

Identification of Candidate Microsatellite Markers Associated with Agronomic Traits in Rice (*Oryza sativa* L.)

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A DIVERSE collection of Egyptian and exotic rice genotypes, were evaluated for agronomic traits. Subsequently in order to assess the allele diversity of quantitative trait loci (*QTLs*) attributed to agronomic traits. The genotypes were characterized using a set of 23 microsatellite markers. In total, 24 significant marker-trait associations *QTLs* were identified; 2 for heading date, 10 for plant height, 2 for panicle length, 4 for number of panicles per plant, 1 for number of filled grains per panicle, 1 for 1000-grain weight and 4 for grain yield per plant. More of these *QTLs* were located on chromosomes 2 and 7. Association analysis of SSR markers showed 4 markers *RM6*, *RM118*, *RM151* and *RM307* had significant association with most of agronomic traits. Detection of *QTLs* for agronomic traits at different chromosomes indicated that these characters are controlled by multiple loci. Higher R^2 values were obtained for most traits and ranged from -0.366* to 0.695** for grain yield per plant and plant height, respectively. Genetic analysis identified the best rice microsatellite markers attributed to agronomic traits and they can be informative for improvement of agronomic traits through marker-assisted selection. Breeders can use this information to design crosses that assemble new potentially durable combinations of genes/*QTLs* to improve rice genotypes.

Keywords: Agronomic traits, Allele size, Marker-trait associations, Microsatellite, *Oryza sativa*, *QTLs*, Rice

Rice (*Oryza sativa* L.) is the second most important staple food crop for more than half of world's population (Delseny *et al.*, 2001 and Feng *et al.*, 2013). Grain yield is one of the most important and complex traits in cereal crops that does not evolve independently but shows correlations with other yield components traits. Thus, breeders have to consider correlated traits in breeding

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programs. Grain yield and its related traits are quantitatively inherited and controlled by many genes with small effects subject to environmental effects (Inostroza *et al.*, 2009 and Shi *et al.*, 2009). Genetic improvement of yield remains a major breeding objective to meet the ever increasing demand for food. In rice, grain weight is one of the three main yield components, the other two being number of panicles per unit area and number of grains per panicle (Fan *et al.*, 2009). Hence, estimation of the positions and effects of quantitative trait loci (*QTLs*) for agronomic traits related to yield is vitally important for marker-assisted selection for yield improvement (Li *et al.*, 2011). *QTLs* related to yield have been identified through classical linkage mapping approaches (Moncada *et al.*, 2001, Jiang *et al.*, 2004, and Suh *et al.*, 2005). With a few exceptions, most of these *QTLs* have not been successfully validated or consistently used in crop improvement (Bernardo, 2008).

Classical approaches are too simplistic to effectively model most of the genetic variation for complex traits, because they are unable to reflect the genetic realities of these traits (Cooper *et al.*, 2005 and Holland, 2007). In rice marker-assisted selection (MAS) is a promising technique to enhance traits with economic and agricultural value (Yamamoto *et al.*, 2009). DNA markers for genes/*QTLs* determined agronomic traits allow breeders to precisely select plants with beneficial traits in breeding programs. Marker-assisted selection (MAS) enhanced the cropping potential of an elite cultivar by enabling the development of versions of the cultivar with diverse heading dates. Four *QTLs* for heading date *Hd6*, *Hd1*, *Hd4*, and *Hd5* were introgressed (Takuchi *et al.*, 2006). Association mapping, has been practiced a number of plant species (Agramma *et al.*, 2007; Mazzucato *et al.*, 2008, Zhu *et al.*, 2008 and Xu *et al.*, 2014). Association mapping has the potential of simultaneous discovery of gene loci responsible for multiple traits with no need to develop permanent segregating populations.

As association mapping exploits the historical recombination events that have occurred during establishment of the sample population, higher mapping resolution could be obtained than that possible in small bi-parental experimental crosses (Flint-Garcia *et al.*, 2005). This strategy has an attractive advantage in the ability to detect the comparative effects of multiple alleles at each genetic locus that exists in crop germplasm. Association mapping is a powerful tool for identifying quantitative trait loci (*QTL*), because it takes advantage of accumulated historic recombination events in natural populations. Therefore, it should be promising for identifying causative polymorphisms of complex traits (Stich *et al.*, 2008). In particular, this approach is superior when genotypes are selected from breeding populations or collections (Thornsberry *et al.*, 2001 and Kraakman *et al.*, 2004).

However, association mapping is complicated by population structure in most germplasm sets (Flint-Garcia *et al.*, 2003). Blast resistance gene *pi21* was found to be linked with gene(s) associated with inferior eating quality (Fukuoka *et al.*, 2009). Zhang *et al.* (2005) successfully conducted whole-genome

association analysis between microsatellite markers and multiple agronomic traits using discriminant analysis (DA) in 218 inbred lines of rice. Iwata *et al.* (2007) associated RFLP markers with the width and length of milled rice grains in a set of 332 rice germplasm using Bayesian method. SSR markers and their allele diversity are useful to effectively distinguish rice genotypes. This approach is now being used to differentiate rice germplasm with different sources of mineral elemental contents and phenotypic traits (Zeng *et al.*, 2009). Also, this approach used to differentiate rice germplasm for grain weight *GW2* (Dixit *et al.*, 2013), through the use of microsatellite markers. *QTLs* mapping of many important agronomic traits, a major goal in plant breeding, requires informative markers in an intra-specific context. The objectives of this study were to i) detect chromosomes that control agronomic traits, ii) determine candidate rice microsatellite markers associated with agronomic traits on a diverse collections, iii) study genetic variation for agronomic traits, (iv) provide useful information for a study on possible SSR functions and (v) demonstrate the utility of MTAs for agronomic traits.

