ISAAA

Peer Reviewed

Pocket K

Biotech Rice

Global Knowledge Center
on Crop Biotechnology

Pocket Ks are Pockets of Knowledge, packaged information on crop biotechnology products and related issues available at your fingertips. They are produced by the Global Knowledge Center on Crop Biotechnology (http://www.isaaa.org/kc). For more information, please contact the International Service for the Acquisition of Agri-biotech Applications (ISAAA) SE*Asia*Center c/o IRRI, DAPO Box 7777, Metro Manila, Philippines.
Tel: +63 2 580 5600
Fax: +63 2 580 5699
Telefax: +63 49 5367216
E-mail: knowledge.center@isaaa.org

May 2010 (No. 37)

ISAAA
International Service for the Acquisition of Agri-biotech Applications

Rice is the staple food of the two billion people living in Asia and Africa, providing 40 to 70% of the total food calories. The past green revolution over three decades ago has provided enough food and livelihood which averted the looming hunger and famine then. With the imminent doubling of the world population in 2050, world food production should be increased by 50% especially the cereal staples[1]. Numerous scientific initiatives and strategies were developed towards increased food production especially on rice. One of these is the International Program on Rice Biotechnology (IPRB) of the Rockefeller Foundation which has provided funds since 1984 to foster cutting-edge genetics research aimed at helping rice farmers in the developing world. Most of the rice experts and rice research laboratories in the developing countries were trained and supported by the Program[2].

Initial studies to develop biotech rice were started in the early 80's as tissue culture experiments: playing with media components including hormones and complex amino acids and sugars; explant sources; culture conditions; and regeneration strategies. This period overlapped with the development of different genetic engineering procedures for rice. Particle bombardment and *Agrobacterium tumefaciens*-mediated transformation were considered the most efficient in expressing reporter genes: beta glucuronidase (*gus*A) and the green fluorescent protein gene (*gfp*); and selectable marker genes: herbicide and antibiotic resistance genes.

Pest and Disease-Resistant Biotech Rice

With the discovery and availability of pest resistance genes within the IPRB program, biotech rice was developed to improve rice's resistance to the devastating pests yellow stem borer, bacterial blight, blast, and sheath blight. Stemborer infestation of rice farms especially in the wet season poses extreme damage to as much as 30%[3]. Stemborer resistance breeding has been a difficult endeavor for the breeders since there is no high level of resistance in the rice gene pool and screening for resistance has always been a problem. A number of laboratories developed different local varieties to contain the *Bt* genes (*cry*1Ab, 1Ac 1Aa, 2A, 1B, or a combination of these genes) for resistance against lepidopteran pests[4,5,6]. The first field testing of the Bt rice was conducted in China in 1998[7,8]. However, no Bt rice has been commercialized legally as yet. In late 2009, China's Ministry of Agriculture released biosafety certificates for Bt rice Huahui No. 1 and Bt Shanyou 63 with possible widescale planting in 2012[9].

Rice stemborers.
(Photo courtesy of IRRI.)

Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *Oryzae* can cause up to 50% yield loss in severe pathogen attacks. With the discovery, identification and cloning of the *Xa*21 gene in the wild rice *Oryza longistaminata* which confer broad-spectrum bacterial blight resistance, a new strategy was unfolded[10]. A number of rice varieties including IR64, IR72, IR50, CO39, Pusa Basmati-1, IR68899B, MH63, BPY5204 and some Chinese lines were genetically-engineered to contain the gene[11,12]. Field testing of some transgenic lines were conducted in China and the Philippines but no commercialized lines has been out so far[13,14].

Efforts to develop rice for resistance to sheath blight were conducted by incorporating genes coding for chitinase and glucanase enzymes that metabolize the fungal cell wall, and other pathogenic-related proteins[15,16]. Increased activity of the introduced chitinase and glucanase were induced with fungal elicitors, however, field experiments need to be undertaken to determine efficacy against the pathogen.

Simultaneously, a collaborative effort to complete the DNA sequence of the rice genome was forged between private and public institutions[17]. In February 2001, the entire rice DNA sequence was completed and has been shared to facilitate the understanding of the rice genetic structure and associated proteins to enable rice breeders to produce more nutritious, productive and resource efficient rice.

