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Gene expression and sequence analysis of known yield genes in high yielding varieties of rice (*Oryza sativa* L.)

E.N. Suresh*, L.N. Vamireddy*, N.P. Eswara Reddy and M. Reddi Sekhar

Acharya N.G Ranga Agricultural University, INDIA

Abstract

The rice yield is governed mainly by three major traits viz. number of tillers per plant, grain number per panicle, and grain size which are directly associated with rice grain productivity. Rice yield is a complexly inherited trait governed by many genes/QTLs. As of now more than 34 genes have been molecularly cloned and characterized using the donors only from China and Japan (Xu *et al*., 2016). However, the expression of these genes and sequence variation need to be validated before directly using them in rice breeding programmes. In the present investigation, an attempt has been made to validate the gene expression and sequence variation of the important cloned genes governing yield traits in known donor varieties from India. In the current study, 21 *indica* rice genotypes have been selected. Among them 15 were high yielding and 6 were low yielding. The phenotypic data of the important yield and its component traits such as plant height, number of tillers per plant, panicle length, number of grains per panicle, biological yield, harvest index, etc. have been recorded. The analysis of variance (ANOVA) for the 21 rice genotypes for all the agronomic traits revealed highly significant differences among the entries for all the characters except for panicle length, grain length, and grain width. The samples of flag leaf and young panicle tissues were collected for RNA isolation and the isolated RNA was converted into cDNA. Semi qRT PCR gene expression analysis in the selected rice genotypes revealed differential expression of all yield genes. The *Gn1a* gene expression with low or nil gene expression such as NLR33892, Ranjit, BPT2601, BPT5204 and Rasi can be used as donors for *Gn1a* gene introgression into low yielding varieties. The gene *OsSPL14* from high grain number varieties with high gene expression can be used as donor varieties viz. MTU1064, Dee-geo-woo-gen, Rasi, BPT5204, and MTU1010 for *OsSPL14* gene from Indian rice germplasm. The high *GIF1* gene expression varieties with high grain weight such as BPT 2678 and Basmati370 are the potential donors for grain weight/grain filling trait. The high grain weight varieties with high *GW8* expression such as MTU3626, IR-8, Dee-geo-woo-gen, MTU1010, MTU1001, and INRC10192 can be used as donors from Indian rice germplasm. The *Ghd7* high gene expression was observed in both high grain number genotypes such as NLR33892, BPT2678, Taichung Native-1, MTU1121, Ranjit and low grain number genotypes IR-8, MTU3626, MTU1001, MTU1064, Tetep, NLR34449 in flag leaf. In case of young panicles, high expression was recorded in high grain number genotypes such as NLR33892, Ravi003, BPT2678, MTU1121, Ranjit and in low grain number genotypes such as IR-8, MTU3626, MTU1001, MTU1064, Tetep, and NLR34449. The high gene expression was observed in both flag leaf and young panicles in both early flowering varieties and late flowering varieties with few exceptions. The high gene expression was observed in both flag leaf and young panicles in both tall plants and dwarf plants. The high grain number, tall plant and late flowering varieties with high *Ghd7* expression such as NLR33892, BPT 2678 and Ranjit can be used as donors from Indian rice germplasm. In the present investigation, besides gene expression analysis, the DNA sequence variation of most differentially expressed yield genes such as *Ghd7, DEP1* and *Gn1a* have also been analyzed in all high yielding and low yielding varieties. The overlapping primers covering the entire gene length including 1000 base pair upstream have been designed and sequenced. The whole genome DNA sequence of *Ghd7* gene (3918 base pair) was resequenced in all 21 rice genotypes. In all 21 rice gentotypes, a total of 104 SNPs and 141 indels were detected. It was found that certain nucleotide variations are unique to high grain number varieties such as MTU1121, MTU1010, and Dee-Geo-Woo-Gen. However, there are no nucleotide variations which are common to their respective either low or high grain number varieties INRC10192, Tetep, and MTU1001. There are no nucleotide variations found specific to either tall or semidwarf varieties MTU7029 (G/A) at 1426 and MTU1001 (T/C) at 1425 base pair positions of the gene. There were no nucleotide variations specific to either early or late flowering varieties. The whole genome DNA sequence of *DEP1* gene (4363 base pair) was resequenced in all high yielding and low yielding rice genotypes. In all

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varieties studied, 99 SNPs and 338 indels were detected in *DEP1* gene. The whole genomic DNA sequence of *Gn1a* gene (6476 base pair) was resequenced in all genotypes. In total, 97 SNPs and 121 indels were detected in the 4837 base pair alignment. Of these, one SNP (MTU1064) and 28 indels were detected in the promoter region. To conclude, an attempt has been made to identify the Indian donor varieties comprising of important yield genes based on consistent gene expression and sequence analysis with reported donors. The shortlisted donors for the yield traits can be used right away in the rice breeding programmes. The marker-assisted stacking of different yield genes from these multiple donors into a single elite variety could pave the way for designing high yield varieties suitable to different ecologies of India.

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