (90%) was highly resistant with 1.0-1.5 resistance scale, 3 plants (6%) falling to 1.6-4.5 scales and 2 plants (4%) were moderately resistant with 4.6-6.5 scale. Reaction of the genotypes to rice blast revealed that 46%(23 plants) was highly resistant, 42%(21 plants) (resistant) and 12% (moderately resistant) as detailed in Table 2. There was no symptoms of the four diseases in 74%(37 plants) of the 50 lines tested. The plants that showed symptoms of the three diseases had scores of not more that 3 on a 1 to 9 scale.

The backgrounds of the two parental lines, Milyang23 and *Oryza glaberrima,* from which the 50 genotypes were generated are as follows; The recurrent parent in the backcrossing, Milyang23 is a high yielding Indica-type variety with the productivities of 6.32? 6.13 tons/ha in milled rice

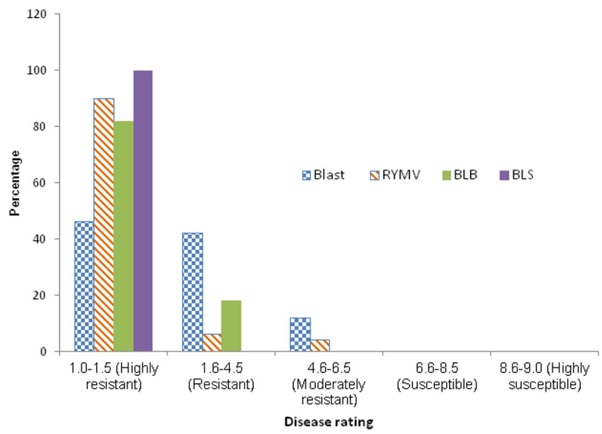

Fig. 1. Resistance reactions of the fifty double-haploid lines derived from a BC_4F_1 (Milyang23*5 /*O. glaberrima*) for each of the four diseases, RYMV, BLS, BLB and BL according to the five resistance ratings from the highly resistant to the highly susceptible

and it has a good grain quality with a 19.5% of low amylose content. but it is susceptible to plant hoppers. *O. glaberrima* is landraces in the West Africa which have multiple resistance to several biotic and abiotic stresses including rice yellow mottle virus and the African rice gall midge as well as unfavorable soil and temperature conditions. In Uganda, *O. glaberrima* is known to show multiple biotic stresses of rice. *O. glaberrima* formed the backbone of the development of the famous NERICA rice varieties through interspecific hybridization and anther culture.

In this study, we tried to develop the high-yielding Milyang 23 type rice varieties which have the resistances to major diseases and insect pest of Africa such as RYMV, BLB, and BL etc by using *O. glabrrima* germplam resistant donors. Backcrosses and anther culture was adopted to speed up the production of the genetically fixed lines in the BC_4F_1 plants, and they were screened for the resistances to major diseases in Uganda.

As results, the distribution of the disease scores was skewed to the resistance reaction indicating that the genotypes are generally resistant to RYMV, BLB, BLS and BL. There was high number of genotypes with low levels of scores of disease suggesting that there was adequate variability to warrant selection. In the case of RYMV, this finding is contrary to observations by Lado et al (2011) who found that all germplasm available in Uganda showed symptoms of RYMV. Furthermore, Kouassi (2005) reported that rice plants infected within 20 days after planting exhibit most of the typical RYMV symptoms, may stop growing, and eventually die. The populations used in the current study were different, therefore new sources of resistance for use as immediate resistant donors have been could be available. Similarly, Lussewa at al. (2012) found that most rice cultivated in Uganda showed symptoms of BLB. This new sourse of resistance could be helpful in solving the problem of BLB in the country.

Currently, rice blast and bacterial leaf streak are devastating rice diseases in Uganda. Results of this study that up to 92% and 88% of the lines did not show any symptom of the two diseases respectively, suggests that the new populations are a new source of resistance to rice blast and bacterial leaf streak.

This new source of resistance could be useful for setting the basis of rice breeding in Uganda. The prelimnary finding of this research that 74% of the germplasm were resistant to the four diseases suggests that these new lines could be used for improving rice in Uganda.

However, the results reported here are preliminary and are from an on-station trial. Tests in other agroecological zones to evaluate the effect of environment on prevalence of the diseases are needed in order to identify genotypes with stable performance in the whole country. Further these sets of genotypes need to be screened further using by innoculation using representative ioslate of the four diseases, RYMV, BLB, BLS and BL.

