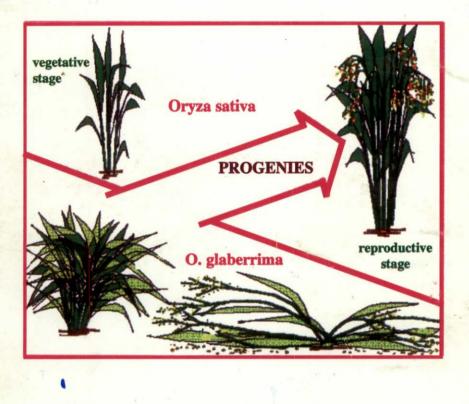
# **INTERSPECIFIC HYBRIDIZATION** : Progress and Prospects

# **HYBRIDATION INTERSPECIFIQUE** : Progrès et Perspectives



Proceedings of the Workshop : Africa / Asia Joint Research on Interspecific Iybridization between the African and Asian Rice Species (O. glaberrima and O. sativa), VARDA, M'bé, Bouake Côte d'Ivoire, December 16-18, 1996.

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dited by/Edité par : Monty P. Jones Michael Dingkuhn David E. Johnson Samson O. Fagade

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### About WARDA

The West Africa Rice Development Association (WARDA) is an intergovernmental research association with a mandate to conduct rice research, training and communications activities for the benefit of the West African region. Formed in 1971 by 11 West African countries with the assistance of the UNDP, FAO and ECA, WARDA now has 17 member countries. The Association is a member of the network of 16 international agricultural research centres supported by funds from donors of the Consultative Group on International Agricultural Research (CGIAR).

The Headquarters of WARDA are in M'bé, Côte d'Ivoire and the Association also maintains regional research sites near St. Louis, Senegal and at IITA, Ibadan in Nigeria

### A propos de l'ADRAO

L'Association pour le développement de la riziculture en Afrique de l'Ouest (ADRAO) est une organisation inter-gouvernementale ayant pour mandat de mener des activités de recherche rizicole, de formation et de communications pour la région ouest-africaine. Créée en 1971 par onze pays membres, sous le parrainage du Programme des Nations-Unies pour l'Alimentation et l'Agriculture (FAO) et de la Commission Economique pour l'Afrique (CEA), l'Association regroupe aujourd'hui 17 pays membres. L'Association est membre du réseau de 16 centres internationaux financés par le Groupe consultatif pour la recherche agricole internationale (GCRAI).

Le siège de l'ADRAO se trouve à M'bé, Côte d'Ivoire et l'Association a également des sites de recherche près de St. Louis, Sénégal et à IITA, Ibadan, au Nigéria.

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### INTERSPECIFIC HYBRIDIZATION: Progress and Prospects Hybridation Interspecifique : Progrès et Perspectives

Edited by/Edité par

Monty P. Jones

Principal Breeder/Coordinator, Interspecific Hybridization Project WARDA, M'bé, Bouaké Côte d'Ivoire

Michael Dingkuhn Program Leader/System Analysist Rainfed Rice Program WARDA, M'bé, Bouaké Côte d'Ivoire

David E. Johnson Weed Scientist WARDA, M'bé, Bouaké Côte d'Ivoire

and

Samson O. Fagade

Agro-Consultant WAFCO (Nig.) Ltd Ibadan Nigeria

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### WARDA/ADRAO 1997

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### Foreword

Rice is the most important food crop of the developing world and rice genetic improvement through breeding has been an effective mechanism for delivering the benefits of science and technology to hundreds of millions of resource poor people. In West Africa, there has been a dramatic growth in rice consumption and production in recent years. Production however is still unable to keep pace with the demand. Increasing rice production in a sustainable way is therefore a major challenge for research and development activities in the sub-region.

Over the years, rice improvements efforts in the sub-region had concentrated on the utilization of introduced *O. sativa* from Asia. Success in varietal improvements has been minimal due to limited resistance of *O. sativa* to many of the stresses that affect rice in the sub-region. Less attention had been paid to the exploitation of the wide genetic variability available in the indigenous *Oryza* species, which is gradually being lost due to this neglect.

However, the African rice species, *O. glaberrima*, in spite of its low yield potential, has many other characteristics valuable to breeders and to farmers. It is resistant to many of the prevailing stresses and has local consumer-acceptable cooking and eating qualities. Farmers in marginal and deep water areas still utilize these varieties along with the introduced *O. sativa*. WARDA scientists recognizing this valuable pool of genetic variability, initiated an interspecific hybridization program to combine the good traits between *O. sativa* and *O. glaberrima*.

This work has led to the development of promising new varieties that could significantly increase farmers yields in a sustainable manner. The enormous potential of this hybridization of Asian and African rice species has yet to be fully exploited. Moreover, apart from O. glaberrima, Africa is blessed with having seven others, of the known twenty Oryza species, originating from the region. Several of them are crossable with O. sativa and are being utilized especially as sources of resistance genes to rice pests and diseases in Asia and Latin America. Environment and biodiversity conservation could be readily achieved in the upland ecosystem with the availability of high and stable yielding varieties under improved fallow management. Farmers adoption of this technology would not require such high investments and purchase of inputs associated with other rice production systems. To further advance the work on interspecific hybridization for the improvement of rice in Africa, WARDA brought together key scientists on the subject for a three day workshop at M'bé, Bouaké, WARDA's headquarters in Côte d'Ivoire, from 16-18 December 1996.

The international workshop reviewed the current status of interspecific hybridization for rice in general and for *O. sativa* and *O. glaberrima* crosses in particular. It also elaborated a strategy for joint research efforts in Africa and Asia based on complimentary expertise and potential usefulness of interspecific rice progenies in both regions. This volume presents in two parts, the proceedings of the workshop and the follow up of the workshop deliberations.

In part one, presentations made by scientists from different institutions and countries are provided. These highlighted progress on current research activities in interspecific hybridization. Emphasis were on methodologies, problems encountered such as incompatibility barriers and possible solutions for overcoming them. Interesting and promising results on introgression of useful traits from *O. glaberrima* or wild species into elite breeding lines were focused in these presentations. These should provide valuable reference to scholars, researchers and developers alike on the potentials and challenges offered by this research. The participants (Appendix I) also brainstormed on future research and strategies for collaboration. Based on these discussions, the participants strongly recommended immediate support for a joint research project on hybridization between African and Asian rice species aimed at developing improved rice varieties for increased rice production in Africa and other parts of the rice growing world. The workshop recommendations were the subject of a strategic meeting in Abidjan, Côte d'Ivoire on 20 December 1996 with donor representatives from Japan, United States of America and the United Nations Development Programme Technical Cooperation among Developing Countries (UNDP/TCDC) (Appendix II). Part two presents the donors views and realization of the technical workshop recommendations.

The funding and technical support provided by donors for these activities are well appreciated and acknowledged. The United Nation Development Program Technical Cooperation among Developing Countries (UNDP/TCDC) funded the hosting of the International Workshop. The government of Japan, Ministry of Foreign Affairs financed the strategic planning meeting and is providing core funds for each of the fiscal years 1996 through 1999 to support the project on interspecific hybridization between African (O. glaberrima) and the Asian (O. sativa) rice species.

> Kanayo F. Nwanze Director General WARDA

## Introductory Remarks : Overview of the Rice Sector and Interspecific Hybridization of African and Asian Rice Species

### Kanayo F. Nwanze

Director General West Africa Rice Development Association

Mr. Chairman, Distinguished participants, Ladies and gentlemen.

Strengthening the capacity of sub-Saharan Africa in sustainable rice production through technology generation and technology transfer is WARDA's raison d'être, and one of the most important ways of meeting our goals is by rice genetic improvement through breeding.

Wide hybridization and biotechnology can significantly strengthen rice breeding programs and help produce new varieties with higher yield potential and greater yield stability. These should improve the efficiency of rice production and allow the expansion of rice-growing areas.

In less than a decade, research on rice biotechnology has moved from a position of neglect to one where some people now consider rice a model plant for cereal research. Tissue culture techniques such as anther culture, embryo rescue and the use of somaclonal variants have contributed to the release of new rice varieties. These technologies are of proven benefit to rice breeding and through research are becoming applicable to a broader range of rice cultivars and breeding objectives.

Most molecular genetic techniques are still being developed, but progress has been more rapid with rice than any other cereal. An RFLP map is now available and several genes of economic importance have already been tagged with markers, paving the way for marker-aided selection work. Regeneration of fertile interspecific hybrids between *Oryza sativa* and *O. glaberrima* have been achieved at WARDA.

Some very useful traits are found in our indigenous African rice species *Oryza glaberrima*. It has been cultivated in West African for some 3500 years, and, in spite of its low yield potential, rice farmers here today continue to sow perhaps as much as 15 percent of their rice fields to *O. glaberrima*. This is because it is able to withstand adversity, such as drought, blast disease, soil acidity and iron toxicity, and to compete with and suppress weeds. As well, it has good grain quality.

I have been requested to set the stage for wide-crossing research in West Africa by providing a background overview of the West Africa rice sector. Let us begin by first getting our bearings. Where is rice produced ?

Because rice can be grown under a large range of conditions, production can be found all across the region. The most important production zone is in the humid/sub-humid zone where rice is grown all along the continuum from the uplands down the slopes through the hydromorphic transition and into the valley bottom wetlands. Upland systems continue farther north where lower rainfall levels and the presence of wide, low valleys has led to the predominance of lowland rice systems in valley bottoms or on seasonally flooded plains along larger streams and rivers. Rice is also grown in irrigated schemes, the largest and best known of which are scattered across the Sahel, notably the Senegal River valley, the office du Niger in Mali, and SEMRY in Cameroon. Finally, rice has long been grown in the mangrove zone along the western coast using techniques specially adapted to that rather complex ecosystem.

On average, every country in the sub-region consumes more rice than it produces, and so must import rice to some degree. Import dependency is a critical issue for a number of countries along the west coast.

Rice consumption continues to grow at a rapid pace. Demand for rice in West Africa has been growing at the rate of 6% per annum since 1973, and now amounts to over 8 million tons per annum. Increased consumption is due both – and about equally – to

population growth (more mouths to feed) and to the increased share of rice in West Africans' diets (more per mouth).

Demand for rice in West Africa has been growing faster (at 6%) than for any other staple food. According to the FAO data upon which these figures are based, the traditional staples have in the meantime barely kept pace with population growth.

Over the same period, production of rice in West Africa has been expanding rapidly, growing at 5.1% per year, faster than any of the other principal staple food crops. What is worrisome about this expansion, however, is that 70% of the growth has been due to increasing the area cultivated in rice, and only 30% due to improved productivity in the form of higher yields. Nearly all of the expansion has been in the two major ecosystems: the upland and the lowlands. We are already seeing the results of pressure on the uplands as fallow periods are reduced causing fertility to decline. Thus continued growth by increasing the area obviously cannot be sustained over the long term, and emphasis will need to be given to further improving productivity.

Despite the growth in production already achieved, demand for rice has risen even faster, leading to increased dependence on imports. Over the past twenty years, imports of rice have grown at the alarming rate of an average 9% a year. Much of that growth took place in the late 1970s, and growth has decelerated considerably since. However, the cost of importing rice remains a heavy burden on trade balances in the region, requiring over \$800 million in foreign exchange each year.

The statistical trends that I have been citing provide only part of the picture of what has been happening in the West Africa rice sector. Over the past decade, the very fabric of the sector has also been changing dramatically as governments have implemented structural adjustment reforms. Previously, the rice sector in most countries had been heavily controlled by the state, In many cases, the state set official prices for producers and consumers, maintained monopoly control of rice imports (providing an important source of government revenues), provided input supplies at subsidized prices, set up and subsidized the operation of industrial rice mills, and even controlled rice marketing channels. Such policies often sent confusing signals to producers and other operators in the rice sector, and the incentives created often encouraged inefficiency.

Under structural adjustment, the state has withdrawn its heavy intervention in the rice sector and rice markets have been universally liberalized. Private operators have taken over many of the production and marketing functions formerly handled by the state. The result appears to be encouraging more efficient supply of rice to consumers. This is evident, especially, in the rapid expansion of small-scale rice milling which is able to process rice much cheaper than the old state-run mills could, and the disappearance of many overly mechanized production systems.

The transition to free markets, which is still on-going here in Côte d'Ivoire, has certainly not been smooth. Major constraints remain to be tackled. Key problems include: the seed industry, which in many cases has been difficult to privatize and has ceased operating; and agricultural credit, which also the private sector cannot provide. Also governments are finding it difficult to know how to formulate appropriate policies in this new free market environment, both due to the general scarcity of hard information since few countries have on-going surveys set up, and due to the lack of analytical tools and experience. At the same time, national research systems and extension services have in many cases been weakened by the reforms and budget crises.

Given this context, I would like to finish by making a few comments about the role of wide-crossing research. First, the potential impact of successful research in this area is immense. Developing new varieties that perform well in water-constrained, low-input production systems will benefit most, the single most important rice production ecology in West Africa: the uplandlowland Continuum in the humid/sub-humid zone. The beauty of the technology is that it is seed-based and does not necessarily require purchased inputs, and so can be readily adopted by farmers – many of whom are women – who are producing primarily for their own consumption rather than the market. This also means that it can be adopted regardless of how government policy is affecting the market. Moreover, once it has been transferred to farmers, there is no need for further investment in infrastructure to maintain it. But beyond the already large expected impact for West African rice farmers, the wide-crossing effort also promises large rewards for rice farmers in marginal environments around the world. Breeding efforts elsewhere have concentrated mainly on favorable irrigated environments, and correctly so due to the higher returns to research in those environments. Wide-crossing research incorporating robust African materials is likely to provide varieties particularly adapted to the more marginal environments and resource-poor farmers in these other regions. I am sure that you will share my excitement imagining Africa returning the favor to Asia and Latin America with such a contribution.

### Good-will Message to the International Workshop on Interspecific Hybridization between African and Asian Rice Species

### **Denis Benn**

Director of the UNDP/Special Unit for Technical Cooperation among Developing Countries (TCDC)

Ladies and Gentlemen, Distinguished Researchers and, Participants to this important workshop.

The Special Unit for TCDC would like to extend its very best wishes and congratulations to WARDA and those gathered here today to deliberate in a very real sense on a practical matter of putting more food on the table in Africa.

We very much regret that we will not be with you today. The Special Unit's participation has been hampered by the unexpected cancellation of the mission of the Regional Officer for Africa due to unforeseen circumstances. We are however with you in spirit in Bouaké.

This three-day Joint Africa-Asia Workshop on Interspecific Hybridization using Cultivated and Wild Rice Species brings together research scientists drawn largely from Africa and Asia not only to cross-fertilize ideas but to set the stage to literally crossfertilize a diversity of rice genetic resources native to Africa with related varieties from Asia. As we understand it, this valuable work will enable the transfer of genes between the two continents that would result in widening the genetic base of improved rice germplasm possessing superior characteristics and traits capable of withstanding the hardships and stresses of difficult rice-growing environments, particularly in Africa. This enterprise represents a concrete expression of South-South and triangular cooperation which is an important aspect of the work we support in SU/TCDC.

Our co-sponsorship of this workshop is therefore consistent with our efforts to promote technical cooperation among developing countries and encouraging the transfer of know-how and expertise. In terms of our mandate emanating from the report on New Directions for TCDC which was approved by the High Level Committee on the Review of TCDC and endorsed by the UN General Assembly in 1995, there is now increased focus on supporting TCDC Africa-Asia initiatives such as those envisaged in the Bandung Framework for Asia-Africa Cooperation which was adopted at the Asia-Africa Forum held in Bandung, Indonesia in December 1994.

Indeed, WARDA was one of the African Centers of Excellence which participated in that Forum and I am pleased to note that the origins of this practical cooperation on which we are embarked today stemmed from recommendations made in Bandung.

The issue of food security in Africa remains at the top of the agenda of regional priorities. Africa's ability to attain selfsufficiency in food will depend, among others, on its applied research efforts to improve agricultural productivity. Clearly Africa cannot do this alone, and would require inputs from other regions with relevant experiences. Research is generally a very costly undertaking requiring in most instances a long-term time-frame in order for results to bear fruits. Nevertheless, it is sometimes feasible for the time-frame to be contracted through technological leap-frogging and the establishment of strategic alliances among institutions undertaking similar or related research.

As you are aware, in today's rapidly globalising economy, there is a discernible reduction in official development assistance (ODA) to developing countries. On the other had, foreign direct investment, which has been growing significantly in the Latin America and Asia region, has been less significant in sub-Saharan Africa, despite the well-documented efforts of the region in liberalizing its economy as a pre-condition to induce investment inflows. These developments have in turn emphasized the need for developing countries to increase cooperation among themselves as a strategy in support of their development efforts and a means of ensuring their equitable participation in the emerging global order.

What we in UNDP are attempting to do through the use of TCDC as a modality for promoting South-South Cooperation is to facilitate technical exchanges such as is provided through this Forum. We also play a catalytic role in organizing the matching of capacities and needs of developing countries' institutions in priority development sectors. Most importantly, a critical area of our support is access to information - the lack of which has constituted a major constraint to TCDC in particular and South-South cooperation in general.

In an effort to deal with the problem, we have established the TCDC-Information Referral System (TCDC-INRES), a database which currently contains in excess of 4000 institutions in developing countries offering over 20,000 training programs and expert services. We are currently updating, diversifying and enriching this database as a multi-dimensional information system in the near future.

These therefore are some of the TCDC instruments viz. the organization of strategic fora, matching capacities and needs and TCDC-INRES that SU/TCDC provides in order to facilitate South-South Cooperation.

Finally, I would like to convey my appreciation for your fruitful and constructive development in shaping research arrangements among the institutions involved in the research program. I understand that an important outcome of this process is the finalization on the research program involving the West Africa Rice Development Association and the International Rice Research Institute (IRRI) in the Philippines.

I urge you to see the exigencies in practical approaches and concrete results. The task is to identify the most direct route between genetic manipulation on the one hand and the transfer to Africa farmers of varieties that are both high yielding and disease resistant in an effort to increase Africa's food supply.

I am confident that you will achieve this objective.

Thank you.

### **PROGRESS REPORT ON CURRENT RESEARCH**

Ι

**RAPPORTS D'ACTIVITÉS SUR LES RECHERCHES EN COURS** 

### The Potential Role of Low-Management Rice Technologies during the Agricultural Transition in West Africa

Michael Dingkuhn and Thomas F. Randolph West Africa Rice Development Association, B.P. 2551, Bouake 01, Cote d'Ivoire

#### Abstract

This paper describes the current status and perceived evolution of rice production systems in West Africa, and derives technology needs for this sector. Despite the massive transfer of modern Asian rice production technologies since the 1970s, a 'green revolution' has not taken place in West Africa. The rapid growth of rice production, now at 8.5 % per annum, is mainly caused by area expansion, while yield increases have been small. Area expansion is driven by an increasing demand for rice, which continues to exceed regional production. Increasing demand for rice is caused by both population growth and increasing per-capita consumption. This paper discusses the probable causes of the limited success of Asian-type irrigated rice technologies in West Africa, and highlights agroecological risks incurred by current trends: unsustainably shortened fallow periods in upland systems, salinization of irrigated soils in the Sahel, and, generally, increasing pest and disease pressure due to intensification and the broad use of introduced varieties. West African rice systems are undergoing a transition from labor to land limitation. This transition is not taking place simultaneously in all environments. thus requiring different technical solutions. The ongoing paradigm shift in research from fully irrigated systems to technically less demanding concepts is not a full answer to the problem, because the main issue is the fit of technologies to local land or labor limitations and markets. WARDA is directing separate research efforts towards low-management. labor-saving technologies for rural environments, while pursuing different approaches to the sustainable intensification of land-limited systems, such as peri-urban lowlands. Many rural rice production systems in West Africa are currently struggling to intensify production using technologies borrowed from extensive agriculture, thereby aggravating weed pressure and soil infertility, and generating a need for extra labor that is not available. These systems require low-management technologies that would ease the labor gap during the agricultural transition. One such technology is a new weed competitive rice plant

type, derived from interspecific crosses using Asian rice, O. sativa, and African rice, O. glaberrima. Production systems that are already landlimited, on the other hand, are more likely to accept Asian-type rice technologies, but care must be taken to prevent pest outbreaks, which may result from excessive pesticide use, reduced biodiversity and poorly adapted varieties. The general prediction is that a green revolution in West Africa will take place and has already begun, driven by population growth. As in Asia, this revolution will chiefly be based on the lowlands. The associated risks related to pests and diseases might be even more serious than those in Asia, because intensive lowland cropping has had no time to co-evolve with the natural biotic environment. WARDA's recent breakthrough in interspecific hybridization might provide solutions at two levels, (1) the improvement of labor productivity through reduced needs for weeding and other management interventions, and (2) the improved sustainability of intensified systems through durable crop resistance to pests and diseases, particularly in lowland systems that so far depend on introduced materials.

#### Introduction

Rice is the most agro-ecologically diverse of the world's major crops. It is grown under deep and shallow flooding, in the dryland (upland), and in coastal mangrove swamps; it is also grown in the temperate, sub-tropical and tropical climates, and at altitudes exceeding 2000 m (Buddenhagen and Persley, 1978; IRRI, 1997). Given this remarkable adaptability of the *O. sativa* species, it is surprising that the success of rice as a staple crop was based on the most controlled of all conceivable field-based crop management systems. The cultivation of irrigated rice, with seedlings transplanted individually from a nursery into puddled, levelled and flooded basins, is one of the most productive and ecologically sustainable crop production systems ever developed. The 'green revolution', although it did not invent it, was based on this genial system.

Early efforts to promote intensive rice cultivation in West Africa tried to faithfully repeat the green revolution. But despite major public investments in irrigation infrastructure since the early 1970s, large rice irrigation systems did not live up to expectations, and, in many cases, have been sustained by political rather than economic objectives. More importantly, introduced intensive rice production systems rarely multiplied or spread spontaneously through indigenous initiatives. This stands in marked contrast to the rapid increase in regional demand for rice during the same period, which led to a dramatic and still ongoing increase in rice imports. Only during the past decade did rice production in West Africa take off, growing by 8.5% annually since 1983. But this increase was mostly driven by area expansion, with yields growing at a distinctly unrevolutionary rate of only 1.9% (WARDA, 1996ab; IRRI, 1997).

The disturbing message is not that the green revolution did not take place in West Africa. It is disturbing, however, that much more than catalytic efforts were made to trigger such a technological revolution, and that these efforts were evidently guided by a vision for which, at least at the time, there was no effective local demand. More recent agricultural research and development concepts have been more modest, addressing the resource poor farmer in his indigenous setting characterized by as many constraints as opportunities, and leaving little scope for positive intervention. In its rousseauistic extreme, this vision may turn out to be irrelevant as well. In this paper, we will try to crudely outline opportunities for intensified, sustainable production of rice in West Africa, and discuss some possible approaches to realize them.

#### **Consumption trends for rice in West Africa**

Changes in the preferences of West African consumers have created a wide and growing imbalance between regional rice supplies and demand. (Statistical figures cited in the following are taken from WARDA, 1996a). Since 1973, regional demand has grown at an annual rate of 6.0%, driven by a combination of population growth (2.9% growth rate) and substitution away from the region's traditional coarse grains. The consumption of traditional cereals, mainly sorghum and millet, has fallen by 12 kg per capita, and their share in cereals used as food from 62% in the early 70s to 50% in the early 90s. In contrast, the share of rice in cereals consumed has grown from 15% to 25% over the same period. Much of this dramatic shift occurred in the late 70s and 80s. Growth in regional rice consumption however, remains high; the FAO projects the rate will be 4.5% through the year 2000. This means that the total volume of rice consumed in West Africa is likely to increase by an additional 70% by the end of this decade

The most important factor contributing to the shift in consumer preferences away from traditional staples and toward rice is rapid urbanization and associated changes in family occupational structures. As women enter the work force, the opportunity cost of their time increases and convenience foods such as rice, which can be prepared more quickly, rise in importance. Similarly, as men work at greater distances from their homes in the urban setting, a greater proportion of meals are consumed from the market where the ease of rice preparation has given it a distinct advantage. These trends have meant that rice is no longer a luxury food but has become a major source of calories for the urban poor. Urban consumption surveys in Burkina Faso, for example, have found that the poorest third of urban households obtain 33% of their cerealbased calories from rice. For that same group, rice purchases represent 45% of their cash expenditures on cereals, a share that is substantially higher than for other income classes. Similar results have been obtained in several other West African states demonstrating that rice availability and rice prices have become a major determinant of the welfare of the poorest segments of West African consumers who are least food secure.

The increasing role of rice in the nutrition of urban populations also renders rice a political crop, its supply and prices affecting social stability. Past public investments in (and subsidies for) rice production were explicitly motivated by self-sufficiency concerns, particularly under the impression of the drought catastrophes in the Sahel during the 1970s and 80s. The high rice prices during the world food (grain) crisis of the mid-70's reinforced self-sufficiency objectives. But it is likely that the high priority generally given to rice by national agricultural authorities in West Africa also reflects the high importance governments attach to satisfying the demands of urban populations, on which political stability depends most.

### **Rice production and rice imports**

In comparison with the rapid growth in demand, regional rice production rose at an annual rate of 4.1% from 1973 to 1983. Although this rate was high compared to the performance of other major crops, it meant that regional rice production only barely exceeded population growth, and was meeting only two-thirds of

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the increments to demand. Since 1983, regional rice production has taken off and grown at an annual rate of 8.3%, significantly faster than demand. The source of the increases in rice production carries important danger signals, though, suggesting that such growth is not likely to be sustainable. Regional rice yields, which average only 40% of the world mean, have risen at only 1.9% per year since 1983. The major source of growth has been the expansion of cultivated area which has grown at a remarkable 6.5% annually over the period.

The widening gap between regional supply and demand has been met by imports. The rapid increase in demand and much slower growth in production from 1973 to 1983 contributed to a dramatic jump in imports rising at over 20% annually from 0.6 million tons in the early 70s to 2.2 million tons a decade later. Since 1983, growth in demand has decelerated and domestic production has improved, leading to a much more modest 2.2% annual increase in imports which averaged 2.6 million tons in the early 90s. The FAO projects imports to increase to 4 million tons by the year 2000 costing more than \$800 million in scarce foreign exchange. Imports of this magnitude represent a major brake on broader development efforts.

#### Typology of rice eco- and production systems in West Africa

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If Asian rice production is commonly, but not entirely correctly, associated with vast monocultures of irrigated rice, no such stereotype exists for West Africa. Its rice ecosystems, now covering about 4.1 million hectares, are extremely diverse and are usually categorized by hydrology and agro-ecological zone. The most abundant system in terms of area is traditional rainfed upland rice (2.3 M ha) located in the moist savanna and humid forest environments, and mostly extensively cultivated with low inputs between long bush fallow periods. Mean yields are about one t/ha. The other extreme in terms of hydrology and cropping intensity is double or single cropped irrigated rice, which is found in irrigation schemes of all sizes and in all agro-ecological zones from the Sahel to the humid forest (ca. 0.5 M ha, about half of it in the Sahel, based on major rivers). Irrigated rice yields average between four and five t/ha in most West African countries, but individual farmers in the Sahel achieve the biological yield potential of up to 10 t/ha in a single season (Dingkuhn and Sow, 1997a). Another distinct environment is the coastal mangrove swamps, where traditional farmers make use of seasonal flushes of fresh water to grow a rice crop with extremely low inputs (0.2 M ha).

The remaining 1.1 million hectares of estimated ricelands are located in lowlands, are rainfed, and are more difficult to classify. The least well characterized systems are located in floodplains in the savanna and Sahel zones, where seasonal inundations may be shallow and enable growing a medium-tall, fairly high yielding rice variety; or floods are deep and necessitate elongating deep-water rices, or even floating rices. Much more is known on the approximately 0.6 million confirmed hectares of rainfed lowland rice grown at the bottoms of inland valleys in the savanna and forest environments. These systems, in francophone countries called 'bas-fonds', are widely considered to have the greatest potential for both area expansion and sustainable intensification.

#### Some basic determinants of intensification and economic sustainability

As indicated earlier, there has recently been a paradigm shift among donors and researchers from high-input irrigated systems to systems with less perfect water control, which require less investment and behavioral adjustment by the farmer. It is now a widely held view that large and technically demanding production systems are poorly adapted to conditions in sub-Saharan Africa, and that is why they easily fall into disuse.

This simplification is almost certainly wrong. There are many examples of highly complex and integrated agricultural production systems, some of them indigenous, and some of recent origin, such as lowland cultivation found in some West African peri-urban environments. The failure of many expensive irrigation schemes is not due to technical complexity or their 'newness', but rather, to disregard of farmers' objectives and communal organization (Diemer and Huibers, 1991; Le Gal, 1997). Rice producers in West Africa generally pursue multiple activities to ensure food security in a variable environment. Depending on the context, rice farmers grow a number of other crops and are employed in a wide range of other activities, both farm and nonfarm, for subsistence and cash income (Richards, 1985; Becker and Diallo, 1991; Braun *et al.*, 1989). Labor, and not land, is in most cases limiting production, and has to be scheduled and allocated to the various seasonal activities. Among the few exceptions are periurban environments, which are limited by land, and therefore, sometimes show high crop intensities reminiscent of Asian lowlands (M. Becker, WARDA, 1997 pers. comm.). Consequently, the introduction of new production systems to sparsely populated rural areas in West Africa has to take into account the competition for labor during any given period of the year. It also has to allow for a high degree of individuality and temporal flexibility for farmers participating in a scheme (Le Gal, 1997).

From the above, it might be hypothesized that introduced 'rice-only' production systems which are secure enough not to necessitate multiple objectives, and which provide a steady flow of income throughout the year, should be sustainable. We are not aware of this hypothesis having ever been tested, but it is interesting to note that some irrigation schemes in Burkina Faso (e.g., Vallee du Kou) and in Niger, originally introduced by Chinese aid workers, have maintained a rice cropping intensity of 2.0 and high yields since two decades or more. Although farmers in the high-intensity rice production systems in Niger are known to have maintained some upland based cropping activities, irrigated rice has become their main livelihood, and the tight activity calendars associated with rice double cropping are generally respected.

Based on the rejection of the notion that 'technically advanced production systems don't work in Africa', we will now take a fresh look at the physical potential for increased rice production.

#### Sustainability problems related to current patterns of intensification

As can be expected from a production environment that is mostly limited by labor and not by land, the physical resources for rice production in West Africa are far from being fully exploited. In the economically most dynamic 444rice ecosystems, namely, irrigation in the Sahel and rainfed lowlands in the humid and subhumid zones, less than ten percent of the potentially arable land is being used for rice cultivation, and most of the land is not cropped at all. By contrast, upland rice cultivation is in many areas reaching the agro-ecological limits of intensification, at least on the basis of the current resource and crop management practices (WARDA, 1996b).

The current patterns of intensification and rapid area expansion for rice in West Africa are likely to be unsustainable in the future. From an agro-ecological point of view, intensification is frequently happening at the wrong places, and with inappropriate methods, while existing, more suitable land resources are not being used. In the case of inland valleys, under-used and comparatively resilient lowlands are frequently found in the direct neighborhood of over-used uplands. In particular, system sustainability is being threatened by the following developments:

i) Shortened bush fallow periods in uplands, caused by increasing population, result in extreme weed pressure and soil degradation. Yields decrease and labor requirements for weed control increase, resulting in a decline in labor productivity. Sustaining the resource base under shortened fallow would require modified cultural practices and investment in resource base quality, thereby implying substantial behavioral changes by the farmer. Extension mechanisms for upland rice based systems are not available in many countries (WARDA, 1995 & 1996b).

ii) Irrigated rice systems in the Sahel, many of which have recently been turned over from public to farmer management or the private sector, have been expanding fast recently. However, the private investors frequently spend less on drainage facilities, thereby accelerating soil salinization and alkalinization. The current boom in Sahelian irrigated rice production may not be sustainable in the long term, unless farmers and scheme management can be competently advised and motivated to manage the land, and not only the crop (Bertrand *et al.*, 1993).

iii) Maintenance is a major determinant of the ecological sustainability of intensified production systems. Irrigated and rainfed lowland rice systems in the savanna and forest zones, for which improved water control was provided essentially free to farmers or donor projects, are frequently not maintained by the users, resulting in physical degradation. The root causes are numerous, but generally socio-economically based. Design and location of the schemes may not be adapted to farmers' objectives

and labor resources, or the systems may require too much technical coordination among producers.

Probably most importantly, intensification of rice systems iv) may incur serious risks of pest and disease outbreaks in all ecosystems, but particularly in the lowlands. Production systems that are already land limited, e.g. those in densely populated or peri-urban areas, are likely to accept Asian-type rice technologies. These are traditionally based on semidwarf indica rices, which are high-yielding but cannot build on locally evolved and adapted African germplasm. Care must therefore be taken to prevent pest outbreaks, which may result from excessive pesticide use, reduced biodiversity of the systems, and poorly adapted crop varieties. The ecological risks, particularly related to pests and diseases, might be even more serious than those experienced during the green revolution in Asia, because intensive lowland cropping in Africa has had no time to co-evolve with the natural biotic environment. African rice gall midge (ARGM) and rice yellow mottle virus (RYMV) are two examples of crop pests and diseases that are indigenous to Africa, but economically important only in production systems that are based on indica-type lowland rices (WARDA, 1995 and 1996b).

We conclude that a vast physical potential exists for the expansion and intensification of lowland rice based systems across the agro-climatic zones in West Africa. Upland rice may have limited scope for either, except through substitution of other crops, or if natural resource management options are found that would render short-fallow systems more sustainable on poor soils. This evaluation is preliminary, pending a quantitative assessment of fertile upland areas in West Africa, such as some that are based on Schist or volcanic base rock, and whose topography is not too prone to erosion.

But even the existing lowland and irrigated systems are in many cases prone to physical degradation, which seems to be mainly a result of poor fit of the introduced systems versus farmers' objectives and resources. In the Sahel, this development will be more difficult to correct, because soil degradation (alkalinization) is frequently irreversible, and its prevention, on the basis of the introduced scheme structures, requires continuing (or increased) investment (Bertrand *et al.*, 1993). It may simply not be possible to sustainably produce rice in the Sahel in the absence of costly irrigation and drainage facilities, which the private sector cannot provide. In the more humid environments, the long-term ecological consequences of system degradation are less important, and betteradapted systems, once available, can gradually substitute or supplement the existing ones.

Another important conclusion to draw is that development projects are ill-advised if guided mainly by the physical potential for production (which is vast for lowland and hydromorphic areas in Africa). Ex-ante potential impact analyses should use the probability of adoption as the starting point, based on socioeconomic fit (farmers' objectives, availability of labor, and expected productivity of labor). Land productivity (yield) may in many cases be a less important criterion for adoption, unless land is limiting. Prospects for impact in terms of national or regional production do not affect the farmers, and should only be considered for decisions at the strategic level.

Strategic policy decisions must also take into account interdependency of upland and lowland systems within small watersheds and inland valleys. The best way to reduce intensification pressure on the uplands may be to partly substitute it with lowland cultivation. This would require a better understanding of why farmers are currently so little involved in lowland cultivation. Only on the basis of this knowledge, incentives for major behavioral change among farmers can be developed (the alternative probably being accelerated migration to urban centers).

An important challenge faced by rice researchers in the region is, therefore, to develop lowland cultivation methods that are profitable, safe for human health, provide local food and income security, require minimal initial investment, and allow for sufficiently flexible individual calendars for labor use. These systems must build on varieties that have horizontal resistance to the major local biotic stresses, which is not the case with the currently cultivated lowland indica rices. The improved systems would probably differ significantly between rural and peri-urban lowlands, because they would respond to differential land and labor availability. In recognition of this, WARDA has identified the 'sustainable intensification of lowland rice based systems' as one of its top research goals, which is being addressed in a project focusing on the intensification gradient between peri-urban and rural lowlands. A complementary project aims at the 'stabilization

of upland rice-based systems under shortened fallow' (WARDA, 1997).

#### Research for today's or tomorrow's farmers?

Agricultural research and development efforts must be based on clear descriptions of the targeted farmer and his environment. These typologies may be explicit or implicit, but by definition must not be static: any intervention aims at impact, which can only be achieved if systems change, including the behavior of the farmer himself. Past development projects covered the whole range from introducing totally alien systems which actually required creating a different farmer (eg, a full-time rice farmer investing all resources in a single enterprise), to romantic concepts of leaving the sociocultural context untouched.

Development interventions, in order to achieve a positive change, have to profoundly affect farmers' technical decision making and resource utilization, but have to answer their basic concerns and fit within their available resources. In West Africa, national researchers and policy makers frequently refer to the 'modern farmer' who need to be created for development to take off. This vision is as unpragmatic as the opposite notion that the indigenous traditional farmer must be preserved. In fact, the rapid population growth in Africa will continue to change rural life and livelihood, and the role of agricultural research must be to exploit this change positively, rather than allowing demographic forces to mine and degrade the resource base (example of over-exploited uplands). Research must anticipate the changes and develop technical opportunities tailored for the next generation of problems, but sufficiently compatible with farmers' current objectives to be adopted now.

### The concept and potential role of low management technologies

WARDA's concept of low-management technologies hinges on a weed competitive, and therefore labor-saving, plant type. This concept emerged from two recent research outputs, the successful hybridization of *O. sativa* with *O. glaberrima* genotypes (Jones et *al.*, 1997), and the identification of labor availability as a major

constraint to increased rice production in rural environments (Richards, 1985). Since the commonly available technologies for intensification of rice systems (such as transplanting and irrigation in the lowland, and high-input, near-continuous cropping in the upland) are either labor-intensive or require mechanization, the transition from extensive to intensive rice cropping in West Africa is currently facing a technological bottleneck.

The low-management plant type, in association with efficient crop and resource management options, is intended to ease the agricultural transition while limiting costs to the environment. The physiological basis for the plant type has been laid by Dingkuhn *et al.* (1996 & 1997ab), Fofana *et al.* (1995) and Johnson *et al.* (1997), and molecular and genetic studies are under way to characterize the genetic resources and mechanisms that will be used for the plant type's realization. These research activities are accompanied in several West African countries by farmer participatory plant type validation and varietal selection. This program has focussed during the past few years on upland environments, but is now being extended to hydromorphic and lowland environments.

The development of economic and environment-friendly crop and resource management options, which will complement the plant types, depends strongly on the ecosystem. Weed competitive and resource efficient rices for the upland may liberate labor resources and other production means, which can then be reinvested in intensification, but they do not necessarily prevent mining of natural resources (although increased and more stable returns to investment at the farm level might encourage the use of fertilizer inputs, which would significantly stabilize 'mined' uplands). Efforts to develop economic approaches to improved fallow management are therefore also under way.

In labor-limited lowland systems, weed competitive plant types should ideally be complemented with low-cost alternatives to irrigation and transplanting, which are both highly labor intensive technologies. Irrigation and transplanting are primarily weedcontrolling devices, which build on the unique adaptation of the rice crop to reduced soil conditions. Along with high-yielding varieties, irrigation and transplanting formed the technological basis for the green revolution in the densely populated plains of monsoonal Asia. As labor becomes increasingly costly, however, these systems are reverting to direct seeding in combination with intensive herbicide use (De Datta, 1986; De Datta and Nantasomsaran, 1991; Erguiza *et al.*, 1990). This modern, highly intensified and mechanized use of direct-seeding has little in common with the traditional direct-seeded systems, except for the common objective to minimize demand on labor. Most lowland rice systems in West Africa, however, are still in the process of moving from traditional direct-seeding to transplanting.

For the labor-limited lowland rice systems in West Africa, an alternative paradigm to the labor-intensive irrigation/transplanting package is conceivable. It might be based on direct seeding, only partial water control and the use of weed-competitive plant types. This could build on the traditional rice cultivation systems in the floodplains of the savanna zone, which are generally based on dry direct-seeding and varieties that are adapted to both aerobic and anaerobic soil conditions. Traditional O. glaberrima landraces have been displaced from these systems by the non-shattering and lodging resistant O. sativa cultivars (Carpenter, 1978). These cultivars, however, have not been bred specifically for this environment, and, in most cases, cannot fully exploit the system's natural resources because they are either too short-strawed and insufficiently weed competitive, or not sufficiently tolerant to waterlogged conditions. The concept of low-management plant types might therefore fill an important technology gap in lowlands with variable hydrology.

The third environment to be targeted by interspecific hybrids are the intensified, and not necessarily labor-limited, lowlands. In these environments, major endemic pests such as African rice gall midge (ARGM) and diseases such as rice yellow mottle virus (RYMV) owe their growing economic importance to the predominant cultivation of highly susceptible indica rices. *Oryza glaberrima*, by contrast, has a rich reservoir of host resistance genes for these and other pests, which can be incorporated into new interspecific varieties (WARDA, 1996b).

Lastly, it should be mentioned that weed competitive rices will be increasingly needed by the agricultural sector in industrialized nations, where ecological concerns demand reduced herbicide use, and where agriculture competes for water with the industry and urban centers. Research on low-management plant types in Africa can therefore be expected to have major spill-overs beyond the continent.

### **Conclusion and outlook**

The green revolution did not take place in West Africa because its land-saving technologies, which are so successful in Asian lowlands, are unattractive to sparsely populated rural environments. In the labor-limited upland rice production systems of West Africa, extensive (shifting) cultivation remains more attractive than lowland cultivation as long as land resources remain abundant. The increasing pressure on these lands, caused by population growth, however, is now resulting in resource degradation, and thus, in a decrease in labor productivity. This is probably the main driving force behind the rapid expansion of rainfed lowland rice cultivation across West Africa. The 'peri-urbanization' of an increasing share of the lowlands also favors informal wetland development, with market access and a rapidly growing urban demand for rice acting as driving forces.

With population growth and urbanization remaining high in West Africa, it can be predicted that the extremely rapid growth in rice production during the past ten years, which reflects in part increased areas under lowland rice, is only the beginning. But it should not be concluded that a belated repetition of the Asian green revolution will now take place in Africa. Topography, soils, rainfall distribution and traditions are not the same as in Asia, and are generally less favorable to highly controlled culture conditions. As in Asia, Africa's green revolution in the lowlands will require major contributions from rice breeders and other scientific disciplines.

