POTENTIAL OF BIOTECHNOLOGICAL TOOLS FOR RICE IMPROVEMENT

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Abstract

A major concern of the government of Sri Lanka is to improve rice production through the use of modern and appropriate technologies, of which biotechnology has been identified as a thrust area for development and integration. Sri Lanka must raise its present low and stagnating yield level of 3.7 t/ha to about 4.1 t/ha by the year 2005. Moreover, the cost incurred for use of pesticides and fertilizer as well as for disease and weed control must be reduced in order to make rice production profitable. Other challenges faced by rice scientists include the reduction in the time taken to produce a variety, efficient selection procedures for abiotic and biotic stress tolerance and improvement of the nutritional value of the grain. Research must, therefore, focus on these problems and identify those to which biotechnology can be used as a solution or as a tool. The production of hybrid rice has been identified as a possible solution to further increase the yield potential of our rice varieties. Recombinant DNA technology can assist in the production of hybrid rice using male sterility. DNA fingerprinting techniques can be used to characterize traditional and new rice varieties which will be used as potential parents in new breeding programmes, in addition to identifying DNA polymorphisms that could be used as molecular markers in the selection procedures, especially in selecting for stress tolerance (salinity and drought) and for selecting for resistance to pests (gall midge, brown plant hopper and thrips) and diseases (blast and blight). Biotechnological tools can also be used to produce biopesticides and biofertilizers, the use of which could further reduce the cost of production. Anther culture techniques could help plant breeders to significantly reduce the time taken to produce a new variety. Research to identify techniques to convert waste from rice fields into useful products using improved strains of microbes will need to be initiated. The production of transgenic rice, especially for herbicide resistance and for improvement of nutritional quality, will have to be initiated as soon as national biosafety procedures are in place. In the same context, DNA identification techniques will have to be established to check for GMOs and GMFs at the quarantine stage. For the successful use of biotechnology in rice production, it will be of prime importance to establish a national center for biotechnology, as well as to develop resources (both infrastructure and manpower) at regional centers, and to establish national, regional and international linkages including the private sector.

INTRODUCTION

Rice is the most important food crop of the country. Agricultural land under rice and other temporary crops amount to about 24% of total land area of Sri Lanka (Dept. of Census & Statistics, 1997). The major concern of the government of Sri Lanka is to improve agricultural production through the use of new and appropriate technologies, of which biotechnology has been identified as a thrust area for development and integration. It is now recognized that both conventional and modern technologies have to be combined in order to achieve future food security. Research and development in agricultural biotechnology, including the development of transgenic crops, has been recognized by the international scientific community to be essential and an important element of a broad strategy to meet this challenge in this new millennium (UNIDO,1998; Rice Biotechnology).

Biotechnology has been used extensively in rice all over the world (Rice Genes 5.0 Database). There are 11 genetic maps of rice, more than 100 genomic clones and 3 sets of comparative homeologies. There are more than 800 references specific to genetic maps. Investigations of traits at the molecular level have identified more than 350 QTLs, 216 phenotypic traits and more than 2500 polymorphisms. Nearly 5,000 molecular markers identified consist of RFLP, AFLP, Microsatellites, RAPDs and isozymes. These have been used to identify Asian gall midge biotypes in India. In China, 3 genes, OSA1, OSA2 and OSA3 related to salinity stress have been identified. Several QTLs controlling tiller and leaf characteristics have been mapped. Molecular markers are being developed for seed quality traits such as amylose content, gel consistency and gelatinization temperature using RAPDs, AFLP and Microsatellites. The complete sequencing of the rice genome is expected soon.

During the last two decades, the challenges to improvement of rice production have been the increase in cost of production, low and stagnating yields, declining area under cultivation and declining manpower (ASDA, 2000). With a per capita consumption of about 100 kg per year and a limited annual cultivated rice land area of about 830,000 ha, Sri Lanka must raise its present average yield level of 3.7 tons/ha to about 4.1 t/ha by the year 2005. The low and stagnating yields of the present varieties limit the scope for increasing production. In all the major rice growing areas, yields are stagnant, whilst there is a significant yield gap between potential (10 t/ha) and realized (3.7 t/ha) yields at farmer level (ASDA, 2000).

Sri Lanka should produce at least 3.4 million tons of rice to feed its predicted population of about 20 million people in 2005. In addition, high cost is incurred for use of pesticides and fertilizer as well as for disease control. Moreover, yield losses due to weeds alone exceed losses caused by other biotic constraints. The use of herbicides is the most common technique used by farmers to control weeds in rice fields. Other challenges faced by rice scientists include the long time taken to produce a variety (~ 8-10 years), problems of selection for stress conditions such as drought and salinity, improvement of nutritional value of the grain, and use of biofertilizer and biopesticides.

