Producing Transgenic Rice with Improved Traits and Yield – How Far Have We Come?

Wong Kiing Fook, CLEMENT[a], Mui Yun, WONG[a,b], Kadir, JUGAH[a], Mahmood, MAZIAH[b,c]*

[a]Department of Plant Protection, Faculty of Agriculture,
Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.
[b]Institute of Tropical Agriculture,
Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.
[c]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia.
*maziahm@upm.edu.my

Abstract – Improving rice production is of current global concern so that food security is maintained especially in developing nations where rice remains as the staple food. With the aid of molecular biology, various isolated genes conferring to abiotic, biotic and herbicide stress tolerance has been successfully transferred into rice. Attempts have also been made to enhance grain yield, nutritional characteristics, fragrance and photosynthetic capacity of rice. The success of a commercialized transgenic rice largely depends on the biosafety and environmental risks assessments as these information translates into consumers' acceptance towards genetically modified (GM) rice. As the renowned Golden Rice has received the green light for field trial in the Philippines and Bangladesh, this would serve as a catalyst for better acceptance of GM food crops. A brief case study on the commercialization of transgenic BT rice in China will also be discussed. The review aims to bring useful insights for future endeavors in improving traits for rice through genetic engineering.

Keywords: Genetic Engineering, Rice, Transgenic, Traits, Yield

Introduction

The introduction of high-yielding semi dwarf wheat and rice varieties through plant breeding, together with large doses of nitrogen fertilizer application for yield improvement marked the success of the first green revolution in averting large scale famine in the semi-arid of the South Asia (Guimarães, 2009). As the world population is estimated to reach 8 billion by 2025, the second green revolution of genetic engineering of food crops has come into play so that grain production is in par with the global demand

(Sakamoto & Matsuoka, 2004). 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. Likewise, the availability of donor genes isolated from a diverse pool of organisms has made it possible for rice to harbour genes from organisms of other kingdoms, ranging from viruses, bacteria, fungi, insects, animals to humans. With such abundant knowledge that is easily accessed nowadays, scientists are able to push their research prospects beyond improving the agronomical and stress tolerance traits in rice. Other important traits such as heavy metal toxicity tolerance, photosynthetic capacity, rice grain nutrition and fragrance have begun to receive heightened attention in these recent years. Examples of traits that have been genetically improved in rice will be discussed comprehensively in the subsequent sections. In addition, these improved rice traits through the transgenic technology has also been summarized in Table 2 – 5.

In the modern world, genetically engineered or transgenic crops have become an integral part of the agriculture industry. More than 28 countries have cultivated and commercialized transgenic cereal crops as shown in *Table 1*. 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 as shown in Table 1.

Table 1: Some commercialized transgenic cereal crops cultivated around the world

Cereal crops	Trait[1]	Developer	Countries
*BtShanyou 63 rice	IR	Huazhong Agricultural University (China)	China
*Huahui-1 rice	IR	Huazhong Agricultural University (China)	China
BtXtraTM maize	IR	Mosanto	Australia, Canada, Japan, New Zealand, Philippines, South Korea, Taiwan, USA
EnlistTM maize	HT	Dow AgroSciences LLC	Australia, Canada, Mexico, New Zealand, South Africa, Taiwan, USA
EnogenTM maize	MPQ	Syngenta	Australia, Canada, Japan, Mexico, New Zealand, Philippines, Russian Federation, South Korea, Taiwan, USA
GenuityDroughtGardTM Maize	AST	Mosanto	Australia, Canada, Japan, Mexico, New Zealand, Taiwan, USA
Herculex XTRATM Maize	HT, IR	Dow AgroSciences LLC and DuPont	Canada, EU, Japan, Mexico, Philippines, South Africa, South Korea, Taiwan, Turkey, USA
YieldGardTM Plus Maize	IR	Mosanto	EU, Japan, Mexico, Philippines, South Africa, South Korea, Taiwan, USA

InVigorTm Maize	PCS	Bayer CropScience	Canada, USA
MaveraTM Maize	MPQ	Renessen LLC	Japan, Mexico, USA
OptimumTM Maize	HT	DuPont	Argentina
Power CoreTM Maize	HT, IR	Mosanto and Dow AgroSciences LLC	Argentina
Roundup ReadyTM Wheat	HT	University of Florida	Australia, Colombia, New Zealand, USA

Abbreviations: IR-Insect resistant; PCS- Pollination Control System; HT-Herbicide tolerant; MPQ-Modified Product Quality; AST-Abiotic Stress Tolerance
*Transgenic rice is currently planted on a field trial scale
Source: Bakshi & Dewan, 2013

Improved stress tolerance - biotic stress tolerance

Insect pests tolerance

The susceptibility of rice towards various biotic stresses such as insects, fungal, bacterial and viral pathogens is an alarming issue that should receive proper management. China, being the world's most populated country, has taken the first step in cultivating transgenic insect-resistant rice for consumption. The transfer of specific *Bacillus thuringiensis* (Bt) genes (Cry1Ab/Ac) has produced BtShanyou 63 and Minghui 63 hybrid rice. It took China 30 years to commercialize Bt rice that is resistant to Lepidopteran insects after obtaining biosafety clearance from the local Ministry of Agriculture (Bakshi & Dewan, 2013). Experience has shown that Bt rice has the potential to generate profits of US$4 billion with an average yield increase of 8 percent and 80% decrease in insecticide usage (Huang et al., 2005). Currently, the amount of area planted with Bt crops worldwide expanded from 1.1 million hectares in 1996 to 66 million hectares in 2011, with an estimated of cumulative total of more than 1 billion acres (Tabashnik et al., 2013).