At least during its early phases, the expected boom in regional rice production is likely to draw from diverse hydrological environments and water management systems, and the cultivated area will continue to grow faster than the yields. During this period, it will be important to provide farmers with low-cost water management technologies and low-management plant types, as an incentive to increase and intensify the cultivation of lowlands. In the upland, low-management (e.g., weed competitive) but input responsive rice varieties are needed to improve yield stability and create incentives for resource-poor farmers to replace nutrients extracted from the soil in short-fallow systems. A long-term recovery of destabilized upland systems would also require more substantial investments in resource base quality. WARDA scientists are currently developing weed competitive and highly input responsive (but not input-dependent) rice types for various water limited ecosystems, based on interspecific crosses between Asian rice, *O. sativa*, and African rice, *O. glaberrima* (Jones *et al.*, 1997; Dingkuhn *et al.*, 1997). These technologies are expected to substantially increase rice yields in labor limited production systems during the transition from extensive to intensive rice production, while providing incentives for farmers to use fertilizers.

In the long run, the role of high-yielding and highmanagement semidwarf rices is expected to increase because farmers will find ways to improve water control as systems intensify, land will become scarce even in the rainfed lowlands, and more labor will become available due to population growth. In short, the boom in West African rice production is likely to continue, and will in the medium term demand low-management (labor-saving), but input responsive rice types and management methods for various hydrological environments. In the long term, riceland will become increasingly scarce, and emphasis will shift, as previously observed in Asia, towards land saving technologies. Since these developments will not take place simultaneously in the various parts of West Africa (e.g., peri-urban vs. rural systems), research must provide the respective technology options at an early stage, and assist in transferring them to the environments where and when they are needed.

WARDA's concept of low-management technologies, although evolved from upland-based research, is likely to be applicable to the following situations: (1) labor-limited upland rice based systems, particularly where shortened fallow periods have led to increased weed pressure; (2) systems in lowlands located in inland valleys and floodplains, where the classical technology package of irrigation / transplanting may be impractical for socio-economic or hydrological reasons; and (3) intensified, high-input systems prone to endemic pests and diseases, where the use of host resistance genes from *O. glaberrima* might prevent major pest outbreaks.

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# Wide Hybridization for Rice Improvement: Alien Gene Transfer and Molecular Characterization of Introgression

# D.S. Brar and G.S. Khush

International Rice Research Institute P.O. Box 933. Manila, Philippines

#### Abstract

The wild species are important reservoirs of useful genes for resistance to biotic and abiotic stresses. In the past, successful gene transfers had been made from wild species into crop plants for resistance to some pests and diseases. These wild species offer a potential for transfer of useful genes for rice improvement.

The objectives of this work are to: widen rice introgression by transferring useful genes from wild species into cultivated species, obtain a precise characterization of alien introgression, determine genome relationships of *Oryza* species and screen wild species of *Oryza* for apomixis.

The approach utilized included identification of useful variability in the wild species germplasm, production of hybrids between elite breeding lines and wild species through direct crosses and embryo rescue; backcrossing and evaluation of progenies; monitoring of introgression; gene tagging and locating introgressed segments on chromosomes.

Hybrids were produced between elite breeding lines and eleven wild species as well as between *O. sativa* and *O. glaberrima*. Four breeding lines from *O. sativa* x *O. officinalis* crosses, which are resistant to brown plant hopper, have been released as varieties for commercial cultivation in Vietnam. Other highlights included the development of monosomic alien addition lines (MAALs) in five species and two new cytoplasm male sterile lines - IR66707A and IR69700A. No clear evidence of apomixis was obtained when wild species were screened. In addition, successes in the tagging of genes with molecular markers were achieved.

# Introduction

The wild species are an important reservoir of useful genes for various economically important traits such as disease and insect resistance, increased tolerance for abiotic stresses, new sources of cytoplasmic male sterility (CMS), and improved quality characteristics. In the past, several successful gene transfers have been made from wild species into crop plants, notably those for rust resistance in wheat, grassy stunt and brown planthopper (BPH) resistance in rice, mildew and crown rust resistance in oats and resistance to several pests in tomato.

The genus *Oryza*, to which the cultivated rice belongs, has 23 wild species; Oryza sativa (2n = 24, AA) is the predominant cultivated species, while *O. glaberrima* (2n = 24, AA) is only cultivated in western Africa. The wild species of rice have either 2n = 24 or 48 chromosomes with AA, BB, BBCC, CC, CCDD, EE, GG and HHJJ genomes. Rice grown under a wide range of agroclimatic conditions and several factors such as unfavorable temperature, water and soil conditions, and diseases and insects, affect its productivity. Genetic variability for some traits such as resistance to tungro virus, sheath blight and yellow stemborer, and salinity tolerance is limited in the cultivated germplasm, and wild species offer potential for the transfer of useful genes for rice improvement.

#### Objectives

The main objectives of our wide hybridization program in rice are:

- widening the rice gene pool by transferring useful genes for disease and insect resistance and tolerance to abiotic stresses, and new CMS sources from wild species;
- precise characterization of alien introgression using RFLP markers and tagging of introgressed alien genes;
- determine genomic relationships of Oryza species, and
- screen wild species of Oryza for apomixis.

# Approaches

- Identification of useful variability in the wild species germplasm.
- Production of hybrids between elite breeding lines of rice and wild species through direct crosses and/or through embryo rescue.
- Backcrossing with the current rice parent followed by embryo rescue to produce fertile progenies.
- Evaluation of advanced disomic progenies for transfer of useful trait(s).
- Monitoring alien introgression using isozyme and RFLP markers. Chromosomal location of gene(s) using monosomic alien addition lines (MAALs).
- Tagging of introgressed alien genes with molecular markers
- Location of introgressed segments on chromosomes through in-situ hybridization.

# Results

# Production of interspecific hybrids

Through embryo rescue, hybrids have been produced between elite breeding lines of rice and 11 wild species representing genomes such as BBCC, CC, CCDD, EE, FF, GG and HHJJ, and also with *Porteresia coarctata* (Table 1). In addition, a series of hybrids have been produced between rice and different A genome wild species through direct crosses. Recently, some hybrids have also been produced between *O. sativa* and *O. glaberrima*.

# Gene transfer from wild species

A number of useful genes from wild species have been transferred into elite breeding lines of rice (Table 2). Additional wide cross progenies are being produced and evaluated for transfer of resistance to tungro, yellow stemborer, sheat blight and tolerance to acid sulfate conditions. From the cross of *O. sativa* x *O. officinalis*  four breeding lines resistant to BPH have been released as varieties for commercial cultivation in Vietnam.

Cross-combination		
Female parent	Male parent	
<i>O. sativa</i> (AA) (2n = 24)	x O. officinalis (CC) 2n	= 24
	x O. eichingeri (CC) 2n	= 24
	x O. rhizomatis (CC) 2n	= 24
	x O. minuta (BBCC) 2n	= 48
	x O. latifolia (CCDD) 2n	= 48
	x O. alta (CCDD) 2n	= 48
	x O. grandiglumis (CCDD) 2n	= 48
	x O. australiensis (EE) 2n	= 24
	x O. brachyantha (FF) 2n	= 24
	x O. ridleyi (HHJJ) 2n	= 48
	x O. granulata (GG) 2n	= 24
	x Porteresia coarctata 2n	= 48

**Table 1.** Hybrids produced between cultivated rice (*Oryza sativa*) and wild species of *Oryza* and related genera following embryo rescue.

# Development of MAALs

Monosomic alien addition lines (MAALs) have been developed in 5 species:

Species	N° of MAALs
O. officinalis (CC)	12
O. minuta (BBCC)	7
O. latifolia (CCDD)	11
O. australiensis (EE)	8
O. brachyantha (FF)	7

#### Development of new CMS sources

Two new CMS lines - IR66707A and IR69700A, having cytoplasm of *O. perennis* and *O. glumaepatula*, respectively, and the nuclear background of IR64 have been developed. The CMS source of these lines is different from WA cytoplasm, the most commonly used source in hybrid rice breeding. Search for restorers is underway.

#### Screening wild species for apomixis

The pistil clearing and callose fluorescence techniques were used to screen more than 100 accessions of tetraploid wild species for apospory and diplospory types of apomixis. No clear evidence of apomixis was obtained.

# Cytogenetic and molecular characterization of alien introgression

- Introgression for allozymes have been detected from various wild species.
- Genes for BPH resistance and earliness have been introgressed from *O. australiensis* into rice. The gene(s) for BPH resistance is/are linked with RG457 of chromosome 12 at a distance of  $3.69 \pm 1.29$  cM.
- In collaborative programs, genes for bacterial blight introgressed from *O. longistaminata* and from *O. minuta* for bacterial blight and blast resistance have been targged with molecular markers. RFLP analysis revealed introgression from 11 of the 12 chromosomes of *O. officinalis* into the rice genome.
- Isozyme and RFLP analyses indicate that alien gene transfer occurs through crossing over rather than substitution of a complete chromosome or a chromosome arm.

#### Molecular characterization of Oryza genomes

Two new genomes have been identified and designated as GG for diploid *O. meyeriana* complex and HHJJ for the tetraploid *O. ridleyi* complex based on molecular divergence analysis.

Donor wild species	Accession
(Genome)	number
O. nivara (AA)	101508
O. longistaminata (AA)	-
	100896
O. minuta (BBCC)	101141
O. latifolia (CCDD)	100914
O. australiensis (EE)	100882
O. brachyantha (FF)	101232
O. minuta (BBCC)	101141
O. officinalis (CC)	100896
	101141
O. latifolia (CCDD)	100914
O. australiensis (EE)	100882
O. granulata (GG)*	100879
O. officinalis (CC)	100896
O. perennis (AA)	104823
O. glumaepatula (AA)	100969
O. brachyantha (FF)*	101232
O. ridleyi (HHJJ)**	100821
O. minuta (BBCC)*	101232
O. rufipogon (AA)*	105908
O. officinalis (CC)**	105220
O. rufipogon (AA)*	CB 751
O. rufipogon (AA)*	
	<ul> <li>O. longistaminata (AA)</li> <li>O. officinalis (CC)</li> <li>O. minuta (BBCC)</li> <li>O. latifolia (CCDD)</li> <li>O. australiensis (EE)</li> <li>O. brachyantha (FF)</li> <li>O. minuta (BBCC)</li> <li>O. officinalis (CC)</li> <li>O. minuta (BBCC)</li> <li>O. latifolia (CCDD)</li> <li>O. australiensis (EE)</li> <li>O. latifolia (CCDD)</li> <li>O. australiensis (EE)</li> <li>O. granulata (GG)*</li> <li>O. officinalis (CC)</li> <li>O. perennis (AA)</li> <li>O. glumaepatula (AA)</li> <li>O. brachyantha (FF)*</li> <li>O. ridleyi (HHJJ)**</li> <li>O. minuta (BBCC)*</li> <li>O. rufipogon (AA)*</li> <li>O. rufipogon (AA)*</li> <li>O. rufipogon (AA)*</li> <li>O. rufipogon (AA)*</li> </ul>

# Table 2. Progress in the transfer of genes from various wild species into rice

# Future research priorities

- Transfer of genes for resistance to yellow stemborer, tungro, bacterial blight, sheath bligh and tolerance to salinity and acid sulfate conditions.
- Molecular characterization of alien introgression using RFLPs and in situ hybridization.
- Tagging of genes introgressed from wild species into cultivated rice.
- Screening of wild species germplasm for apomixis using pistil clearing and histological techniques.

# Acknowledgement

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# Current Status of Rice Interspecific Hybridization at CIAT

# César P. Martinez<sup>1</sup>, J. Tohme<sup>1</sup>, J. Lopez<sup>1</sup>, J. Borrero<sup>1</sup>, S.R. McCouch<sup>2</sup>, and W. Roca<sup>1</sup>

<sup>1</sup>CIAT.Rice Program and Biotechnology Research. Unit. A.A. 6713. Cali, V, Colombia <sup>2</sup>Assistant Professor. Cornell University. Plant Breeding. Dpt.252 mEmerson Hall. Ithaca, N.Y.

#### Abstract

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The current world rice production, of approximately 575 million tons, needs to increase by 20% to meet estimated demand in the next decade. The research challenges to meet these needs include:

(i) developing varieties and farming systems that yield far more than is currently produced, while bearing in mind that the long-term viability of the agricultural systems and the resource base, upon which they depend, must be protected;

(ii) greater international cooperation and new strategies for plant improvement, streamlining of biotechnological and other classical approaches are needed;

(iii) developing highly nutritious, productive and resource efficient crop varieties and

(iv) sustainable increased production through exploitation of wider genetic variation, abundant in nature.

Plants of the wild species of *O. rufipogon, O. glaberrima* and *O. barthii* were hybridized with improved rice cultivars.  $F_1$  plants were backcrossed to the recurrent parents and resulting  $BC_1F_1$  were evaluated for desirable agronomic traits in order to narrow population down to a few best individuals. Further backcrossing and selections were done. These populations of  $BC_2F_2$  populations were selected for field trials and molecular characterization. Embryo rescue was used to overcome sterility and embryo abortion.

Transgressive segregation was observed in field trials with several lines having between 5 and 15% higher yield than the recurrent parent. Similar preliminary results were obtained for disease resistance from some of the interspecific hybrid progenies. This was an indication that introgression from the wild species into elite lines could contribute positively, not only to yield but also to stress resistance. Population development is still at different stages from the 36 crosses made. The more advanced lines have been backcrossed twice to the recurrent parents.

# Introduction

Rice forms the nutritional basis for much of the world's population. This cereal is planted on 148 million hectares worldwide and is the world's most important food crop. Globally, rice provides 20% of energy and 15% of per capita protein. It is estimated that by the year 2025, some 8.3 billion people will live on earth and that 50% of them will be rice eaters. Therefore, current world rice production (approx. 575 million tons) must be increased by 70% to meet this demand. More than 90% of world's rice is grown and consumed in Asia, while Latin America's rice production represents 3.5% of the total: over 70% of rice production in Latin America (LAC) comes from irrigated and rainfed lowland ecosystems. Rice production in LAC increased from 9.9 to 18.8 million tons from 1966 to 1994, while modern semidwarf varieties combined with appropriate management practices produced a 76% regional average yield increase, for the irrigated and rainfed lowland sectors, from 2.5 to 4.4 t/ha.

Rapid population growth in Asia and LAC puts increasing pressure on the already strained food-producing resources of these regions. By the year 2010, a food deficit in excess of 100 million tons each year is predicted, unless the world agricultural community is able to develop crop varieties and farming methods that yield far more than is currently produced. In addition, longterm viability of our agricultural system and the resource base upon which they depend must be protected. New paradigms of international, regional and interinstitutional cooperation and new strategies for plant improvement are needed. Highly productive, nutritious, and resource-efficient crop varieties must be developed.

Ongoing plant improvement efforts must be streamlined using a mixture of biotechnological and classical approaches. Familiarity with a variety of approaches to genetic enhancement of crop plants will be increasingly essential in agricultural research.

Early plant domestication by man followed by modern intensive breeding of crop varieties by plant breeders has narrowed down the genetic base in many crops (Simmonds 1976; Ladizinsky 1985; Tanksley and Nelson 1996). This problem is more critical in self-pollinated crops, like rice (Wang et al. 1992). The reduced genetic variation among improved commercial cultivars make them more vulnerable to biotic and abiotic stresses, and this could explain the slower rate of genetic progress achieved by plant breeders (Tanksley and Nellson 1996). This problem is particularly critical as it relates to yield, and has culminated in the phenomenon known as the 'yield barrier'.

There is an urgent need to sustainably increase rice production, but the reduced genetic variation in rice works against it . In LAC, irrigated rice breeding has depended on a genetic core of 12 landraces (Cuevas, 1993). In Colombia, average yield of irrigated rice has not changed since 1970 (Fedearroz, 1993). Numerous studies have indicated that after initial improvements obtained by using this core of germplasm, followed by the introduction of a set of plant traits necessary for local adaptation, the yield potential of irrigated rice in Latin America has reached a plateau (CIAT medium term plan, 1993-1998). At IRRI, the last major advance in rice yield potential was obtained with the development of IR8, and for the last 30 years breeding efforts have been largely devoted to improving pest and disease resistance, quality and general adaptation, while maintaining potential yields at constant levels. A new plant type has been developed at IRRI (Peng et al. 1994) with an increased yield potential. However, it is not yet ready for commercial planting and more breeding is needed to improve its insect and disease resistance and grain quality.

Fortunately, genetic variation is abundant in nature; the wild ancestors and related land races of most crop species can still be found in situ and/or in germplasm banks around the world. According to Tanksley and Nelson (1996), plant scientists have been unable to exploit the majority of this genetic potential for two reasons: (i) difficulty in identifying genes for yield and quality in wild germplasm and (ii) linkage drag. In the case of rice (Vaughan and Sitch, 1991), the genus Oryza consists of approximately 20 wild species and two cultivated species (O. sativa and O. glaberrima). Occasionally, some of the wild species have been used in breeding programs, specially at IRRI, and most of the time as sources of genes for disease and insect resistance. On the other hand, two well-saturated maps of rice are available (Cause et al., 1994; Kurata et al., 1994). These maps contain closely linked, codominant loci that can be monitored for linkage to genes controlling traits of economic importance (Tanksley et al., 1989); Paterson et al., 1991). These maps if used in conjunction with traditional breeding methods, can allow breeders to locate and selectively transfer genes that will improve yield, quality, and adaptability to different production constraints (Xiao *et al.*,1995; Ahn *et al.*, 1993; Nakamura *et al.*, 1994; McCouch and Doerge, 1995). More recently, Tankseley and Nelson (1996), proposed a new method called 'advanced backcross QTL analysis' for combining QTL analysis with variety development. Plant breeders have at their hands a powerful tool to enhance their germplasm.

The purpose of this paper is to present the status of interspecific hybridization at CIAT.

#### Materials and Methods.

#### Population development

Plants in each of the wild species O. rufipogon, O. glaberrima, and O. barthii were hybridized to plants of each of the improved rice cultivars (recurrent parent) listed in Table 1. Single crosses were obtained and grown in the greenhouse at CIAT in early 1994. Three F<sub>1</sub> hybrid plants were backcrossed to the improved cultivars, using the latter one as the female parent; approx. 100-180 BC<sub>1</sub>F<sub>1</sub> seeds were obtained per cross combination. The resulting BC<sub>1</sub>F<sub>1</sub> plants were transplanted (30x50 cm) and evaluated based on phenotype: Negative phenotype selection for undesirable agronomic traits (spreading plant type, excessive shattering, long awns, dark-color grains, high sterility, etc) was used to narrow the selection down to the best (40-50) individuals (Fig.1). Each selected BC1 individual was backcrossed again to the recurrent parent and approximately thirty BC<sub>2</sub>F<sub>1</sub> seeds were produced. Twenty BC<sub>2</sub> seeds from each of the selected BC<sub>1</sub> plant were sown in wooden trays in the screenhouse and later transplanted (30x40 cm) under irrigated conditions. A negative phenotypic selection was applied again and best individuals per cross were selected and harvested individually to generate BC<sub>2</sub>F<sub>2</sub> seed. Approximately 220-300 BC<sub>2</sub>F<sub>1</sub> plants were selected per cross combination for field testing. Each selected BC<sub>2</sub>F<sub>1</sub> plant was evaluated for twelve agronomic traits including days to heading and maturity, plant height, panicle length, panicles per plant, spikelets per panicle, grains per panicle, seed set rate, spikelets per plant, grains per plant, 100-grain weight and yield per plant. Based on field observations and genetic potential only three

populations (BG90-2/O. rufipogon, O. Llanos 5/O. rufipogon, and Caiapo/O. rufipogon were chosen for field testing.

100-180 F.BC. seeds

BG90-2/O.rufipogon O. LLANOS 5/O.rufipogon CAIAPO/O. rufipogon	NEGATIVE SELECTION $\checkmark$ Best 40-50 plants $\checkmark$ $F_1BC_2$ : 900-1000 seeds
MOLECULAR CHARACTERIZATION: PARENTS, $F_1$ : DONE $F_2BC_2$ FAMILIES: UNDERWAY QTL ANALYSIS: BEST FAMILIES $\rightarrow$ 3ER BC $\rightarrow$ NILS	<ul> <li>Image: A state of the state of the</li></ul>

Fig. 1. Flow chart followed at CIAT

Field trials ( $BC_2F_2$  families)

The 300 BC<sub>2</sub>F<sub>2</sub> families derived from the crosses BG-90-2/O. rufipogon and Caiapo/O. rufipogon, and the 220 families from the cross O. Llanos 5/O. rufipogon were planted in replicated yield trials in four sites in Colombia (CIAT-Palmira and CIAT-Santa Rosa, Villavicencio, La Libertad Exp. Station, Villavicencio, and Saldana. Tolima). The Caiapo/O. rufipogon cross was planted under upland-savanna conditions. and the other under irrigated/rainfed conditions. Transplanting (20x30 cm) was used at CIAT-Palmira, while direct seeding was used elsewhere. A completely randomized design with two replications, 2 row-plot, 5 m. long was used. Data on the 12 agronomic data described elsewhere, including plot yield/family were taken.

Parent	Accessio	on		
	No.	Source	Origin	Note
Donor				
O. rufipogon	105491	IRRI	Malaysia	Ancestor of O. sativa
O. glaberrima	103544	IRRI	Mali	Cultivated in Africa
O.barthii	104119	IRRI	Chad	Relative of O. sativa
Recipient (Reci	urrent par	ent)		
Cypress		Louisiana	USA	Tropical japonica, quality
Lemont		Texas	USA	Tropical japonica, quality
RU9403006 (Je	efferson)	Texas	USA	Tropical japonica, quality
Oryzica Llanos	5	CIAT	Colombia	Indica; resistant to P.Oryzau
BG90-2		CIAT	Sri Lanka	Indica; high yield
Morelos A88		CIAT	Mexico	Good combining ability
Oryzica 3		CIAT	Colombia	Indica; high yield
O. sabana 6		CIAT	Colombia	Tropical japonica; upland
P. turipana 7		CIAT	Brazil	Tropical japonica; upland
Progresso		CIAT	Brazil	Tropical japonica; upland
Caiapo		CIAT	Brazil	Tropical japonica; upland
CT6196-33-11	-1-3	CIAT	Colombia	Tropical japonica; upland

Table 1. Plant materi	als used i	n interspecific h	nybridization at CIAT
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# Molecular characterization

Parental surveys filters containing *O. rufipogon*, Fanny, O. Llanos 5, BG90-2, Caiapo and, O. Sabana 6, and the corresponding  $F_1$ 's were prepared using four restriction enzymes (Eco RI, Eco RV, Hind III, and Dra I). Approximately 140 markers from the rice molecular framework linkage map were selected at 10-20 cm intervals throughout the genome. A set of 50 mapped rice microsatellite markers, which were developed at Cornell University, is also being used to complement the RFLPs in QTL analysis. DNA from each  $BC_2F_2$  family of the BG90-2/*O. rufipogon* cross have already been extracted.

O. rufipogon	BG90-2//2 * BG90-2 (3) MORELOS A88//2* MORELOS A88 (3) ORYZICA 3//2* ORYZICA 3 (3) ORYZICA LLAMOS 5//2* ORYZICA LLANOS 5 (3) LEMONT//LEMONT (2) RU94030006//2*RU94030006 (3) CYPRESS//2*CYPRESS (3) ORYZICA SABANA6//2* ORYZICA SABANA 6 (3) ORYZICA TURIPANA 7 (1) PROGRESSO (1) CT6196-33-11-1-3 // CT6196-33-11-1-3 (1) CAIAPO//2* CAIAPO (3)
O. barthii	BG90-2//2 * BG90-2 (2) MORELOS A88//2* MORELOS A88 (3) ORYZICA 3//2* ORYZICA 3 (3) ORYZICA LLAMOS 5// ORYZICA LLANOS 5 (2) LEMONT//LEMONT (2) RU94030006//RU94030006 (2) CYPRESS//2*CYPRESS (2) ORYZICA SABANA6// ORYZICA SABANA 6 (2) ORYZICA TURIPANA 7// ORYZICA TURIPANA(2) PROGRESSO (1) CT6196-33-11-1-3 // CT6196-33-11-1-3 (2) CAIAPO (1)
O. glaberrima	BG90-2//2 * BG90-2 (2) MORELOS A88 (1) ORYZICA 3//ORYZICA 3 (2) ORYZICA LLAMOS 5 (1) LEMONT (1) RU94030006//2* RU94030006 (3) CYPRESS (1) ORYZICA SABANA6// ORYZICA SABANA 6 (2) ORYZICA TURIPANA 7// ORYZICA TURIPANA(2) PROGRESSO (1) CT6196-33-11-1-3 (1) CAIAPO (1)

Table 2. Interspecific crosses made between several improved irrigated and upland rice cultivars, and three wild species of rice

(2) = First backcross made; in some cases second backross are in replicated yield trials

Second backcross made; in some cases replicated yield trial (3) = with F2BC2 progenies

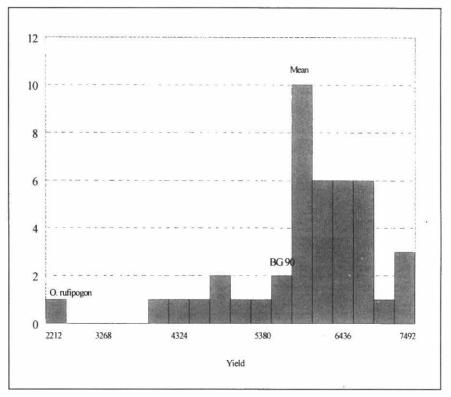


Figure 2. Transfer of novel genes from wild Oryza. Yield of O.rufipogon, BG-90 and selected BC<sub>2</sub>F<sub>2</sub> families

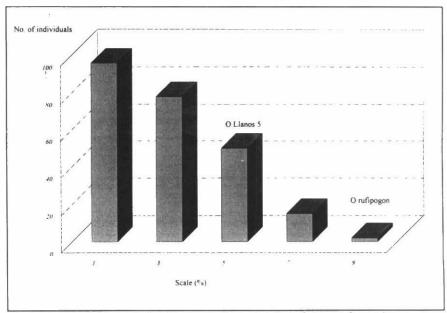


Figure 3. Evaluation of F2BC2- Oryza lanos 5 / O. rufipogon for resistance to hoja blanca virus

## **Results and Discussion**

#### Probe selection

Data indicated that 90 probes out of 140 clones were polymorphic (64%); polymorphism was greater between *O. rufipogon* and the tropical japonica cultivars Fanny, O. sabana 6 and Caiapo, compared to *O. rufipogon* and the indica cultivars, O. Llanos 5 and BG90-2. Screening of  $BC_2F_2$  families with polymorphic RFLP clones and microsatellites is underway.

#### Population development

A total of 36 crosses (Table 2) was made using the parental lines listed in Table 1; population development from these crosses is underway but at different stages; the more advanced populations have gone through two rounds of backcrossing to the recurrent parent. Although all of the donor wild rice belong to the same genome AA as cultivated rice and crossability should not be a problem, however there were some sterility problems and embryo abortion in some cases specially in crosses with *O. barthii*. Failure of embryo development was observed 10-15 days after pollination,. Therefore, embryo rescue was also used in the backcrossing scheme if progenies had a desirable phenotype.

# Yield Trials

These evaluations were conducted during June-October 1996. Data on main agronomic traits are still being recorded and processed and only partial information generated in CIAT-Palmira is presented. The distribution of grain yield (kg/ha) of 38 BC<sub>2</sub>F<sub>2</sub> families (BG90-2/O. rufipogon) chosen in the field, based on phenotype and plot yields (40 plants, two replications) is illustrated in Fig. 2. Transgressive segregation was observed, with several lines having between 5 and 15% higher yield than the recurrent parent BG90-2. This preliminary data is in agreement with reports on work being conducted in China at the Hunan Hybrid Rice Research Center (McCouch, S. 1995) and in South Korea. These data from different groups working with diverse recurrent parents suggest that DNA introgressed from *O. rufipogon* can contribute positively to yields in elite rice cultivars. Furthermore, data from China (McCouch, S. 1995) suggest that two QTLs found in chromosomes 1 and 2 of *O. rufipogon* are responsible for this yield increase.

Further work at CIAT will indicate whether or not we are dealing with the same QTLs reported by the Chinese group. OTL analysis performed in populations developed with O. glaberrima and O. barthii will show they carry the same QTLs for yield as found in O. rufipogon. If each wild rice posses specific positive alleles for yield, then rice breeders will have a tremendous breeding strategy for increasing yield in a systematic and pyramided manner. that is, in a step-wise process. On the other hand, 220 BC<sub>2</sub>F<sub>2</sub> families derived from the cross Oryzica Llanos 5/O. rufipogon were evaluated under field conditions at CIAT-Palmira using a highly viruliferous insect colony of Tagosodes oryzicola. Distributions of disease incidence (%) based on a 0-9 scale (0=no disease symptom; 9=>70% diseased plants) is presented in Fig.3. Transgressive segregation for rice hoja blanca virus resistance can be observed. with approx. 50% of the families falling in the categories 1 and 3. This preliminary data from an un-replicated experiment seem to suggest that positive alleles for rice hoja blanca virus form O. rufipogon could be contributing to increased resistance to this particular virus disease. More testing is needed to confirm this result.

In summary, preliminary data support the hypothesis that DNA introgressed from *O. rufipogon* can contribute positively not only to yield in elite rice cultivars, but also to stress resistance. This information also provides the basis for implementing the method proposed by Tanksley and Nelson 1996 referred to as 'advanced backcross QTL analysis' for the simultaneous discovery and transfer of valuable QTLs from wild germplasm into elite breeding lines.

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# Characterization and Utilisation of Oryza glaberrima Steud. in Upland Rice Breeding

Monty. P. Jones, A. Audebert, S. Mande and K. Aluko

West Africa Rice Development Association (WARDA), Rainfed Rice Program, Bouake, Cote d'Ivoire

#### Abstract

Oryza glaberrima Steud. is a potential source of useful genes for a range of economically important characters. During the 1992 and 1993 growing seasons, 1130 accessions of O. glaberrima and 591 accessions of O. sativa L. were characterized at the West Africa Rice Development. Association (WARDA) at M'bé, Côte d'Ivoire for 43 morphological traits. A number of accessions with desirable morphological traits and resistance to stresses such as drought and blast have been identified and are now being utilized in the breeding program. Progenies from interspecific hybridization of O. sativa and O. glaberrima are showing high sterility in the F<sub>1</sub> hybrids and early generations. However, fertility was obtained (5-95%) and retained after two to four backcrossings in some populations. Although true intermediates between O. glaberrima and O. sativa were generally rare, their occurrence in some populations was as high as 30%. Desirable morphological traits introgressed from O. glaberrima into segregating BC, F<sub>3</sub> populations included early maturity (75-100 days), rapid seedling growth and high tillering. To exploit the potential of O. glaberrima in interspecific breeding, it is necessary to better understand its physiology and ecology. It will also be necessary to characterize alien gene introgression through isozyme analysis and molecular markers to examine the crossability, genetic homoeology and nuclear stability of hybrid progenies.

# Introduction

Rice is grown under a range of agro-climatic conditions in West Africa. Grain yields are constrained by unfavorable weather, water and soil conditions, a number of diseases and some major insect pests. Variability in resistance to drought, soil acidity, rice blast, rice yellow mottle virus (RYMV), stem borers and African rice gall midge is limited in the widely cultivated species, *Oryza sativa*. In addition to *O. sativa*, the Asian domesticated species, *O. glaberrima*, the indigenous domesticated species is also grown by African farmers.

Wild species of *Oryza* indigenous to Africa include *O. barthii* A. Chev., *O. longistaminata* A. Chev. et Roehr., *O. punctata* Kotschy ex Steud., *O. eichingeri* A. Peter and *O. brachyantha* A. Chev. et Roehr. These species are diploid except *O. punctata*, which is both diploid and tetraploid (Takeoka, 1965; Chang, 1975; Ng et *al*, 1983). The chromosome number, genome grouping and geographical origin of the cultivated and wild *Oryza* species are given in Table 1.

The African cultivated rice, *O. glaberrima*, is known to have been selected and cultivated in parts of West Africa more than 3500 years ago (Jacquot, 1977; Carpenter, 1978). It probably developed independently, domesticated from a wild progenitor, *O. barthii* (syn. *O. breviligulata*) (Jacquot, 1977). It is believed to have originated or been first domesticated in the upland delta area of the Niger river, whence it spread through the upper Niger valley and delta, and then westwards to the Senegal valley and Casamance (Ng et al., 1991).

Species	2n	Genome	Origin
O. sativa(cultivated)	24	AA	Asia
D. glaberrima cultivated)	24	A <sup>g</sup> Ag	West Africa
O. stapfii (weed species)	24	A <sup>g</sup> A <sup>g</sup>	West Africa
O. barthii	24	A <sup>g</sup> A <sup>g</sup>	West Africa
O. longistaminata	24	A <sup>1</sup> A <sup>1</sup>	Tropical Africa
O. brachyantha	48	FF	West & Central
O. eichingeri	24	CC	Africa
	48	BBCC	East & Central
O. punctata	24	BB	Africa
	48	BBCC	•
D. schwein furthiana	48	BBCc	Tropical Africa

Table 1. The species of Oryza in Africa

Source: Adapted from Ng et al. (1983), Takeoka (1965)

Oryza glaberrima is cultivated in deep water, shallow water, mangrove swamps, hydromorphic conditions and upland conditions. Studies of its genetic diversity indicate that there are two major groups, the floating type and the upland type (Jacquot, 1977). In some areas in West Africa, farmers still grow mixed proportions of O. glaberrima and O. sativa (approximately 15-20% of the total rice area), while in other areas O. glaberrima is grown alone (10-15%). Under poor management conditions, the yield of O. glaberrima can exceed that of O. sativa.

Although *O. glaberrima* has never been subjected to modern varietal improvement, some cultivars are resistant to drought, blast, soil acidity and other stresses (WARDA 1992). It also has excellent vegetative growth which helps to suppress weeds and good grain quality(Koffi, 1980). *O. glaberrima* varieties compete more effectively with weeds than *O. sativa*, as they tend to produce more tillers (Koffi, 1980). Varieties capable of suppressing weeds are required in West Africa, where weeding is generally manual, but in many cases effective weed control is impossible due to shortage of or competing demand for labor.

Unfortunately, O. glaberrima has many undesirable traits which result in low yield potential. Its grain shatters easily and has long dormancy. The weak stems lodge easily. Past efforts to transfer useful genes from O. sativa into O. glaberrima have had limited success, primarily because of sterility barrier between the two species. However, gene flow between O. glaberrima and O. sativa or O. longistaminata has been analyzed at ORSTOM by Second (1984) and De Kochko (1987), who recommended the exploration of O. glaberrima as a source of new variability in upland rice. Although the grain yield of O. glaberrima is generally low, the species can be used in hybridization with O. sativa to incorporate genes for resistance to major stresses as well as for vigorous vegetative growth.

WARDA breeders recently initiated research to better understand useful traits of *O. glaberrima* germplasm and to exploit them in hybridization program for the upland and rainfed lowland ecosystems.

# Materials and methods

Morpho-agronomic characterization of O. glaberrima and O. sativa accessions

The WARDA working collection includes 1721 upland accessions (1130 *O. glaberrima*, 316 improved *O. sativa* and 275 traditional *O. sativa*). The *O. glaberrima* and traditional *O. sativa* accessions were collected from 14 African countries between 1985 and 1990 (Table 2).

Country	O. sativa	O. sativa Frequency		
	No.	(%)	O. glaberrima Frequency	
			No.	(%)
Burkina Faso	-	-	14	1.2
Cameroon	-	-	20	1.7
Chad	2	0.7	7	0.6
Côte d'Ivoire	138	50.1	28	2.4
Ghana		-	18	1.5
Guinea Bissau	19	7.0	-	-
Conakry	96	35.0	12	1.0
Liberia		-	579	51.2
Mali	2 <b>/=</b> 2	-	84	7.4
Nigeria	-	-	287	25.4
Senegal	10	3.6	51	4.5
Sierra Leone	-	-	23	2.0
Tanzania	10	3.6	-	-
The Gambia	-	-	5	0.4
Total	275	100	1130	100

 Table 2. Origin and number of accessions of Oryza sativa (traditional) and O.
 glaberrima collected, 1985-90

In June of 1992 and 1993, all 1721 accessions were evaluated for a range of morphological and agronomic traits, including seedling vigor, growth duration, plant height, panicle per m<sup>2</sup> and grain shape. The evaluation was conducted at Gagnoa in central west Côte d'Ivoire. Located in the forest zone at 205 m above sea level, 05E57'W and 06E08'N, the Gagnoa site is characterized by an ultisol and a bimodal rainfall pattern (March to May, and September to November); the average annual rainfall and temperature are 1586 mm and 26°c, respectively. In the 1992 and 1993 seasons, the soil was plonghed and harrowed in February, followed by seed dibbling of each entry at a spacing of 25 x 25 cm in (1 x 5 m) single plots. The seeds were sown after a basal application of 20 kg N/ha, 36 Kg  $P_2O_5$ /ha and 36 Kg  $K_2O$ /ha. Urea was applied at a rate of 30 kg N/ha at 35 and 70 days after sowing (DAS). The herbicide Ronstar was applied 1 DAS at a rate of 4 liters ha. For weed assessment, hand weeding was done on 18 and 40 days after sowing and weed weighed on plot basis. The morphoagronomic traits of the test entries were evaluated according to the IRRI Standard Evaluation System (IRRI 1988) for Rice.

#### Evaluation for resistance to major stresses

#### Drought resistance

In December 1993, 410 *O. glaberrima* and 50 *O. sativa* accessions, previously identified as resistant to drought (WARDA 1992), were screened in the field to assess varietal differences in resistance and recovery abilities under drought conditions in the upland ecosystem at M'bé. Located in the Guinea savanna climatic zone at 5E06'W and 7E52'N, the rainfall pattern at M'bé is bimodal (April to June, and September to October). The average annual rainfall and temperature are 1146 mm and 25°c, respectively.

All materials were screened in the dry season for response to drought stress during the vegetative and reproductive stages. Two sets of test and check varieties were subjected to 35 days of moisture stress from 15 days after sowing (DAS) exposing the entries to stress during the vegetative stage or during the reproductive stage from 45 DAS. This allowed for a comparison of drought resistance at both stages. The entries were sown in 1 m rows, with resistant (OS6) and susceptible (IR20) check varieties repeated after every 20 entries. Each entry was rated at 2-weekly intervals for a number of traits, including seedling vigor, plant height, leaf tip burn, and leaf rolling and unfolding ability. The entries were visually scored at weekly intervals for drought resistance during the period of drought stress and recovery ability after the stress is relieved, using a 0-9 scale (IRRI 1988) as follows:

Description	Scale
No symptoms	0
Slight tip drying	1
Tip-drying extending up to	
1/4 length in most leaves	3
One-fourth to 1/2 of leaves fully	
fully dried	5
More than 2/3 of all leaves fully	
dried	7
All plants apparently dead	9

# (1) Drought resistance

(2) Recovery score

Description	Score
90-100% of plants recovered	1
70-89% of plants recovered	3
40-69% of plants recovered	5
20-39% of plants recovered	7
0-19% of plants recovered	9

# Blast disease resistance

The most important disease of rice in West Africa is rice blast, caused by the fungus *Pyricularia oryzae*. Sources of durable resistance to blast have been found in very few landraces of *O. sativa*. To identify new sources of resistance, 126 *O. glaberrima* accessions were evaluated for field reaction to the disease at m'bé in 1993. Each entry was sown in single plots (2 x 5 m rows), with the check variety, Moroberekan, replicated after every 20 entries. The entries were scored for blast resistance at 30, 37, 44 and 51 DAS, using a 0-9 scale as follows:

% infected plants	Scale
0 1-10	0
11-30	3
31-50	5
51-70	. 7
71-100	9 9

#### Hybridization

During 1992 a program of wide crossing between *O. sativa* and *O. glaberrima* was undertaken to incorporate genes for rapid vegetative growth and drought resistance into improved *O. sativa* varieties. In 1993 we continued the search for new donors of *O. sativa* (*japonica* and *indica*) and *O. glaberrima* origin with desirable traits in order to broaden and diversify the genetic base of new breeding materials. Fifteen successful single crosses and backcrosses were made to incorporate genes for appropriate plant type to compete with or suppress weeds and for blast, drought and insect resistance. Backcrosses and  $F_1$ ,  $F_2$  and  $F_3$  populations were grown in the field and modified bulk and pedigree selections were made.

#### **Results and discussion**

Diversity of O. glaberrima and O. sativa

Seedling vigor

The characterization work showed that there is very wide variation in important morphological and agronomic traits within both the *O. glaberrima* and the traditional and improved *O. sativa* accessions. For example, wide variation in seedling vigor was observed within each varietal group (Figure 1). However, over 90% of the *O. glaberrima* and traditional *O. sativa* accessions screened scored 3 or less, indicating very high seedling vigor. Among the improved *O. sativa* materials, only 5% scored 3 or less. A few accessions of O. glaberrima, such as 1B19, CG14, IG10, CG20 and Biyagero, had very rapid vegetative growth and produced a large number of tillers and droopy lower leaves within 20-30 DAS, resulting in rapid ground cover and high leaf area index of about 4. These varieties had low weed biomass from their plots at 18 DAS and/or at maturity and were capable of suppressing weeds. For example, weed weight ranged from 120 kg per hectare in Biyagero and CG14, two O. glaberrima varieties, to 380 kg per hectare in ITA 257, an improved O. sativa at 18 DAS and from 410 in CG14 to 1560 kg per hectare in another improved O. sativa, WAB32-80 at maturity. Varieties capable of suppressing weeds are required in West Africa, where 80% of farmers in upland and rainfed lowland areas are unable to ensure timely and complete weeding because of family labor and farm resources constraints. It was also noted that O. glaberrima has the ability to compensate for weed competition in the early stages of crop growth by producing up to eight late tillers per plant stand between 40 and 60 DAS after the crop has been weeded. Koffi also reported in 1980 that varieties of O. glaberrima compete more effectively with weeds than O. sativa, as they tend to produce more tillers.

#### Growth duration

The number of days from planting to maturity for all accessions and the frequency distribution in each varietal type is given in Figure 2. Over.80% of the traditional *O. sativa* accessions had medium to long duration (120-140 days). Among the improved *O. sativa* accessions, the distribution covered a narrow range with most entries between 80 and 120 days. In the *O. glaberrima* accessions there was a much wider range with about 85 days between the minimum and maximum values. We identified several *O. glaberrima* accessions with very early maturity of about 75 DAS to maturity; these included Gbondobai, OG7, Zaria(3), 113 90 and UG24(2), all of which will be exploited in the breeding program as donors for earliness. The merit of early-maturing rice varieties is that they may escape drought in drier areas, while in more humid areas they will escape late-season fungal diseases such as blast, glume discoloration and sheath rot.

