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RESEARCH ARTICLE

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Correlation between allele sizes of microsatellites and phenotypic variations in rice landraces

Abstract Yunnan is one of the largest centers of genetic diversity in the world. Allele size of microsatellites associated with phenotypic traits of rice landraces in Yunnan, Southwest China, was investigated based on 20 SSR markers and 23 phenotypic traits, as well as eight mineral elements in brown rice within the core collection of 629 accessions; and there was a significant correlation for 182 ($r = 0.083^{*} - 0.438^{**}$) of 620 pairs among these markers and traits, as well as elements. Surprisingly, there was a significant correlation for 94 of 180 pairs between the allele size of microsatellites and grain traits, and 48 of 160 pairs between allele size of microsatellites and panicle traits. In these rice landraces, 309 alleles were detected, with an average of 15.5 alleles per marker, ranging from 5 (RM60) to 40 (RM257). There was a significant correlation between the allele size of 20 SSR markers and some phenotypic traits, such as the significant correlation of 17 ($r = -0.085^{*} - 0.438^{**}$) pairs between the allele size of RM224 and 23 phenotypic traits, as well as eight elements. The allele size of microsatellites was more associated with grain or panicle traits than that of plant traits or element contents in brown rice. Grain length/width ratio and 1–2 internode length, as *indica-japonica* classification traits, in which two traits were closely associated with the allele size of 14 SSR markers ranging from 0.089^{*} to -0.438^{**}. Therefore, allele size of SSRs was associated with phenotypic traits (especially in grain traits), as well as elemental contents in brown rice.

Keywords allele size of microsatellite, phenotypic traits, mineral elements, correlation, rice landraces

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1 Introduction

Rice (*Oryza sativa* L.) is not only the most important food crop and staple food for 50 percent of the world population, but also it is a model for monocotyledonous plants. Phenotypic traits, especially quantitative ones, are among the most important and least understood traits. Genetic variability of the simple sequence repeats (SSRs) markers is valuable for understanding the phenotypic traits, including fingerprinting genotypes, analyzing genetic diversity, determining variety identity, marker-assisted breeding, phylogenetic analysis, and map-based cloning of genes (Shen et al., 2004), as well as key component of any breeding program for broadening the gene pool of rice. Expansion of a (GA) dinucleotide at a microsatellite locus associated with domestication of rice (Ramakrishna et al., 1998). So far, 2740 SSR markers have been genetically mapped in rice, which amounts to one SSR marker every 157 kb (McCouch et al., 2002). Microsatellites are associated with several genes that control agronomic traits. For example, the allele of RM5 marker on chromosome 1 in *O. rufipogon* was associated with an 18% increase in grain yield per plant (Xiao et al., 1996), and RM5303 was linked with a low silicon rice (*Lsi1*) gene (Ma et al., 2006). Historically, SSRs were used as genetic markers but were functionally unimportant. However, recent research has

shown that SSRs have many important functions in terms of development, gene regulation, and evolution (Lawson and Zhang, 2006). SSRs within genes can be subjected to stronger selective pressure than other genomic regions because of their functional importance (Li et al., 2004). Functional markers are derived from polymorphic sites with gene involved in phenotypic trait variation (Andersen and Lübberstedt, 2003). The distribution of SSRs appears highly nonrandom and varies a great deal in different regions of the genes in the genomes (Lawson and Zhang, 2006). To date, there exist 16 genes cloned by Chinese scientists and 33 genes fine-mapped ones (Jiang et al., 2007). *Ghd7*, encoding a CCT domain protein, isolated from an elite rice hybrid, has major effects on an array of traits in rice, including number of grains per panicle, plant height, and heading date (Xue et al., 2008).

Yunnan is not only one of the largest centers of genetic ecological diversity in the world (Zeng et al., 2003; Zhang et al., 2006) and is the center of origin of Asian *O. sativa* (Zeng et al., 2007), but also the genetic differentiation center of *indica* and *japonica* subspecies of Asian cultivated rice (Zeng et al., 2007). It is located in the center of the genetic diversity of Asian cultivated rice—an area that encompasses southwest China, Nepal, Bhutan, Northeast India, Myanmar, Laos, and Northern Thailand (Chang, 1976; Gao et al., 2002; Zeng et al., 2003). In the present study, we examined the allele size of 20 SSR markers and the content of 8 mineral elements and 23 phenotypic traits using 629 accessions of core collection for rice landraces from Yunnan, Southwest China. Our objectives were to reveal the allele size of SSRs associated with mineral elemental contents in brown rice and phenotypic traits, especially in grain traits, aid to QTL mapping and provide useful information for study on possible SSR functions, as well as the reference evidence of markers associated for seeking the relationship between microsatellites and phenotypic traits in rice.

