アフリカ向けイネ品種の改良を目指した JIRCAS での研究開発
JIRCAS's Research Activities Targeting at Improvement of Rice Varieties for Africa

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要　約

独立行政法人国際農林水産業研究センター (JIRCAS)は、世界、とりわけ発展途上地域の安定的な食糧生産に農業研究を通じて貢献することをミッションに掲げている。発展途上地域では干ばつ等により農業生産が脅かされる機会が増加しており、干ばつなどの環境ストレス耐性を重要な目標として研究を推進している。JIRCAS は日本政府と国連開発計画による「アフリカ・アジア共同研究：アフリカ・アジア稲の品種交配研究プロジェクト」に 1998 年から参画し、西アフリカ稲開発協会(WARDA)に研究者を派遣してきた。ここでは、アフリカのイネ品種の改良に関連して JIRCAS で進行中の研究課題について紹介する。

WARDA で開発された NERICA は現在アフリカ各地への普及が図られている段階であるが、普及には、純正な種子が大量に準備されていることが前提となる。NERICA の種子増殖体系をアフリカで構築するために JIRCAS 所属の JICA 専門家である池田氏が Benin の WARDA で育種家種子から原々種の生産方法の体系化に取り組んでいる。育種家種子の評価中にいくつかの NERICA では種子の均一性が担保されていないことが明らかとなり、種子増殖体系確立とともに育種家種子からの純系選抜をも併行して進めている。

乾燥、冠水はアフリカのイネ栽培では大きな制限要因であり、これらに耐性を示すイネ遺伝資源の選抜を行うために JIRCAS は2人の研究者を WARDA および IRAG (ギニア)に派遣している。この研究の目的は耐性を示す遺伝資源の選定と、耐性に関連する DNA マーカーの獲得であり、両者は、耐性品種を育成する際の有力なツールとなる。

環境ストレス耐性の分子的機構の解明に関する研究により、種々の環境ストレスに対して耐性を示す遺伝子を単離した。現在、この遺伝子を国際農業研究機関と共同で各種作物に導入中であるが、このたび、JIRCAS 独自の研究課題としてこの遺伝子を NERICA に導入するプロジェクトを開始した。

これらの研究活動を通じて、すでに育成された NERICA、あるいは将来開発される環境ストレス耐性品種がアフリカ各地で栽培され、食糧の安定化に繋がることを期待している。

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Abstract

Holding its mission to contribute to global agriculture, especially in developing regions, through research in agricultural sciences, JIRCAS has been conducting many research activities in various areas of agriculture. Among traits needed for such regions, JIRCAS has been placing its top priority in abiotic stress tolerance in a number of crops.

Since 1998, JIRCAS has dispatched its scientists to Africa Rice Center (WARDA), as a member of Collaborative Projects of Africa/Asia Joint Research on Interspecific Hybridization between African and Asian Rice Species supported by Japanese Government and UNDP. In this article, our on-going research activities dealing with improvement of rice varieties in Africa will be introduced.

NERICA (New Rice for Africa) developed by WARDA is now in the phase of dissemination to various cultivation regions in Africa. One of the pre-requisites for dissemination of new varieties is availability of ample amount of certified seeds. Dr. Ikeda who is a specialist of JICA and a member of JIRCAS has been establishing a seed propagation system for NERICA in WARDA at Benin. During a course of evaluation of each variety, some of NERICA varieties have been found to be not always homogeneous in the breeder seeds, which will cause serious problems in practical cultivation. Therefore, along with developing the seed propagation system, selection for pure lines has to be pursued.

Two scientists of JIRCAS have been conducting collaborative research on drought tolerance and submergence tolerance of rice with WARDA (Nigeria) and Institut de Recherche Agronomique de Guinee (IRAG). Wide ranges of rice germplasm including Oryza glaberrima have been subjected to evaluation for tolerance of drought or submergence. As one of the indicators of drought tolerance, rooting depth is used. Goals of these projects are 1) identification of germplasm with the tolerance, and 2) acquisition of DNA markers linked to such tolerance, both of which can be a powerful tool in breeding programs targeting for drought and submergence tolerance of rice varieties in Africa.