Materials and Methods

Plant materials and phenotypic variation

A diverse collection of 22 Egyptian and exotic rice (*Oryza sativa* L.) genotypes were randomly selected and used to establish the experimental materials for this investigation. The genotypes were supplied by Agricultural Research Center [(ARC), Giza, Egypt], International Rice Research Institute, (Los Banos, Philippines) and National small grain collection, (USDA, ARS, USA). Details of rice genotypes presented in Table 1.

Genomic DNA isolation

DNA was extracted from fresh seedling leaves for each genotype following a modified CTAB method (McCouch *et al.*, 1988). Based on the rice microsatellite genetic linkage map of Akagi *et al.* (1996) and Temnykh *et al.* (2000), 23 rice microsatellite (RM) markers were selected to represent the entire rice genome. The loci, chromosomal location, primer sequence, annealing temperature (°C) and fragment size are presented in Table 2.

Field experiment

This investigation was carried out at the Experimental Farm of the Rice research and Training Center (RRTC), Sakha, Kafer El-Sheikh, Egypt. Divers collections utilized in this study were grown during the two rice succession growing seasons, 2011 and 2012. The experiment was arranged in a randomized complete block design (RCBD), with three replicates. Each rice genotype was transplanted into a plot; each plot comprised two rows, 5 meters long and contained 25 hills. Ordinary cultural practices for rice production were applied.

TABLE 1. List of rice cultivars used in the study.

No	Genotype Name	Origin	Source of seed	Subspecies Group
1	IR 20	Philippines	IRRI	<i>Indica</i>
2	IR 22	Philippines	IRRI	<i>Indica</i>
3	IR 24	Philippines	IRRI	<i>Indica</i>
4	IR 50	Philippines	IRRI	<i>Indica</i>
5	IR 64	Philippines	IRRI	<i>Indica</i>
6	IR 74	Philippines	IRRI	<i>Indica</i>
7	Bala	India	IRRI	<i>Indica</i>
8	IET 1444	India	IRRI	<i>Indica</i>
9	Arabi	Egypt	USDA, USA	<i>Japonica</i>
10	Agamy M1	Egypt	USDA, USA	<i>Japonica</i>
11	Nahda	Egypt	USDA, USA	<i>Japonica</i>
12	Yabani M1	Egypt	USDA, USA	<i>Japonica</i>
13	Yabani M7	Egypt	USDA, USA	<i>Japonica</i>
14	Yabani 15	Egypt	USDA, USA	<i>Japonica</i>
15	Yabani lulu	Egypt	USDA, USA	<i>Japonica</i>
16	Giza 14	Egypt	USDA, USA	<i>Japonica</i>
17	Giza 171	Egypt	ARC, Egypt	<i>Japonica</i>
18	Giza 172	Egypt	ARC, Egypt	<i>Japonica</i>
19	Giza 177	Egypt	ARC, Egypt	<i>Japonica</i>
20	Giza 178	Egypt	ARC, Egypt	<i>Indica/ Japonica</i>
21	Giza 181	Egypt	ARC, Egypt	<i>Indica</i>
22	Gz 1386-5-4	Egypt	ARC, Egypt	<i>Indica</i>

TABLE 2. SSR markers, chromosomal location, motive, annealing temperature (°C), repeat category and expected fragment size.

No.	SSR markers	Chromosomal location	Motif	Annealing temperature (°C)	Repeat category	Expected Fragment size (bp)
1	RM 5	1	(GA)14	55	di	84
2	RM151	1	(TA)23	55	di	197
3	RM6	2	(AG)16	55	di	163
4	RM154	2	(GA)21	60	di	106
5	RM22	3	(GA)22	55	-	194
6	RM55	3	(GA)17	55	di	213
7	RM307	4	(AT)14(GT)21	55	complex	104
8	RM161	5	(AG)20	60	di	116
9	RM 413	5	(AG)11	50	di	65
10	RM133	6	(CT)8	60	di	224
11	RM162	6	(AC)20	60	di	130
12	RM11	7	(GA)17	55	di	115
13	RM118	7	(GA)8	60	di	106
14	RM408	8	(CT)13	55	di	109
15	RM433	8	(AG)13	50	di	215
16	RM215	9	(CT)16	55	di	126
17	RM285	9	(GA)12	55	-	205
18	RM271	10	(GA)15	55	di	65
19	RM 474	10	(AT)13	55	di	195
20	RM552	11	(TAT)13	55	tri	153
21	RM144	11	(ATT)11	55	tri	208
22	RM19	12	(ATC)10	55	tri	195
23	RM277	12	(GA)11	55	di	108

Evaluation of agronomic traits

In total, 7 traits were scored for each genotype. The symbolization of *QTLs* follows the rules of MacIntosh *et al.* (2003) (Table 3). Ten guard bands of each genotype from each replicate were selected randomly to determine characteristic phenotypes, including heading date (*Hd*), plant height (*Ht*), panicle length (*Pl*), number of panicles per plant (*NoP*), number of filled grains per panicle (*Nofg*), 1000-grain weight (*Tgw*) and grain yield per plant (*Gyp*).