2 Materials and methods

2.1 Plant materials

The 629 accessions from core collection of Yunnan rice landraces came from 100 counties in 16 prefectures in Yunnan Province. The collection was established in our laboratory. These accessions represented over 90% phenotypic diversity of 6121 accessions in Yunnan rice landraces (Zeng et al., 2007).

2.2 Morphological traits

Field experiment was conducted on a farm in Xinping (24°N, 102°E, elevation of 500 m above sea level) county of Yunnan Province. All materials were sown in midseason (April–August) of double-cropping rice zone characterized by high temperature. Five-row plots were planted with 20 plants per row with a spacing of 10 cm × 20 cm. Twenty-three phenotypic traits including six plant traits (plant height, tillers per plant, panicles per plant, days to heading, flagleaf length and width), nine grain traits (1000-grain weight, grain length and width, grain length/width ratio, grain thickness, rice length and width, rice thickness, and shattering), eight panicle traits (panicle length, 1–2 internode length, awn length, filled grains per panicle, blighted grains per panicle, total grains per panicle, seed setting rate, and grain density) were examined at appropriate stages during plant growth. Days to heading for each accession was recorded when the first panicle emerged the sheath of flagleaf, and other 22 phenotypic values of each accession were represented by the mean values from 10 individual observations according to 'Characters to be studied and standard for rating of rice genetic resource' (Institute of Crop Germplasm Research of Chinese Academy of Agricultural Science, 1986, unpublished literature).

2.3 Mineral element content

The experimental plots were established on red mudstone lowland rice soil. Soil analysis showed average pH 5.53, organic matter 2.96 %, available phosphorus 10.08 $mg \cdot kg^{-1}$, available nitrogen 108.47 $mg \cdot kg^{-1}$, available potassium 61.42 $mg \cdot kg^{-1}$, available calcium 1505 $mg \cdot kg^{-1}$, and available magnesium 310 $mg \cdot kg^{-1}$. Total P, N, and K content were 0.053%, 0.176%, and 1.99%, respectively. Average available micronutrient level of Cu, Zn, Fe, and Mg in the soil was 3.80 $mg \cdot kg^{-1}$, 1.45 $mg \cdot kg^{-1}$, 297 $mg \cdot kg^{-1}$, and 22.75 $mg \cdot kg^{-1}$, respectively.

The concentrations of P, K, Ca, Mg, Fe, Zn, Cu, and Mg in 629 rice seeds harvested from the experimental plots were tested with two replications in Supervision and Testing Center for Farm Products Quality, Ministry of Agriculture of the People's Republic of China. A 0.5 g sample of each brown rice was precisely weighed and put into a beaker with 5 mL of nitric acid and 1 mL of perchloric acid, which was heated to nitrify and decompose rice sample until the solution became clear. The clear solution was continuously heated and evaporated to dryness. The residue was dissolved with 5 mL of 1 $mol \cdot L^{-1}$ hydrochloric acid and transferred to a 50-mL graduated bottle. The sample was reduced to ashes at high temperature or decomposed with warm acid decomposition. The residue was dissolved in acid solution in order to transform the elements into inorganic ion. This solution was diluted and analyzed with an ICP-AES spectrometer (Zeng et al., 2008)

2.4 Microsatellites

Twenty SSR markers covering all 12 chromosomes were analyzed on the basis of the published rice microsatellite

framework map. The repeat motifs, primer sequences, and chromosomal position for these markers could be found in the RiceGenes database (http://www.gramene.org/microsat/RMprimers.html). Microsatellite primer pairs were obtained from Seagon Bioengineer Limited Company, China. The 30-day seedlings of each accession were used to extract the genomic DNA with a modified 1% CTAB method (Doyle and Doyle, 1990). The PCR amplifying procedure was that of Panaud et al. (1996) with slight modification and subsequently run on 8% denatured polyacrylamide gel at 70 W. For the same marker, all runs after the first run included not only the samples but also the checks and a standard molecular weight marker-PUC19 DNA digested by *Msp*I. When all the samples were run completely, checks were run with another standard molecular weight marker 10 bp DNA ladder from Invitrogen, and the molecular weight for each allele was estimated. All gels were stained with silver method (Bassam et al., 1991). In the case of null alleles in these species, PCR amplification was repeated to exclude failed PCR reaction. More than two alleles per locus occurred for some accessions. In such cases, we amplified and run them again and selected the alleles that stably occurred in the two replications.

