

## Genetic dissection of biomass production, harvest index and panicle characteristics in *indica-indica* crosses of Iranian rice (*Oryza sativa* L.) cultivars

\*Hossein Sabouri<sup>1</sup>, Atefeh Sabouri<sup>2</sup>, Ahmad Reza Dadras<sup>3</sup>

<sup>1</sup>Department of Plant Production, Gonbad High Education Center, P.O. Box 163 Golestan, Gonbad, Iran

<sup>2</sup>Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Guilan,  
P.O. Box 41635-1314, Rasht, Iran

<sup>3</sup>Former student in Azad University of Lahijan

\*Corresponding author: Saboriho@yahoo.com

### Abstract

Improvement of rice (*Oryza sativa* L.) yield through breeding requires a good understanding of genetic factors that control component traits. In the current study, traits associated with this multiplicative relationship were investigated in a field trial with F<sub>2</sub> and F<sub>2:3</sub> population of rice. One-hundred-and-ninety-two individuals and families derived from a cross between two genetically divergent high-yielding varieties, Khazar (KHZ, *indica* type with a large biomass) and Tarommahalli (TAM, *indica* type with a low biomass), were grown in Rice Research Institute of Iran and Gonbad High Education Center to detect QTLs for biomass and related traits. Result of mapping on F<sub>2</sub> population showed that QTLs controlling GWP (qGWP-3a and qGWP-3b) overlapped with the QTLs for number of SNP (qSNP-3a and qSNP-3b) and the QTL for HI (qHI-3). Five QTLs were mapped for harvest index. These QTLs were showed the large effects on the harvest index and explained 21.35 to 31.28% of the total phenotypic variance. Result of F<sub>2:3</sub> population showed that QTLs controlling GWP (qGWP-2, qGWP-7 and qGWP-12) overlapped with the QTLs for number of SNP (qSNP-2, qSNP-7 and qSNP-12). Clustering of the QTLs for different traits at the same markers interval in both of mapping population was observed for GWP, HI and SNP. These result implied that HI, might partly share a common genetic basis with GWP and SNP. Our result indicated that breeding methods of QTLs pyramiding by using marker-assisted selection could be very useful for the development of new varieties with a high level of grain yield.

**Keywords:** biomass; grain yield; rice (*Oryza sativa* L.); QTL mapping.

**Abbreviation:** KHZ\_ Khazar variety; TAM\_ Tarommahalli variety; PH\_ plant height; HI\_ Harvest index; GWP\_ weight of grain panicle; SNP\_ spikelet number of panicle; LFL\_ length of flag leaf; WFL\_ width of flag leaf; BI\_ biomass; QTL\_ Quantitative trait loci; CIM\_ composite interval mapping

### Introduction

Rice (*Oryza sativa* L.) is the world's second most important cereal crop that providing half of the feed for world population and more than 90 percent of rice produced is consumed in Asia. Therefore, it is necessary to increase rice production (Yoshida, 1981). Most of the earlier studies have been used potential classic plant breeding method to improve

traits contributing to yield (Brondani et al., 2002). Yield, yield components and agronomically useful traits for example rice grain number, grain weight, plant height, biomass and harvest index have continuous phenotypic distributions, implying that many genes with relatively minor effects, termed quantitative traits loci (QTLs) control them (Falconer

and Mackay, 1996). Using of DNA markers enabled researchers to identify QTLs affecting quantitative traits (Liu, 1998). A great deal of QTL mapping for these traits, have been conducted using various mapping populations derived from inter-specific crosses (Xiong et al., 1999; Moncada et al., 2001; Thomson et al., 2003) *indica-japonica* inter-subspecific crosses (Yu et al., 1997; Lu et al., 1996; Xiao et al., 1996; Redona and Mackill 1998; Sasahara et al., 1999; Yagi et al., 2001; Xing et al., 2002; Mei et al., 2003, 2005). These studies were showed that detected QTLs for the traits related to yield were distributed throughout all rice chromosomes.