Apart from the Bt gene, genes that encodes for lectin and proteinase inhibitor are some alternatives considering the fact that insect resistance to Bt proteins still remains as a painstaking issue worldwide. The overexpressed *Allium sativum* leaf lectin gene (ASAL), which codes for the mannose binding homodimericprotein in garlic, revealed entomotoxic effects to a few species of major sap-sucking planthoppers in transgenic rice (Bharathi et al., 2008). Improvements were made later by sexual crosses between transgenic rice lines harbouring the ASAL gene and *Galanthusnivalis* lectin (GNA) genes leading to transgene pyramided rice lines with improved resistance to sap-sucking insects (Bharathi et al., 2011). A yam tuber lectin, *Dioscoreabatatas* tuber lectin 1 (DB1) gene expressed in transgenic rice also showed resistance of up to 30% towards brown planthopper (Yoshimura et al., 2012). Similar to Bt proteins and lectins, insects are killed when they ingest plant proteinase inhibitors produced within the vegetative tissues upon chewing and wounding. The average body length and weight of the rice stripe stem borer larvae were reduced to nearly 50% and 61% in transgenic rice harbouring the potato proteinase inhibitor gene (PINII-2x) (Bu et al., 2006). Interestingly, the inducible expression of a gene-fusion encoding two proteinase inhibitors from maize proteinase inhibitor (MPI) and potato carboxypeptidase inhibitor exhibited a dual resistance towards striped stem borer and blast disease (Quilis et al., 2014).

Fungal and bacterial disease tolerance

Both fungal diseases – blast (causal agent: *Magnaporthe grisea*) and sheath blight (causal agent: *Rhizoctonia solani*); as well as bacterial leaf blight (causal agent: *Xanthomonasoryzae*pv. *oryzae*, Xoo) are the most serious threats to rice productivity. The most extensively used genes isolated either from plants or microbes are those from the pathogenesis-related (PR) protein family such as chitinase and β, 1-3 glucanase. The Gns1 (which encodes for ß, 1-3 glucanase) overexpressing transgenic plants showed resistance upon inoculation of virulent *M. grisea* (Nishizawa et al., 2003). Chitinase genes from Trichoderma fungus or *Streptomyces griseus* have also been used to increase rice resistance towards the blast and sheath blight disease (Itoh et al., 2003; Liu et al., 2004; Shah et al., 2009). Endogenous rice resistance genes conferring resistance towards *M. grisea* (Pid-2, Pi54) and Xoo (Xa3, Xa26) have also been successfully transformed into rice (Chen et al., 2010; Li et al., 2012). High level of resistance towards certain race or strains of pathogens was often observed in transgenic rice inserted with such resistant genes.

Genes that prime the expression of specific salicylic acid (SA) and jasmonic acid (JA)-responsive endogenous genes during fungal and bacterial infection provide another practical approach for engineering broad-spectrum disease resistant rice. The overexpression of AtNPR1 gene from *Arabidopsis thaliana* in transgenic rice showed enhanced resistance towards blast and bacterial blight by triggering a cascade of signalling response in the SA pathway (Quillis et al., 2008). Transgenic rice overexpressing the transcription factors like the OsNPR1 gene from rice also conferred resistance to bacterial blight by activating both the SA and JA dependent pathways (Yuan et al., 2007). The activation of the OsMPK6 gene from the protein kinase family also showed enhanced resistance to bacterial blight with an upregulation of SA signalling genes during infection (Shen et al., 2010). Recently, the rice HPL3 that encodes for hydroperoxidelyase has been characterized to activate both the SA and JA pathways, which subsequently showed improved disease resistance towards bacterial blight when expressed in transgenic rice (Tong et al., 2012).

Several other genes that correlated to ethylene, gibberellin, auxin, brassinosteroids and abscisic acid production were reviewed to be responsible to rice immunity against diseases (Yang et al., 2013). As an example, the resistance towards rice blast and sheath blight was improved in transgenic rice overexpressing ACS2 gene, a key enzyme in ethylene biosynthesis (Helliwell et al., 2013). Another notable example is the hormone gibberellin (GA) that plays a key role in regulating plant stature. It was recently discovered this hormone might modulate the pathogenesis of bacterial and fungal pathogens. Qin et al. (2013) reported that the rice gibberellin 20-oxidase gene (OsGA20ox3), which was involved in the GA biosynthesis pathway showed enhanced susceptibility to blast and bacterial blight in transformed rice. Conversely, the RNAi-OsGA20ox3 lines showed resistance to both pathogens since rice defense genes were upregulated in gene-silenced lines. This suggested that the downregulation of GA-biosynthesis associated genes might be needed in order to activate the rice defense response against bacterial blight.

Viral disease tolerance

Common viral diseases that infest rice crop in Asia includes the rice tungro bacilliform or spherical virus (RTBV/RTSV) and rice stripe virus (RSV). Engineering virus-resistant rice plants can be achieved by two strategies, namely protein and RNA-mediated resistance. Both of these strategies exploit the virus-encoded proteins or RNA to interfere with the crucial steps in the viral infection cycle. The transgene expression of a coat protein gene from RTBV reduced the accumulation of viral DNA, followed by a reduction of tungro symptoms which includes stunted growth and leaf discoloration (Ganesan et al., 2009). The application RNA-mediated strategy, for instance, the RNA-interference (RNAi) technology was reported in rice plants transformed with vector containing the anti-sense or untranslatable genes from RTBV and RSTV genomic DNA. The expression of these genes will trigger the RNA silencing defense machinery which ultimately, degrade and delay the accumulation and transmission of the viral RNA (Verma et al., 2012). Undoubtedly, tremendous efforts have been dedicated in developing and screening for disease resistance in transgenic rice but it seems that there is yet any commercial release of transgenic lines so far with an exception of the large scale cultivation of Bt rice.

