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Analysis of F_2 Population of Intraspecific CC Genome *Oryza* by using SSR Marker

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Abstract

Of the 22 Oryza species, three are diploid CC genome species and six are allotetraploid species sharing the CC genome. The diploid CC genome species have useful genes such as those conferring resistance to brown plant hopper, white back plant hopper, bacterial blight and yellow dwarf virus disease. This work describes SSR marker analysis of progeny derived from crosses in *O. eichingeri* (CC genome). Twelve Microsatellites or Simple Sequence Repeats (SSR) markers have been used to analyze 82 F_2 progeny plants from an intraspecific cross, O. *eichingeri* (Uganda) × O. *eichingeri* (Sri Lanka). The markers were distributed on 8 of the 12 linkage groups. Most of the SSR markers had the expected distribution of plants segregating for the parental or heterozygous condition except for RM109 which was present in more individuals with the genotype of the parent from Sri Lanka than the heterozygous or Uganda parent. The F_2 population showed transgressive segregation for days to heading. Some characters had a normal distribution such as internode color and leaf size. Other characters showed segregation toward one of the parents viz. panicle type toward the Uganda parent and auricle color and leaf morphology toward the Sri Lankan parent.

Introduction

The genus *Oryza* consists of about 22 species. Of these three are diploid CC genome species and six are allotetraploid species sharing the CC genome. More species in the genus *Oryza* have the CC genome than any other *Oryza* genomes (AA genome eight species, BB genome four species, EE genome one species, FF genome one species, GG genome two species, HHJJ genome three species and HHKK genome one species). The CC genome is closely related to AA genome (Vaughan et al. 2003; Aggarawal et al. 1997). Thus the CC genome is central to understanding the genus *Oryza*.

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CC genome *Oryza* species are grouped in the *O. officinalis* complex. The *O. officinalis* complex consists of species with BB, CC, EE genomes and allotetraploid species with BBCC and CCDD genomes. The diploid CC genomes species such as *O. eichingeri* have useful genes such as resistance to brown plant hopper, white back plant hopper, bacterial blight and yellow dwarf virus disease (Brar and Khush 1997; Heinrichs 1985; Muniyappa and Raju 1981). Transfer of brown plant hopper resistance from *O. eichingeri* to *O. sativa* has been successful (Yan et al. 2001). Antifungal substances have been identified from leaves of *O. officinalis* (CC genome) that inhibit germination of blast fungus spores (Neto et al. 1981). Bacterial blight resistance found in *O. officinalis* is non-allelic for resistance to the same bacterial blight race found in *O. sativa* (Brar and Khush 1997). These examples suggest that the CC genome is a rich source of useful genes not found in the AA genome gene pool. However, the spectrum of useful genes in species with the CC genome is not known since they have not been broadly and systematically evaluated.

From the rice genome sequence available from the database, it is possible to design specific primers for desired sites. The public availability of the rice genome sequence has dramatically altered the strategies used for marker development and has removed many limitations to understanding diversity in *Oryza*. Specific PCR markers have been used to map genes and in marker assisted selection at a number of loci. Sequence Microsatellites or Simple Sequence Repeats (SSR) are tandem arrays of short nucleotide repeats with 1 - 5 bp (Wu and Tanksley 1993) and are widely dispersed in all eukaryotic genomes. Dinucleotide repeats $(AT/TA)_n$ and $(GA/CT)_n$ are commonly found in vascular plants. SSR are co-dominant, highly polymorphic, abundant and randomly distributed markers in genomes. SSR markers can be easily amplified by PCR and are probably selectively neutral (Akagi et al. 1997). The technical efficiency and multiplex potential of SSR markers make them useful for many forms of high throughput mapping, genetic analysis and marker assisted plant improvement strategies.

