Current Status of Molecular Rice Breeding in Vietnam

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Introduction

Biotechnology is considered as a tool originated from many disciplines including molecular biology, genetics, microbiology, bio-informatics, biochemistry. Biotechnology has been applied in agriculture as a breakthrough measure to aim at developing a sustainable agriculture. The development of DNA-based techniques and their application to plants opened new possibilities for rice. In Vietnam rice is a staple diet and therefore it received considerable attention in improving its productivity and quality. Recently, efforts are underway to use biotechnological means to improve crop production, with rice receiving attention. Considering the costs involved in setting up biotechnology based breeding systems, the country needs to develop a strategy in furthering its adoption.

In Vietnam policy are being devised to enhance the cultivation of rice from 4.3 million ha to 7.6 million ha and production has increased from 15.7 million tons in 1985 and 34.5 m tonnes in 2003. Average rice yield obtains 4.67 t/ha today. The idea is to feed the current population in Vietnam of 81 million people. However, with the onslaught of

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urbanization and other reasons up to 4.0 million ha may be reduced. Beside that, water shortage, salt intrusion will become serious problems and a big challenge for human life. Rice is one of the most important food crop in the country providing 67 per cent calories on an average to an individual in the country. Rice production was increased.

The Vietnamese Government has set up a National Biotechnology Programme through regulation 18/CP (projection from 2000 to 2010) to look into the need of trained manpower and investment for research and development. The budget has been increased year by year (Table 1, and 2), it depends to the economic development of the country. Especially, rice biotechnology projects were supported by the Rockefeller Foundation at initial years, and ARBN (Asian Rice Biotechnology Network), ICGEB (International Center for Genetic Engineering and Biotechnology). Vietnam has to plan both short-term and long-term training systematically to obtain specialists on genomics and others to implement the development. Attentions on rice biotechnology applications have been paid to: (1) gene transformation to improve the traits in which the conventional breeding is not available, (2) double haploid lines through anther culture, (3) DNA recombinant techniques, (4) marker-assisted selection in rice breeding, (5) genetic diversity assessment, did this happen in Vietnam or elsewhere. Again, give references.

Table 1: Budget for national biotechnology programme

Period	Budget (USD / year)
1981-1985	20,000
1986-1990	1,000,000
1991-1995	1,500,000
1996-2000	2,500,000
2001-2002	2,000,000
2003-2004	75,500,000(planned)

Table 2: Funding by national budget for biotechnology project (2003-2004)

	VND $ billion	per cent
Education	150	2.5
Equipment	500	8.2
Agriculture	3,500	57.2
Medicine	1,300	21.5
Industry	250	4.1
Environment	140	2.3

Human resource has been promoted recently: one million USD covers scholarship for training abroad/year in addition of training in the country, including 4 per cent of scholars doing biotechnology. Key national projects on biotechnology (2004-2010) are identified as Project 1: Human Resource; Project 2: Establishment of national key lab; Project 3: Strengthening R&D capacity; Project 4: Biotechnology for sustainable agriculture; Project 5: Biotechnology for public health; Project 6: Biotechnology for industry. There are six national key lab on biotechnology are being set up. However, we have more equipment but less experts now. The scientific manpower must be strengthened and the agenda for biotechnology research must be clear to meet the demand of development.

Rice Monoculture and Low Competitiveness in Rice Markets

Vietnam had obtained one million tonne of paddy increased per year from 1990-2000; however, the income per ha has been a constant (around $US 1010 / ha in 2002). It means that rice-based farming systems are not diverse and not effective. Agro-economic structure conversion is considered to aim at diversify various sources of farmer income and to enrich biodiversity. During last decade, exporting rice became a target of rice production in Vietnam, and the amount of exported rice has been used to access the balance between rice supply and demand. Therefore, it is necessary to evaluate the comparative advantage of rice production in Vietnam. All efforts by rice workers in the country are paid attention: (1) to close yield gap, (2) to sustain current yield, and (3) to break yield ceiling by various ways (including hybrid rice and new plant type). Beside that grain quality improvement is always recommended with the emphasis on intermediate amylose content, long grain, less chalky varieties to sustain current yield, rice improvement has been focused to release new genotypes with stable tolerance to the target stresses and favourable soils for rice culture in two big granaries of the country as alluvial soils are 1.18 m Ha (30.1 per cent) and 0.91 m Ha (48.5 per cent) in Mekong Delta and Red River Delta (Table 3), respectively.

In acid sulfate soils, low pH, aluminum toxicity, iron toxicity, and low phosphorous are considered as main limited factors for rice growing. Currently, water management and agronomic practices have been recommended. Some improved genotypes have been identified to tolerate to drought, salinity, acid sulfate but not stable.