Microsatellite markers analysis

A total of 23 rice microsatellite markers were selected for genotyping as given in Table 2. SSR markers were chosen on the basis of their proximity to genome specificity and according to information available in the Rice Genesdatabase (<http://www.gramene.org/microsat/Rmprimers.html>) most of the marker positions within chromosomes were based on the published rice microsatellite from work map. Microsatellite amplifications, polymerase chain reaction and fragment analysis for SSR markers were performed according to Akagi *et al.* (1996) and Temnykh *et al.* (2000). Rice microsatellite (RM) designation, chromosomal location, motif, annealing temperature (°C) and fragment size location in 'IR 36' (bp) of the amplified loci were reported by Akagi *et al.* (1996) and Temnykh *et al.* (2000).

Statistical analysis

Data on each of the 7 agronomic traits were separately correlated to each of the 23 polymorphic rice microsatellite markers. When a genotype 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 sizes. Correlation was determined by applying Pearson's method. Statistical significance was defined at $P < 0.05$. The coefficient of determination (R^2) was estimated for each of SSR markers using SPSS 10.5 software (SPSS, Inc., Chicago, USA).

Results*Association analysis*

Significant association was observed for 17 of the 23 polymorphic microsatellite markers with at least one of the 7 agronomic traits and the 23 markers identified with $R^2 > 10\%$ for traits (explained more than 10% of the phenotypic variation for each trait) (Table 3). In total, 24 marker-trait associations, significant *QTLs* for agronomic traits were identified. More *QTLs* were located on 2 and 7 chromosomes. The *QTLs* were distributed across 12 chromosomes, ranging from 1 *QTL* on chromosomes 6, 8, 9, 10 and 12 to 4 *QTLs* located on chromosomes 2 and 7, respectively. In this study, the microsatellite markers *RM6*, *RM118*, *RM151* and *RM307* were appropriate MTAs to improve agronomic traits because most of agronomic related traits such as *Hd*, *Ht*, *Pl*, *NoP*, *Nofg*, *Tgw* and *Gyp* were significant with these

microsatellite markers. A higher R^2 values were obtained for most agronomic traits and ranged from -0.366* to 0.695** for grain yield per plant and plant height, respectively. In total, 24 detected *QTLs*, 14 *QTLs* for *Hd*, *Ht*, *Pl*, *Nop*, *Nofg*, *Tgw* and *Gyp* might be the same as that obtained in earlier studies.

TABLE 3. Association of microsatellite markers with agronomic traits.

Trait	<i>QTL</i> symbol	Chromosome	Marker	R-value
Heading date (<i>Hd</i>)	<i>QHd.RRTC.1.1</i>	1	<i>RM151</i>	-0.471*
	<i>QHd.RRTC.2.2</i>	2	<i>RM006</i>	-0.387*
Plant height (<i>Ht</i>)	<i>QHt.RRTC.2.1</i>	2	<i>RM006</i>	-0.512**
	<i>QHt.RRTC.2.2</i>	2	<i>RM154</i>	0.427*
	<i>QHt.RRTC.3.3</i>	3	<i>RM022</i>	-0.506**
	<i>QHt.RRTC.3.4</i>	3	<i>RM055</i>	-0.362*
	<i>QHt.RRTC.5.5</i>	5	<i>RM161</i>	0.694**
	<i>QHt.RRTC.5.6</i>	5	<i>RM413</i>	-0.440*
	<i>QHt.RRTC.6.7</i>	6	<i>RM162</i>	0.695**
	<i>QHt.RRTC.7.8</i>	7	<i>RM011</i>	-0.645**
	<i>QHt.RRTC.8.9</i>	8	<i>RM433</i>	-0.632**
	<i>QHt.RRTC.12.10</i>	12	<i>RM019</i>	-0.610**
Panicle length (<i>Pl</i>)	<i>QPl.RRTC.4.1</i>	4	<i>RM307</i>	-0.426*
	<i>QPl.RRTC.7.2</i>	7	<i>RM118</i>	-0.600**
Number of panicles per plant (<i>Nop</i>)	<i>QNop.RRTC.1.1</i>	1	<i>RM005</i>	-0.409*
	<i>QNop.RRTC.2.2</i>	2	<i>RM006</i>	0.436*
	<i>QNop.RRTC.7.3</i>	7	<i>RM118</i>	-0.470*
	<i>QNop.RRTC.11.4</i>	11	<i>RM552</i>	-0.420
Number of filled grains per panicle (<i>Nofg</i>)	<i>QNofg.RRTC.1.1</i>	1	<i>RM151</i>	0.506**
1000-grain weight (<i>Tgw</i>)	<i>QTgw.RRTC.10.1</i>	10	<i>RM271</i>	-0.412*
Grain yield per plant (<i>Gyp</i>)	<i>QGyp.RRTC.4.1</i>	4	<i>RM307</i>	-0.373*
	<i>QGyp.RRTC.7.2</i>	7	<i>RM118</i>	-0.366*
	<i>QGyp.RRTC.9.3</i>	9	<i>RM215</i>	-0.491*
	<i>QGyp.RRTC.11.4</i>	11	<i>RM552</i>	-0.73*

The statistics shown refer to the coefficient of determination (R^2), Only SSR markers with significant marker-trait association are given.

