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Molecular Research in Rice

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Abstract

The molecular genetic basis of agronomical important traits in rice to develop novel rice cultivars showing good agronomic performance and strong climate resilience in the future, we have to identify further important genes, elucidate their molecular functions, and design desirable genotypes based on individual and interaction effects of those genes. In addition to the traits described in the previous and present Special Issues, there are many other agronomical important traits that should be explored using molecular genetics and biological research. In addition, the wide genetic diversity that exists in rice genetic resources including wild rice species (e.g., *Oryza rufipogon Griff.*) has not been fully exploited in genetic analysis and breeding programs.

Keywords: Breeding programs; Breeding programs; Molecular biology techniques

Introduction

Rice (*Oryza sativa L.*) is that the most significant food crop within the world, being a staple food for over half the world's population. The importance of rice as a crop and as a model species promoted overseas collaborations resulting in the formation of the International Rice ordination Sequencing Project, and rice became the primary crop ordination to be sequenced in 2004. When the discharge of the primary complete rice ordination of 'Nipponbare', researchers have created many reference genomes for alternative rice cultivars together with '93-11', 'IR64', 'Zhenshan 97', and 'Minghui 63', and picked up giant sets of whole-genome sequence knowledge comprising over 3000 rice cultivars distributed worldwide [1-3].

Rice grain yield consists of 4 main components: the amount of panicles per plant, the amount of grains per raceme, the proportion of ripening grains, and 1000-grain weight. Changes in raceme design are related to improved grain yield. In rice, raceme design is especially determined by the spike and branch arrangement. Spikelets and branches are initiated and developed from inflorescence meristems.

Developing rice dwarf varieties has been widely considered as one of the most important achievements in rice breeding history. Another breakthrough achievement was to harness heterosis by developing and growing hybrid rice. Heterosis or hybrid vigour refers to a situation in which the hybrids perform better than their parents, and this has been exploited to improve crop production for nearly a century. Exploitation and utilization of heterosis in rice was first initiated by Prof. Longping Yuan in the 1960s, and a significant progress was made in 1970 due to the discovery of a cytoplasmic male sterility (CMS) line from wild rice (*Oryza rufipogon*). Five years later, large-scale hybrid seed production using a three-line sys- tem was fully established, making it feasible to commercially produce hybrid rice. Several additional CMS lines were later identified and successively exploited, which had greatly expanded the germplasm pool of CMS. The subsequent establishment of the two-line hybrid system broadened the use of hybrid vigour both within and between subspecies, and this technology further increased rice yield by 5%–10% compared to the three-line system. In 1996, the Chinese government launched a nationwide "Super Rice Breeding Program", with an ultimate goal to further boost rice yield through an improved understanding of the theory and practice of hybrid development. In recent field tests, Super Hybrid Rice has set a new world record by reaching an average yield over [4-6].

Materials and Method

Understanding of the genetic basis for varied abiotic stress resistance and tolerance is very important for rice cultivation, as a result of these stress conditions cause important decreases in yield and grain quality. Bang et al. Li et al. showed that the verticillated Primary Branch one (VPB1) cistron is preferentially expressed within the inflorescence plant tissue and is related to management of primary branch arrangement. The VPB1 cistron encodes a BELL like homeodomain (BLH) macromolecule that alters the expression of alternative cistrons concerned in inflorescence plant tissue identity and hormone-signaling pathways by binding to the promoter region of the OsBOP1 gene. When compared with the conventional ones because of theirhigh harvest index, resistance to lodging, and improved response to fertilizers. Characterised the Rice plastid RNA- binding macromolecule one (OsCRP1) cistron, that is crucial for the stabilization of RNAs from the NAD(P)H dehydrogenase complicated in rice plastid. They clearly indicated that the OsCRP1-overexpressing lines showed higher cyclic negatron transport activity and elevated ATP levels for chemical change, compared with wild-type plants. In addition, the OsCRP1-overexpressing lines considerably increased drought- and cold-stress tolerance. Rumored that the Rice WRKY Transcription issue 55 cistron is concerned each in drought responses and in plant growth regulation. They created OsWRKY55-overexpression lines, and discovered that the overexpression lines showed quicker water loss and a larger accumulation of oxide (H2O2) and superoxide radicals (O2−) in leaves underneath drought-stress conditions, and consequently diminished drought resistance compared with wild-type plants. They discovered macromolecule–protein interactions of the OsWRKY55 macromolecule with mitogen-activated protein kinases (MAPKs) OsMPK7, OsMPK9, OsMPK20-1, and OsMPK20-4 that would be iatrogenic by drought conditions, and showed binding activity to the promoter region of the OsAP2-39 cistron that controls cell size and

