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# Usefulness of the cloned and fine-mapped genes/QTL for grain yield and related traits in *indica* rice breeding for irrigated ecosystems

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## ABSTRACT

Many genes/QTL for grain yield (GY) and yield related traits in rice have been cloned or fine-mapped in the last three decades. A collection of *indica* elite breeding lines and cultivars assembled in IRRI was used to test the usefulness of 39 well characterized yield related genes/QTL. The population of lines was phenotyped for GY and ten yield related traits under eight environments of three locations including Jiangxi, and Sichuan in China, and six season (2) and nitrogen rate (3) combinations in IRRI and genotyped using 46 markers tightly linked to the 39 target genes/QTL and 53 SSR markers evenly distributed on the genome. Using the 53 random SSR markers identified two major subpopulations. Association analyses were separately carried out for the whole population and the two subpopulations. All the 39 target genes/QTL were associated with two or more measured traits including traits not previously reported. *GW6* and *Gn1a* were associated with nine and eight traits, respectively. *Ghd7*, *qSPP7*, *SCM2*, and *SPP1* were associated with seven traits. *GIF1* and *Ltn* were associated with six traits. *GS3*, *GW2*, *gw3.1*, *htd1*, *Nop(t)*, *qGY2-1*, and *qPH6-1* were associated with five traits. *D10*, *d27*, *DEP2*, *DWL1*, *Gnp4*, *Gw1-1*, *GW3*, *gw5*, *MOC1*, *PAP2*, *qGL7*, *qGL7-2* and *qGN4-1* were associated with four traits, *D88*, *Ghd8*, *GS5*, *Gw1-2*, *IPA1*, *qSH3* and *RPH* were associated with three traits. *ep3*, *gw8.1*, *gw9.1* and *qPDS3* were associated with two traits. A total of 16 genes/QTL were found to be associated with GY. *GS3*, *GW1-1* and *d27* were associated with GY in two testing environments and the others were only in one environment. Sixteen, six and ten genes/QTL were associated with panicle number per plant, grain number per panicle and thousand grain weight, respectively. Significant gene-by-environment interaction was present for all the studied genes/QTL. GY could not be well predicted using the markers significantly associated with the measured traits or all target markers based on stepwise multiple linear regression analysis. The adjusted coefficient of determination ranged from 0.024 to 0.191 for the final selected models considering the associated markers only and from 0.039 to 0.261 for the final selected models considering all target markers. Nevertheless the known genes might be explicitly utilized in developing more efficient selection criteria for enhancing selection accuracy.

## 1. Introduction

Rice (*Oryza sativa* L.) is one of the most important crops in the world. It has played a central role in human nutrition and been cultured for nearly 10,000 years (Molina et al., 2011). Great progress has occurred in rice production in the last decades due to the adoption of green revolution technology. Paddy rice production has increased from 257 million tons in 1966 to 744.4 million tons in 2014 (Khush, 2005; FAOSTAT, 2014). As world population grows, the global rice demand is estimated to rise from 439 million tons (milled rice) in 2010 to 496 million tons in 2020 and further

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*Abbreviations:* DTF, days to flowering; GIF, grain incomplete filling; GL, grain length; GN, grain number per panicle; GS, grain size; GW, grain width; GY, grain yield; HD, heading date; IPA, ideal plant architecture; PB, primary branches per panicle; PH, plant height; PN, panicle number per plant; SN, spikelet number per panicle; SR, seed setting rate; SB, secondary branches per panicle; TGW, thousand grain weight; TN, tiller number; WLT, withered leaf tip.

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increase to 555 million tons in 2035 (GRiSP, 2010). At least 50% of the increase of rice production in the past has been due to the adoption of new cultivars. It is expected that breeding will still be one of the key approaches for further increasing in rice productivity. Rice breeders today face the challenge of how to increase productivity by effectively integrating well-established conventional breeding methods with new approaches offered by rapid advances in molecular marker technology and genomics.

For quantitative traits such as yield, many genes of small effects and environmental factors collectively determine the trait performance. The favorable alleles are likely to be spread across more than two lines, therefore requiring the assembly of alleles from different sources to a single inbred line in order to achieve significant improvement (Ye, 2010). Recurrent selection (RS) is a well established conventional breeding method which is designed to gradually increase the frequency of desirable alleles while maintaining genetic variability for future selections (Hallauer, 1985). RS is a cyclical breeding strategy involving three main steps: selection, evaluation and recombination of the best performing selections. Multiple genotypes are inter-mated to increase the chance of creating novel allelic combinations. Through multiple cycles of recombination between selected genotypes, linkage blocks are broken down and favorable genes are accumulated while the genetic diversity remains (Châtel et al., 2008).