*, ** Indicate significance at the probability levels of 0.05 and 0.01, respectively.

(*Hd*) heading date, (*Ht*) plant height, (*Pl*), panicle length, (*NoP*), number of panicles, (*Nofg*), number of filled grains (*Tgw*) 1000-grain weight and (*Gyp*) grain yield per plant.

*Marker-traits associations (MTAs) analysis**Heading date (Hd)*

Correlation analysis indicated that there was a significant correlation in two *QTLs* ($r = -0.387^*$ to -0.471^*) of the 23 traits pairs between microsatellite allele size and *Hd* (Table 3). These two *QTLs* were designated as *QHd.RRTC.1.1* and *QHd.RRTC.2.2*. *Hd* showed a significant correlation with the allele size of *RM6* and *RM151* on chromosomes 2 and 1, respectively.

Plant height (Ht)

For *Ht*, the correlation analysis indicated that there was a significant correlation in ten *QTLs* ($r = -0.440^*$ to 0.695^{**}) of 23 pair traits between microsatellite allele size and *Ht* (Table 3). These ten *QTLs* were designated as *QHt.RRTC.2.1*, *QHt.RRTC.2.2*, *QHt.RRTC.3.3*, *QHt.RRTC.3.4*, *QHt.RRTC.5.5*, *QHt.RRTC.5.6*, *QHt.RRTC.6.7*, *QHt.RRTC.7.8*, *QHt.RRTC.8.9* and *QHt.RRTC.12.10*. *Ht* showed a significant correlation with the allele size of *RM6*, *RM154*, *RM22*, *RM55*, *RM161*, *RM413*, *RM162*, *RM11*, *RM433* and *RM19* on chromosomes 2, 3, 5, 6, 7, 8 and 12, respectively.

Panicle length (Pl)

From MTAs, there was a significant association with two *QTLs* ($r = -0.426^*$ to -0.600^{**}) of 23 pair traits between microsatellite allele size and *Pl* (Table 3). These two *QTLs* were designated as *QPl.RRTC.4.1* and *QPl.RRTC.7.2*. *Pl* had a significant association with allele size of *RM307* and *RM118* on chromosomes 4 and 7, respectively.

Number of panicles per plant (Nop): As for *Nop*, there was a significant correlation in four *QTLs* ($r = -0.409^*$ to 0.436^*) of 23 pair traits between microsatellites allele size and *Nop* (Table 3). These four *QTLs* were designated as *QNop.RRTC.1.1*, *QNop.RRTC.2.2*, *QNop.RRTC.7.3*, and *QNop.RRTC.11.4*. *Nop* showed a significant correlation with some allele size of *RM5*, *RM6*, *RM552* and *RM118* on chromosomes 1, 2, 11 and 7, respectively.

Number of filled grains per panicle (Nofg): A significant MTAs only in one *QTL* ($r = 0.506^{**}$) of 23 pair traits was obtained between microsatellites allele size and *Nofg* (Table 3). These *QTL* was designated as *QNofg.RRTC.1.1*. *Nofg* showed a significant correlation with the allele size of *RM151* on chromosome 1.

1000-grain weight (Tgw) : Concerning the *Tgw*, there was a significant correlation in one *QTL* ($r = -0.412^*$) of 23 pair traits between microsatellites allele size and *Tgw* (Table 3). These *QTL* was designated as *QTgw.RRTC.10.1*. *Tgw* showed a significant correlation with allele size of *RM291* on chromosome 10.

Grain yield per plant (Gyp): There was a significant correlation in four *QTLs* ($r = -0.366^*$ to 0.491^*) of 23 pair traits between microsatellites allele size and *Gyp* (Table 3). These four *QTLs* were designated as *QGyp.RRTC.4.1*, *QGyp.RRTC.7.2*, *QGyp.RRTC.9.3*, and *QGyp.RRTC.11.4*. *Gyp* showed a significant correlation with allele size of *RM215*, *RM307*, *RM552* and *RM118* on chromosomes 9, 4, 11 and 7, respectively.

Discussion

Yield is the most important and complex trait for genetic improvement in cereal crops, and marker-assisted selection enhances the improvement of efficiency. Marker-trait associations (MTAs) offers a very good tool for rice breeders to obtain high yield. Selection can be done on the markers associated with the targeted traits by using DNA-markers. Once, the phenotypic traits are fixed, breeders can evaluate large numbers of progenies for yield performance in a conventional way. A combination of MTAs and conventional evaluation could significantly improve the breeding program efficiency, its process and new released cultivars (Collard and Mckill, 2008).