2.5 Statistical methods

The mean of two replications for each mineral element concentration in brown rice and each phenotypic value per accession based on 10 individual observations were calculated. Given that some accession showed its heterozygosity at a certain SSR locus, the molecular weight for that SSR marker in that accession was represented by the mean of two allele size. Correlation was determined by applying Pearson's method; Pearson correlation coefficient was measured between the molecular weight of 20 SSR markers and eight mineral elements, as well as 23 measured phenotypic traits of 629 accessions. Statistical significance was defined as $P < 0.05$. Statistical analysis was performed on a personal computer using SPSS software. All analyses were performed using SPSS 10.5 software (SPSS, Inc., Chicago, USA).

3 Results

3.1 Number and size of alleles of microsatellites

Allele size of microsatellites and its linked traits are shown in Table 1. There was very high polymorphism distributed over 12 chromosomes. A total of 309 alleles of 20 SSR markers for 629 accessions in Yunnan rice landraces were detected, and the number of alleles per marker was RM257(40) > RM247(28) > RM253(22) > RM241 (21) > RM224(18) = RM235 > RM263(17) = RM234 = RM223 > RM255(15) > RM258(13) > RM81A(12) = RM225 = RM18 > RM5(11) = RM249 > RM244 (9) > RM221(8) > RM60(5), ranging from 5 to 40, with an average of 15.5 alleles per locus. This is distinctly higher than 5.8 alleles per locus reported so far (http://www.gramene.org/microsat/microsats.txt). The overall size of PCR products amplified using 20 SSR primer pairs ranged from 95–137 bp (RM235) to 123–210 bp (RM257) (Table 1). The molecular size difference between the smallest and the largest allele for a given SSR locus varied from 8 bp (RM60) to 88 bp (RM253).

3.2 Allele size of microsatellites and mineral element content in brown rice

Correlation analysis indicated that there was a significant correlation only in 23 ($r = 0.083^{*} - 0.144^{**}$) of 160 pair traits between the allele size of microsatellites and mineral content (Table 2). P content in brown rice showed a significant correlation with the allele size of RM81A, RM253, RM232, RM234, and RM244, comparatively, indicating that P content was tightly associated with panicle and grain traits. K content was significantly correlated with the allele size of RM235, RM253, RM225, RM5, RM81A, and RM244, indicating that K content was associated with yield traits (Zeng et al., 2005). Ca content was significantly correlated with the allele size of RM247 and RM255 and thus tightly associated with plant and grains traits. Mg content was significantly correlated with the allele size of RM225, RM244, and RM18, indicating its tight association with yield traits (Zeng et al., 2005). Fe content was significantly associated with the allele size of RM225, which was linked with nitrogen use efficiency, but Zn content had no correlation with any of the 20 SSR markers. Cu content was significantly associated with the allele size of RM81A, RM253, RM60, and RM247. Mn content was correlated with the allele size of RM60 and RM225.

3.3 Allele size of microsatellites and plant traits

Correlation analysis indicated that there was a significant correlation only in 17 ($r = 0.084^{*} - 0.200^{**}$) of 120 pair traits between allele size of microsatellites and plant traits (Table 3). Plant height displayed a significant correlation with the allele size of RM224, RM225, RM60, and RM263. Tillers per plant had a significant correlation with the allele size of RM257, RM18, RM5, RM224, and RM247, among which RM18 and RM224 were also linked with tillers per plant. Panicles per plant displayed significant correlations with the allele size of RM258 related with spikelet fertility (Cui et al., 2004). Days to heading displayed a significant correlation with the allele size of RM223 ($r = 0.200^{**}$), RM232 (0.143^{**}), RM224 (-0.113^{**}), and RM60 (-0.084^{*}), of which RM60 was also linked with days to heading (Thomson et al., 2003). Flagleaf length displayed significant correlations with the allele sizes of RM257 and RM224.

Table 1 Allele size of microsatellites in Yunnan rice landraces and its linking gene or traits