Microsatellites have been used for studies of parentage (Rao et al. 2000), genetic mapping and breeding (McCouch et al. 1997), gene flow, genetic diversity and population differentiation (Byrne et al. 1996; Cho et al. 2000). The present investigation was carried out for analysis of F_2 population of intraspecific CC genome *Oryza* by using SSR primer which will be helpful for gene mapping of CC genome of *Oryza.*

Materials and Methods

Two accessions of *O. eichinger*i Peter (W1519 from Uganda and SL6 from Sri Lanka) were selected to develop a CC genome mapping population. The hybrid

between these accessions was produced by Sanchez et al. (2004) at the National Institute of Agrobiological Science (NIAS), Tsukuba, Japan. A single crossed hybrid was selfed and F_2 seeds were collected. Eighty-two F_2 plants were grown in the greenhouse as a CC genome mapping population during 2004.

DNA extraction and SSR marker analysis: Total genomic DNA was isolated from the parental lines, F_1 and F_2 plants, using the BioRobot EZ1 and EZ1 DNA kits. 12 SSR markers were used for analysis of F_2 population. Their map locations, primer sequences are given in Table 1.

The PCR reactions were performed as described by Wu and Tanksley (1993). The polymorphism between W1519 and SL6 with respect to these markers was assessed following PCR conditions: a denature period of 2 min at 94°C followed by 35 cycles of 15s at 94°C, 30s at 55°C, 1 min at 68°C and 3 min at 68°C for final extension. Reactions were carried out in a volume of 10 µl**.**

The amplified primer PCR products were separated in 6% in polyacryalmide gel. The gels were stained with ethidium bromide (0.5 µg/ml) and photographed under UV light (Sambrook and Russel 2001) and the banding patterns were recorded.

Results and Discussion

Twelve SSR markers were used to genotype 82 F_2 plants from an intraspecific cross between *O. eichingeri* (Uganda) and *O. eichingeri* (Sri Lanka). These were distributed on eight of the 12 linkage groups (based on the genome map for rice) (Table 1) (McCouch et al. 2002)). SSR band pattern of intraspecific CC genome hybrids and the F_2 population are shown in Fig. 1. This figures shows that 22 F_2 progeny have P_1 like band (e.g. lane 2 arrow). On the other hand 15 F_2 progeny show P_2 like band (e.g. lane 1). The rest 45 show a heterozygous distribution of bands (e.g. lane 5). The extra hand in heterozygote is probably not due to SSR allelic diversity and was ignored. Based on SSR banding pattern it was found that most of the SSR markers had the expected distribution of plants segregating for the parental or heterozygous condition except for RM109 that had more individuals with the genotype of the parent from Sri Lanka than heterozygous for Uganda parent (Fig. 2).

The F_2 population showed transgressive segregation for days to heading. Ten plants flowered earlier and another 13 later than the two parents (Fig. 3). Segregation for other characters is shown in Figs. 4 - 8. The results revealed various segregation patterns for the characters analyzed. Some characters had a normal distribution such as the internode colour (Fig. 5) and leaf size (Fig. 7). Other characters showed segregation towards one of the parents like panicle type (Fig. 4) towards the Uganda parent and auricle color (Fig. 6) and leaf morphology (Fig. 8) towards the Sri Lankan parent. Similar segregation pattern