The association between markers and genes/*QTLs* controlling the targeted traits must be first established prior the marker associated selection (MAS) process. This study was conducted to identify *QTLs* correlated with agronomic traits in rice. Microsatellite markers used were well distributed amongst the 12 chromosomes, (Cho *et al.*, 2000 and Temnykh *et al.*, 2000). Heading date (*Hd*) is a major determinant of the regional and seasonal adaptation of rice varieties. Data presented in this study clearly identified two *QTLs* for *Hd* associated with two microsatellite markers *RM6* and *RM151* on chromosomes 1 and 2, respectively. Yamamoto *et al.* (2000), have a putative *QTL* for heading date (*Hd*) on chromosomes 2 and 3. For heading date, the *QTL* associated with *Hd* (*QHd.RRTC.2.2*) on chromosome 2, might be the same as that found recently (Yamamoto *et al.*, 2000). Plant height (*Ht*) is one of the most important traits related to plant status and yield potential. In the current study, a total of ten *QTLs* were identified for plant height on chromosomes 2, 3, 5, 6, 7, 8 and 12. There were several reported molecular marker based genetic analyses of plant height in rice, which detected a number of *QTLs* on chromosomes 1, 2, 4, 5, 6, 9 and 11 (Lin *et al.*, 2011), Huang *et al.* (1996) analyzed *QTLs* for plant height in five rice populations and identified 13 major dwarfing genes were located in close proximity to these *QTLs*. Moreover, Yu *et al.* (2002) detected four *QTLs* for *Ht* on chromosomes 1, 5, 7, and 11. More recently, a gene for *Ht* on chromosome 5 was cloned using a map based cloning strategy (Ashikari *et al.*, 1999) and on chromosomes 1, 3 and 7 (Hittalmani *et al.*, 2003). With regard to *Ht*, 7 *QTLs* (*QHt.RRTC.1.1*, *QHt.RRTC.2.2*, *QHt.RRTC.3.3*, *QHt.RRTC.3.4*, *QHt.RRTC.5.5*, *QHt.RRTC.5.6* and *QHt.RRTC.7.8*) from 10 detected were located on chromosomes 2, 3, 5, 6, 7, 8 and 12, respectively. The *QTLs* on chromosomes 2, 3, 5, 6 and 7 respectively, might be the same as that found by Ashikari *et al.* (1999), Yu *et al.* (2002), Hittalmani *et al.* (2003) and Lin *et al.* (2011).

For *Pl*, two *QTLs* were associated with two rice microsatellite markers *RM307* and *RM118* on chromosomes 4 and 7. Ahamadi *et al.* (2008) found *QTLs* for *Pl* on chromosomes 2, 4, 11, and 12. In previous studies, the *QTL* (*QPl.RRTC.4.1*) for *Pl* on chromosome 4 might be the same as that found by Ahamadi *et al.* (2008). Concerning the *Nop*, four *QTLs* were associated with microsatellite markers *RM5* *RM5* *RM6* *RM118* and *RM552* on chromosomes 1, 2, 7 and 11. Hittalmani *et al.* (2003) reported one *QTL* was detected for *Nop* on chromosomes 1, 4 and 12. Also, Zhu *et al.* (2011) detected *QTL* for *Nop* on chromosome 1. In this case, the *QTL* (*QNop.RRTC.1.1*) for *Nop* on chromosome 1 might be the same as that found recently by Hittalmani *et al.* (2003) and Zhu *et al.* (2011). Large number of well-filled grains per panicle (*Nofg*) is an important yield component trait in rice. In this study, *QTL* for *Nofg* was identified by SSR marker *RM151* on chromosome 1. Ahamadi *et al.* (2008) detected a total of three *QTLs* for panicle grain number was on chromosomes 1 and 12. In this case, the *QTL* (*QNofg.RRTC.1.1*) for the number of filled grains on chromosome 1 might be the same as that found earlier by Ahamadi *et al.* (2008). 1000-grain weight (*Tgw*) is an important factor affecting grain yield as well as grain quality in rice. In the present study, *QTL* for 1000-grain weight was associated with SSR marker *RM271* on chromosome 10. Tang *et al.* (2013) found a number of *QTLs* for 1000-grain weight on chromosomes 2, 3, 5, 6, 8 and 10. In this case, the *QTL* (*QTgw.RRTC.10.1*) for 1000-grain weight on chromosome 10 might be the same as that found recently by Tang *et al.* (2013). (*Gyp*) is a complex trait consisting of several yield components. It is of great importance to reveal the genetic relationships between *Gyp* and its yield components at the *QTL* level for multi-trait improvement in rice. In the present study, four *QTLs* for *Gyp* were associated with microsatellite markers *RM307*, *RM118*, *RM215* and *RM552* on chromosomes 4, 7, 9 and 11. Liu *et al.* (2008) detected 10 *QTLs* for *Gyp* on chromosomes 1, 2, 3, 4, 7, 8, and 12. The *QTLs* (*QGyp.RRTC.4.1* and *QGyp.RRTC.7.2*) for *Gyp* on chromosomes 4 and 7 might be as that found by Liu *et al.* (2008).

Marker trait association (MTA) is new approach in cereal genetics and particularly in rice. In contrast to conventional bi-parental mapping, which can only analyze allelic differences between two parents, association mapping attempts to scan genetic variation across a wide spectrum of genotypes. The present study underlines the value of genetic basis of agronomic traits even with a relatively small collection of genotypes. A substantial number of MTAs for the whole set of agronomic traits were detected. Many loci were detected that coincide with known major genes/*QTLs* for agronomic traits, indicating the power of association mapping. Additionally, potential novel loci were identified that may help to better understand the architecture of complex genetic traits. Based on marker approach, the novel loci provide opportunities for further improvement of rice. Breeders can use this information to design crosses that

assemble new, potentially durable combinations of agronomic traits genes/*QTLs* to improve rice genotypes.

