Turkish Journal of Agriculture and Forestry

Volume 35 | Number 5 Article 4

1-1-2011

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TÜBİTAK

Research Article

Turk J Agric For
35 (2011) 481-490
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doi:10.3906/tar-1001-645

Heritability for some agronomic characters of rice (*Oryza sativa* L.) and their linked microsatellites identification

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Received: 01.02.2010

Abstract: The objectives of this study were to estimate the narrow sense heritability of the examined characters and to find their linked simple sequence repeats (SSRs) using bulked segregant analysis (BSA) in rice (*Oryza sativa* L). $F_{2:3}$ families of the cross between 'Mousa-Tarom' and '304' were used in an augmented design with 6 replications. Narrow sense heritability of panicle length and the culm number were low (<0.18), and of the brown rice characteristics such as length, width, and shape were very high (>0.84). The SSR primers RM16589 and RM17166 on chromosome 4 showed linkage with the gene(s) controlling culm length, plant height, leaf width, culm number, grain width, and grain shape. On chromosome 11, the SSR primer RM26063 showed linkage with plant height, panicle length, leaf width, grain length, grain width, and grain shape. The SSR primer RM26291 showed linkage with culm number, grain width, culm, panicle, and leaf and grain length, and the RM26509 to the grain shape. Chromosomes 1, 4, 5, and 11 had genes effective on the grain shape. The chromosomes 3, 5, and 11 carried the genes that controlled the leaf width. The linked SSR primers to the corresponding characters are helpful to select the traits in breeding programs and to find more tightly linked markers with the controlling genes of the desirable traits.

Key words: Bulked segregant analysis, narrow sense heritability, rice (*Oryza sativa* L.), SSRs

Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world and feeds over half of the global population. Lack of sufficient investment to improve varieties and yield is one of the factors that has delayed the increase in rice grain production. To defeat this challenge and meet the growing demand in this manner, both classical and molecular plant breeding methods have to be used (Smith and Dilday 2003). One of the demerits of classical plant breeding is transferring the unwanted genes together with the desired ones (Collard et al. 2005). With finding linked markers to the loci controlling the desirable traits, using molecular breeding methods, it is possible to select the plants with desirable traits and with few or no unwanted traits. Once the linked markers to genes or quantitative trait loci (QTLs) of interest are identified, prior to field evaluation of a large number of plants and at the early stages of growth, breeders may use specific DNA marker alleles as a diagnostic

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tool to identify plants carrying the genes or QTLs (Collard et al. 2005). Tightly linked DNA markers to the important genes may be used as molecular tools for marker-assisted selection (MAS) in plant breeding, which helps in more efficient, reliable, and commercial phenotype selection compared to the classical plant breeding methods (Collard et al. 2005).

More recently, microsatellites or simple sequence repeats (SSRs) have been developed for major crop plants, which are predicted to lead to even more rapid advances in both marker development and implementation of breeding programs (Garland et al. 2000). Being co-dominant and PCR-based, SSRs have been preferentially used for evaluating genetic diversity and relationships among closely related rice varieties or accessions. SSRs are locus-specific, and therefore it is possible to identify the chromosomal locations of the gene(s) controlling the traits, based on linked SSR markers (Neelu et al. 2006).

Several methods have been proposed in order to increase the efficiency of genotypic evaluations with the use of as few individuals as possible, when searching for a controlling QTL or gene of the related traits. Lander and Botstein (1989) have proposed a selective genotyping method, whereby one evaluates the segregating population phenotypically. Michelmore et al. (1991) have employed a similar idea in bulked segregant analysis, which is most commonly used with PCR-based markers. Both these methods are more applicable to traits for which one or two major genes are of interest. These methods have less power for detecting genes with more minor effects. They are also used for mapping genes, affecting only a single trait (Beavis 1994; Wang and Paterson 1994; Garland et al. 2000; Collard et al. 2005).

Different traits may be important to increase the rice grain production. The considered traits may include short plant height, strong culms, moderate tillering, short and erect leaves, large and compact panicles, and early maturation (Paterson et al. 2005). Tillering in rice is one of the most important agronomic characters for grain production (Smith and Dilday 2003), because the tiller number per plant determines the panicle number, a key component of grain yield (Yan et al. 1998). The prime characters among the various grain quality characters in deciding the overall grain quality in rice are grain length, width, shape, and its weight (Gravois and Helms 1996; Tan et al. 1999). Rice grain features such as length, width, and shape have a direct effect on the marketability, and therefore on the commercial success of modern rice cultivars (Redona and Mackill 1998). Most of the grain quality characters of rice are controlled by QTLs showing continuous phenotypic variation (Yano and Sasaki 1997; Yoshida et al. 2002).