Table 3: Problem soils in rice culture areas in Mekong Delta (MD) and Red River Delta (RRD)

Soil type	Area (million ha)		Percentage (%)	
	MD	RRD	MD	RRD
Acid sulfate	1.60	0.11	40.8	5.95
Salinity	0.74	0.09	18.9	5.23
Peat soils	0.02	0.002	20.0	0.10
Grey soils	0.13	0.15	3.4	8.02

For biotic stresses, brown plant hopper is mainly considered as the most important pest in rice production. Due to the change of population or biotype, in addition to the heavy intensification, current rice genotypes are not stably resistant for a long period. Introgression of target genes from wild rice species into cultivars is being implemented.

Blast disease is also the most important biotic stress. Many isolates were collected in Vietnam, and gene *Pi-2* is considered to control almost virulent races in Vietnam beside other genes. Farmers are misusing fungicides and other chemicals in their rice field to break the natural balance and create new races so that no current cultivars are showing their stable resistance after two seasons

Bacterial leaf blight is the main problem in North at both main seasons, and in South in monsoon season. Genotypes with *xa-5, xa-13, Xa-7, Xa-4, Xa-21* can control the virulent bacteria in Vietnam (Buu and Lang 1998, Buu *et al.* 1999).

Sheath blight is considered as important in both North and South, in any season. No genotype has been identified so far to be tolerant to this disease. Chemical and agronomical practices are used to manage it. Beside that, in Winter-Spring season, there are case worm, stem borer, and in Summer-Autumn, thrips, gallmidge, rice bug. Brown plant hopper break which occurred in 1978-1979, 1985-86, 1992 with million ha in South, is an impressive event in rice production history. Integrated pest management (IPM) has been launched through many campaigns with the respond by farmers, policy makers, and scientists.

Natural calamities such as typhoon, storm, flood are also risks in rice production every year. Three basic climate regimes are found in Vietnam:

- In the North, especially in the interior, the temperature are subtropical, cold tolerance is needed in Spring season, flood type is sudden flood in monsoon, submergence tolerance is required.

Shifting seasonal wind patterns result in dry winters and wet summers.

- The Central and Southeastern areas are in tropical monsoon climate, with high temperature and abundant precipitation, with the emphasis on typhoon in coastal Central.

In the Southwest, distinct wet and dry periods are evident, but stagnant flood is typical from August to November. Post-harvest losses of rice in Vietnam range from 13 to 16 per cent as compared to 10-37 per cent in Southeast Asian countries, mainly due to harvesting, drying, storage and milling. Post-harvest losses vary by seasons and regions with 15 per cent in South Coastal Central, 13 per cent in Mekong Delta, and 10 per cent in Red River Delta.

Research and Its Impact

Ministry of Agriculture and Rural Development (MARD) has many research institutes and centres undertaking responsibility of food research including CLRRI. Programme "KN01-Food Crop Research" had 19 projects of which 18 are implemented by MARD, and "KC18-Biotechnology" with 18 projects of which 4 are implemented by MARD

Total researchers for food crops in whole country is 819 while the researchers with PhD degree 145 (17.7 per cent) and the researchers with MSc. Degree 124 (15.0 per cent) while the post-graduated researchers account for 32.7 per cent in terms of food crops. Varietal improvement has provided farmers with the best materials avialable from pure line selection, introductions, and local hybridization. About 5,000 accessions of local rices and hundred populations of four wild rice species: *Oryza rufipogon, O. nivara, O. officinalis, O. granulata* have been collected, catalogued and evaluated. This resource material has provided donors for biotic and abiotic stresses (Buu *et al.* 1997). Rice germplasm evaluation assited by DNA markers has been conducted at some institutions in Vietnam, to supply a reliable information to rice breeders while selecting appropriate materials. The extra early rice varieties with 80-90 days duration, and early genotypes (91-105 days) created a new strategy to escape flood by growing them before and after flooding to increase more rice seasons in the Mekong Delta. Growing rice areas have increased from 2.2 million ha in previous 1990's to 3.9 million ha/1.7 million ha of cultivated areas up to now due to improving short duration genotypes. The shortcoming has maintained for many years that they never mind the quality of their seeds so that seed technology should be mentioned in further development strategy.

Vietnam has become a rice exporter, and grain quality improvement has just set up as top priority in rice improvement program. Intermediate amylose, intermediate gelatinization temperature, no chalkiness, long slender grain are the criteria of breeding objectives in terms of grain quality improvement. We successfully released some high yielding varieties (HYVs) with long grain shape and translucence. All efforts have been done to improve intermediate amylose content. The effect of waxy and non-waxy gene dosage, and endosperm appearance is continued to study. Seven new varieties released in 2002 with the emphasis on grain quality and high yield as DT122, NX30, CH5, P1, MT58-1 in Red River Delta and Northern parts AS996, OMCS2000 in Mekong Delta and Southern parts.