markers	629 accessions		repeat motif*	linking traits of microsatellites from *http://www.shigen.nig.ac.jp/rice/oryzabase/update/html*	main references
	no. allele	size of allele/bp			
RM5	11	103–121	$(GA)_{14}$	yield-enhancing, grains per panicle, percent germination, head rice grain, grain length/width ratio	Xiao et al., 1996; Thomson et al., 2003; Liang et al., 2004
RM81A	12	101–120	$(TCT)_{10}$	grain yield per plant, 1000-grain weight, plant height	Cui et al., 2004
RM211	8	142–162	$(TC)_4T_3C_3(TC)(CT)_2$	yield-improving, grain width, days to heading	Miyata et al., 2007
RM263	17	149–197	$(CT)_{34}$	broken rice grain, crushed rice grain, 1000-grain weight, spotted leaf	Septiningsih et al., 2003; Xu et al., 2004
RM60	5	160–168	$(AATT)_5AATCT(AATT)$	high-tillering dwarf 1, days to heading, shattering	Thomson et al., 2003; Septiningsih et al., 2003
RM232	15	137–164	$(CT)_{24}$	1000-grain weight, panicles per plant, panicle length, grains per panicle, grain yield per plant, cracked grain	Tan et al., 2007; Yoshida et al., 2002
RM241	21	111–148	$(GA)_{31}$	Panicles per plant, plant height	Thomson et al., 2003; Cui et al., 2004
RM255	15	112–159	$(AGG)_5(AG)_2(GA)_{16}$	1000-grain weight, panicle size, panicles per plant, panicle neck diameter	Xu et al., 2004; Yoshida et al., 2002
RM249	11	121–166	$(AG)_5A_2(AG)_{14}$	tillers per plant, panicles per plant, grains number per plant, grain thickness, 1000-grain weight, panicle length	Pradeep et al., 2005; Miyata et al., 2007; Abdelkhalik et al., 2005
RM225	12	124–151	$(CT)_{18}$	nitrogen use efficiency, grain breadth	http://www.cropscience.org.au/icsc2004/poster/3/4/1/1296_senthilvels.htm
RM253	22	98–187	$(GA)_{25}$	percent head rice, amylase content, alkali spreading score, protein content, grain length, grain length /width ratio, days to maturity, heading date, awn length	Xiao et al., 1996; Linh et al., 2006
RM18	12	150–172	$(GA)_4AA(GA)(AG)_{16}$	frizzle panicle, grain yield, tillers per plant, grains per panicle, heading date	Duan et al., 2003
RM234	17	110–170	$(CT)_{25}$	days to heading, days to maturity, maximum root length	Thomson et al., 2003; Miyata et al., 2007
RM223	17	137–170	$(CT)_{20}$	panicle per plant, days to heading, panicle length;	Pradeep et al., 2005; Miyata et al., 2007
RM257	40	123–210	$(CT)_{24}$	cold resistance, heading date	Miyata et al., 2007
RM244	9	149–164	$(CT)_4(CG)_3C(CT)_6$	fertility restorer genes	Jing et al., 2000
RM258	13	131–152	$(GA)_{21}(GGA)_3$	spikelet fertility, 1000-grain weight, tiller number, heading date	Cui et al., 2004; Miyata et al., 2007
RM224	18	124–162	$(AAG)_8(AG)_{13}$	plant height, tillers per plant, panicle length, root-shoot ratio	Miyata et al., 2007
RM235	18	95–137	$(CT)_{24}$	panicles per plant	Thomson et al., 2003
RM247	28	124–184	$(CT)_{16}$	phosphorus deficiency, plant height	Thomson et al., 2003

Note: *http://www.gramene.org/microsat/ssr.html.

Table 2 Correlation coefficients between allele size of microsatellites and mineral concentration contents

markers	P	K	Ca	Mg	Fe	Zn	Cu	Mn
RM5	−0.011	0.087*	0.080	−0.022	−0.017	−0.001	0.036	−0.013
RM81A	−0.144**	−0.086*	−0.014	0.003	0.003	0.076	−0.129**	0.033
RM211	−0.049	0.002	−0.043	0.015	−0.026	0.008	0.036	0.029
RM263	0.002	0.001	−0.058	0.035	0.001	0.064	0.076	0.042
RM60	0.012	−0.028	0.046	0.024	0.042	−0.061	−0.096*	−0.091*
RM232	0.101*	−0.018	0.007	0.042	−0.015	0.006	0.001	−0.002
RM241	0.001	0.006	−0.047	−0.012	−0.056	0.007	0.012	0.046
RM255	0.060	0.074	0.091*	0.047	−0.005	0.023	−0.023	0.051
RM249	−0.041	0.036	0.018	−0.022	−0.051	−0.037	−0.062	−0.066
RM225	−0.075	−0.087*	−0.059	−0.101*	−0.083*	0.037	−0.027	−0.093*
RM253	−0.104*	−0.092*	−0.009	−0.063	0.024	0.015	−0.103*	0.002
RM18	0.035	−0.021	0.025	0.088*	−0.013	0.017	−0.013	0.033
RM234	0.096*	−0.026	0.042	−0.020	0.007	−0.006	−0.047	0.014
RM223	0.019	0.052	0.044	0.019	−0.009	0.002	0.027	−0.025
RM257	−0.002	0.037	−0.052	−0.010	−0.020	0.028	−0.024	−0.046
RM244	−0.092*	−0.085*	0.016	−0.091*	−0.030	−0.002	−0.065	−0.011
RM258	0.076	−0.036	−0.031	0.020	−0.012	0.039	0.028	−0.004
RM224	−0.034	0.006	−0.024	−0.034	0.009	0.033	0.042	−0.034
RM235	0.053	0.110**	−0.007	0.058	0.024	0.036	−0.042	0.047
RM247	0.029	0.059	0.101*	0.047	0.051	0.010	−0.084*	0.041

Note: * and ** represent significant correlation at 5% ($r_{0.05}$ = 0.0813) and at 1% ($r_{0.01}$ = 0.1062), respectively (n = 629).