Xu et al. (2004) dissected the relationship between three traits of the primary sink size-spikelet number per panicle, panicle number per plant, and 1000-grain weight, and their 10 component traits in 292 F<sub>13</sub> recombinant inbred lines using a complete linkage map. Some QTLs had large effects on panicle number (*QPn4*), panicle branching and length (*QPbn3a*, *QPbn3b* and *QPbl4*), grain length and volume (*QGl3*, *QGl5* and *QGv2*), and grain shape (*QGs1* and *QGs7*). Zhang et al. (2004) mapped QTLs related to grain yield, biomass, harvest index, spikelet number and plant height in a double haploid population of rice. qGY-3, qBM-1-2, qHI-11, qSNP-3-2 and qPH-2 detected as major QTL, for grain yield, biomass, harvest index, spikelet number and plant height and explained 23.3, 28.1, 22.6, 27.2 and 28.7% of total phenotypic variation, respectively.

Guo et al. (2005) mapped QTLs related to yield, tillers per plant, grain per plant and grain weight using of conditional and unconditional QTL mapping methods. Unconditional QTL mapping detected a QTL for grains per plant that explained 14.84% of total phenotypic variation.

Xie et al. (2008) Using of a series of BC<sub>3</sub>F<sub>4</sub> nearly isogenic lines (NILs) derived from a cross between the Korean *japonica* cultivar Hwaseongbyeon and *Oryza rufipogon* (IRGC 105491), mapped seven QTLs for 1,000-grain weight, spikelets per panicle, grains per panicle, panicle length, spikelet density, heading date and plant height. All seven QTLs were additive, and alleles from the low-yielding *O. rufipogon* parent were beneficial in the Hwaseongbyeon background.

Zhao et al. (2008) simultaneously mapped unconditional and conditional QTLs for tiller numbers at various stages by using single segment substitution lines in rice. Fourteen QTLs for tiller number, distributing on the corresponding substitution segments of chromosomes 1, 2, 3, 4, 6, 7 and 8 were detected. Both the number and the effect

of the QTLs for tiller number were various at different stages. The tiller number at a specific stage was determined by sum of QTL effects estimated by the unconditional method, while the increasing or decreasing number in a given time interval was controlled by the total of QTL effects estimated by the conditional method.

Hong et al. (2008) detected QTLs yield traits (panicles per plant, number of filled grains per panicle, total number of spikelets per panicle, spikelet fertility, 1000-grain weight, and grain yield per plant ) on the short arm of rice chromosome 6 in F<sub>2:3</sub> population consisting of 221 lines was derived and grown in two trial sites. Most of the QTLs for yield components acted as additive QTLs, while the three QTLs for grain yield had dominance degrees of 1.65, 0.84 and -0.42, respectively.

However, a few studies reported QTL mapping of agronomic traits in *indica-indica* (Lin et al., 1996; Zhuang et al., 1997) or *japonica-japonica* crosses (Yamagishi et al., 2002). Obviously, the possibility of obtaining fertile hybrids and the potential for identifying and transferring useful genes for agronomic traits are important factors in success of breeding programs that are based on the interspecific crosses (Brobdani et al., 2002). Crossing *indica-indica* varieties will cause to fertile hybrid. We studied mapping of biomass, yield and yield component traits in a F<sub>2</sub> population derived from two Iranian *indica* varieties. The identification of QTLs represents the first step toward dissecting molecular basis of naturally occurring genetic variation for complex traits such as agronomic traits.

The objectives of this study were: (1) To identify putative QTLs for biomass and panicle characteristics in *indica-indica* Iranian rice crosses. (2) To identify SSR markers linked to the traits.

## Materials and methods

### Field experiment

Field experiments were conducted in Rice Research Institute of Iran (2006) and Gonbad High Education Center (2008). 192 individual of F<sub>2</sub> population developed from the 17 cross between Taromahalli (TAM), a traditional *indica* cultivar and Khazar (KHZ), an improved *indica* cultivar. In first experiment (2006), the seeds of F<sub>2</sub> population and their parental cultivars were soaked in distilled water at 30°C for 2 days, after surface-sterilization with 70% ethanol solution and 1% sodium hypochlorite solution. Germinated seeds were sown on a paddy

