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Can we create “Elite Rice”—a multifunctional crop for food, feed, and bioenergy production?

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Abstract

Because arable land is limited, land use for food and bioenergy production remains a controversial issue. If food crops can generate high yields and the biomass can also be used effectively for both animal feed and bioenergy production feedstock, conflicts over land use can be reduced. Rice is an important crop; as a worldwide staple food with abundant residuals (polysaccharide-rich straw) after grain collection, this crop plant is attractive as a renewable raw material for bioenergy and feed production. Here, we address current issues and discuss promising methods for improving rice plant characteristics suitable for food, feed, and bioenergy production. Advanced genetic engineering techniques can be used to precisely manipulate the mechanisms regulating grain production, cellulose and lignin content, and stress tolerance. In addition, genetic modification of the mechanisms controlling glycoside hydrolase expression can enhance biomass saccharification for bioenergy production and improved animal digestibility. We also address the issue of nutrient recycling associated with rice straw utilisation for biofuel production.

Keywords: Rice, Grain yield, Stress tolerance, Cellulose content, Straw, Bioenergy, Lignin, Quantitative trait loci, Glycoside hydrolase, Nutrient recycling

Owing to fossil fuel depletion and energy security concerns, bioconversion of lignocellulosic materials to alternative energy sources such as ethanol is a current global research focus [1,2]. Agricultural wastes such as corn stover, sugarcane bagasse, and wheat straw and dedicated energy crops such as poplar, switchgrass, miscanthus, and sweet sorghum are being evaluated as potentially important feedstocks for cellulosic ethanol production; these substrates are likely of interest because of their abundance and availability in leading bioenergy nations [3].

In Asia, rice is an important cereal crop, as most inhabitants rely on it as a staple food. Rice not only provides up to 76% of the caloric intake of the southeast Asian population, but also supplies more than 21% of food needs worldwide [4]. In some countries such as China, India, and Thailand, rice is a principal commodity playing a significant role in improving the economy. According to FAO statistics, global production of (paddy) rice was 719.74 million tonnes in 2012; Asia alone produced 651.58 million tonnes, accounting for 90% of the world's rice output.

China is the largest rice producer, generating 205.99 million tonnes in 2012, followed by India with a yield of 152.60 million tonnes. On the other hand, the leading rice traders are Thailand and Vietnam, which exported 10.67 and 7.11 million tonnes of rice, respectively, to the international market in 2011 [5]. To meet predicted demand by the world's growing population, rice production is projected to increase by up to 40% by 2030 [4,6]; this increased production is expected to supply food to more than two billion people [7]. Consequently, the improvement of rice traits such as grain yield, disease resistance, stress tolerance, and nutritional value (i.e. vitamins and vaccines) through genetic manipulation is an important strategy to ensure an adequate rice supply, reduce food security concerns, and provide rice growers with market opportunities [8,9]. The usefulness of genetically modified rice is exemplified by the β -carotene (provitamin A)-rich rice known as “Golden Rice”, which was created to help alleviate human vitamin A deficiency, especially in impoverished and rural populations [10].

An abundance of by-products, especially rice straw, remain in the field following rice grain collection. Based on the quantity of harvested paddy rice mentioned above and assuming a 1:1 grain:residue ratio [11], Asia generated

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approximately 651.58 million tonnes of the world's net total of 719.74 million tonnes of rice straw in 2012. Although rice straw can be used as feed, its abundance necessitates its disposal prior to the next season's cultivation; thus, farmers usually remove it by fire. The open-air burning of rice straw negatively affects the environment and the health of local residents. Considering its abundance, availability, polysaccharide content, and non-food status, rice straw is potentially an excellent substrate for Asian ethanol production [12]. However, the native form of rice straw is difficult to deconstruct into fermentable sugars owing to the recalcitrance of plant cell walls evolved to withstand microbial attacks [13]. Readily decomposable rice straw is therefore needed.

With the current availability of whole genome sequences, rice can be used as a leading model system for producing a multifunctional crop for the following reasons: (i) rice has a relatively small genome (390 Mb) compared with other cereals; (II) the rice genome is syntenic with the genomes of other cereals; and (III) transformation is conveniently performed [7], facilitating genetic manipulation.