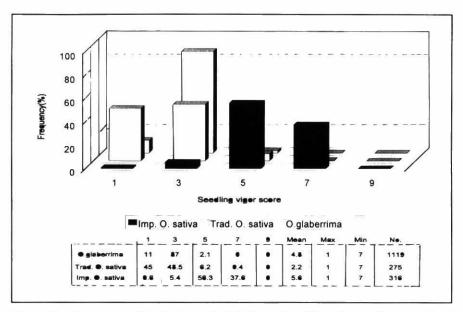


Figure 1. Frequency distribution and statistics of seedling vigor of improved and traditional O. sativa and O. glaberrima

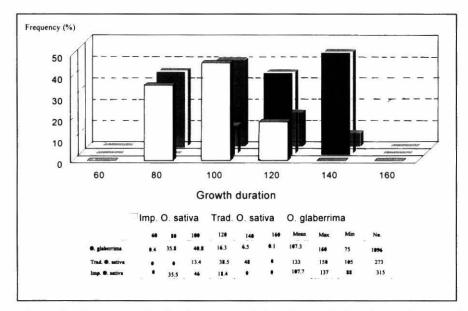


Figure 2. Frequency distribution and statistics of growth duration of improved and traditional *O. sativa* and *O. glaberrima* 

#### Plant height

The traditional O. sativa accessions are generally tall-statured types with over 120 cm. (Figure 3). Such varieties can lodge easily, especially under high soil fertility conditions. Loss of grain due to lodging in traditional O. sativa varies from 10% to over 50%, depending upon the time at which the crop lodges (WARDA, 1992). Most of the O. glaberrima and improved O. sativa accessions are intermediate-statured types, with plant height varying between 80 to 100 cm. Although other results have shown that most of the improved varieties are resistant to lodging (Jacquot, 1977), O. glaberrima materials have a weak and brittle culm that sometimes causes them to lodge easily during the late vegetative stage of growth. Some O. glaberrima materials, such as Old Ayoma, Fufore-Yola, UG75(1), UG75(2), NG26(3), CG66, Katsina Ala and Shendam, that were rated as resistant to lodging are characterized by short to intermediate stature of 80 to 100 cm and a thick leaf sheath. These materials can serve as donors for lodging resistance.

# Number of panicles

Considerable variability was observed in the number of panicles for each varietal type (Figure 4). Panicle production was highest in improved *O. sativa* accessions, with an average of 277 panicles per m<sup>2</sup>. The average number of panicles per m<sup>2</sup> in *O. glaberrima* and traditional *O. sativa* were 221 and 166, respectively. However, we identified several *O. glaberrima* and traditional *O. sativa* accessions with a higher number of panicles than in the improved varieties. The accessions Barta Tazo(1), Demsa-Yola, MF13(1), Dan Mauya and Barta(9) all had more than 400 panicles per m<sup>2</sup> and are potential donors of high panicles.

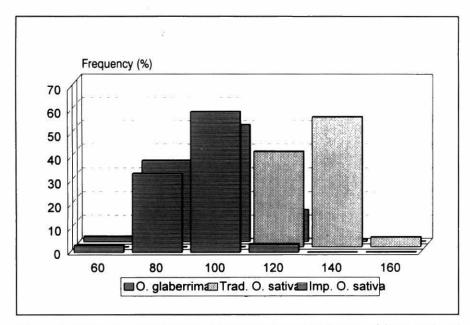


Figure 3. Frequency distribution and statistics of plant height of improved and traditional O. sativa and O. glaberrima

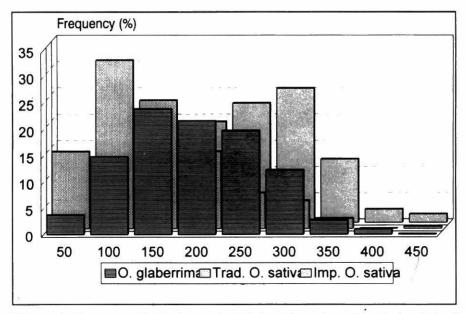


Figure 4. Frequency distribution and statistics of number of panicules /m<sup>2</sup> of improved and traditional *O. sativa* and *O. glaberrima* 

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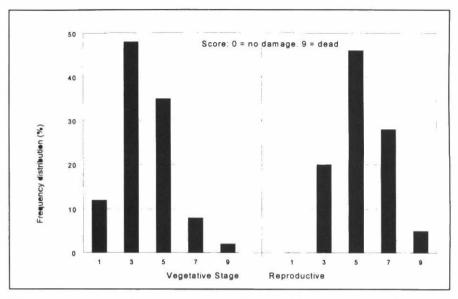


Figure 5. Frequency distribution of scores for drought resistance in 200 selections after 4 weeks of exposure to drought during the vegetative and reproductive stages, 1992

#### Evaluation for resistance to major stresses

#### Drought resistance

Rice grown in upland and hydromorphic conditions in the humid forest, forest-savanna transition and savanna zones of West Africa may be subjected to varying degrees and durations of drought stress during the crop cycle. This results in yield reduction and sometimes total crop failure. To improve yield stability, drought resistance is therefore required in rice varieties for upland and hydromorphic environments.

O. glaberrima and O. sativa accessions varied in their response to drought stress imposed during the vegetative stage. There was considerable variation in visual scores for seedling vigor, tillering ability, leaf tip burn and drought resistance among the accessions (IRRI, 1988). The resistant check, OS6, gave an average rating of 3 for the visual drought score. There were 32 O. glaberrima and 30 O. sativa accessions with visual scores of 0 or 1 and these were rated as highly resistant. Two hundred and seventeen O. glaberrima and 18 O. sativa accessions with visual scores of 2 or

3 were rated as moderately resistant. The remaining entries exhibited low resistance.

For comparative purposes, 15 *O. sativa* varieties currently recommended by national agricultural research systems (NARS) for cultivation in West Africa or recently developed for drought resistance were included in the screening. These varieties were all classified as moderately resistant or susceptible, with visual scores of 3. Several widely cultivated local varieties, including Lac 23, ROK 6, Moroberekan and M22, were also classified as moderately resistant.

The results for seedling vigor, tillering ability and leaf tip burn showed that most upland improved and traditional rice varieties of *O. sativa* origin have low or moderate drought resistance. The finding that *O. glaberrima* accessions had higher resistance than some of the best improved *O. sativa* varieties indicates the potential for producing improved varieties with better resistance through hybridization, using *O. glaberrima* as a parent.

Figure 5 shows the distribution of drought resistance scores in the vegetative and reproductive stages. A positive correlation between resistance scores at the two stages (r = 0.189, P < 0.001) was obtained although some varieties resistant in the vegetative stage were susceptible in the reproductive stage. There was a highly significant correlation between resistance scores, after 4 weeks of moisture stress at the reproductive stage, and seedling vigor (r = 0.22, P < 0.001), and a negative correlation (r = 0.391, P < 0.001) between resistance in the reproductive stage and leaf-rolling ability. The results indicate that varieties with good resistance at the reproductive stage had good seedling vigor and high leaf-rolling ability during stress in the vegetative stage.

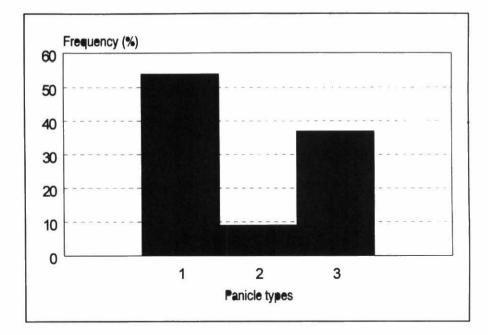


Figure 6. Frequency distribution of panicle types of 300 F2 individuals from the cross between WAB 56-104 (*O.sativa*) and CG 14 (*O.glaberrima*); 1. Progenies with primary and secondary branches as in the *O. sativa* parent. 2. Progenies with primarey branches and very few secondary branches towards the panicle base. These are intermediates between *O.sativa* and *O. glaberrima* panicle types. 3. Progenies with mainly primary branches on the panicle as in the *O.glaberrima* parent.

#### Blast disease resistance

Leaf and neck rice blast occurs in most areas where rice is grown in West Africa. Of the 123 *O. glaberrima* entries screened, 14 varieties with scores of 3 or less were found to be resistant, 46 with scores between 3.1 and 5 were moderately resistant and 63 susceptible. A number of the resistant *O. glaberrima* entries, including TOG 6701, TOG 7264, TOG 6690 and TOG 7386, had symptoms similar to those in *O. sativa* landraces, OS6, Moroberekan, Lac 23 and 63-83, classed as having durable resistance to blast (Bidaux, 1978). Durable resistance to blast was recognized by the slow progress of the disease which may be due to several factors such as reduction in infection efficiency, sporulation capacity, lesion size and infection period, and prolonged incubation period (Ahn and Ou, 1982, 1984., Villareal *et al.*, 1981) In extensive studies conducted by Bidaux in Côte d'Ivoire, it was concluded that many traditional *O. sativa* 

upland rice varieties have a non-specific and polygenic resistance similar to horizontal or durable resistance (Bidaux, 1978). This resistance was different from the race-specific monogenic or vertical resistance found in most improved *O. sativa* materials.

# Interspecific hybridization

Interspecific hybridization for plant improvement is complicated by the presence of incompatibility barriers, such as hybrid inviability, which hinder heterogenetic recombination (Brar and Khush, 1986). For example, since 1992, 48 crosses were made involving O. glaberrima and O. sativa. Seven of these crosses were successful as hybrids were obtained and  $BC_1$  and  $BC_2$  progenies were produced by successive backcrossing to the O. sativa parents. After two backcrosses,  $F_1$  to  $BC_2F_3$  progenies were monitored. Crosses involving O. sativa and O. glaberrima had 90 to 95% spikelet sterility in the F<sub>1</sub> generation. Therefore, only 5% seed set from the 6783 spikelets pollinated was obtained in F<sub>1</sub> hybrids from which the following O. glaberrima accessions were used as one of the parents: CG14, CG20, T2, YG230 and YG170. The F<sub>1</sub> hybrids more closely resembled the O. glaberrima parents than the O. sativa parents indicating dominance of the O. glaberrima traits. The hybrids had rapid vegetative growth, intermediate ligule, long awns, purple apiculus and tillered profusely. Fertility was retained and improved to between 86 and 98% after two backcrossings. Figure 6 shows that the distribution of panicle types of the  $BC_{2}F_{1}$  genotypes was strongly skewed towards the parental types. This shift was considerably accelerated towards the O. sativa panicle type with primary and secondary branches when backcrossing was continued with the O. sativa recurrent parent. Although true intermediates with ligule size of 15 to 25 mm compared to 40 to 46 mm in O. sativa and 4 to 6 mm in O. glaberrima were generally rare, their occurrence in some populations, such as WAB449 and WAB450, was as high as 30%. Seed shattering in the intermediates was high and occurred as early as the milk stage in some populations.

The fertility of true intermediates was generally low, suggesting a gradual elimination of heterozygotes and thus a gradual reduction of recombination. However, for a few crosses, such as WAB449 and WAB450, fertility increased with each generation suggesting that some amount of *O. glaberrima* genetic material had been introgressed into the new plants. Desirable morphological traits introgressed from *O. glaberrima* into 74 populations selected from WAB450 included early maturity of between 75 to 100 days, rapid seedling growth and high tillering. Fixed populations with these desirable traits have been selected for yield trials. Most progenies, including some of the intermediates, were characterized by a weak culm as they lodged early and easily like the *O. glaberrima* parents. However, several intermediate types also had a stiff culm, enabling them to resist lodging.

In the immediate future we will focus on identifying useful variability in the *O. sativa* and *O. glaberrima* species for tolerance/resistance to major biotic and abiotic stresses such as blast, drought and acidity and in producing additional hybrids between the breeding lines and *O. glaberrima*, particularly those possessing useful variability for resistance to abiotic stresses. Introgressed segments of chromozomes from *O. glaberrima* to *O. sativa* will be tagged with molecular markers.

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# Using Backcrossing and Doubled Haploid Breeding to Generate Weed Competitive Rices from *O. sativa* L. X *O. glaberrima* Steud. Geneplools

# Monty P. Jones<sup>s\*</sup>, Michael Dingkuhn<sup>+</sup>, Gabriel K. Aluko and Mande Semon

Principal Breeder<sup>\*</sup>, Team Leader/Systems Analyst<sup>\*</sup> and Research Assistants West Africa Rice Development Association, 01 BP 2551, Bouaké, Côte d'Ivoire.

# Abstract

Weed competition is the most important yield-reducing factor in upland, hydromorphic and rainfed lowland rice environments in Africa. Many of the commonly grown tropical *japonica* and *indica* varieties have a high yield potential, but they compete poorly with weeds. By contrast, the indigenous cultivated *Oryza glaberrima* Steud. landraces are highly competitive due to high tillering ability, vigor and leaf area during vegetative growth. But their yield potential is low because of *O. glaberrima's* specific panicle type and tendency to lodge. High specific leaf area (SLA), greater initial assimilate partitioning to leaves, high leaf N concentration and higher tillering rates contribute to the rapid vegetative growth of *O. glaberrima* cultivars.

The competitive abilities of these cultivars results in greater yield stability, an important factor on farmers' fields. Rice breeders at WARDA recently developed stable and fertile progenies from O. sativa and O. glaberrima crosses through backcrossing and doubled haploid breeding (DHB). Backcrossing with the O. sativa parents increased fertility and helped combine the O. sativa and O. glaberrima features. The use of DHB to generate large proportions of doubled haploids from *japonica x O*. glaberrima BC<sub>3</sub>F<sub>1</sub> hybrids, helped to overcome constraints associated with the conventional breeding of these species, such as : (1) slow fixations of the lines, (2) frequent partial sterility of the progenies and (3) low recovery of useful recombinants. The rapid fixation through DHB has the additional advantage of retaining genes which would otherwise be lost through conventional selection. The frequency of callusing anthers of four hybrids ranged from 29 to 48% while the regenerating capacities of the microspore-derived calli varied from 1.2 to 22%. The number of calli forming green plants ranged from 0.37 to 7.2.

Most of the anther cultured-derived lines were homogenous. Nevertheless, 3% of the lines appeared unstable over the selfing generations, thus suggesting a possible resurgence of recessive heterozygous mutation. While the *O. glaberrima* parent CG14 had two to three times the leaf area index (LAI), and 1.5 to 2 times the tiller number of WAB 56-104, the *O. sativa* parent, some progenies had intermediate SLA, LAI, leaf N content and assimilate partitioning patterns. They combine the superior vigor of CG14 with the panicle structure and study stems of WAB 56-104. Some progenies achieved a high SLA and therefore early groundcover (*O. glaberrima* type) during the vegetative phase followed by a low SLA (*O. sativa* type) during the reproductive phase, resulting in yields of up to 5 tons ha<sup>-1</sup> under improved management conditions and 3 tons ha<sup>-1</sup> in traditional low input systems.

#### Introduction

Crop improvement scientists at the West Africa Rice Development Association (WARDA) systematically evaluate germplasm from both within and outside Africa, generate breeding materials, select superior lines and test early and advanced breeding materials onstation and on-farm. WARDA's strategy for upland rice improvement is to combine specific agro-ecological adaptations of local upland varieties with the yield potential of introductions.

Targeting the numerous constraints limiting yields of upland rice (drought, insects and diseases, weeds and low input cultural practices), conventional breeding programs have been active over more than three decades to improve the performance of upland rice varieties (Takeoka, 1965; Bidaux, 1978; Kofi, 1980; and Arradeau, 1992) in West Africa. The gains from this research have been limited, in part because Oryza sativa L., the most widely cultivated rice species in West Africa, has limited resistance to many of the stresses that affect upland rice in the region (WARDA, 1992). Although advanced selections from these intra-specific breeding programs mostly out perform farmers' traditional varieties on-station under relatively high input conditions, they perform poorly when cultivated under the low input systems which dominate upland farming in West Africa. In an effort to break this pattern, WARDA initiated interspecific hybridization program in 1992 to introgress important traits between O. sativa and O. glaberrima thereby, increasing the genetic variability within each type.

Interspecific hybridization for plant improvement is complicated by incompatibility barriers. These include hybrid inviability which hinder heterogenetic recombination (Brar and Khush, 1986; Jena and Khush, 1990). Past efforts to transfer useful genes from *O. glaberrima* into *O. sativa* were also constrained by sterility in the early progenies of crosses (Second, 1984). Despite these barriers, natural gene flows between *O. glaberrima* and *O. sativa* or *O. longistaminata* have been reported and analysed by several workers (Takoka, 1965; Second, 1984 and De Kochko, 1987), who recommended the exploration of *O. glaberrima* as a source of new variability for upland rice improvement.

Rice scientists have been aware of Oryza glaberrima for many decades (Carpenter, 1978; Brar and Khush, 1986; and De Kochko, 1987). Among eight other species indigenous to Africa (Table 1), O. glaberrima is known to have been selected and cultivated in parts of West Africa more than 3500 years ago (Jacquot, 1977; Bidaux, 1978; and Carpenter, 1978). Because O. glaberrima has survived without help, or interference of man, the species has developed adaptive or protective mechanisms for resisting major biotic and abiotic stresses. O. glaberrima represents a reservoir of useful genes for resistance to diseases and insect pests as well as tolerance to acid soils, iron toxicity, drought, unfavourable temperatures and excess water (Table 2). This paper presents procedures for developing fertile interspecific progenies from O. sativa and O. glaberrima crosses as well as results of preliminary evaluation of yield potential and other agronomic traits under upland conditions.

# Materials and methods

Morpho-agronomic characterization of O. glaberrima and O. sativa accessions

Among the working collection kept by WARDA, 1,721 accessions of improved (316) and traditional (275) *O. sativa* and *O. glaberrima* (1130) were evaluated over two years (1991 and 1992) for a range of morphological and agronomic traits including seedling vigour, growth duration, plant height, panicle per m<sup>2</sup> and grain shape. These characters were measured using the standard evaluation system developed by the International Rice Research Institute (IRRI, 1988).

The trials were carried out at WARDA main research station at M'bé about 15km North of Bouake, located in the Guinea savannah at 5/06'W, 7/52'N with a bimodal rainfall pattern separated by a relatively short dry period. The first humid period occurs in April-June, and the second in September-October. The average annual rainfall and temperature were 1146 mm and 25.15/c respectively (minimum 23.3/c, maximum 27.3/c). The soil is well drained, deep sandy clay to clay with overlying carapace between 80 cm and 120 cm.

Species	2n	Genome	Origin
O. sativa			
(cultivated	24	AA	Asia
O. glaberrima			
(cultivated)	24	A <sup>g</sup> A <sup>g</sup>	West Africa
O. stapfii (weed			
species)	24	A <sup>g</sup> A <sup>g</sup>	West Africa
O. bathii	24	A <sup>g</sup> A <sup>g</sup>	West Africa
O. longistaminata	24	A <sup>1</sup> A <sup>1</sup>	Africa
0. brachyantha	48	FF	East & central
			Africa
O. eichingeri	24	CC	Tropical Africa
	48	BBCC	
O. punctata	24	BB	Tropical Africa
	48	BBCC	
O. schwein	48	BBCCA	Tropical Africa
furthiana			

Table 1. The species of Oryza in Africa

Source : Adapted from Takeoka (1965)

Soil preparation was done by tractor in June of each year. The entries were grown in single plots of 4 by 5 m rows, spaced 25 cm apart. Seeds were dibbled in the rows at 25 cm apart. The compound fertilizer 10-18-18 NPK was applied at the rate of 100 kg/ha. Urea was later applied at the rate of 40 Kg N/ha each at 30 and 60 days after sowing. Pre-emergence application of Ronstar was made at a rate of 4 litres/ha and hand weeding was done later as often as necessary.

Interspecific hybridization

On the basis of the morpho-agronomic characterization, eight *O. glaberrima* parents that had the best combination of traits and interspecific genetic compatibility with the best *O. sativa* upland rice varieties developed by WARDA, were selected for wide hybridization in 1992. Both conventional and doubled haploid breeding were then used to break the sterility barrier.

 Table 2. The species of Oryza in Africa with traits of economic importance

Species	Useful traits
O. sativa	High grain yield
O. glaberrima	Early vegetative vigor, drought tolerance, GLH resistance
O. barthii •	Drought avoidance, bacteria blight resistance
O. longistaminata	High pollen productions, Long stigma, drought tolerance, source of CMS.
O. brachyantha	
O. eichingeri	Tolerance to laterite soils, yellow stemborer, leaf folder and whorl maggot resistance
O. punctata	Shade tolerance, BPH, GLH and WBPH resistance
1	BPH resistance

Source: Adapted from Brar, P. S & G.S. Khush, 1986.

### **Conventional Breeding**

Forty eight crosses were carried out at WARDA between *O. sativa* and *O. glaberrima* lines. Seeds of seven crosses that produced a few fertile grains were carefully collected and the  $F_1$  progenies produced were successively backcrossed to the *O. sativa* parents. After two backcrosses, individuals from these  $F_2$  generations then went through pedigree selection, where seeds from the most promising

individuals were selected and grown to produce the  $F_3$  generation. Selection continued until varietal traits were fixed after an additional six generations.

#### Doubled haploid breeding

On a parallel track, anther culture techniques were used to increase the success of obtaining fertile plants and to shorten the number of cycles required for the fixation of particular traits. Some of the F<sub>2</sub> progenies resulting from the crosses mentioned above were passed through anther culture. Once the F, plants had grown and were in the booting stage the tillers were removed and taken into the laboratory. Anthers were removed from the spikelets, and added to a modified N<sub>6</sub> growth medium (Chu, et al., 1975) containing 0.5mg l<sup>-1</sup> of Kinetin, 2mg l<sup>-1</sup> of 2,4D, 5% maltose and 150ml l<sup>-1</sup> of coconut milk. Calli emerged from the anthers three to eight weeks after plating. The calli were transferred to an MS medium (Murashige and Skoog, 1962) when they were about 2 mm in size. After 3-4 weeks, they developed into small plantlets. The green plantlets were then transferred into an MS medium with half the concentration of chemicals to which is added multi effective triazole (MET), a hardening chemical. This strengthened the plants allowing them to be transferred to grow in the soil.

# Field evaluation of promising interspecific progenies:

Two field experiments were conducted in 1995 at WARDA's Mbé experimental station to study the fertility, genetic stability and agronomic traits of newly fixed *O. sativa* x *O. glaberrima* progenies. In the first experiment, a mix of twenty-six genotypes derived from conventional breeding and anther culture were evaluated in a replicated yield trial under high and low input levels of management on an exhausted upland soil that had been under short fallow (3 years) after three years of rice cropping. The entries included three *O. sativa* cultivars, IDSA6, WABC165 and WAB56 104; the *O. glaberrima* parent, CG14; and 22 newly fixed progenies. For high input treatment, the land was disc-plowed and harrowed to facilitate good soil tilt. Whereas for the low input, it was slashed and most of

the trees were removed. Minimum destumping was practiced and the soil was scarified with a hand hoe.

The test entries were planted in 2.5m x 5m plots in a randomized complete block design with four replications. The seeds were hand-drilled uniformly along the rows made at a uniform spacing of 25cm apart and were covered by passing a hand rake parallel to the direction of the furrows. Fertilizer was applied according to levels of management. For the high input, a basal application of 200 Kg/ha NPK 10-18-18 was followed by 40 Kg/ha of N (urea) each at 30 and 60 days after sowing (DAS). The low input treatment received only 20 Kg/ha each of N (urea) at 23 and 43 DAS.

Ronstar was applied as a pre-emergence herbicide under highinput at 4 liters per ha a day after sowing, and later hand weeded twice to check weed growth in the experiment. For the low input treatment, hand weeding was done at 21 and 42 days after sowing. Data collected included seedling vigor at 30 DAS, grain yield per plot and grain moisture content.

In the second experiment, four newly fixed lines, WAB450-I-B-P-160-HB, WAB450-24-3-2-P18-HB, WAB450-2-3-P33-HB and WAB450-I-B-P-31-HB, and their parents, WAB56 104 and CG 14, were evaluated in a preliminary yield trial conducted in a fertile upland field that had been under long (fallow > 6 years). The trial was conducted in a randomized complete block design of six replications with nitrogen applied at five rates between zero and 120 Kg N/ha.

# **Results and discussion**

#### Diversity of African Rice Germplasm

Success in crop improvement depends on the diversity of the genetic resources for genes responsible for various traits. We found relatively wider variation for some traits such as growth duration in the *O. glaberrima* than in the traditional and improved *O. sativa* varieties (Table 3). Thus revealing the valuable genepools in the *O. glaberrima* germplasm that can be utilized. The *O. glaberrima* varieties generally possess many traits that make them well adapted to traditionally practised shifting cultivation. For example, most *O. glaberrima* varieties had rapid and profuse vegetative growth with

droopy lower leaves which helps to suppress weeds by quickly outgrowing and shading them (Jennings et al., 1979). This result is consistent with previous reports obtained with morphological markers in rice them (Jennings et al., 1979; Koffi, 1980). This character is particularly important for West African rice production since weeding is usually done manually, and effective weed removal may be impossible due to labor shortages or competition with other activities (WARDA, 1992; 1993). Some O. glaberrima were found to have more tillers and panicles than O. sativa. However many of the O. glaberrima accessions lack secondary branching on the panicles. This limits the number of spikelets and therefore the number of grains per panicle. As a result, most O. glaberrima produce few grains per panicle, generally between only 75 and 150 (Takeoka, 1965: WARDA, 1994). This compares to O. sativa which, because it has secondary branches, can respond to improved fertility producing up to 250 or more grains per panicle. Hence, O. glaberrima has many undesirable traits which result in low yield potential. Grain shattering, and long dormancy are some of the problems. In large part, it is due to these negative traits that farmers are rapidly replacing O. glaberrima by O. sativa varieties.

#### Interspecific hybrids

# Conventional breeding

Out of 48 wide crosses, seven were successful and produced few fertile seeds (>5%) in the F1 generation. The resulting  $F_1$  progenies were observed to have high sterility(> 95%). Similar results were obtained by Jena and Khush (1990) who reported 1.3% seed set when trying to introgress genes from *O. officinalis* to *O. sativa*. However, up to 5% seed fertility was obtained in  $F_1$  hybrids for which the following accessions had been used as the *O. glaberrima* parents: CG14, CG20, T2, YG230 and YG170.

The  $F_1$  plants showed a number of traits similar to their *O.* glaberrima parents, including the lack of secondary branching of panicle, and early seedling vigor. Fertility was retained and improved to between 30-65% after two backcrossings. The backcross plants were morphologically diverse.

Character	O.glaberrima	O. sativa (traditional)	O. sativa (Improved)
Seedling Vigor (DAS)*	7-1	7-1	7-1
Plant height (cm)	65-138	80-182	65-138
Growth Duration (days)	75-160	105-150	95-160
Number of panicles/m <sup>2</sup>	46-470	6-476	49-477
Grain length to width ratio	2-5	2-5	2-5
Number of grain per panicle	75-150	99-260	140-280
1000 Kernel weight(g)	6-38	6-38	13-39

**Table 3:** Range of variation on some selected characters of *O. sativa* and *O. glaberrima* recently characterized for 44 traits at WARDA Mbé station.

Traits absent in either parents or in earlier generations, such as purple leaf sheath, awn and apiculus, were observed among the backcross populations, presumably resulting from new gene combination. Backcrossing with the *O. sativa* parents not only increased fertility, but also helped to combine the *O. sativa* and *O.* glaberrima features producing the second or  $F_2$  generation of plants. The  $F_2$  population divided itself into 2 or 3 distinct morphological groups. The majority of the population resembled either one or the other parent, and with spikelets fertility higher than 70 %. The morphological similarity to a parent suggests that only a limited amount of features of the other parent was introgressed.

Although true intermediates between *O. sativa* and *O. glaberrima* were generally rare, their occurrence in some populations, such as WAB449 (WAB56-104 x T2) and WAB450 (WAB56-104 x CG14), was as high as 30%. Some of the intermediates have a ligule length of 15-25 mm, compared to about 6 mm in *O. glaberrima* and 45 mm in *O. sativa*. Various degrees of awning and shades of purple apiculus were also observed in the intermediates. Most importantly, some intermediates combine the high yield potential of *O. sativa*, a result of high spikelet number caused by secondary branches on the panicle, with many useful traits

of *O. glaberrima*. The latter include rapid vegetative growth with droopy lower leaves enabling them to compete successfully against weeds, high tillering, short duration, with plants maturing between 75 to 100 days, and superior grain quality. This results are consistent with previous studies (Jacquot, 1977; Koffi, 1980)

The seed fertility of true intermediates was generally low, around 40% in the  $F_2$  after two backcrossings, suggesting a gradual elimination of heterozygotes and thus a gradual reduction of recombination. However, in the two crosses mentioned above, fertility did increase with each generation suggesting that significant amounts of *O. glaberrima* genes had been introgressed into the new plants.

Table 4. Anther culturability of four distinct hybrids

Hybrids	Type of cross	Anther plated	Callusing anthers		Calli regenerating green plants <sup>a</sup>		Calli regenerating albino plants	
		No.	%	No.	%	No.	%	
WAB 450	JxG	3500	1666	47.6	37	2.2	56	3.4
WAB 515	JxI	2500	893	40.6	48	5.4	65	7.3
WAB 585	JxJ	500	161	32.2	36	22.4	53	32.9
WAB 668	IxJ	1900	555	29.2	07	1.2	23	4.1
						Victoria Canada Canada		1

<sup>a</sup>Number of green shoot forming-calli per 100 anthers inoculated J=Japonica I=Indica G=Glaberrima

In the repeated selection process within the intermediates, desirable traits were retained and improved, and fertility rates were increased in each subsequent generation, achieving up to 100% in the  $F_7$  generation. Seed shattering has been greatly reduced, and the selection of plants with thick culms has solved the problem of lodging.

Table 5: Frequency	of	spontaneous	recovery	of	haploid,	dihaploid	and
polyploid ty	pe	anther cultur	re plants				

Hybrids	Type of cross	% Survi	Total of plant	Haploid plants		Diploid plants		Polyploid	
				No.	%	No.	%	No.	%
WAB 450	JxG	96	37	17	46	11	30	9	24
WAB 515	JxI	96	48	22	45	10	21	16	33
WAB 585	JxJ	95	36	9	25	27	75	0	0
WAB 668	IxJ	96	7	3	43	2	28	2	28
				A	40		39		22

J=Japonica

I=Indica

# G=Glaberrima

#### Doubled haploid lines

Anther culture was used to generate large populations of doubled haploids (DH) from japonica x *O. glaberrima*  $BC_2F_1$  populations and to overcome some general constraints to wide crosses such as: (1) slow fixation of the lines, (2) frequent partial sterility of the progenies and (3) low recovery of useful recombinants. Also, japonica x *O. glaberrima* populations are generally less amenable to androgenesis than are intrajaponica crosses, producing more albino, haploid and partially sterile diploid plants.

Anther culturability of four hybrids is given in Table 4. The frequency of callusing anthers ranged from 2902 to 47.6% while the regenerating capacities of the micros-pore derived calli varied from 1.2 to 22.4%. The number of calli forming green plants ranged from 0.37 to 7.2. The modified N6 medium resulted in the production of a large number of fast growing , white, compact and lobed calli with fairly high regenerability.

In most cases, several plantlets were regenerated from different sectors of a single callus. Twenty-two percent of the plantlets developed through anther culture were green, and these were retained for use in the next stage of the process. The green plants developed spontaneously into three distinct groups, depending on the number of chromosomes present. Fifty-two percent of the plants developed into haploids, 41% into double haploids, while 7% came out as polyploids having chromosome levels beyond the normal diploid state (Table 5). Both haploid and diploid plantlets were obtained from the same callus indicating the possibility of chromosome duplication during callus growth, or during meristem differentiation.

Fifteen percent of the spontaneously doubled haploid lines displayed only partial fertility. Sterility might have been caused by aneuploidy of the regenerated plants or fixation of sterility genes. However, several genetically stable  $F_1$  and  $F_2$  anther culture derived doubled haploid populations had 96 to 100 percent fertility. This technology is good in that it ensures the retention of genes which otherwise might have been lost through conventional selection methods.

# Field performance of interspecific progenies

The successful introgression of genes from *O. glaberrima* into *O. sativa* has not only resulted in a significant broadening of the genetic base of cultivated rice, it has also produced new plant prototypes with extremely interesting agronomic traits. In the first experiment, the interspecific progenies responded to fertilizer input levels in the same way as the *O. sativa* cultivars. Under high input conditions, 11 progenies were the top yielders, with average grain yields ranging from 3.4 to 3.8 tha<sup>-1</sup> (Fig 1). A new progeny, WAB450-24-2-3-P33-HB, produced the highest plot grain yield of 4.7 t ha<sup>-1</sup>. Under low input management, 16 of the newly fixed progeny outyielded the *O. sativa* check cultivar and had grain yields similar to or better than the *O. glaberrima*, indicating their adaptation to low input conditions.

In the second experiment, at only 80 kg nitrogen per hectare, the new upland plant type produced average yields of up to 5.6 tons per hectare, which is significantly greater than both of its parents sown in the same trial. And under zero nitrogen, the new plant type produced a yield of up to 3.9 tons per hectare.

The high transgressive yields observed in the progenies in both trials resulted in part from panicles with secondary branches inherited from the *O. sativa* parent giving them the ability to respond

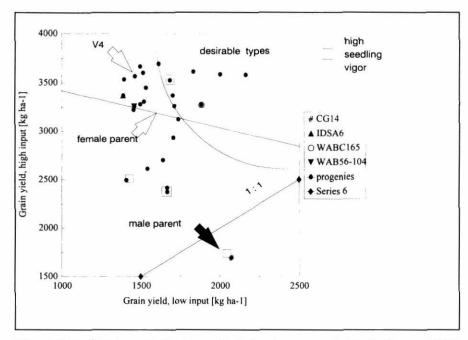


Figure 1. Performance of *O.sativa* x *O.glaberrima* progenies under low and high input levels.

to added inputs by producing a greater number of spikelets yielding up to 400 or more grains per panicle. In addition, most progeny showed transgressive segregation with substantially larger panicles and number of spikelets per panicle than either of their parents. For example, all four progenies had more secondary branches than either of the parents (Fig 2).

In tests conducted during 1995, seedling vigor ratings varied between 1 (extra vigorous) in the *O. glaberrima* and 5 (normal seedlings) in the *O. sativa* parent. Ten progenies scored 1 or 2 while 11 scored 2.5 (Fig 3). Early vegetative vigor showed the ability of the new plant types to rapidly establish ground cover filling the space between plants and rows.

Table 6. The plant type concept (Preliminary)

The projected plant type combines good resource/input responsiveness with high yield stability under a broad range of upland and hydromorphic conditions. It has the following properties:

Vegetative growth phase: Weed competitiveness through	<ul> <li>+ superior growth vigor</li> <li>+ rapid N uptake</li> <li>+ drought resistance</li> <li>+ rapid ground cover through:</li> <li>+ thin leaves (high SLA)</li> <li>+ droopy leaves</li> <li>+ high partitioning of C</li> <li>+ high tillering</li> </ul>
<b>Reproductive/ripening growth phases:</b> High assimilate sink capacity through	<ul><li>+ secondary panicle branches</li><li>+ high tiller (panicle) number</li></ul>
High assimilate source capacity through	<ul> <li>+ thick dark green leaves</li> <li>+ erect leaves</li> <li>+ stiff culms</li> </ul>
High harvest index through	<ul> <li>+ intermediate plant height</li> <li>+ big panicles</li> <li>+ lodging resistance</li> <li>+ no grain shattering</li> </ul>
General robustness: Resistance to biotic stresses, such as	<ul> <li>+ weed competition</li> <li>+ blast disease</li> <li>+ parasitic nematodes</li> </ul>
Resistance to abiotic stresses, such as	<ul><li>+ soil acidity and P deficiency</li><li>+ drought</li></ul>

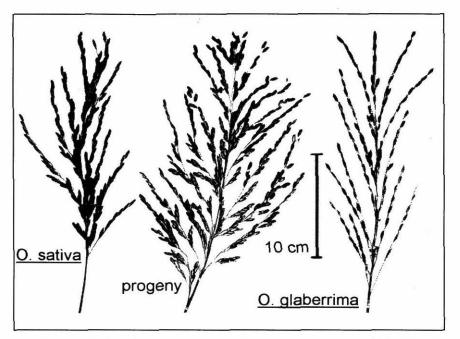


Figure 2. Panicle types observed for the O. sativa line WAB56-104 the O. glaberrima cultiva CG14 and an interspecific progeny WAB450-24-3-2-P186HB.

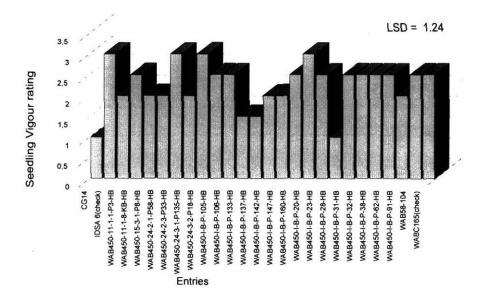


Figure 3. Seedling vigor of *O.sativa*, *O.glaberrima* and interspecific lines tested under low-input management, (1 = high vigor, 9 = low vigor).

The O. glaberrima parent, CG14, had a two fold (zero N) to three fold (80 kg N) higher leaf area index (LAI) than the O. sativa parent, WAB56-104 (Fig 4). This was associated with faster leaf canopy establishment and N responsiveness. The progenies were intermediate. The differences in LAI could be explained by specific leaf area (SLA), or the leaf surface that was produced with a given amount of dry matter (Fig 5). At any given growth stage and input level, a significant positive correlation across genotypes was found between LAI and SLA. The SLA therefore, was a major determinant of early vigor and ground cover. The progenies partly inherited the O. glaberrima parent's high SLA during early growth, theoretically improving competitiveness with weeds, and from the O. sativa parent, the rapid decrease in SLA towards the reproductive stage, theoretically enabling high leaf photosynthetic rates and high yield capacity (Fig 6).

High initial SLA (thin leaves) and droopy leaves, followed by a rapid decrease in SLA and dark green leaves, were identified as the major components of the new interspecific, weed competitive plant type. We are fine-tuning our plant type concepts to fit specific stress environments and resource situations using modelling tools and indepth characterization of parental and derived materials.

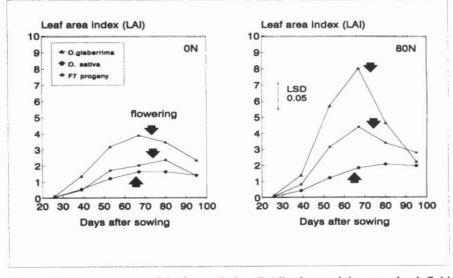


Figure 4. Time courses of leaf area index (LAI) observed in an upland field fertilized with 0 and 80 kg N/ha for interspecific rice progeny (WAB 450-24-3-2-P18-HB) and its parents WAB 56-104 (O.sativa) and CG14 (O.glaberrima)

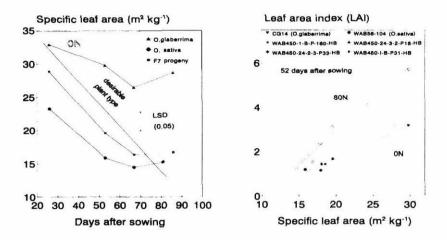


Figure 5. Left:Time courses of specific leaf area (SLA) for an inter-specific rice progeny (WAB450-24-3-2-P18-HB) and its parents WAB56-104 (O.sativa) and CG14 (O.glaberrima). The broken line indicates the ideal SLA for a high yielding, weed competitive plant types. Right: Relationship between leaf area index (LAI) and SLA across inter-specific progenies and their parents, as observed during the late vegetative stage in an upland field fertilized with two nitrogen levels.

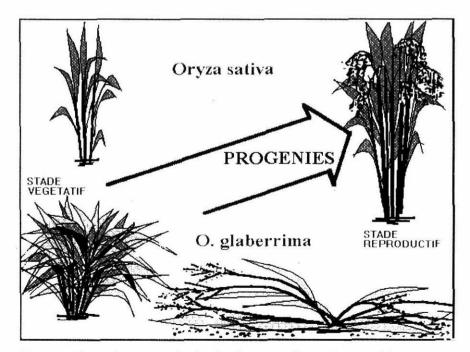


Figure 6. The basic concept for the development of a weed competitive, interspecific rice varieties.

### Conclusion and outlook

The present study provided morpho-physiological evidence for trait introgressions from *O. glaberrima* into an *O. sativa* background. Most of these introgressions are of potentially high adaptive value in a resource-limited production environment, particularly in weed prone upland fields. They include: (1) rapid vegetative growth and leaf area development, at least in part caused by high initial SLA and partitioning of much assimilate to leaves during early growth; and (2) droopy leaves during early growth stages, theoretically resulting in a high extinction coefficient for solar radiation, and thus, high light use efficiency and suppression of weeds. Expressed in combination with agronomically useful traits derived from *O. sativa*, such as large panicles, sturdy stems and erect foliage during reproductive stages, these traits are expected to improve yield stability at a high level of potential yield.

Preliminary studies indicate that weed competitiveness is not the only adaptation that new plant types can draw from *O. glaberrima*. Although we know little about the underlying mechanisms, it is already evident that at least one of the *O. glaberrima* parents in this study, CG14, is highly drought resistant. Many *O. glaberrima* landraces are also highly resistant to blast, rice yellow mottle virus and the African rice gall midge.

A series of studies has been initiated at WARDA to morphophysiologically and genetically characterize these traits, trace introgressions through molecular markers, develop models to compose and test environment specific plant type concepts, and identify compatible donors for the traits constituting the new plant types.

The new plant type concepts, as well as the screening tools that will contribute to their realization, will take into account the dynamic nature of morpho-physiological traits. For example, plants will be screened for a droopy, profuse foliage with high SLA during early growth stages, and erect leaves with much lower SLA during reproductive stages. In other words, the new plant types will resemble *O. glaberrima* during early and *O. sativa* during later growth stages (Fig. 6). Preliminary results indicate that this ambitious objective is indeed feasible.

#### Acknowledgements

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# The Physiological Basis for Developing Low-Management Upland Rice Plant Types

Michael Dingkuhn, David E. Johnson, Monty P. Jones, and A. Sow

West Africa Rice Development Association, 01 BP 2551, Bouaké, Côte d'Ivoire.