**Table 1.** Phenotypic value of parents and F<sub>2</sub> and F<sub>2:3</sub> population of measured in rice.

Trait <sup>a</sup>	Parents (2006) and F <sub>1</sub>			Parents (2008) and F <sub>1</sub>			F <sub>2</sub> (mean ±S.D.)	F <sub>2:3</sub> (mean ±S.D.)
	TAM	TAM	F1	KHZ	TAM	F1		
	(mean ±S.D.)	(mean ±S.D.)	(mean ±S.D.)	(mean ±S.D.)	(mean ±S.D.)	(mean ±S.D.)		
GWP	0.57±0.15	3.98±0.56	1.60±0.44	0.62±0.48	3.88±0.93	1.560±0.32	1.36±1.88	1.28±1.67
SNP	100.50±0.71	152.00±0.64	143.61±0.33	104.50±0.61	160.00±0.75	149.45±0.54	139.85±3.78	137.95±3.99
PH	160.8. ±3.61	117.90±1.15	149.89±1.77	159.8. ±3.74	121.90±1.23	147.56±2.65	142.01±13.69	139.21±13.76
HI	33.65± 2.04	41.76±1.98	43.45±0.98	32.55± 2.04	44.56±1.68	47.33±1.55	38.33±12.44	35.44±14.32
LFL	37.19±0.51	23.37±0.74	35.78±0.35	34.29±0.51	26.77±0.46	36.34±0.37	29.09±5.35	31.59±5.72
WFL	1.79±0.05	0.98±0.13	1.43±0.16	1.49±0.65	1.03±0.23	1.43±0.20	1.39±0.22	1.62±0.34
BI	50.90±10.36	117.40±13.74	98.88±0.8.76	50.80±10.77	120.40±12.84	97.92±6.98	90.20±30.07	92.71±32.88

<sup>a</sup> plant height (PH), Harvest index (HI), weight of grain panicle (GWP), spikelet number of panicle (SNP), length of flag leaf (LFL), width of flag leaf (WFL) and biomass (BI) in the Taromahalli /Khazar F<sub>2</sub> and F<sub>2:3</sub> population.

field. The plants were transplanted into a paddy field with single planting per hill. The space between hills was 25 cm. In second experiment (2008), 192 F<sub>2:3</sub> families were used for traits phenotyping. After sterilization of seeds, 192 F<sub>2:3</sub> families were grown as spaced plants and transplanted at four rows. Distance of 25 cm between plants on a row and the rows were 25 cm apart. Only the 28 plants in the middle of each row were used for traits scoring. The field managements followed the normal agricultural practice in both of place and year.

Phenotypic measurements were performed for seven traits according to SES system at both years as follows: Grain weight for main panicle per plant (GWP), Length and width flag leaf (LFL and WLF), Plant height (PH) measured in centimeters from the soil surface to the tip of tallest panicle excluding the awns, Spikelet Number of main panicle per plant (SNP), The biomass (BI) per plant referred as to the above-ground biomass (grain yield and total plant straw) and harvest index (HI) was calculated as the ratio of grain yield to biomass.

### Construction of SSR linkage map

Leaves from the main stem of each plant examined were sampled, and genomic DNA was extracted according to CTAB method (SaghiMaroof et al., 1994). Marker analysis using 365 SSR markers were conducted to identify polymorphic markers from available rice SSR markers (McCouch et al, 2002; Chen et al., 1997; Temnykh et al., 2000). Polymorphic primers between two parents were used for whole F<sub>2</sub> population screening. A chi-square test was performed on each marker to verify the expected 1:2:1 segregation ratio. The expected genotypic frequency was 0.25 for homozygote A (used for Taromahalli alleles), 0.5 for heterozygous H (used

for Taromahalli and Khazar alleles, spontaneously), and 0.25 homozygote B (used for Khazar alleles). A SSR linkage map of F<sub>2</sub> population was constructed using MapmanagerQTX (Manly and Olson, 1999), and the genomic distance (cM) were calculated from recombination value using Kosambi function (Kosambi, 1944). QTLs affecting relative parameter were sought with composite interval mapping (CIM) using QTL Cartographer v 2.5 (Basten et al., 2001) and a threshold LOD of 2.5 were used for test to claim the presence of QTL. Automatic cofactor selection using a forward/backward regression was performed using QTL cartographer v 2.5. For each QTL, the position, the additive effect and the percentage of phenotypic variation explained were estimated.

## Results

### Result of phenotypic evaluation

Some F<sub>2</sub> progenies and F<sub>2:3</sub> families showed extreme performance for nearly all traits, with a higher value than that of the better parent and smaller than (Fig 1, 2) that of the worse parent, indicating transgressive segregation for all traits in the populations.