Some genotypes tolerant to biotic stresses like brown plant hopper, blast were commonly used. The exploitation of gene pool from wild rice species fruitfully obtained a true introgression of desirable traits into HYVs such as AS996 (IR64/*Oryza rufipogon*) which is tolerant to some major biotic and abiotic stresses, short duration, yiel yield, wide adaptibility. The inbred rices from CLRRI now occupy more than 60 per cent of cultivated rice areas in the Delta. Hybrid rice has been quickly developed in Red River Delta with 480,000 ha in 2001, and 530,000 ha in 2003. Beside three-line system varieties introduced from China such as San You, Vietnam Hybrid Rice Research Centre has tried to develop HYT83, HYT84 in Spring, 25A / PM3 in monsoon season. Two-line system with gene *tgms-2, tgms-2, tgms-3* is also exploited to develop promising varieties.

Available Applications on Rice Biotechnology

1. Biodiversity

Germplasm conservation is the first step for application of biotechnology. Because genetic erosion becomes serious everywhere in the world including Vietnam. By the DNA recombinant technology, genetic diversity is possibly assessed Domestic and exotic germplasms have been collected and preserved including landraces and wild species They need to be characterized and evaluated both phenotyping and genotyping with a cumbersome work. Due to the current applications on biotechnology such as detection of candidate genes, gene profile, allele mining techniques, they can enhance the process of germplasm conservation for evaluation and utilization. Database management will be facilitated by bio-informatics development. Genetic variation will be also enhanced through double haploid (DH) populations, advanced backcross (AC), recombinant inbred lines (RIL), nearly isogenic lines (NIL), introgressive lines from wide hybridization. Map-based cloning is encouraged as a key strategy in genome analysis.

2. Cloning

Development of vectors such as plasmid, cosmid, BAC (bacterium artificial chromosome) has facilitated to clone target genes, to approach genome libraries, or cDNA libraries, to form gene constructs for transformation.

BAC clone library has been set up to identify target genes as *Xa-21, Bph-10* cDNA libraries for target traits which control tolerance to major biotic and abiotic stresses have been carried out.

3. Marker-assisted selection (MAS)

Marker-assisted selection has been considered as a good strategy since 1995 for crop improvement in developing countries. PCR-based markers such as RAPD, AFLP, STS, microsatellite can be used to detect target genes in parent and progenies lines. “Fine mapping” is the key of the strategy through a careful selection of parental materials with typical genotype of susceptible and resistant one to a target objective.

Cuu Long Delta Rice Reseach Institute (CLRRI), Agricultural Genetics Research Institute (AGI) have focussed MAS to fruitfully investigate resistant rice genotypes to brown plant hopper, blast, and bacterial leaf blight.

4. Introgression of target genes from wild rices

Target genes from wild species related to a given crop can be introgressed into cultivars through embryo rescue technique in term of two different genomes. Chromosome manipulation can be facilitated by FISH (fluorescent *in-situ* hybridization) in terms of wide rice hybridization. One variety was released through crossing between IR64 x *Oryza rufipogon* collected in Dong Thap Muoi (strongly acid sulfate soil). It has been well developed in less-favorable areas in the Mekong Delta.

5. QTL analysis and QTL mapping

Most of economic traits are controlled by polygenes, and some complex genes e.g. blast resistance are controlled by both major and minor genes. They require quantitative trait loci (QTL) mapping such as drought, salt tolerance, P deficiency tolerance, aluminum toxicity tolerance, etc. GxE interaction is a challenge for QTL analysis, it needs a well-modified software to interpretation of outputs.

PCR-based markers can be simply used but they have to cover all chromosomes with closer linkage between genes and markers.

6. Cellular techniques and gene transformation

Anther culture can be applied to exploit homozygosity quickly and micro propagation is also considered as a progressive technique in developing countries.

The development of DNA-based techniques, generally known as biotechnology, and their application to plants opened new possibilities for seed businesses. Genetically modified crops (GMC) have received some great achievements recently, but they have also received a lot of criticisms.

Two different viewpoints in well-developed countries on GMO. They are often conflict viewpoints. Instead of in developing countries, their common attitudes are hesitation and waiting because they do not own any GM crop serving to export purpose and they do not need to import GM crops.

GMO should be scientifically investigated and need more time to conclude. Risk assessment is requested.