Research must, therefore, focus on the above problems in rice production and identify those to which biotechnology can be used as a solution or as a tool, whilst maintaining soil fertility and conserving the environment.

Biotechnology has given the world more nutritious food, and crops resistant to insects, diseases and herbicides by "single season breeding". At present, there are 3 million hectares planted to transgenics worldwide. Among the Asian countries of the region, India, Philippines, Thailand, Vietnam, Singapore and Malaysia have conducted field trials of transgenic crops. Korea has created a strain of transgenic rice that could increase yield by 26 %; China has proposed to produce transgenic rice with fewer stalks / plant but twice as many kernels/stalk, with increased protein in the grain, and rice that needs less water. The world has been introduced to "Golden Rice" with enhanced pro-vitamin A. Therefore, there is a great potential in the use of appropriate transgenic rice varieties whilst maintaining strict safety standards.

OBJECTIVES

The objectives of this paper is to identify the problems in rice production to which biotechnological tools could be used, the use of biodiversity, the problems of implementation that will include development of resources both infrastructure and manpower, the importance of linking with local, regional and international institutes, and the method of monitoring the progress based on strict time schedules, whilst working within national biosafety regulations.

METHODOLOGY

The information required for identifying the appropriate problems was received from the CARP Committee of Biotechnologists, NSF, EDB and from the institutes and research stations of the NARS. Information regarding the use of biotechnology in the region and globally is available on the internet.

The biotechnological tools available for use in rice improvement and production are tissue culture including anther culture, genomic analysis, DNA typing/fingerprinting and transgenic plant production. Moreover, these tools can be used to produce biopesticides, biofertilizer and methods to convert agricultural waste to useful products. To make use of these various techniques to improve rice production, it is necessary to acquire knowledge and to establish relevant databases, to establish local, regional and international linkages, in order to establish collaborative research and sharing of resources for which a bioinformatics center is a definite requirement.

Local problems in rice production and the application of bio-technological tools

In order to identify the biotechnological tools for use in rice production, it is necessary at first to identify the problems encountered in rice production and improvement in Sri Lanka. The following problems have been identified by the rice breeders as well as by the National Committee of Biotechnologists and Breeders of CARP (Council for Agricultural Research Policy). The potential tools that could be used in an attempt to solve these problems are also discussed.

(i) Yield gap

The present, the average yield level is 3.7 t/ha whereas the potential yield is 10 t/ha (ASDA, 2000). The present average has to be increased to about 4.1 t/ha by the year 2005. This can be accomplished by further increasing the yield potential of our rice varieties. The low and stagnating yields of the present varieties limit the scope for increasing production. One method of achieving this is by producing high yielding rice hybrids using male sterility technique. Male sterility genes can be transferred by using genetic engineering techniques, and monitored by using molecular markers.

Another reason for the low and stagnating yields is the narrow genetic base available to the breeders. This can be overcome by introducing foreign germplasm, as well as specific traditional varieties for specific purposes, into the breeding programmes. Genes from wild rices and landraces can also be employed, such as in the case of YLD genes identified in *O. rufipogon* and transferred to a cultivated variety (UNIDO, 1998). In this process, molecular markers should be identified to assist in selecting superior plants during the selection procedure. DNA typing techniques such as RAPDs and Microsatellites can be employed for this purpose.

(ii) High cost of production

High cost of production is incurred in the use of pesticides, fertilizer and in the use of chemicals for disease control. Moreover, the use of herbicides for weed control increases cost of production further. Biotechnology presents many techniques that can be used to reduce cost of production.

The production and use of biopesticides and bioherbicides, which are derived from natural material, should be encouraged. They are less harmful, affects only the target organisms, required in small quantities and decompose quickly, and are therefore, environmentally friendly and safe. There are more than 700 such products available in the global market. They consist of microbial pesticides made up of bacteria, fungi, viruses and even protozoans, plant pesticides having plant material such as neem, and biochemical pesticides using naturally occurring substances such as sex pheromones, scented plant extracts *etc.* Research should be initiated to identify active ingredients for use as biopesticides and bioherbicides from the vast biodiversity available in Sri Lanka.

In the same manner, research should be initiated to study, identify and isolate ingredients from our biodiversity including beneficial microbes, algae *etc.* that could be used in the production of biofertilizer for use in rice soils.

Research should be initiated to use biotechnological tools/kits for rapid and accurate disease diagnosis.

Molecular marker technique should be used to select disease and pest tolerant genotypes early in breeding programmes. Molecular makers for bacterial leaf blight and blast, as well as for gall midge, brown plant hopper and thrip resistance should be identified to assist rice breeders in their selection programmes. This type of marker assisted selection should be a priority in rice breeding programmes.