### Population structure and linkage map

In rice, high density molecular linkage maps have been constructed (Chen et al., 1997; Temnykh et al., 2000; McCouch et al., 2002), and utilized for studying quantitative traits related to agronomical characters (Xiao et al., 1996., Lu et al., 1996., Redona and Mckill 1998; Ishimaura et al., 2001; Mei et al., 2003; Mei et al., 2005; Guo et al., 2005). Biomass, yield and yield component in rice has been studied extensively (Moncada et al., 2001; Xing et al., 2002;

**Table 2.** putative QTLs for traits in the F<sub>2</sub> population derived from TAM and KHZ.

Traits	QTL <sup>a</sup>	Chr.	Flanking markers	LR	a <sup>b</sup>	d <sup>c</sup>	PEV <sup>d</sup>	Dpe <sup>e</sup>
BI	qBI-5	5	RM440-RM5140	11.63	14.5	6.06	21.44	KHZ
PH	qPH-1	1	RM8097-RM3475	11.74	-4.05	-2.03	16.3	KHZ
	qPH-5	5	RM440-RM5140	15.23	-5.23	-0.94	21.48	KHZ
LFL	qLFL-2	2	RM8254-RM262	16.08	-1.21	-1.47	15.58	KHZ
	qLFL-4	4	RM5642-RM6589	14.09	2.36	-2.37	22.06	TAM
WFL	qWFL-7	7	RM5481-RM11	13.21	-0.02	-0.1	4	KHZ
GWP	qGWP-2	2	RM3865-RM3294	15.67	0.25	0.16	26.41	KHZ
	qGWP-3a	3	RM416-RM6832	15.02	-0.47	0.09	31.23	TAM
	qGWP-3b	3	RM6832-RM5626	14.63	-0.46	0.15	31.39	TAM
	qGWP-5	5	RM421-RM480	24.17	-0.52	0.25	33.84	TAM
	qGWP-12	12	RM7626-RM1337	11.56	-0.29	-0.13	27.31	TAM
SNP	qSNP-2a	2	RM3865-RM3294	22.27	9.96	10.64	18.22	KHZ
	qSNP-2b	2	RM3294-RM7426	20.17	9.62	10.23	18.09	KHZ
	qSNP-3a	3	RM416-RM6832	13.92	-18.78	4.97	26.08	TAM
	qSNP-3b	3	RM6832-RM5626	14	-18.51	6.94	26.39	TAM
	qSNP-4	4	RM5642-RM6589	12.77	3.37	-19.02	21.64	KHZ
	qSNP-5	5	RM421-RM480	15.74	-16.37	9.61	25.96	TAM
	qSNP-12	12	RM7626-RM1337	12.28	-10.41	-7.08	24.72	TAM
HI	qHI-2	2	RM3865-RM7426	13.79	2.36	2.84	21.35	KHZ
	qHI-3	3	RM416-RM5626	16.11	-7.21	3.8	31.28	TAM
	qHI-4a	4	RM5642-RM6589	18.48	1.31	-8.28	22.51	KHZ
	qHI-4b	4	RM5473-RM551	12.01	4.24	-7.31	26.96	KHZ
	qHI-5	5	RM421-RM480	15.52	-5.3	-0.49	24.7	TAM

<sup>a</sup> QTLs are named by abbreviations plus chromosomal number

<sup>b</sup> Additive effect

<sup>c</sup> dominance effect

<sup>d</sup> percentage of total phenotypic variance explained by the QTL

<sup>e</sup> direction of phenotypic effect, TAM and KHZ indicate TAM and KHZ, respectively.

Thomson et al., 2003; Septiningsih et al., 2003; Zhang et al., 2004). In this study, out of the 365 SSR markers tested, 85 produced polymorphic bands between the two parents and 74 primers amplified clear and scorable bands for F<sub>2</sub> individuals. A linkage map based on F<sub>2</sub> population was constructed, which covered a total of 1231.50 cM with an average two locus interval of 19.83 cM (Fig. 3). The position of most SSR markers on chromosomes was similar to the previously reported maps but the distance between markers were not similar.