### Abstract

Weed competition is the most important yield reducing factor in upland and hydromorphic rice environments in West Africa, where labor resources are typically insufficient for effective weed control. Many of the commonly grown tropical japonica varieties have a high yield potential but compete poorly with weeds. Indigenous *Oryza glaberrima* landraces are highly competitive due to early tillering and vigor, but their yield potential is low because of *O. glaberrima*'s specific panicle type and tendency to lodge. A recent breakthrough at WARDA in generating fertile progeny from *Oryza sativa* x *O. glaberrima* crosses gives rice breeders access to a broader range of germplasm. By combining the *O. sativa* panicle type with the vegetative growth characteristics of *O. glaberrima*, the productivity of weed-prone upland rice systems might be improved.

Two field and one pot experiments during 1995 and 1996 served to characterize growth and yield potential of CG14 (O. glaberrima), WAB56-104 (O. sativa) and their progeny. During the 1995 wet season and the 1996 dry and wet seasons, the lines were seeded in a well drained upland field in Côte d'Ivoire with supplemental sprinkler irrigation. A randomized complete-block design with three replications was used, with cultivar and nitrogen levels as sub-plots. Specific leaf area (SLA), leaf area index (LAI), leaf chlorophyll content (SPAD method) and tiller number were measured at 2-wk intervals until flowering. Grain yield and yield components were measured at maturity. In all environments, CG14 produced two to three times the LAI and tiller numbers as WAB56-104. This was associated with a high SLA and low leaf chlorophyll content. Grain yields of CG14 did not respond to N inputs, although the sink potential did. The difference was mainly caused by grain shattering. The progenies had intermediate LAI, SLA and leaf chlorophyll content, but their tiller numbers, resistance to lodging and yields were similar to WAB56-104. Across lines, LAI and SLA were significantly correlated. This relationship also confirmed for a wider range of rice cultivars. A pot experiment demonstrated that leaf net CO<sub>2</sub> assimilation rates (A<sub>max</sub>) followed a common linear function of areal leaf chlorophyll content across cultivars. The main common cause of differential LAI and  $A_{max}$  appeared to have been genotypic patterns of SLA, which might be an important determinant of vigor and weed competitiveness. First empirical evidence is presented on the effect of SLA on weed competitiveness.

The possibility is discussed of combining, in a single line, high SLA during vegetative growth (for weed competitiveness) with low SLA during the reproductive growth

phase (for high yield potential), to produce an efficient plant type for lowmanagement conditions.

#### Introduction

Average yields in West Africa's two million hectares of upland rice fields are about one ton grain per hectare (WARDA 1993). Considering that the most commonly grown cultivars, such as the tropical-japonica types Moroberekan or OS6, have a yield potential of about four tons, the yield gap is substantial. It reflects the multiple stresses that affect the crop and are largely beyond the control of resource poor farmers. Weed competition is estimated to be the most important yield reducing factor, followed by drought, blast and soil acidity and general soil infertility. Farmers traditionally keep these stresses at the lowest possible level through long periods of bush fallow. More recently, however, population growth has led to a dramatic reduction of fallow periods in many areas, and thereby, aggravated weed pressure and soil infertility (WARDA 1995).

Substantial progress has been made in improving the tolerance of upland rice to soil acidity (e.g., IAC165 from CIAT which is a parent of many of WARDA's acidity tolerant WAB lines [WARDA 1996]), but the weed competitiveness of *O. sativa* upland rices remains poor. By the contrast, the African indigenous *Oryza glaberrima* upland cultivars are highly weed competitive due to early vigor and ground cover (Koffi, 1980; WARDA 1996; Fofana et al., 1995; Johnson, 1995). Many also resist drought, blast and other stresses. But they have been marginalized by the widespread adoption of Asian rice, *O. sativa*, which has a higher yield potential (Carpenter, 1978).

O. glaberrima, of which only cultivated forms are known, is now sporadically grown in the upland for its superior grain quality, and in deep water ecosystem where the species' flood tolerant landraces still have a comparative advantage. The species has never been used systematically in rice breeding because of the sterility barrier with *O. sativa* (Bauharmont et al. 1985), its panicle type which has very few secondary branches, its weak stems that easily lodge, and its tendency to shatter grain as soon as it matures. Consequently, the marginalization of *O. glaberrima* has resulted in a major, but unquantifiable loss of biodiversity.

This paper explores the physiological basis for developing high yielding, strongly weed competitive upland rice plant types drawing from the morpho-physiological characteristics of *O. sativa* and *O. glaberrima*, based on a recent breakthrough in hybridizing the two species (WARDA 1995 & 1996; Jones et al., 1997). The focus was on the physiological characterization of selected parent and progeny materials with respect to growth, yield and photosynthetic potential under differential N resources and nonlimiting water resources; and on physiological traits that might determine weed competitiveness. In conjunction with ongoing experiments on drought resistance, this study will provide the information needed to define environment specific 'low management' plant types as guidance to breeders.

# Materials and methods

Growth and yield analyses of four O. sativa (upland-adapted improved tropical japonica) x O. glaberrima (upland-adapted landrace from Senegal) F7 progenies and their parents were conducted during the 1995 wet season on WARDA's research station at Mbe (7° 52' N, 5° 6' W; 300 m alt.) in Ivory Coast. The male parent material was CG14 (O. glaberrima) (V1) and the female parent, WAB56-104 (O. sativa) (V2). The progenies were WAB450-1-B-P-160-HB (V3), WAB450-24-3-2-P18-HB (V4), WAB450-24-2-3-P33-HB (V5) and WAB450-1-B-P31-HB (V6). These lines had previously been selected for detailed characterization from breeding trials on the basis of phenotypic acceptability and yield, but not the physiological parameters discussed here. During the 1996 dry and wet seasons, the experiment was repeated with the same parent materials and one of the progeny, V4. Seed was sown on 25 June (wet season 1995), 19 December 1995 (dry season 1996) and 2 July 1996 (wet season 1996). For the dry season 1996 experiment, however, only data on specific leaf area (SLA) are reported here, which is probably the only parameter that was not affected by the

damage caused by rabbits during that season.

The materials were grown on a well-drained alfisol following one crop of maize and six years of bush fallow, using a two-factorial randomized complete-block design with three replications. The factors were cultivars and nitrogen levels (0, 40, 80, and 120 kg N/ha as urea in the WS 1995; 0 and 80 kg N/ha in the DS and WS 1996), half of which was applied basally and the other half, topdressed 40 days after seeding (DAS). To all plots, 100 kg P/ha as triple superphosphate and 50 kg K ha<sup>-1</sup> as KCl were applied basally. The crop was not limited by moisture due to supplementary boom irrigation (2 to 3 times a weeks to restore soil moisture to field capacity). Plots measured 3 x 5 m, including a yield area measuring 2 m x 2.5 m. Weather data for the two wet seasons are presented in Table 1.

Dry seed was dibbled at a rate of three seeds per hill with a spacing of 0.25 m x 0.25 m. Seedlings emerged about 5 days later. After establishment, the crop was thinned to two plants per hill. At 14-day intervals, plant height, tiller number, the dry weight of bulk green laminae, dead laminae, stems and panicles, and leaf area index (LAI) were destructively determined using four hills. On the same dates, specific leaf area (SLA) was measured on randomly sampled [exception: sampling of flag leaves at flowering], fully expanded, healthy laminae, based on in-situ area measurement with a LI-3000 (Li-Cor, Lincoln, Nebraska) leaf area meter and subsequent dry weight measurement. The areal chlorophyll content of the same leaves was measured with a SPAD chlorophyll meter (Minolta, Tokyo). SPAD measurements give a unitless but highly reproducible parameter that is linearly correlated with areal leaf nitrogen content (Peng et al. 1993). The product of the SPAD parameter and green LAI gives a relative estimate of N present in the leaf canopy on a ground area basis.

At maturity, grain yield and yield components were measured as described by Dingkuhn and Le Gal (1996). In the 1995 wet season experiment, we also counted the number of primary, secondary and tertiary branches and estimated the degree of shattering by counting the number of attached and missing spikelets for a random sample of 10 panicles per plot at maturity. Across treatments and within lines, total spikelet numbers were regressed against the DW growth observed during the reproductive growth phase. **Table 1.** Weather data recorded at the experimental site, expressed for meteorological decades as means of daily values (temperature, solar radiation [Rs], evaporation from Class-A pan [E(pan)], and wind speed) or cumulative values (rainfall). Mbé, Ivory Coast

Month	Decade	Temper (°C)	rature	(N	Rs 1J m <sup>-2</sup> d <sup>-1</sup> )	E(pan) (mm)	Rain (mm)	Wind (m s <sup>-1</sup> )
Wet sease	on 1995							
		mean	min	max				
June	1	25.2	22.3	29.3	18.4	4.95	49.9	0.99
	2	25.7	22.5	29.8	16.9	4.09	13.8	1.27
	3	25.3	22.5	29.2	16.4	4.27	13.5	1.25
July	1	24.5	21.8	28.0	14.0	3.53	2.6	0.87
	2	24.3	22.0	27.7	17.5	2.98	25.2	0.83
	3	24.4	22.3	27.2	15.8	3.48	10.0	1.27
August	1	24.2	22.0	27.3	13.0	2.81	24.4	1.00
-	2	24.2	21.0	29.1	15.6	2.59	128.7	0.11
	3	24.6	21.6	29.3	15.2	2.97	28.0	0.18
Septembe	er 1	24.9	21.7	29.8	16.4	3.42	102.0	0.11
	2	24.5	21.3	29.4	17.7	3.08	74.2	0.22
	3	24.1	21.1	29.3	16.3	3.26	19.9	0.25
October	1	24.6	21.2	31.0	16.1	3.72	27.0	0.81
	2	24.9	20.9	32.0	15.5	3.73	22.0	0.65
	3	24.9	20.9	32.3	16.3	3.40	23.0	0.62
Wet seas		26.5	20 4	22.6	15.5	3.22	44.2	0.89
June	1	26.5	22.4	33.6	15.5	3.22 4.04	44.2 60.2	0.89
	2 3	27.4 26.3	22.8 22.6	34.6 32.9	17.7 14.7	4.04 3.68	70.8	1.05
Inly		26.3	22.6	32.9	14.7	3.08	43.0	0.99
July	1	26.2	22.4	32.8	15.1	3.14		1.08
	2 3	26.0	22.4	32.8	14.9	2.83	38.8 0.40	1.08
August	3 1	25.5	22.1	30.5	12.0	2.63	0.40 87.9	0.99
August		25.7	22.0	30.9	12.7	3.00	87.9 45.1	0.99
	2 3	25.0	22.1	30.4	13.5	2.93	45.1 51.8	1.10
Septemb		25.5	22.9	30.7	12.6	2.93	51.8	1.03
Septemb	2	25.5	22.3	31.5	12.0	2.42	3.8	1.05
	2 3	25.6	22.4	31.9	12.6	2.30	3.8 79.9	0.74
October	1	25.0	22.0	32.6	12.0	2.27	79.9	0.74
October	2	25.9	21.8	32.8	13.8	2.90	40.1	0.73
	2	23.7	21.4	52.0	14.7	2.09	40.1	0.05

The slope of this relationship is commonly considered a genotypic constant and is used to predict yield potential (Kropff et al., 1994). The reproductive phase was calculated according Penning de Vries et al. (1989) as a fraction of the thermal time (degree-days) from germination to flowering, with germination occurring at stage 0, flowering at stage 1.0, and panicle development from stages 0.65 to 1.0.

Two additional experiments were conducted to test hypotheses derived from the main experiment. The first served to validate the relationship between LAI and SLA for a wider range of cultivars under irrigated lowland conditions. Twelve genetically diverse lines ( O. glaberrima: CG14 and IG10; O. sativa lowland-adapted indicas: Suakoko 8 and Bouake 189; O. sativa upland-adapted tropical japonicas: WAB56-104, Moroberekan and OS6; O. sativa upland/lowland adapted tropical japonicas: IDSA6 and Azucena; interspecific progenies: V4, WAB450-I-B-P-122-1-1 and WAB450-I-B-P-26-1-1) were sown on 22 June 1996 in a seedbed nursery and transplanted into large seed multiplication plots (irrigated paddy fields) at 20 DAS. From each plot (cultivar), three 6-hill subplots were randomly selected to measure SLA and LAI. Fertilizer rates were 40 kg N.ha (urea), 20 kg P/ha (triple superphosphate) and 20 kg K/ha (KCl). At maximum tillering (51 DAS) and flowering stages. LAI was measured by collecting the leaves from 6 hills per sample and passing them through a conveyor-belt type leaf area meter (LAI-3000; Li-Cor, Lincoln, Nebraska). SLA was measured on the same sample by determining the dry weight as well.

The second experiment, which aimed at determining the maximum leaf CO<sub>2</sub> exchange rates of leaves having different SLA and chlorophyll content, used CG14, WAB56-104 and V4 plants sown on 7 November 1996 in drained pots (three plants and 15.9 l soil per pot) using soil from the main experimental site, which was kept at field capacity. The experiment had a two-factor, randomised split-plot design with four replications (pots), three cultivars and two N-fertilizer levels. Pots were fertilized with zero or 0.3 g N per pot as urea, and 0.15 g of each P and K per pot as triple superphosphate and KCl, and grown in the open under ambient atmospheric conditions. At 33, 41, 56 and 68 days after sowing (DAS), leaf stomatal conductance and net photosynthetic rate (LiCor 6200; Li-Cor, Lincoln, Nebraska), chlorophyll content (SPAD) and SLA were measured sequentially on the same leaf (three youngest-fully expanded leaves per pot) between 0900 and 1500 hours at light

saturation, using supplemental white light from a slide projector to raise the ambient photosynthetically active radiation (PAR) to 1800-2000  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>.

Preliminary data are also presented from an upland rice-weed competition trial conducted at the same site during the 1996 wet season, in which rice lines were grown in single rows, with single rows of competition species planted at both sides at 25-cm distance. The competitor species were (1) the natural weeds of the site, (2) a creeping cowpea, (3) maize [removed after two months of competition], (4) a short-duration [WAB56-50] and a medium-duration upland rice [OS6], and a highly weed competitive *O. glaberrima* landrace [IG10]. A competition index was formed by dividing yield under competition by yield under monoculture.

#### **Results and discussion**

### Dry matter growth and tillering

Shoot dry matter accumulation during the exponential growth period (vegetative phase) was similar among the lines under zero-N inputs, but more rapid in CG14, the *O. glaberrima* parent, than WAB56-104, the *O. sativa* parent, when N was applied (Fig. 1). The interspecific progeny V4 presented an intermediate behavior. From 60 to 80 DAS onwards, however, dry matter accumulation of CG14 slowed considerably or even decreased, probably as a result of lodging (which occurred only in CG14 at about heading stage with no N applied, and 2 weeks earlier at 80 kg N ha<sup>-1</sup>) and early leaf senescence. Regardless of the N rate, 8% of shoot dry matter consisted of dead leaves at flowering in CG14, as compared to 4% to 5% in V4 and less than 2% in WAB56-104 (data not presented).

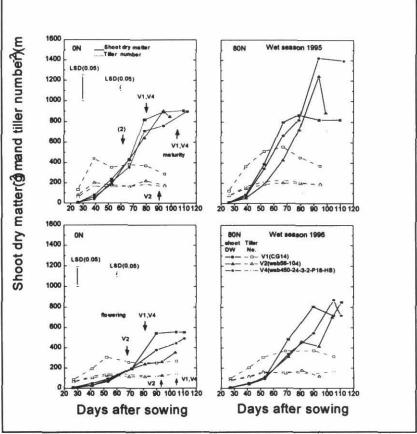


Figure 1. Time courses of shoot dry weight and tiller number during the 1995 (top) and 1996 (bottom) wet seasons.

Dry matter production was much lower in the WS 1996 than in the WS 1995, which might have been a result of lower solar radiation (Table 1) or decreasing soil fertility during three consecutive seasons (wet season 1995, dry and wet seasons 1996), which could not be fully corrected with NPK application. This phenomenon, which is commonly observed in West African upland soils, is one of the main reasons for shifting cultivation in the area (Nye and Greenland, 1960; WARDA, 1996).

CG14 produced about twice as many tillers as WAB56-104 and the progeny V4, which had similar tillering patterns and rates (Fig. 1). Application of N did not increase the rate of production of tillers in CG14, but sustained tillering for a longer period and delayed the onset of tiller abortion, resulting in a larger number of tillers at the end of the season. As derived from shoot dry matter and tiller number, the average weight of CG14 tillers was only about half that of WAB56-104 and V4. The low tillering rate of V4 as compared to its *O. glaberrima* parent might be partly due to high investment of assimilates in its sturdy, lodging-resistant, stems. In fact, V4 partitions a greater fraction of the available assimilates to stems and leaf sheaths, as compared with CG14 (Dingkuhn et al., 1997)

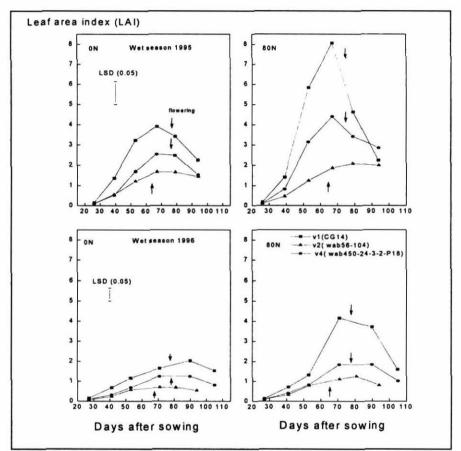


Figure 2. Time courses for green leaf area index during the 1995 (top) and 1996 (bottom0 wet seasons.

Despite the low weight of the individual tillers, CG14 developed the greatest leaf area per tiller among the test lines. The leaf area index (LAI) of CG14 was two to three times greater than that of WAB56-104 in all treatments and seasons (Fig. 2). The progeny V4 had an intermediate LAI, and the other three interspecific progenies tested (not presented) had LAIs between that of V4 and the *O. sativa* parent, WAB56-104. The LAI of CG14 responded more strongly to N inputs than that of the other lines tested, and dropped by 50% from the 1995 WS to the 1996 WS, during which solar radiation was

lower (Table 1) and soil fertility might have been affected by intensive cropping. Consequently, CG14 used available resources to produce an extremely leafy canopy and dense population of tillers, which should theoretically result in superior weed competitiveness. In fact, this *O. glaberrima* landrace has been reported to suppress weeds far better than a number of other rice lines, including WAB 56-104 (Fofana et al., 1995; WARDA, 1996).

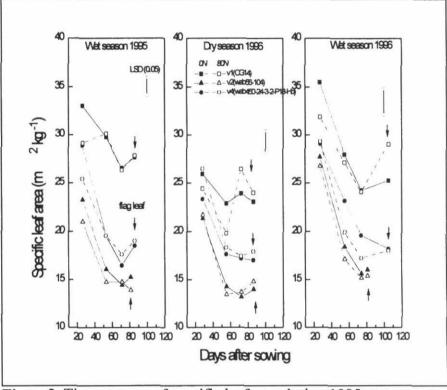


Figure3. Time courses of specific leaf area during 1995 wet season and 1996 dry and wet seasons.

Leaf area index, chlorophyll content and specific leaf area

The leaf canopies of CG14 had a pale-green (but not yellowish) appearance as compared with the other lines tested. This was due to a low chlorophyll content of CG14 leaf laminae (data not presented) which, in turn, was associated with a high specific leaf area (SLA; Fig. 3). The ability of CG14 to produce a large leaf area on the basis of the same N resources and, at least in the zero-N treatment, the same shoot biomass as the other lines, was thus associated with a low level of resources invested per unit leaf area. The nitrogen

concentration on a DW-basis of CG14 laminae was not lower than that of the other lines tested, however, indicating that the pale-green color of CG14 leaf canopies was not caused by N-deficiency (data not presented).

Specific leaf area was a remarkably stable trait across treatments and seasons, except for a marked reduction of SLA in CG14 during its early development stages in the dry season (Fig. 3). Independent observations indicated that CG14 and some other O. glaberrima landraces are physiologically sensitive to dry, hot winds (data not presented), which might be related to their thin leaves, or high SLA. Nitrogen rate had no significant effects on SLA except during seedling stage (first sampling date in each season), during which N application consistently, and in some cases significantly (P < 0.05), increased SLA. In all treatments, SLA decreased strongly between seedling stage and flowering. The SLA of the progeny V4 was generally between that of its parents and, at most sampling dates, significantly different from both. Whether the pronounced droopiness of the leaves of many O. glaberrima landraces caused by high SLA, anatomical properties, or both, is not known. The environmental stability of SLA and the strong differences among lines indicate that this parameter can be used as a trait in plant breeding.

Across the two parents and four progeny, linear correlations were found between SLA and LAI (positive) and leaf chlorophyll (negative) 4). The slopes of these (Fig. content relationshipsdepended on N inputs. Consequently, LAI depended on available resources in all lines tested, but more leaf area could be produced with a given level of resources (N, assimilates) if the genotypic SLA was high. The validity of this theory was tested in a separate experiment using 12 genetically and ecologically diverse rice lines and cultivars that were transplanted in a paddy field (Fig. 5). Across the lines, a significant correlation (P < 0.05) was observed between LAI and SLA towards the end of the vegetative growth phase (51 DAS). The correlation became weaker as the canopy was closed and light interception neared 100% at flowering stage (data not presented).

The degree to which individual lines deviated from the LAI vs SLA regression line was associated with eco-physiological adaptation. Cultivars that tolerate flooding well (CG14, Suakoko 8, Bouake 189) developed a comparatively high LAI, whereas typical upland-adapted tropical japonica rices (OS6, Moroberekan and WAB56-104), which do not grow well in anaerobic soil, had small LAI (exception: IDSA6, a tropical-japonica rice with wide hydrological adaptation). Three interspecific progenies from the CG14 x WAB56-104 cross (common denomination WAB450) behaved more like their *O. sativa* tropical-japonica parent.

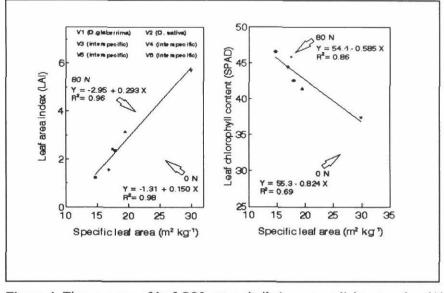


Figure 4. Time courses of leaf CO2 net assimilation rate at light saturation (A), leaf chlorophyll content (SPAD) and specific leaf area (SLA) during the 1997 dry season.

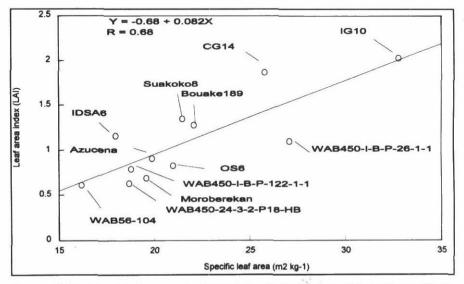


Figure 5. Relationship between leaf area index (LAI) and specific leaf area (SLA) at 51 days after sowing for 12 diverse rice lines.

#### Potential net CO<sub>2</sub> assimilation

In order to evaluate the cost of large SLA (and thus, low leaf chlorophyll content) in terms of maximum leaf  $CO_2$  assimilation rates ( $A_{max}$ ), leaf gas exchange rates were measured in potted plants under light saturation. The phenological decrease of SLA and the cultivar differences in SLA and leaf chlorophyll content were extremely similar to those observed in the field (Fig. 6). CG14, which had the largest SLA, had significantly lower  $A_{max}$ than WAB56-104, while the progeny V4 had intermediate rates. Furuya et al. (1994) also observed low  $A_{max}$  in several *O. glaberrima* landraces, as compared with *O. sativa* cultivars.

Two local checks, the high-yielding lowland cultivar Bouake 189 and the traditional upland cultivar Moroberekan, had nearidentical gas exchange characteristics and were similar to the V4 interspecific cross. It appears therefore that the two parents of the interspecific cross represented extreme types in terms of gas exchange characteristics and SLA.

When plotting  $A_{max}$  against leaf chlorophyll content across lines, development stages, and N rates, one common linear function was found for the parents and the interspecific progeny (Fig. 7). It appears therefore that, despite strong cultivar differences in SLA and chlorophyll content,  $A_{max}$  depended on these parameters in the same way. We therefore hypothesize that the marked differences in growth behavior and resource use observed between the parents and the interspecific progeny are the result of only a few traits, such as SLA. This hypothesis needs further investigation.

# Sink potential and yield

Grain yields of CG14 did not respond to N inputs, where WAB56-104 and the interspecific progenies responded strongly, particularly in the WS 1996 crop, which had the lowest growth rates (Fig. 1) and mean yields (Table 2). The yields of CG14, however, were greater in 1996 than in 1995, probably due to a positive association of lodging with biomass production. CG14 lodged in all seasons and treatments, but at an earlier development stage when N was applied than when it was not, and it lodged at an earlier stage in the WS 1995 than in the WS 1996 (data not presented). The interspecific

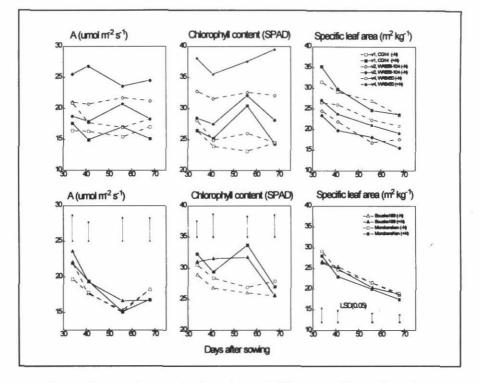
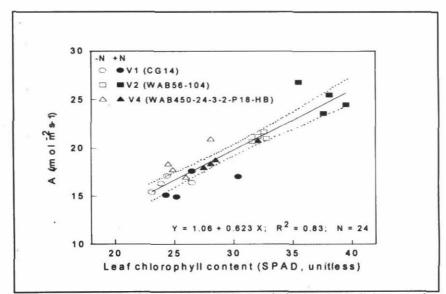
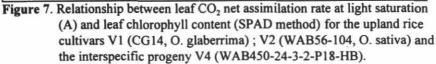


Figure 6. Relationship between leafarea index (LAI) and specific leaf area (SLA), and between areal leaf chlorophyll content (SPAD method) AND sla, at 52 days after sowing, during the 1995wet season.





progeny,which generally did not lodge, had yield patterns similar to the *O. sativa* parent, WAB56-104. In all test lines, and at all N rates, between 75% and 90% of the tillers bore panicles (data not presented). Consequently, the superior tiller production of CG14 translated into a about twice as many panicles (between 190 and 262 m<sup>-2</sup> in the WS 1995, depending on the treatment) than in WAB56-104 and the progenies (between 93 and 149 m<sup>-2</sup>).

Because lodging and grain shattering generally affect yield but not the sink potential of the crop, the absence of any yield response to N rate in CG14 does not necessarily mean that its sink potential did not respond to resources. Sink potential is defined here as product of spikelet number per unit area and the potential weight of the individual grains. Grain weight is a very stable parameter in rice, in comparison with most other cereals (Yoshida, 1981), and sink potential of rice is mainly a function of spikelet number. (In this study the individual grain weight of the cultivars was 26.6 mg for CG14, 28.2 mg for WAB56-104 and 29.0 mg for V4, and varied by less than 1 mg between seasons and N levels.) Spikelet number is usually correlated with the amount of above-ground biomass formed between development stages 0.65 (about panicle initiation) and 1.0 (flowering) (Kropff et al., 1994).

We observed a significant correlation across the two parents and the V4 progeny between spikelet number per ground area and dw growth between stages 0.65 and 1.0 (Fig. 8). Consequently, the sink potential of the three lines responded similarly to the resources acquired by the plant, and the absence of a yield response to N in CG14 was mainly due to shattering. Grain shattering had a leveling effect on yields, because it was more pronounced in fertilized than in unfertilized plots. Spikelet sterility was not significantly different among the lines. **Table 2.** Grain yield at 14% moisture for some interspecific upland rice progeny (V3 to V6) and their parents, V1 (*O. glaberrima*) and V2 (*O. sativa*), fertilized with zero, 40, 80 and 120 kg N ha<sup>-1</sup>. Due to missing data, SAS analysis provided CV but not LSD values. Mbé, Ivory Coast

			Grain yield (t ha-1)			
	0N	40N	80N	120N	Mean	SE
1995 Wet season			******			
VI (CG14)	2.52	2.55	1.52	2.21	2.20	0.23
V2 (WAB56-104)	3.59	5.16	4.95	4.79	4.62	0.23
V3 (WAB450-1-B-P-160-HB)	3.26	4.77	5.22	5.37	4.66	0.21
V4 (WAB450-24-3-2-P18-HB)	3.70	4.91	5.44	4.97	4.76	0.23
V5 (WAB450-24-2-3-P33-HB)	3.90	3.04	5.57	4.69	4.30	0.21
V6 (WAB450-I-B-P3I-HB)	4.19	4.99	5.17	4.90	4.81	0.21
Mean	3.53	4.24	4.65	4.49	4.23	
SE		0.18	0.21	0.18	0.18	
CV (%) 17.6						
1995 Wet season						
V1 (CG14)	3.30		3.49		3.40	0.25
V2 (WAB56-104)	2.33		4.40		3.37	0.33
V3 (WAB450-24-3-2-P18-HB)	2.55		4.37		3.46	0.29
Mean	2.73		4.09		3.41	
SE	0.23		0.24			
CV (%) 18.4						

#### A strategy for combining weed competitiveness with high yield potential

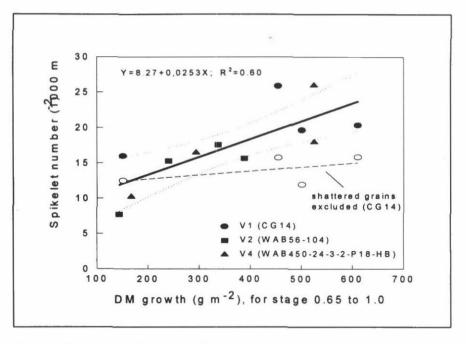
*Oryza glaberrima* landraces are known to be highly weed competitive, but unresponsive in terms of grain yield to inputs (Koffi, 1980). Although the present study did not evaluate weed competitiveness, the results indicate that the landrace CG14 was able to produce a large number of tillers even under low external resources, and to achieve ground cover much more rapidly that the other lines tested. These characteristics have been correlated with competitiveness with weeds of upland rice (Fofana *et al.*, 1995) and of wheat (Lemerle et al., 1996). Even in environments that did not give CG14 a significant growth advantage (e.g., zero N inputs during the WS 1995; Fig. 1), CG14 produced a much larger LAI

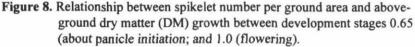
than the other lines (Fig. 2). This phenomenon could be explained with genotypic differences in SLA.

Simulations with the rice growth model ORYZA\_1 (Kropff *et al.*, 1994) confirmed that the LAI depends linearly on SLA during the exponential growth phase, during which light interception (and not yet light-use efficiency) limits canopy  $CO_2$  assimilation rates  $(A_{canopy})$ . In such situations, the gain in light interception resulting from a larger SLA (and larger LAI, as a result) seems to be equal to or greater than the loss in terms of lower leaf photosynthetic rates. When N resources were low, high SLA enabled a higher LAI while dw growth was not affected. When N was applied, high SLA increased both LAI and dw.

The simulation studies also confirmed our observation that the initial growth advantage drawn from high SLA in CG14 could not be carried through to the flowering stage. High crop growth rates (CGR) at heading and flowering are generally considered necessary to achieve a high harvest index and yield (Tanaka *et al.*, 1966; Yoshida, 1981). Simulations with ORYZA\_1 revealed that large SLA permits a high CGR during the periods when leaf area limits growth, but reduces CGR as soon as the canopy is closed (and light interception cannot be further increased). In the latter situation, the A<sub>max</sub> of sun-exposed leaves drives A<sub>canopy</sub>, and large SLA (in association with low N and chlorophyll content per leaf area) becomes a disadvantage.

The challenge in the development of weed-competitive plant types is to achieve that characteristic without losing the gains in yield potential achieved with modern cultivars (Johnson, 1995). We hypothesize that an 'optimum' plant type for low-management conditions, weed-competitive yet high-yielding, should have a large SLA during exponential growth, and a small SLA during linear growth. Such patterns are generally found in rice (Figs. 3 and 6), but would need to be further enhanced through breeding. Plant types behaving like CG14 during the vegetative growth phase (large SLA, droopy leaves, large LAI and light interception) and WAB56-104 during the reproductive phase (small SLA, erect and dark-green leaves, modest LAI) might substantially reduce physiological tradeoffs between weed-competitiveness and yield potential. Our results also indicate that yields of O. sativa x O. glaberrima progenies having the suggested phenological patterns of SLA would not be necessarily limited by spikelet number, if they are resistant to lodging and grain shattering.





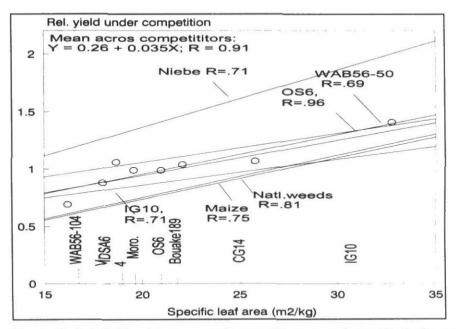


Figure 9. Relatioships between weed competitiveness and specific leaf area (SLA) for 8 test lines exposed to competition with 6 competitor species/varieties.

Validating the strategy: First results on effects of SLA on weed competitiveness

Preliminary results from a field trial with controlled competition confirmed that SLA is indeed a major physiological determinant of weed competitiveness (Fig. 9). Eight rice lines with different SLA competed with six competitor species (including rices). Regardless of the competitor species, a significant correlation was found between relative yield under competition (yield under competition divided by the yield observed in monoculture) and SLA. Rice competed well with a creeping cowpea (Niebe), but poorly with the natural weed flora, maize or IG10, a highly weed competitive *O. glaberrima* landrace.

As already observed in previous studies, the *O. glaberrima* landraces CG14 and IG10 proved excellent competitors. Surprising, however, was the good weed competitiveness of Bouake 189, a high-yielding semidwarf type adapted to irrigated culture. Possibly, the high tillering and leaf area growth of this variety in the absence of water stress gave it an advantage over upland rices such as WAB56-104 or IDSA6. Consequently, this study needs to be extended to drought prone situations, in which potential vigor, as enabled by high SLA, is not necessarily the main determinant of weed competitiveness. Nevertheless, it seems extremely likely that SLA is an important factor in rice weed competitiveness.

The distribution of SLA among fixed inter-specific progenies is gaussian, with a single maximum situated between the values observed for the parent materials, and tailing significantly towards both these values (Fig. 10). We conclude that SLA is a heritable trait occurring in sufficient diversity to be used in breeding.

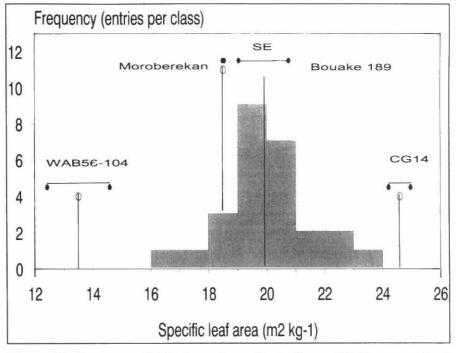


Figure 10. Frequency distribution of specific leaf area (SLA) among fixed interspecific progenies.

#### Conclusion

The phenological patterns of growth, tillering, LAI and SLA were characterized under different N inputs for an *O. sativa* and an *O. glaberrima* parent and some interspecific progeny. Compared with the *O. sativa cultivar* WAB56-104, the *O. glaberrima* landrace CG14 had extremely large LAI, SLA and tillering rates in all treatments and seasons, but superior dw accumulation only when N was applied to the crop. The interspecific progeny had intermediate LAI, growth rates, leaf chlorophyll content and SLA. Their tillering rates, however, were as low as those of WAB56-104, and were possibly a result of the increased assimilate demand of their sturdy, lodging-resistant, stems. The physiological cost of lodging resistance in upland rice needs further study.

We found SLA to be a major determinant of early growth vigor and LAI. High SLA was probably the direct cause of the low chlorophyll content, and therefore,  $A_{max}$ , observed in CG14; and the indirect cause of its high LAI. We conclude that high-yielding, weed-competitive, rices should have a large initial SLA to accelerate leaf area development, followed by a rapid decrease in SLA during

the reproductive growth period, in order to ensure high leaf photosynthetic rates. Breeding for such a plant type might be possible on the basis *O. sativa* x *O. glaberrima* crosses, specific selection for the desired dynamics of SLA, and selection against lodging and shattering types. The potential of this concept for effectively combining weed competitiveness

with high yield potential, particularly under water-limited conditions, needs to be further validated, and a greater diversity of *O. sativa* and *O. glaberrima* accessions characterized to fully exploit the available genetic resources.

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# Exploitation and Utilization of Interspecific Hybrid Vigor between Oryza sativa and O. glaberrima

Tao Dayun, Hu Fengyi, Yang Guifen, Yang Jiangyi, Tao Hong

(Yunnan Academy of Agricultural Sciences, Kunming 650205, The People's Republic of China)

#### Abstract

Rice is the basic food for half the world's population which is presently growing at about 2% per annum. Over 400 million tons additional rice will be required by the year 2020 to meet the rice demand. However, production growth rate has been slowing down due to reduced investments in irrigation development and management. New rice varieties with higher yield potential and stability will be required to increase yields particularly in unfavorable environments constituting 40% of area planted to rice worldwide.

Innovative and new rice breeding strategies aimed at developing improved varieties such as the super high yield *indica-japonica* hybrid in Japan and elsewhere were initiated. Heterosis in rice has been accomplished commercially in China and other countries with *indica/indica* or *japonica/japonica* crosses where 15-26% yield increase have been obtained. Interspecific hybrid rice with *O. sativa* and *O. glaberrima* has been proposed, and there is evidence of interspecific heterosis.

In 1995 at Sanya, Hainan Province, China, 13 accessions of O. sativa and O. glaberrima were used for interspecific reciprocal crosses. It was found that for both species, there were some crossability differentiation which was greater for O. sativa. Crossability was higher when O. glaberrima was used as female parent. Spikelet fertility of  $F_1$ hybrid ranged between 1 and 15% in a total of 309 cross combinations. The interspecific hybrids usually have longer awns and more tillers per plant than their parents. It is proposed that it might be useful to use O. glaberrima as female parent in interspecific hybridization.

# Introduction

Rice is the basic food for half the world, now and well into the future (IRRI, 1993). The population of rice consumers is presently increasing at the rate of about 2 % annually. The number of rice eaters will probably increase by 57 percent and rice requirements by 70 % during the next 25 years. To feed these people, an additional 700 million tons of rice will have to be produced annually by 2020. But growth in production has begun to slow down because of lack in investment in the development and maintenance of irrigation, and reaching of the yield ceiling by many farmers in the irrigated system. At the same time, modern varieties and technology have had very little impact on increasing the yields of rice under unfavorable (rainfed lowland, upland, and flood-prone) environments which constitute 40 % of the area planted to rice(Khush, 1996). The increased demand for rice will have to be met from less land, with less water, less labor and less pesticides (IRRI, 1993). Among numerous factors required to increase rice yield, new varieties with higher yield potential and yield stability will play the leading role. The future challenge of rice improvement are two fold (Khush, 1996):

-developing varieties for the irrigated areas with high potential and yield stability.

- developing improved varieties with tolerance for biotic and abiotic stresses and with higher yield in unfavorable environments.

To meet the two-fold challenge, some development strategies of rice breeding and new frontier projects were envisaged and launched. These include the super-high yield breeding projects using *indica / japonica* hybridization in Japan (Kushibuchi,1988), and the super-high yield breeding through exploitation and utilization of *indica / japonica* intersubspecific heterosis (Kush, 1996; Yuan and Mao, 1991). Others include the new plant type concept for tropical *japonica* rice (Kush, 1995), developing a perennial upland rice plant (IRRI, 1993), and introgression of biotic and abiotic resistances from wild rice species (Brar and Khush, 1995). All these strategies could contribute in complementary ways to facing the challenge.

Heterosis in rice has been extensively exploited commercially. China alone contributes about 20 % of the world's rice production by exploiting this phenomenon, which has helped the country to increase rice production by 200 million tons from 1976 to 1991. Hybrids also showed heterosis for cold tolerance at seedling stage, drought tolerance, salt tolerance, ratooning ability and deepwater tolerance (Virmani, 1994). Thus, hybrid rice development is indispensable in meeting the two-fold challenge in the future, not only under favorable conditions but also under certain stress environments.

So far, exploitation of heterosis in rice has been accomplished commercially in *Indica / Indica* or *Japonica / Japonica* crosses in China and the Democratic People's Republic of Korea, where yields can be increased by 15-20 % ovrt the levels obtained with the best inbred rices being cultivated by farmers in the tropics, subtropics, and temperate regions.

Prospects of further enhancing the yield heterosis level by at least 20 % beyond that of *Indica-Indica* and *Japonica-Japonica* crosses are being exploited in Japan, China and IRRI. Additionally, Yuan and Mao (1991) envisaged distant hybrids (interspecific or intergeneric hybrids) to increase yield of rice enormously. Based on preliminary observations with *Oryza glaberrima* in Yunnan, Tao *et al.* (1996) proposed the utilization of hybrid vigor and fertility of cultivated interspecific hybrid rice, *Oryza sativa* and *O. glaberrima*.

#### **Literature Review**

#### Relationship between the genus Oryza and other grasses

The genus Oryza, which consists of 2 cultivated rice species, Oryza sativa L. and O. glaberrima Steud., with about 20 related species, are in the grass family Poaceae. From Watson's classification (1985), rice and its relatives are quite unrelated to other major cereals such as maize, wheat, and sorghum, hybridization between them would be extremely difficult (Vaughan, 1994).

Under the family *Poaceae*, rice belongs to the tribe *Oryzeae*, which consists of 12 genera. Even within *Oryzeae*, there are only two cereal crops, *O. sativa* and *O. glaberrima*. Thus, from biosystematic points of view, exploitation of interspecific heterosis between *O. sativa* and *O. glaberrima* is the sole choice.