Successful changing of the traits in a population by hybridization is predictable only through information about the heritability of those traits. Heritability of the most metric traits is low (Falconer and Mackay 1997). Narrow sense heritability was 0.8 for grain length and 0.55 for grain width, with no fluctuation in their estimation, from the F_2 to F_4 generations (Kato 1990). Narrow sense heritability for plant height was 0.75 (Boonhong 1997), and broad sense heritability was 0.74 for grain length, 0.74 for grain breadth, and 0.89 for grain shape (Rabiei et al. 2004). At least 17 Mendelian genes for rice tillers have been identified by mutant analyzing (http://www.gramene.org/db/mutant/search.core), and numerous QTLs for rice tillers have been located on 10 rice chromosomes (Liu et al. 2009).

The reported narrow sense heritability was low (<0.2) for culm length (Horie et al. 1964), panicle length (Horie et al. 1964) and culm number (Kato 1990; Surek and Korkut 1998; Surek and Beser 2005). Medium narrow sense heritability ($0.2 < h^2 < 0.5$) was reported for tiller number (Horie et al. 1964; Gravais and McNew 1993), high h^2 ($0.5 < h^2 < 0.8$) for brown rice width (Kato 1990) and plant height (Boonhong 1997), and very high h^2 (>0.8) for brown rice length (Kato 1990). Genetic postulates on grain length vary from mono, di, or tri to polygenic inheritance. Grain width showed polygenic inheritance (Govindaraj et al. 2005). According to Yoshida et al. (2002), 4 to 5 genes were effective on grain length and width in rice.

Several researchers had studies on finding SSRs linked with the different agronomic traits of rice. Their reports are given in Table 1. On the other hand, previous results have shown that the heritability of the traits is cross- and generation-dependable.

The aims of the present study were to estimate the narrow sense heritability (h^2) of some agronomic characters and detection of their linked SSR markers in rice (*Oryza sativa* L.), using the bulked segregant analysis.

Table 1. The traits, the reported linked SSR markers or marker interval, chromosome number (Chr. #), and the references cited.

Trait(s)	Linked marker interval	Chr. #	References
Culm length	RM5- RM488, RM529, Rm5916, RM2614, RM7097, RM255, RM335- RM518, RM4447, RM481, RM8036, RM333, RM229- RM21, RM235	1, 2, 3, 4, 6, 7, 10, 11, 12	Sato et al. 2004, Yoon et al. 2006, Onishi et al. 2007, Kwon et al. 2008
Culm (Tiller) number	RM206, RM283-RM562, RM579-RM9, RM526-RM425, RM135-RM55, RM168-RM571, RM252- RM3276, RM6972-RM3170, RM133-RM136, RM133-RM587, RM508-RM225, RM481, RM515-RM210	1, 2, 3, 4, 5, 6, 7, 8, 11	Yoshida et al. 2002, Onishi et al. 2007, Liu et al. 2009, Ma et al. 2009
Grain length	RM438- RM341, RM251-RM554, RM255, RM437-RM289, RM204, RM539- RM121, RM481-RM125, RM72-RM515, RM229, RM229- RM21	2, 3, 4, 5, 6, 7, 8, 11	Yoshida et al. 2002, Rabiei et al. 2004, Yoon et al. 2006
Grain shape	RM290- RM550, RM475-RM263, RM251-RM554, RM411-RM16, RM241, RM437- RM289, RM50- RM539, RM478, RM481-RM125, RM256-RM230, RM502- RM264, RM72-RM515, RM202- RM536	2, 3, 4, 5, 6, 7, 8, 11	Rabiei et al. 2004, Yoon et al. 2006, Kwon et al. 2008
Grain width	RM84- RM259, RM213- RM166, RM233A- RM8, RM279-RM555, RM290- RM550, RM16- RM411, RM7-RM251, RM241, RM13-RM289, RM31, RM437-RM289, RM50-RM121, RM527-RM3, RM125- RM11, RM478, RM481-RM125, RM256-RM230, RM502- RM264, RM434-RM201, RM224- RM144	1, 2, 3, 4, 5, 6, 7, 8, 9, 11	Yoshida et al. 2002, Rabiei et al. 2004, Abdelkhalik et al. 2005, Yoon et al. 2006, Kwon et al. 2008
Panicle length	RM539- RM121, RM320, RM210- RM256, RM215, RM288-RM205, RM5652, RM311- RM467, RM167- RM120, RM21-RM206, RM332	2, 4, 5, 6, 7, 8, 9, 10, 11	Xiao et al. 1998; Thomson et al. 2003; Marri et al. 2005; Yoon et al. 2006, Yan et al. 2007; Ahmadi et al. 2008; Kwon et al. 2008; Liu et al. 2008
Panicle number	RM485- RM236, RM241- RM348, RM279- RM555, RM241-RM349, RM349- RM280, RM153, RM50, RM241- RM349, RM481, RM224- RM144	2, 4, 7, 11	Yoon et al. 2006; Onishi et al. 2007; Ahmadi et al. 2008; Liu et al. 2008
Plant height	RM6333, RM5652	1, 9	Yan et al. 2007; Chen et al. 2009
Seedling height	RM262- RM263, RM175-RM22, RM270- RM17	2, 3, 12	Abdelkhalik et al. 2005; Han et al. 2007