Table 2: Examples of transgenic rice produced for improved biotic stress tolerance

Gene(s) inserted	Trait(s)	Remarks	Reference
PINII-2X	Insect resistance	Improved tolerance to rice stripe stem borer	Bu et al. (2006)
ASAL	Insect resistance	Improved tolerance to planthoppers	Bharathi et al. (2008)
Cry1Ab/Ac	Insect resistance	Strong resistance to stem borers and leaf folders	Lu (2010)
ASAL, GNA	Insect resistance	Improved resistance to sap-sucking insects	Barathi et al. (2011)
DB1	Insect resistance	30% of resistance towards brown planthopper	Yoshimura et al. (2012)
MPI, potato carboxypeptidase inhibitor	Insect resistance	Dual resistance towards striped stem borer and blast fungal disease	Quilis et al. (2014)
ChiC	Fungal disease resistance	Improved resistance towards blast	Itoh et al. (2003)
Gns1	Fungal disease resistance	Improved resistance towards fungal blast disease	Nishizawa et al. (2003)

Ech42, nag70, gluc78	Fungal disease resistance	Improved resistant to blast and sheath blight	Liu et al. (2004)
OsNPR1	Bacterial disease resistance	Improved resistance towards bacterial blight	Yuan et al. (2007)
AtNPR1	Fungal and bacterial disease resistance	Improved resistance towards blast and bacterial blight	Quillis et al. (2008)
Cht42	Fungal disease resistance	Improved resistance towards sheath blight	Shah et al. (2009)
Pi-d2	Fungal disease resistance	Resistant towards blast	Chen et al. (2010)
OsMPK6	Bacterial disease resistance	Improved resistance towards bacterial blight	Shen et al. (2010)
Xa3/Xa26	Bacterial disease resistance	Race-specific resistance towards bacterial blight	Li et al. (2012)
HPL3	Bacterial disease resistance	Improved resistance towards bacterial blight	Tong et al. (2012)
ACS2	Fungal disease resistance	Improved resistance towards blast and sheath blight	Helliwell et al. (2012)
OsGA20ox3	Fungal and bacterial disease resistance	Improved tolerance towards blast and bacterial blight	Qin et al. (2013)
RTBV coat protein	Viral disease resistance	Reduced accumulation of RTBV RNA	Ganesan et al. (2009)
RSTV RNA	Viral disease resistance	Delayed accumulation of RSTV RNA and low virus transmission	Verma et al. (2012)

Abiotic stress tolerance

Climate change is often related to yield loss in rice cultivation due to unfavourable environmental regimes. Common transgenic approaches typically address single abiotic stress. As the understanding of

the rice regulatory mechanism towards abiotic stress is rapidly progressing, so is the isolation of genes that could confer to a multitude of abiotic stress. Increased accumulation of low molecular weight solutes (glycinebetaine, proline, trehalose, polyamines and late embryogenesis abundant proteins, LEA) in transgenic rice in response to, and to counteract the effects of salinity, drought and temperature stress has been reported. The accumulation of glycinebetaine in transgenic rice overexpressing spinach choline monooxygenase confers enhance tolerance to salinity and temperature stress (Shirasawa et al., 2006). The overproduction of proline in transgenic rice using the mutagenized P5CS – P5CSF129A gene showed enhanced salt stress tolerance without subjected to feedback control in the proline biosynthetic pathway (Kumar et al., 2009). The rice trehalose-6-phosphate synthase (OsTPS1) gene demonstrated a wide range of abiotic stress tolerance including, cold, drought and salinity in transgenic rice which accumulates trehalose and proline (Li et al., 2011b). Salt and drought tolerant rice was also obtained by the overexpression of the rice OsLEA3-2, OsLEA3-H and OsLEA3-S genes (Xiao et al., 2007; Duan & Cai, 2012). The functional study of genes that encode for polyamines only began recently. Though no transgenic studies has been done on rice, preliminary studies indicated that polyamine accumulation, characterization of a rice polyamine uptake transporter (OsPUT1), and the down-regulation of S-adenosylmethionine decarboxylase genes could be applied as potential abiotic-stress tolerance genes in future (Mulangi et al., 2012; Chen et al., 2014).

Expression of stress-responsive genes is regulated by a group of protein known as the transcription factors. In the genetic modification of rice, there are countless transcription factors (TF) been isolated and characterized in which many of these genes were reported to confer to various abiotic stress(Kumar et al., 2013). The most notable ones are those from the DRE-binding proteins (DREBs) domain which binds specifically to the dehydration-responsive element/C-repeat (DRE/CRT) cis-acting element in the promoters that drive the expression of abiotic-stress responsive genes. Recent discoveries on transgenic rice overexpressing barley HvCBF4, maize ZmCBF3, rice OsDREB1F and OsDREB2A showed improved tolerance towards salinity, drought and low temperatures (Oh et al., 2007; Wang et al., 2008; Xu et al., 2011; Mallikarjuna et al., 2011). Emerging transcription factors such as those from the leucine zipper protein (bZIP), zinc finger protein, MYB and NAC families have been studied for their functional role in modulating expression of stress-related genes in transgenic rice (Huang et al., 2009b; Song et al., 2011; Zou et al., 2012; Xiong et al., 2014). In a compendious review, Todaka et al. (2012) highlighted specific functions of each TFs and their expression studies in transgenic rice. Despite the wide array of TFs available, the crosstalk between rice transcription regulatory network and abiotic stress is still unclear as signaling mechanisms in plants remains as a diverse and complex response to be understood holistically.

The characterization of stress signaling pathways in rice such as the mitogen-activated protein kinase (MAPK) and calcium/calmodulin-mediated pathways have been actively progressing with several novel genes been isolated (Saeng-ngam et al., 2012; Singh & Jwa 2012) As an example, the rice calmodulin gene, OsCam1-1 acts as a salt stress sensor by accumulating ABA thereby conferring salt tolerance in rice

(Saeng-ngam et al., 2012). Salt and drought stress were also overcome in transgenic rice by overexpressing the rice OsMAPK44 (Jeong et al., 2006). In contrast, expressing the OsMAPK33 increased rice sensitivity towards salt stress (Lee et al., 2011). Besides that, the reactive oxygen species (ROS) regulatory pathway is also activated in plants under stress. Several instances of transgenic rice overexpressing *E. coli* catalase (KatE) gene, rice isoflavone reductase-like gene, and lastly the co-expression of two C4 photosynthesizing enzymes (maize pyruvate orthophosphate dikinase – PPDK and maize C4-specific phosphoenolpyruvate carboxylase – PCK) demonstrated tolerance towards drought, salinity and ROS (Nagamiya et al., 2007; Kim et al., 2010; Gu et al., 2013).