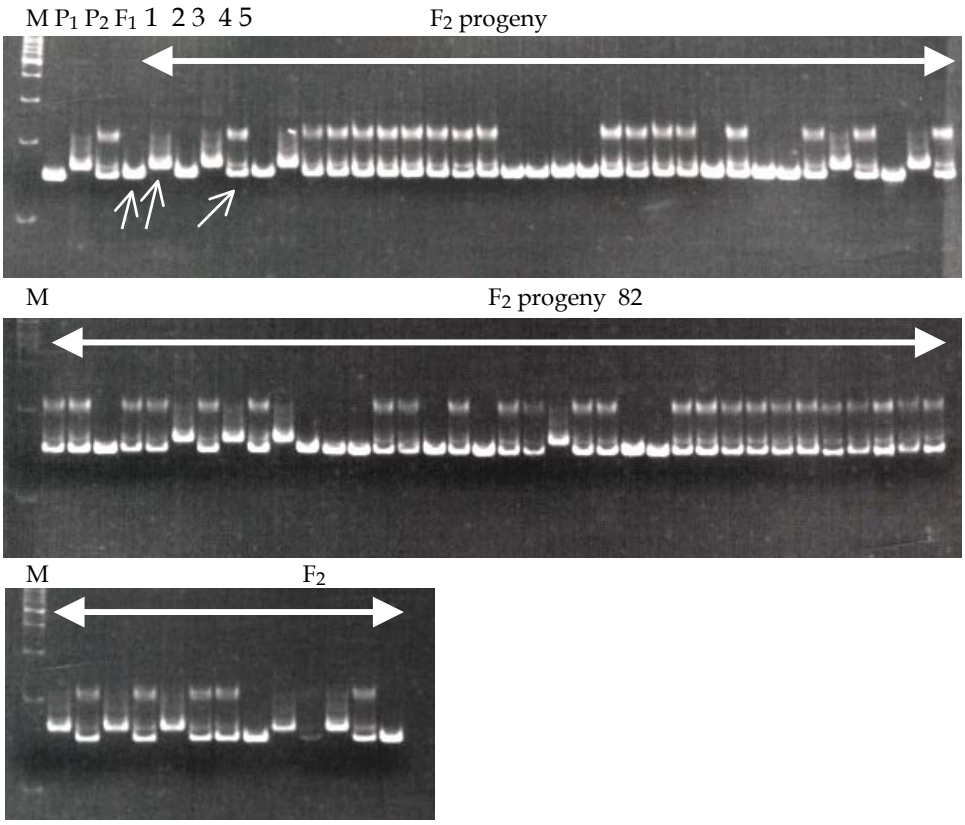


Fig 1. SSR band pattern of intraspecific CC genome hybrids and the F_2 population by primer RM 283 (M = 100 bp marker, P_1 = W1915 and P_2 = SL6).