REFERENCES

[1,12,14] Rice Biotechnology: A need for developing countries. 2004 Swapan K Data Agbioforum 7(1&2):31-35.

[2] Rice Biotechnology: Rockefeller to End Network After 15 Years of Success 1999. Dennis Normille (http://www/sciencemag.org/)

[3] IRRI Annual Report, 1999. Los Baños, Laguna

[4] Achieving successful deployment of Bt Rice. 2004. Sasha Ming High, Michael B. Cohen, Qing Yao Shu, and Illimar Altosaar.TRENDS in Plant Science 9:6:286-292.

[5] Translational fusion hybrid Bt genes confer resistance against yellow stem borer in transgenic elite Vietnamese rice (*Oryza sativa* L.) cultivars. 2006. N. H. Ho, N. Baisakh, N. Oliva, K, Datta, R. Frutos, and S.K. Datta. Crop Scie. 46:781-789

[6] Bt rice harbouring cry genes controlled by a constitutive or wound-inducible promoter: protection and transgene expression under Mediterranean field conditions. 2004. J.C. Breitler, J.M. Vassal, Maria del Mar Catala, D. Meynard, V. Marfa, E. Mele, Monique Royer, Isabel Murillo, Blanca San Segundo, E. Guiderdoni, and Joaquima Messeguer. Plant Biotechnology Journal 2(5) :417-430.

[7] Transgenic rice plants with a synthetic *cryiAbi* gene from *Bacillus thuringiensis* were highly resistant to eight lepidopteran rice pest species. Q. Shu, G. Ye, H. Cui, X. Chang, Y. Xiang, D. Wu, M. Gao, Y. Xia, C. Hu, R. Sardana, and I. Altosaar. Mol. Breed. 6: 433-439.

[8] Transgenic IR72 with fused *Bt* gene *cry1(b)/cry1A(c)* from *Bacilus thuringiensis* is resistant against four lepidopteran species under field conditions. 2001. Plant Biotechnol. 18:125-133.

[9] Crop Biotech Update 4 December, 2009.

[10] A receptor kinase-like protein encoded by the rice disease resistance gene *Xa*21. 1995. Song, W.Y. Song,G.L. Wang, L.L. Chen, H.S. Kim, L.Y. Pi, T. Holsten, B. Wang, W.X. Zhai, H. Zhu, C. Fauquet, and P.C. Ronald, Science 270:1804-1806.

[11] Transgenic elite indica rice varieties, resistant to *Xanthomonas oryzae* pv. *Oryzae*. 1998. S Zhang, W-Y. Song., L. Chen, D-L. Ruan, N. Taylor, P. Ronald, R. Beachy and C. Fauquet C. Molecular Breeding 4:551-558.

[13] Field performance of *Xa21* transgenic indica rice (*Oryza sativa* L.), IR72. 2000. Theoretical and Applied Genetics. 101:15-20.

[15] Genetic engineering of rice for resistance to sheath blight. 1996. W. Lin, C.Anuratha, S. Datta, K. Potrykus, S. Mathukrishnan and S.K. Datta. 1995. Bio/Technology 13:686-691.

[16] Improving rice to meet food and nutrient needs: Biotechnological approaches. 2002 S.K.Datta and G.S. Khush. Journal of Crop Production. 6(1):229-247.

[17] The map-based sequence of the rice genome. 2005.International Rice Genome Sequencing Project. *Nature*, Vol. 436, pp. 793-800.

[18] http://www/agbios/com/dbase.php?action=ShowProd&data=CL121%2C+Cl141%2C+CFX51

[19] Water management in irrigated rice: Coping with water scarcity. 2007. B.A.M. Bouman, R.M. Lampayan, and T.P. Tuong. http://dspace.irri.org:8080/dspace/bitstream/10269/266/2/9789712202193_content.pdf

[20] Improvement of water use efficiency in rice by expression of HARDY, an *Arabidopsis* drought and salt tolerance gene. 2007. A. Karaba, S. Dixit, R. Greco, A. Aharoni, K.R. Trijatmiko, N. Marsch-Martinez, A. Krishnan, K. N. Nataraja, M.Udayakumar, and A. Pereira. PNAS. 104(39):15270-15275.