(iii) Abiotic stress tolerance

Molecular marker technique should also be used to produce rice varieties tolerant to drought and saline conditions. PCR-based DNA markers for characteristics related to drought and salinity tolerance such as root characters, hydraulic conductivity, as well as high yield, should be identified and used in breeding programmes. Foreign varieties, as well as traditional varieties, landraces and wild rices should be assessed for these characters and used in breeding programmes together with marker assisted selection.

(iv) Long time to produce a new variety

It takes nearly 8-10 years to produce a new variety of rice, during which period conditions can change, including market requirements, Biotechnological tools such as anther culture can reduce this time period to less than half by producing dihaploid populations. The development of tissue/anther culture techniques will also enable the production of intergeneric somatic hybrids as well as inter-sub-species hybrids through protoplast fusion.

(v) Low nutritional value of grain

The nutritional value of the rice grain can be improved by using our traditional varieties which harbour excellent grain quality characters such as oiliness, flavour, medicinal traits *etc.* in the breeding programmes using molecular markers to assist the breeders. Moreover, transgenic varieties such as golden rice can be used in the breeding programmes to transfer high nutritional value to our recommended varieties.

(vi) Agricultural waste

Biotechnological tools can be used to convert agricultural waste into biogas as well as biofertilizer. Moreover, techniques can be used to convert agricultural waste into other useful products such as alcohol.

(vii) Transgenic rice

Many transgenic rice plants carrying numerous new features are being field tested around the world. It is proposed that a herbicide tolerant transgenic rice variety would help in lowering the cost of production as less chemical herbicides will be used. Transgenic rice research and testing will be initiated once the national biosafety regulations are established.

(viii) Bioinformatics

In order to carry out the application of biotechnological measures to improve rice, it is of prime importance to establish a knowledge system by means of setting up a bioinformatics center.

It should establish relevant local databases with access to regional and international databases, develop data warehouses, data designs and data mining from single and multiple databases and mirror sites and establish partnerships with local, regional and international software companies.

CONCLUSION AND SUGGESTIONS

The problem of reducing the yield gap will have to be accomplished by producing higher yielding varieties including hybrids. The major problem of producing hybrids will be to introduce male sterility. This can now be done through recombinant DNA techniques. Yields can also be improved by providing pest & disease resistant genes as well as genes resistant to herbicides. Transgenic plants have been produced that have such genes.

The production of transgenic plants should be carried out at a central laboratory, provided national biosafety procedures are in place. In the case of production of transgenic plants it will be necessary to link up the institutes concerned and obtain assistance from institutes of the regional countries (India, Thailand, Malaysia, China), international institutes (IRRI, CGIAR etc.) and other international bodies such as ISAAA, ICGEB, ISNAR *etc.*, as well as the private sector.

The problem of long time required for development of a variety can be overcome by producing dihaploids using anther culture techniques. This has been carried out at IRRI, and University of Birmingham, UK. Therefore, tissue culture facilities will have to be developed at Batalagoda, as well as the other rice breeding stations.

The genes for resistance to pests (gall midge, brown plant hopper, thrips), diseases (blast, blight), drought and salinity, as well as for grain quality can be searched for in our biodiversity including the traditional varieties as well as in foreign varieties. DNA markers will have to be identified for this purpose to assist breeders in their selection programmes. Therefore, DNA fingerprinting techniques will have to be developed to assist in this MAS procedure. DNA fingerprinting for characterization of the national rice germplasm collection will be a prerequisite for this.

The use of biofertilizer and biopesticides using local material will have to be developed in order to reduce the cost of production further. Research into conversion of waste from rice fields into useful products using improved strains of microbes will need to be initiated.

The introduction of GMOs and GMFs without legal permission has created much concern to people of developing countries such as Sri Lanka. In view of this, it will be necessary to provide DNA identification techniques at plant quarantine stage.

It is of great importance to establish and develop a central laboratory for bioinformatics in order to establish local databases relevant to rice biotechnology as well as to assist the scientists to obtain and exchange information.

It is recommended that a National Center for Biotechnology be established to undertake transgenic research and other expensive techniques (such as DNA synthesis and DNA sequencing). It can also have the bioinformatics laboratory. Tissue culture and DNA fingerprinting facilities should be developed at the relevant institutes/stations where they are required.

It is recommended that an Apex Body of Biotechnologists be appointed to approve and monitor priority research, advise higher authorities, establish national, regional and international linkages, establish and develop required resources, and disseminate knowledge among the scientific community as well as the general public. It should explore the possibilities of using the local biodiversity (which will involve patenting possibilities, benefit sharing, documentation of traditional knowledge *etc.*), which would also encourage the participation of private sector industries, both local and foreign. This Apex Body can survey the resources available and those required in order to use the wide variety of modern techniques available for the benefit of the rice farming community and the consumers of Sri Lanka.

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