References

- Agrama, H.A., Eizenga, G.C. and Yan, W. (2007)** Association mapping of yield and its components in rice cultivars. *Mol Breed*, **19**: 341–356.
- Ahamadi, J., Fotokian, M.H. and Fabriki-Orang, S. (2008)** Detection of *QTLs* influencing panicle length, panicle grain number and panicle grain sterility in rice (*Oryza sativa* L.). *J. Crop Sci., Biotech*, **11** (3) : 163-170.
- Akagi, H., Y. Yohozeki, A. Inagaki, A. Nakamura and Fujimura, T. (1996)** A co-dominant DNA marker closely linked to the rice nuclear restorer gene *Rf-1*, identified with inter-SSR fingerprinting. *Genome*, **39**: 1205-1209.
- Ashikari, M., Wu, J., Yano, M., Sasaki, T. and Yoshimura, A. (1999)** Rice gibberellin-insensitive dwarf mutant gene dwarf 1 encodes the α -subunit of GTP-binding protein. *Proc. Natl. Acad. Sci., USA* **96**:10284-10289.
- Bernardo, R. (2008)** Molecular markers and selection for complex traits in plants: learning from the last 20 years. *Crop Sci.*, **48**:1649-1664.
- Cho, Y.G., Ishii, T., Temnykh, S., Chen, X., Lipovich, L., McCouch, S.R., Park, W.D., Ayres, N. and Cartinhour, S. (2000)** Diversity of microsatellites derived from genomic libraries and gen bank sequences in rice (*Oryza sativa* L.). *Theor. and Appl. Genet.*, **100**(5): 713-722.
- Collard, B.C. and Mackill, D.J. (2008)** Marker assisted selection: An approach for precision plant breeding in the twenty-first century. *Philos. Trans. Roy. Soc. Lond B Biol. Sci.*, **363**: 557-572.
- Cooper, M., Podlich, D.W. and Smith, O.S. (2005)** Gene-to-phenotype models and complex trait genetics. *Aust. J. Agric. Res.*, **56**: 895–918 .
- Delseny, M., Salses, J., Cooke, R., Sallaud, C., Regad, F., Lagoda, P., Guiderdoni, E., Ventelon, M., Brugidou, C. and Ghesquière, A. (2001)** Rice genomics: Present and future. *Plant Physiol Biochem*, **39**(3/4): 323-334.
- Dixit, N., Dokku, P., Amitha Mithra S.V., Parida, S.K., Singh, A.K., Singh, N.K. and Mohapatra, T. (2013)** Haplotype structure in grain weight gene *GW2* and its association with grain characteristics in rice. *Euphytica*, **192** (1): 55-61.
- Fan, C., Yu, S., Wang, C. and Xing, Y. (2009)** A causal C-A mutation in the second exon of *GS3* highly associated with rice grain length and validated as a functional marker. *Theor. Appl. Genet.* **118**: 465-472.
- Feng, Y., Zhai, R. R., Lin, Z. C., Cao, L. Y., Wei, X. H. and Cheng, S. H. (2013)** *QTL* analysis for yield traits in rice under two nitrogen levels. *Chin. J. Rice. Sci.*, **27** (6): 577-584. (in Chinese with English abstract).

- Flint-Garcia S.A., Thornsberry, J.M. and Buckler, E.S. (2003)** Structure of linkage disequilibrium in plants. *Annu. Rev. Plant. Biol.*, **54**: 357-374 .
- Flint-Garcia, S.A., Thuillet, A.C., Yu, J., Pressoir, G., Romero, S.M., Mitchell, S.E., Doebley, J., Kresovich, S., Goodman, M.M. and Buckler, E.S. (2005)** Maize association population: a high-resolution platform for quantitative trait locus dissection. *Plant J.*, **44**:1054-1064 .
- Fukuoka, S., Saka, N., Koga, H., Ono, K., Shimizu, T., Ebana, K., Hayashi, N., Takahashi, A., Hirochika, H., Okuno, K. and Yano, M. (2009)** Loss of function of a proline-containing protein confers durable disease resistance in rice. *Science.*, **325**: 998-1001.
- Hittalmani, S., Huang, N., Courtois, B., Venuprasad, R., Shashidhar, H.E., Zhuang, J.Y, Zheng, K.L., Liu, G.F, Wang, G.C., Sidhu, J.S., Srivantaneeyakul, S., Singh, V.P, Bagali, P.G., Prasanna, H.C., McLaren, G. and Khush, G.S. (2003)** Identification of *QTL* for growth and grain yield-related traits in rice across nine locations of Asia. *Theor. Appl. Genet.*, **107**: 679-690.
- Holland, J. (2007)** Genetic architecture of complex traits in plants. *Curr. Opin. Plant. Biol.*, **10**:156-161
- Huang, N., Courtois, B., Khush, G.S., Lin, H.X., Wang, G.L., Wu, P. and Zheng, K.L. (1996)** Association of quantitative trait loci for plant height with major dwarfing genes in rice. *Heredity*, **77**:130-137.
- Inostroza, L., Pozo, A.D., Matus, I., Castillo, D., Hayes, P., Machado, S. and Corey, A. (2009)** Association mapping of plant height, yield, and yield stability in recombinant chromosome substitution lines (RCSLs) using *Hordeum vulgare* subsp. *spontaneum* as a source of donor alleles in a *Hordeum vulgare* Subsp. *vulgare* background. *Mol. Breed*, **23**: 365-376.
- Iwata, H., Uga, Y., Yoshioka, Y., Ebana, K. and Hayashi, T. (2007)** Bayesian association mapping of multiple quantitative trait loci and its application to the analysis of genetic variation among *Oryza sativa* L. germplasms. *Theor. Appl. Genet.*, **114**:1437-1449 .
- Jiang, G.H., Xu, C.G., Li, X.H. and He, Y.Q. (2004)** Characterization of the genetic basis for yield and its component traits of rice revealed by doubled haploid population. *Acta Genet Sinicavol*, **31**: 63-72 .
- Kraakman, A.T., Niks, R.E., Van den Berg P.M., Stam, P., Van and Eeuwijk, F.A. (2004)** Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. *Genetics*, **168**: 435-446 .
- Li Y, Fan C, Xing Y, Jiang Y, Luo L, Sun L, Shao D, Xu C, Li X, Xiao J, He Y, Zhang Q (2011)** Natural variation in GS5 plays an important role in regulating grain size and yield in rice. *Nat. Genet.*, Doi:10.1038/ng.977.