#### Evolution and genetic consideration

The African rice, *O. glaberrima*, is endemic to West Africa; and was developed in Africa independently of the origin of *O. sativa* in Asia (Porteres, 1956, cf. Nayar, 1973). The two species have common ancestor (Chang, 1976; Oka, 1964; Pental *et al.*, 1985). The two rice cultigens are thought to have same genome (A) as the chromosome pairing in their  $F_1$  meiosis is normal (Morinaga *et al.*, 1957); while sterility amongst them is controlled by genetic mechanism (Sano, 1986).

#### Biodiversified consideration

O. glaberrima has some complementary useful traits for O. sativa, such as strong vegetative vigor, resistance to RYMV (Thottapilly et al., 1993), blast (Silue et al., 1991), Sheath blight (Wasano et al., 1986), stalk-eyed fly (Alam, 1986), stemborers and hispids (Sauphanor, 1985), yellow stemborer (Mandras, 1991), and tolerance for low phosphorus (Monde et al., 1991).

#### Crossability and fertility of hybrids

Hybrids are obtained without any difficulty between *O. sativa and O. glaberrima* (Chu *et al.*, 1969; Morishima *et al.*, 1962). There is also no hybrid disability Morishima *et al.*, 1962, 1963). The  $F_1$  seeds, after dormancy has een broken, germinate normally, and the plants also grew normally (Morishima *et al.*, 1962, 1963). But the  $F_1$  hybrids between *O. glaberrima* and *O. sativa* are generally sterile and only occasionally show slight fertility (Bauharmont 1962, Nayar 1973; Morinaga and Kuriyama 1957; Morishima *et al.*, 1963; Nayar 1958, cf. Nayar 1973 ).

However, Ramanujam (1938) reported that the cross sativaglaberrima was highly fertile. Bauharmont *et al.* (1985) reported that spikelet fertility of Asian rice D34 and African rice Lib142 and CG70b was from 9.56 to 94.99 percent. Richharia *et al.*(1962) reported that in F<sub>1</sub> hybrids between *O. glaberrima* and *O. sativa (O. glaberrima* X PTB.10), the pollen sterility was only 25 %, and postulated that the sterility was of the same nature as that found in intersubspecific.hybridization of *O. sativa*. Recently, Second (1986) also pointed out that an approximately equal genetic distance was found between *O. glaberrima* and the "ancestral" *indica* and *japonica* in the three combinations.

#### **Existence of interspecific heterosis**

Intergenomic hybrids obtained by embryo rescue, in Oryza sativa / O. officinalis (Jena and Khush, 1986); O. sativa / O. minuta (Amante et al., 1992), and O. sativa / O. australiensis (Multani et al., 1994), grew vigorously, so did hybrids between O. sativa and O. glaberrima (Morishima et al., 1962, 1963; Yabuno, 1977). Is it heterosis of vegetative growth?

#### **Preliminary Results**

In Spring 1995 at Sanya, Hainan Province, 13 accessions of *Oryza* glaberrima and 13 cultivars of *O. sativa* were used to make reciprocal interspecific hybridization (Tables 1 and 2).

#### **Table 1.** Crossability difference among accessions of *Q* glaberrima with *Q* sativa

Accessions of	No. of	Seed	Range
O. glaberrima	combination	set (%)	(%)
IRGC100127	23	38	8-72
IRGC100140	11	46	4-77
IRGC102203	21	28	0-62
IRGC102254	22	29	0-71
IRGC102321	15	41	0-74
IRGC102360	10	41	9-67
IRGC102375	18	31	3-68
IRGC102502	16	35	4-73
IRGC102541	18	34	0-80
IRGC102571	14	38	0-79
IRGC102602	13	32	2-71
IRGC104165	12	28	0-68
IRGC103944	19	33	0-80

There were some differences of crossability among the various accessions of *O. glaberrima*, but the difference was greater among

different cultivars of *O. sativa*. This shows that crossing barriers exist between the two cultivated species, *O. sativa* and *O. glaberrima*, in varying degrees. For both species of *O. sativa* and *O. glaberrima*, there existed some crossability differentiation.

Designation of	No.of cross	Seed Set	Range
O. sativa		(%)	(%)
Guichao No. 2	16	50	26-78
IR24	16	33	2-72
IR36	16	32	0-72
Jiangchengkugu	23	25	0-69
IRAT216	17	40	0-79
Dianrui 457	24	27	0-67
Dianrui 324	15	40	21-61
IRAT104	3	35	24-45
Reimei	21	39	3-80
Xinan 175	14	38	5-72
IRGC101855	18	35	10-72
IRGC103464	10	22	2-56
IRGC103616	16	40	17-80

**Table 2.** Cultivar difference of crossability of O. sativa

 with O. glaberrima.

The degree of differentiation for *O. sativa* was, however, greater than that for *O. glaberrima*. It was also found that the crossability was higher when *O. glaberrima* was use female parent than that when *O. sativa* was female with a few exceptions (Table 3). Therefore, it could be true that cytoplasm of *O. sativa* has some adverse effect on crossability besides inducing male sterility (Sano, 1986). It should then be possible, and it is now a fact, for cytoplasm of *O. glaberrima* to induce male sterility when interacting with the nucleus of *O. sativa* (Carnahan *et al.*, 1972; Lo and Yuan 1990; Yang and Lu 1989).

With regards to spikelet fertility of hybrid  $F_1$ , among a total of 309 cross combinations observed in 1995 and 1996, one cross (Reimei / IRGC102502) had 15 %, while four crosses had between 1 and 5 percent, the remaining 304 combinations had below 1 percent (Figure 1).

Because of strong photoperiod sensitivity of most designations of *O. glaberrima* used and high spikelet sterility of hybrids. comparative heterosis of interspecific hybrids was not observed. But the interspecific hybrids usually had longer awn and more tillers per plant compared with their parents.

Accession of		O. gla	aberrima		O. sati	va l	
O. sativa	/O. sativa				O. glaberrima		
	Averag (%)	No. of cross	Range	Averag (%)	No. of cross	Range	
Guichao No. 2	49	8	30-75	51	8	26-78	
IR24	48	9	21-72	11	8	0-31	
IR36	46	8	28-71	16	9	0-36	
Jiangchengkugu	33	13	0-69	16	10	0-28	
IRAT216	50	10	8-79	25	7	0-74	
Dianrui 457	41	12	0-67	13	12	0-30	
Dianrui 324	40	8	26-61	40	7	18-60	
IRAT104	41	2	38-45	24	1	-	
Reimei	54	11	22-80	24	10	5-73	
Xinan 175	43	7	24-72	33	7	13-54	
IRGC101855	51	8	26-72	23	10	10-41	
IRGC103464	32	3	2-56	17	7	4-36	
IRGC103616	33	7	17-59	43	9	2.6-80	

**Table 3.** Reciprocal difference of crossability between O. sativa and O. glaberrima.

# **Future Endeavor and Discussion**

•Are there any interspecific heterosis, heterobeltiosis, and standard heterosis?

•Are there any real fertile interspecific cross combinations? If the answer is "yes", why not exploit and utilize them ?

•Improvement of *O. glaberrima* for traits such as good plant type, resistance to lodging, photoperiod insensitivity, intermediate or low amylose content.

•Since cytoplasm of *O. glaberrima* usually has less adverse effects on crossability and hybrid fertility(Sano, 1986), with outcrossing favoring characters (blooming earlier, longer period from opening to closing of florets, high stigma exertion rate), it might be more plausible to use *O. glaberrima* as female parents when breeding for interspecific hybrids. •It is quite necessary to confirm the resistance of *O. glaberrima* to yellow stem borer and ShB, then introgress them to *O. sativa*. Resistance to RYMV and stalk-eyed fly are of local importance in

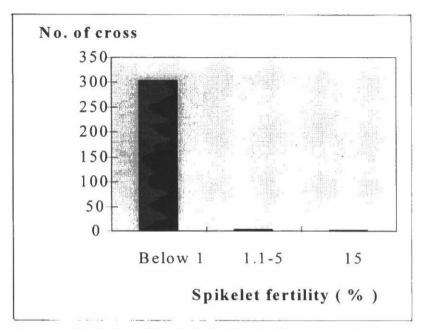


Figure 1. Distribution of F<sub>1</sub> spikelet fertility for 309 cross combinaison.

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# Amélioration du riz *Oryza glaberrima* à Travers la Culture de Tissus

#### Selome Yawovi DOGBE

Institut National des Cultures vivrières BP 2318 Lome TOGO

### Resumé

La capacité de 11 cultivars d'Oryza glaberrima de former des cals et de régénérer des plantes a été examinée. Les explants testés sont des embryons mûrs sur un milieu de composition MS. L'évaluation des plantes de génération R0 et R1 a été effectuée respectivement en serre et en champ. Elle a permis de démontrer l'apparition d'une grande variabilité au sein des populations obtenues. Les mutants identifiés diffèrent des cultivars dont ils sont issus par un ou plusieurs caractères.

Certains variants ont présenté des caractéristiques intéressantes qui pourraient servir de critère d'amélioration de l'espèce. Celles qui sont les plus importantes sont, la résistance à l'égrenage et la présence de ramifications secondaires au niveau des panicules.

Cette étude aura permis de démontrer qu'il sera possible d'exploiter la variation somaclonale comme une source additionnelle de variabilité utilisable dans un programme d'amélioration variétale.

#### Abstract

The capacity of 11 cultivars to form callus and to regenerate plants were analyzed. The tested explants are ripe embryos on a MS medium. The evaluation of plants from RO and RI generation was undertaken on-farm and in the field, respectively. Through this analysis, it was possible to demonstrate the appearance of a great variability among the populations obtained. The identified mutants differrd from the cultivars from which they were derived by one or several traits.

Some variants presented interesting characteristics which could be used as criteria to improve the germplasm. The most important ones are resistance to shattering and the presence of secondary branches on the panicles.

Through this study, it was possible to demonstrate that the somaclonal variation could be exploited as an additional source of variability which can be used in a varietal improvement programme.

### Introduction

Le riz africain, *Oryza glaberrima* est cultivé depuis des milliers d'années par les populations rurales. Malgré l'introduction de variétés modernes performantes de l'espèce *Oryza sativa*, les paysans continuent de cultiver cette espèce dont la productivité reste faible. Les rendements obtenus ne dépassent guère 300 à 500 kg ha<sup>-1</sup>. Le refus des paysans d'abandonner cette espèce a pour origine les avantages comparatifs qu'elle possède du point de vue qualité organoleptique et nutritionnelle par rapport à l'espèce sativa.

Les efforts d'amélioration du rendement des cultivars de cette espèce, par les méthodes culturales n'ont guère connu de succès; car la plante répond mal à l'apport d'intrants. D'autres recherches orientées vers les hybridations interspécifiques ont été entreprises mais peu de résultats convaincants ont été obtenus. Car le transfert des caractères intéressants à l'espèce et leur maintien sont rendus improbables par le phénomène de délétion dans les descendances hybrides.

Or, on sait depuis quelques années que les plantes regénérées aux dépens de cultures in vitro, font montre de modifications utilisables pour la sélection. Ce phénomène appelé variation somaclonale est particulièrement importante chez le riz. Il permet de disposer de variants qui ne diffèrent de la variété de départ que par un nombre restreint de caractères (Nishi et al 1969; Oono 1984; Huang et al 1985). L'utilisation de la variation somaclonale pour la sélectin du riz est actuellement très avancée pour l'espèce *O. sativa*, mais très limitée pour *O. glaberrima*.

L'objectif de cette étude d'évaluer la capacité de formation de cals et de régénération chez des cultivars de l'espèce *O. glaberrima*, de détecter les variants et de sélectionner ceux qui, tout en conservant les qualités du matériel de départ exhiberaient des caractéristiques amélioratrices du rendement.

#### Matériel et méthodes:

#### Matériel végétal

Il est constitué de 11 cultivars d'O. glaberrima, 7176 TOG, 6629 TOG, 6630 TOG, 6631 TOG, 6597, 6589 TOG, 6472 TOG, 6216 TOG, 5897 TOG, 5860 TOG, 5406 TOG, issus des collections

maintenus à l'Adrao en plastique noir.

#### Culture de tissus

Les graines de paddy sont décortiquées manuellement. Les cargos obtenus sont désinfectés dans une solution de formol à 0,8 %, sous agitation constante pendant 40 minutes. Puis ils sont trempés dans une solution d'hypochlorite de calcium à 5 %. A partir des cargos désinfectée, des embryons sont prélevés en conditions stériles et placés dans des boîtes de pétri de 9 mm de diamètre, à raison de 5 embryons par boîte. Les boîtes contenant 25 ml de milieu de callogenèse sont mis à l'obscurité à 25°c pendant 10 à 15 jours pour l'induction de cals. La callogenèse est initiée dans un milieu Murashige et Skoog à pH 5,7 plus mg/l de 2,4 D (Dichloro-acide-phénoxyacétique), 1 mg/l de ANA (Naphtalène acide acétique), 1 mg/l de BAP (Benzyl-aminopurine), 20 mg/l de cystéine, 40 g/l de saccharose et 6 g/l d'agarose.

Les cals formés sont repiqués dans des boîtes de pétri contenant un milieu de néoformation dont la composition est: 1/2 dose MS, 0,01 mg/1 2,4 D, 1 mg/1 ANA, 1mg/1 BAP, 20 mg/L cystéine, 40 g/l saccharose, 6 g/l d'agarose. Les boîtes sont exposées à la lumière de 2000 à 3000 lux pendant 16 heures sur 24. Le séjour des boîtes dans ces conditions variant suivant les cultivars, dès l'apparition des zones chlorophylliennes à la surface des cals, ils sont disséqués, et des ébauches de plantules ayant 2 à 3 feuilles qui entourent une tigelle portant de fines radicelles blanches et plumeuses sont isolées. Ces plantules sont ensuite repiquées dans des tubes contenant 40 ml de milieu semblable à celui de callogenèse plus ajout de 0,2 mg/l de IBA (Acide indole-3 butyrique). Les tubes sont placés à la lumière de 2000 à 3000 lux pendant 16 heures sur 24 dans des conditions de température de 25 à 29°c et d'hygrométrie de 65 à 75 %.

Lorsque les jeunes plantes sont suffisamment développées dans les tubes, elles sont transférées en serre dans des pots en plastique noir contenant du terreau. Les plantes sont alors suivies jusqu'à maturité.

#### Evaluation de la variation

Les plantes de la génération R0 et celles de la génération R1 sont évaluées respectivement en serre en 1994 et en champ en 1995. Les observations ont porté sur les caractéristiques morphophysiologiques. Les caractères qualitatifs sont évalués à partir des notations visuelles et les caractères quantitatifs sur la base de mensuration.

### Résultats

### Callogenèse et regénération des plantes

Les données concernant l'aptitude à la callogenèse et à la regénération de plantes des cultivars testés sont présentées au tableau 1. Tous les cultivars évalués ont produit des cals sauf le cultivar TOG 7176. La capacité de callogenèse est fonction du génotype. Les valeurs obtenues varient entre 8 et 86 %. Les cultivars TOG 6216 et TOG 6629 sont plus performantes avec des taux de 86 et 78 % respectivement. Le cultivar TOG 5897 a la plus faible capacité.

Les données concernant la regénération sont basées sur le nombre de plantules obtenues. Le taux de regénération chez les 10 cultivars qui ont produit des cals est en moyenne de 64,70 %. Le minimum, 26,08 est enregistré chez le cultivar TOG 6630. Le cultivar TOG 5406 est particulièrement prolifique avec un taux de regénération de 100 %. Le cultivar TOG 5897 qui a produit des cals (8 %) n'a pas regénéré de plantules.

Le taux de survie des plantules après sevrage a été également calculé. Il varie entre 21,21 et 66,67 %. Les meilleures performances proviennent des plantules issues des cultivars des embryons des cultivars TOG 6597 (66,67 %), TOG 6631 (66,25 %), TOG 6630 (50 %), TOG 6472 (47,65 %) et TOG 5406 (47,29 %).

#### Evaluation de la variation somaclonale chez les plantes R0

Environ 373 plantes R0 provenant de 6 génotypes ont été observées en serre. La détection de la variabilité a porté sur des caractères qualitatifs et des caractères quantitatifs. Les résultats de l'analyse des données recueillies sont présentés au tableau 2. Il en ressort que les populations présentent une grande variabilité pour toutes les caractéristiques évaluées. Le degré de variabilité est fonction de l'origine de la plante et du caractère.

Les caractères qualitatifs: au niveau de la couleur de l'apex, il a été observé une variabilité chez les plantes appartenant à 5 cultivars. Le type d'apex identifié est l'apex normal et l'apex coloré en brun ou en rouge. Les plantes provenant du cultivar TOG 6216 portent toutes des grains à apex normal. Pour l'aristation, il a été noté que 14,77 % des plantes provenant de TOG 5406 et 75,75 % des plantes d'origine TOG 6629 ont des grains barbus. Les mutations touchent également la couleur des grains qui va du noir à la couleur paille. On a dénombré 10 % de plantes dont les grains sont de couleur fauve.Au niveau du caryopse, il a été également dénombré de 10 à 57 % de plantes dont les grains sont à caryopse blanc.

Cultivars (TOG) 7176 6629 6630 6631 6597 6589 6472 6216 5897 5860 5406 % cal/cult 0 78 46 70 18 62 74 86 0 52 72 % plt/cal 0 41,9 84,7 26 45,7 33,3 56,7 76,7 76,2 100 0 % plt/emb0 66 12 32 6 26 42 0 40 14,8 66 21,2 56,2 66,7 38,4 47,6 % survie 0 50 36,3 0 40 47,2

**Tableau 1.** Taux (%) de callogenèse et regénération de 11 cultivarsO. glaberrima 1994

Cultivars				C.V. (	%)	
	LFP	Haut	TT	TF	LP	LG
6216 TOG	31,2	0,4	31	25,6	24,9	55,2
5406 TOG	29	13,4	30	32,3	13,9	32,4
6589 TOG	18,5	2,4	59,2	57,3	12,7	76,1
6472 TOG	4	8,8	31,3	31,3	24,9	46,5
6631 TOG	21,8	13,1	42,2	37,1	5,9	53,6
6629 TOG	28,8	2,1	31	25,6	24,9	47,8

**Tableau 2:** Variabilité au niveau de six (6) caractères des plantes R0 (1994)

Tableau 3. Fréquence des mutations en R1 (1995)

				Cultivars (TOG)		
	6616	5406	6589	6472	6631	6629
TT	4,7	6,9	2,3	0,1	7,5	2,1
LFP	33,5	47,3	13,2	24,7	17,5	12,9
HAUT	43,5	52,8	36,7	39,4	18,8	23,4
CYCLE	5,8	11,6	7,5	8,9	12,1	3,4
LP	15,2	18,4	23,6	32,8	4,5	2,4
NG/P	6,8	5,2	11,6	18,4	3,9	13,1
LG	2,3	1,8	4,3	7,4	1,5	9,3
P1000G	3,4	2,6	4,7	6,4	2,1	5,7
Moyenne	14,4	18,3	17,8	17,7	0,5	9

# Les caractères quantitatifs

la variabilité de 6 caractères a été calculée suivant la méthode de coefficient de variation. D'une manière générale, les plus forts

coefficients de variation sont enregistrés au niveau des caractères tallage total, tallage utile, longueur de la feuille paniculaire, longueur du grain. Le caractère hauteur de la plante est le moins variable avec un coefficient de variation de 7,05 %.

Au sein des plantes issues d'un même cultivar, la capacité de tallage total, et utile est très variable chez TOG 6589 (59,24 % et 57,30 %). La longueur de la feuille paniculaire est également très variable chez les plantes d'origine TOG 6216, TOG 6472 et TOG 6629 (24,91 %). La variabilité est plus importante pour le caractère longueur de la feuille paniculaire parmi les plantes provenant de TOG 6629 (28,80 %). Les valeurs obtenus pour la longueur des grains vont de 26,5 à 76,8 %.

#### Evaluation des plantes R1

Les plantes de la génération R1 suivies au cours de la campagne 1995 ont permis d'appréhender le degré des variations. Les données sont présentées au tableau 3. On note que les mutations sont plus fréquentes parmi les populations d'origine TOG 5406 (18,3 %), TOG 6589 (17,8 %) et TOG 6472 (17,6 %). Les mutations sont plus fréquentes au niveau de la hauteur (35,5 %), de la longueur de la feuille paniculaire (35,7 %).

Des observations plus fines ont permis de déceler des ramifications secondaires parmi les plantes des populations provenant de TOG 6472, et TOG 5406. D'autres mutants possédant un bon niveau de résistance à l'égrenage ont été également identifiés chez les populations dérivées de TOG 6216, TOG 5406, et TOG 6472.

#### Conclusion

A partir des embryons mûrs de l'espèce *O. glaberrima*, il est possible d'induire des cals et de régénérer des plantes. L'aptitude à l'organogenèse est fonction du cultivar. Les plantes regénérées et leurs descendances présentent des différences par rapport au cultivars dont elles dérivent pour plusieurs caractères. Chaque plante néoformée a accumulé de zéro à plusieurs mutations qu'elle peut transmettre à sa descendance. Le nombre de mutations est fonction aussi bien du cultivar que du secteur du cal qui est à l'origine de la différenciation de la plante (Quiberon et al 1986). Fukui (1983) a

également décrit chez le riz l'apparition de quatre mutations récessives qui intéressent sectoriellement le cal somatique lors de sa croissance. Chez le plantain, Vuylsteke et Swennen (1990) ont observé que la variation somaclonale allait de 0,5 à 69,1 % en fonction du cultivar. Les modifications détectées qui touchent aussi bien les caractères qualitatifs que quantitatifs, ont été aussi signalées chez le riz espèce O. sativa (Zarkri, 1986) et chez d'autres graminées notamment le blé (Larkin et al, 1984).

La fréquence de la variation dépend aussi du caractère. Dans notre cas, nous avons remarqué que les caractères les plus variables sont la longueur de la feuille paniculaire, la hauteur de la plante et la longueur de la panicule. Ces observations rejoignent celles de Sun et Zheng (1990) qui faisant la synthèse des recherches concernant la variation somaclonale chez *O. sativa* ont fait ressortir que certains caractères sont plus variables.

Il est aussi fréquent de trouver une similitude chez des variants issus de cultivars différents. C'est le cas de la coloration blanche du caryopse et de la coloration de l'apex des grains.

L'observation des plantes regénérées a fait apparaître l'induction de mutations chez la plupart des caractères pris en compte. Cette variation somaclonale mise en évidence chez les plantes provenant des cultivars d'*O. glaberrima* constitue un atout majeur pour l'amélioration de cette espèce.

Les variants qui combinent un certain nombre de caractères agronomiques nouveaux intéressants tels que la coloration blanche du caryopse, un bon niveau de résistance à l'égrenage, la présence de ramifications secondaires chez la panicule seront suivis dans un processus de sélection. D'autre part il pourra être envisagé des croisements entre les variants somaclonaux intéressants *O. glaberrima* et des variétés de l'espèce *O. sativa*.

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# Application des Marqueurs Moléculaires et de la Cartographie Génétique à l'Utilisation du Potentiel de l'espèce Africaine de riz Cultivé (*O. glaberrima* Steud.) en Amélioration des Plantes

M.N. Ndjiondjop<sup>1</sup>, M. Lorieux<sup>1</sup>, J. Séquier<sup>1</sup>, D. Fargette<sup>2</sup>, G. Reversat<sup>3</sup>, G. Second<sup>1</sup> & A. Ghesquière<sup>1</sup>

<sup>1</sup>ORSTOM/LRGAPT, BP 5045, 34032 Montpellier Cedex 1, France

<sup>2</sup>CIRAD/LPRC, BP 5035, 34032 Montpellier Cedex 1, France

<sup>3</sup>ORSTOM/LEST, 32, Avenue Henri Varagnat, 93143 Bondy Cedex, France

#### Résumé

Un projet d'utilisation des ressources génétiques de l'espèce africaine de riz cultivé, O. glaberrima Steud. est développé par l'ORSTOM en collaboration avec l'ADRAO en Côte d'Ivoire. Une population issue d'un recroisement entre une variété irriguée d'O. sativa, IR64 et la variété d'O. glaberrima Tog5681 a été développée pour être cartographiée avec des marqueurs moléculaires à base PCR (STS, microsatellites). Cette population servira de point de départ pour réaliser un programme d'introgression systématique qui sera dirigé par des marqueurs moléculaires cartographiés au cours de recroisement successifs en utilisant IR64 comme parent récurent. L'objectif est d'obtenir l'introgression de petits fragments d'O. glaberrima dans le même fond génétique d'O. sativa sous formes de lignées isogéniques plus propices à l'évaluation de la diversité génétique utile provenant de l'espèce africaine de riz cultivé. Deux caractères modèles ont été choisis pour réaliser leur transfert chez O. sativa : la résistance au virus de la panachure jaune du riz (RYMV) et la résistance au nématode Hetérodera sacchari. Les premiers résultats concernant l'évaluation polymorphisme entre les parents et la cartographie des marqueurs sur la population de recroisement sont présentés et montrent que de fortes distorsions de ségrégation sur le chromosome 6 peuvent rendre compte de l'action d'un gène de stérilité sporo-gamétophytique sur ce chromosome. Néanmoins, l'évaluation de quelques individus de la population de recroisement montre que le transfert des caractères cibles est faisable. Grâce à ce projet, l'identification et la caractérisation des reproductives rend définition barrières possible la de ponts interspécifiques qui permettront de mieux gérer les ressources génétiques d'*O. glaberrima* tant sur le plan de leur utilisation que sur le plan de leur conservation.

#### Introduction

# O. glaberrima, une ressource génétique pour l'amélioration variétale du riz

O. glaberrima Steud. est une espèce de riz cultivé dont l'importance économique est limitée à l'Afrique de l'Ouest. Deux agroécotypes principaux sont observés, un type flottant tardif et photosensible cultivé dans les plaines inondables et un type dressé précoce et insensible à la photopériode qui est cultivé en culture pluviale ou en zone de bas fonds modérément inondée (Bezançon 1993). L'évaluation de la diversité des riz sauvages et cultivés en Afrique a confirmé clairement qu'O. glaberrima a été domestiqué en Afrique de l'Ouest à partir de l'espèce sauvage annuelle O. breviligulata A. chev. et roehr (= O. barthii A. Chev), (Second 1982) bien longtemps avant l'introduction d'O. sativa L. dans cette même région (Portères 1950). Depuis, l'espèce africaine de riz cultivé a été progressivement remplacée par O. sativa à cause de son faible potentiel de production dû à une grande sensibilité à la verse et à l'égrenage spontané. O. glaberrima se rencontre encore néanmoins en culture pure dans certaines conditions écologiques marginales (riz flottant au Tchad et au Mali, riziculture de mangrove en Guinée maritime). Les évaluations génétiques d'O. glaberrima concordent pour affirmer que sa diversité est faible quelque soit les marqueurs étudiés : isozymes (Second 1982), RFLP de l'ADN nucléaire (Wang et al. 1991), ou cytoplasmique (Dally et Second 1990; Second et Wang 1992). Au contraire, la domestication indépendante de deux lignées divergentes d'O. rufipogon Griff. a donné naissance chez le riz cultivé asiatique O. sativa à deux grands groupes variétaux indica et japonica fortement structurés sur le plan génétique et analogues à deux sousespèces (Second 1982).

De nombreux échantillons d'*O. glaberrima* ont été collectés par l'ORSTOM-CIRAD, l'ADRAO et l'IITA pendant les 20 dernières années (Ng et al. 1983). Ils ont constitué le matériel de base pour l'étude de la diversité génétique (Second 1982), des barrières de stérilité F1 avec *O. sativa* (Pham et Bougerol 1989) et des relations génétiques avec *O. breviligulata* (Bezançon 1993). L'ADRAO en Côte d'Ivoire détient actuellement une collection de base de plus de 1300 échantillons issus de ces prospections et qui font l'objet d'une caractérisation et d'une évaluation agronomique permanente (Jones et al. 1994). De nombreuses caractéristiques intéressantes ont été identifiées dans certaines variétés : résistance au virus de la panachure jaune du riz (RYMV), (Attere et Fatokun 1983; John et al. 1985), résistance aux insectes : Diopsis thoracica (Alam 1988; Sauphanor 1985), résistance à des insectes foreurs des tiges (Chilo zacconius et Maliarpha separatella, B. Vercambre, données non publiées) résistance à la cecidomyie africaine du riz provoquée par Orseolia oryzivora (Jones et al. 1994). Également, O. glaberrima présente des résistances à certaines espèces de nématodes endoparasites (Diomandé 1984; Reversat et Destombes 1997). Enfin, O. glaberrima présente aussi des caractéristiques de développement végétatif précoce qui lui permettent d'être plus compétitif vis à vis des adventices (Jones et al. 1994) et une meilleure adaptation à des stress abiotiques comme la salinité, la sécheresse, la toxicité ferrique (Sano et al. 1984). Malgré toutes ces caractéristiques intéressantes, l'utilisation des riz africains s'est souvent limitée à l'obtention des premières générations de recroisement sans jamais réussir à transférer un seul trait favorable chez O. sativa. Cette difficulté d'utiliser O. glaberrima en amélioration des plantes tient à la très forte stérilité pollinique observée chez les hybrides interspécifiques (Pham et Bougerol 1989; Sano 1985; Oka 1974). Des modèles génétiques basés sur des systèmes d'interaction sporo-gamétophytique ont été proposés pour rendre compte de la rémanence de la stérilité et de l'accélération du retour vers les types parentaux chez les plantes dérivées d'hybridations interpécifiques (Sano 1985). De fait, l'isolement reproductif entre les deux espèces semble être extrêmement strict et ne permet pas l'observation d'essaims d'hybrides spontanés lorsque les deux espèces sont en sympatrie. Récemment, l'ADRAO en Côte d'Ivoire a développé avec succès les techniques d'haplométhode sur des plantes issues de recroisement et a obtenu du matériel fixé qui est en cours d'évaluation agronomique (Jones et al. 1996).

# Les possibilités offertes par les marqueurs moléculaires cartographiés pour contrôler les introgressions à partir d'O. glaberrima

réaliser l'introgression des La difficulté de caractères d'O. glaberrima peut être réexaminée désormais grâce à de nouveaux moyens méthodologiques. Le développement des marqueurs moléculaires directement issus de l'ADN a permis de construire des cartes de liaisons génétiques très complètes dans le cas du riz (Causse et al. 1994; Kurata et al. 1994). Ces cartes constituent maintenant un outil précieux pour étudier les phénomènes de restriction à la recombinaison, de distorsion de ségrégation, et les relations morphologie-viabilité dans les produits d'hybridations entre les deux espèces de riz cultivé. Depuis deux ans l'ORSTOM a initié avec l'ADRAO un projet d'introgression génome d'O. glaberrima. L'objectif est systématique du d'identifier et de suivre à travers un programme de recroisement assisté par les marqueurs moléculaires l'introgression de petits fragments d'O. glaberrima d'environ 15-20 cM dans un même fond génétique d'O. sativa. A terme, il s'agit de construire ces introgressions sous forme de lignées "contigs" ou les fragments introgressés se chevaucheront pour permettre de représenter la totalité du génome d'O. glaberrima (fig.1). Cette approche systématique et non ciblée a priori semble appropriée pour i) détecter et cartographier toutes les caractéristiques intéressantes venant d'O. glaberrima, ii) informer valablement sur les mécanismes et les gènes intervenant dans les barrières de reproduction, iii) proposer à terme une nouvelle ressource génétique sous de formes de lignées fixées distribuables à n'importe quelle institution nationale ou internationale de recherche, iv) intégrer ce matériel dans un schéma global mieux gérer les permettant de ressources génétiques d'O. glaberrima sur le plan de leur utilisation en amélioration des plantes comme sur le plan de leur conservation. Les premiers résultats concernant le développement de cette stratégie originale sont présentés.

# Résultats

# Développement du matériel génétique approprié

La première étape de ce projet a eu comme objectif d'établir une population issue d'un recroisement interspécifique dont la cartographie permettra d'établir une carte de liaison génétique de base et de choisir les individus pour lesquels les recroisements seront poursuivis afin d'initier le processus d'introgression systématique. Pour cela, la variété IR64 qui est une variété irriguée sélectionnée à l'IRRI a été retenue comme parent O. sativa. Le choix du parent O. glaberrima s'est orienté sur une variété particulière, photosensible qui présente le grand intérêt de manifester une résistance totale au virus de la panachure jaune du riz (RYMV). Ce choix permettait ainsi d'envisager comment un caractère de résistance pourrait être transférer chez O. sativa au delà des barrières reproductives séparant les deux espèces. Plusieurs hybrides interspécifiques ont été obtenus. Ces hybrides sont complètement mâle-stériles et ont été intensivement recroisés avec IR64 comme parent récurent pour aboutir à une population de taille non limitante (183 individus) et initier la cartographie des marqueurs.

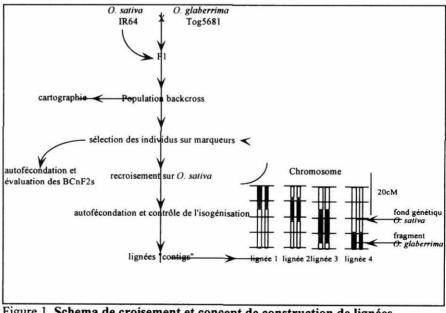


Figure 1. Schema de croisement et concept de construction de lignées isogéniques d'O.glaberrima sous forme de lignées "contigs".

Cette population se caractérise par une très forte stérilité pollinique. Une fraction importante de plantes présentent des graines en autofécondation mais celles-ci s'accompagnent dans la majorité des cas d'une détérioration plus ou moins importante de l'albumen. Seules 3 plantes ont donné des descendances de graines normales en autofécondation. Il semble ainsi qu'en plus des phénomènes de stérilité, les barrières reproductives peuvent comporter des termes supplémentaires plus tardifs lorsque les fécondations mettent en jeu l'autopollen des plantes car les recroisements de deuxième génération avec IR64 donnent des graines normales. Sur le plan morphologique, la population backcross est fortement déviée vers des plantes sensibles à la photopériode, pigmentées avec un type de panicules semblable à celui d'O. glaberrima. Tous ces caractères sont fortement associés et permettent d'individualiser un petit groupe de plantes précoces. non pigmentées qui renferme en outre les rares individus fertiles donnant des graines normales en autofécondation.

#### Polymorphisme et cartographie des marqueurs moléculaires

Le choix des marqueurs s'est orienté délibérément vers des marqueurs à base PCR qui seuls peuvent être compatibles avec les quantités d'analyses et la rapidité d'exécution requises pour un tel programme. Ces marqueurs sont des STS (Sequences tagged-sites) correspondant à l'amplification de l'ADN entre deux amorces spécifiques déterminées aux extrémités de sondes RFLP cartographiées et issus d'ADN génomique ou d'ADNc. Ces marqueurs présentent comme les marqueurs RFLP l'avantage d'être locus-spécifique et codominant. En outre. si les électrophorèses ne permettent pas de séparer les produits d'amplification et d'identifier directement un polymorphisme entre les deux parents, ces produits peuvent être digérés par des enzymes de restriction de l'ADN pour générer de nouvelles bandes et augmenter ainsi la probabilité de distinguer les parents. Une première série de 63 marqueurs STS (Inoue et al. 1994) correspondants à des sondes d'ADN génomique cartographiées sur la carte de liaison intraspécifique de (Kurata et al. 1994) a été systématiquement testée sur les parents du croisement. Une deuxième série de 250 STS localisés sur la carte de référence

interspécifique (Causse et al. 1994) est en préparation à l'IRRI (Ghareyazie et al. 1993) et une centaine d'entre elles ont pu être également testées sur les parents. Le polymorphisme de longueur séquence unique (SSLP - Simple Sequence Length de Polymorphism) est aussi une autre source de marqueurs particulièrement bien adaptés à ce programme. Ce polymorphisme provient d'un nombre variable de répétitions de courtes séquences nucléotidiques (di, tri ou tétra-nucléotidiques) et les marqueurs microsatellites contenant ces séquences sont très fréquents et largement distribués dans le génome du riz (Panaud et al. 1995; Wu Tanksley 1993). Ils présentent aussi l'avantage d'être et codominants et peuvent être amplifiés par leurs amorces PCRspécifiques. En outre, ces marqueurs manifestent des variations alléliques beaucoup plus élevées que les marqueurs RFLP et RAPD ce qui permet de les utiliser même dans des croisements proches. 120 marqueurs microsatellites ont déjà été identifiés cartographiés sur le croisement de référence (IR64 x Azucena), (McCouch et al. 1996). Les séquences des amorces d'autres microsatellites cartographiés sont également disponibles (Akagi et al. 1996).

**Table 1.** Bilan du polymorphisme observé pour des marqueurs à base PCR (STS et microsatellites) entre les parents du croisement interspécifique (*O. sativa* x *O. glaberrima*)

Origine et nombre de marqueurs testés	Polymorphisme (direct)	Polymorphisme (après. digestion)	Marqueurs utilisables en cartographie
65 STS (génomique)	12,3%	33.8%	14
90 STS (génomique + cDNA)	6,7%	-	3
20 STS (génomique + cDNA)	-	35%	3
44 microsatellites	88,7%		36
Total			56

Les premiers résultats concernant l'évaluation du polymorphisme entre les parents sont présentés dans le tableau 1.

Compte tenu du temps de divergence et de l'isolement reproductif entre les deux espèces de riz cultivé (Second 1982), un polymorphisme significativement plus élevé que celui observé par (Ghareyazie et al. 1993) parmi les variétés d'O. saliva était attendu. Néanmoins, le polymorphisme détecté par les STS est faible même en augmentant la résolution avec des gels d'acrylamide. Le polymorphisme est augmenté légèrement en digérant les produits d'amplification mais seuls un petit nombre de marqueurs de ce type seront utilisables en cartographie. Au contraire, les premières évaluations sur les marqueurs microsatellites montrent un polymorphisme très élevé qui permettra la cartographie d'une majorité d'entre eux. La cartographie des marqueurs a commencé et donne déjà des informations intéressantes sur les phénomènes de distorsion de ségrégation dans cette population. Ainsi, le STS G30 sur le chromosome 6 montre une distorsion extrême en faveur de l'allèle porté par O. glaberrima puisque la fréquence de celui-ci est supérieure à 92%. Cette distorsion suggère que, chez les hybrides F1, la stérilité agit au niveau femelle et peut traduire la proximité d'un gène de stérilité sporo-gamétophytique de type "gamete eliminator" chez O. glaberrima. Ce gène pourrait correspondre au gène S10 identifié par (Sano et al. 1994) à proximité immédiate du gène wx sur l'extrémité du chromosome 6. En effet, le marqueur G30 est à moins de 10 cM de wx sur la carte intraspécifique de Kurata et al. (1994). En outre, les rares plantes présentant l'allèle venant d'O. sativa pour le marqueur G30 appartiennent toutes à ce petit groupe de plantes précédemment individualisé sur le plan morphologique.

Évaluation de la diversité utile d'O. glaberrima en vue de son transfert chez O. sativa

Parallèlement au développement de la population de cartographie, les parents ont été évalués comparativement pour différents caractères comme un préalable à l'évaluation ultérieure des descendances et à la recherche de marqueurs de ces caractères. Deux caractères agronomiques particulièrement importants pour la riziculture africaine ont été étudiés : la résistance au virus de la panachure jaune du riz (RYMV) et la résistance au nématode *Heterodera sacchari*. Étant donné la forte stérilité des plantes issues de recroisement et pour éviter la perte des génotypes en cas de forte sensibilité, 20 individus ont été dédoublés à un mois et demi pour être évalués pour la résistance au RYMV. De même, des boutures de noeuds (3 à 5 par individus) ont été prélevées sur 21 plantes âgées de 3 mois et demi pour fournir le matériel nécessaire à l'évaluation de la résistance à *Heterodera sacchari*.

# Résistance à la panachure jaune du riz causé par le RYMV

La panachure jaune du riz a été identifiée pour la première fois au Kenya (Bakker 1971) et s'est rapidement répandue dans l'ensemble du continent Africain et à Madagascar où elle est à l'origine de dégâts très importants en riziculture irriguée (Awoderu 1991). L'agent de cette maladie est un sobemovirus qui est transmis par différentes espèces de coléoptères (Chrysomelidae spp), (Hull 1988). Le RYMV a un pouvoir infectieux très élevé, il est présent chez de nombreuses espèces de graminées sauvages y compris l'espèce sauvage de riz O. longistaminata A. Chev. & Roehr. et peut être en plus transmis mécaniquement (Thresh 1991). Dans ces conditions, l'identification et le transfert de résistances naturelles représentent une solution réaliste pour lutter contre ce virus. L'évaluation de la résistance variétale est basée sur des inoculations artificielles à des stades précis à partir de souches de virus de référence multipliées sur des variétés très sensibles (BG90-2). La concentration en virus est ensuite mesurée par des test-Elisa (Clark et Adams 1977) plus ou moins longtemps après l'inoculation et le contenu en virus peut être confronté avec l'observation des premiers symptômes. Plusieurs variétés d'O. glaberrima ont été testées comparativement à deux variétés d'O. sativa de référence, IR64 (sensible) et Azucena (résistante).

Lorsque les lignées sont testées très précocement, (inoculation 10 jours après semis et test-Elisa 7 jours après inoculation), la concentration virale est très élevée chez IR64 alors qu'elle est faible chez Azucena. Parmi les différentes variétés d'*O. glaberrima* qui ont été testées, seule la variété Tog5681 montre un contenu en virus extrêmement faible même avec une souche originaire du Burkina Faso réputée plus agressive que les autres souches de virus. Le suivi de la concentration virale au cours du temps montre des évolutions très différentes suivant les lignées testées. Chez une variété très sensible comme IR64, le contenu en virus est déjà maximum au 7e jour après l'inoculation et les premiers symptômes sont nettement visibles à partir du 10e jour.

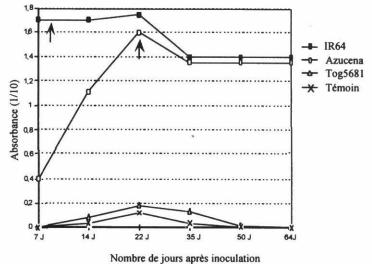


Figure 2: Evolution de la concentration virale en fonction du temps chez deux lignées témoins d'O. sativaet chez la lignée résistante **d**. glaberrimaTog5681. La fléche indique l'apparition des premiers symtômes.