Table 3 Correlation coefficients between allele size of microsatellites and plant traits

markers	chromosome	plant height	tillers per plant	panicles per plant	days to heading	flagleaf length	flagleaf width
RM5	1	−0.019	−0.089*	0.037	0.054	0.037	0.064
RM81A	1	0.042	0.059	0.057	0.013	0.137	−0.047
RM211	2	−0.052	−0.023	0.012	−0.019	−0.001	0.012
RM263	2	−0.085*	−0.028	−0.015	−0.036	−0.049	−0.098*
RM60	3	−0.109**	−0.002	0.022	−0.084*	0.058	−0.020
RM232	3	0.057	0.043	0.066	0.143**	0.070	−0.043
RM241	4	0.039	−0.070	0.058	0.078	0.021	0.006
RM255	4	0.029	0.070	0.042	0.071	0.065	0.009
RM249	5	0.044	0.033	−0.100*	0.0423	0.047	0.005
RM225	6	−0.140**	−0.015	−0.001	−0.0275	−0.031	0.042
RM253	6	0.012	−0.028	0.077	0.0641	0.033	−0.010
RM18	7	0.045	0.095*	0.055	0.0275	0.068	−0.078
RM234	7	0.022	−0.022	−0.077	−0.0104	0.037	0.043
RM223	8	0.037	−0.008	−0.068	0.2001**	0.052	−0.053
RM257	9	0.028	0.126**	0.006	0.0417	0.138**	0.055
RM244	10	0.042	0.076	0.053	0.0501	0.058	0.008
RM258	10	−0.039	−0.026	−0.108**	0.0114	−0.043	0.008
RM224	11	−0.151**	−0.085*	−0.042	−0.1134**	−0.086*	0.021
RM235	12	0.023	−0.052	0.044	0.0228	−0.032	−0.048
RM247	12	−0.006	0.082*	0.049	−0.0140	0.047	−0.012

Note: * and ** represent significant correlation at 5% ($r_{0.05}$ = 0.0813) and at 1% ($r_{0.01}$ = 0.1062), respectively (n = 629).

3.4 Allele size of microsatellites and grain traits

Correlation analysis indicated that there was a significant correlation in 94 ($r = -0.085^{*} - 0.424^{**}$) of 180 pair traits between allele size of microsatellites and grain traits (Table 4). 1000-grain weight displayed a significant correlation with the allele size of six SSR markers ranging from 0.089^{*} (RM5) to -0.190^{**} (RM224). RM258 (-0.151^{**}) was also linked with the 1000-grain weight. There was also a significant correlation in 27 ($r = 0.089^{*} - 0.424^{**}$) of 40 pair traits among allele size of microsatellites and grain size (length and width), among which RM5 and RM211 were also associated with grain size and yield improvement (Xiao et al., 1996). Grain length/width ratio was one of *indica-japonica* classification traits (Zeng et al., 2000), and it had a significant correlation with the allele size of 14 SSR markers ranging from 0.089^{*} (RM249) to -0.420^{**} (RM234). RM5 was linked with grain length/width ratio, and three SSR markers (RM18, RM247 and RM258) were associated with subspecies differentiating DNA markers (Cheng et al., 2004). There was a significant correlation in 30 ($r = -0.087^{*} - 0.349^{**}$) of 60 pair traits among allele size of microsatellites and rice size (length, width and thickness).

3.5 Allele size of microsatellites and panicle traits

Correlation analysis indicated that there was a significant correlation in 48 ($r = 0.082^{*} - 0.438^{**}$) of 160 pair traits between the allele size of microsatellites and panicle traits (Table 5). The 1–2 internode length, as one of *indica-japonica* classification traits (Zeng et al., 2000), displayed a significant correlation with the allele size of 14 SSR markers ranging from 0.094^{*} (RM249) to -0.438^{**} (RM234), and three SSR markers (RM18, RM247, and RM258) associated with subspecies differentiating DNA markers (Cheng et al., 2004). There was a significant correlation in 16 ($r = -0.082^{*} - 0.349^{**}$) of 80 pair traits among the allele size of microsatellites, seed setting rate, and panicle composition traits (filled grains per panicle, blighted grains per panicle, and total grains per panicle), among which four seed setting traits were significantly correlated with the allele size of RM225 which in turn was linked with nitrogen use efficiency. Seed setting rate was significantly correlated with the allele size of RM257 associated with cold tolerance.