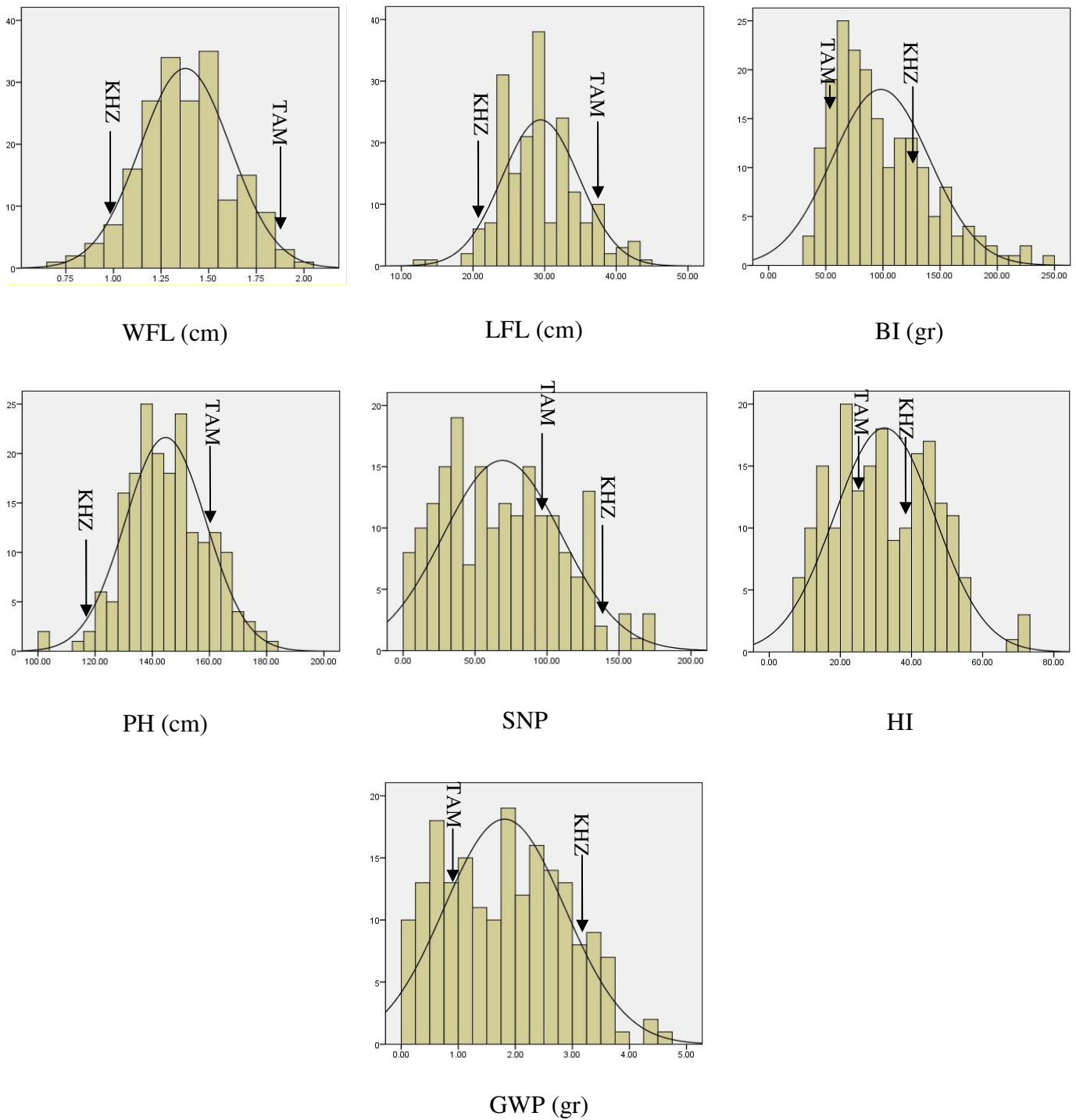
### QTL mapping

Chromosomes 1, 2, 3, 4, 7 and 8 had relatively good marker coverage based on the comparison of their physical length in pro-metaphase and on the total number of marker mapped. In other chromosome, fewer markers were mapped and this would certainly restrict the power to detect significant associations. In first year of experiment, QTLs associated with 7 traits were detected on 7 chromosomes (1, 2, 3, 4, 5, 7 and 12) and in second year, QTLs related to these traits

were mapped on 9 chromosomes (1, 2, 3, 4, 5, 7, 8, 11 and 12). Significant marker/trait associations were detected on chromosome 5, 11 and 12 even with limited marker coverage. 23 and 24 QTLs associated with GWP, BI, HI, SNP, PH, LFL and WFL were detected (Table 2 and 3) and phenotypic variation explained by a single QTL varied from 4 to 34% and 17.33 to 29.28%, in 2006 and 2008, respectively.