Materials and Methods

Population development

A cross between Mousa-Tarom (high grain quality) and line 304 (high yielding) was crossed to obtain a population of 193 $F_{2:3}$ families, for use. The plants were transplanted at the 5-leaf growth stage, with 25 × 25 cm spacing in an augmented design, with 6 replications and 3 control cultivars. The field experiment was conducted at Khanmirza region (altitude 1564 m, longitude 50°49′, latitude 31°31′, mean annual temperature 15.4 °C, with clay-loam soil type), Chaharmahal and Bakhtiari provinces, Iran. Each plot consisted of 4 rows with 60 plants per plot. Evaluation of the characters was conducted according to the Standard Evaluation System for rice (SES) manual, provided by the International Rice Research Institute (IRRI 2002). The examined characters comprised seedling height, plant height, culm length, panicle length, leaf length, leaf width, and brown rice length, width, and shape (length/width ratio).

Bulked segregant analysis

Bulked segregant analysis (Michelmore et al. 1991) was performed, in conjunction with SSR analysis, to

identify the SSR markers linked to the controlling genes of the characters. Two bulks, made by mixing equal amounts of DNA from the first 5 high extreme plants and 5 low extreme plants of the $F_{2:3}$ families, for all characters, were subjected to polymorphism screening using SSR markers. The selection of 5 plants per bulk expected to prevent heterozygosity in the bulks (Govindaraj et al. 2005).

DNA extraction

Genomic DNA of parental lines as well as the $F_{2:3}$ families was extracted from about 0.5 g of frozen leaf tissue from the bulked leaf samples using the CTAB method (Murray and Thompson 1980) with slight modifications. The DNA was spooled out, washed twice with 70% ethanol, and dissolved in TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) containing 30 μg mL^{-1} RNase-A, and incubated at 37 °C for 60 min. Twenty percent (v/v) sodium acetate (3 M, pH 5.5) was added and disturbed, and extracted with chloroform-isoamyl alcohol (24:1 v/v). DNA was re-precipitated, dissolved in TE buffer, and checked for its quality, using 0.8% agarose gel electrophoresis, and its quantity using a spectrophotometer (Biophotometer, Eppendorf Inc.).

SSR primers selection and PCR reaction

Eighty-five SSR primers were chosen on all chromosomes at around 16-20 cM distances based on the reported rice SSRs (IRGSP 2005). The map position, original source, and repeat motifs for these markers can be found in the rice genes database (http://www.gramene.org/microsat/RM_primers). The polymerase chain reaction (PCR) was conducted in a reaction volume of 20 μL containing 1 × PCR buffer, 200 μM of each dNTP, 0.4 μM of each primer, 1.6 mM $MgCl_2$, 0.5 units *Taq* DNA polymerase (BioFlux, Japan Inc.), and 50 ng of template DNA. The temperature cycling conditions were 5 min at 95 °C, followed by 35 cycles of 94 °C for 1 min, 55- 60 °C, based on annealing temperature of primers, for 1 min, and 72 °C for 2 min; this was followed by a final elongation at 72 °C for 10 min and a 10 °C hold. To detect polymorphism, the PCR-amplified products of all used SSR markers were evaluated on a 3% agarose gel (0.5 × TBE).