4 Discussion

4.1 Size of alleles for SSR markers associated with elemental contents

The overall size of PCR products amplified using 20 markers for 629 accessions in Yunnan rice landraces ranged from 95–137 bp (RM235) to 123–210 bp (RM257) (Table 1). Mineral elements played an important role in forming genetic diversity (Zeng et al., 2006). Twenty three pairs ($r = 0.083^{*} - 0.200^{**}$) of 160 pairs between the allele size and mineral element contents, such as P, Cu, and K content in brown rice displayed a significant correlation with the allele size (molecular weight) of RM81A and RM253, and meanwhile, P content was associated with allele size of other markers (RM232, RM244, and RM234), and K content associated with allele size of other markers (RM5, RM255, RM244, and RM235). Recently, there are some evidences for QTL linked to the three SSR markers (RM81A, RM253, and RM244) associated with morphological traits of plants. RM81A is linked with grain yield per plant, 1000-grain weight, and plant height (Cui et al., 2004), RM253 is associated with percent head rice, amylase content, alkali spreading score, protein content, grain length, heading date, awn length, days to maturity and grain length/width ratio (Xiao et al., 1996; Linh et al., 2006), with RM244 associated with fertility restorer genes (Jing et al., 2000), RM232 associated with 1000-grain weight, panicles per plant, panicle length, grains per panicle, and grain yield per plant (Tan et al., 2007), and RM234 associated with days to heading, days to maturity and maximum root length (Thomson et al., 2003; Miyata et al., 2007). There existed significant correlations ($P < 0.01$) between P content in brown rice and 11 traits (protein content, rice width, panicle length, filled grains per panicle, blighted grains per panicle, seed setting rate, plant height, tillers per plant, days to heading, grain width, and thickness) and between K content in brown rice and 8 traits (amylose contents, alkali spreading score, gel consistency, protein content, plant height, tillers per plant, grain thickness, and panicle length) (Zeng et al., 2005). The genetic diversity investigated based on eight mineral contents in brown rice from Lincang, Simao, Xishuangbanna, and Dehong Prefectures has the similar trend with the genetic diversity of rice landraces of Yunnan revealed based on morphological traits and isozyme and SSR markers (Zeng et al., 2006). Therefore, we suggest that these eight elemental concentrations of P, K, Ca, Mg, Fe, Zn, Cu, and Mn in brown rice are associated with the allele size of rice landraces in Yunnan Province, and further understanding the relationship of mineral elements associated with origin of life is needed. As far as we know, this is the first report on genotypic variations in the eight elemental concentrations in brown rice associated with the allele size of SSR markers.

4.2 Allele size of SSR markers associated with phenotypic traits

Seventeen pairs ($r = 0.083^{*} - 0.144^{**}$) of 120 pairs between the allele size and plant traits, such as some markers associated with plant height (RM263, RM60, RM225 and

Table 4 Correlation coefficients between allele size of microsatellites and grains traits

markers	1000-grain weight	grain length	grain width	grain length /width ratio	grain thickness	rice length	rice width	rice thickness	shattering
RM5	−0.089*	0.273**	−0.195**	0.294**	−0.130**	0.148**	−0.220**	−0.133**	−0.134**
RM81A	0.059	0.189**	−0.186**	0.233**	−0.029	0.010	−0.194**	−0.209**	−0.187**
RM211	−0.080	−0.121**	0.101*	−0.134**	0.025	−0.134**	0.079	−0.032	0.092*
RM263	−0.183**	−0.287**	0.127**	−0.246**	0.077	−0.224**	0.194**	0.052	0.070
RM60	−0.079	−0.045	−0.068	0.019	0.016	−0.078	−0.025	−0.024	−0.010
RM232	−0.028	−0.020	−0.053	0.027	−0.045	0.025	−0.048	−0.026	−0.045
RM241	0.001	0.064	−0.089*	0.089*	−0.041	0.050	−0.067	−0.066	−0.013
RM255	0.057	0.180**	−0.162**	0.212**	−0.125**	0.073	−0.178**	−0.134**	−0.089*
RM249	−0.008	0.041	−0.056	0.066	0.024	−0.023	−0.069	−0.047	−0.092*
RM225	−0.141**	−0.155**	0.103*	−0.174**	0.025	−0.126**	0.124**	0.028	−0.011
RM253	−0.069	−0.056	−0.001	−0.035	0.011	−0.087*	0.035	−0.019	0.030
RM18	0.046	0.198**	−0.148**	0.221**	−0.079	0.148**	−0.166**	−0.028	−0.051
RM234	−0.073	−0.079	0.014	−0.060	0.074	−0.079	0.068	0.006	0.090*
RM223	0.013	−0.032	0.037	−0.026	−0.012	0.012	0.055	0.033	−0.012
RM257	0.009	0.214**	−0.239**	0.291**	−0.144**	0.138**	−0.241**	−0.151**	0.085*
RM244	0.037	0.210**	−0.159**	0.230**	−0.142**	0.116**	−0.159**	−0.109**	−0.135**
RM258	−0.151**	−0.226**	0.135**	−0.216**	−0.032	−0.152**	0.160**	0.049	0.067
RM224	−0.190**	−0.424**	0.242**	−0.420**	0.173**	−0.349**	0.348**	0.189**	0.113**
RM235	−0.027	−0.129**	0.236**	−0.239**	0.143**	−0.052	0.209**	0.167**	0.086*
RM247	0.027	0.244**	−0.188**	0.265**	−0.089*	0.176**	−0.190**	−0.087*	−0.068