### QTL for biomass (BI)

In first experiment, one QTL was mapped on chromosome 5 for biomass. This QTL with a LR score of 11.63, showed large effect on the BI and explained 21.44% of the total phenotypic variance. In qBI-5, allele from KHZ increased BI by 14.50. The dominant effect of qBI-5 was 6.06 gr (Table 2). Two QTLs were detected on chromosomes 3 and 5, using of F<sub>2,3</sub> families. In qBI-3 and qBI-5 alleles from KHZ increased BI and showed large effects on the BI with explaining 22.11 and 23.52% of the total phenotypic variance (Table 3).



**Fig 1.** Histogram related to plant height (PH), Harvest index (HI), weight of grain panicle (GWP), spikelet number of panicle (SNP), length of flag leaf (LFL), width of flag leaf (WFL) and biomass (BI) in the Taromahalli /Khazar  $F_2$  population.

**Table 3.** putative QTLs for traits in the F<sub>2:3</sub> population derived from TAM and KHZ

Traits	QTL <sup>a</sup>	Chr.	Flanking markers	LR	a <sup>b</sup>	d <sup>c</sup>	PEV <sup>d</sup>	Dpe <sup>e</sup>
BI	qBI-3	3	RM1022-RM6283	14.24	18.52	10.67	22.11	KHZ
	qBI-8	5	RM440-RM5140	17.22	3.14	-12.17	23.52	KHZ
PH	qPH-8	5	RM440-RM5140	18.38	4.25	8.32	23.35	TAM
LFL	qLFL-3	3	RM1022-RM6283	17.85	-2.24	-3.28	20.00	KHZ
	qLFL-4	4	RM5642-RM6589	15.72	2.57	2.86	18.98	TAM
	qLFL-8	8	RM6208-RM8264	12.13	1.13	-3.79	23.44	TAM
WFL	qWFL-1	1	RM466-RM314	18.14	0.15	0.15	20.40	TAM
	qWFL-8	8	RM6208-RM8264	13.20	0.13	-0.15	18.94	TAM
	qWFL-11	11	RM144-RM1341	17.05	-0.076	-0.03	22.58	KHZ
GWP	qGWP-1	2	RM3865-RM3294	12.789	0.27	0.32	19.42	KHZ
	qGWP-1	1	RM466-RM314	13.28	0.16	0.20	19.45	KHZ
	qGWP-1	3	RM416-RM6832	13.86	-0.17	0.23	19.70	TAM
	qGWP-1	5	RM421-RM480	21.74	-0.31	0.46	22.62	TAM
	qGWP-7	7	RM5481-RM11	23.47	0.05	-0.66	24.22	KHZ
	qGWP-5	11	RM144-RM1341	13.47	-0.36	0.13	22.30	TAM
	qGWP-11	12	RM7626-RM1337	15.44	-0.02	0.51	27.80	TAM
SNP	qSNP-1	2	RM3865-RM3294	11.92	12.41	9.58	18.41	KHZ
	qSNP-1	3	RM1022-RM6283	14.31	14.11	8.76	21.12	KHZ
	qSNP-1	3	RM416-RM6832	11.79	7.62	7.65	18.22	KHZ
	qSNP-1	3	RM6832-RM5626	22.04	14.38	14.48	20.84	KHZ
	qSNP-7	7	RM5481-RM11	21.43	1.05	-24.16	23.75	KHZ
	qSNP-11	12	RM7626-RM1337	17.79	-0.9105	21.38	29.28	TAM
HI	qHI-2	2	RM3865-RM7426	13.69	6.4828	8.74	17.33	KHZ
	qHI-10	4	RM5473-RM551	19.14	8.7659	-4.61	19.06	KHZ

<sup>a</sup> QTLs are named by abbreviations plus chromosomal number

<sup>b</sup> Additive effect

<sup>c</sup> dominance effect

<sup>d</sup> percentage of total phenotypic variance explained by the QTL

<sup>e</sup> direction of phenotypic effect, TAM and KHZ indicate TAM and KHZ, respectively.

### *QTLs for plant height (PH)*

Two QTL for PH were identified on chromosome 1 and 5, in 2006. The QTLs qPH-1 and qPH-5 with a LR score of 11.77 and 15.22 showed the large effects on the PH and explaining 16.30 and 20.48% of the total phenotypic variance, respectively. In both QTLs alleles from KHZ decreased PH by -4.05 and -5.24 cm (Table 2), whereas, one QTL was detected on chromosome 5 that QTL allele from TAM with large effect and explaining 23.25% of the total phenotypic variance increased PH by 4.25 cm, in 2008 (Table 3).