[21] Arabidopsis CBF3/DREB1A and ABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. 2005. S-J. Oh, S.I. Song, Y.S. Kim, H-Y. Jang, S.Y. Kim, M.Kim, Y-K. Kim, B.H. Nahm, and J-K Kim. Plant Physiology 138:341-351.

[22] Expression of a bifunctional fusion of the *Escherichia coli* genes for trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase in transgenic plants increases trehalose accumulation and abiotic stress tolerance without stunting growth. 2003. I-C. Jang, S-J. Oh, J-S. Seo, W-B. Choi, S.I. song, S.H. Kim, Y.S. Kim, H-S Seo, Y.D. Choi, B.H. Nahm, and J-K. Kim. Plant Physiology. 131:516-524.

[23] Food composition and nutrition tables. 6th ed. 2000. S.W. Souci, W. Fachmann, H. Kraut. Food Quality Control, World Food Programme. http://foodquality.wfp.org/FoodProcessing/Cerealmilling/Nutritionalimpact/tabid336/PageContentID/458/Default.aspx.

[24] Engineering the provitamin A (b-carotene) biosynthetic pathway into carotenoid-free) rice endosperm. Y. Xudong, S. Al-Babili,. A. Klöti, J. Zhang, P. Lucca, P.Beyer, I. Potrykus. Science 287:303-305.2000,

[25] Improving the nutritional value of Golden Rice through increased pro-vitamin A content. 2004. J.A. Paine, C. A. Shipton, S. C. Rhian, M. Howells, M. J. Kennedy, G.Vernon, S.Y. Wright, E. Hinchliffe, J. L. Adams, A. L. Silverstone, and R. Drake. Nature Biotechnology 23(4), 482-487

[26] Transgenic rice is a source of iron for iron-depleted rats. 2002. L.E. Murray-Kolb, F. Takaiwa, F. Goto, T. Yoshihara, E. C. Theil, and J.L. Beard. Journal of Nutrition.132:957-960.

[27] Regulation of lysine synthesis and catabolism in rice. 2008. Q-Q. Liu, M-L. Chan, R-X. Duan, H-X. Yu, M-H. Gu, S.S. M. Sun. Abstract in Plant Genomics in China, PGCIX. (http://www.plantgenomics.cn/abslist.cgi?absid=789).

[28] Rice-based mucosal vaccine as a global strategy for cold-chain and needle-free vaccination. 2007. T. Nochi, H. Takagi, Y. Yuki, L. Yang, T. Masumura, M. Mejima, U. Nakanishi, A. Matsumura, A. Uozumi, T. Hiroi, S. Morita, K. Tanaka, F. Takaiwa, H. Kiyono. PNAS. 104(26):10986-10991.

[29] When expressing lactoferrin and lysozyme has antibiotic-like properties when fed to chicks. 2002. B.D. Humphrey, N. Huang, K.C. Klasing. J. Nutrition. 132:1214-1218.

[30] The rice squad. 2002. Nature 416: 576-578. April 11, 2002.

[31] Apomixis: The plant breeder's dream. 2001. (http://www.grain.org/seedling/?id=2

Problem on the lack of irrigation in the rice paddies is aggravated by the presence of the noxious weeds that affect the normal growth and yielding capacity of rice. Weed control measures usually include application of herbicide combinations, crop rotation, flooding and tillage which are expensive, labor intensive, and harmful to the environment and non-target humans and animals. The development of glufosinate-resistant biotech rice in 1999 was a welcome weed control measure. Glufosinate ammonium is a natural, broad-spectrum, contact herbicide that controls a wide range of weed species through the inhibition of the glutamine synthetase enzyme consequently preventing photosynthesis. It is highly degradable, has no residual activity, and has very low toxicity for humans and wild fauna. Glufosinate-resistant rice has been approved for commercialization in the USA, Canada, and Mexico[18].