Au contraire, la résistance d'Azucena et des variétés considérées comme résistantes chez O. sativa se traduit par une progression plus lente de la concentration virale et une apparition plus tardive des symptômes associée à un impact plus faible de la maladie sur la floraison et la fertilité (Ghesquière et al. 1997). Dans ces conditions, la variété Tog5681 présente un contenu en virus très faible qui reste stable pendant toute la vie de plante même longtemps après la floraison et sans jamais montrer de symptômes ni d'effet sur la croissance des plantes inoculées (fig. 2). Ce type résistance chez O. glaberrima qui s'apparente à une quasi "immunité" n'est pas fréquente et seules de rares lignées d'O. glaberrima la manifeste (Paul et al. 1995). L'étude de l'hérédité de la résistance au RYMV chez O. glaberrima à partir d'une analyse diallèle a montré par ailleurs que cette résistante était de nature récessive avec une forte composante génétique additive (Paul et al. 1995). Des boutures d'hybrides F1 (IR64 x Tog5681) et de 20 individus issus de recroisements sur IR64 ont été réalisées et testées suivant des conditions similaires aux parents. Tous les individus testés montrent des concentrations virales très fortes et du même ordre qu'IR64. Les résultats confirment donc bien la nature récessive de la résistance provenant d'*O. glaberrima*. Celle-ci implique vraisemblablement peu de gènes au vu de l'homogénéité de la réponse des plantes issues de recroisement.

# Résistance à Heterodera sacchari

Le nématode Heterodera sacchari a été décrit pour la première fois comme parasite de la canne à sucre (Luc et Merny 1963) mais c'est sur le riz pluvial et inondé qu'il a été identifié comme un pathogène très important dans différents pays Afrique (Merny 1970; Babatola 1983a). L'évaluation variétale n'a pas permis d'identifier de variétés résistantes chez O. sativa (Babatola 1983b) ; en revanche, des variétés résistantes à plusieurs souches de ce parasite ont été identifiées chez O. glaberrima (Reversat et Destombes 1997). L'évaluation de la résistance repose sur des inoculations par un inoculum connu de juvéniles fraîchement éclos sur de jeunes plantules cultivées sur milieu stérile dans des microserres ou des tubes individuels qui permettent d'assurer un isolement complet de chaque individu. Au bout de 5 semaines (1ere génération) ou 11 semaines (2eme génération) de contact avec l'inoculum, les kystes visibles sur les racines sont dénombrés et disséqués à l'aide de permanganate de potassium qui provoque leur éclosion (Reversat 1981). L'évaluation peut être complétée par une mise du système racinaire sous asperseur qui permet de récupérer les juvéniles. La population finale obtenue peut être ainsi comparée avec l'inoculum (population initiale) et permet d'apprécier le coefficient de multiplication du nématode sur l'espèce ou la variété-hôte considérée. L'évaluation d'une collection représentative de riz (43 variétés d'O. sativa et 21 d'O. glaberrima) montrent que toutes les variétés d'O. sativa sont très sensibles à Heterodera sacchari quelque soit leur origine géographique ou leur type variétal avec des coefficients de multiplication allant de 11 à 25. Au contraire, une proportion importante de variétés d'O. glaberrima (70%) est résistante avec un coefficient de multiplication inférieur à 1 (Reversat et Destombes 1997). Cette résistance chez le riz cultivé africain s'observe également chez son ancêtre sauvage direct O. breviligulata puisque 7 souches sur les 9 testées sont résistantes (Reversat et Destombes 1997). L'étude des lignées d'O. sativa et d'O. glaberrima impliquées dans les croisements cartographiés a été répétée dans une nouvelle évaluation plus précise impliquant 7 répétitions ; celle-ci a confirmé à nouveau l'absence de kystes visibles sur les différentes lignées d'O. glaberrima testés et en particulier sur le parent O. glaberrima Tog5681 (Tab. 2).

Tableau 2 : Evaluation de la résistance à l'espèce de némadode *Hérodera sachari* chez*O. sativa, O. glaberrima* et des descendances interspécifiques 5 semaines après inoculation.

Lignées ou descendances	Nb d'individus ou génotypes	présence de de kystes	population finale de nématodes
O. sativa			
Moroberekan	7	Présence	2098
IR64	7	Présence	1644
Azucena	7	Présence	2186
0. glaberrima			
CG14	7	Absence	1,8
CG20	7	Absence	1,4
SG329	7	Absence	0,3
Tog5673	7	Absence	0,3
Tog5681	7	Absence	0,1
BC1 : (IR64 x Tog5681) x R64	3	Absence	0
(boutures)	9	Présence	167
1 BC1F2	13	Absence	0
(graines)	4	Présence	221

Le test de boutures prélevés sur un échantillonnage de 21 plantes issues du recroisement interspécifique montre que certains individus ont un comportement analogue à *O. glaberrima* suggérant que cette résistance pourrait être dominante. Ces résultats doivent être pris avec prudence car l'hétérogénéité des boutures entraîne un développement plus ou moins tardif des racines qui peut ne plus correspondre avec la période d'infectivité de l'inoculum et induire une classification erronée d'individus dans la classe des plantes

résistantes. Néanmoins, la descendance de l'une des rares plantes fertiles a pu être testée suivant le protocole standard sur graines et confirme bien une ségrégation entre des plantes résistantes et sensibles en accord avec un gène de résistance dominant.

## Discussion

L'identification des barrières reproductives représente un facteurclé dans un programme d'introgression avec O. glaberrima, car la restauration de la fertilité est indispensable d'une part pour évaluer les descendances interspécifiques vis à vis des caractères cibles et d'autre part pour continuer les recroisements avec les plantes retenues. Chez le riz, de très nombreux gènes de barrière reproductive avant des modes d'action variables ont été décrits. Ainsi des gènes gamétophytiques dupliqués expliquent la stérilité pollinique F1 observée dans les croisements entre variétés indica et japonica d'O. sativa (Oka 1974). Dans les combinaisons avec O. glaberrima, les systèmes de stérilité sporo-gamétophytique ont pour caractéristique de pouvoir intervenir également sur le gamétophyte femelle et donc pour conséquence de pérenniser la stérilité même au cours des recroisements successifs. Loin d'être dispersés sur le génome du riz, de nombreux gènes de stérilité intervenant dans les barrières reproductives sont localisés sur le chromosome 6. La distorsion observée pour le marqueur G30 témoigne vraisemblablement de la présence d'un de ces gènes de stérilité sporo-gamétophytique. Lorsque l'on compare la position de marqueurs communs aux différentes cartes de liaison génétique disponibles (wx, G30, C, Est-2), la zone distale du chromosome 6 est très sensiblement plus petite dans le recroisement entre O. sativa et O. glaberrima par rapport à ce que l'on observe entre les mêmes marqueurs sur les cartes de référence intraspécifique ou même interspécifique. Sur le plan morphologique, la forte déviation de la population est également conforme avec la présence de gènes gouvernant certains caractères concernés par les distorsions sur le chromosome 6 (gène de photosensibilité Se-1, gène C, codant pour le chromogène qui permet la pigmentation anthocyanique de certains organes de la plante), QTLs intervenant sur la ramification des panicules (Pham 1990). Ainsi, la liaison génétique de ces caractères avec des gènes de stérilité extrêmement puissants peut rendre compte des relations morphologie-viabilité et des

restrictions à la recombinaison sur les secteurs chromosomiques concernés.

Théoriquement, l'introgression chez O. sativa d'un caractère lié à un "gamète eliminator" présent chez O. glaberrima sera favorisée au cours des recroisements successifs mais sera lié à une stérilité très forte. Inversement si le gène provoquant la stérilité est présent chez O. sativa, il ne sera pas possible d'introgresser un gène utile issu d'O. glaberrima. Les travaux de (Sano 1985) à partir de lignées isogéniques suggèrent la présence simultanée de tels gènes sporo-gamétophytiques à la fois chez O. sativa et O. glaberrima. L'observation de graines dont l'albumen est détérioré dans les autofécondations des plantes issues de recroisement peut témoigner de phénomènes de complémentation entre ces gènes et des effets de dosage défavorable dans l'albumen triploïde. De fait, la restauration de la fertilité des croisements entre O. sativa et O. glaberrima varie beaucoup suivant les combinaisons mises en jeu. Certaines variétés d'O. glaberrima de Casamance (Sénégal) sont plus aptes à donner des descendants fertiles alors que d'autres variétés sont très difficiles à croiser et ceci quelque soit le parent O. sativa concerné. En outre, il semble exister une relation entre l'aptitude à l'androgénèse et la possibilité de donner des descendants fertiles. Les croisements avec les variétés pluviales du groupe *japonica* sont plus faciles et permettent une restauration de la fertilité nettement meilleure que les combinaisons avec des variétés indica (Jones et al. 1996). La comparaison des distorsions de ségrégation entre descendances issues naturellement de recroisement et issues d'haplométhode pourrait renseigner valablement sur l'acquisition de la stérilité par les microspores et si l'androgénèse permettrait de court-circuiter certaines barrières reproductives. Enfin, il est observé que des descendances interspécifiques fixées peuvent être recroisées plus facilement que ce soit avec des variétés très difficiles à croiser d'O. glaberrima ou des variétés indica d'O. sativa (M. Jones, comm. pers.). Ceci suggère donc qu'au cours des générations avancées, les "gametes eliminator" sont fixés et peuvent jouer un rôle efficace de ponts interspécifiques vis à vis d'autres lignées d'O. glaberrima.

La cartographie du génome d'*O. glaberrima* permet de mettre en évidence des points chauds renfermant des gènes de barrière reproductive dont l'identification est indispensable pour déterminer l'utilisation potentielle de l'espèce africaine de riz en introgression avec *O. sativa*. Les résultats soulignent également

l'importance de travailler sur des effectifs élevés permettant par marqueurs moléculaires de détecter de rares recombinants favorables pour lesquels la fertilité ne serait plus limitante. Ceux-ci peuvent fournir les descendances nécessaires pour l'évaluation des caractères intéressants et l'étude de leur hérédité dans un contexte génétique interspécifique. Dans cet objectif, une vingtaine d'individus ont été recroisés avec IR64 pour fournir la deuxième génération tout en limitant la dérive en génome d'O. glaberrima. La cartographie génétique a permis déjà d'identifier différents chromosomiques pour lesquels segments un processus d'introgression est envisageable grâce aux marqueurs moléculaires. L'obtention de lignées d'introgression fixées connues et la caractérisation des barrières reproductives prennent tout leur intérêt dans la mesure où d'une part il n'est pas envisageable de reproduire cette approche systématique sur un grand nombre de lignées différentes d'O. glaberrima et où d'autre part, les caractères utiles susceptibles d'être introgressés sont très largement dispersés dans les variétés d'O. glaberrima. L'utilisation de ponts interspécifiques bien caractérisés offre à terme deux avantages en gestion des ressources génétiques : premièrement, celui de pouvoir constituer des populations sources dont la diversité génétique serait plus facilement mobilisable en amélioration des plantes (prebreeding) et deuxièmement celui de fournir les bases rationnelles d'une conservation dynamique des ressources génétiques d'O. glaberrima sous forme vivante (conservation in situ ou conservation à la ferme).

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# Interspecific Hybridization between *O. sativa* and *O.longistaminata* to Develop a Perennial Upland Rice

# Véronique Schmit

Plant Breeding, Genetics and Biochemistry Division International Rice Research Institute, P.O.Box 933, Manila, Philippines.

# Abstract

The objective of developing a perennial upland rice is to contribute to limiting erosion problems in South-East Asia, focusing on areas where slash and burn is still used, and where, because of population increase, the fallow period is too short to allow restoration of soil fertility. The possession of traits such as strong rhizomes which allow survival during the dry season, vigorous regrowth at the beginning of the rainy season and resistance to nematodes made the African wild species (*O. longistaminata*) attractive for the research. *O. longistaminata* has been little used in breeding programs perhaps because of the crossing barrier which causes sterility. Another potential donor of perenniality is *O. rufipogon*.

A special scheme of crossing was designed to account for the mode of transmission of the rhizomes, while avoiding the crossing barrier. A series of backcrossings were made to obtain hybrids with rhizomes. After further intercrossing, recurrent selection was applied to pool interesting genes and eliminate unwanted traits.

A total of 718 hybrid seeds from backcrosses of *O. sativa* and 783 seeds from backcrosses on *O. longistaminata* were obtained showing a success rate varying from 1.4 to 5.2% for *O. sativa* and 1.1 to 15.6% for *O. longistaminata*. For the complex crosses, 435 hybrid seeds were obtained.

Preliminary results show that the hybrids germinate well but seeding vigor is lower in backcrosses with O. longistaminata. None of the F1 plants in the backcross with O. sativa produced rhizomes, though they were vigorous and presented high variability for many traits. On the other hand, the F1 plants in the backcross with longistaminata had long panicles, long awns and rhizomes, but only few seeds have been obtained at this initial stage. Domestication of wild perennial species, through improvement of agronomic traits of O. longistaminata populations introgressed with genes from O. sativa, will be utilized as an alternative method to inter-specific hybridization for developing perennial upland rice. This will be in collaboration with WARDA.

# Introduction

Wild relatives of rice are a valuable source of interesting genes for the cultivated species. They have been used mainly as a source of resistance genes to pests and diseases, in the case of O. longistaminata as a donor of the Xa-21 gene of resistance to bacterial blight (Khush et al. 1990) This African wild species belongs to the same genome (A) as cultivated rice, O. sativa and O. glaberrima. However it is separated from the other species of the A genome by a crossing barrier which causes the abortion of the hybrid embryos. For that reason, perhaps, the species has been little used in breeding programs. O. longistaminata is an allogamous species with, generally, a high rate of self-incompatibility, the cross pollination being favoured by the morphological traits of the reproductive organs (anther and stigma length and exertion). These traits are useful in the production of hybrid rice and have been successfully transferred to O. sativa (Taillebois, 1983; Taillebois and Guimaraes, 1987). In developing a perennial rice for the upland ecosystem, other traits of this wild perennial species are of interest: it has strong rhizomes which allow survival during the dry season and vigorous regrowth at the beginning of the rainy season; it possesses resistance to nematodes, an important trait in a perennial crop; the allogamy would confer a better adaptability to highly variable environments like the uplands.

The long-term objective in developing a perennial upland rice is to limiting erosion problems in Southeast Asia, focusing on areas where slash and burn is still used and, because of population increase, the fallow period is too short to allow restoration of soil fertility. Our target area will be where the slopes are moderate to steep, the rains are heavy and the dry season not too long nor too severe. A perennial rice would be planted in hedgerows or as a cover crop, improving the stability of the upland subsistence farming systems and providing a source of food as well. The costs in work and external inputs would be minimal.

Attempts have been made in different species of the *Poaceae* family to develop perennial varieties (cultivars), and two main strategies have been used:

- interspecific hybridization of the cultivated species with wild perennial relatives to transfer the perennial traits

- domestication of the wild perennial species through agronomical improvement of wild populations, preferably introgressed with genes from the cultivated species.

The first technique has been used in wheat, rye and sorghum with different degrees of success (see Wagoner, 1990, for a review, for rye: Reimann-Philipp (1996), for sorghum: Kulakow and Ennis (1988), Piper and Kulalow (1993). The domestication of perennial grasses has been intended during the last 15 years in the U.S. and has been concentrated on four species: intermediate wheatgrass (*Thinopyron intermedium*), wildrye (*Leymus arenarius*, L. racemosus), saltgrass (*Distichlis palmeri*) and eastern gamagrass (*Tripsacum dactyloides*). Saltgrass and intermediate wheatgrass are already sold to consumers (Wagon).

In rice, the efforts of Chinese scientists to introduce perenniality in irrigated rice varieties with the idea of creating a perennial hybrid rice has not led so far to the selection of improved varieties (Li Qinxiu, personal communication). Although various wild rice species are perennial (table 1), *O. longistaminata* is an ideal potential donor of perenniality because of several reasons: the rhizomes allow resistance to drought (Second, 1985; Second et al. 1977); it belongs to the same genome as *Oryza sativa* and once the initial F1 hybrid has been obtained, it can be easily backcrossed to both parents; populations naturally introgressed with genes from *O. sativa* are available; hybrid material has already been developed by ORSTOM and was available to start the research program.

Species	Chromosomes	Genome	Habitat	Preferred light
	number	group		Requirement
O. rufipogon	24	AA	Vegetative	Full sun
			crown and	
			stoloniferous	
O. longistaminata	24	AA	Rhizomatous	Full sun
O. officinalis	24	CC	Usually	Partial shade to full
			rhizomatous	sun
O. rhizomatis	24	CC	Rhizomatous	Full sun
O. eichingeri	24	CC	Vegetative	Partial shade
			crown	
O. minuta	48	BBCC	Stoloniferous	Partial to full shade
O. punctata				
(tetraploid)				
O. alta	48	CCDD	Vegetative	Full sun
			crown	
O. grandiglumis	48	CCDD	Vegetative	Full sun
			crown	1999 - 1999 -
O. latifolia	48	CCDD	Vegetative	Full sun
5 68 6	5.2		crown	
O. australiensis*	24	EE	Rhizomatous	Full sun
O. schlechteri	48	unknown	Stoloniferous	Partial to full shade
O. ridleyi	48	unknown	Vegetative	Full sun
			crown	
			and	
			stoloniferous	
O. longiglumis	48	unknown	Vegetative	Full shade
			crown	
			(n ?)	
0. granulata	24	x	Vegetative	Full shade
			crown	
O. meyeriana	24	x	Vegetative	Full shade
			crown	

Table 1. Wild perennial species of the genus Oryza, their chromosome number, genome group, habitat and light requirements

\*biennial-perennial

Source: Vaughan (1994)

**Table 2.** Phenotype and genotype of *O. sativa* and *O. longistaminata* parents, the F1 and the backcrosses.

Parent or hybrid	Genotype <sup>1</sup>	Presence of rhizomes
О.	<u>D1d2</u>	Rhizomes
longistaminata	D1d2	
O. sativa	d1D2	none
	d1D2	
F1	d*d2	none
	d1d2	
BCS	d*d2, d1D2	none
	d1D2, d1D2	
BCL	(a) d*d2	(a) Rhizomes (b) late
	(b) d1D2	abortion resulting in
	D1d2	shrunken seed
	D1d2	

 $^{1}$ d\* = inactivated D1

Another potential donor of perenniality is *O. rufipogon*. Its resistance to drought is being investigated and the preliminary results are promising.

In *O. longistaminata*, the reproductive barrier is due to the action of 2 complementary dominant lethal genes that cause the abortion of the embryo (Chu and Oka, 1970; Ghesquière, 1988) D1D1 is present in *O. longistaminata* and D2D2 is present in *O.sativa*, and induce differential levels of albumen deterioration according to their dosage in the triploid endosperm. D1D1D2 induces early deterioration with no visible seed. D2D2D1 produces late deterioration resulting in shrunken seeds.  $F_1$  hybrids can be obtained through embryo rescue and show no rhizomes, high tillering, photoperiod insensitivity, low male fertility, variable female fertility, and continuous production of panicles.

The genes controlling the transmission of rhizomes are tightly linked if not identical to the genes controlling the reproductive barrier. Ghesquière (1988, 1991) proposed the intervention of an inactivating transposable element in the genetic control of the reproductive barrier, assuming that in F1 the D1 allele becomes inactive and is no longer complemented by D2. The fact that the rhizomes are lost in F1 and show nearly unilateral inheritance in their backcross offspring supports that hypothesis: in the backcrosses on *O. longistaminata*, the rhizomes are restored; in backcrosses on *O. sativa* and their subsequent selfed progenies, the rhizomatous habit is definitely lost (Table 2).

The development of a perennial rice is a long-term research. Many traits have to be pooled in order to create a crop adapted to specific farming systems, with resistance to pests and diseases and tolerance to abiotic stresses like drought and acid soils, frequent in the uplands of Southeast Asia. However, the exploitation of the variability of wild perennial species like *O. longistaminata* and *O. rufipogon* will certainly lead to short term outputs.

# Material

The materials we used in crosses were obtained from ORSTOM and IRRI. ORSTOM provided us with the F1 (BS125 x WL02) an almost completely sterile hybrid (7.8% pollen fertility); some *O. longistaminata* accessions and several BC<sub>L</sub>F<sub>2</sub> plants obtained through backcrossing of the F<sub>1</sub> to different *O. longistaminata* accessions; and selfing of the BC<sub>1</sub>F<sub>1</sub>. This material is listed in tables 3 and 4, as well as some characteristics. At IRRI, the upland rice breeding program provided us with seeds from 10 elite upland varieties from different origins, which are listed in table 5.

# Methods

#### Crosses

Due to the mode of transmission of the rhizomes, a special scheme of crossing has been designed in order to keep the rhizomes while avoiding the crossing barrier. The scheme we are following, as well as the possible genotypes and phenotypes of the products of those crosses, are presented in table 6. The F1 is backcrossed on the 10 upland cultivars, used as pollen parent. Then the BC<sub>s</sub> hybrids are crossed with the BC<sub>L</sub>F<sub>2</sub> provided by ORSTOM and presenting a reasonable pollen fertility (Table 4) and a good expression of rhizomes. The BC<sub>s</sub> hybrids are used as female parent to avoid lethal genes complementation in the product of the crosses and early embryo abortion. After obtaining the complex BC<sub>s</sub>A x BC<sub>L</sub>F<sub>2</sub> hybrids, individual hybrid plants with rhizomes will be selfed and the F2 progeny will be evaluated in the field in order to select plants with rhizomes and interesting agronomic traits. These plants will be intercrossed and a recurrent selection will be applied in order to try to cumulate the interesting genes and to eliminate the unwanted traits

The  $F_1$ , *O. longistaminata* and  $BC_LF_2$  plants received from ORSTOM were multiplied trough cuttings and planted in big pots. In the case of the backcrosses on the  $F_1$ , emasculation is not necessary because of the sterility of the  $F_1$ . In further steps, the mother plant is hand emasculated using forceps. The crossed panicles are bagged to avoid contamination with foreign pollen and shattering of the seeds.

# Evaluation for resistance to tungro and nematodes

Beside the on-going screening of various *O. longistaminata* and O. rufipogon accessions from the IRRI genebank for perenniality and resistance to drought, the *O. longistaminata* used as donor of perenniality have been screened for resistance to tungro and to nematodes, in collaboration with the phytopathology division.

Table 3.Pollen fertility and rhizomes expression in the O.longistaminataaccessions used in the crossing program

O. longistaminata accession	Origin	Pollen fertility (%)
SL313-13	Senegal	35.7
SL313-13	Senegal	16.6
UL59-1	Cameroun	Not yet flowering
DLO1-1	Burundi	21.6
WL02-2	Botswana	77.5

Hybrid number <sup>1</sup>	O. longistaminata used	Pollen fertility	Rhizomes
	for the backcross	(%)	expression <sup>2</sup>
BCLAF <sub>1</sub> -3	SL313-19	7.4	
BCLAF1-5*	SL313-19	47.2	++
BCLAF6-1*	SL313-19	5.3	++
BCLAF6-2*	SL313-19	14.3	++
BCLAF6-5*	SL313-19	10.0	
BCLAF10-1*	SL313-19	13.8	++
BCLAF15-2	AL2b-1	0.5	
BCLAF18-4*	SL313-13	33.1	++
BCLAF19-2*	SL313-13	10.9	++
BCLAF19-4*	SL313-13	6.4	++
BCLAF20-1*	AL2b-1	19.0	++
BCLAF21-2	UL59-1	1.3	++
BCLAF21-5*	UL59-1	7.8	++
BCLAF21-6	UL59-1	4.5	
BCLAF21-7	UL59-1	0.5	++

**Table 4.** Pollen fertility and rhizomes expression in the  $BC_LF_2$  individuals used in the crosses

<sup>1</sup> selfed backcross on O. longistaminata \*hybrids used as pollen parent in the complex crosses

<sup>2</sup> ++ = very typical rhizomes; -- = few very short rhizomes, or rhizomes developing lately

Table 5. Characteristics	of	the	upland	cultivars	selected	for	the	breeding
program								

Variety	Isomyc group	Characteristics
IRAT 216	VI	Improved, selected in Africa, adaptability, good traits of upland cultivars but close to irrigated ones, medium height
IR60080-46A	VI	Improved, selected at IRRI, resistant to blast, good vigor
IR63371-38	VI	Improved, selected at IRRI, resistant to blast, good vigor
IR63380-08	VI	Improved, selected at IRRI, resistant to blast, good vigor
Azucena	VI	Traditional, aromatic, well adapted
Cuiabana	VI	Improved, selected in Brazil, resistant to blast and drought, earliness, good grain shape
IRAT104	VI	Improved, selected in Africa, close to traditional varieties, medium height, resistant to blast
IRAT212	VI	Improved, selected in Brazil, short, earliness, resistant to blast
WAB56-50	VI	Improved, selected in Africa, adaptability, resistant to blast
Vandana	I/II	Good Indian variety, resistant to drought, yield stability

# **Results and discussion**

Most of the material planted at the end of July 1995 started to flower in October 1995, except UL59-1, which could not be used in backcrosses. This accession is strongly photoperiod sensitive and was still in the booting stage at the end of November 1996. Backcrosses of the F1 on *O. sativa* and *O. longistaminata* were performed from November 1995 to July 1996.

The complex crosses between  $BC_s$  and  $BC_LF_2$  are being carried out since the beginning of June 1996.

# Hybrid seeds obtained

We obtained a total of 718 hybrid seeds from backcrosses on *O.* sativa and 783 seeds from backcrosses on *O. longistaminata*. The success rates vary from 1.4 to 5.2% for *O. sativa* and from 1.1 to 15.6% for O. longistaminata according to the cultivar/accession used. It is higher for *O. longistaminata*, with an average of 10.9% than for *O. sativa* with an average of 2.6%. These results are different from those obtained by Causse (1989) who backcrossed a natural hybrid between *O. sativa* and *O. longistaminata*, considers like a F1, with 10 cultivars of upland rice from Africa and Brazil. She obtained higher rates of success, between 7 and 16% according to the variety of *O. sativa* used. This difference could be due to the use, in our case, of a true F1 hybrid as male parent and of different upland rice cultivars, to the different methodology used or also to different ecological conditions while crossing. The detailed results are presented in annex 1.

For the complex crosses, 435 hybrid seeds have been obtained so far and the results per female parent (BCS) are presented in table 7. The detailed results (per different BCS parent and male parent) are presented in annex 2.

Female parent	Male parent	Progeny	Genotype(s)	Rhizomes ?
F1	O. sativa	BCS	d1(d*)d1d2D2, d1(d*)d1D2D2	No No
F1	O. longistaminata	BCL	d1(d*)D1d2d2 d1(d*)D1d2d2	Yes Late abortion
BCL	Selfing	BCLF2	D1D1d2d2 d1(d*)D1d2d2 d1(d*)d1d2d2	Yes Yes No
BCS	BCLF2 with Rhizomes	F1 (BCSxBCLF2)	d1(d*)D1d2d2 d1(d*)D1d2D2 d1(d*)d1(d*)d2d2 d1(d*)d1(d*)d2D2	Yes Late abortion No No
F1 with rhizomes (BCSxBCLF 2)	Selfing	f2	D1D1d2d2 d1(d*)D1d2d2 d1(d*)d1(d*)d2d2	Yes Yes No

**Table 6.** Crosses being carried out to develop a perennial upland rice, possible genotypes, and phenotypes of the progeny

**Table 7.** Identification of complex crosses, number of panicles crossed and hybrid seeds obtained.

Female parent	No panicles	No spikelets	No seeds	% success
F1 x Azucena	46	1934	59	3.1
F1 x IR63371-38	105	4310	47	1.1
F1 x IRAT216	71	3019	72	2.4
F1 x IR60080-46A	27	1036	16	1.5
F1 x IR63380-08	51	1970	92	4.7
F1 x IRAT104	53	2008	70	3.5
F1 x WAB5650	61	2482	38	1.5
F1 x Cuiabana	16	640	11	1.7
F1 x IRAT212	28	920	30	3.3
Total	458	18319	435	2.4

The low success rate obtained in some combinations of the BCS x BCLF2 crosses can be explained by the crossing barrier between *O. longistaminata* and *O. sativa* (late abortion when dominant alleles of the two complementary lethal genes are present in the developing hybrid seed) and by the low pollen fertility of the male parents.

# Preliminary observations on the hybrids

In general, the hybrid seeds germinate well, but the seedling vigor is lower in BCL and complex hybrids than in BC<sub>s</sub> and more young plants died in an early stage. In backcrosses of the F1 on WLO2 (*O. longistaminata*), 101 (82%) out of 123 seeds germinated but only 32 plants were obtained (26%).

The BC<sub>s</sub>F1 plants are generally very vigorous and present a high variability, notably in plant height, panicle length, panicle ramification, awning and shattering. None of them shows rhizomes and they don't seem to be photoperiod sensitive. They flower abundantly and show a continuous production of tillers. Many of them present long and exerted stigma that would be useful for the development of hybrid rice, as well as long anthers. Pollen fertility is also variable (table 8). Only 15.2% of the plants can be considered as sterile (% fertile pollen <20) and most of the hybrids (62.9%) had a pollen fertility

Parents		No plants	Average % pollen fert.	Nber plants with % fertile pollen =				
Female	Male			0-20	20-40	40-60	60-80	>80
F1	Azucena	35	40.8	5	12	13	5	0
	Cuiabana	11	34.4	3	4	3	1	0
	IR60080-46A	11	47.4	0	5	2	4	0
	IR63371-38	30	33.4	7	14	7	1	1
	IR63380-08	19	55.3	1	5	4	6	3
	IRAT104	21	40.7	4	7	6	3	1
	IRAT212	7	63.1	0	1	2	2	2
	IRAT216	24	52.9	2	3	12	5	2
	WAB5650	20	42.9	5	2	10	1	
Total	BCS	178	45.7	27	53	59	28	11
				15.2%	29.8%	33.1%	15.7%	6.2%
F1	DL01-1	1	42.4	0	0	1	0	0
	SL313-13	18	31.8	7	5	4	2	0
	SL313-19	1	23.8	0	1	0	0	0
	WL02-2	10	44.4	2	1	4	3	0
Total	BCL	30	35.6	9	7	9	5	0

Table 8. Percentage of fertile pollen in F	1 x O.	sativa (BCS) and F1 x O.
longistaminata (BCL) hybrids	,	

between 20 and 60%. This is again different from the results obtained by Causse (1989): she observed that 65% of the plants had a fertility of less than 20% and the average for all the plants was 18%%. In our case, the average is higher, with 45.7%.

Variability is also observed in heading date. The parental O. sativa cultivars flowered between 13 and 14 weeks after transplanting, while in BC<sub>s</sub>F1 the number of weeks between transplanting and heading varies from 5 to 23 weeks (Fig. 1). As we are using the BC<sub>s</sub> in crosses it is difficult to evaluate the female fertility of the plants. Some panicles were however bagged and seeds harvested from selfing. When the pollen fertility is good, there seem to be no problem to obtain BC<sub>s</sub>F<sub>2</sub> seeds.

The  $BC_LF1$  plants are very high and generally higher than the  $BC_sF1$ . They show generally long panicles, long awns, and rhizomes. The number of weeks between transplanting and heading varies between 11 and 28 weeks. Up to now, only very few seeds have been obtained from BCL plants.

Although it is too early to evaluate the rate of seed setting, according to Ghesquière (1988), the BC<sub>L</sub> plants are able to produce a lot of pollen and many seeds from selfing if the pollen fertility is good. However, it is possible that the backcross on *O. longistaminata* causes a restoration of the self-incompatibility. In our case, the self-incompatibility of some of the *O. longistaminata* parents was recorded in the greenhouse: DLO1-1, SL313-13 and WL02-2 showed a percentage of self-compatibility of 10.3, 0 and 2.7% respectively.

The percentage of sterile plants is higher in  $BC_LF1$  than in  $BC_sF1$ , with 30% of the plants showing less than 20% fertile pollen. The average pollen fertility of all the BCLF1 is also lower than in BCS, with 35.6% fertile pollen. However, 46.7% of the plants show a percentage of fertile pollen comprised between 40 and 80%, and seed setting should not be a problem.

The first complex F1 hybrids have been planted recently, and no observation is available so far.

#### Evaluation for resistance to tungro and to nematodes in O. longistaminata

From the 4 accessions evaluated for tungro, all were susceptible to the *rice tungro bacilliform virus* (RTBV), which is the main causal agent of the tungro symptoms and one accession was also susceptible to the *rice tungro spherical virus* (RTSV).

The results of the tests for nematode resistance showed that 1 accession (WL02, represented by 2 individuals: WL02-2 and WL02-15) is highly resistant to *Meloidogyne graminicola*, the nematode species causing the most important damages in rice crops, as well as one individual from the SL313 accession (SL313-13). SL313-19 showed a variable reaction and DL01-1 was susceptible.

Up to now, no high resistance level to *Meloidgyne graminicola* has been found in *O. sativa* (JC Prot, personal communication). This result is being confirmed at present. As WLO2 has been used as parent in the F1 and in backcrosses, the F! has been included in the confirmation evaluation, as well as the *O. sativa* parent, BS125, a landrace form Guinea-Bissau, which was included as a check. Results will be available very soon.

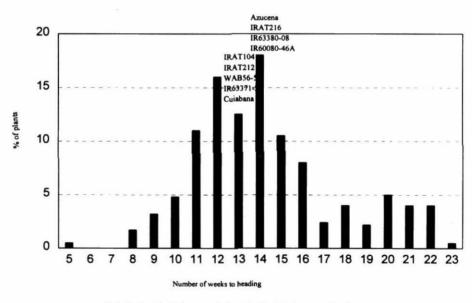


Fig 1. Number of weeks from transplanting to heading in backcrosses on O. sativa

# **Conclusion and future research**

The results of the breeding program are very preliminary, and more time is needed to produce interesting hybrid material for selection of a perennial rice. The F2 from the complex hybrids will be planted in the field and evaluated for rhizome expression and perenniality.

Up to now, we have focused on *O. longistaminata* as a source of perenniality, for the reasons presented before. We have been observing several accessions of *O. longistaminata* and *O. rufipogon* in the greenhouse for more than one year, including a dry period of 3 months. *O. longistaminata* populations have a good tolerance level. In *O. rufipogon*, variability was observed within and between accessions and individuals with good tolerance to drought were selected. These plants will be tested for regeneration of stem cuttings and the best ones will be used in crosses with *O. sativa*. We are also planning to compare the different strategies for perenniality: ratooning (or cespitose base, vegetative crown), production of stolons and presence of rhizomes.

At IRRI, the first methodology, interspecific hybridization, is used to develop a perennial upland rice. An alternative is to domesticate the wild perennial species through improvement of the agronomic traits of *O. longistaminata* populations introgressed with genes from *O. sativa*. This approach is going to be used in collaboration with WARDA: the first step is to evaluate *O. longistaminata* in the field in order to identify introgressed populations or individuals, which will be selected and evaluated for interesting agronomic traits. At the same time, it is a good opportunity to seed increase and rejuvenate the collection for future needs.

A mapping population is being developed in order to identify the genes responsible for perenniality and related traits. To obtain a population segregating for perenniality, the F! has been backcrossed on *O. longistaminata* WL02. The backcrossed plants are being selfed to produce the F2 segregating generation. That population will be mapped using molecular markers, mainly AFLP, and then used for gene tagging. It is hoped that the mapped population will also be useful to identify markers linked to resistance to *M. graminicola*.

*O. longistaminata* has been used as a donor of allogamous traits to develop parents for hybrid rice production (Taillebois and Guimaraes, 1987). An allogamous upland rice is also an option breeders are interested in because of the better adaptability to variable environments like the uplands conferred by allogamy.

At IRRI, BCSF2 hybrid will be tested in upland conditions next season. The hybrid rice program is also interested in plants with long exerted stigma, to incorporate that trait I male sterile and maintainers lines used for the production of hybrid rice.

#### Acknowledgments

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Cross combination	No panicles crossed	Tot no	No hybrid	%success
		spikelets	seeds	
F1 x Cuiabana	90	3698	104	2.8
F1 x IRAT212	119	5283	74	1.4
F1 x IRAT216	28	1158	41	3.5
F1 x IRAT104	37	1860	72	3.9
F1 x IR63380-08	29	1265	47	3.7
F1 x IR60080-46A	36	1767	35	2.0
F1 x IR63371-38	31	1408	73	5.2
F1 x Azucena	35	1915	88	4.6
F1 x WAB5650	82	3760	76	2.0
F1 x Vandana	108	5092	108	2.4
Total F1 x O. sativa	595	27206	718	2.6
F1 x DL01-1	20	1297	14	1.1
F1 x SL313-13	66	3301	381	11.5
F1 x SL313-19	2	142	2	1.4
F1 x WL02-2	56	2470	386	15.6
Total F1 x O. longistaminata	144	7210	783	10.9

Annex 1. F1 x O. sativa and x O. longistaminata crosses, number of panicles crossed and hybrid seeds obtained

nybr	id seeds obtain	ed			
Female parent	Male parent	No panicles	No spikelets	No seeds	% success
F1 x Azucena	BCLAF6-1	2	119	0	0
F1 x Azucena	BCLAF6-2	24	1010	28	2.8
F1 x Azucena	BCLAF6-5	1	42	0	0
F1 x Azucena	BCLAF10-1	12	465	23	4.9
F1 x Azucena	BCLAF18-4	5	230	8	3.5
F1 x Azucena	BCLAF21-5	2	68	0	0
F1 x IR63371-38	BCLAF6-1	1	60	0	0
F1 x IR63371-38	BCLAF6-2	54	2077	17	0.8
F1 x IR63371-38	BCLAF6-5	4	135	0	0
F1 x IR63371-38	BCLAF10-1	20	819	18	2.2
F1 x IR63371-38	BCLAF18-4	22	1096	10	0.9
F1 x IR63371-38	BCLAF21-5	4	123	2	1.6
F1 x IRAT216	BCLAF6-2	33	1428	7	0.5
F1 x IRAT216	BCLAF6-5	1	24	6	25.0
F1 x IRAT216	BCLAF10-1	14	572	46	8.0
F1 x IRAT216	BCLAF18-4	18	806	8	1.0
F1 x IRAT216	BCLAF19-2	2	83	2	2.4
F1 x IRAT216	BCLAF21-5	3	106	3	2.8
F1 x IR60080-46A	BCLAF6-2	15	567	4	0.7
F1 x IR60080-46A	BCLAF6-5	1	25	0	0.7
F1 x IR60080-46A	BCLAF10-1	10	369	12	3.3
F1 x IR60080-46A	BCLAF18-4	1	75	0	0
F1 x IR63380-08	BCLAF6-2	21	725	27	3.7
F1 x IR63380-08	BCLAF6-5	1	48	5	10.4
F1 x IR63380-08	BCLAF10-1	12	520	54	10.4
F1 x IR63380-08	BCLAF18-4	16	646	6	0.9
F1 x IR63380-08	BCLAF21-5	1	31	0	0.5
F1 x IRAT104	BCLAF6-2	30	1131	36	3.2
F1 x IRAT104	BCLAF6-5	1	30	4	13.3
F1 x IRAT104	BCLAF10-1	11	428	26	6.1
F1 x IRAT104	BCLAF18-4	10	388	4	1.0
F1 x IRAT104	BCLAF21-5	1	31	0	0
F1 x WAB5650	BCLAF6-2	28	1123	5	0.4
F1 x WAB5650	BCLAF6-5	5	179	4	2.2
F1 x WAB5650	BCLAF10-1	10	394	18	4.6
F1 x WAB5650	BCLAF18-4	14	632	10	1.6
F1 x WAB5650	BCLAF6-19	14	47	0	0
F1 x WAB5650	BCLAF21-5	3	107	1	0.9
F1 x WAB3030 F1 x Cuiabana	BCLAF6-1	1	43	0	0.9
F1 x Cuiabana	BCLAF6-1 BCLAF6-2	5	173	2	1.2
F1 x Cuiabana	BCLAF10-1	4	144	3	2.1
F1 x Cuiabana	BCLAF18-4	6	280	6	2.1
		9	216	2	0.9
F1 x IRAT212	BCLAF6-2	2	81	4	4.9
F1 x IRAT212	BCLAF6-5	6	223	24	10.8
F1 x IRAT212	BCLAF10-1		400	0	0
F1 x IRAT212	BCLAF18-4	11			

Annex 2: Identification of complex crosses, number of panicles crossed and hybrid seeds obtained

# Amélioration de la Productivité des Variétés Locales de *Oryza. glaberrima* steud. Par Croisements Intraspécifique et Interspécifique avec *Oryza sativa* Linn. au Bénin

**P. Adeyemi\* et S.R.Vodouhé\*\*** \*Agriculturalist - Genetics Rice Breeding, SRCV \*\* Rice Breeder - Director of SRCV

#### Abstract

Improvement in the production of local varieties of *O. glaberrima* is a main potential factor in to increasing yields of rice varieties in Benin. Production improvements in *O. glaberrima* focused attention on this species which has been neglected compared to *O. sativa*. It is, however, a well appreciated staple food for the Gnindé of Atacora in Northern Benin, because it gives them health and vitality.

In order to assess the behavior of local cultivars and varieties introduced by WARDA, INERA and IER, in the framework of the future process of genetic and agricultural improvement at the Food crop Research Station (SRCV) of INA, the agronomic characteristics of the collections were observed and measured.

From the results, 24 varieties were selected at the end of two sets of field trials which were Man Monron and TOG 562 which gave 4.95 and 4.92 t/ha respectively among intraspecific and interspecific crosses between the promising varieties are planned for future activities. The methodology to be used will be the genealogical breeding to develop varieties that will then be released to rice farmers.

# Introduction

Le riz est, après le blé la denrée de grande consommation pour laquelle le Bénin dépend presque exclusivement des importations massives pour satisfaire ses besoins.

La consommatiom très faible dans les années 1960, a augmenté à un rythme très accéléré passant de 8 kg par tête d'habitant en 1994. Plus de 85% des besoins de consommation (environ 50.500 tonnes par an) sont importés malgré l'existence d'un potentiel élevé de production (plus de 130.000 hectares de terres irrigables, auxquelles s'ajoutent de vastes étendues de terres hydromorphes ou exondées aptes à la riziculture) (INRAB, 1996).

Le riz cultivé provient de deux origines: le riz d'origine africaine (*Oryza glaberrima* Steud.) peu productif a tendance à regresser en superficie malgré les nombreuses qualités qu'on lui reconnait tandis que celui d'origine asiatique (*O. sativa L.*) connait une expansion et se rencontre aujourd'hui sur tous les continents.