Statistical analysis

The test of normality was performed for all characters with the Shapiro-Wilks test through the STATISTICA 7.0 software (Statistica 2004). The collected data from the check cultivars were subjected to analysis of variance (ANOVA) using a randomized complete block design. The F_3 generation analysis for all corresponding characters was conducted using one-way ANOVA based on the Kearsey and Pooni (1996) method. There were 4 parameters, namely V_A (additive component of genetic variance), V_D (dominant component of genetic variance), V_{EC} (common environmental variance), and V_E (environmental component of variance), but only 2 statistics, σ^2_w and σ^2_B. Therefore, it was necessary to ignore 2 parameters, and as the environmental variation (V_E) was clear, V_D and V_{EC} were set at 0 (Kearsey and Pooni 1996; Amiri-Fahliani et al. 2010). In the ANOVA table of the F_3 generation, the between (MS_B) and the within (MS_W) families variance sources of variation had expectations $\sigma^2_w + r\,\sigma^2_B$ and σ^2_w, respectively, in which r was equal to the number of individuals per family. Using the expectation values of σ^2_w and σ^2_B given for the F_3 generation (Kearsey and Pooni 1996), $\sigma^2_B = \frac{MS_B - MS_W}{r}$ and $\sigma^2_w = MS_W$ (Amiri-Fahliani et al. 2010). Supposing V_D and V_{EC} were zero, then $V_A = \sigma^2_B$ and $V_E = \sigma^2_w - {}^1/_2\, V_A$ (Kearsey and Pooni 1996). Weighted least squares (WLS) were used in multiple variable regression methods to estimate the standard errors (SE) of parameters (Amiri-Fahliani et al. 2010).

Narrow sense heritability of characters was evaluated as the ratio of additive genetic variance (V_A) to phenotypic variance (V_P) (Falconer and MacKay 1997; Singh 2005) as

$$h^2 = V_A / V_P$$

Results

The effect of blocks was not significant statistically based on the ANOVA of control cultivars for corresponding characters (data not shown), and thus it was unnecessary to adjust the data obtained from the $F_{2:3}$ families.

The 2-parameter model based on V_A and V_E was adequate for the traits in variance analysis (data not shown). Using one-way ANOVA, additive variance (V_A) was significant for all the traits ($P < 0.05$) (Table 2). The amount of V_E was not significant only for the brown rice shape ($P > 0.05$) (Table 2). Narrow sense heritability (h^2) of all corresponding characters has given in Table 2. It was low (<0.18) for panicle length and culm number, and very high (>0.84) for brown rice features. In addition, plant height, culm length, and seedling height had high heritability (≥0.5).

The means of parents and of selected bulks of high and low extremes for all evaluated characters are given in Table 3. The parents Mousa-Tarom and 304 had close means for seedling height and panicle length. Under our experiment conditions, a significant difference could not be detected between parents for seedling height and panicle length ($P > 0.05$, t-test) (Table 3). However, the differences between selected bulks of high and low extremes were statistically significant ($P < 0.01$) for both seedling height and panicle length characters. In view of the mean of parents and selected bulks for each character, transgressive segregation was seen for all characters (Table 3).

Using the BSA method for all corresponding characters, the linked SSR marker(s) to each character were detected (Table 4). As the SSR markers have a distinct position on the chromosomes of rice, the approximate position or at least the arm of the chromosome carrying the gene(s) controlling the corresponding characters could be detected (Tables 4 and 5). The SSR primers RM16589 and RM17166 on chromosome 4 showed linkage with gene(s) controlling culm length, plant height, leaf width, culm number, grain width, and grain shape (Table 4). On chromosome 11, the primer RM26063 showed linkage with plant height, panicle length, leaf width, grain length, grain width, and grain shape. In addition, the primer RM26291 (RM3625) on chromosome 11 showed linkage with culm length, panicle length, leaf length, culm number, grain length, and width, and RM26509 showed linkage with grain shape. For grain shape, chromosomes 1, 4, 5, and 11 carried its controlling genes. Leaf width seemed to be under control of the genes on chromosomes 3, 5, and 11 (Table 4).