In lieu of transcription factors and signaling pathways, plants also adapt to stress by maintaining the cellular ion homeostasis particularly through the aquaporins and potassium/sodium channel proteins. Aquaporins (water channels) are membrane proteins that facilitate water and low molecular weight compounds transport across plasma membrane. Specific aquaporins from the plasma membrane intrinsic protein (PIP) family, have been reported to improve tolerance of transgenic rice towards cold (rice OsPIP1;3 gene), salinity (barley HvPIP2;1 gene) and drought (rice RWC3 gene) (Lian et al., 2004; Katsuhara, 2007; Matsumoto et al., 2009). The Na+/H+ ion antiporter, driven by the electrochemical gradient of protons produced by the H+-ATPase and H+-pyrophosphatase, extrudes sodium ions from the cytoplasm in exchange for protons to avoid excessive accumulation of sodium ions. Transgenic rice seedlings co-expressing the *Suaeda salsa* SsNHX1 encoding for Na+/H+ and *Arabidopsis* AVP1 (vacuolar H+-PPase) showed tolerance to salinity with higher photosynthetic capacity and ROS scavenging activity (Zhao et al., 2006). Similarly, the overexpression of *Pennisetum glaucum* PgNHX1 gene in transgenic rice developed extensive root system without yield penalty under saline condition (Verma et al., 2007). Until now, literatures on genetic manipulation of these transporter genes into rice are relatively limited. Much work is still being dedicated to dissect the regulatory function of these transporters during plant stress (Brini & Masmoudi, 2012).

As opposed to drought and salinity stress, submergence tolerance in rice is important to certain flood-prone areas. The isolation of the submergence gene (SUB1A) from the rice cultivar Flood Resistant 13A (FR13A) has kicked off various research efforts in producing submergence-tolerant cultivars either by marker-assisted breeding or via transgenic approaches (Bailey-Serres et al., 2010). Intolerant rice cultivars genetically engineered with the SUB1A gene showed similar characteristics to resistant cultivars by conserving carbohydrate consumption, reducing ethylene production, limiting gibberellin-mediated node elongation and the ability to regrowth upon de-submergence (Fukao & Bailey-Serres, 2008). Further investigations showed that transgenic rice harbouring the SUB1A gene was found to have improved drought tolerance as well (Fukao et al., 2011). A recent study on the overexpression of the ethylene gene, OsETOL1 in rice increased carbohydrate metabolism so that the energy production can be utilized to elongate shoots above water surface during submergence (Du et al., 2014).

Rice grain yield declines by 10% for every temperature increase of 1°C (Peng et al., 2004). Novel genes conferring to heat tolerance to rice plants presents a promising approach in producing transgenic rice plants capable of maintaining or increasing yield under high heat environment (Zou et al., 2011). Introducing genes encoding for heat shock proteins (Athsp101 and OsHSP101) into rice produced heat tolerance Basmati rice (Agarwal et al., 2003). Overexpression of rice SBPase resulted in enhanced growth and photosynthesis activity in relation to heat tolerance in transgenic rice plants (Feng et al., 2007). The gene knockout of rice OsGSK1 gene also generated heat tolerance rice cultivar (Koh et al., 2007). Wu et al. (2009) discovered that the expression of the OsWRKY11 gene under the control of HSP101 promoter also enhanced rice tolerance towards heat and drought with more than 80% survival as compared to the wild type. Recently, the overexpression of OsMYB55 leads to heat tolerance transgenic rice with enhanced amino acid metabolism and increased grain yield (El-kereamy et al., 2012).

The presence of mineral nutrients in soil, either in excess or lacking, is determined by the soil pH. Soil with high pH (calcareous soil) contain limited iron which can be ameliorated by overexpressing the mutational reconstructed yeast ferric chelate reductase in transgenic rice plants (Ishimaru & Nishizawa 2008). Phosphate deficiency due to imbalance soil pH was relieved through overexpression of the rice transcription factor OsPTF1, rice protein kinase OsMAPK2, phosphorus-starvation tolerance 1 (PSTOL1) and rice proton translocating pyrophosphatase genes, which transgenic rice showed improved uptake of phosphate ions (Gaxiola et al., 2011; Gamuyao et al., 2012; Hur & Kim, 2014). Alternately, low pH soils contain high concentrations of aluminium that can inhibit root growth. The co-expression of two genes namely STAR1 and STAR2 was necessary to form a complex with the function of a bacterial-type ABC transporter in order to detoxify aluminium in transgenic rice (Huang et al., 2009a). A putative abscisic acid, stress and ripening (ARS-5) gene was expressed only in transgenic japonica instead of indica rice upon exposure to aluminium. Further downstream analysis postulated this gene could be a transcription factor that collectively regulates the expression of genes responsible for aluminium detoxification (Arenhart et al., 2013).

Heavy metal accumulation in soils may be introduced through anthropogenic activities such as mining, overuse of fertilizers, metal-based pesticides and other industrial activities. Plants especially rice tends to accumulate heavy metals such as cadmium (Cd) which could cause serious health ailments including renal dysfunction (Honda et al., 2010). In addition, indica rice accumulates higher concentration of cadmium in grains and shoots as compared to japonica varieties (Uraguchi & Fujiwara, 2009). Because of these reasons, the importance of reducing heavy metals content in the rice grain has gained interest recently to promote better human health (Azevedo et al., 2012). Rice genetically engineered to overexpress genes like the OsHMA3 gene showed reduced Cd accumulation (Ueno et al., 2010). Low-Cd transgenic rice was also produced by knockdown of certain genes in regulating Cd uptake, for instance, the OslRT1 and OsLCT1 genes (Lee &An, 2009; Takahashi et al., 2011). Besides cadmium, the effects of other heavy metals inclusive of zinc, copper, mercury, nickel, arsenic and lead on rice plants should not be overlooked as these elements could hamper productivity. There has been only single report by Lee et

al. (2009) that overexpression of the OsNAS3 gene showed improved tolerance towards excessive zinc, copper and nickel toxicities.

