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The Application of Biotechnology in Rice (Oryzae sativa) Quality Improvement

Adugna Mosissa Kajela (MSc)

Abstract
Cereals are one of the important foods and rice is important cereal grain and one of the most consumed staple foods around the world next to wheat. From the importance this crop provides nutritional, food security, economic and cultural importance is the major ones in the world. Rice is an autogamous plant propagating through seeds produced by self-pollination and also propagated by asexual propagation especially to generate transgenic crops by using tissue /cell culture techniques. Rice is a diverse crop that grows in different ecosystems. The Green Revolution saved millions of people with additional food but could not prevent hunger and poverty because of certain limitations and inadequate management available to take full advantage of the Green Revolution. Current gene revolution should provide wide scope for the application of biotechnology across ecosystems and crop barriers. Biotech rice has been developed using tissue culture, genetic engineering and molecular marker to address concerns that focus on the profitability of rice farming such as pest and disease resistance, abiotic stress tolerance and value-adding rice through nutritional improvement. In addition, basic studies to increase rice yield are underway including the incorporation of genes in the C4 pathway, a more efficient converter of light energy and carbon dioxide into food assimilates. Moreover, basic research on rice production of cloned seed has been started and promising results are being generated. This will considerably reduce the cost of production of hybrid rice, an important breeding strategy in rice production.
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1. INTRODUCTION

Rice is a monocot plant belonging to the genus *Oryza*in family Poaceae, cultivated for more than 10,000 years (Sasaki 2001). The genus consists of 26 species (Khush 1997) out of which 24 are wild and only two (*Oryza sativa* L. and *O. glaberrima*Steud) are cultivated. The cultivated rice (*Oryza sativa*) is divided into three subspecies *indica, javanica* and *japonica* (Datta *et al*., 2003). The two main subspecies grown in Asia are *indica* and *japonica*. 80% of cultivated rice in the world comprises *Indica* rice (*Ramesh et al*., 2009; Tie et al., 2012) and is grown mainly in South and South-East Asian countries (Zhang *et al*., 2005). Greater than half of the world's population depend on rice as staple food (Hadiarto and Tran, 2011). In Asia more than 90% of the population use rice as main food (Khush and Brar 2001; Ziegler and Barclay , 2008). By 2020 eight billion populations are expected from today's global population of six billion.

Hence, we must produce 25–40% more rice with less land and water and with a reduced use of agrochemicals. Rice yield has been slow for the last three decades, despite the improved varieties and technologies in place. Therefore, biotechnology particularly genetic engineering may provide ample capacity to improve rice yield and plant protection, enable rice to grow in drought and saline conditions, and lead to more nutritious rice for reducing malnutrition (Datta, 2004). Rice is the second broadly cultivated cereal in the world, after wheat (Pazuki and Sohani, 2013). There is an everyday increasing demand of rice production as the rice consumers are increasing at the rate of 1.6-1.8% every year (Karthikeyan *et a*l., 2009; Shobarani *et al*., 2010). According to Savary *et al*. (2000), pests, diseases and weeds are causes for 24–41% loss of rice yield annually. In the developing world, the nutritional improvement of rice can also help in decreasing the problem of malnutrition (Bajaj and Mohanty, 2005). Hence, the use of biotechnological tools is the novel, powerful and effective way to combat such problems.

2. LITERATURE REVIEW

2.1. Overview of Biotechnology Application in Rice

According to the report by Program for Biosafety Systems (PBS) and ABSPII (2004) in the twentieth century, breeding became more complicated, as the characters that breeders select for include increased yield, disease and pest resistance, drought resistance and enhanced flavor traits are passed from one generation to the next through genes, which are made of DNA (Wegrzyn *et al*., 2003). All living things including the fruits, vegetables and meat that we eat contain genes that tell cells how to function. Currently, scientists have studied and identified genes that are responsible for traits that will improve rice production (PBS and ABSPII, 2004). Starting from the ability to identify genes that confer advantages on some crops, and the ability to work with such characteristics very precisely, biotechnology enhances breeders' ability to make improvements in crops and livestock. Improvements have been possible using biotechnology that is not possible with traditional crossing of related species alone (Herdt, 2006). Biotechnology applying on different crops in the world while rice is one of the crops that have been improved by biotechnology (Wang *et al*.2007). Rice is also used for animal feed and provides the major source of

income for rural people; high quality rice brings in additional income (Mohanty, 2013). Agricultural Biotechnology is used by;