With respect to food supply, rice yield improvements are needed. Grain number, and hence crop productivity, is related to plant height, as rice plants suffer from lodging under wind and heavy rain. Rice dwarf mutants are currently agronomically desirable because short stature confers enhanced lodging resistance, leading to higher yields [14,15]. Some dwarf mutants also produce erect leaves, allowing higher planting densities; a greater volume of products can consequently be obtained in the same cultivation area used to grow conventional bent-leaved rice cultivars [16,17]. In terms of bioenergy feedstock, however, dwarfism is deleterious because it reduces biomass yield. A recent approach using quantitative trait loci (QTLs) was proposed to improve lodging resistance in rice [18]. Through application of QTL analysis and positional cloning, the gene regulating culm strength—known as *STRONG CULM2* (*SCM2*)—has been identified and isolated. *SCM2* confers lodging resistance, and a near-isogenic line containing this gene has been found to exhibit increased culm strength and spikelet number as a consequence of pleiotropic effects [18]. A QTL-based strategy can thus serve as a rice improvement alternative to induction of dwarfism in rice plants. Cytokinin oxidase (OsCKX2)—an enzyme that degrades the phytohormone cytokinin—is related to rice grain number and may be useful for increasing grain production. Reduced expression of the OsCKX2-encoding gene *Gn1a* results in cytokinin accumulation, increasing thereby the number of reproductive organs and potentially enhancing grain yields [7]. Taken together, generation of a multi-purpose non-dwarf rice plant, featuring high grain yields for food and high biomass production for bioenergy feedstock and even ruminant feed, is achievable if the molecular mechanisms

(genes) that regulate grain productivity and biomass production can be elucidated.

Because ethanol-fermenting microorganisms preferentially metabolise glucose, an increase in content of biomass polysaccharides, especially cellulose, should lead to an increase in glucose for improved microbial utilisation. The targeting of cellulose biosynthesis is one means of increasing cellulose concentration. Cellulose is synthesised at the plasma membrane by a cellulose synthase (CESA) complex, which has been identified in rice. The distribution and movement of CESA complexes in the plasma membrane is related to cellulose length [19]; cellulose content can perhaps be increased by controlling these factors. The sucrose synthase-encoding gene *Susy* is a potential alternative for increasing cellulose content, as its overexpression in poplar has been found to increase cellulose content by 2–6% without triggering any adverse effects on plant growth [20]. Enhanced photosynthesis can also increase cellulose production. Photosynthetic rate is positively linked to canopy carbon dioxide (CO₂) fixation rate and productivity [1]. For example, the cultivation of rice under elevated CO₂ levels results in higher yields of biomass [21]. These approaches can provide fermentable sugar-rich biomass for ethanol production.

Lignin is important for plant growth because it provides strength and structural support and confers biotic stress resistance. Nevertheless, the presence of lignin in rice straw limits polysaccharide digestibility in animals. Lignin also hampers the ethanol production process: through their negative effects on ethanol-fermenting microorganisms, lignin degradation products obstruct the fermentation of sugars into ethanol [3]. Reduction of lignin content is therefore of interest for development of multifunctional rice plants. Down-regulation of genes involved in lignin biosynthesis may be used to reduce lignin content and alter lignin structure [22]. For example, repression of cinnamate-4-hydrolase (C4H) using an RNA interference approach lowered lignin content. C4H transgenic rice plants grew normally, with minimal impact observed on agronomic traits [23]. On the other hand, abnormal phenotypes due to reduced lignin content, such as dwarfing, colouration, and xylem collapse through loss of mechanical support, have also been observed. Additionally, insect resistance and plant fitness have been found to be negatively affected by lignin modification and silencing of the lignin biosynthesis pathway. The integration of phenolic derivatives or peptide cross-links upon lignification has been suggested to form more hydrolysable lignin without loss of structural strength or plant fitness [3]. Finally, the mapping of QTLs influencing lignin content can be applied—with the aim of reducing lignin content and improving forage quality—during marker-assisted selection for rice breeding [24].

Rice plant *in vivo* expression of cell-wall deconstructing enzymes (CDEs), including cellulases and xylanases, would

be highly profitable for cellulosic ethanol and animal feed production. CDE production in transgenic rice can lower exogenous loading during biomass saccharification. Additionally, the rice plant can provide larger volume capacity and higher protein yields compared with submerged microbial fermentation, thus reducing enzyme costs. Under the conditions needed for CDE expression during growth, however, problems involving plant cell damage, loss of strength, and abnormal phenotypes have appeared. In this context, three promising solutions have been suggested to overcome these issues. First, localisation or compartmentalisation of enzymes in non-cytosolic compartments, such as vacuoles, mitochondria, and chloroplasts, has been proposed to prevent substrate-enzyme interaction on plant cell walls (consisting largely of polysaccharides) and to reduce interference due to cytosolic metabolic activity [25]. A second idea involves the use of a chemically inducible promoter to express CDEs at the appropriate time. In this approach, a combined sequence consisting of a signal peptide derived from a plant cell-wall protein and a chemically induced promoter would be spliced into a target CDE gene, such as a cellulase gene. The resulting construct would then be used to transform rice plants. Spraying plants with the chemical inducer before harvesting would trigger cellulase expression, with the enzyme being targeted to the cell wall where it would begin degrading cellulose. The enzyme would remain active after harvesting and continuously liberate glucose until subsequent ethanol processing [1]. A third possible solution involves expression of thermophilic CDEs in rice to prevent cell-wall damage during growth. Such enzymes would be optimally functional at elevated temperatures (i.e. active above 50°C), with limited biological activity at normal rice growth temperatures. The expression of thermophilic CDEs in rice would consequently have reduced or negligible impact on plant growth and strength [3,26]. As an example, a thermophilic cellulase of *Acidothermus cellulyticus*, the endo-glucanase Cel9B, has been successfully expressed in normally growing rice; the enzyme accounted for 4.9% of total soluble proteins and exhibited biological activity towards AFEX-pre-treated rice straw [27]. With respect to animal feed, non-starch polysaccharides in cereal cell walls, particularly xylan, are a primary problem: they negatively affect ruminal digestibility and solubilise in broiler chicken intestinal tracts, thereby increasing gut content viscosity. The addition of exogenous xylanases to animal feed has been shown to improve digestibility and increase energy intake [28,29]. If xylanase can be expressed in rice and remain functionally active during harvesting and storage, feed processing, or even in ruminant and broiler intestinal tracts, it would enhance xylan degradation into sugar monomers or short-chain oligomers and thus decrease gut viscosity. Additionally, the xylo-oligomers (comprising 2–6 monomer units) might function as a