- Lin, Y.R., Wu, S.C., Chen, S.E., Tseng, T.H., Chen, C.S., Kuo, S.C., Wu, H.P. and Hsing, Y.I. (2011)** Mapping of quantitative trait loci for plant height and heading date in two inter-sub specific crosses of rice and comparison across *Oryza* genus. *Botanic. Stud.*, **52**: 1-14.
- Liu, G.F., Yang, J., Xu, H.M., Hayat, Y. and Zhu, J. (2008)** Genetic analysis of grain yield conditioned on its component traits in rice (*Oryza sativa* L.). *Aust. J. of Agricult. Resea.*, **59**: 189-195
- Mazzucato, A., Papa, R., Bitocchi, E., Mosconi, P., Nanni, L., Negri, V., Picarella, M.E., Siligato, F., Soressi, G.P., Tiranti, B. and Veronesi, F. (2008)** Genetic diversity, structure and marker-trait associations in a collection of Italian tomato (*Solanum lycopersicum* L.) landraces. *Theor. Appl. Genet.*, **116**: 657-669
- McCouch, S.R., G Kochert, Z. H. Yu, Z.Y. Wang, G.S. Khush, W.A. Coffman and S.D. Tanksley (1988)** Molecular mapping of rice chromosome. *Theor. Appl. Genet.*, **76**: 815-829.
- McIntosh, R. A., Y. Yamazaki, K. M. Devos, J. Dubkovsky, W. J. Rogers. and R. Appels. (2003)**. MacGene 2003-Catalogue of gene symbols for wheat. CD, In: "*Proc 10th Int Wheat Genet Symp*", Paestum , Italy .
- Moncada, P., Martinez, C.P., Borrero, J., Chatel, M., Gauch, H., Guimaraes, E., Tohme, J. and McCouch, S.R. (2001)** Quantitative trait loci for yield and yield components in an *Oryza sativa* × *Oryza rufipogon* BC₂F₂ population evaluated in an upland environment. *Theor. Appl. Genet.*, **102**:41-52.
- Shi, J., Li, R., Qiu, D., Jiang, C., Long, Y., Morgan, C., Bancroft, I., Zhao, J. and Meng, J. (2009)** Unraveling the complex trait of crop yield with quantitative trait loci mapping in *Brassica napus*. *Genetics*, **182**:851–861
- Stich, B., Möhring, J., Piepho, H.P., Heckenberger, M., Buckler, E.S. and Melchinger, A.E. (2008)** Comparison of mixed-model approaches for association mapping. *Genetics*, **178**: 1745–1754 .
- Suh, J.P., Ahn, S.N., Cho, Y.C., Kang, K.H., Choi, I.S., Kim, Y.G., Suh, H.S. and Hong, H.C. (2005)** Mapping of *QTLs* for yield traits using an advanced backcross population from a cross between *Oryza sativa* and *O. glaberrima*. *Korean J. Breed*, **37**: 214–220 .
- Takeuchi, Y., Ebitani, T., Yamamoto, T., Sato, H., Ohta, H., Hirabayashi, H., Kato, H., Ando, I., Nemoto, H., Imbe, T. and Yano, M., (2006)** Development of isogenic lines of rice cultivar Koshihikari with early and late heading by marker-assisted selection. *Breed Sci.*, **56**: 405-13 .
- Tang, S., Shao, G., Wei, X., Chen, M., Sheng, Z., Luo, J., Jiao, G., Xie, L. and Hu, P. (2013)** *QTL* mapping of grain weight in rice and the validation of the *QTL* qTGW3.2. *Gene.*, **527**(1): 201-206