Au Nord Bénin dans les départements de l'Atacora et du Borgou, certains paysans riziculteurs cultivent toujours des variétés de *O. glaberrima*. Parmi les raisons évoquées par ces derniers, on note:

-la résistance à la sécheresse,

-la rusticité et la capacité de l'espèce à lutter contre les mauvaises herbes,

-la résistance à la pyriculariose et à l'helminthosporiose,

-la bonne vigueur des plants.

La valorisation des collections de ressources génétiques de *O. glaberrima* par une amélioration génétique et agronomique de ses variétés au Bénin permettra d'ouvrir et de mener à bien la gestion de cette espèce.

Pour les Gninde de l'Atacora, la production du riz *O. glaberrima*, est effectué essentiellement dans les flancs collinaires, les plaines alluviales et les bas-fonds non aménagés en culture pure ou associée à d'autres spéculations telles que le maïs ou l'igname.

Cette production a toujours évolué en dents de scie par manque de variétés de *O. glaberrima St.* réellement améliorées. Une amélioration même très sommaire de la productivité de cette espèce, favorisera sa valorisation et sa diffusion à grande échelle.

# Objectifs

# Objectifs généraux

Les potentialités génétiques du riz africain *O. glaberrima* sont encore mal connues et peu utilisées (Berger, 19980; Goli, 1979; Miezan, 1985).

Contrairement à l'espèce *O. sativa, O. glaberrima* n'a fait l'objet d'aucun travail d'amélioration intraspécifique. Des tentatives d'amélioration interspécifique engagées depuis les années 1960 n'ont donné aucun résultat jusqu'à tout récemment, quand l'ADRAO a réussi à obtenir des descendants hybrides fertiles assez performants (Jones, 1996).

Mais en dehors de l'exploitation des gènes de *O. glaberrima* pour améliorer *O. sativa*, il s'avère nécessaire d'envisager également l'amélioration de la productivité de l'espèce africaine elle-même pour satisfaire la demande de nombreux paysans qui y sont très attachés.

# Objectifs spécifiques

Les travaux entamés depuis deux campagnes (1995 - 1996, 1996 - 1997) pour le programme de recherche rizicole à INA portent essentiellement sur l'évaluation botanique, morphologique et agronomique de O. glaberrima.

La collection installée au cours de la campagne 1996 - 1997 est composée de cultivars locaux collectés et testés en 1995, et des variétés de *O. glaberrima* introduites de l'ADRAO (Côte d'Ivoire), du Programme riz INERA (Burkina Faso) et du Programme riz IER (Mali) soit 150 variétés au total.

Le programme prévu pour trois ans grâce à l'appui financier ADRAO/INRAB vise les objectifs suivants:

- bonnes caractéristiques agronomiques des plantes,
- bonnes qualités de grains,
- rendement potentiel en grains élevé,
- stabilité de rendement dans le temps.

Pour atteindre ces objectifs, il est prévu en plus de la sémection directe de matériels dans la collection, de faire des croisements interspécifiques entre les meilleures variétés de *O. glaberrima*. dégagées des tests de comportement. Des croisements intraspécifiques entre les meilleures variétés de *O. glaberrima*. et celles de *O. sativa*. en vue de l'introgression dans *O. glaberrima*. de certains traits favorables pour une bonne productivité. La sélection généalogique sera notre principal outil de travail.

# Matériel et methodes

# Matériel

Les caractéristiques (climat et sols) de la station d'INA où les travaux ont été conduits sont présentés en annexe.

# Cultivars

En 1995, deux missions de prospection et de collecte au niveau du Nord Bénin (Atacora et Borgou) (carte 1 et tableau 1) ont permis d'obtenir un nombre total de 22 cultivars locaux appartenant à l'espèce *O. glaberrima*. Ces cultivars ont été testées la même année.

**Tableau 1.** Cultivars locaux de *O. glaberrima*. collectés dans les départements de l'Atacora et du Borgou (Benin)

No	m vernaculaire	Groupe	Localité	S/Préfecture	Départem.
	des variétés	ethnique			
1.	YAPISSI	BATONOU	GNEKERE BANSOU	KOUANDE	ATAKORA
2.	Ατακουαμα	OTAMARI	KOUPAGOU	BOUKOUMBE	ATAKORA
3.	M'BAHI - 1	FOULFOULBE	DOUAYAKOU	NATITINGOU	ATAKORA
4.	M'BAHI - 2	BATONOU	PEPEYAKOU	NATITINGOU	ATAKORA
5.	IMONDA	BATONOU	N'KOHIN	BOUKOUMBE	ATAKORA
6.	MARO GOUDO	BATONOU	ORUKAYO	KOUANDE	ATAKORA
7.	MONRI SWA - B	BATONOU	ALIBORI-2	BANIKOARA	ATAKORA
8.	OROU KPEHINTE	BATONOU	KOKEY	BANIKOARA	ATAKORA
9.	MONRI SWA - B	BATONOU	INA	DEMBEREKE	BORGOU
10.	NAN NONROU	BATONOU	INA	DEMBEREKE	BORGOU
11.	TOBOROU SARJ	BATONOU	INA	DEMBEREKE	BORGOU
12.	MONRI SWA-1-2	BATONOU	INA	DEMBEREKE	BORGOU
13.	BARO MONRI	BATONOU	TOBRE	PEHOUNKO	ATAKORA
14.	BAROU MONRI	BATONOU	GAMEZI GOUROU	PEHOUNKO	ATAKORA
15.	MON WINGA	NATIMBA	KPESSINE	KOUANDE	ATAKORA
16.	<b>I</b> MONHA IKONFII	OTAMARI	KOUYA	BOUKOUMBE	ATAKORA
17.	<b>IMOUAN IWONHI</b>	GNINDE	KOUTCHATIE	BOUKOUMBE	ATAKORA
18.	YAMONRI YO'MBE	GNINDE	Tassayota	BOUKOUMBE	ATAKORA
19.	MONTCHERE	BATONOU	SEPOUNGA	TANGUIETA	ATAKORA
20.	BOBOGOUDA	OTAMARI	DIKONGUIN	BOUKOUMBE	ATAKORA
21.	MON TAFFIRA	DZERMA	BIRNI	KOUANDE	ATAKORA
22.	MONRI WONKA	BATONOU	INA	BEMBEREKE	BORGOU

En 1996, ils ont été repris en test avec 129 variétés introduites de l'ADRAO, de l'INERA et de l'IER soit un total de 151 cultivars. Une variété de grande culture dans la région

(INARIS-88) a été utilisée comme témoin et intercalée entre chaque lot de dix (10) variétés dans le test.

# Méthodes

Le dispositif utilisé dans les deux campagnes a été le DITER modifié. 151 variétés ont été installées en tout.

En plus du test de la collection, les six (6) meilleurs cultivars dégagés pendant la campagne 1995-1996 ont été semés dans un dispositif de bloc de Fisher à quatre (4) répétitions (essai variétal) au cours de la campagne 1996-1997.

Le semis est effectué le 3 juillet 1996 pour l'essai variétal et les 3 et 4 juillet pour le test, après un labour de 25 cm de profondeur suivi de deux pulvérisages croisés.

Chaque variété est semée sur 3 lignes de 0,25 m entre lignes et 0,20 m sur la ligne. Quatre (4) à six (6) grains paddy ont été enfouis par poquet de 3 cm de profondeur pour la collection, tandis que pour l'essai variétal, chacune des six variétés a occupé une parcelle élémentaire de 6 m x 3 m répétée quatre (4) fois.

La fumure de fond utilisée au semis de 100 kg de phosphate d'ammonique (PA) + l'hectare soit 120 g par parcelle élémentaire pour l'essai variétal et 15 g par ligne pour le test, complétés par 50 kg d'urée à l'hectare. La fumure de couverture, constituée de 100 kg d'urée à l'hectare est utilisée au tallage maximum du riz.

# Observations

Les observations suivantes ont été faites:

- % de levée (
- tallage (60 jours),
- cycle à 50% floraison,
- cycle à maturité,
- hauteur à maturité;
- % verse;
- stérilité des épillets;
- -poids de 1000 grains;
- rendement en grains (en kg/ha)

Pour la collection, certaines observations plus fines ont été effectuées (type de plants, type de grain (couleur de glumelle, présence ou absence de pilosité, aristulation), existence de seconds branchements sur la panicule, longueur de la ligule).

# **Résultats et discussions**

#### Tests de la collection

Les observations faites sur la morphologie des plantes et les caractéristiques agronomiques des variétés ont permis d'identifier certaines variétés *d'O. glaberrima* assez intéressantes.

#### Aspects morphologiques

# Les feuilles

La paille de la plupart des variétés testées est haute. Elle varie de 84,8 cm (5650 TOG) à 163,4 cm (6126 TOG). La variété témoin (INARIS - 88) par contre de taille moyenne (100-125 cm ). Le chaume est fort et épais sur certaines variétés. Les feuilles sont larges et raides pourvues de poils épars ou pratiquement inexistants. L'observation à la loupe binoculaire de la pilosité des feuilles face supérieure et face inférieure montre que les rayures longitudinales des faces supérieures foliaires les rendent plus raides au toucher, cependant les faces inférieures dépourvues de rayures sont lisses. La plus grande masse foliaire est constatée dans les parties basses des plantes (Vodouhé, 1995). Ces feuilles sont retombantes ce qui permet aux variétés de l'espèce *O. glaberrima* (N'Cho, 1992), de bien lutter contre les adventices et de tolérer la sécheresse modérées.

# La panicule

La longueur des panicules est fonction du développement de la plante et des facteurs abiotiques (attaque des maladies et incidence des insectes). Chez un grand nombre de variétés la longueur des panicules est comprise entre 20 cm et 30 cm de même que chez le

témoin INARIS-88. Du point de vue de la forme, presque toutes les panicules sont lâches que celles du témoin. Le nombre de grains par panicule est faible comparativement au témoin. Les branches secondaires ou racemules sont assez rares contrairement à ce qui s'observe sur les panicules des variétés de *O. sativa* où l'on trouve des ramifications secondaires. Ce caractère doit être amélioré car il contribue beaucoup à l'amélioration de la productivité.

L'épillet et le caryiopse

Les glumelles de la plupart des variétés de la collection sont garnies de poils vers leur sommet. Cependant certaines glumelles observées sont glabres. Les épillets de plusieurs variétés sont mutiques néanmoins on observe parfois une tendance générale à l'aristulation. Les épillets et caryopses sont épais et lourds. Le rapport format des grains des échantillons observés se situent bien dans la classification quantitative de la plante (Malle, et Dellecolle, 1977) :

- grains minces > 3,0: 29 variétés au total
- grains moyens 2,1 3,0: 94 variétés
- gros grains < 2,1:22 variétés.

Caractéristiques agronomiques de la collection

# Levée

La levée a été homogène chez toutes les variétés malgré l'effet néfaste des pluies sur certaines variétés (18 au total) le jour du semis. Le pourcentage de levée varie en moyenne de 90 à 95% pour les variétés testées contre 98% pour le témoin. (Tableau 2).

Le tallage

Pour ce caractère, les observations ont été menées à deux différents stades de développement des plantes à savoir :

- nombre de talles à 30 jours après semis (JAS),

- nombre de talles à 60 JAS.

L'analyse des résultats obtenus (Tableau 2) montre que le nombre de talles est lié au stade de développement des plantes et strictement lié aux variétés. Il apparaît aussi que les variétés de O. glaberrima tallent moins que celles de *O. sativa*, néanmoins dans le lot des 22 variétés retenues, les variétés (6209 TOG, 5440 TOG, 5477 TOG, 5997 TOG, Nan Nonrou, 5976 TOG ont un nombre de talles à 60 jours après semis largement supérieur au témoin (Tableau 2)

Quant à l'essai variétal comparatif, trois variétés (Maro Goudo, Azakouama, M'vah-1) tallent mieux que le témoin (Imonda) et sont plus précoces que ce dernier (voir cycle au Tableau 2)

# Hauteur des plantes à maturité

Toutes les variétés sélectionnées sont de grande taille par rapport au témoin. Par contre, les variétés (5573 TOG, 5440 TOG, Monri - SWA, 5635 TOG et 6205 TOG peuvent être classées parmi les variétés de taille intermédiaire (Arraudeau et Vergara, IRRI/IRAT, 1992).

# Réponse à la verse

La verse peut signifier :

- tiges faibles
- trop forte densité de semis,
- variété haute
- fortes pluies et orages,
- problème de sol,
- trop d'engrais (Araudeau et Vergara, 1992).

La taille haute des plantes a beaucoup favorisé la verse dans le cas de la collection. Un accent doit être mis sur ce caractère dans l'amélioration des variétés de *O. Glaberrima*. Le pourcentage de verse obtenu au niveau des deux essais varie de 0 à 100%. Une seule variété n'a pas connu de verse parmi les variétés sélectionnées dans le test. Il s'agit de la 5635 TOG.

Variétés	Levée	Talla ge sur 5 touf fes	Date s à 50% florai son	Date à maturi té (jour)	Haute ur (cm)	Verse (cm)	Poids de 1000 grain (g)	Nbre panic. Pleine sur 5 touf fes	Stérilité paniculaire Nbre panic. Pleine sur 5 touffes
NARIS-88	98	53	82	115	114.4	0	26.3	12.2	1.6
l'émoin)	93	84	91	132	132.2	100	22.6	24	3
440 TOG	93	84	74	120	132	25	23.6	20.2	1.2
477 TOG	90	35	91	125	144	100	23.2	18	7
MONDA	95	43	92	122	132	15	25.3	14.8	1
538 TOG	93	53	78	115	136.4	98	27	17.6	1.6
522 TOG	76	53	67	132	124.4	10	31.4	17	0.4
573 TOG	95	51	69	94	150	100	29.3	21	6
574 TOG	89	61	104	115	152	` 20	21.9	20	2.2
NAN NONROU	95	29	94	120	134.4	100	27.2	16	2
596 TOG	93	31	84	123	136.8	98	29.1	18.2	1.2
5642 TOG	95	25	60	82	126.2	0	27.2	18.4	2
5635 TOG	95	32	89	115	127.6	100	21.7	16.4	0
509 TOG	92	40	79	115	148.4	100	30.6	20.4	1
OROU	89	44	95	125	151.7	100	26.4	16.2	1.2
<b>KPEHINTE</b>	93	38	100	132	149.8	100	25.2	13.2	1.8
5682 TOG	96	70	98	135	155.2	95	29	18.2	1.8
5693 TOG	98	58	50	140	147.6	50	27.9	20	6
5976 TOG	94	44	101	137	140.4	100	23.9	18	2.2
5997 TOG	93	44	77	115	128	95	28	15.8	2.2
MON WINGA	92	32	86	115	149.6	21	23.5	18.4	1.4
5700 TOG	96	42	68	91	126.8	50	23.6	22	3.2
5199 TOG	94	88	71	94	138.4	98	24	24.4	1
5202 TOG 5209 TOG MONRI SWA-I-1	89	52	77	103	123.2	50	24.3	15	2.6
Moyenne	92.5	49.4	82.4	117.2	137.2	67.71	25.9	18.1	2.2

Tableau 2 : Performances agronomiques des variétés sélectionnées. INA, 1996

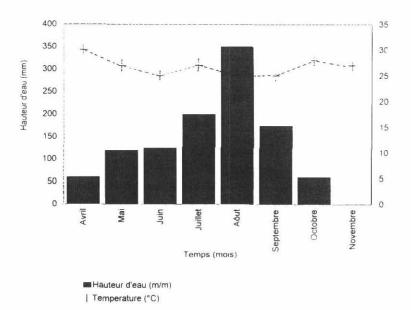


Figure 1. Pluviometrie et température de la campagne d'essai. INA, 1996

# Cycle

## Date à 50% de floraison

Le cycle à 50% floraison varie de 60 jours après semis (5635 TOG) à 104 jours après semis chez le cultivar Na Nonrou. Il est égal à 82 jours chez INARIS - 88. Dans le lot des variétés sélectionnées, on trouve (5635 TOG, 5574 TOG, 5573 TOG et 6209 TOG) qui sont précoces. Les tardives sont constituées de Nan Nonrou (104 jours), 5997 TOG (108 jours), Mon Winga, (108 jours) (Tableau 2)

#### Cycle des plantes à maturité

Toutes les variétés retenues ont en moyenne un cycle supérieur ou égal à celui du témoin. Les variétés précoces ont donné 82 jours (5635 TOG), 91 jours (6205 TOG), 94 jours (5574 TOG, 6209 TOG). Les tardives sont les variétés (5440 TOG, 5573 TOG, 5693 "TOG). Elles sont arrivées à maturité 132 jours après le semis pour les trois premières variétés et à 135, 137 jours après semis pour les deux restantes. On conclut en attendant que les bas-fonds à forte rétention d'eau dans lesquels la hauteur d'eau reste élevée, ont un effet négatif non seulement sur l'épiaison des plantes, mais ne leur permet pas d'écourter leur cycle de développement.

### Aptitude à l'égrenage des panicules

Aucune des variétés n'a résisté à l'égrenage. A la maturité et avant la récolte, une simple pression mécanique faite à la base de la panicule vers le sommet, nous permet d'infirmer que les variétés de *O. glaberrima* égrènent facilement comparativement aux variétés de *O. sativa*. Il serait souhaitable que la récolte du paddy intervienne un peu plus tôt dans les champs à dominance de variétés de *O. glaberrima*.

### Poids de 1000 grains

Chaque stade de croissance contribue au rendement en grains des variétés de riz de bonnes pratiques culturales sont nécessaires pour

permettre aux variétés de *O. glaberrima* d'exprimer leur potentiel de rendement. Dix (10) variétés ont donné chacune un poids grains paddy supérieur à celui du témoin INARIS -88 (26,5 g). Pour le test de collection, il s'agit de 5522 TOG (27,OG), 5573 TOG (31,4G), 5574 TOG (29,3G), 5596 TOG (27,2g), 5642 TOG (29,1g), 5635 TOG (27,2g) Orou Kpehinte (30,0g), 5976 TOG (29,0g), 5997 TOG (27,9 g), 5700 TOG (28,0g).

### Stérilité paniculaire

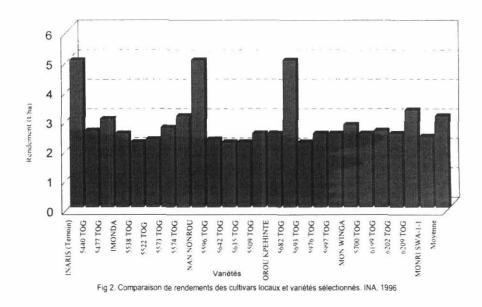
### a) Nombre de panicules

Pour ce caractère agronomique observé sur cinq touffes de plantes quelque jours après la maturité, toutes les variétés sélectionnées ont donné chacune un nombre de panicules pleines supérieur à celui du témoin. Une seule variété la 5693 TOG (13,2) a donné un nombre de panicules pleines assez proche de celui de témoin (12,2).

Le nombre de panicules vides sur cinq touffes varie de 0,0 (5509 TOG) à 7,0 (Imonda). Néanmoins sur les variétés 5997 TOG (6,0), et Imonda, il n'a pas eu d'attaque de maladies ni d'incidence des insectes. Il est souhaitable que les nouvelles variétés à créer aient les gènes de résistance aux maladies et insectes comme la 5509 TOG.

### Rendement en grains

Les résultats de deux campagnes (Tableau 3) montre que les variétés de *O. glaberrima* sont en général moins productives que celles de *O. sativa*. cultivées dans les mêmes conditions. Par contre les variétés Nan Monrou (4,95 t/ha), 5682 TOG (4,92 t/ha) ont été plus proches du témoin INARIS-88 (5 t/ha). Les autres variétés ont donné un rendement allant de 2,16 t/ha (5442 TOG), à 2,85 t/ha (5477 TOG) (Fig.2). Aucune variété n'a excédé le témoin Imonda (2,35 t/ha) dans l'essai variétal comparatif (Tableau 6). Nan Nourou et 5682 TOG sont intéressants et devront être retenus dans un programme d'amélioration de la production de la productivité d'*O. glaberrima*.



#### Essai variétal

Les résultats de l'essai variétal sont présentés au tableau 5. La hauteur de paille a varié de 93 cm chez le témoin Imonda à 111 cm chez Maro Gouda, ce qui indique qu'elles sont toutes de taille courte à moyenne. Les cycles sont également moyens (103 à 116 jours). Les différences entre le témoin et les testées sont significatives pour ces caractères. Les variétés Imonda et

Atakouama ont les grains les plus lourds 30,5 et 30,1 pour 1000 grains, suivies de Maro-Gouda. Ces trois variétés ont d'ailleurs les meilleurs rendements 2,31; 2,26 et 2,25 t/ha dans le même ordre (Tableaux 4 et 5)

Variétés	Rendement (t/ha)	
INARIS-88 (Témoin)	5.00	
5440 TOG	2.57	
5477 TOG	2.85	
IMONDA	2.35	
5538 TOG	2.19	
5522 TOG	2.23	
5573 TOG	2.69	
5574 TOG	2.88	
NAN NONROU	4.95	
5596 TOG	2.40	
5642 TOG	2.21	
5635 TOG	2.21	
5509TOG	2.50	
OROU KPEHINTE	2.50	
5682 TOG	4.92	
5976 TOG	2.19	
5976 TOG	2.35	
5997 TOG	2.49	
MON WINGA	2.73	
5700 TOG	2.23	
6199 TOG	2.45	
6202 TOG	2.52	
6209 TOG	3.26	
MONRI SWA-I-1	2.28	
Moyenne	2.79	

**Tableau 3.** Comparaison de rendements des cultivars locauxet variétés sélectionnés. INA, 1996.

		Nb jr 50%	Nb jr			Poids
Cultivars	Tal.	Flor	100%	Ht	Cycle	1000
	60		Flor.	cm	(J)0	grains
	JAS					(g)
Imonda (Témoin)	35.5b	94.5a	101.3a	93.4b	116a	30.5a
Maro Goudo	38.7ab	89.3c	92.5c	111.3c	108c	29.0b
Atakouama	44.0a	83.3d	88.3d	107.2a	103c	30.1a
M`bahi-2	26.5c	92.7b	96.8b	105.6a	109b	27.0d
Yapissi	32.3bc	84.8d	91.5c	105.2a	103c	28.0c
M'bahi-1	38.3ab	84.3d	88.8d	105.6a	103c	26.0c
ppds<0.05	7.8	1.6	1.3	7.4	0.7	0.9

Tableau 4 : Comparaison des caractères agronomiques des six cultivars

## Tableau 5. Comparaison de rendements des cultivars locaux sélectionnés. INA, 1996.

Variété	Rendement (t/ha)		
IMONDA(témoin)	2.31 a		
MARO GOUDO	2.25 a		
ATAKOUMA	2.26 a		
M'BAHI-2	1.14 b		
YAPISSI	1.13 b		
M'BAHI-1	0.31 c		
Moyenne	1.56		
CV(%)	4.5		

dispositif en bloc de fisher à 4 répétitions les moyennes suivies d'une même lettre ne sont pas significativement différentes au seuil de 5% par le test de duncan.

### **Conclusion et Perspectives**

Les cultivars locaux et les variétés introduites se sont bien comportés en écologie de bas-fond à INA. Il faut également préciser qu'au cours de la campagne de culture de ces variétés, il n'a pas du tout eu d'attaque de maladies, ni d'incidence des insectes comparativement à ce qui s'observe sur les variétés de *O. sativa.* De même la présence des Diopsis dans le bas-fond au stade de tallage maximum des plantes n'a eu aucune incidence sur ces variétés nullement inquiété jusqu'à la récolte. Tout ceci nous a permis de sélectionner vingt cinq (25) variétés à utiliser dans la suite du programme (voir Tableau 8). Certaines de ces variétés sont retenues pour les croisements entre variétés de l'espèce *O. glaberrima* que nous envisageons. Par contre, Imonda, Atakouama et Marogouda seront épurées et remis à la disposition des producteurs en attendant la sortie de descendants plus performants.

Année	Pluvio métrie	Moyennes annuelles	Différence	Nbre Jours de pluies
1986	846	1200	-354	75
1987	843	1200	-357	75
1988	1357	1200	+157	77
1989	1280	1200	+80	84
1990	874,3	1200	-325,7	68
1991	1194,4	1200	-0,6	84
1992	1070,2	1200	-129,8	79
1993	969	1200	-231	65
1994	1478,9	1200	+278,9	88
1995	899,8	1200	-300,2	70
1996	1072,1	1200	-127,9	67

**Tableau 6.** Evolution de la pluviométrie à INA de 1986 à 1996

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### Annexe

### Climat

La station d'INA appartient à la zone agro-écologique soudanoguinéenne caractérisée par deux saisons: une saison de pluies (Avril à Octobre) et une saison sèche (Novembre à Mars) (Tableau 3). Le total enregistré cette année est de 1072,1 mm en 67 jours (fig.1).

La tenpérature moyenne annuelle de la station est d'environ 26°C pour les maxima (SRCV-INA, 1985). Elle varie considérablement au cours de la journée surtout de novembre à janvier, période de la saison où souffle l'harmattan.

L'évapotranspiration potentielle varie d'une année à l'autre (Igue et Youssouf, 1995). Les valeurs minimales sont enregistrées en décembre-janvier et juillet-août (103 à 107 mm) et les valeurs minimales emn mars-avril-mai (1326-140 mm).

Sol

Les sols de bas-fond à INA sont de consistance très dure à l'état sec, plastique à l'état humide. La pénétration radiculaire est limitée à l'horizon humifère (Youssouf, 1990). Ces sols sont sablo-limono-argileux et argilo-limoneux en profondeur.

# Utilization of *Oryza glaberrima* Genetic Resources for Lowland Rice Improvement

B.N.Singh<sup>1</sup>,A.T.Maji<sup>2</sup>,N.Q.Ng<sup>3</sup>, C.Paul<sup>1</sup>, C.Williams<sup>1</sup> and M.N.Ukwungwu<sup>2</sup>

1 WARDA c/o International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria 2 National Cereals Research Institute (NCRI), Badeggi, Bida Niger State, Nigeria 3 Genetic Resource Unit, IITA, Ibadan, Nigeria

Abstract

West African cultivated rice, *Oryza glaberrima* and other five wild rice species are spread across the African continent. They possess many useful genes for resistance to several biophysical stresses. For lowland rice improvement at Ibadan, *O.glaberrima* lines from the Genetic Resources Unit of IITA, were screened for drought and African rice gall midge (ARGM) resistance. Screening for drought resistance was carried out with 1,200 lines at vegetative stage in an augmented design. One hundred and twenty three lines with drought tolerance similar to the check, Salumpikit were further evaluated in a replicated experiment. Eighteen lines had better drought resistance and recovery than Salumpikit.

In another screening trial for ARGM, 150 lines were screened in the field and screenhouse in separate experiments. Four immune lines viz., TOG 7442, 7106, 7206 and 6489 were identified. Genetic analyses of resistant lines to ARGM is under progress. Five other RYMV immune lines viz., TOG 5674, 5675, 5681, 7235, and 7291 from previous screening are being studied for genetic analyses. Single, double and backcrosses between *O. sativa* and *O. glaberrima* lines showed a high degree of sterility in  $F_2$  and backcross populations. There is a need to use anther culture and other innovative breeding techniques to incorporate useful genes from *O. glaberrima* and other wild species into high yielding indica rices.

### Introduction

Oryza glaberrima is a West African domesticated cultivated rice. There are five other wild rice species viz., Oryza barthii, Oryza longistaminata, Oryza brachyantha, Oryza eichingeri, and Oryza punctata present in Africa (Chang, 1976). O. barthii is an annual species while O. longistaminata is perennial and rhizomatous. O. longistaminata is the most widespread of all the Oryza species across the Africa (Ghesquiere, 1986; Ng et al.,1991). Due to its higher yield potential, O. sativa has now replaced most of the cultivated O. glaberrima rice, except in the Niger river flood plains and some of the drought prone uplands and rainfed lowlands. In many fields they are often grown as mixture.

IITA's Genetic Resources Unit has a collection of 2,489 entries of *O. glaberrima* and 270 entries of wild rices from 22 countries in Africa (Ng *et al.*,1991). IRRI has the duplicate set of these collections from IITA, Ibadan, Nigeria. Prior to IITA, many collection missions from Japan, IRAT, ORSTOM and WARDA have been conducted, but these collections are probably not available now. Genetic erosion of *O. glaberrima* and other African wild rice species has been taking place over the years and there is a need to collect the land races and the natural hybrids from both the explored and unexplored areas (Chang, 1989., Abifarin, 1991; Ng et al.,1991). *O. glaberrima* is ocassionally becoming weed in *O. sativa* crops.

*O. glaberrima* as species should be considered for improvement of its production potential. *O. glaberrima* lacks secondary branches in its panicle, and shatters at maturity. But genetic variability exist for other desirable traits and they can be improved *per se*. Under poor management conditions some *O. glaberrima* cultivars have shown better yield than *O. sativa*. Many of the accessions mature in less than 90 days. Early maturity is another trait which can be utilized for developing drought escape in elite indica rice.

At IRRI, African rice lines and wild species have been screened for green leaf hopper (*Nephotettix virescens*), rice tungro virus, white backed plant hopper (*Sogatella furcifera*), brown plant hopper, salt tolerance, and drought resistance and recovery ability. Many landraces are known to have resistance to these stresses (IRRI,1976., 1977; Akbar *et al.*,1987; Chang, 1989). At IITA, screening was carried out for resistance to rice yellow mottle virus (RYMV), stalk eyed fly (SEF), white stem borer (*Maliarpha separatella*), and at WARDA in Liberia, for iron toxicity tolerance. Lines with immunity to RYMV and better resistance than *O. sativa* for drought and leaf hopper have been observed. (Chang, 1976; Virmani,1976; Attere and Fatokun, 1983; Alam, 1988; Abifarin, 1991 b; Paul, 1993; Thottappilly and Rossel, 1993). *O. glaberrima* has also shown resistance to white backed plant hopper which is not found in Africa. Out of 681 *O. glaberrima* lines tested, 45% were found resistant (Chang, 1989).

Efforts have been made in the past to incorporate useful genes from the African rice and wild taxa into *O. sativa* genetic background through conventional breeding methods. Hybrid sterility and aberrant chromosome pairing in hybrids of *O. glaberrima* with *O. sativa* crosses have been described (Chang, 1964; Sano et al., 1980; Sano, 1986). In the present paper, research work on identifying genes for resistance to some bio-physical stresses at Ibadan lowland rice station of WARDA in the past five years are summarized.

### Screening for drought resistance

One thousand two hundred accessions of O. glaberrima from the Genetic Resources Unit of IITA were screened in the 1992 dry season in the screenhouse to identify drought resistant lines in two experiments. In experiment I, all the lines were screened in a non replicated augmented design with five O. sativa checks, among which cultivar Salumpikit was the most resistant check. Others include OS 6, IRAT 13, ITA 235 and IR 20 as susceptible check. The crop was grown with irrigation for 30 days and thereafter irrigation was withdrawn. The reaction of the lines at three levels of water stress is shown in Table 1. The drought reaction was scored using the standard evaluation scale SES (IRRI, 1988). After 23 days of drought stress, when IR 20 had permanent writing, 18 % of the glaberrima lines had similar or better score than the tolerant check, Salumpikit. The experiment was irrigated after 23 days of water stress and scored for recovery ability at 3 and 10 days after rewatering (Maji, 1994). The result showed that 7 % of the glaberrima lines recovered faster than Salumpikit after three days. This number increased to 15 % at 10 days after re-watering (Table 1).

In the second experiment, 123 *O. glaberrima* lines selected from different score groups in experiment I were re-screened in a randomized complete block design with 4 replications. Eighteen lines were better than Salumpikit in drought resistance after 25 days of drought stress, while 14 lines showed better recovery within 24 hours of re-watering (Table 2). These *Oryza glaberrima* lines therefore offer scope for utilization in drought tolerance improvement for *O. sativa* lines (Maji and Singh, 1993).

**Table 1.** Percentage distribution of 1,200 *Oryza glaberrima* lines according to vegetative stage drought tolerance and recovery scores in a screenhouse at three stages of drought development and two stages of recovery at IITA-Ibadan, 1992 dry season (Salumpikit, score 5 and IR20, score 9 for drought tolerance and recovery).

Score SES		ought Tolerar % frequency			y ability Juency
	Days of v	ater stress		Days water	
	9	17	23	3	10
1	1.3	0.6	0.0	0.2	14.7
3	15.4	7.8	09	6.5	34.5
5	52.8	67.8	16.8	34.5	25.6
7	28.0	23.1	55.0	41.0	14.6
9	2.5	0.7	27.2	17.9	10.5
Total	100.0	100.0	100.0	100.0	100.0

\*There was 54 mm rain on the 10th day of drought making the distribution at 9 and 17 days almost similar

### African rice gall midge (ARGM)

Series of experiments were conducted both in the field and in cages to screen *O. glaberrima* lines for ARGM resistance. In 1995 wet season, the first experiment was conducted at three locations in Niger state of Nigeria. Fifty lines of *O. glaberrima* were screened

Accession	Origin	Drought tolerance			Recorvery
					after
					drought
			of water stre		
		10	15	25	
TOG 5389	Nigeria	2.5	3.0	4.0	3.0
TOG 5406	-do-	1.8	2.5	4.0	3.0
TOG 5491	-do-	2.5	2.5	3.5	3.5
TOG 5493	-do-	1.5	1.5	2.5	2.0
TOG 5502	-do-	2.5	2.5	4.0	5.5
TOG 5897	Liberia	1.8	3.0	4.0	3.0
TOG 5983	Nigeria	2.0	2.5	4.0	20
TOG 7106	Mali	2.5	3.0	3.0	4.0
TOG 7176	Senegal	2.0	2.0	2.0	1.5
TOG 7206	Côte d'Iv.	2.5	3.5	4.0	2.5
TOG 7216	Mali	1.5	3.0	4.0	3.5
TOG 7265	Cameroun	1.5	2.5	4.0	4.5
TOG 7353	Nigeria	1.5	2.5	3.0	4.5
TOG 7977	Ghana	1.5	2.5	3.5	3.0
TOG 8005	U.Volta	2.3	3.5	4.0	3.0
TOG 8040	Nigeria	2.5	2.5	4.0	3.0
TOG 8050	-do-	1.5	3.5	4.0	3.5
TOG 8056	-do-	2.5	3.5	4.0	4.0
Salumpikit	Philippines	2.5	3.5	5.0	3.5
IR 20	IRRI	6.5	9.0	9.0	9.0
IRAT 13	Côte d'Iv.	3.0	6.5	8.5	7.0
OS 6	Nigeria	3.8	8.3	9.0	9.0
ITA 306	IITA	4.0	6.0	7.5	6.5
Lsd (0.05)		1.4	1.8	2.0	2.6
CV [%]		35.1	26.7	23.2	38.4

**Table 2.** Eighteen best ranked accessions of *O. glaberrima* for drought tolerance and their recovery ability score (SES, IRRI, 1988).

in the field under natural infestation at Anfani and Gadza, and in a paddy screenhouse at NCRI Badeggi, in a single non replicated design. Five *O. sativa* checks were grown at every 10th row. The checks showed susceptible reaction at all the locations. Location scores and across location means showed that all *O. glaberrima* lines were better than the checks. The average ARGM infestation across three locations was 44 % hill infestation and 8 % tiller infestation. One of the *O. glaberrima* lines, TOG 7442 was

completely free of galls in the three locations, while two other lines, TOG 7106 and TOG 7206 were slightly infested at one and two locations, respectively (Table 3). These three lines and others were grown in seedboxes and tested by artificial infestation in cages in two other experiments in the 1996 dry and wet seasons at IITA, Ibadan. The three lines mentioned above were found to be highly resistant, recording no galls in the two experiments (Tables 4 and 5).

In another experiment, 100 lines of *O. glaberrima* were screened in a paddy gall midge screen house with two *O. sativa* checks using an augmented design. The two checks recorded 30 % and 60% tiller infestation for checks NAHTA 8, ITA 306, at 40 days after transplanting (DAT), respectively. Forty-six lines were scored zero at 40 DAT. These results demonstrate that a high level of resistance to ARGM exists in *O. glaberrima* lines and hence a resource for a genetic solution to the midge problem in sub Saharan Africa.

#### Rice Yellow Mottle Virus (RYMV)

In 1980, two accessions of O. glaberrima were reported to be resistant to RYMV (IITA, 1981). Attere and Fatokun (1983) reported 13 O. glaberrima accessions as being resistant to RYMV. Later, based on the ELISA test, Thottappilly and Rossel (1993) reported five accessions viz. TOG 5379, 5674, 5681, 7235 and 7291 as immune to RYMV. Paul (1993) studied the genetics of resistance in three O.glaberrima lines viz., TOG 5674, 7177 and 7291. Resistance was observed to be controlled by recessive genes. However, allelic studies were not carried out and it was not known whether the genes were the same or different for varietal improvement. In 1995, a project was started to characterize the nature of resistant genes and the allelic relationship in five immune lines viz., TOG 5674, 5675, 5681, 7235 and 7291. All the five lines were crossed to TOG 8040, a susceptible line. F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> lines are being grown to study the gene action. Diallel crosses (excluding reciprocals) are being made to know whether the genes are allelic

**Table 3.** Mean reaction of selected rice lines to natural field infestation of African rice gall midge at three locations in Niger state of Nigeria 1995 wet season. B in parenthesis shows the seed from Bouaké and IB from Ibadan

		Percentage infe	station
Designation	Hill	Tiller	SES
			*
CG 14 [B]	44.0	5.7	5
CG 17	30.3	3.7	3
CG 20	56.7	10.3	5
CG 10	53.0	7.0	5
T2	78.8	2.7	3
ITA 306	93.8	36.0	9
TOG 5860	64.3	19.6	7
TOG 6216	16.7	3.0	3
TOG 6216	36.7	5.3	3
IR 46	85.0	3.1	3
TOG 6508	34.0	5.8	5
TOG 6589 [B]	32.7	6.0	5
TOG 6589 [B]	76.0	11.2	7
TOG 6597 [IB]	27.0	3.6	3
TOG 6629	28.0	4.2	3
CISADANE	81.3	3.9	3
TOG 6629	32.8	5.7	5
TOG 6631 [B]	37.3	5.6	5
TOG 6631 [B]	40.0	6.0	5
TOG 6648 [IB]	30.3	5.7	5
IR 72	88.8	23.5	7
TOG 7106 [IB]	6.7	0.5	1
TOG 7106 [B]	9.3	0.9	1
TOG 7206	3.0	3.6	3
TOG 7442	0.0	0.0	0
Mean	44.2	8.3	
Range	0-93.8	0-36.0	
CV [%]	42.2	58.9	

\* SES, IRRI. 1996

	Percer	ntage Infestation	on score*
Designation	Hill	Tiller	Score*
TOG 5524	25.0	12.5	5
TOG 5681	21.6	8.3	3
TOG 5950	35.0	5.2	1
TOG 6374	92.9	38.7	7
TOG 6346	26.4	5.8	3
TOG 6508	12.5	3.5	1
TOG 6542	64.0	1.5	1
TOG 6597	19.7	5.0	1
TOG 6631	5.1	2.7	1
TOG 7106	0.0	0.0	0
TOG 7198	49.6	14.1	5
TOG 7206	0.0	0.0	0
TOG 7442	0.0	0.0	0
TOG B7684	58.0	16.5	5
CV [%]	77.2	1.0	

**Table 4.** Reaction of O.glaberrima lines to ARGM infestation incages, IITA, Ibadan, 1996 dry season.

\*SES - IRRI (1988)

**Table 5**: Mean reaction of O. glaberrima lines to ARGM in cages,May, 1996, at IITA, Ibadan.

	Percen	tage Infestation so	core*
Designation	Hill	Tiller	Score*
TOG 7106	0.0	0.0	0
YOG 6539	25.0	8.4	3
TOG 6489* (SB)	0.0	0.0	0
TOG 6472	58.4	25.0	7
TOG 6629	8.4	3.0	1
TOG 7206	0.0	0.0	0
TOG 6582	59.1	8.3	3
TOG 7677	12.5	4.2	1
TOG 6489 [LB]	27.3	11.0	5
TOG 6374	43.2	14.8	5
TOG 6342	47.2	19.7	5
TOG 6730	60.0	29.5	7
TOG 7442	0.0	0.0	0
TOG 7198	40.7	12.7	5
CV [%]	99.8	105.2	

\*Poor germination

or nonallelic amongst the parents. Effort will be made to pyramid these genes in O. *glaberrima* and also transfer them to elite *O. sativa* lines.

### Hybridization between O. sativa indica and O. glaberrima rices

To utilize the O. *glaberrima* germplasm in the lowland breeding programme, single, double and backcrosses were made with different donors for blast, drought and gall midge resistance at Ibadan, Nigeria. From 1991 to 1995, 150 crosses were made with O. *glaberrima* as one of the parent (WARDA, 1996). In the majority of the crosses, the O. *glaberrima* line was used as female and indica as male. Reciprocal crosses were also made to transfer desirable traits. Most of the parents used were indica lowland lines.  $F_1$  plants were very vigorous but there was 99.9% sterile spikelets. Few seeds which were of shattering nature in the panicles were made in two cycles. However, the seed set in backcross generations were still very low. Three-way crosses involving two indica lines also had very poor seed set.

### **Conclusion and Outlook**

The results of screening for drought, African rice gall midge and rice yellow mottle virus resistance have shown that a high degree of resistance exist in the *O. glaberrima* lines. The  $F_1$  plants of crosses with *indica* lines, however, had a high degree of sterility. There is a need to isolate the useful genes from *O. glaberrima*, using recent biotechnological tools like RFLP, gene tagging, cloning and transfer of these to high-yielding germplasm.

Repeated backcrossing, use of tropical japonica and indicajaponica lines and anther culture to develop doubled haploid lines will also improve the chances of gene transfer to high-yielding lines. Genetic variability exists for many useful traits like early maturity, grain quality, secondary branches in panicles, plant type and non-shattering. *O. glaberrima* as a species should also be considered for improvement.

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### Bilan de Prospections et Utilisation des Espèces Traditionnelles en Amélioration des Plantes

**Coulibaly M.Y.** Institut des Savanes Bouaké, Côte d'Ivoire

### Abstract

A number of rice collection missions were undertaken from 1974 to 1991 in western, central and southern Africa. Some of the countries visited were Senegal, Mali, Burkina Faso, Côte d'Ivoire, Guinea, Tchad. and Zambia. A total of 3610 accessions were collected as follows:

2.288 O. sativa
805 O. glaberrima
166 O. longistaminata
16 O. brachyanta
18 O. punctata
12 O. eichingeri

### Introduction

Le bilan des prospections de riz réalisées en Côte d'Ivoire depuis 1974 comporte la synthèse sur l'ensemble des prospections et sur le point de la situation des échanges de matériel.