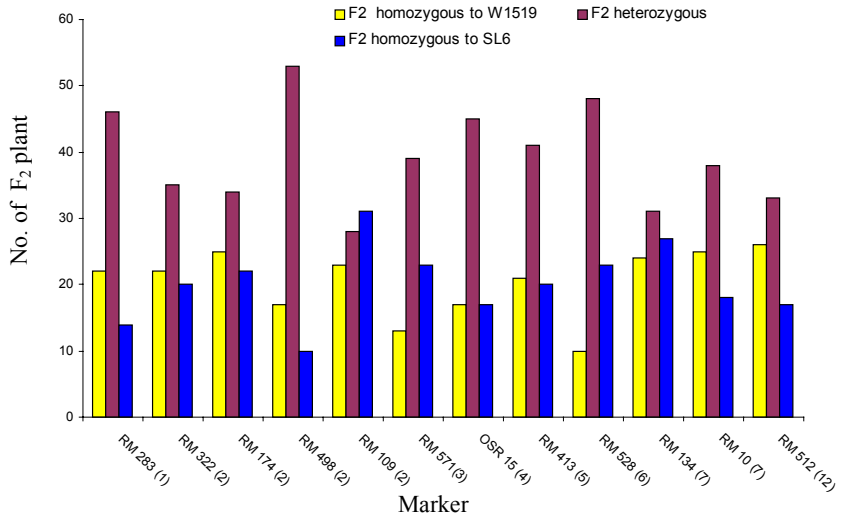
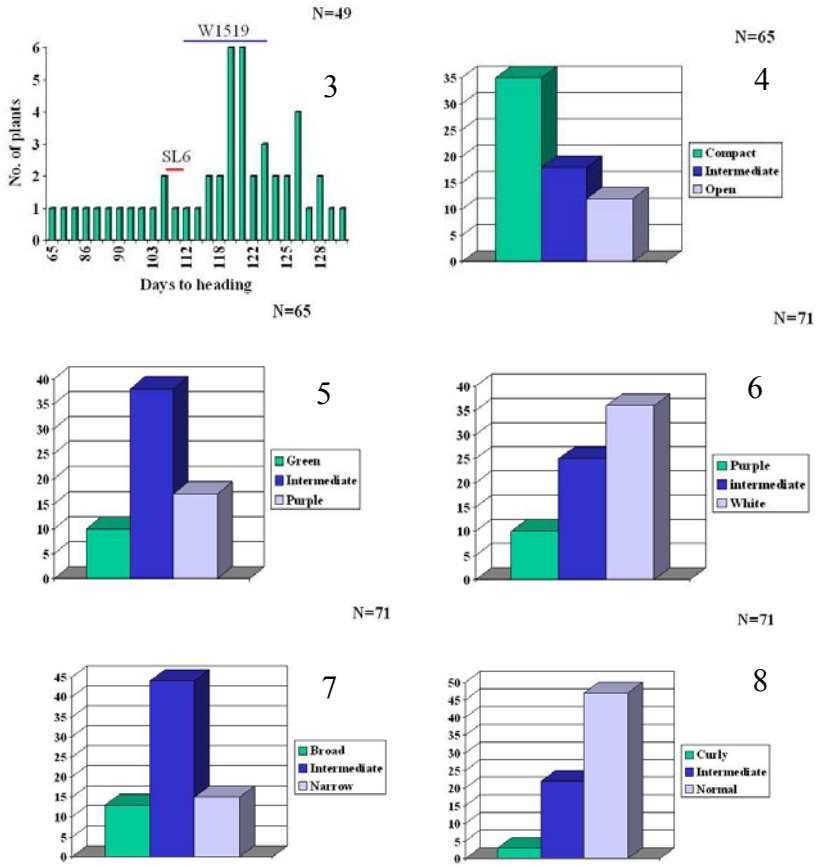


Fig. 2. Distribution pattern of F_2 population in different SSR marker.

Table 1. SSR primer pairs used for CC genome *Oryza* F_2 population DNA amplification study.

Primer code	Location on chrom. No.	SSR motif	No. of alleles	Size length
RM 283	1	(GA)18	3	147 - 155
RM 498	2	(AG)12	4	211 - 219
RM 109	2	(AG)16	6	89 - 101
RM 174	2	(AGG)7 (GA)10	7	207 - 222
RM 322	2	(CAT)7	2	106 - 112
RM 571	3	(GT)11 (AG)13	5	182 - 194
OSR 15	4			
RM 413	5	(AG)11	6	69 - 105
RM 528	6	(GA)17	5	232 - 260
RM 10	7	(GA)15	5	159 - 177
RM 134	7	(CCA)	2	84 - 93
RM 512	12	(TTTA)5	3	214 - 218



Figs. 3 - 8: 3. Segregation in days to heading in F_2 mapping population. 4. Segregation in panicle type in F_2 mapping population. 5. Segregation in internode colour in F_2 mapping population. 6. Segregation in auricle colour in F_2 mapping population. 7. Segregation in leaf size in F_2 mapping population. 8. Segregation in leaf morphology in F_2 mapping population.

has been reported in mapping population of rice, corn and barley (Mc Couch et al. 1987; Helentjaris et al. 1986 and Heun et al. 1991). The trait internode colour and leaf size showed monogenic pattern of inheritance where purple colour was dominant over green colour of internode and broad leaf shape was dominant over narrow leaf shape. In both the cases non-significant χ^2 value ranged from 0.046 to 0.690 at 5 per cent level of probability. The results obtained will be incorporated with others to enable the marker linked to resistance or interesting characters or the CC genome of *Oryza* to be compared with the genome map of rice.

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