- Temnykh, S., Park, W.D., Ayres, N., Cartinhour, S., Hauck, N., Lipovich, I., Cho, Y.G., Ishii, T. and McCouch, S.R., (2000)** Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theor. Appl. Genet.*, **100**: 697-712.
- Thornsberry, J.M., Goodman, M.M., Doebley, J., Kresovich, S., Nielsen, D. and Buckler, E.S. (2001)** *Dwarf8* polymorphisms associate with variation in flowering time. *Nat Genet*, **28**: 286-289
- Xu, F., Tang, F., Shao, Y., Chen, Y., Tong, C. and Bao, J. (2014)** Genotype environment interactions for agronomic traits of rice revealed by association mapping. *Rice Sci.*, **21**(3): 133–141
- Yamamoto, T., Yonemaru, J. and Yano, M. (2009)** Towards the understanding of complex traits in rice: substantially or superficially? *DNA Res.*, **16**:141–54.
- Yamamoto, T., Lin, H., Sasaki, T. and Yano, M. (2000)** Identification of heading date quantitative trait locus Hd6 and characterization of its epistatic interactions with Hd2 in rice using advanced backcross progeny. *Genetics*, **154**: 885–89.
- Yu, S.B., JX, Li, JX, Xu CG, Tan YF and Li XH (2002)** Identification of quantitative trait loci and epistatic interactions for plant height and heading date in rice. *Theor. Appl. Genet.*, **104**: 619-625.
- Zeng, Y., Zhang, H., Yang, S., Du, J., Pu, X., Wang, L., Liu, J., Xiao, F. and Li, Z. (2009)** Correlation between allele sizes of microsatellites and phenotypic variations in rice landraces. *Front. Agric. China.*, **3**: 130-139.
- Zhang, N., Xu, Y., Akash, M., McCouch, S. and Oard, J.H. (2005)** Identification of candidate markers associated with agronomic traits in rice using discriminant analysis. *Theor. Appl. Genet.*, **110**: 721–729.
- Zhu, C.S., Gore, M., Buckler, E.S. and Yu, J.M. (2008)** Status and prospects of association mapping in plants. *Plant Genome*, **1**: 5–20
- Zhu, J., Zhou, Y., Liu, Y., Wang, Z., Tang, Z., Yi, C., Tang, S., Gu, M. and Liang, G. (2011)** Fine mapping of a major *QTL* controlling panicle number in rice. *Mol. Breeding*, **27**: 171-180.

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تحديد المعلمات الجزيئية المرتبطة بالصفات المحصولية في الأرز (*Oryza sativa* L.)

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يُعد استخدام المعلم الجزيئي الميكروستاليت (microsatellite) هام لتحديد
الأليلات المرتبطة بالصفات المحصولية في الأرز. ولقد أجريت هذه الدراسة
بهدف:

تحديد الكروموسومات التي تتحكم في وراثة الصفات المحصولية.
تحديد المعلمات الجزيئية الميكروستاليت المرتبطة بالصفات المحصولية باستخدام
مجموعة متنوعة من أصناف الأرز المصرية والأجنبية.
دراسة التباين الوراثي للصفات المحصولية.
توفير المعلومات الهامة للاستفادة من الأهمية الوظيفية للمعلم الجزيئي
الميكروستاليت.
اثبات فائدة استخدام الأنتخاب باستخدام المعلمات الجزيئية للصفات المحصولية.

استخدم في هذه الدراسة ثلاثة وعشرون بادئاً بالإضافة إلى سبعة صفات
مورفولوجية هي ميعاد التزهير، طول النبات، طول السنبل، عدد السنبيلات لكل
نبات، عدد الحبوب بكل سنبل، وزن الألف حبة، محصول النبات الفردي وذلك
لتحديد الأليلات المرتبطة بالصفات المحصولية. وفيما يلي ملخص لأهم النتائج:
تم تحديد ٢٤ موقعاً للصفات الكمية (QTLs)، وكانت موزعة على الصفات
المحصولية كما يلي: موقعين لصفة ميعاد التزهير، عشرة مواقع لصفة طول النبات،
موقعين لصفة طول السنبل، أربعة مواقع لصفة عدد السنبيلات على النبات،
وموقع واحد لصفة عدد الحبوب بكل السنبل، موقع واحد لصفة وزن الألف حبة،
وأربعة مواقع لصفة محصول النبات الفردي. ووجد أن معظم هذه المواقع موجود
على الكروموسومين ٢، ٧.

أظهرت النتائج باستخدام بادئات التكرارات المتسلسلة البسيطة، أن أكثر
المعلمات الجزيئية ارتباطاً بمعظم الصفات المحصولية هي *RM118*، *RM6*،
RM151، *RM307*. كما أن وجود مواقع الـ *QTLs* المرتبطة بالصفات
المحصولية على كروموسومات مختلفة مما يدل على أنه قد يتحكم في وراثته عديد
من المواقع الوراثية.

أوضحت النتائج أن أعلى قيمة لمعامل الارتباط (R^2) لمعظم الصفات
وتراوح ما بين (0.366^*) إلى (0.695^{**}) لصفة محصول النبات الفردي
وطول النبات على التوالي.

حدد التحليل أفضل معلم جزيئي للأرز نسبة إلى الصفات المحصولية، وأنه
يمكن لمربي الأرز تحسين الصفات المحصولية من خلال الانتخاب بمساعدة تلك
المعلمات الجزيئية، كما يمكن لمربي النبات استخدام هذه المعلومات لتحديد الهجن
التي تجمع تراكيب جينية *QTLs* جديدة، لتحسين التراكيب الوراثية في الأرز.