

## Transgenic Rice: Advancements and Achievements

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### Letter to the Editor

Rice is a staple crop and 90 percent of it is produced and consumed in Asia [1]. Viewing the widespread popularity and consumption of rice grain and its products, writers believe that “There may be homes where wheat and maize haven’t been cooked ever, but there will no home where rice hasn’t been cooked ever”. Green revolution has transformed rice production globally from 257 million tons to 718 million tons since 1996 to 2011 [2]. Unfortunately, even after such a vast achievement in the advancement of rice production, still 800 million people have to sleep with their stomach empty [2]. According to the current projection in growth rate it has been estimated that the world population will have a jump of 20.7% by 2030 and thus the jump in the food need is self-understood. Global rice demand is estimated to rise from 676 million tons in 2010 to 763 million tons in 2020 and to further increase to 852 million tons in 2035. This is an overall increase of 176 million tons in the next 25 years [3]. This additional need of rice production may only be achieved by increasing the productivity by supplementing conventional breeding methods with newly emerging techniques like genetic engineering. In this array countless breeding programmes have been initiated with one ultimate objective of future rice food security. Though the contribution of conventional breeding methods in enhancing the rice production by means of providing better yielding varieties, even under stress conditions is remarkable and cannot be denied, time has come to marinate the traditional breeding methods with the recent advancements in the genetic engineering. It has been almost two decades since the first transgenic rice came into existence. Since then a tremendous progress has been achieved by genetic engineers in developing more frequent and routine genetic transformation protocols by means of direct DNA transfer [4] or Agrobacterium mediated genetic transformation. Recombinant DNA technology has resulted in creation of transgenic rices with novel genetic traits and for resistance to biotic and abiotic stresses. High throughput transformation protocols for rice [5], activation tagging and insertional mutagenesis have bearing for enhancing transformation efficiency [3]. This advancement in technologies has facilitated researches in introducing several agronomically and economically important trait including nutritional improvements, which may not have been possible through conventional breeding. A major milestone, which has cherished these advancements of technologies in rice, is the availability of fully sequenced rice genome [6], that has not only opened the doors for the rice improvement but also for the improvements in other cereals such as maize and wheat [7]. It has been recommended that these advances in rice and other crops will realize a second green revolution through genetic engineering of food crops [8]. The advancements in rice genomics have helped researchers in developing more consistent technologies for the rice genetic transformation. Form last few years the focal point of agricultural researchers has also been shifted to comprise use of rice as

model monocot system, [3] which is not just because of the availability of rice genome sequence, but its small genome size of only 389 mb, availability of exceedingly dense physical and molecular maps [9,10] and simplicity in genetic transformation [11].

Among the objectives for increasing rice yields, foremost are the development of varieties which can tolerate biotic and abiotic stresses which are very common in the current climate change scenario [4,12]. The infestation of plant brown hopper (BPH) in rice leads to an annual loss of over billion dollars, which is an issue of great worry. Infestation of insect-pests and diseases not only lead to monetary losses but also affects the health of flora and fauna significantly, due to use of agro-chemicals especially in the developing and under-developed countries, since they have a lack in the regulations of the proper chemical uses in crops. The noteworthy efforts of researchers made introduction of foreign genes that provides a wide range of protection towards a variety of insects and pathogens, possible. These approaches are not only cost effective but also safer to the flora and fauna as they lessen the use of chemicals. In this array, the first ever known accomplishment of plant scientists was production of Bt-rice plants, which were created by introducing a synthetic Cry gene through particle gun mediated gene transformation, which principally provided resistance against lepidopteran pests. Subsequently, the enthusiasm of researchers helped them in unravelling other modifications of Cry gene (Cry1A, Cry1B, Cry1C, Cry1Ab, Cry9B) and gna and ltr (both providing resistance against hemipteran and coleopteran pests). Lui et al. [13] cloned and characterized bph3 gene, a cluster of three genes encoding plasma membrane-localized lectin receptor kinases (OsLecRK1-OsLecRK3). The bph3 was found significantly involved in providing resistance against BPH on transferring in susceptible varieties. In past few years numerous R genes have been cloned, in this sequence Pi-ta was the initial R gene cloned in laboratories, providing a wide range of resistance towards *Manophthora gracie*. Subsequently several R genes such as Xa1, Xa21, Xa26 providing resistance against bacterial blights were cloned. Recently, Chao et al. [14] identified a MYB transcription factor gene *OsAMYb* from rice landrace Heikezijing, which encodes a protein with 283 amino-acid residues belonging to R2R3-type MYB transcription factor family, they found that this novel gene was harbouring the rice plants with blast resistance with a novel trend in transgenic rice. Resistant against rice tungro bacilliform virus (RTBV), while rice tungro spherical virus (RTSV) assists the transmission of viruses by vector green leafhopper, *Nephotettix virescens*, has also been achieved by using two different novel strategy namely coat mediated resistance and replicase mediated resistance [15].