Table 3: Examples of transgenic rice produced for improved abiotic stress tolerance

Gene(s) inserted	Trait(s)	Remarks	Reference
AtHsp101, OsHsp101	Heat tolerance	Enhanced tolerance to heat stress	Agarwal et al. (2003)
OsPIP1;3	Cold tolerance	Enhanced tolerance to cold stress	Lian et al. (2004)
OsMAPK44	Salinity and drought tolerance	Enhanced tolerance to salinity and drought stress	Jeong et al. (2003)
Choline monooxygenase	Salinity and heat tolerance	Enhanced tolerance to salinity and temperature stress	Shirasawa et al. (2006)
SsNHX1, AVP1	Salinity and ROS tolerance	Enhanced tolerance to salinity, ROS stresses and improved photosynthetic capacity	Zhao et al. (2006)
OsSBPase	Heat tolerance	Enhanced heat tolerance with improved photosynthetic capacity	Feng et al. (2007)
HvPIP2;1	Salinity tolerance	Enhanced tolerance to salinity stress	Katsuhara et al. (2007)
OsGSK1	Heat tolerance	Enhanced tolerance to heat stress	Koh et al. (2007)
KatE	Drought tolerance	Enhanced tolerance to drought stress	Nagamiya et al. (2007)
HvCBF4	Cold, drought and salinity tolerance	Demonstrated a wide range of tolerance to cold, drought and salinity stress without growth penalty	Oh et al. (2007)
PgNHX1	Salinity tolerance	Enhanced tolerance to salinity stress without yield penalty	Verma et al. (2007)

OsLea1-3	Drought tolerance	Enhanced tolerance to drought stress	Xiao et al. (2007)
SUB1A	Submergence tolerance	Enhanced tolerance to submergence stress	Fukao & Bailey-Serres (2008)
Yeast ferric chelate reducatase	Soil alkalinity tolerance	Enhanced tolerance to alkaline soils	Ishimaru & Nishizawa (2008)
OsDREB1F	Cold, salinity and drought tolerance	Enhanced tolerance to cold, salinity and drought stresses	Wang et al. (2008)
STAR1, STAR2	Aluminium toxicity tolerance	Enhanced tolerance to aluminium toxicity via detoxification	Huang et al. (2009a)
ZFP245	Cold, drought and ROS tolerance	Enhanced tolerance to cold, drought and ROS stresses	Huang et al. (2009b)
P5CSF129A	Salinity tolerance	Enhanced tolerance to salinity stress	Kumar et al. (2009)
Os1RT1	Cadmium toxicity tolerance	Reduced accumulation of cadmium in rice	Lee & An (2009)
OsNAS3	Heavy metals toxicity tolerance	Enhanced tolerance to zinc, copper and nickel toxicities	Lee et al. (2009)
RWC3	Drought tolerance	Enhanced tolerance to drought stress	Matsumoto et al. (2009)
OsWKRY11	Heat and drought tolerance	Enhanced tolerance to heat and drought stress with improved yield	Wu et al. (2009)
Isoflavone reductase	Salinity tolerance	Enhanced tolerance to salinity stress	Kim et al. (2010)
OsHMA3	Cadmium toxicity tolerance	Reduced accumulation of cadmium in rice	Ueno et al. (2010)
SUB1A	Submergence and drought tolerance	Enhanced tolerance to submergence and drought stress	Fukao et al. (2011)

OsMAPK2	Phosphate deficiency tolerance	Improved uptake of phosphate ions	Gaxiola et al. (2011)
OsTPS1	Cold, drought and salinity tolerance	Demonstrated a wide range tolerance to cold, drought and salinity stresses.	Li et al. (2011b)
OsDREB2A	Salinity stress	Enhanced tolerance to salinity stress.	Mallikarjuna et al. (2011)
OsNAC5	Cold, drought and salinity tolerance	Enhanced tolerance to cold, drought and salinity stresses	Song et al. (2011)
OsLCT1	Cadmium toxicity tolerance	Reduced accumulation of cadmium in rice	Takahashi et al. (2011)
ZmCBF3	Cold, drought and salinity stress	Demonstrated a wide range of tolerance to cold, drought and salinity stress without yield penalty	Xu et al. (2011)
OsLEA3-2	Drought and salinity stress tolerance	Enhanced tolerance to drought and salinity stress	Duan & Cai (2012)
OsMYB55	Heat tolerance	Enhanced tolerance to heat stress with improved yield	El-kereamy et al. (2012)
OsPTF1	Phosphate deficiency tolerance	Improved uptake of phosphate ions	Gamuyao et al. (2012)
OsCam1-1	Salinity tolerance	Enhanced tolerance to salinity stress	Saeng-ngam et al. (2012)
PPDK, PCK	ROS tolerance	Enhanced tolerance to ROS generated during abiotic stress	Gu et al. (2013)
OsETOL1	Submergence tolerance	Enhanced tolerance to submergence stress	Du et al. (2014)
PSTOL1	Phosphate deficiency tolerance	Improved uptake of phosphate ions.	Hur & Kim (2014)
OsMYB48-1	Drought and salinity	Enhanced tolerance	Xiong et al. (2014)

tolerance	to drought and
	salinity stresses

Herbicide tolerance

Herbicides are classified into two classes – the selective ones that kill weeds but not crops and the non-selective ones that eliminate all types of plants. Recent transgenic technology has opened possibilities of isolating genes that confer resistance towards the non-selective herbicides in order to reduce undesirable herbicide resistant weeds that could hamper rice productivity. Glyphosate and glufosinate were known as the widely used 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 a G6 gene, which encodes for Epsp synthase isolated from *Pseudomonas putida* showed resistance to 8 g/l of glyphosate (which is the commercial resistance level) during field trial (Zhao, Lin, & Shen, 2011). Transgenic rice engineered with the optimized codon of the Cp4-Epsp synthase gene showed 2.7 times 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. The cleavage of the transit peptide occurs once the gene is transferred across the chloroplast membranes.

Another emerging group of herbicide is known as the acetolacetate synthase (ALS)-inhibiting herbicides that inhibits the biosynthesis of branched-chain amino acid such as leucine, isoleucine and valine. 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). Overexpression of a novel rice cytochrome P450 gene, CYP72A31 also exhibited resistance towards another ALS-inhibiting herbicide, bispyribac sodium (Saika et al., 2014). However, further downstream analysis of the gene flow of herbicide resistance gene from transgenic rice to its weed relative red rice has shed light that the transgene was detected in the red rice (Busconi et al., 2012). Indeed, stringent guidelines and proper agricultural management in administering proper herbicide dosages are necessary to mitigate the spread of such transgenes into weedy rice populations.