Note: * and ** represent significant correlation at 5% ($r_{0.05}$ = 0.0813) and at 1% ($r_{0.01}$ = 0.1062), respectively (n = 629).

Table 5 Correlation coefficients between allele size of microsatellites and panicle traits

markers	1–2 internode length	panicle length	awn length	filled grains per panicle	blighted grains per panicle	total grains per panicle	seed setting rate	grain density
RM5	−0.251**	−0.013	−0.055	−0.039	0.022	−0.035	−0.013	−0.029
RM81A	−0.369**	0.098*	−0.130**	0.005	0.045	0.055	−0.029	0.010
RM211	0.156**	−0.021	0.041	−0.114**	0.129**	0.019	−0.120**	0.010
RM263	0.298**	−0.045	0.061	−0.035	0.088*	0.050	−0.035	0.085*
RM60	−0.016	−0.013	0.019	−0.057	0.016	−0.075	−0.047	−0.019
RM232	0.017	0.039	−0.020	−0.009	0.070	0.015	−0.061	0.049
RM241	−0.049	−0.001	−0.025	−0.016	−0.043	−0.003	0.042	−0.057
RM255	−0.262**	0.036	−0.093*	0.029	−0.009	0.022	0.039	0.009
RM249	−0.074	0.038	0.009	−0.009	−0.015	−0.010	0.054	−0.025
RM225	0.113**	−0.035	0.046	−0.158**	0.133**	0.044	−0.149**	0.006
RM253	0.006	0.035	−0.039	0.022	0.045	0.042	−0.027	0.036
RM18	−0.259**	−0.011	−0.036	0.015	−0.066	−0.025	−0.082*	−0.057
RM234	0.052	0.020	0.061	−0.036	0.039	0.043	−0.028	−0.004
RM223	0.094*	0.100*	0.059	−0.074	0.088*	−0.005	−0.110**	−0.031
RM257	−0.285**	0.036	−0.111**	0.046	−0.042	0.060	0.090*	−0.023
RM244	−0.293**	0.032	−0.055	0.062	−0.041	0.033	0.046	0.023
RM258	0.279**	0.003	0.130**	−0.054	0.099*	−0.072	−0.087*	0.044
RM224	0.438**	−0.057	0.090*	−0.040	0.098*	0.071	−0.072	0.086*
RM235	0.303**	−0.005	0.070	−0.080	0.135**	0.055	−0.134**	0.030
RM247	−0.257**	0.083*	−0.101*	0.060	−0.034	0.025	0.056	−0.005

Note: * and ** represent significant correlation at 5% ($r_{0.05}$ = 0.0813) and at 1% ($r_{0.01}$ = 0.1062), respectively (n = 629).