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Received: 01-Jul-2022, Manuscript No. rroa-22-71018; **Editor assigned:** 04-Jul-2022, PreQC No. rroa-22-71018 (PQ); **Reviewed:** 18- Jul-2022, QC No. rroa-22-71018; **Revised:** 22- Jul-2022, Manuscript No. rroa-22-71018(R); **Published:** 29-Jul-2022, DOI: 10.4172/2375-4338.1000314

Citation: Silveira F (2022) Molecular Research in Rice. J Rice Res 10: 314.

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plant height. Used marker-assisted choice to introduce the Sub1A cistron and develop breeding lines with sturdy submersion tolerance the developed lines.

Exhibited fascinating agronomical traits together with high grain yield and quality, and showed sturdy drought and submersion tolerance. Abiotic stresses iatrogenic by high or low tem- peratures also are a very important issue for rice cultivation as a result of they because important decreases in grain yield and qualitydetected 2 quantitative attribute loci (QTLs) for the determination of flowering time in line with seasonal temperature conditions. The Hd16 cistron is one amongst the genetic factors related to strength of flowering time to environmental fluctuations. These analysis efforts are useful in dissecting the genetic basis of stress resistance and tolerance in future breeding programs. China has been a major player of the rice genome research, contributing to sequencing and resequencing genomes of many cultivated and wild rice varieties. A wealth of genomic data combined with fast-growing biotechnologies greatly facilitated gene discovery and functional analyses. Prof. Jiayang Li and his team successfully cloned MONOCULM 1 (MOC1), a key regulator controlling rice tiller number. Thereafter, Chinese scientists have made great strides in isolating dozens of key genes relevant to important agronomic traits. Examples of such genes include the plant.

Data-Analysis

In this study, to generate transgenic rice, the plasmid BU9-3301 transferred to the indica rice sterile line Guangzhan 63-4S via modified *Agrobacterium*-mediated transformation to generate transgenic. The BU9-3301 vector was created utilizing pCAMBIA3301 as the backbone and homologous recombination of the *BPH9* gene obtained from Pokkali. The progeny plants were grown in a greenhouse with a photoperiod of 12:12 h light: dark at 28/24 °C and 70% relative humidity. The putative transgenic lines were confirmed via PCR using the primers specific for *bar* and *BPH9* genes, and 20 positive rice transgenic lines were obtained Subsequently, T1 were screened by testing resistance. Finally, an individual T1 transgenic rice line that showed the best tolerance to bastes herbicide and resistance to BPH was selected and named H23**.**Quantitative real-time PCR was used to validate the expression of *BPH9* and *bar* genes of Guangzhan 63-4S and the T3 generation of H23. In tillering stage, elongating stage, and mature stage, the leaves, stems, roots, and leaf sheath were harvested for total RNA isolation; moreover, the total RNA of young embryos in mature stage was also extracted. In addition, samples took three independent plants as one replicate, and three biological replicates were performed. *BPH9* and *bar* genes qPCR validation were taken with a CFX96™ Real-Time System (BIO-RAD) using Roche FastStart Universal SYBR Green Master (Rox) (Roche). $2^{-\Delta\Delta CT}$ relative quantification method was selected for data analysis and *Actin1* was reference gene. The primers for qPCR validation were designed with primer 5.0 software and are listed for generating a transgenic rice resistance to glufosinate and BPH, the vector with stacked *bar* and *BPH9* gene was transferred to the indica rice sterile line Guangzhan 63-4S via the, an excellent transgenic rice line, H23, was identified with best tolerance to glufosinate and BPH.

