

Short Communications

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Marker Validation in F₂ Population of Rice (*Oryza sativa* L.) for Water and Nitrogen Use Efficiency

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ABSTRACT

The new challenge of irrigated lowland rice is to sustain the needed increase in rice yields while reducing the requirements for water and N per unit of grain production. Validation of markers helps in determining the reliability and practical applicability of the markers in predicting the phenotype. In the present study, Simple sequence repeats (SSR) markers flanking WUE and NUE traits reported to explain high percentage phenotypic variation for their respective traits in RILs population from a cross Swarna x WAB450 were used for validation in F₂ population individuals of BPT5204 x WAB450 and BPT5204 x Mysore Mallige crosses for the same traits for which they are already reported to be associated. Single marker analysis was done to with the help of student's t test and by single factor ANOVA. Among the markers chosen for validating across the population, only few were found to be significantly associated with WUE and NUE traits in F₂ mapping population individuals. This probably may be due to fact that these were detected in other mapping populations with different genetic background, and because the marker intervals may be quite large. The SSR markers RM518 and RM225 are associated with Water Use Efficiency and Nitrogen Use Efficiency in F₂ mapping population of BPT5204 x WAB450 and BPT5204 x Mysore Mallige respectively. The per cent contribution of the significantly associated markers to total phenotypic variance were 48.26 and 38.74 percent with a p-value of 0.0135 and 0.0306 located on chromosome number 4 and 6 respectively. Low R² values indicates large environmental component of variations. It can be further exploited in hybridization programme with MAS for improving WUE and NUE traits of rice genotypes.

Keywords

Oryza sativa, F₂ population, Water and nitrogen use efficiency and marker validation

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Introduction

The emphasis on increasing rice grain yield of irrigated lowland rice when water and N are amply supplied will gradually diminish in importance as concerns mount about reduced water availability for irrigated rice and the environmental impact from N losses. The new

challenge is to sustain the needed increase in rice yields while reducing the requirements for water and N per unit of grain production. Newly developed crop management strategies have been very effective in maintaining rice yield with reduced input of water and N, resulting in great WUE and NUE. However, it is always more difficult for farmers to adopt a new crop management technology than

planting new varieties.

Validation of markers helps in determining the reliability and practical applicability of the markers in predicting the phenotype. The present investigations were carried out to map markers for Water Use Efficiency and Nitrogen Use Efficiency and related traits in mapping population obtained from two crosses *viz.*, BPT5204 x WAB450 and BPT5204 and Mysore mallige. With a view of identifying SSR markers associated with WUE, NUE and their related traits, 88 SSR primers were surveyed between the parents.

Materials and Methods

Raising F₂ generation

Seeds were collected from a single F₁ hybrid plant and F₂ generation (260 plants) was raised along with the parents in the field during *kharif* 2013 in F3-Block at Agricultural Research Station, Gangavati.

Parental polymorphism survey

Simple sequence repeats (SSR) markers flanking WUE and NUE traits reported to explain high percentage phenotypic variation for their respective traits in RILs population from a cross Swarna x WAB450 were used for validation in F₂ population individuals for the same traits for which they are already reported to be associated. DNA of the Swarna, WAB-450 (NERICA-L-20), Mysore Mallige and ADT-43 were extracted using the protocol described in Dellaporta *et al.*, (1983) and analyzed for polymorphism using Rice Microsatellite (RM) primers. A total of 88 RM primers were used to survey the parental polymorphism. The primers were chosen based on their distribution in the genome and earlier reports. Polymorphic primers were noted. A primer was considered polymorphic, if it amplified different band in WAB450 and Swarna.

Genotypic evaluation of mapping population

The selected genotypes along with available checks and parental lines were screened with 88 polymorphic Microsatellite markers to study their segregation pattern. The population was scored for the presence or absence of the WAB450 alleles in homozygous or heterozygous state.

Single Marker Analysis (SMA)

Single marker analysis was performed to tag and confirm potential SSR markers linked to the traits of WUE and NUE based on phenotypic and genotypic data pertaining to the F₂ populations, which is based on simple linear regression method (Haley and Knott, 1992).