적 요

농진청에서 수행하고 있는 아프리카 국제협력프로그램인 KAFACI 사업의 일환으로 우간다 등 8개국에 '통일형 다수성 벼 품종개발' 사업을 추진하고 있다. 이를 위해 아프리카에서 정상적인 생육과 수량을 나타내는 다수성 통일형 품종인 밀양 23호와 아프리카의 재래종인 *O. glaberrima*를 이용하여 밀양 23호의 유전적배경을 보유한 근동질 계통인 BC_4F_1을 육성하고, 약배양을 통해 유전적 고정계통을 육성하였다. 이 중 50개 계통을 우간다에서 생물검정을 실시한 결과, 아프리카에서 문제시되는 Yellow Mottle Virus (RYMV), Bacterial Leaf Streak(BLS), 흰잎마름병 및 도열병에 복합저항성인 계통으로 판명되었다. 특히 RYMV에 대한 저항성은 저항성원이 결여되어 있는 병으로 본 연구를 통해 육성한 계통들은 향후 아프리카에 적응하는 내병성 다수성 품종개발에 유용한 재료를 활용될 것으로 생각된다.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institute of Crop Science (PJ009262), Rural Development Administration, Republic of South Korea. We are thankful to Government of Uganda and Alliance for Green Revolution (AGRA) for supporting training that lead to generation of these new lines, evaluation of the new lines.

REFERENCES

Esperance M., R. Edema, J. Lamo, P. Gibson, P. Rukundo. 2015. Combining ability for resistance to rice yellow mottle virus disease in interspecific and intraspecific rice genotypes. African Journal of Crop Science 3(1):102-107

Habarurema I., R. Edema, P. Gibson, J. Lamo, G. Asea, Y. Séré, and Gasore E.Y. 2013. Population diversity of rice bacterial leaf blight isolates in Uganda. *Journal of Plant Science and Research* 3(1):1-9

IRRI (International Rice Research Institute), 2002. Standard Evaluation System for rice (SES). Los Banos, Laguna, The Philippines, 3rd Edition, p. 23.

Kouassi N. K., P. N'Guessan, L. Albar, C. M. Fauquet, and C. Brugidou. 2005. Distribution and characterization of rice yellow mottle virus: A threat to African farmers. Plant Dis., 89:124-133

Lado, M., R. Edema, and J. Lamo. 2011. Inheritance of resistance to RYMV in selected rice cultivars in Uganda: Determining the mode of inheritance for resistance to a prevalent RYMV strain in Uganda *LAP Lambert Academic Publishing*. ISBN 978-3-8433-8683-8, paperback, 104 pp

Lamo, J., J. Imanywoha, G. Bigirwa, M. Walusimbi, D. Kyetere, J. Kikafunda, and T. Kalule. 2010. First NERICA rice released in Uganda tops farmer's rankings. *International Rice Research Notes* IRRN 35:0117-4185.

Lamo, J., P. Tongoona, J. Derera, P. Okori, M. Laing, B. Manneh, M. Semon, and M. Sie. 2014. Participatory varietal selection of new generation of high-performing varieties of upland rice for rapid adoption in Uganda. *in proceedings* of the South Africa Plant Breeders Association Conference 2014. P26.

Lussewa, R. K., R. Edema, and J. Lamo. 2012. Inheritance of bacterial leaf blight resistance in crosses involving interspecific and intraspecific rice genotypes In *Third RUFORUM Biennial Meeting 24 - 28 September 2012, Entebbe, Uganda* 2 261-265.

MAFAP, 2013. *Rice in Uganda: Supporting producers but penalizing consumers*. Policy Brief. Available at: http://www.fao.org/mafa

Munganyinka, E., R., Edema, J. Lamo, and P. Gibson. 2012. Heritability of resistance to rice yellow mottle virus disease and of yield related attributes in segregating generations of rice. In *Third RUFORUM Biennial Meeting 24 - 28 September 2012, Entebbe, Uganda* 2 271-275.

Ochola, D. and G. Tusiime. 2011. Pathogenicity of Rice Yellow Mottle Virus and the Potential Sources of Resistance against the Disease in Eastern Uganda. *Asian Journal of Plant Pathology*, 5, pp.1-5.

Onasanya, A., M. M. Ekperigin, F. E. Nwilene, Y. Sere, and R. O. Onasanya. 2009. Two pathotypes of *Xanthomonas oryzae* pv. *oryzae* virulence identified in West Africa. *Current Research in Bacteriology*, 2(2): 22-35 p: FAO FAO, MAAIF and NARO.

Sere, Y., A. Onasanya, A. Afolabi, H. D. Mignouna, and Akator, K. 2007. Genetic diversity of the blast fungus, *Magnaporthe grisea* (Hebert) Barr. in Burkina Faso. *African Journal of Biotechnology* 6(22): 2568-2577.

Traoré, O., A. Pinel-Galzi, F. Sorho, S. Sarra, M. Rakotomalala, E. Sangu, Z. Kanyeka, Y. Séré, G. Konaté, and D. Fargette. 2009 A reassessment of the epidemiology of Rice yellow mottle virus following recent advances in field and molecular studies. Virus Research 141, 258-267.

Mogga M. L., J. Lamo, G. ASEA, P. Gibson, and R. Edema. 2012. Reaction of rice cultivars to a virulent rice yellow mottle virus strain in Uganda. African Crop Science Journal, Vol.20, Issue Supplement s1, pp.51 - 57

Zouzou, M., T. H. Kouakou, M. Kone, and S. Issaka. 2008. Screening rice (Oryza sativa L.) varieties for resistance to rice yellow mottle virus. Scientific Research and Essay 3(9):416-424.