As results of extensive molecular work on stress tolerance, JIRCAS has identified DREB (dehydration responsive element binding protein) genes which confer tolerance to abiotic stresses including drought, high salinity and low temperature. JIRCAS is collaborating with a number of International Agriculture Research Institutes to develop abiotic stress tolerant varieties. Recently, JIRCAS has initiated its internal project to develop drought tolerant NERICA by introducing these genes.

Through these research activities, both conventional and biotechnological, JIRCAS is hoping that the existing NERICA as well as new stress tolerant varieties to be developed would contribute towards increased rice acreage in Africa.

Introduction

Under the mission to contribute to global agriculture, especially in developing regions, through research in agricultural sciences, JIRCAS has been conducting many research activities in various areas of agriculture. Among traits needed for such regions, JIRCAS has been placing its top priority in abiotic stress tolerance in a number of crops.

Since 1998, JIRCAS has dispatched its scientists to Africa Rice Center (WARDA), as a member of Collaborative Projects of Africa/Asia Joint Research on Interspecific Hybridization between African and

Asian Rice Species supported by Japanese Government and UNDP. In this article, our on-going research activities dealing with improvement of rice varieties in Africa will be described.

Research activities targeting genetic improvement of rice adapted for African ecosystems can be classified into the following 3 phases. The first phase is targeting dissemination of the already developed NERICA varieties. In the first phase, higher priority should be placed in establishment of seed propagation system as well as in accumulation of data on agronomic performance of each variety. The second and the third phase are aiming at improvement of drought tolerance of rice in Africa, because among many stresses, drought has been considered to be major constraints for rice production in Africa. In the second phase, conventional approach is taken and in the third phase molecular approach is employed to attain the targeted goal.

Phase 1 Research on the existing NERICA varieties

NERICA (New Rice for Africa), developed by WARDA by hybridization between *Oryza sativa* and *O. glaberrima* and backcrossed with *O. sativa,* is now in the phase of dissemination to various cultivation regions in Africa. One of the pre-requisites for dissemination of new varieties is availability of ample amount of certified seeds.

Generally, seeds for farmers for a new variety will be produced in the following steps; 1) breeders' seed, 2) foundation seeds, 3) registered seeds, and 4) certified seeds from which seeds for farmers will be finally propagated. Dr. R. Ikeda who is a specialist of JICA and a member of JIRCAS has been establishing a seed propagation system for NERICA in WARDA at Benin. For establishment of an overall seed propagation system for NERICA, WARDA and ARI (African Rice Initiative) are taking a role to establish a system for breeders' seeds and foundation seeds. During a course of evaluation of breeders' seed of each variety, some of NERICA varieties have been found to be not always homogeneous (Ikeda 2006), which will cause serious problems in practical cultivation. Typical off-types observed during the evaluation in the field include, dwarf, abnormal panicle, sterility and albino (Table 1). It is not certain at this moment whether these off-types are resulted from segregation or simple mixture with other variety. Since the breeders' seed must be homogeneous in all aspects, the first step for establishment of seed propagation system for NERICA is to select a genetically homogeneous line within a variety.

Another important point for dissemination of a new variety is to accumulate data on overall agronomic performance of each variety. Since such date is limited for NERICA, systematic survey will be definitely necessary involving field evaluations at multiple sites.

Phase 2 Improvement of drought tolerance by conventional approach

According to FAOSTAT, total acreage of rice field in Sub-Saharan Africa is about 5 million ha. About 80% of the rice field is so-called rain-fed field (Table 2). Among many constraints to rice production, drought has been regarded to be most serious one. In order to improve the productivity in these regions as well as to expand rice cultivation area, it is essential to develop rice varieties with higher tolerance to drought.