The potential benefits of using molecular markers linked to the genes of interest in breeding programs, which have changed from phenotype-based toward a combination of phenotype- and genotype-based selection, have attracted much attention for more than three decades (Bernardo, 2008; Tester and Langridge, 2010). The success of marker-assisted selection (MAS) for traits of simple inheritance in many crops including rice has motivated rice breeders to search for QTL for complex traits, which account for a large proportion of phenotypic variation (major QTL) (Guo and Ye, 2014). Many yield-related genes/QTL have been identified and some of them are fine-mapped or cloned (Xing and Zhang, 2010). The effects of the well characterized genes/QTL were usually tested using specific populations. The use of these well-characterized genes/QTL in improving yield has started. However, significant improvement of GY in farm environments has yet been reported (Guo and Ye, 2014). A promising MAS method, known as genomic selection (GS) or genome-wide selection, has been recently introduced for using all trait-affecting genes to improve quantitative traits (Hayes et al., 2013; Meuwissen et al., 2001). GS uses genome-wide markers to predict the breeding (genotypic) values of the selected candidates. Once an accurate prediction model is developed using a reference population with genotyping and phenotypic observations, the model is then used to select genotypes within the selection population with genotyping data only. Simulation and empirical studies in self-pollinated crops, including wheat, barley and oat, have demonstrated the great potential of GS in improving quantitative traits (Jannink, 2010).

The irrigated rice breeding program at the International Rice Research Institute (IRRI) has a mission to develop new rice germplasm that produces high and stable yields across a broad range of environments. IRRI breeding lines have been directly released for production or used as crossing parents in many breeding programs in different countries. IRRI is adopting a new integrated breeding strategy to break yield barrier (GRiSP, 2010). The strategy utilizes RS to quickly pyramid the major QTL that have been proven useful in the breeding population in the first few selection cycles and maintain the genetic variation contributed by the many minor genes to be explored in later cycles and explores GS for reducing the length of breeding cycles and the cost of expensive phenotyping. A series of studies have been conducted to obtain essential information for designing more efficient mating and selection schemes of this general breeding strategy. As part of this effort, 392 advanced lines and cultivars from many breeding programs in different countries representing the genetic diversity of breeding gene pools for irrigated ecosystems were collected to be used as part of the base population for future breeding at IRRI. This population was phenotyped via multi-environment trials (METs) in South-east Asia and genotyped using markers for well-characterized genes/QTL and genome-wide markers generated by genotyping by sequencing. Data collected so far has been used to analyse GEI for GY (Liang et al., 2015), investigate the factors affecting head rice yield and chalkiness (Zhou et al., 2015) and test the usefulness of important known genes/QTL for grain quality traits (Zhao et al., 2015). In this paper, the usefulness of 39 fine-mapped or cloned genes/QTL for GY and yield related traits was tested through association analysis. Prediction of GY with markers for these well characterized genes/QTL was investigated. The results can be used together with information from other studies to optimize the proposed integrated breeding strategy. The information gained will also be valuable to other rice breeding programs willing to exploit the well characterized genes/QTL for GY and related traits.

## 2. Materials and methods

### 2.1. Plant materials and phenotyping

Three hundred and ninety two rice lines developed for the irrigated lowland ecosystem were used in this study to achieve a large amount of genetic diversity. The majority of the lines were from IRRI (223). A good number of lines were also from PhilRice (31), CIAT (17), China (13) and Vietnam (11) (Supplementary Table 1). Phenotyping was conducted in Jiangxi (JX) and Sichuan (SC) in China and IRRI headquarters (Los Baños, Philippines). The experiments in JX and SC were for one crop season in 2012. At IRRI, the experiment was carried out in the dry season (DS) and wet season (WS) of 2012. Three nitrogen fertilizer application rates, no nitrogen, low (90 kg $\mathrm{ha}^{-1}$), and high (180 kg $\mathrm{ha}^{-1}$), were used to create three artificial environments in the DS, designated as DS1, DS2 and DS3, respectively. Similarly, three nitrogen fertilizer application rates, no nitrogen, low (45 kg $\mathrm{ha}^{-1}$), and high (90 kg $\mathrm{ha}^{-1}$), were used to create three artificial environments in the WS, designated as WS1, WS2 and WS3, respectively. Grian yield (GY) and ten related traits including grain number per panicle (GN), panicle number (PN), thousand grain weight (TGW), spikelets number per panicle (SN), seed setting rate (SR), number of primary branches per panicle (PB), number of secondary branches per panicle (SB), tiller number (TN), days to flowering (DTF) and plant height (PH), were tested. All 11 traits were measured for the five experiments at IRRI except DS2. In DS2, DTF, PH, PN, GY, and TGW were tested. GY, DTF, GN, PH, PN, TGW and SR were measured in SC, while GY, DTF, GN, PH and SR were measured in JX. Detailed trial description and trait measurement were given in Liang et al. (2015).

### 2.2. Markers and genotyping

Two sets of markers were used. The first set consisted of 53 random SSR markers evenly distributed on the whole genome (Supplementary Table 2). The second set consisted of 46 target markers, including SSR, STS and Indel markers which were tightly linked to 39 cloned or fine-mapped genes/QTL for GY or yield related traits (Table 1). Those target markers were initially chosen from the original publications, in which the known genes/QTL were reported. If there was no polymorphism in our population for any marker, another marker close to the initial marker was chosen. A total of 46 polymorphic markers for the 39 target genes/QTL were developed and screened for the population.

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