Apart from pests and pathogens, abiotic stress (dehydration, salinity, submergence, mineral deficiency, extreme temperature etc.)

negatively affects the rice production [2]. Bray et al. [16] mentioned that several abiotic stresses cumulatively lessen global crop production by 50% on an average. Production of 1 kg of rice seed requires 3,000 to 5,000 L of water, with less than half that amount needed for 1-kg seed production in other crops such as maize or wheat [17]. The severity in reduction of crop production has forced the plant scientists to engineer for abiotic stress tolerance. Since the initiation of genetic engineering in rice, till now, there has been a lot of progress and advancement in development of transgenic rice for abiotic stress tolerance. Both post genome sequence era and advancement in transgenic strategies with great ease have encouraged the plant scientists to understand the basics abiotic stresses in plants. Great progress has been accomplished by rice researches in developing tolerance headed for several abiotic stresses through transgenesis. Plant scientists have achieved a better position in reducing the losses by identifying and introducing endogenous and foreign genes. Introduction of genes such as, OsHsfA7, OSrab7, OtsA, OsGlyII and many more which have been isolated from rice had provided multiple abiotic stress tolerance towards drought, submergence, salinity and cold [4,18-24] at different level of gene expressions. Novel genes HVA1 and codA providing a protection from both drought and salinity have been isolated and transferred to rice from barely and *Arthro bacter globiformis* [25,26]. In 2015, Das and Mishra [27], reported an over-expressing gene namely HFBV2 involved in the high degree of salinity tolerance in rice by mean of suppressing the RNAi activities. Further, in the same year Haong et al. [28] provided the evidence of significantly improved level of salinity tolerance by introducing three exogenous anti-apoptotic over-expressing genes of different origins viz. AtBAG4 (*Arabidopsis*), Hsp70 (*Citrus tristeza virus*) and p35 (*Baculovirus*) in transgenic rice and demonstrated traits associated with tolerant varieties including, improved photosynthesis, membrane integrity, ion and ROS maintenance systems, growth rate, and yield components. An overexpressing gene SNAC1 TF providing a certain degree of both salinity and drought tolerance has been isolated from the rice landrace Pokkali of Bangladesh and was transferred into a popular high-yielding variety BRRI dhan 55, which was poorly responsive to tissue culture by the in planta method [29]. As rice is the one of the most consuming food crop, supplementing rice with little more nutritive values by means of foreign gene introduction or by enhancing the expression of endogenous genes may supplement those parts of globe which are facing an inadequate level of nutritional resources per capita. According to Poletti et al., [30] various strategies for biofortification in rice have been evaluated including both breeding and genetic engineering. Production of provitamin A-enriched rice popularly known as “golden rice” is a landmark, which was developed by Ye et al. [31] by introducing an entire  $\beta$ -carotene biosynthesis pathway into rice endosperm in a single transformation step [31]. The miracle became reality because of advances in genetic engineering techniques that enabled Ye et al. [31] to isolate psy (for phytoene synthase) and crt1 (for phytoene desaturase) from daffodil and bacteria respectively and let them introduce in rice genome successfully. Despite the landmark achievement of “golden rice” rice has also been furnished with genes governing the synthesis of human lactoferrin [32] and ferritin to meet the requirements of iron by infants and adults. Rice with significantly improved level of essential amino acid such as glycine [33]; lysine [34]; tryptophan [35]; cysteine [36] and methionine [37] have also been developed. Beyond these qualities several other qualities such as improved oil content [38]; manipulated starch content by manipulating the waxy locus at chromosome 6 in both indica and japonica subtypes [39] using RNAi technology [40] and gene targeting technology [41], had already been achieved by plant scientists. In order to secure

human health another landmark achievement through transgenesis is the development of plant-based oral vaccines [42]; especially in rice, probably the concept of developing these oral vaccines is behind stability and resistance to digestion in mammalian stomachs, of rice grains. Reports of testing the efficiency of rice-based vaccines for infectious and autoimmune diseases on mice and several other animals are already available. Azegami et al. [43] tested the efficiency of a rice-based oral vaccine called Mucorice-CTB for its safety and stability. The reports of the efficacy testing of same vaccine (Mucorice-CTB) are also available on humans and primates, successfully. Even though quite a few studies have depicted transgenic rice plants with enhanced biotic and abiotic stress tolerance during field trials, additional research is necessary to unravel the regulatory mechanism of complex trait response and tolerance under field conditions.

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