Drought tolerance is a very complex trait. The expression of drought tolerance depends on interaction of different morphological (earliness, reduced leaf area, leaf rolling, wax content, rooting system, and reduced tillering), physiological (reduced transpiration, high water use efficiency, stomatal closure and osmotic adjustment) and biochemical (accumulation of compatible solutes, increased nitrate reductase activity and increased storage of carbohydrates) characters (Mitra 2001). Tobita et al. (2001) found that xylem exudation rate can be used as a criterion to discriminate drought resistant and susceptible rice varieties. With regard to rooting system, the importance of a deep root system has been repeatedly emphasized in rainfed rice (Nguyen et al. 1997, Ito et al. 1999). Among the traits related to drought tolerance, we have selected rooting depth as an indicator of drought tolerance.

Wide range of rice germplasm consisting of the core collection of IRRI, the core collection of National Institute of Agrobiological Sciences (NIAS) in Japan, upland rice varieties provided by Ibaraki Agricultural Center, and 86 lines of *O. glaberrima* from WARDA was first evaluated in Bouake, Cote d'Ivoire, for drought tolerance at seedling stage using the standard evaluation system (IRRI 1996). The varieties classified as drought tolerant were subjected to measurement of root depth in the field. Six drought tolerant varieties such as Azucena, Black Gora showed deeper roots compared with drought sensitive varieties, indicating positive correlation between drought tolerance and rooting depth. Under this evaluation, NERICA 1 - 4 and its parental lines were not regarded to have deeper roots in the experimental field at WARDA (Sakagami and Tsunematsu 2003).

Furthermore, root depth at the reproductive stage was evaluated for approximately 600 rice germplasm including *O. glaberrima*. Based on the deepest root length at the stage of 2 weeks after heading, top 100 germplasm has been selected. Second and third rounds of the evaluation are now on-going at WARDA Nigeria Station. After identifying germplasm with deeper root, correlation with drought tolerance at the reproductive stage will be further examined. Also, hybridization between germplasm with deep root and with shallow root will be made for development of the segregating population which will be used for QTL analysis for rooting depth.

Phase 3 Molecular approach to improve drought tolerance

JIRCAS molecular group has been conducting research to elucidate molecular mechanisms of abiotic stress tolerance of plant. The group has revealed the complex regulatory network of gene expression in response to drought, high salinity and cold stresses using a model plant, *Arabidopsis*. The *Arabidopsis* RD29A gene encoding a LEA (late embryogenesis abundant)-like protein responds to dry, high salinity and cold stresses strongly. Through the yeast one hybrid method, a transcription factors that bind to DRE *cis*-acting element on RD29A promoter was isolated from *Arabidopsis* and named DREB (Dehydration Responsive Element Binding protein).

Gene constructs consisting of constitutive promoter 35S or stress inducible promoter RD29A from *Arabidopsis* linked to DREB1A were introduced into *Arabidopsis*. The resultant transformants showed increased level of tolerance to freezing, drought and high salt concentration. In the case of 35S promoter, transformants showed retarded growth. However, the use of stress inducible promoter, RD29 from *Arabidopsis*, minimized the negative effect on the growth retardation. DREB1 can activate more than 40 target stress-inducible genes under stress condition. Over-expression of DREB1 activated strong expression of the target genes which, in tern, resulted in an increased tolerance of freezing and drought stresses. (For review; Nakashima and Yamaguchi-Shinozaki 2005, Umezawa et al. 2006)

Orthologs for DREB1 were found in rice genome and termed OsDREB. Over-expression of OsDREB1A in *Arabidopsis* resulted in higher tolerance to high salinity and freezing stress (Dubouzet et al., 2003). Furthermore, when OsDREB1A under control of a constitutive promoter was introduced into rice, the resultant transgenic rice showed improved tolerance to drought (dehydration for 9 days), high salinity (250mM NaCl for 3 days) and low temperature (2℃ for 93 hr) stresses (Ito et al. 2006).