prebiotic beneficial to animal health. Furthermore, the feed formulation would not require supplementation with exogenous xylanases, thus reducing production costs.

Apart from improving characteristics suitable for food, feed, and bioenergy purposes, the agronomic performance of rice is of primary concern. Tolerances to drought and submergence are essential for rice production in south and southeast Asia because drought and flash flooding—a rapid and extreme flow of high water into a normally dry area, or rapid water-level rise in a stream above a predetermined flood level [30]—occur more frequently in these regions. A few QTLs against these stresses have been identified and are candidates for breeding [18]. Additionally, the development of rice plant protection against weeds, insects, and pathogens is needed, with built-in resistance to these biotic stresses highly advantageous. Transgenes conferring herbicide and insect resistance, such as lectin genes, *Bacillus thuringiensis* endotoxin, avidin, and rice cysteine protease inhibitor, could be introduced into rice to facilitate crop damage prevention [31].

Concerns have been raised regarding the use of crop residues for biofuels and its effect on nutrient recycling [32,33]. It has been shown that excessive removal of residues from soils may lead to nutrient depletion and soil quality degradation. In this regard, using rice straw for ethanol production is believed to have less adverse effects on nutrient recycling. Rice is typically harvested by cutting machines, which leave the short stems and root systems in the field when the straw is taken for ethanol production. The remaining root system can retain organic matter and soil water and preserve soil structure, whereas stem organic matter can be released into the soil after tillage for subsequent cultivation. Because of the excessive quantities involved, farmers habitually dispose of rice straw in fields by burning. Although straw burning can return carbon into the atmosphere, it releases soot and smoke that cause air pollution and human health problems. This practice in addition leads to loss of plant nutrients present in straw, including nitrogen, potassium, and sulphur, as well as soil nutrients and microbiota [34]. When straw is used for ethanol production, straw carbon not converted into ethanol can be released through microbial fermentation into the atmospheric carbon pool as CO₂, which plants can fix via photosynthesis. After saccharification and fermentation, the remaining materials, which include fibre, proteins, and minerals in the straw together with microbial cells, can be returned to the field as soil amendments or fertilisers to supply the soil with organic and inorganic matter. The straw residue is present in a structurally degraded form; it is thus readily assimilated and mineralised by soil microorganisms, while the residual proteins (enzymes used in saccharification) and cell debris can supply the soil with available nitrogen. Moreover, the degraded structure of the straw residue is beneficial to soil: it can

reduce the soil nitrogen deficiency due to microbial nitrogen immobilisation that typically occurs when native straw is incorporated into soils. To solve this problem, various authors have previously proposed that straw decomposition be enhanced by inoculation with fungi or bacteria or by applying additional inorganic nitrogen to the soil [34,35].

If the entire rice plant (and those of other cereal crops) can be utilised effectively, i.e. the grains used for food and the tillers for feed and bioenergy, the conflict over land use for food and bioenergy production can be reduced and waste generation minimised. The recycling of residual straw and microbial cells/proteins into fertilisers or soil amendments after ethanol production is a potential method for returning nutrients to the soil. Most importantly, the use of standard, good agricultural practices must be maintained throughout the rice cultivation season to increase grain yield for food and straw biomass for the bioenergy supply chain. Because grain from multifunctional transgenic rice would be used as a staple food, concerns regarding genetically modified food might be raised by consumers. A possible means to avoid this uneasiness is to use advanced genetic manipulation techniques, i.e. QTL-based methods, to precisely edit or modify only the rice genome rather than importing foreign genes from other species. The creation of a multifunctional rice is a challenging task; nonetheless, as we have discussed, opportunities exist to make it a reality. This promising endeavour can have profound bioeconomic benefits.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

PP and KR wrote the manuscript, with PP being the main contributor. Both authors read and approved the final manuscript.

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