Result and Discussion

Southern blot analysis was performed to determine the copy number and stability of the exogenous genes in H23, using Guangzhan 63-4S as the control. *Eco*RI and *Xba*I were selected for the *bar* gene to digest. *Eco*RI has two restriction sites in the BU9-3301 vector, one in the T-DNA insert and the other on the backbone After *Eco*RI digestion, the positive plasmid BU9-3301 vector generated a single band of about 5.1 kb, while the inbred line Guangzhan 63-4S, which served as the negative control, generated no bandA. The T2 and T3 generations of H23 showed two bands at approximately 4.3 kb and 10 kb indicating two copies of the *bar* gene in H23. Meanwhile, the T-DNA insert has four *Xba*I expected band size of 12.5 kb was obtained with the positive A [7-9]. The T2 and T3 generations of H23 generated two bands, one of and the other of 12 kb, in line (Figure 1). These observations confirmed to Analysis was carried out with various tissues in different development stages., *BPH9* and *bar* show significantly higher expression in H23 than in 63–4S. *BPH9* is an endogenous rice gene; the qPCR results displayed *BPH9* showed very low expression level in donor 63-4S. On the contrary, it highly expressed in the H23 transgenic line. Moreover, *BPH9* showed extremely high expression level in stem and young embryo of H23 in mature stage is an exogenous gene; as expected, no expression was detected in donor 63-4S. Furthermore, *bar* expressed very well throughout the entire period of H23, especially in leaf. These results demonstrate that *BPH9* and *bar* genes expressed well in the H23 transgenic line. Weeds and pests are the critical limiting factors for growth factor are always crop yield under the modern agricultural system (Table 1). The young leaves of the inbred rice line Guangzhan 63-4S and the T2 and T3 generations of H23 were harvested for to detect the copy number of the foreign genes. Genomic DNA (30 μg) was digested overnight using restriction enzymes, and the well-digested DNA was separated on agarose gel (0.7%) and immobilized on nylon membranes. *Xba*I and *EcoRI*/*Sac*I restriction enzymes were used for the target gene. The primers for the gene-specific PCR product were designed and used for probe

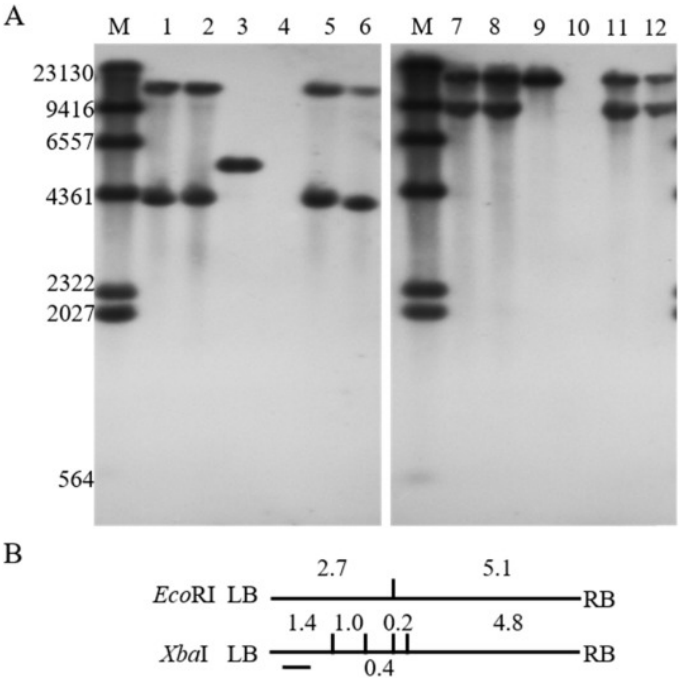

Figure 1: Electron Microscopic view root.