- Tissue culture
- Genetic engineering
- Molecular markers

2.2. Application of tissue culture

Tissue culture is a process of growing cell, tissue, seed node, bud, leaves, meristem and root tips under controlled environment or ascetic environment. Crop improvement by tissue culture method is easier when we compare to conventional plant breeding (Yamada, 1986).Somaclonal variations occur after the tissue culture, which involve a callus stage (Larkin and Scow Croft 1981). Callus is undifferentiated mass of rapidly proliferating cells, which can be obtained by culturing explant source (seed, node, bud, leaves, meristem and root tips etc.) on nutrient medium containing specific growth regulators along with a standard recipe of chemicals. Rashid *et al.* (2000) reported that rice seeds have more potential for callogenesis as compared to node or tip. Successful callus induction of rice seed were reported by many researchers (Gonalz 2000; Navraj *et al*.1999; Marrassi 1996; Valdez *et al*. 1997; Xie *et al*.1995). But the improved method for callogenesis was reported by Rashid *et al.*2000).

2.2.1. Anther culture

Anther culture is used to produce double haploid lines (DH) from crosses for various plant breeding objectives. Niizeki (1997) reported that Niizeki and Oono were the first successful callus induction of haploid from rice through anther culture. Since then, the anther culture technique has been advanced significantly. According to Oono and Niizeki (1971) report, Indica rice is generally regarded as difficult for anther culture. According to Chen *et al,* (1997) and Zhou *et al*. (1983) reported the use of anther culture to improve the fertility by hybridization of japonica/indica species of rice. When the seed set of f1 hybrids was 26-36 % fertile, the back crossing improved this rate to 45-50 %, and the anther culture derived H1 lines from the same crosses exhibited as high as 80 % fertility (Furuhashi, and Yatazawa, 1964). Anther culture does not conceal the phenotypic expression of the recessive genes Zapata *et a*l. (1991).

According to Sood, Siddiq (1978) reported the aromatic culture of anther is controlled by a single recessive gene which is increase the ratio of the produced aromatic lines. Euphytica *et al,* (1996) reported Un-nucleate pollens, the developing stage of pollens is effective in culture and the highest induction ratio was obtained when middle uni-nucleate pollens were used for tissue culture. These pollens can be identified when the length between auricles of flag leaf and penultimate leaf is between 5-7 cm and the colour of glumaceous flower is light green. The reply of genotypes for culturing ability is ordered as from high to low that is glutinous rice, japonica, japonica/indica hybrids, hybrids of indica, indica and non-glutinous rice (Chen *et al*., 1983).The alanine content in anthers affects induction and regeneration process. This was very pronounced in the anthers of Indica type which contains alanine lower than other types. Callus production in this type was promoted by addition of alanine to culture medium. The pretreatment (panicles kept in refrigerator at 10 ºC for 10 days) is believed to cause microspores to unite into uninucleate stage and this is due to respiration reduction for microspores and reduction for consumption of substances.

2.2.2 Callus induction

Somaclonal variations commonly appear after tissue culture, which produce a callus stage (Larkin and Scow Croft 1981). Callus is undifferentiated mass of rapidly proliferating cells, can be obtained by culturing explants source (seed, node, bud, leaves, meristem and root tips etc.) on nutrient medium containing specific growth regulators along with a standard recipe of chemicals. Rashid *et al.* (2000) studied that rice seeds have more potential for callogenesis as compared to node or tip. Successful callus induction from rice seed has been reported by several researchers (Gonalz 2000; Navraj *et al*.1999; Marrassi 1996; Valdez *et al*. 1997; Xie *et al*.1995). But an improved method for callogenesis was reported by Rashid *et al.* 2000). The present study is based on tissue culture techniques carried out in ABI laboratory for callus induction in rice varieties viz. Basmati-370 and Basmati-385. Two types of basal media M.S (Murashige and Skoog1962) and N6 (Nitsch and Nitsch, 1969) supplemented with 2, 4-D (2, 4 dichloro phenoxy acetic acid) alone or in combination with different concentrations of BAP (6-benzylaminopurine) were used for callus induction.