Abiotic Stress Resistance

Rice is a water-loving plant that uses 30% of the freshwater used for crops worldwide – two to three times more water than other food crops[19]. With the imminent water shortage and increased salinity brought by global warming, strategies to develop rice to combat these abiotic stresses were conducted using stress-related genes and transcription factors identified in the model plant Arabidopsis. This include the expression of the *HRD* gene in rice that increased the leaf biomass and bundle sheath cells that would probably contribute to enhanced photosynthesis assimilation, water use efficiency and drought resistance[20]; and the expression of *CBF3/DREB1A* and *ABF3* in rice increased its salinity and drought tolerance 21. Moreover, bacterial genes for trehalose accumulation also increased tolerance to drought, salt, and cold in transgenic rice[22].

Photo courtesy of IRRI.

Nutritional Improvement

Rice is a good source of carbohydrate, proteins, fiber, lipid and fats, minerals (potassium, phosphorous, magnesium, calcium, sodium, copper and iodine) and vitamins (thiamine, riboflavin, niacin, vitamin B6 and folic acid)[23]. In poor countries which have less access to meat and fish, rice is predominantly eaten, thus, important minerals and vitamins are lacking in the diet. This leads to a widespread occurrence of vitamin A and E, iron and zinc deficiency which afflict susceptible children, pregnant and lactating women. Food supplementation and fortification programs conducted were found to be relatively expensive, noncompliance is high, and requires infrastructure for delivery and targeting. A novel approach is biofortification which uses biotechnological tools to incorporate genes for increased amounts of these essential food nutrients. Biotech rice with provitamin A (Golden Rice) has been developed[24,25] and is being used to transfer beta carotene loci into high-yielding local commercial cultivars through marker-assisted back cross breeding in the Philippines, Bangladesh and India. Progress in molecular marker-aided breeding projects the release of golden rice varieties by 2012. Biotech rice with increased ferritin content was found to replenish the hemoglobin and liver iron concentrations in rat experiments suggesting that biotechnological approaches to manipulating ferritin expression of seed iron may contribute to a sustainable solution to global problems of iron deficiency[26].

Ordinary rice (left) and golden rice (right).
(Photo courtesy of www.goldenrice.org)

Rice is devoid of essential amino acids such as threonine, tryptophan, lysine, and methionine. Strategies to improve the lysine content of rice showed that inhibition of lysine degradation through the RNAi approach increased free lysine level, and affected the concentrations of the amino acids related to lysine metabolic pathway, such as threonine and aspartic acid[27]. As plant proteins are the primary sources of all dietary proteins consumed by human and animals and are inexpensive to produce in comparison with meat, improving their quality will make a significant contribution to future needs.

Biopharming in Rice

Rice can be used as a vehicle to produce pharmaceuticals including vaccines. One of these is the development of a rice-based oral vaccine containing the vaccine antigen cholera toxin B subunit (CTB) which accumulates in the protein bodies of the starchy endosperm cells. These are taken up by mucosal cells of the gastrointestinal tracts for the induction of antigen-specific mucosal immune responses with neutralizing activity[28]. In addition, the rice-based CTB vaccine remained stable and maintained immunogenicity at room temperature for more than 1.5 years, and was protected from pepsin digestion in vitro. Other mucosal cell vaccines can be produced in rice to target diseases of the respiratory and gastrourinary tracts and can be administrated economically in the developing countries where need is often the greatest.

Extended use of antibiotics is documented to contribute to the development of antibiotic resistance in commensal bacteria in poulty, pigs, cattle, and humans necessitating the search for alternative strategies. Antibacterial molecules such as lactoferrin and lysozyme were considered and expressed in rice grains through biotechnology. Experimental feeding of broiler chickens fed with rice containing lactoferrin and lysozyme showed that they improve the feed efficiency, histological indices of intestinal health, and increased bacteriostatic activity. This strategy can also be used in maintaining intestinal health and in the prevention of diarrhea in other young animals including human infants[29].

Biotech Rice and the Future

Biotech rice has been developed to address concerns that focus on the profitability of rice farming such as pest and disease resistance and abiotic stress tolerance; value-adding rice through nutritional improvement; using it as a vehicle to produce pharmaceutical products; and as an instrument to provide environmental protection and reduce global warming.
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[30]. Moreover, basic research on apomictic rice or the production of cloned seed has been started and promising results are being generated[31]. This will considerably reduce the cost of production of hybrid rice, an important breeding strategy in rice production.