Results and Discussion

Simple sequence repeats (SSR) markers flanking WUE and NUE traits reported to explain high percentage phenotypic variation for their respective traits in RILs population from a cross Swarna x WAB450 were used for validation in F₂ population individuals for the same traits for which they are already reported to be associated. The choice of male and female parent is critical in order to improve their special combining ability in selected crosses. Some studies have also warned of the danger of assuming that marker-QTL linkage will remain in different genetic backgrounds or in different testing environments, especially for complex traits (Rohlf, 1993). Even when a single gene controls a particular trait, there is no guarantee that DNA markers identified in one population will be useful in different populations, especially when the population originates from distinctly related germplasm (Rohlf, 1993).

Single marker analysis was done to with the

help of student's t test and by single factor ANOVA. Single marker analysis used for this study, though the simplest in detecting QTLs associated with single markers of the phenotypic data based on genotype at each marker position to test for the presence of one or more QTL have been reported to be adequate and efficient, giving rise to essentially similar results using marker intervals (Davarsi *et al.*, 1993). The results have been given in the below table. A few SSR markers have been found to be associated with WUE and NUE in different population background. In the present study, it has been found that SSR markers RM518 and RM224 are associated with Water Use Efficiency in F₂

mapping population obtained from the cross BPT5204 x WAB450. The per cent contribution of the significantly associated markers to total phenotypic variance was 48.26 and 23.52 per cent with a p-value of 0.0135 and 0.0306 respectively. RM225 and RM525 markers showed significant association with Nitrogen Use Efficiency in the background of F₂ population from a cross BPT5204 x Mysore Mallige. The SSR marker RM225 describes the phenotypic variance of 38.74 per with a p-value of 0.0218. The marker RM525 explained the phenotypic variance of 19.15 per cent with p-value of 0.0371.

Single Factor ANOVA

One-way ANOVA			
Trait	Locus	Probability	R ² Value
WUE	RM518	0.0135	48.26
WUE	RM224	0.0306	23.52
NUE	RM225	0.0218	38.74
NUE	RM525	0.0371	19.15

Validation of markers helps in determining the reliability and practical applicability of the markers in predicting the phenotype (Collard *et al.*, 2005). Among the ten markers chosen for validating across the population, only few were found to be significantly associated with WUE and NUE traits in F₂ populations of the crosses, BPT5204 x WAB450 and BPT5204 x Mysore Mallige respectively. This probably may be due to fact that these were detected in other mapping populations with different genetic background, and because the marker intervals may be quite large. Low R² values indicates large environmental component of variations.

Markers validation on independent populations of different genetic background is essential in determining the effectiveness and reliability of the markers to predict

phenotypic (Cakir *et al.*, 2003; Collins *et al.*, 2003; Koyamana *et al.*, 2001; Li *et al.*, 2001; Lin *et al.*, 1998 and Speilmeyer *et al.*, 2005) which indicates whether or not a marker could be used in routine screening for MAS (Prevost and Wilkinson 1999; Speilmeyer *et al.*, 2005). Markers should also be validated by testing for the presence of the markers on a range of cultivars and other important genotypes (Speilmeyer *et al.*, 2005; Tanksley, 1993). QTL analysis is based on the principle of detecting an association between phenotype and genotype of markers. Markers are used to partition the mapping population into different genotypic groups based on the presence or absence of a particular marker locus and to determine whether significant differences exist between groups with respect to the trait being measured (Young *et al.*, 1996; Lin *et al.*, 2000).

Future line of work

The SSR markers RM518, RM224 and RM225, RM525 are found to be associated with Water Use Efficiency and Nitrogen Use Efficiency which can be further exploited in hybridization programme with MAS for improving WUE and NUE traits of rice genotypes. It is necessary to identify more markers in the marker regions by further fine mapping for efficient marker assisted selection for root morphological, Water Use Efficiency and Nitrogen Use Efficiency traits.

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