Table 4: Examples of transgenic rice produced for improved herbicide tolerance

Gene(s) inserted	Trait(s)	Remarks	Reference
G6	Herbicide tolerance	Resistant to the commercial applicate rate of glyphosate (8 g/l)	Zhao et al. (2011)
NAT	Herbicide tolerance	Resistant towards butafenacil	Park et al. (2013)

| Cp4-Epsp | Herbicide tolerance | Demonstrated 2.7 times higher resistance to both glyphosate and glufosinate | Deng et al. (2014) |
| CYP72A31 | Herbicide tolerance | Resistant towards bispyribac sodium | Saika et al. (2014) |

Improvement of grain yield and quality

Grain yield improvement

Overwhelming increase of global population is one of the major concerns in most plant breeding programmes to produce rice plants that generate copious grain yield. Addressing yield gaps directly by means of genetic engineering is a promising way. Transgenic rice plants with enhanced nutrient acquisition towards specific nutrient sources such as the ammonium (NH4+) and phosphate (Pi) ions have shown improved growth and yield. Ranathunge et al. (2014) demonstrated that the insertion of OsAMT 1;1 gene has increased the membrane permeability of rice roots towards NH4+ under optimal and suboptimal application of NH4+, thereby enhancing the nitrogen use efficiency for growth and yield. Similar observations were also observed in transgenic rice overexpressing the tobacco high affinity phosphate transporter (NtPT1) gene in which phosphate accumulation led to an increase in grain yield (Park et al., 2010). Besides improving rice nutrient uptake, rice yield is a complex trait directly associated to a range of physiological features ranging from the grain size, grain weight, panicle, tiller and spikelet number. The on-going effort of discovering novel genes that govern these traits has generated transgenic rice plants harbouring genes such as the microRNA OsmiR397 that improves yield as much as 25% in the field trial, by increasing the grain size and number of panicle branching (Zhang et al., 2013). The transgenic rice lines incorporated with the TIFY11B gene showed greater carbohydrate accumulation in the vegetative organ than that wild type, suggesting that enhanced carbohydrate assimilation has led to increase grain size and yield (Hakata et al., 2012). Other than that, the introduction of the SPIKE gene into indica rice cultivars has improved several rice physiological features including the spikelet number, root system, leaf size and the number of vascular bundles. An overall improvement in the plant architecture exhibited an increase in grain yield (Fujita et al., 2013).

Improved photosynthetic capacity for increased grain yield

Rice plants come from the C3 family that suffers from inefficient photosynthetic mechanism. The main enzyme in the photosynthetic pathway, ribulose bisphosphate carboxylase/oxygenase (RubisCO) catalyzes the fixation of both CO_2 and O_2. In C3 plants like rice, when the ratio of O_2 is higher than CO_2, the RubisCO's oxygenase activity then dominates leading to the energy consuming photorespiration pathway so that the produced toxic phosphoglycolate is broken down (von Caemmerer et al., 2012). Comparing to C4 plants, these plants are able to concentrate CO_2 which acts as a reservoir for the Rubisco enzyme because of their two-celled C4 system. Such efficient C4 photosynthesis machinery fascinates

scientists to embark on this daunting journey to engineer rice with overexpressed C4 enzymes with the hope of increasing photosynthetic capacity and also grain yield.

Numerous experimental attempts have been initiated through insertion of C4 specific genes, whether expressed singly in combination into rice. Transgenic rice harbouring the PEPC gene showed a 25-fold increase of PEPC activity and more than two-fold increase carbonic anhydrase activity leading to an increase in CO_2 uptake, carboxylation efficiency and improved tolerance towards photo-oxidative stress due to excess light energy. Furthermore, the expression of PEPC and both PEPC and PPDK genes in transgenic rice produced 22 to 24% more grains than the wild type plants (Jiao et al., 2002). Zhang et al. (2009) also indicated the combined expression of both PEPC and PPDK genes in rice showed enhanced photosynthetic rate, higher water use efficiency and lower stomatal conductance during high temperature and excess light which led to 9.29 to 10.91% increase in grain yield. The photosynthetic rate of the transgenic rice was further increased by the exogenous supply of $NaHSO_3$ and ATP that the actual mechanism has not been described in detail in this study (Zhang et al., 2009). The expression of the other photosynthesis-related genes such as the large isoform RubisCO-activase (RubisCO-A) in transgenic rice, an light-radiation inducible enzyme that activates RubisCO by removing any inhibitory sugar phosphates from the active sites of RubisCO, showed increased photosynthetic rate and shortened period of heading time (Wu et al., 2006; Wu et al., 2007).

Other than manipulating the expression of the photosynthetic enzymes, the mutated rice homolog of the DE-ETIOLATED 1 (OsDET1) expressed in transgenic rice was discovered to be responsible to enhance total chlorophyll pigments in rice leaves with increased sensitive to light and its nuclear-localized expression was found to be under circadian control (Huang et al., 2013). During photosynthesis, the conversion of CO_2 and inorganic phosphate (Pi) into triose-P is then metabolized into sucrose for utilization by the developing sink tissues and some portion retained as starch in the chloroplast. Both sucrose production and starch synthesis process contribute to the recycling of Pi to synthesis ATP and thereby, reducing feedback regulation of photosynthesis. As wild type rice plants synthesize low leaf starch, transgenic rice harbouring the potato large subunit of ADP-glucose pyrophosphorylase (AGPase) upreg1 gene demonstrated elevated leaf starch levels, improved CO_2 assimilation during photosynthesis, and increased number of panicles leading to more grains per panicle (Gibson et al., 2011). This study has provided helpful insights the relationship between starch synthesis, CO_2 assimilation and plant growth suggesting another alternative in improving rice photosynthesis. A novel chloroplast-localized of photoassimilate defective1 (phd1) gene was introduced in transgenic rice in which, its overexpression leads to increase photosynthetic capacity, plant biomass and grain yield (Li et al., 2011a). The functional role of phd1 was suggested to be synthesizing galactolipids for the thylakoid membrane so as to maintain proper chloroplast biogenesis and photosynthetic activity which implicates its key role in rice photosystem.