Results from the evaluation of transformed *Arabidopsis* and rice at greenhouse-level indicate that transcription factor genes, DREB1, has great potential to generate crops which show tolerance to abiotic stresses, such as drought, high salinity and freezing, at practical field level. To investigate to what extent transformants carrying DREB genes exhibit tolerance to abiotic stresses at field level, JIRCAS has been carrying out collaborative research with a number of international research institutes including International Rice Research Institute (IRRI), International Center for Tropical Agriculture (CIAT), International Maize and Wheat Improvement Center (CIMMYT), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and International Center for Agricultural Research in the Dry Areas (ICARDA), covering many different economically important crops such as rice, wheat, soybean, lentil, groundnut, and chickpea.

Since an ultimate goal of the collaborative research is placed in generation of transformants carrying a single and an intact copy of a transgene, a transformation protocol should enable to generate large number of independent primary transformants. The reason why a large number of independent transformants are necessary derives from the fact that any available transformation systems currently available are not a perfect system in the sense that the following parameter cannot be controlled; 1) intactness of transgene (without modification or fragmentation of transgene), 2) copy number of transgene (somehow related to number of locus for transgene), 3) location of insertion of transgene in host genome (resulting in positional effect), and 4) suppression of somaclonal variation during dedifferentiation phase (if tissue culture phase involved).

Although examples of large-scale transformation are very limited, one example can be found in a paper by Hu et al. (2003). In their attempt to generate elite transformants of herbicide tolerant wheat, they found the frequency of such elite line as 0.8% and 0.2 % of primary transformants, by *Agrobacterium* and biolistic transformation method, respectively. Therefore, a high-throughput transformation system is certainly one of important pre-requisites for obtainment of elite transformants.

JIRCAS has recently started its internal transformation work aiming at improvement of drought tolerance of rice in Africa, including NERICA. Since there has been no prior work on transformation system of NERICA which is an interspecific hybrid between *O. sativa* and *O. glaberrima*, we are now working on establishment of transformation system which would fulfill the requirements listed above.

Conclusion

Through these research activities, both conventional and biotechnological, JIRCAS is hoping that the existing NERICA as well as new stress tolerant varieties to be developed would contribute to not only stable production of rice but also expansion of rice cultivation areas in Africa.

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Table 1 Off-types found in NERICA varieties.

NERICA Variety	Frequency of Off-type (%)	Example of Off-type
1	0.25	Drarf, Abnormal panicle
2	7.39	Early maturity, Dwarf, Semi-sterility
3	0.99	Early maturity, Dwarf, Abnormal tillering, Semi-sterility
4	--	Mixture
5	4.83	Broad leaf, Narrow leaf, Semi-sterility, Abnormal tillering
6	6.33	Dwarf, Early maturity, Semi-sterility, Abnormal tillering
7	0.36	Dwarf, Plant elongation, Narrow leaf, Abnormal tillering, Semi-sterility

Source: Ikeda (2006)

Table 2 Cropping System of Rice in Sub-Saharan Africa

Cropping System	Area (%)*	Major Constraints
Rainfed Lowland	42.6	Iron toxicity, Floods, Insects, Diseases, Drought
Upland	37.1	Drought, Weeds, Disease (Blast), Pests, Low inputs
Irrigated	13.9	Diseases, Insects, Pests, Weeds, Nutrient deficiency, Toxicity
Deepwater	4.8	
Mangrove	1.6	

* After Nguyen and Tran (2002)

質疑応答
Question and Answer Session

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司会：　松本　哲男　　Chair person: Tetsuo Matsumoto
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Professor, ICCAE, Nagoya University

松本（司会）：

私のほうから質問させてもらってもいいですか。先ほどローカルのものと比較して乾燥のストレスに対する抵抗性の問題ですが、NERICAは一般的に弱いと考えていいのでしょうか？

それから、そこに入ってくる今度新しく出る乾燥遺伝子のdrought toleranceは、先ほどの説明ですと、塩とかまた、温度との関係でストレス耐性が高まるという効果があると思うのですが、それもやはり同じ遺伝子がtolerance関係のものをすべてコントロールしていると考えていいのでしょうか、あるいは生理的な仕組みがそうなっていると考えてよろしいのでしょうか？