Discussion

Comparing the obtained results from the 2-parameter model of multiple regression (data not shown) and the ANOVA method (Table 1) of variance, a component analysis showed that the results derived from the ANOVA method of Kearsey and Pooni (1996) were completely similar to the results obtained from the 2-parameter model of the multiple regression method. This was not out of suspense, because the 2-parameter model of multiple regression method is similar to the ANOVA method with ignoring the dominance and V_{EC} components of variance. Based on the adequacy of the 2-parameter model of regression (data not shown) it could be

Table 2. Variance components and their ± standard errors of evaluated characters, and narrow sense heritability resulting from ANOVA of $F_{2:3}$ families based on Kearsey and Pooni's (1996) method.

Parameters	Culm Length (cm)	Plant Height (cm)	Panicle Length (cm)	Seedling Height (cm)	Culm Number	Leaf Length (cm)	Leaf Width (mm)	Brown Rice Length (mm)	Brown Rice Width (mm)	Brown Rice Shape[1)]
V_A	41.851**	48.280**	1.579**	18.012**	4.683**	19.863**	0.779**	0.180**	0.025**	0.137**
	± 5.743	± 6.769	± 0.303	± 2.460	± 0.864	± 2.958	± 0.109	± 0.023	± 0.003	± 0.017
V_E	29.361**	48.306**	8.083**	15.970**	21.496**	34.649**	0.898**	0.034**	0.003*	- 0.015
	± 3.581	±4.562	± 0.404	± 1.612	± 1.092	± 2.387	± 0.076	± 0.012	± 0.002	± 0.009
h^2(Narrow)	0.5877	0.4999	0.1634	0.5300	0.1789	0.3644	0.4643	0.8419	0.8840	1.0000

[1)] Grain length/width ratio. * $P < 0.05$, and ** $P < 0.01$

Table 3. Selected bulks, the means of parents and selected bulks, and the *t*-test results for evaluated characters.

Character	Parents Mean		t-test	Selected Bulks Mean		t-test
	Mousa-Tarom	304		Low Extreme	High Extreme	
Seedling Height (cm)	31.05	28.90	ns	17.26	38.06	**
Culm Length (cm)	86.28	68.71	**	62.06	89.26	**
Plant Height (cm)	108.60	89.54	**	82.72	112.04	**
Panicle Length (cm)	22.32	20.83	ns	17.74	24.41	**
Culm Number (cm)	16.60	13.18	*	12.80	29.60	**
Leaf Length (cm)	75.70	65.48	**	54.52	75.32	**
Leaf Width (cm)	11.45	13.08	**	9.95	13.96	**
Brown Rice Length (mm)	6.57	5.59	**	5.62	7.26	**
Brown Rice Width (mm)	2.09	2.50	**	1.94	2.56	**
Brown Rice Shape	3.17	2.24	**	2.27	3.66	**

ns non-significant, * $P < 0.05$, and ** $P < 0.01$

Table 4. SSR markers, chromosome number carrying the marker, chromosome (Chr.) position (bp), and bulked characters.

SSR markers	Chr. number	Chr. Position[1)]	Bulked characters
RM12243 (RM3694)	1	3276	Grain shape
RM15838	3	13729	Panicle length, Leaf length, Grain length
RM16589	4	31369	Culm length, Plant height, Leaf width, Culm number, Grain width, Grain shape
RM17166	4	72253	Leaf width
RM18265	5	13442	Grain width
RM18421	5	248	Leaf width, Grain width, Grain shape
RM21943 (RM6420)	7	21277	Culm number, Grain width
RM26063	11	30190	Plant height, Panicle length, Leaf width, Grain length, Grain width, Grain shape
RM26291 (RM3625)	11	29	Culm length, Panicle length, Leaf length, Culm number, Grain length, Grain width
RM26509	11	77891	Grain shape

[1)] Chromosome position of the marker in base pairs (bp) distance from the beginning of short to long arm.

stated that there was no reason for the existence of a dominant or epistatic effect of the controlling genes for any of the traits under study (Kearsey and Pooni 1996).

The amounts of narrow sense heritability obtained for the characters in this study were in agreement with previous reports for both grain length (Kato 1990; Rabiei et al. 2004, Vanaja and Babu 2006) and grain width (Rabiei et al. 2004, Vanaja and Babu 2006), but for grain width it was not in agreement with Kato (1990). High h^2 of seedling height showed that this character could be selected in early generations of segregation, and this was contrary to the report by Takahashi (1984). The low h^2 of panicle length was in agreement with a previous report (Smith and Dilday 2003). Breeding for traits with low h^2 (<0.2) is difficult because low h^2 means that the phenotype is not highly correlated with the genotype. In other words, the contribution of the environmental conditions is relatively high in such traits (Singh 2005). As the variance could not be negative, it could be postulated that a contradictory event, such as the negative amount of V_E for brown rice shape (Table 2), occurred because of experimental errors. A low h^2 of culm number and panicle length showed high environmental effects on these traits. Similar results of low h^2 for culm number have also been reported previously (Hoshikawa 1989; Moldenhauer et al. 1994; Gravois and Helms 1996). A number of environmental factors such as manuring, planting density, light, temperature, and water supply influenced the tillering power of plants (Smith and Dilday 2003). Therefore, to get the desired culm number and panicle length in the farm, farmers could use adequate fertilizers.