L'utilisation des espèces traditionnelles, comme *O. glaberrima*, suppose réaliser les étapes suivantes :

1) Description et regroupement (classification numérique)

des échantillons collectés en populations phénotypiquement homogènes: par l'observation de nombreux caractères tout au long du cycle de la plante, afin de regrouper des individus ayant des profils de caractères voisins qui peuvent être génétiquement proches. Le but est de constituer des groupes dans lesquels les individus se ressemblent génotypiquement plus entre eux qu'entre groupes afin d'étudier ces groupes à partir de quelques individus pris au hasard dans chaque groupe.

- Evaluation du matériel : Etude de la variabilité génétique des caractères qui intéressent le généticien et le sélectionneur.
- 3) Etude génétique de ces groupes dans des croisements intra et interspécifiques.

### Bilan des principaux résultats

Le bilan comporte la description de l'ensemble des échantillons des prospections entreprises par l'IRAT, l'ORSTOM, et l'IDESSA ; opérés de manière ponctuelle dans les zones équatoriale et tropicale du continent africain :

Afrique Occidentale : Sénégambie en 1974 ; Mali en 1974, 1975, 1977 ; Haute Volta en 1976 ; Côte d'Ivoire en 1976, 1977, 1980, 1989, 1990, 1991; Sénégal en 1977 ; Guinée-Bissau en 1979; Guinée-Conakry en 1979.

Afrique Centrale : Tchad et Nord-Cameroun en 1977 ;

Afrique Australe : Zambie en 1978 ; Tanzanie en 1979.

Ces prospections ont permis de collecter un nombre important d'échantillons appartenant aux différentes espèces cultivées:

*O. Sativa* : cultivars traditionnels de l'espèce asiatique d'introduction ancienne

O. glaberrima : espèce cultivée africaine

*O. breviligulata* : espèce comportant 2 formes : une spontanée et une adventice.

O. longistaminata : espèce allogame à rhizomes.

O.punctata, O. brachyanta, O. eichingeri.

Au total 3610 échantillons ont été collectés dans 12 pays africains dont 7 d'Afrique Occidentale, 2 d'Afrique Centrale et 3 d'Afrique du Sud-Est.

Les cultivars des deux espèces cultivées constituent l'essentiel des échantillons prospectés (78,5%) repartis ainsi : O. sativa (49,2%) et O. glaberrima (29,3%). Les espèces sauvages et adventices (O. breviligulata et O. longistaminata) constituent 19,8% des échantillons. Quelques espèces ne possédant pas le génome A des riz cultivés représentent 1,7% de l'ensemble des échantillons (O. punctata, O. brachyanta, O. eichingeri) Chaque pays hôte de prospection dispose d'un lot des principales espèces collectées et se doit de le conserver soigneusement en constituant une ressource génétique nationale. Les autres échantillons sont répartis entre l'ORSTOM, l'IRAT, et l'IITA pour la conservation à court et moyen termes; l'IRRI pour la conservation à long terme l'IBPGR et l'ADRAO. Dans l'ensemble, la distribution aux organismes intéressés n'a pas suivi le rythme des prospections. Seuls les échantillons collectés à partir de 1977 ont été transmis en quasi totalité au gervex : 86% des échantillons de Côte d'Ivoire, 94% des échantillons de Guinée-Conakry.

### Approche du probème

### A - Choix des objectifs

Quand on fait appel à des espèces peu évoluées, un choix se présente :

. on utilise une caractéristique particulière (compétivité)

pour inclure dans une variété donnée par les méthodes de la sélection classique:

. on retient les meilleurs génotypes (sélection massale)

. on augmente la variabilité (recombinaison)

. on veut synthétiser un individu (idéotype) à partir des individus prospectés qui peuvent présenter chacun un ou plusieurs caractères intéressants mêlés à des caractères défavorables. Le simple jeu des recombinaisons paraît illusoire pour créer cet individu où plusieurs géniteurs seront utilisés.

					Espèces prospecté	es	
PAYS PRO	OSPECTES	Année	Prospecteurs	О.	Ο.	О.	
				sativa	glaberrima	breviliguta	
	Ensemble	1974	Bezançon/Bozza	-	101		53
MALI	Delta	1975	Bezançon/Second	8	41		
	intérieur						
	du Niger						
	Périphérie	1977	Bezançon/Bozza	1		25	1
	du Delta						
	Gambie	1975	Second	31	283	9	30
SENEGAL	Région du	1977	Bezançon/Bozza	1	3	12	8
	fleuve						
	Sénégal						
	Casamance	1978					
HAUTE		1976	Bozza/Second	5	42	3	5
VOLTA							
	Ouest	1975	Bozza	75			
	Centre						
	Nord-						
	Ouest						
	Nord-Est	1976	Bezançon				
8	Nord						
	0	1075					
COTE	Ouest (du	Nov. 1977					
<b>D'IVOIRE</b>	Nord au						

### Tableau 1 : Espèces prospectées

	Centre-	Oct. 1980	Ghesquière/Miezan				
	Ouest						
	Centre	1989					
	Sud-Ouest	1990	Coulibaly	450	25		
	Nord	1991					
TCHAD	R. Sud	1977	Borg/Second	16	2	49	2
CAMEROU	Nord	1977	Borg/Second	14	16	14	7
N							
ZAMBIE	Botw	1977	Bezançon/Second	20		4	
GUINEE	Bissau	1978	Borgel/Bozza	134	47	2	4
TANZANIE		1979	Miezan/Second	54	3	6	
GUINEE	Conakry	1979	Bezançon/Koffi	296	75	5	
GUINEE	Conakry	1982	Koffi/de Kochko	277	97	11	
TOTAL				2288	805	142	163

.

193

				Espèces prospectées				
PAYS PROSPECTES		Année	Prospecteurs	A*	B*	C*	D*	Total
	Ensemble	1974	Bezançon/Bozza	8	2			164
MALI	Delta int.	1975	Bezançon/Second	36				138
	du Niger							
	Périphérie	1977	Bezançon/Bozza	5	7			41
	du Delta							
	Gambie	1975	Second	44				397
SENEGAL	Région du	1977	Bezançon/Bozza	7				31
	fleuve							
	Sénégal							
	Casamance	1978	Bezançon/Bozza					
HAUTE		1976	Bozza/Second					63
VOLTA								
	Ouest	1975	Bozza					75
	Centre			2		6	1	60
	Nord-Ouest	Nov.	Bezançon/Koffi					
	Nord-Est	1976						
	Nord							

TOTAL				166	16	18	12	3610
		1982	Koffi/de Kochko					397
GUINEE	Conakry	1979	Bezançon/Koffi	13				379
TANZANIE	Nie	1979	Miezan/Second	12	2	8	4	89
GUINEE	Bissau	1978	Borgel/Bozza	7				194
ZAMBIE	Botw	1977	Bezançon/second	10				34-
CAMEROUN	Nord	1977	Borgel/Second	10	3	1	I	66
TCHAD	R. Sud	1977	Borgel/Second	8	1	3		91
	Nord	1991						
	Sud-Ouest	1990	Coulibaly					475
*	Centre	1989						
	Ouest							
	Centre-	1980	Ghesquière/Miezan					58
	Sud)							
D'IVOIRE	Nord au							
COTE	Ouest (du	1977	Koffi/Yoboué					409
	Nord							
	Nord-Est	1976						
	Nord-Ouest	Nov.	Bezançon/Koffi					

A\*=O. longistaminata B\*=O. brachyanta C\*=O.punctata D\*=O.eichingeri

### B - Intérêt des prospections

Les prospections permettent de constituer des groupes d'individus proches génétiquement qui subissent les mêmes pressions de sélection pour un milieu donné (les facteurs du milieu peuvent avoir des répartitions spatiales variables : type dressé, type flottant, concurrence avec parasites-photopériode) et présentent une certaine variabilité (multiallélisme).

Chaque groupe est caractérisé par ses fréquences alléliques qui résultent des pressions sélectives qui agissent sur lui dans un milieu donné ; ces pressions retiennent des groupements qui se maintiennent, préférentiellement au cours des générations : les linkats ; chaque individu de la population porte un arrangement allélique particulier au sein du linkat sélectionné (multiallélisme).

Il convient d'être prudent car en fonction des pressions sélectives il peut exister différents types de multiallélismes :

- type dressé : feuilles larges - fines ; tiges mince.

- type flottant : feuilles étroites - épaisses ; tiges épaisses, verse.

### Méthode de sélection

Afin d'éviter de perdre à chaque croisement des recombinaisons intéressantes, la recombinaison de nombreux caractères peut être substituée par la juxtaposition de linkats après une première sélection se faisant au niveau de chaque linkat.

La recombinaison intéresse un ou quelques loci dans lesquels on échange des allèles. Les linkats sont sélectionnés avec une bonne balance interne. Grâce au multiallélisme, il est possible de retenir au sein d'une population l'individu présentant le meilleur arrangement allélique pour le comportement recherché. L'existence et la transmission d'un ou plusieurs linkats sont analysées dans les populations.

L'AGC est testée en mettant la structure à étudier en présence d'autres structures (type polycross).

Cette structure propre à une population est représentée par quelques individus pris au hasard dans cette population.

### The Effect Of Weed Competition On Cultivars Of *Oryza Glaberrima*, *O. Sativa* And An Interspecific Hybrid Rice.

David E. Johnson, Monty P. Jones, Michael Dingkuhn and Moussa C. Mahamane West Africa Rice Development Association, 01 BP 2551, Bouake Côte d'Ivoire

Abstract

Competition from weeds is a major constraint to rice production in the uplands and rainfed lowlands of West Africa. Rice cultivars which are more competitive with weeds would make a major contribution to improving the returns to traditional farming In a field experiment in 1996, a cultivar of Oryza systems. glaberrima, two O. sativa cultivars and an O. sativa x O. glaberrima interspecific hybrid were grown with and without competition with weeds. The growth of weeds and rice was recorded at regular intervals throughout crop duration. The O. glaberrima had less weed growth than the improved O. sativa cultivars by the end of the crop growth. The O. glaberrima produced a larger number of tillers, more leaves, and a higher leaf area index and specific leaf area than the O. sativa cultivars. These characteristics make important contributions to competitiveness with weeds. The interspecific hybrid shared some, but not all of these characteristics with the O. glaberrima.

### Introduction

Upland rice is the major rice production system in West Africa, accounting for about 40% of the total rice area, and the crop is largely grown by farmers with scarce resources. In a farm survey,

weeds were the most cited rice pest and almost 80% of farmers questioned stated they would increase the rice area grown if weeds were less of a problem (Adesina *et al.* 1994). Similar situations are found elsewhere in the world, where upland rice provides the staple for 100 million people (Arraudeau, 1995), including some of the poorest farmers and those in the most fragile ecosystems. In Asia, Latin America and Africa, yield losses in rice caused by weeds are thought to be 15-21% (Oerke *et al.*, 1995) with the highest losses tending to occur in the upland and rainfed lowland systems.

In the humid forest zone of West Africa, rice tends to be grown after the forest has been cut and burnt, and following one or two crops the land is left as fallow. In the savanna zones, the land may be cropped for longer periods before being left to fallow. Increasing demands for land is causing cropping intensification, with either reduced periods of fallow or extended periods of cultivation, and levels of weed infestation are increasing as a result. Farmers usually weed the rice crop at least once, though due to the demands of other crops or labour shortage, this is often delayed. Where weed infestation is particularly severe, a farmer may abandon a portion of the field to focus efforts where there is less weed growth. Commonly, farmers rely on family labour and hand weeding to control the weeds in the crop. Only a small proportion of farmers rely on herbicides to control weeds in rice (Adesina *et al.* 1994).

Asian farmers who replaced traditional, tall rice cultivars with short statured improved cultivars reported that more time was required for weeding, either suggesting that more weeds grew with the modern varieties, or that farmers were expecting a higher return from weeding (Moody, 1979). Differences in the response of rice cultivars to weed competition have been recorded mainly in Asia (Moody, 1979; Garrity *et al.*, 1992), but also in Africa (Merlier and Deat, 1978; Fofana *et al.*, 1995). Studies on different rice plant types in Latin America concluded that high vegetative vigour and tall stature were major factors influencing the competitive ability of rice plants with weeds (Kawano *et al.*, 1974). Similar findings have recently been reported for wheat (Lemerle *et al.* 1996).

In earlier studies in West Africa, within a wide selection of plant types grown in the region, including *Oryza glaberrima*, and traditional and improved *O. sativa*, lower weed weights at harvest

were correlated with those rice cultivars with more tillers and higher leaf area indices in the vegetative stages of growth (Fofana *et al.*, 1995). The *O. glaberrima* while not producing the highest yields under clean weeded conditions, were able to produce better yields and were more competitive with weeds than the majority of the traditional and improved *O. sativa* cultivars tested. The low yield potential, the tendency for the grains to shatter and the crop to lodge has resulted in *O. glaberrima* being replaced in the majority of the cropping systems by *O. sativa*. The competitiveness of *O. glaberrima* with weeds however, is one of the attributes which makes this species attractive for use in breeding programmes (Dingkuhn *et al*, 1996), and recent advances in plant breeding has enabled *O. sativa* to be crossed with *O. glaberrima* (Jones *et al.*, 1997).

Weed competitiveness is an important criteria for the selection, at the West Africa Rice Development Association, for rice cultivars able to produce high and stable yields under low input conditions. Weed competitive rice cultivars will have an important role to play in the upland, hydromorphic, and direct seeded systems of West Africa, where the losses due to weeds tend to be greatest. In order to gain a better understanding of crop growth in competition with weeds, detailed studies were conducted. In these, the cultivars used were representative of distinct plant types of relevance to the plant breeders.

### Methods

The experiment was sited on a free-draining Alfisol, 300 m amsl, near Bouaké in Côte d'Ivoire (5' 06 W 7' 52 N) and grown during the main wet season and sown on June 29, 1996. The site is within the derived savanna zone and had been cleared from natural vegetation in 1994, and maize was subsequently grown. The land was ploughed and harrowed at the end of the dry season in preparation for the experiment. Fertilizer applications to all plots were 20 kg P and 50 kg K ha<sup>-1</sup> to the seedbed, and 46 kg N ha<sup>-1</sup> in two equal split applications between 28 and 56 days after emergence (DAE). Overhead sprinkler irrigation was used to supplement rainfall, to ensure the rice received a minimum of 200 mm of rainfall per month during the experiment. Mean maximum and minimum temperatures (1981-94) for the area were 28.1 and 20.8  $^{\rm o}$  c.

The experiment comprised two factors, rice cultivars and weed management, in a complete randomized block design, with 6 replications. The rice cultivars were: IG10, an Oryza glaberrima; Moroberekan, a traditional O. sativa upland japonica; IDSA 6, an improved O. sativa upland japonica, and V4 (WAB 450-24-3-2-P18-HB) a hybrid O. glaberrima x O. sativa from parents other than those above. The weed management regimes were: W0 =weed free throughout crop, with an application of oxadiazon 0.875 kg ha<sup>-1</sup> followed by hand weeding; W14 = maintained weed free 0-14 DAE by hand weeding, after which weeds allowed to grow; W28 = one hand weeding 28 DAE. The experiment included plots with no rice where weed growth was monitored, and these were managed as for the rice except that in W0, the plots were unweeded. To make the weed growth in the experiment more uniform, 1.5 g of seed of each Ageratum convzoides and Digitaria horizontalis were broadcast on each plot.

Plots were 5 m x 3.5 m and were divided into an area for destructive sampling and another for non-destructive data collection and harvest data. Rice was dibble sown in holes 25 cm apart, with 3 seeds per hole, thinned to two seedlings at 7 DAE. At 21, 35, 49, 63, 77 and 91 DAE, the number of green leaves and number of tillers from 10 rice hills was recorded, and the leaf area index of the canopy measured with a LI2000 meter (Licor Inc., Lincoln, USA). At these dates, in two 0.25  $m^2$  sample areas, the rice plants were cut, separated into stem, green and dead leaf, and weeds were cut at ground-level, separated by species. Specific leaf area (SLA), or leaf area for given dry leaf weight, was measured for the rice cultivars in all treatment combinations. Relative growth rates (RGR) for the exponential and linear growth phases were calculated following Hunt (1978). At harvest, tiller and panicle numbers, the proportion of filled and empty grains and rice plant dry weights, divided into stem and leaf weights, were recorded from 10 hills (0.6  $\text{m}^{-2}$ ) and grain weight was taken from a  $4\text{m}^{-2}$  area.

### Results

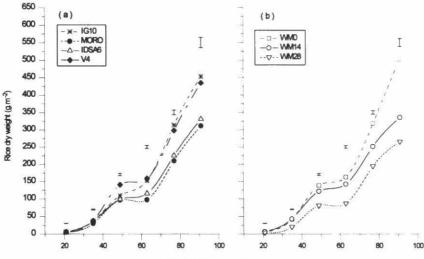
The interaction effects of cultivar and weeding regime were not significant for LAI, SLA, tiller number, rice or weed weight. Across weed management regimes at 49 DAE, V4 had the highest rice plant weight followed by IG10, and by 91 DAE these cultivars had greater plant weight than either Moroberekan or IDSA 6 (Fig. 1). The relative growth rates of the rice between 0-35 DAE, did not differ between cultivars, but across cultivars were least for the plots weeded once at 28 DAE, with values for 10.5, 10.5, and 8.3 % day<sup>-1</sup>, S.E  $\pm$  0.18 for treatments W0, W14 and W28 respectively. Between 35-63 DAE, the *O. sativa* cultivars had lower growth rates of 4.2% for IDSA6 and 4.3% for Moroberekan compared to 5.3 and 5.4% day<sup>-1</sup> (S.E  $\pm$  0.23) for the hybrid and IG10 respectively.

**Table 1.** Specific leaf area (SLA) of rice, weed weight and rice grain yield, main effects of cultivar (across three weeding regimes), Côte d'Ivoire, 1996.

	SLA M <sup>-2</sup> kg	Weed wt g m <sup>-2</sup>	Grain Yield g m <sup>-2</sup>	Relative Yield <sup>1</sup>
	DAE			
cultivar	56	91	_	
IG10	32	58	238	0.74
Morobereka n	22	91	184	0.54
IDSA 6	21	78	197	0.60
V4	21	83	223	0.57
No rice		202	-	-
$SE^2$	0.4	10.2	9.0	0.038

<sup>1</sup> Mean grain yield of plots with weeds (W14 & W28) as a proportion of weed-free plots W0.

<sup>2</sup> Based on 61 df



Days After Emergence (DAE)

Fig 1. The evolution of rice shoot dry weight, main effects of cultivar (a) and main effects of weed management regimes(b), 1996, Côte d'Ivoire. (1 = SE of the means).

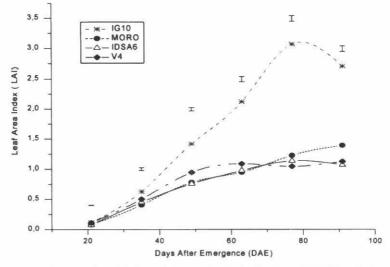


Fig 2. The evolution of leaf area index, main effects of cultivar, 1996, Côte d'Ivoire. (I = SE of the means)

Across weed management regimes, at 21 DAE, IG10 and V4 had the higher LAI than Moroberekan or IDSA6, though by 49 DAE, IG10 had 50% greater leaf area than V4 and almost twice the leaf area of the two *O. sativa* cultivars (Fig. 2). At 49 DAE, IG10 and V4 had almost identical green leaf biomass, and some 22% more than Moroberekan and IDSA 6, which were also very similar (data not presented). The SLA of IG10, however, was

about 50% higher than Moroberekan, IDSA6, or V4. Between 21-35 DAE, IG10 partitioned a higher proportion of the increasing biomass to leaf production rather than stem, with leaf partitioning coefficients for IG10, Moroberekan, IDSA6 and V4 of 0.506, 0.489, 0.468 and 0.432, SE  $\pm$  0.0099. Across weed management regimes, IG10 produced the most tillers, followed by IDSA6, V4 and Moroberekan, and further, IG10 produced the first tillers at 15 DAE, or about 5 days before the other cultivars (Fig. 3).

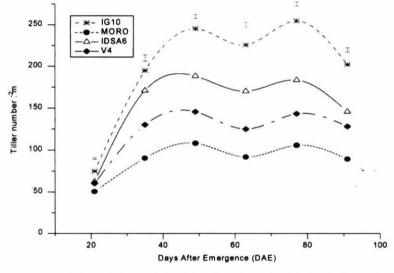


Fig 3. The evolution of tiller number, main effects of cultivar, 1996, Côte d'Ivoire. ( I = SE of the means )

Moroberekan at 120.1 cm was the tallest cultivar, at 84 DAE, followed by V4 117.6, IG10 107.3 and IDSA6 104.1, S.E  $\pm$  2.04. Across weeding regimes, the rice was shortest in W28, and W0 and W14 did not differ in height; there were no interaction effects. At 91 DAE, there was more than twice the weed growth in those plots where no rice was sown, compared to where rice had been sown and where the weeding regimes were identical. The least weed growth occurred in the plots sown to IG10, and this was about a third of the weed growth recorded in plots where rice had not been sown. Across the weeding regimes, V4 and IG10 had a higher grain yield than Moroberekan and IDSA6. Under weed free conditions, there were no significant differences between the cultivars, with V4 giving the highest value. The grain yield in competition with weeds relative to the clean weeded plots (relative yield), was greater with IG10 than the other cultivars.

### Discussion

The O. glaberrima, IG10, had the lowest weed growth in the plots and the most stable yield across weeding regimes among the cultivars grown and this competitive ability with weeds is consistent with the findings of Fofana *et al.*, (1995). In these subsequent and more detailed studies it has been shown that IG10

has high RGR in the vegetative stages of the crop and partitions a higher proportion of the increasing biomass to the leaves compared to other cultivars. The high SLA of IG10 compared to the other cutivars, enables the production of a greater leaf area for a given leaf biomass and gives a considerable advantage in the early establishment of a leaf canopy. The *O. glaberrima* x *O. sativa* hybrid, V4, shared a number of characteristics with IG10, such as the high biomass accumulation, and early establishment of a high LAI, but did not however have the advantage of the higher SLA. In the clean weeded plots V4 produced the highest grain yield, but this was considerably reduced as a result of competition with weeds.

Koffi (1980) reported the high tiller production of O. *glaberrima* and a capacity to produce tillers after a late hand weeding, which was not found in O. *sativa*. Under the conditions that prevail in many low input situations, tiller production is an important characteristic. Tillering enables the rice plant to adapt to variable plant populations and "expand into gaps" in the crop area, a situation common in upland fields where establishment can be variable. An ability to tiller early, facilitates rapid ground cover, while late tillering enables the crop to recover after a late weeding. In this experiment, V4 did not show the high tillering capacity of IG10, and was intermediate between IDSA6 and Moroberekan. Future work will seek to identify the ability, among other O. *sativa* x *O*. *glaberrima* hybrids, for increased tiller production under low input situations.

It is clear that the *O. glaberrima* genepool has the potential to introduce a range of characteristics such as rapid establishment of a crop canopy, good suppression of weeds and good yield stability in

competition with weeds. To introduce these characteristics into hybrids of *O. sativa* and *O. glaberrima*, without sacrificing the potential for high grain yield in good growing conditions and resistance to other pests and diseases remains a major task. The breeding programme to develop suitable "low management" plant types is currently producing a large number of progeny from *O. sativa* and *O. glaberrima* parents. Early studies indicate that among the progeny there is substantial variability in their adaptation to low input situations and competition from weeds. To fully exploit these materials, improved mass screening methods for weed competitiveness are required to identify desirable plant types at an early stage in the breeding process. As such methodologies do not exist currently, this is the subject of intensive study at WARDA.

### Acknowledgement

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# **III PROSPECTS:** STRATEGIC PLANNING MEETING ON

THE INTERSPECIFIC HYBRIDIZATION OF RICE IN AFRICA

Perspectives: Réunion sur la stratégie de la Planification de l'hybridation Interspecifique du riz en Afrique

1



# **Official Opening Address**

## Mamadou Diomandé

Director, Centre Ivoirien de Recherche Technologique (CIRT) and member, WARDA Board of Trustees, Representing the Ministry of Higher Education, Research and Technology Innovation, Government of Côte d'Ivoire

Your Excellency, distinguished participants, ladies and gentlemen.

Professor Saliou Touré, the Honorable Minister of Higher Education, Research and Technology Innovation of the Government of Côte d'Ivoire, sincerely wished to chair this important international scientific meeting. But alas he is not able to be here today due to other professional obligations. I have the privilege of having been designated by him to represent him and deliver this message.

I present my warmest welcome to all participants of this strategic planning meeting organized in the context of the 'Common Agenda' signed between the Governments of Japan and the United States of America with the objective of enhancing South-South partnership to accelerate agricultural development in Africa. Indeed, the enhancement of South-South partnership in the field of scientific research and technology transfer is a key element in the strategy of the Ministry of Higher Education, Research and Technology Innovation of the Government of Côte d'Ivoire, under the dynamic direction of the President Henri Konan Bédié. We are thus very pleased to participate in this meeting. The theme chosen could not be more appropriate: the creation of new rice varieties through interspecific crossings of *Oryza sativa*, rice of Asian origin, and *O. glaberrima*, rice of African—particularly West African – origin.

Since the 1970-1980s, Côte d'Ivoire has been well aware of the real and potential importance of the African *O. glaberrima* and traditional varieties of *O. sativa*, which are still widely cultivated by our rice farmers, particularly in upland rice production. Côte d'Ivoire has, through the genetics laboratory of ORSTOM (France) based in Diopodoume at the time, and IDESSA (Institute des savanes, Côte d'Ivoire), gathered a very wide range of *O. glaberrima*, O. sativa and other *Oryza* species obtained through different collecting missions of genetic resources, across Côte d'Ivoire and in other West African countries (Burkina Faso, Mali, Niger, Guinea, Guinea-Bissau, Senegal, and the Gambia), and in central and eastern African countries (including Cameroon, Chad and Tanzania).

Preliminary results of the work done on this important plant resource very rapidly revealed real capacities in *Oryza glaberrima* for resistance to stresses: insects, nematodes, drought, etc. Although some of these results have been well known for more than a decade, and in spite of WARDA's progress in this field, the attempts to transfer these important traits to *O. sativa* have not yet produced the desired results when using traditional methods.

With the emergence of biotechnology, new and more effective instruments can now be used for a better exploitation of interspecific crossings of *Oryza* plant types in general, and *O. sativa* and *O. glaberrima* types in particular.

It is in this context that this meeting is of the utmost importance. It is the first time a planning meeting involving several key research institutes of Africa, Asia and America has been organized to define policies and strategic directions with the aim of accelerating the creation of interspecific varieties and ensure their rapid transfer to African, Asian and America farmers.

I am delighted by such progress and simply express my sincere congratulations. As regards to our contribution, Côte d'Ivoire actively participants in this common endeavor. I hope that you will continue to acknowledge and include national research systems, which are endowed with capacities and infrastructures that will certainly be useful for the success of this challenging project. Before concluding, permit me to thank the Government of Japan which graciously accepted to finance these high level research activities. I would also like to thank the united Nations Development Programme (UNDP) for its constant support to the technological development of Africa. I also convey my congratulations and encouragement to the West Africa Rice Development Association (WARDA) which is slowly but surely contributing to rice self sufficiency and food security in West Africa.

With these comments, I would like to end my remarks and wish you success in yours deliberations. I declare the strategic planning meeting open. Thank you very much.

# **Opening remarks**

His Excellency Mr. Hiromi Sato Ambassador of Japan to Côte d'Ivoire

Mr. Chairman, Distinguished participants, Ladies and gentlemen.

First of all, I would like to extend my warm congratulations to Dr. Kanayo F. Nwanze for his appointment as Director General of the West Africa Rice Development Association (WARDA).

It is a great pleasure for me to participate in this strategic planning meeting on the interspecific hybridization of rice and I would like to thank you for giving me the opportunity to make some remarks on behalf of the Government of Japan.

The Government of Japan, which works towards the economic development and the improvement of the welfare of African countries, continues to strengthen its cooperation with sub-Saharan African countries.

Self-sufficiency in food constitutes, in particular, a priority element in the development of a country. Increasing rice production, in which the countries of this sub-region have shown a great interest, will contribute to reducing the importation of food products, especially rice. This will help improve the deficit of balance of payments.

In addition to increased bilateral food products assistance and the promotion of food production programs under Grant Aid to West African countries, the Government of Japan cooperates with various international organizations, including WARDA.

I would like to elaborate further on our cooperation efforts with WARDA. Financial contributions by Japan to WARDA's activities has been increasing year after year, from US\$200,000 in 1980 to

US\$2 million in 1993. In 1995, Japan disbursed more than US\$2 million to WARDA. Japan is now WARDA's single largest donor.

Japan's cooperation with WARDA dates back to 1978 when we began to provide technical assistance, concentrating in particular on the important area of post-harvest technology. Since then, five Japanese experts have served at WARDA. I am pleased to mention that Dr .Hideo Watanabe, a grain quality expert, has been a member of WARDA's research team since May 1996.

Last January, during my visit to the WARDA headquarters and main research center near Bouaké, I was strongly impressed by the results already obtained in interspecific hybridization between African and Asian rice species. I would like to congratulate all the researchers who are working on this project. I believe this project will greatly contribute towards increasing rice production in this sub-region and in African in general.

According to the United Nations, 33 out of the 48 least-developed countries in the world are in Africa. The promotion of economic development in this region is thus an issue of the highest priority for the international community. While per capita GNP increased by an average of four percent among developing countries as a whole between 1980 and 1992, it decreased by an average of 1,8 percent among African nations. This fact indicates that poverty continues to be a serious problem for this part of the world.

If African nations are to achieve self-sustained growth, the international community must provide them with the support they need to formulate and implement their own appropriate development policies. It is necessary also to assist their democratization efforts and to strengthen the support of the international community in the basic social sectors tat are most likely to be affected when economic reforms are undertaken.

It was from this perspective that Japan hosted the Tokyo International Conference on African Development (TICAD) in 1993. The conference adopted the Tokyo Declaration on African Development, which expressed agreement on the importance of self-help efforts and good governance, and on the need for a 'new global partnership' within the international community for undertaking development activities. As announced at TICAD, Japan has organized follow-up meetings to capitalize on Asian experiences in African development. It has also provided active support to Africa in five priority areas, namely democratization, economic reform, human resource development, environmental protection, and greater effectiveness and efficiency in providing assistance through policy dialogues.

To review the progress made since TICAD and to further drive the momentum of efforts toward African development within the international community, Japan proposes that the second meeting of the Tokyo International Conference on African Development (TICAD II) be held in or around 1998, and its preparatory meeting in 1997, both in Tokyo. Japan would like to have high-level participation from African countries as well as Asian ones in these meetings.

Furthermore to promote the development of human resources in Africa, Japan supports the international community's goal of primary education for all African children by the year 2015. In this context, I am pleased to confirm Japan's intention to provide assistance amounting to US\$100 million over three years for the purpose of expanding education in Africa. Also with regard to development, human resource Japan intends invite to approximately 3000 trainees to Japan from African countries over the next three years for technical training courses, including those in the agricultural sector, and to make available US\$2 million out of its contribution to the Japan-UNDP Human Resources Development Fund to promote South-South cooperation, including Asia-Africa cooperation.

The interspecific rice hybridization project for which we are meeting here today, is being supported through this Fund, with additional Japanese financial support of US\$200,000.

I would like to close by saying that Japan sincerely hopes this meeting will be successful and that Japanese support will promote research and the development of technologies for increased rice production in West Africa through the West Africa Rice Development Association

Thank you for your attention

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# **Opening remarks**

**Mrs. Jocelline Bazile-Finley** United Nations Development Programme Resident Representative Côte d'Ivoire

Mr. Chairman, Your Excellency, Distinguished participants, Ladies and gentlemen.

It is an honor and indeed a privilege for me to address this strategy meeting following the scientific workshop on interspecific hybridization between the African and Asian rice species recently held at the main research center of the West Africa Rice Development Association (WARDA) at M'bé near Bouaké.

I would like to take this opportunity to express my thanks to His Excellency Mr Sato, the Ambassador of Japan and, through him, the Government of Japan for their efforts and continued support to the development of human resources and the promotion of technical cooperation among developing countries. I also wish to thank WARDA for initiating the reflections and discussions of this morning, and placing the emphasis on strategic planning and research as a key development tool in Africa.

The UNDP puts great emphasis on technical cooperation among developing countries (TCDC) as an instrument to promote sustainable human development guidelines adopted by our Board of Governors. TCDC modalities encourage transfer of expertise and know-how among countries not only of the South but through North/South cooperation, to strengthen development perspectives in Africa. An example of this collaboration with the East/South/North was the First Asia-Africa Forum held in Bandung, Indonesia, in December 1994, with an increased focus on supporting TCDC initiatives between Asia and Africa. Rice consumption has increased significantly in most African countries. During the last decades, annual consumption has increased from 12 to 24 kg per person. The weakness of local production capacity obliges these countries to import rice from elsewhere to fill the grape between demand and supply. It is therefore necessary to develop and applied research approach aimed at improving and increasing production of this commodity in Africa.

The UNDP has played an important role since 1971 in the establishment and development of WARDA, in order to allow the institution to promote research, training and communications in the field of rice science with a view to improving productivity of target populations to obtain sustainable and rewarding production.

As you know, the UNDP has put poverty eradication at the center of its priorities to achieve sustainable human development that puts people at the heart of all development efforts. In this context, food security – which is a fundamental element of poverty eradication – gas constituted a major element of the united Nations Special Initiative for Africa. This concern led to the organization of the recent world summit on food security by the FAO to sensitize and mobilize all development partners.

For this reason, I am delighted at the organization of this meeting which brings together distinguished delegates from the scientific world. I would like to strongly stress that you should focus on the identification of a mechanism that will allow small African farmers – of whom women constitute a large proportion – to benefit from what is being done at research centers. There is no doubt that the benefits will help them obtain sustainable agricultural production, increase their revenues in a context of environmental conservation, and reduce poverty at the same time.

It is therefore important that during today's meeting recommendations are made for concrete and tangible activities that will have a real impact for the concerned populations. For challenging road towards a sustainable human development for all.

Thank you.

# Formulation of the Project on Interspecific hybridization between the African and Asian rice species

Monty P. Jones Principal Plant Breeder & Project Coordinator

The strategic planning meeting on the interspecific hybridization of rice developed by the West Africa Rice Development Association (WARDA) was held in Abidjan, Côte d'Ivoire, on December 20, 1996. The meeting was chaired by Professor Ryuichi Ishii of the University of Tokyo, Japan, and member of the WARDA Board of Trustees. Participants are listed in Appendix 2.

# 1. Importance of the meeting

The importance of promoting the interspecific hybrid rice project, which promises to improve productivity of rice in the West African region and beyond, was recognized by all participants. Delegates from the United States of America and Japan commented that this project had an integral place within the larger goals of the Japan/United States Common Agenda for Cooperation in Global Perspective. Within this framework, both Governments had raised an array of issues that are expected to be major challenges facing future generations. The representative of the United Nations Programme/Technical Development Cooperation among Developing countries (UNDP/TCDC) unit also explained that this project was being developed and funded at the request of the UNDP and would be a model of South-South cooperatio0n. The activity emphasizes the benefits that can accrue from exchange of crop genetic diversity.

Participants confirmed that the ultimate goal of this project is not only to conduct research to create interspecific rice germplasm, but to ensure that farmers can access and use the resulting technologies to increase productivity. This will benefit both farmers and consumers in West Africa and beyond.

## 2. Technical assessment

The conclusion of the Scientific Workshop held December 16-18, at the WARDA headquarters near Bouaké, Côte d'Ivoire, was reported. During that workshop, participants held in-depth discussions on various aspects related to wide hybridization of Asian and African rice germplasm including methods, progress, problems and prospects, and developed proposals for future research collaboration benefiting from the immediate input of participants.

Participants acknowledged the significant progress that is being made in wide hybridization. They supported the idea that the project should be proposed by WARDA as one of the UNDP's projects in collaboration with other international agricultural research centers, namely the International Rice Research Institute (IRRI) and the International Center for Tropical Agriculture (CIAT). The participants also endorsed WARDA as the lead center responsible for the coordination and implementation of the project. The American and Japanese representatives said they would consider the provision of experts or other technical support for the project.

To this end, both Japan and the USA had sponsored the attendance of leading rice geneticists at the meeting. The overall assessment of these experts was positive, emphasizing that interspecific hybrids could lead to significant increases in productivity (approaching 20-40%) in some areas, in as little as three years.

Based on the three-day discussions, the participants identified and developed at workplan for the following priority research activities:

1.Development of improved rice varieties resistant to the African rice gall midge (ARGM)

2.Development of improved rice varieties resistant to drought and iron toxicity.

3.Development of rice germplasm with weed competitiveness

4.Development of high yielding Oryza glaberrima germplasm resistant to grain shattering and lodging.

5.Although the technical meeting did not include the disease rice yellow mottle virus (RYMV) in its workplan, the strategic planning meeting participants recommended its inclusion as a research priority activity.

## 3. Implementation

Participants recognized the necessity of monitoring mechanisms to review and evaluate the progress of project activities. Therefore, it was decided that annual meetings should be held to review research achievements, to set work plans, and to approve financial plans for the following year. (Dates and venues will be determined through diplomatic channels.) Participants supported the idea that technology transfer procedures should begin to be put in place as early as possible in the next three years. Appendix 1

# List of Participants to the International Workshop on Interspecific Hybridization of Rice in Africa

## BENIN

## Mr. Adeyemi Pascal

CRI BP. 3 Niaouli (Attogon) Fax. (229) 51 00 05

BRAZIL

**Dr. Paulo Hideo Nakano Rangel** Rice Breedeer (Wild Rice) Crosses EMBRAPA-Centro Natural de Pesquisa de Arroz e Feijao (CNPAF)

## CHINA

## Dr. Tas Dayun

Associate Scientist, Head of Rice Dept. & Upland Rice Program Food Crops Research Institue Ynnan Academy of Agricultural Sciences Kunming 6505205

# **COLOMBIA/CIAT**

**Dr. Cesar Martinez** CIAT Apartado Aero

# **COTE D'IVOIRE Dr. Yaya M. Coulibaly** IDESSA

## FRANCE

**Dr. Alain Ghesquière** ORSTOM

JAPAN

**Prof. Ruyichi Ishii** University of the Tokyo Faculty of Agriculture Crop. Science Laboratory

**Dr. K. Nakamura** Director of Multilateral Cooperation in the Japanese Ministry of Foreign Affairs Tokyo

**Mr. Noro** Ministry of Foreign Affairs Tokyo

# PHILIPPINES/IRRI

Dr. O.S. Brar IRRI

**Dr. Veronique Schmidt** IRRI

SIERRA LEONE

Mr Malcolm Jusu Rice Research Station PMB 736-Freetown Rokupr

# TOGO

**Dr. Sélomé Dogbé** INCV Lomé

USA

**Dr Susan Mccouch** Cornell University Plant Breeding Department Ithaca, New York 14853-1901

**Prof. James (Jim) Oard** Dept. of Agronomy Louisiana State University Baton Rouge Louisiana

Dr. S.K. Reddy REDSO/WCA

WARDA

**Dr. Peter Malton** Director of Research Côte d'Ivoire

**Dr Michael Dingkuhn** Continuum Program Leader Côte d'Ivoire

**Dr Monty P. Jones** Upland Rice Breeder Côte d'Ivoire

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# **Dr David Johnson**

Weed Scientist Côte d'Ivoire

# **B.N. Singh**

Lowland Rice Breeder PMB 5320-Ibadan-Nigeria

# Dr Kouamé Miezan

Irrigated Rice Breeder Sahel Program Leader Senegal

## Appendix 2

# List of Participants to the Strategic Planning Meeting on the Interspecific Hybridization of Rice in Africa

#### Mrs. Jocelline Bazile-Finley

Resident Representative United Nations Development Programme (UNDP) Abidjan, Côte d'Ivoire

#### Mrs. Aissatou Cissé

Chargée de programme UNDP Abidjan, Côte d'Ivoire

#### Dr. Michael Dingkuhn

Continuum Program Leader West Africa Rice Development Association (WARDA) Bouaké, Côte d'Ivoire

#### Dr. Mamadou Diomandé

Directeur Centre Ivoirien de Recherche Technologique (CIRT) Abidjan, Côte d'Ivoire

#### Professor Ruyichi Ishii (chairperson)

Graduate School of Agriculture and Sciences University of Tokyo Tokyo, Japan

## **Dr Monty P. Jones**

Upland Rice Breeder WARDA Bouaké, Côte d'Ivoire

#### Mr. Dougou Keïta

Rice Task Force Research Assistant WARDA Bouaké, Côte d'Ivoire

## Dr. Cesar P. Martinez

Centro International de Agricultura Tropical (CIAT) Cali, Colombia

# Dr. Peter J. Malton

Director of Research WARDA Bouaké, Côte d'Ivoire

## Dr. Kunio Nakamura

Assistant Director Multilateral Cooperation Division Economic Cooperation Bureau Ministry of Foreign Affairs Tokyo, Japan

#### Dr. Kanayo F. Nwanze

Director General West Africa Rice Development Association (WARDA) Bouaké, Côte d'Ivoire

**Dr. James Oard** Louisiana State University Baton Rouge, LA, USA

## Dr. S.K. Reddy

Assistant Director Agriculture, Natural Resources and Environment United States Agency for Internation Development (USAID/REDSO) Abidjan, Côte d'Ivoire

## Mr. Hiromi Sato

Ambassador of Japan Abidjan, Côte d'Ivoire **Mr. Ishihara Shuji** Expert, Centre de formation mécanique Agricole à Grand-Lahou (CFMAG) Japan International Cooperation Agency (JICA) Abidjan, Côte d'Ivoire

## M. Hiroyuki Suzuki

N. First Secretary O. Embassy of Japan Abidjan, Côte d'Ivoire

## Mr Tsuboi Tatsushi

Expert, Centre de formation mécanique Agricole à Grand-Lahou (CFMAG) Japan International Cooperation Agency (JICA) Abidjan, Côte d'Ivoire

## Mr Kunio Tsubota

Japan International Research Center for Agricultural Sciences (JIRCAS) Tsukuba City, Japan