2.3. Genetic engineering

Scientists have learned how to move genes from one organism to another. This has been called genetic modification (GM), genetic engineering (GE) or genetic improvement (GI). Regardless of the name, the process allows the transfer of useful characteristics (such as resistance to a disease) into a plant, animal or microorganism by inserting genes (DNA) from another organism (Li and Gray, 2005). Nearly all crops improved with transferred DNA (often called GM crops or GMOs) to date have been developed and support to increase production and productivity of farmers by reducing crop damage from weeds, diseases or insects (PBS and ABSP11, 2004). Ever since the appearance of the first report on successful production of transgenic rice plants of *Japonica* in 1988

(Zhang *et al*., 1988), a large number of rice varieties have been introduced with agronomical and economically important genes. Direct DNA transfer methods such as protoplasts (Datta *et al*. 1990), biolistic method (Christou *et al*., 1991) and Agrobacterium mediated methods (Hiei *et al*., 1994) are being used regularly in rice transformation in the biotechnology laboratories across the world including India. Transgenic *indica* rice tolerant to biotic stresses such as insect pests and disease causing organisms like viruses, fungi and bacteria have been developed and tested by research group's worldwide (Datta et al. 1996; Lin *et a*l., 1995; Tu *et al*. 1998).

2.3.1. Transgenic rice for modifying yield potential

Starch biosynthesis has great role in metabolism of plant. According to the report of Stark *et al,* (1992), starch levels and dry matter accumulation were enhanced in potato tubers of plants transformed with *glg*C16 gene from *E.coli* encoding ADPGPP. The *glg*C16 gene has been introduced into rice and the yield potential of these lines was evaluated. Ku *et al.* (1999) reported that the transfer of C4 character into C3 rice is to improve efficiency of photosynthesis. However, it is difficult to incorporate genes for C4 traits into C3 plants through traditional plant breeding methods. *Agrobacterium*-mediated transformation has been used to introduce a gene for phosphoenolpyruvate carboxylase (PEPC) from maize to rice which catalyzes the initial fixation of atmospheric CO2 in C4 plants Ku *et al.* (1999).

Most transgenic rice plants showed high level expression of the maize gene; the activities of PEPC in the leaves of some transgenic plants were two to three times higher than those in maize, and the enzyme accounted for up to 12 percent of the total leaf soluble protein. The level of expression of the maize PEPC in transgenic rice plants correlated with the amount of transcript and the copy number of the inserted maize gene. The transgenic rice plants exhibited reduced O2 inhibition of photosynthesis and photosynthetic rates comparable to those of untransformed
plants.

2.3.2. Transgenic rice for insect resistance

Improved rice cultivars are either susceptible to the insect or have only moderate levels of resistance. Fujimoto *et al.* (1993) reported that a truncated d-endotoxin gene, *cry1A (b),* into rice. Transgenic plants in the R2 generation expressing the *cry1A* (*b*) protein showed increased resistance to striped stem borer and leaf folder. Wunn *et al.* (1996) introduced *cry1A (b)* gene into IR 58 through particle bombardment. The transgenic plants in R0, R1 and R2 generation showed resistance to several lepidopteran insect pests, with feeding studies revealing a mortality rate of up to 100 percent for yellow stem borer and striped stem borer larvae.