Comparing the parents with selected bulks (Table 3), it could be interpreted that the controlling genes of seedling height and panicle length were dispersed in the parents. This claim is in accordance with Kearsey and Pooni (1996). The identified, linked SSRs in the present study (bold face) with the orderly, prior reported markers (normal face) have been shown in Table 5. The introduced linked markers

Table 5. Orderly prior (normal face) and newly identified (bold face) linked markers, chromosome number, order, and distance of markers.

Linked Markers	Chromosome number	Order(S-L)[a]	Distance bp (S-L)[b]
RM529, RM12143	1	2143	40,693,740
RM3694, RM12243	**1**	**3276**	**42,652,023**
RM168, RM15749	3	1517	27,907,093
RM15838	**3**	**1606**	**29,284,194**
RM5414, RM518, RM16352	4	102	2,025,820
RM16589	**4**	**339**	**11,328,211**
RM17166	**4**	**916**	**24,623,693**
RM437, RM17964	5	256	
RM18265	**5**	**557**	**11,227,894**
RM18421	**5**	**713**	**15,744,817**
RM11, RM21672	7	898	19,218,640
RM6420, RM21943	**7**	**1169**	**24,756,474**
RM26063	**11**	**122**	**2,274,145**
RM167, RM26176	11	235	4,061,132
RM26291, RM3625	**11**	**350**	**6,591,247**
RM202, RM26402	11	461	8,923,471
RM26509	**11**	**568**	**11,246,668**

[a] Order of the markers from the beginning of short (S) to long (L) arm of each chromosome.
[b] Distance of markers to the beginning of the short arm in each chromosome.

in the present study were not similar to any of the previously reported ones (comparing Tables 4 and 5). In other words, different and new genes may exist for the related characters. Considering the similar markers on the same chromosomes linked to more than one character, it could be inferred that those characters may have different but close controlling genes, or may be a single gene controlling all these traits (pleiotropy). Based on the results from the BSA and the markers distance (<20 cM), it could not be said that the characters with unique linked markers were controlled by the same gene, especially when a 9900 bps sequence size of the genes in rice had been reported (IRGSP 2005). Ultimately, it was seen that some characters were under the control of more than one gene located on different chromosomes. The most interesting finding was that the grain shape, that is, the ratio of grain length to grain width, seemed to have controlling genes different from the genes controlling grain length and width. For example, on chromosome 1 there was an SSR marker (RM12243) that was only linked to the grain shape. Although the grain shape was computed as a ratio here, its controlling gene(s) could affect the grain shape totally as a single character.

Conclusion

Knowing the heritability of the traits is very important for plant breeding projects. In addition, finding sure-linked markers to the related traits is very useful to select the traits, especially those with low heritability or those difficult for selection because of their metric properties. The $F_{2:3}$ families of the cross between 'Mousa-Tarom' and line '304' aimed to estimate the narrow sense heritability of the traits and to find their linked SSRs using bulked segregant analysis. Narrow sense heritability of the panicle length and culm number was very low, and that of the brown rice features was very high. The SSR primers RM16589, RM17166, RM26063, RM26291, and RM26509 showed linkage with the genes controlling the important agronomic characters. The chromosomes 1, 4, 5, and 11 had genes effective on the grain shape. The genes on the chromosomes 3, 5, and 11 controlled the leaf width. The linked markers reported in the present study are applicable to select the traits in breeding programs and to find tightly linked markers to these characters. In addition, they could be used as the basis for selecting the flanking markers of these parts of the chromosomes to find tightly linked markers to the desirable traits.

Acknowledgments

The authors are very thankful to ShareKord University for its financial support of this research. The first author wishes to express his thanks to S. Mousavi-Fard, A. Arminian, and H. Mardaani, for their invaluable assistances in different stages of practices in this study.

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