神代：　まず一番目の質問ですが、WARDAのパンフレットにdroughtについて書いてありますが、乾燥抵抗性はいろいろ複雑なメカニズムもあり、drought escapeであることは確かです。生育期間が短い、つまり成熟まで100日以内でやるということで、乾燥が来る前に全部収穫し終えるので、乾燥にあわずに収穫できるということが、ここでは正しいと思います。しかし、根が長いということなのか、あるいは本当に水の無い、水の少ないところでどうなるかということに関しては、我々のデータ、それから他にもいくつか知っているのですが、必ずしもそれほど強くないと私は思っています。

それからdroughtの話ですが、これは確かにdrought遺伝子を交鎖させると　乾燥、塩、低温、この三つに効きます。これはmoist levelと同じです。この三つに共通していることがありまして、これは非常に細胞の浸透圧が高くなります。というのは水が抜けるからです。そういうことで大丈夫だということで、そこのメカニズムでは確かに効果があります。しかしこの遺伝子を入れたから根が深くなるとか、あるいは気孔の開化温度が変わるかというと、そういったことまでは期待できないと思います。だから、そこは色々な他の性質を組み合わせて総合的に抵抗性を上げていかなければならないと思います。

先ず我々がやらなければいけないことは、遺伝子のデータモードが必要な時に、どのあたりのレンジで耐性を示すのかということをちゃんと記録しなくてはいけないと思っています。

松本（司会）：

他にご質問はありますか？

飯嶋：NERICA が他の品種等と混ざっているものがある可能性を最初のほうのスライドで言及されていましたが、特に NERICA の 4 番が他のものと比べて非常に根も大きいし、乾燥する度合いも高いというのが、私自身も栽培していてそう感じているのですが、これがどうも、他のものと混ざっているというデータが出ていまして。

神代：これはじつは、育種家種子にふたつあったのです。ある人が持っていて名前は忘れたのですが、それが混ざっていたということです。専門家の話によりますと、分けてあればあんなにひどい結果は出ていなかった、もう少しまともだったということです。どちらが良いかはわかりませんが。

飯嶋：そうしますと、他の NERICA に近いようなものが NERICA 4 だったということですね。あの、NERICA 4 はとても非常に何か、ちょっと変わっているなという印象を受けましたので。

神代：どれかと言われると私もよくわからないのですが…。

飯嶋：はい、どうもありがとうございました。

浅沼：Submergence tolerance というのは特殊な条件でそういう問題があるのでしょうか、あるいは、何か環境問題などがあると思うのですが、どういうところでそこに注目されたのですか。

神代：必ずしも特殊な例ではないです。いわゆる lowland rainfed の場合は最初はいやというほど水があって、それから急になくなって最後は乾燥になるということです。要するに水はあるのだけれどその配分がコントロールできないから色々な問題が起きてくるわけです。だから、最初は submergence でやられることもあるわけで、そこに対してもある程度耐性がないと、特に lowland には適さないということで始めたわけです。

浅沼：それは幼苗期の話しということですか？

神代：そうですね、生育初期ですね。

松本（司会）：

神代先生、ありがとうございました。

Profile

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1974 年に京都大学大学院農学研究科（育種学専攻）を修了し、民間企業において植物バイオテクノロジー分野を担当し、葯培養による半数体育種法の確立、非対称細胞融合を用いた核―細胞質置換による雄性不稔性品種の開発、および単子葉植物の形質転換法の開発など研究開発に従事した。研究開発管理部門を経て、2004 年 7 月に JIRCAS の現職に就いた。

Academic career

Mr. Takashi Kumashiro graduated from Graduate School of Kyoto University in 1974 with a major in Plant Breeding.

Professional career

After graduation, Mr. Kumashiro immediately joined a private company where he conducted and was involved in biotechnology-related research projects, including haploid breeding using anther culture, asymmetric protoplast fusion, and development of Agrobacterium-mediated transformation for monocots. With his wealthy experience in management of research and development, Takashi Kumashiro joined Japan International Research Center for Agricultural Sciences (JIRCAS) in 2004 and is currently the Director of the Biological Resources Division.