According to the report of Nayak *et al.* (1997) transformed IR 64 through particle bombardment using *cry1A(c)* gene and identified six independent transgenic lines with high expression of insecticidal crystal protein. The transferred synthetic *cry1A(c)* gene was stably expressed in the treatment 2 of these lines and the transgenic rice plants proved highly toxic for yellow stem borer larvae. Cheng *et al.* (1998) reported that 2600 transgenic rice plants in nine strains through *Agrobacterium* mediated transformation. The plants were transformed with two synthetic *cry1A (b)* and *cry1A(c)* -coding sequences from *Bacillus thuringiensi*. According to the report of Maqbool *et al.* (1998) Bioassays in R1 indicated that the transgenic plants were highly toxic for striped stem borer and yellow stem borer.

2.3.3. Transgenic rice for disease resistance

Sources of resistance to some diseases (blast and bacterial blight) have been identified within cultivated rice germplasm, and improved germplasm with resistance has been developed. Hayakawa *et a.* (1992) reported that CP (coat protein) gene for rice stripe virus was introduced into two *japonica* varieties by electroporation of the protoplasts. The resultant transgenic plants had high levels of CP (up to 0.5% of the total soluble proteins) and exhibited a significant level of resistance to virus infection. The resistance was inherited in the progenies. Zhang *et al.* (1998) introduced *Xa21* using projectile bombardment of cell suspensions of elite *indica* rice varieties: IR 64, IR 72, Minghui 63, and BG90-2. Six of the 55 R0 lines carrying*Xa21* showed a high level of resistance to bacterial blight in subsequent generations. According to the report of Zhu and Lamb, (1991) about six chitinase genes have been identified in rice and are being manipulated to increase the level of resistance to fungal diseases. Lin *et al.* (1995) introduced that 1.1 kilobase rice genomic DNA fragment containing a chitinase gene through PEG-mediated transformation which made the rice plant resistance to fungal diseases.

2.3.4. Transgenic rice for abiotic stress tolerance

Abiotic stresses, such as drought, excess water, mineral toxicities/deficiencies in soil and unfavourable temperature, affect rice productivity. Genetic engineering approaches hold great promise for the development of rice cultivars with higher levels of tolerance to abiotic stresses. Sakamoto and Murata (1998) introduced the *codA* gene for choline oxidase from *Arthrobacterglobiformis*. The *codA* gene was inherited into the second generation of transgenic rice and its expression was stably maintained at levels of the mRNA, the protein and enzyme activity and enhanced tolerance to salt and the cold.

2.3.5. Transgenic rice for Herbicide tolerance

Herbicides is a substance that is toxic to plant used to destroy unwanted vegetation and classified into two classes – the selective ones that kill weeds but not crops and the nonselective ones that eliminate all types of plants.

Glyphosate and glufosinate were known as the widely use d herbicides which giant companies like Mosanto have mass produced transgenic in-bred maize that are resistant to both herbicides (Dunwell, 2013). In recent years, rice has been genetically modified to exhibit resistance to herbicide. For example, glyphosate resistant rice engineered with aG6 gene, which encodes for Epsp synthase isolated from *Pseudomonas putida* showed resistance to 8g/l of glyphosate (which is the commercial resistance level) during field trial (Zhao, Lin, and Shen, 2011). Transgenic rice engineered with the optimized codon of the Cp4- Epsp synthase gene showed 2.7times higher resistance towards both glyphosate and glufosinate than that reported by Mosanto (Deng *et al*., 2014). As Epsp synthase is localized to the plant's chloroplast, both studies included a chloroplast transit peptide to the N-terminus of the gene to guide the translocation of the desired gene to the targeted organelle. Melatonin-rice transgenic rice inserted with the sheep serotonin N-acetyltransferase (NAT) gene demonstrated resistance against butafenacil, an ALS-inhibiting herbicide that kills plant by producing excessive ROS (Park *et al*., 2013).