RM224) and days to heading (RM60, RM232, RM223 and RM224), were associated with the allele size of 4 SSR markers, respectively. Tillers per plant displayed a significant correlation with the allele size (molecular weight) of 5 SSR markers (RM5, RM18, RM257, RM224 and RM247). Panicles per plant displayed a significant correlation with the allele size (molecular weight) of RM249 and RM258. In the previous study, RM224 had been found associated with plant height, tillers per plant, panicle length and root-shoot ratio (Miyata et al., 2007), with RM60 associated with high-tillering dwarf 1, days to heading and shattering (Septiningsih et al., 2003; Thomson et al., 2003). Tillers per plant also displayed a significant correlation with the allele size of 5 SSR markers (RM5, RM18, RM257, RM224 and RM247) and RM249 was associated with tillers per plant, panicles per plant and grains number per plant (Abdelkhalik et al., 2005; Pradeep et al., 2005; Miyata et al., 2007). Furthermore 94 pairs ($r = -0.085^{*} - 0.424^{**}$) of 180 pairs between allele sizes and grain traits, such as nine grain traits displayed a significant correlation with the allele size (molecular weight) of RM5 and RM224. Previous studies showed that RM5 was associated with grains per panicle, percent germination, head rice grain and grain length/width ratio, especially the yield-enhancing genes increased the yield of hybrid rice by 18% (Xiao et al., 1996; Thomson et al., 2003; Liang et al., 2004). Eight grain traits displayed a significant correlation with the allele size (molecular weight) of RM257 and RM244, with RM257 associated with cold resistance and heading date (Miyata et al., 2007). Seven grain traits were significantly correlated with the allele size (molecular weight) of RM255 and RM235, as well as RM247, with RM255 associated with panicle size, panicles per plant, panicle neck diameter, and 1000-grain weight (Xu et al., 2004) and RM235 associated with panicles per plant (Thomson et al., 2003). There were six grain traits significantly correlated with the allele size of four SSR markers (RM81A and RM263, RM225, and RM258). Previous studies also showed that RM263 was associated with broken rice grain, crushed rice grain, 1000-grain weight, and spotted leaf (Septiningsih et al., 2003; Xu et al., 2004), and RM258 was associated with the spikelet fertility, 1000-grain weight, tiller number, and heading date (Cui et al., 2004; Miyata et al., 2007). Five grain traits displayed a significant correlation with the allele size of RM211 and RM18. In addition, RM211 was related with yield-improving, grain width and days to heading (Miyata et al., 2007), while RM18 was associated with frizzle panicle, grain yield, tillers per plant, grains per panicle, and heading date (Duan et al., 2003). Grain length/width ratio and grain width were significantly correlated with the allele size of RM241 etc., which proved to be associated with panicles per plant as well as plant height (Thomson et al., 2003; Cui et al., 2004), with 14 SSR markers ranging from 0.089^{*} to -0.438^{**}, and three SSR markers (RM18, RM247, RM258) were associated with subspecies differentiating DNA markers (Cheng et al., 2004). Grain length/width ratio and 1–2 internode length are two of *indica-japonica* classification traits (Zeng et al., 2000). 48 pairs ($r = 0.082^{*} - 0.438^{**}$) of 160 between allele size and panicle traits, such as four panicle traits, displayed a significant correlation with the allele size of 5 SSR markers (RM211, RM225, RM223, RM258, and RM224), among which RM225 proved to be associated with nitrogen use efficiency and grain breadth (http://www.cropscience.org.au/icsc2004/poster/3/4/1/1296_senthilvels.htm) and RM223 associated with panicle per plant, days to heading and panicle length (Pradeep et al., 2005; Miyata et al., 2007). Three panicle traits displayed a significant correlation with the allele size of four SSR markers (RM81A, RM257, RM235 and RM247), among which RM247 proved to be associated with phosphorus deficiency and plant height (Thomson et al., 2003). In general, allele size (molecular weight) of microsatellites is more associated with grain or panicle traits than plant traits or mineral element content in brown rice.

Correlation analysis indicated that there existed a significant correlation not only in 182 of 620 pairs between the allele size of 20 SSR markers and 23 phenotypic traits, not only in eight mineral elements in brown rice but also in 17 pairs between allele size of RM224 and 23 phenotypic traits and eight mineral elements in brown rice, with RM224 associated with plant height, tillers per plant, panicle length, and root–shoot ratio (Miyata et al., 2007). Up to now, 8558 QTLs controlling various complex traits have been located on different chromosome regions in *Oryza sativa* (http://www.gramene.org/qtl/index.html). However, those published have not clearly discussed the allele size (molecular weight) of microsatellites and phenotypic traits, because of the limited available information about SSR locations on chromosomes. SSRs in different positions of a gene can play an important role in determining protein function, genetic development, and regulation of gene expression (Lawson and Zhang, 2006). These 20 SSR markers for 629 accessions in Yunnan rice landraces were linked to phenotypic traits based on previous study. SSR allele size of core collection associated with phenotypic traits in rice has not been reported so far. Therefore, the data on combined phenotypic traits and allele size (molecular weight) of microsatellites within genes may provide strong evidence on the allele size of SSRs associated with mineral elemental contents in brown rice, and the SSRs on gene regulation and phenotypic traits, especially in grain traits, so as to further seek for the relationship between the molecular weight of microsatellites and phenotypic traits in rice based on markers associated.

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