Table 1: Distribution of ZPX data.

Material	Resistance score	Correct resistance score	Resistance level
H23	5.3 ± 0.4 b	5	moderate resistance
63-4S	6.1 ± 0.6 b	7	susceptible
TN1	9.0 ± 0.9 a	9	highly susceptible
RH	1.7 ± 0.2 c	1	high resistance

Table 2: Distribution of Biological Traits of Xylem.

Material	Plant height (cm)	Effective spike number	Spike length (cm)	Total number of grains per spike	Seed setting rate (%)	Thousand grain weight (g)	Yield (kg/acre)
H23	117.9 ± 1.1 a	14.9 ± 0.8 a	20.4 ± 2.3 a	236.7 ± 17.9 a	69.1 ± 2.9 a	22.9 ± 1.2 a	572.3 ± 37.2 a
63-4S	119.3 ± 1.3 a	13.3 ± 2.3 a	19.6 ± 1.2 a	251.3 ± 11.5 a	63.8 ± 3.0 a	23.1 ± 1.8 a	566.0 ± 23.0 a

preparation Following a previously reported method, hybridization and detection of the target gene fragments on the blots were performed. PCR was performed to identify the transfer DNA (T-DNA) integration site and confirm the presence of the T-DNA insertion fragment in the transgenic rice H23. Genomic DNA was isolated from the young leaves of H23 (T2 and T3 generations) and Guangzhan 63-4S (nontransgenic control) using the cetyltrimethylammonium bromide (CTAB) protocol Further, PCR analysis was carried out using the genomic DNA of H23 and Guangzhan 63-4S as the template and the designed on the basis of the T-DNA sequence of the BU9-3301 vector. PCR products were further purified for sequencing, and the obtained sequence was blasted against that of BU9-3301 to confirm the T-DNA insertion fragment in H23 as showed in table given below (Table 2). The present study generated a transgenic rice H23, with *BPH9* and *bar* stacked genes for BPH resistance and herbicide glufosinate tolerance. Southern blotting and PCR analysis revealed one copy of *BPH9* in the H23 transgenic line. Furthermore, GM rice H23 with glufosinate tolerance and BPH resistance was expected to display advantages under complex farming conditions. H23 showed excellent resistance to BPH in the seedling and mature stages, indicating that *BPH9* is a valuable gene for resistance breeding in rice. Southern blotting analysis detected two copies of the glufosinate-resistance gene, *bar*; however, PCR analysis showed 2 *bar* gene fragments in H23—one is the functional *bar* gene and another is an incomplete nonfunctional fragment of the *bar* gene. Based on the expression analysis of *BPH9* and *bar* genes, both of them showed higher expression in H23 than 63-4S, so the structure of incomplete fragment of *bar* did not influence the *bar* and *BPH9* gene expression, suggesting its nonfunctional nature in H23 transgenic rice. Moreover, the T-DNA insertion did not affect the growth and development of H23, as the agronomic traits displayed no significant difference between H23 and 63-4S. The glufosinate tolerance analysis in the greenhouse showed that H23 was highly resistant to glufosinate. Overall, the molecular assessment demonstrates H23 as an excellent glufosinate-tolerant and BPH.

Conclusion

The present study employed a gene stacking process that combines more than one gene/trait into an individual transgenic plant to meet the increasing cropping demands under complex conditions. The transgenic rice H23 co-expressing *bar* and *BPH9* genes demonstrated both glufosinate tolerance and BPH resistance. The molecular characterization revealed that H23 possessed a single T-DNA cassette on chromosome 3 that was inherited stably. Evaluation of insect resistance and glufosinate tolerance confirmed H23 as an excellent double-resistant transgenic rice. These findings indicate that H23 can satisfy insect management and weed control in the modern rice agricultural system. However, a deregulation study will help for prospective commercial planting.

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