2.3.6. Transgenic rice for improved nutritional quality

The advances of transgenic rice biotechnology began two decades ago with the production of the first transgenic rice plants which then advocated the development of reproducible and high throughput transformation protocols (Hiei *et al*., 1994). Along with the sequencing of the rice genome, transgenic rice research has spurred on with a variety of improved rice with better stress tolerance and nutritional quality being successfully developed. Currently, there are 170 million hectares of land cultivated with transgenic crops in 2012 as compared to the 1.7 million hectares in 1996 (Gupta *et al*., 2013). Currently, commercial cultivation of transgenic rice has yet to reach the massive planting scale of transgenic maize which accounts of more than 15 million hectares around the world (James, 2011). Continuous efforts are on the way to bring transgenic rice into a larger marketable scale which is globally beneficial. These efforts are then translated into milestones achieved in the production and commercialization of transgenic cereal crops with improved agronomical traits Rice contains neither b-carotene (pro vitamin A) nor C40 carotenoid precursors in its endosperm.

Millions of rice consumers who depend on rice for a large proportion of their calories suffer from vitamin A deficiency. Ye *et al.* (2000) reported that the production of transgenic rice (Golden Rice) with the provitamin A (b-carotene) biosynthetic pathway engineered into its endosperm. *Agrobacterium*-mediated transformation was applied to introduce three genes: phytoene synthase (*psy*), phytoenedesaturase (*crt1*) and lycopene cyclase (*lcy*). HPLC (high performance liquid chromatography) analysis revealed the presence of b-carotene in transgenic seeds. Goto *et al.* (1999) introduced the entire coding sequence of the soybean ferritin gene into kita-ake, a rice cultivar via *Agrobacterium*-mediated transformation. The introduced ferritin gene was regulated by the rice seed storage protein glutelin promoter, *GluB*-1, and terminated by the *Nos* polyadenylation signal. Synthesis of soybean ferritin protein was confirmed in each of the transformed rice seeds by western blot analysis, and specific accumulation in endosperm was determined by immunological tissue printing. The iron content of transformed seeds was up to three times higher than in untransformed seeds.

2.4. Molecular Markers systems for Rice

Genetic markers are the biological features that are determined by allelic forms of genes or genetic loci and can be transmitted from one generation to another, and thus they can be used as experimental probes or tags to keep track of an individual, a tissue, a cell, a nucleus, a chromosome or a gene. Genetic markers used in genetics and plant breeding include morphological markers, biochemical markers (alloenzymes and other protein markers) and molecular markers (based on DNA-DNA hybridization (Xu, 2010).DNA markers have developed into many systems based on different polymorphism detecting techniques or methods (southern blotting – nuclear acid hybridization, PCR – polymerase chain reaction, and DNA sequencing), such as RFLP,AFLP, RAPD, SSR, SNP (Collard *et al*., 2005;Farokhzadeh *et al*., 2014).DNA marker technology refers to the application of DNA based markers in breeding programs to improve the selection efficiency (Sarma and Sundaram, 2005).

However, traditional breeding involves selection of individual plants or animals based on visible or measurable traits. By examining the DNA of an organism, scientists can use molecular markers to select plants or animals that possess a desirable gene, even in the absence of a visible trait. Thus, breeding is more precise and efficient. Another use of molecular markers is to identify undesirable genes that can be eliminated in future generations (PBS and ABSP11, 2004). However conventional rice breeding approaches have improved rice cultivars for thousands of years. But the progress is slow, due to the time consuming process, the quantitative nature of most agronomic traits and difficulties in genotype selection (Wang *et al*., 2007). In rice, many PCR based markers like randomly amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), inter-simple sequence repeats (ISSRs) and simple sequence repeats (SSRs) are being used. Among the PCR based DNA markers, microsatellites or simple sequence repeats (SSRs) are highly preferred for gene tagging and mapping efforts due to the high level of polymorphism content and versatility and are preferred due to their reproducibility and amenability for automation (McCouch *et al*., 1997; McCouch *et al*., 2002). More than twenty thousands of SSR markers have been developed in rice so far and their chromosomal location and polymorphism levels have been determined (IRGSP 2005). Due to these reasons, SSRs are the markers of choice for rice

improvement today for MAS application.