For rice transformation, normally immature embryos or calli were used as explants for transformation. Embryogenic suspension cells of indica rice cutivars were successfully used in the *Agrobacterium*-mediated transformation.

Selectable marker has been improved such as *pmi* with mannose selection procedure.

7. Functional Genomics

Since 1999, special term has been created as “functional genomics” to mention a new aspect on genomics in order to detect more candidate genes with their special functions with two major tools: (1) whole genome expression analysis, (2) systemic gene disruption, with the goals as followed:

- to create an information mode to deposit and disseminate information in crop functional genomics,
- to build a public platform to promote access to genetic stocks and phenotypic information,
- to develop databases on phenotypes and mutants with linkage to sequencing laboratories, and
- to initiate partnerships to develop resources for microarray analysis

Attentions on gene discovery should be paid through the collaboration which belongs to the international rice functional genomics consortium (IRRI).

Concluding Remarks

Rice production in Vietnam has successfully increased due to research collaboration with IRRI and others. Vietnam is trying its efforts for setting a sustainable agriculture. All rice research activities aim at diversifying natural resources, maintaining natural balance in rice field. It is so called "integrated management".

Attentions on the biotechnology applications in rice have been paid to: (1) gene transformation to improve the traits in which the conventional breeding is not available, (2) double haploid lines through anther culture, (3) DNA recombinant techniques, (4) marker-assisted selection in rice breeding, (5) genetic diversity assessment. Rice biotechnology has significant potential to help agriculture contribute to the goal of sustainable development:

- to increase the production and productivity of crops
- to increase the nutritional and market values of food
- to enhance the stability and sustainability of agro-ecosystem
- to mitigate the deleterious impacts of agro-ecosystems

Priorities will be considered as (1) marker-assisted selection and (2) using the advantages of gene transformation with clean DNA construction. Vietnam needs to increase capacity building biotechnology to rice improvement and to receive assistance in preparing bio-safety regulations. The integration of biotechnology tools with conventional breeding methods offers new opportunities to increase rice productivity and sustainability, achieve food security and improve nutritional quality. Existing biodiversity of rice varieties and their nutritional composition need to be explored before engaging in transgenic. The potential of the genetic diversity has not been adequately utilized. We need the collaboration to make better use of this potential latest biotechnological methods employed in conjunction with conventional rice breeding program.

Attentions will be paid to enhance rice grain quality to meet the demand of common international preferences on long grain appearance with/or without aroma. Varietal improvement and post-harvest technology should be included. Vietnam needs the collaboration on revising policies with the least distorting effects on the world rice market

IRRI has been working with hybrid rice since 1979. Work continues on increasing cytoplasmic diversity in CMS three-line hybrids. The new two-line system has shown stable pollen sterility. Vietnam is focusing research efforts to develop hybrid varieties with acceptable grain quality and bacterial leaf blight resistance.

Narrowing yield gap is considered as a key strategy contributing significantly to increase rice production in the country. The adoption of rice integrated crop management (RICM) has demonstrated great potential to facilitate the adoption of improved management practice and technology by farmers to improve yield, grain quality, reducing production cost and protect the environment, e.g. current "Three Reduces Project" by IRRI in Mekong Delta (reducing seed rate, N fertilizer, pesticide). Vietnam needs to promote RICM through extension farmer schools following the effective approach, such as *Ricecheck* methods, to narrow the yield gap and enhance food security, to converse some rice farms into more advantageous farming system (i.e. shrimp) but ensuring food security goal. Vietnam needs to formulate policies to encourage incentives and support for the development and transfer of RICM

Resources and policies should be adequate to promote appropriate rice drying, rice storing in small metallic silos, rice cleaning how to increase the quality of commercial rice in the market.

Collaboration Delivery Opportunities

Enhancing the value of international rice gene bank collection by discovering novel alleles for the development of nutritious rice adapted to fragile environments: these approaches will include high-throughput DNA microarray technologies, highthroughput genotyping (HTG) methods which will allow mass screening of gene bank accessions for alleles at many candidate gene loci.

There is also a need to enhance water and land productivity through alternating wetting-drying irrigation and root zone aeration in irrigated rice and development of technologies to harness the productivity potential of salt-affected areas: to (1) introduce salt tolerance genotypes adapted to main salt-affected areas and to different farming systems, and (2) develop integrated crop management suitable to the demands of natural resource management packages. Some perspectives for the dissemination and marketability of environmentally sound rice production technology in the Mekong Delta may be further developed. Vietnam is emerging as a key partner in the global rice research community. Vietnam-IRRI collaboration and other countries from the Mekong River and Asia will address the farming community in its entirety so that Vietnam can be assured of rice security, the income of farming families will increase, and the environment will be protected.

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