Grain quality improvement

Rice is the staple food for most underdeveloped and developed countries where most could not afford costly fruits and vegetables resulting more than 50% of the population suffering from malnutrition (Christou &Twyman, 2004). Hence, nutritional enhancement or biofortification of rice seeds using genetic transformation techniques can directly improve the health status of many poor nations. Biofortification of rice seeds began with the insertion of specific genes responsible for synthesizing vitamin A in the carotenoid pathway. Golden rice was, in fact, being engineered with several different genes like the maize phytoene synthase (PSY) gene so as to maximize the quantity of vitamin A production (Pérez-Massot et al., 2013). *Arabidopsis* Atgtpchi and Atadcs genes co-transformed into transgenic rice produced a 100-fold increase in vitamin B9 (folate) in rice grains as compared to tomato indicating the potential of nutritional enhancement in rice grains (Storozhenko et al., 2007). An early study by Farre´ et al. (2012) demonstrated that the insertion of AtHPPD gene, which catalyzes the first step in vitamin E biosynthesis, could only produce marginal increase in tocotrienol synthesis.

Essential amino acids are elements that could be synthesized de novo by humans. As most cereal crops are poor sources of lysine, tryptophan and methionine, genetic engineered rice with enhanced production of amino acids are desirable (Ufaz & Galili, 2008). Initial attempt of increasing sulfur-rich amino acid in rice grains through the overexpression of sunflower seed albumin (SSA) gene was not successful (Hagan et al., 2003). Following that, transgenic rice overexpressing sesame 2S albumin showed an increase in methionine and cysteine content in rice seeds while increased tryptophan content was found in rice engineered with the OASA1D gene (Lee et al., 2003; Wakasa et al., 2006). Similar to amino acids, most fatty acids can be synthesized de novo by humans but not the health-promoting ω-3 and ω-6 polyunsaturated fatty acids (also known as omega-3 and omega-6 fatty acids. Transgenic rice modified to accumulate high amounts of linoleic acids was achieved only recently by co-expression of genes encoding omega-3 fatty acid desaturase, Δ9–elongase, Δ8-desaturase and Δ5-desaturase which are involved in fatty acid biosynthesis pathway (Cheah et al., 2013).

The bioavailability of inorganic nutrients (minerals) must be absorbed from the soil unlike organic nutrients, which are produced as a result of plant metabolism. It is rather common for populations to be deficient in certain minerals and this has sparked the development of rice tailored to provide all these limiting nutrients. Enhancing iron content in rice grains has become recent interest to combat the increasing incidence of anemia. Zinc, another health-preserving element is also of major interest lately. Various reports have demonstrated that introducing genes that promotes the iron and zinc uptake in transgenic rice such as the rice nictotianamine synthase gene OsNAS3 and rice iron (II) nicotianamine transporter OsYSL2 have shown enhanced iron content in rice grains (Lee et al., 2009; Masuda et al., 2012). In another example, the calcium content in rice seed was manipulated by insertion of Arabidopsis Ca2+/H+ antiporter gene to reduce the incidence of osteoporosis in poorer communities (Yi et al., 2012).

Manipulating phytic-acid or phytate in rice grain is emphasized because these elements are anti-nutrients that bind mineral nutrients and sequester into complexes that cannot be absorbed by human and animal digestive system. The seed-specific gene silencing of inositol 1,3,4,5,6-Pentakisphosphate 2-Kinase (IPK1) and myo-inositol-3-phosphate synthase (MIPS) successfully generated low phytate rice (Ali et al., 2013a; Ali et al., 2013b). Prolamines, largely accumulated as indigestible protein bodies in rice seeds, was also reduced by silencing the 13 kDprolamin genes so as to improve nutritional value of the grain (Kim et al., 2013). The cooking and eating quality of rice can also be improved by reducing the amylose content in rice. Transgenic rice overexpressing the antisense-Waxy gene showed lower amylose content in rice seeds which, otherwise known as the "soft rice" (Li et al., 2009; Yu et al., 2009).

Rice Fragrance

Characteristic fragrance in aromatic rice has begun to gain popularity worldwide. Since then, the incorporation of aromatic traits to produce new fragrant rice hybrid has been extensively carried out. Although a myriad of fragrance volatile compounds has been detected in rice grains, the current principal compound thought to be responsible is identified as 2-acetyl-1-pyrroline with L-proline being its possible precursor (Yoshihashi et al., 2002). Physical allele mapping revealed that the candidate gene is the rice betaine aldehyde dehydrogenase (OsBADH2) gene. The downregulation of this gene in non-aromatic rice plants via the RNAi technology was able to emit fragrance but such transgenic plants suffered from yield loss (Niu et al., 2008; Chen et al. 2012). It is suggested the use of a stronger endosperm-specific promoter could circumvent this limitation (Chen et al., 2012). The road to producing fragrant rice using transgenic approach is still far beyond reach as its biochemical pathway is yet to be elucidated in detail.