Recently, a special class of markers called single nucleotide polymorphisms (SNPs) are gaining predominance in those crop species like rice, whose genomes have been sequenced. A single nucleotide polymorphism (SNP, pronounced as snip) is a DNA sequence variation occurring when a single nucleotide A, T, C, or G in the genome differs between members of a species or paired chromosomes in an individual. McNally *et al*. (2009) undertook genome-wide SNP variation among 20 diverse elite rice varieties and landraces and observed 160,000 non-redundant SNPs. Even though SNP markers are not widely used for marker-assisted breeding (MAB) in rice, in future it is expected that they will replace SSRs as markers of choice. Over the past decade, researchers have developed and applied marker assisted selection (MAS) and quantitative trait locus (QTL) analysis techniques to rice breeding. These approaches promote new germplasm identification and new elite cultivar establishment (Wang *et al*., 2007). As described in the above MAS have over advantages on transgenic and conventional breeding. It can be more efficient, effective and reliable than phenotypic selection.

MAS can shorten the development time of varieties significantly, so in some cases it will be more cost effective than selection based on phenotypes. MAS also allow the breeding of complex traits not feasible through previous conventional methods. Although certainly not the silver bullet for all problems, MAS is a promising approach to conventional plant breeding. When we comparing with transgenic breeding it has over advantages of the Biosafety and Intellectual Property Rights are not major issues, however source of genes restricted to the gene pool of the species and Marker-assisted breeding has a much higher crop improvement potential than genetic engineering. Many ergonomically important rice genes have been tagged with markers and are readily deployed by breeders in breeding programs.

2.4.1. Gene tagging in rice

Gene tagging is the use an insertional mutagen to mark interrupted genes with a unique DNA sequences. This DNA sequence subsequently can be used a target for hybridization or as an annealing site for PCR primer. According to the report of Khush and Brar (1998) two of the most serious and widespread diseases in rice production are rice blast (caused by the fungus *Pyriculariaoryzae*) and bacterial blight (caused by *Xanthomonasoryzae* pv.*oryzae*). The development of varieties with durable resistance to these diseases is the focus of a joint coordinated effort by International Rice Research Institute (IRRI) and National Agricultural Research Systems (NARS) using molecular marker technology.

Several genes for bacterial blight and blast resistance have been tagged with molecular markers many rice improvement programmes now aim to incorporate quantitative or polygenic resistance into rice varieties. Wang *et al.* (1994) reported that identified gene *Pi5*, which confers complete resistance, including nine regions of the genome with quantitative effects on blast resistance. The latter were considered to be putative QTL for blast resistance. Three of the QTL found to be associated with partial resistance in this study had previously been identified as being linked to genes for complete resistance. Analysis of major resistance genes has also been carried out using near isogenic lines (NILs). The NILs have been used for mapping genes *Pi2* and *Pi4*.

2.4.2. QTL mapping

According to the report of Khush and Brar (1998) a number of important characters is determined by loci which have major effects on phenotype. However, most economically important traits, such as yield, quality and tolerance to various abiotic stresses (drought, salinity, submergence etc.), are of a quantitative nature(Khush, and Brar, (2001). Genetic differences affecting such traits (within and between populations) are controlled by a relatively large number of loci, each of which can make a small positive or negative contribution to the final phenotypic value of the traits. Several QTL for traits of economic importance, such as blast resistance (Wang *et al.*, 1994), root traits (Redoña and Mackill, 1996; Yadav *et al.*, 1997; Zheng *et al.*, 2000), submergence tolerance (Nandi *et al.*1997) and yield components (Xiao *et al.*, 1996, 1998), have been mapped with molecular markers.