Table 5: Examples of transgenic rice produced for improved grain yield and quality

Gene(s) inserted	Trait(s)	Remarks	Reference
NtPT1	Grain yield	Phosphate accumulation in rice leading to higher grain yield	Park et al. (2010)
TIFY11B	Grain yield	Improved carbohydrate assimilation for increased grain size and yield	Hakata et al. (2012)
SPIKE	Grain yield	Overall improved rice physiological features leading to increased grain yield	Fujita et al. (2013)
OsmiR397	Grain yield	Improved grain yield by increased grain size and number of panicles	Zhang et al. (2013)

OsAMT1;1	Grain yield	Improved nitrogen use efficiency for growth and grain yield	Ranathunge et al. (2014)
PEPC, PPDK	Photosynthetic capacity	Improved grain yield	Jiao et al. (2002)
RubisCO-A	Photosynthetic capacity	Improved photosynthetic rate and shortened period of heading time	Wu et al. (2006), Wu et al., (2007)
PEPC, PPDK	Photosynthetic capacity	Improved photosynthesis rate and higher water use efficiency leading to higher grain yield	Zhang et al. (2009)
Phd1	Photosynthetic capacity	Improved photosynthesis rate, plant biomass and grain yield	Li et al. (2011a)
OsDET1	Photosynthetic capacity	Improved chlorophyll pigments synthesis	Huang et al. (2013)
SSA	Grain quality	No enhancement of sulfur content in rice grains	Hagan et al. (2003)
Sesame 2S albumin	Grain quality	Enhanced methionine content in rice grains	Lee et al. (2003)
OASA1D	Grain quality	Enhanced tryptophan content in rice grains	Wakasa et al. (2006)
Atgtpchi, Atadcs	Grain quality	Enhanced 100 fold increase of vitamin B9 content in rice grains	Storozhenko et al. (2007)
OsNAS3	Grain quality	Improved iron content in rice grains	Lee et al. (2009)
Waxy	Grain quality	Reduced amylose content in rice grains	Li et al. (2009), Yu et al. (2009)
AtHPPD	Grain quality	Marginal increase in vitamin E content in	Farré et al. (2012)

		rice grains	
OsYSL2	Grain quality	Improved iron content in rice grains	Masuda et al. (2013)
Ca2+/H+ antiporter	Grain quality	Enhanced calcium content in rice grains	Yi et al. (2012)
IPK1, MIPS	Grain quality	Reduced phytate content in rice grain	Ali et al. (2013a), Ali et al. (2013b)
Δ9-elongase, Δ8-desaturase, Δ5-desaturase	Grain quality	Enhanced linoleic acids content in rice grains	Cheah et al. (2013)
13 kD prolamin	Grain quality	Reduced indigestible prolamine content in rice grains	Kim et al. (2013)
PSY	Grain quality	Enhanced vitamin A content in rice grains	Pérez-Massot et al. (2013)
OsBADH2	Rice fragrance	Rice grains emitted fragrance but with yield penalty	Niu et al. (2008), Chen et al (2012)

A Brief Case Study – Commercialization of Transgenic BT rice in China

In 2009, two insect-resistant genetically modified rice lines (Huahui-1 and Bt-Shanyou -63) were granted biosafety certificates by the Ministry of Agriculture (MOA) in China after nearly 10 years of rigorous and strict biosafety assessments (Li et al., 2014). Most farmers welcomed the planting of the transgenic rice due to their potential to reduce pesticide spraying by 50-60%, increase yield by 60-65% and improve the health of farmers (Li et al., 2016). Though so, these two lines have yet been commercialized to the public which is large because of low public acceptance. In a survey, more than half (55%) of the respondents strongly believed that GM crops may cause health and environmental risks. Only 21% know of the technology while the other half of the people have little knowledge or none pertaining to transgenic technology (Qu et al., 2011). This has created a dilemma. On the one hand, the government has invested substantial amount of funding in GM crop development, but on the other hand, the end users or consumers are not prepared to accept GM products due to safety reasons.

Public worries still exist simply because the protocols of the technology remains too technical to be understood. Consumers often do not have access to appropriate channels providing science-based and easy-to-understand information. Consequently, they are misled by activists from non-governmental organizations (NGOs) which utilizes the social media reports to publicize the misconception of GM crops (Lu, 2016). The outreach of scientists in educating the public with essential knowledge of transgenic biotechnology, its benefits derived and biosafety issues is crucially needed in order to bring notable

impact to the public's acceptance of GM crops. In the United States, numerous pro-GM crop NGOs have been actively promoting unbiased scientific information to the community. In fact, the US is one of the country that has the largest area (73.1 million hectares) of transgenic crops since 2010 (Wong & Chan, 2016). The adoption rate amongst consumers keeps elevating yearly and 75% of the processed foods in supermarkets contain GM ingredients (James, 2014). Improving the public understanding on the role of transgenic biotechnology in the food supply has only begun. A guide translated in several languages including Mandarin was developed by the International Food Information Council with the hope of engaging more Chinese readers. Without the support and consumption of the end users, the transgenic BT rice will have no place in China.

Besides implementing biosafety assessments, Lu (2016) suggested that the current biosafety regulation, which was enacted on 2001, has to be updated. As an example, there are no specified threshold for low-level presence of transgenes which could complicate GM labelling and detection. There are also no proper regulations for GM crops that are produced by new transgenic technologies such as stacked multiple genes and gene silencing. The intellectual property (IP) risk management of transgenic crops has often been taken lightly. In the case of BT Shanyou 63, essential information such as nucleic acid and amino acid sequences of the GM rice, detailed methodology as well as material transfer agreements were not disclosed in some patents. Limited patent information could hamper IP analysis and its impact on GM rice commercialization in the international market. If China were to commercialize GM rice globally, the Chinese developer could be sued for infringement when the described technology has already been patented in other countries. Additionally, the GM rice have not been granted with plant variety rights for almost 14 years after application. If it is commercialized domestically, the developer and seed companies will not be guaranteed for economic incentives. Therefore, an efficient IP protection framework for GM rice in China must be developed before its commercialization in domestic and international markets (Liu & Cao, 2014)

Conclusion

With the current easy-to-access rice genome sequence database, countless genes governing economically important traits have been isolated and functionally characterized. Genes that were successfully integrated into the rice genome have shown significant improved traits than the wild types. To date, most transgenic rice production were confined to laboratory or greenhouse scale due to government regulatory reasons. Moreover, a great deal of opposition from the non-government bodies that emphasized on the health threatening aspects of transgenic products has also hampered the commercialization progress. Consumers are still clouded by unverified health and environmental controversies imposed by transgenic crops. Such misconceptions surrounding transgenic rice have to be first properly addressed with clear evidence to the public especially the consumers since they are the key players that drive the commercialization process. The successful commercialization of transgenic rice is also heavily dependent on efficient IP frameworks and biosafety regulations that are frequently updated. In other words, commercializing GM rice is an intricate process which requires collaborative efforts.

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