According to Wang *et al.* (1994) mapped *Pi5* and *Pi7* for blast resistance on chromosomes 4 and 11, respectively. Nine QTL with quantitative resistance to isolate PO6-6 of blast were also identified. Similar research also reported by Li *et al.* (1995) indicated that six QTL contributing to resistance to *Rhizoctonia solani*. These QTL are located on six of the 12 rice chromosomes and accounted for 60 percent of the genotypic variation in the cross Lemont x Teqing. Nandi *et al.* (1997) used AFLP markers and identified four QTL for submergence tolerance on chromosomes 6, 7, 11 and 12 of rice. In addition, a major gene (*Sub1*) for submergence tolerance was localized on chromosome 9. Xiao *et al.* (1996, 1998) analyzed BC2 test cross families from the interspecific cross (*O. sativa* x *O. rufipogon*) and found that *O. rufipogon* alleles at marker loci RM5 on chromosome 1 and RG256 on chromosome 2 were associated with an 18 and 7 percent increase in grain yield per plant. A total of 68 significant QTL were identified and of these, 35 (51%) had beneficial alleles derived from the phenotypically inferior *O. rufipogon* parent.

Moncada *et al* .(2001) used advanced backcross QTL analysis on *O. sativa* x *O. rufipogon* derivatives and found that certain regions of rice genome harbor genes which are useful in a range of environments. Molecular markers can be used to identify QTL from wild species responsible for transgressive segregation. Comparative mapping among different cereals has also increased the efficiency of mapping orthologous QTL. QTL map has

significant application in;

•Identification of QTL exploited in different environments

•Exploitation of complementary QTL for isolation of transgressive segregants, particularly from interspecific crosses

•Identification of orthologous QTL among different species, as the conservation of such QTL among species may provide new opportunities for the manipulation of economic traits

•High resolution of QTL to determine whether QTL are single genes or clusters of tightly linked genes and whether over dominance plays a significant role in heterosis; and

•Cloning of QTL based on high resolution mapping.

2.4.3. Map-based cloning of genes

Map based on cloning is an iterative approach that identifies the underlying genetic cause of the mutant phenotype and by looking the linkage of markers whose physical location in the genome is known. The high density molecular genetic map and the development of BAC and YAC libraries have been important developments leading to the isolation of rice genes such as *Xa1*, *Xa21* and *Pib*. Wang *et al.* (1995a) used a BAC library to identify clones linked to the *Xa21* gene for bacteria blight resistance (BB). Song *et al.* (1995) isolated the *Xa21* gene using positional cloning isolated gene and introduced into rice cultivars through transformation. Similarly, *Xa1* for BB resistance has been cloned through a map-based cloning strategy (Yoshimura *et al.*, 1998). The expression of *Xa1*, unlike that of previously studied resistance genes, was induced by wound and pathogen infection.

At IRRI, a BAC library was constructed from IR 64 genomic DNA (Yang *et al.*, 1997), and it consists of 18 432 clones. BAC clones carrying *xa5* for BB resistance have been identified (Yang *et al.*, 1998). Efforts to clone *xa5* are underway and, once cloned, it will be transferred to elite rice varieties through transformation. Wang *et al.* (1999) cloned the blast resistance gene *Pib*. The expression of *Pib* is induced when there are changes in the environmental conditions, such as temperature and darkness. Sanchez *et al.* (1999) identified BAC contigs flanking the *xa13* locus for BB resistance, while Hayano-Saito *et al*. (2000) identified overlapping BAC clones flanking *Stvb1*, the gene for stripe virus resistance

3. CONCLUSION

Cereals are one of the important foods and rice is important cereal grain and one of the most consumed staple foods around the world next to wheat. From the importance this crop provides nutritional, food security, economic and cultural importance is the major ones in the world. Rice is an autogamous plant propagating through seeds produced by self-pollination and also propagated by asexual propagation especially to generate transgenic crops by using tissue /cell culture techniques. Rice is a diverse crop that grows in different ecosystems. Biotech rice has been developed using tissue culture, genetic engineering and molecular marker to address concerns that focus on the profitability of rice farming such as pest and disease resistance, abiotic stress tolerance and value-adding rice through nutritional improvement. Moreover, basic research on rice production of cloned seed has been started and promising results are being generated. This will considerably reduce the cost of production of hybrid rice, an important breeding strategy in rice production.

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