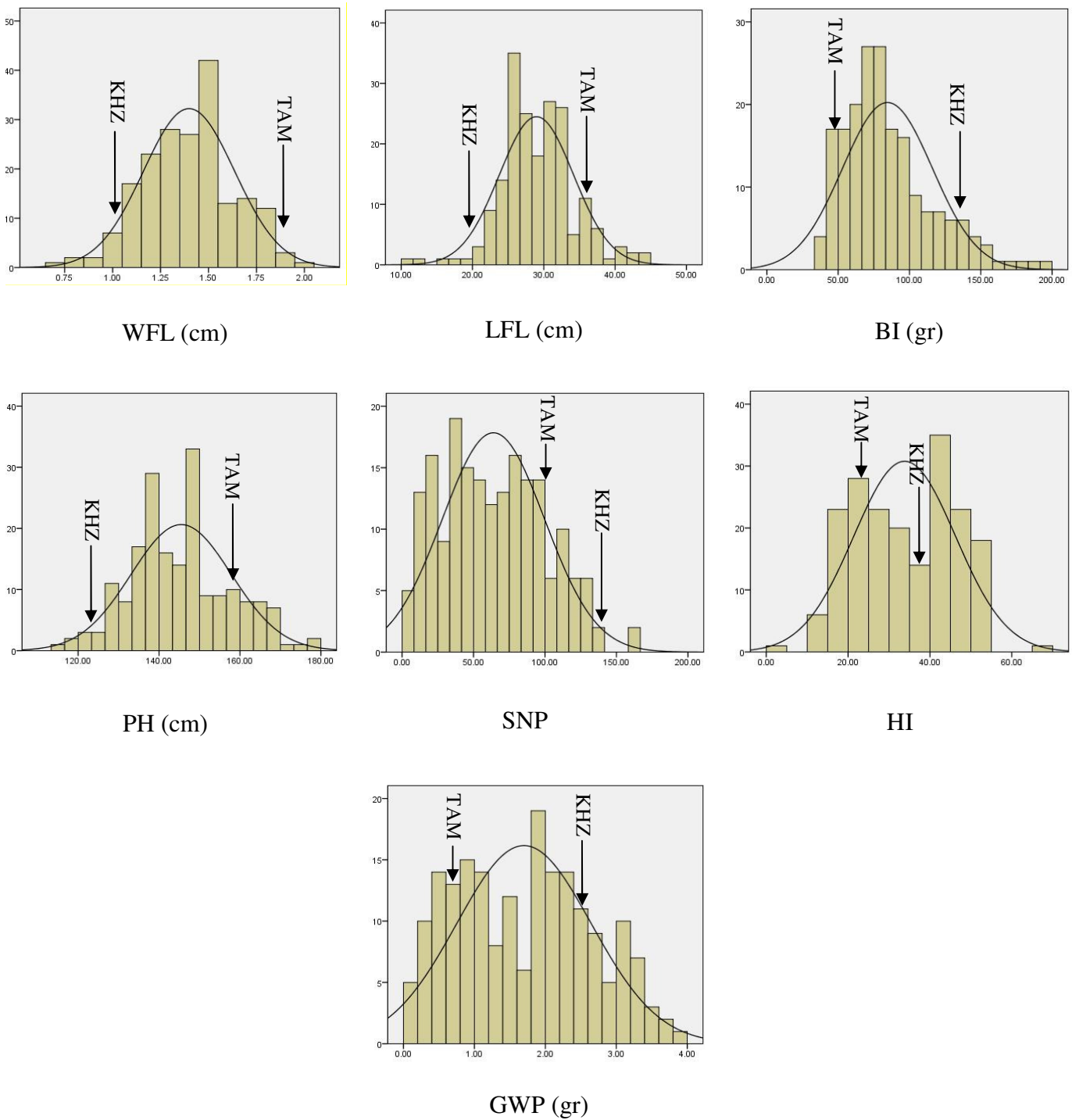
### *QTLs for length of flag leaf (LFL)*

For this trait, two QTLs were mapped at first of experiment. The QTLs, qLFL-2 and qLFL-4 with an LR score of 16.08 and 14.09 showed the large effects on the LFL and explained 14.57 and 22.06% of the total phenotypic variance and had additive effects of -1.21 cm and 2.36 cm for decreased and increased LFL and were from KHZ and TAM, respectively. The dominance effects for QTLs were negative and

showed overdominance for qLFL-2 and complete dominance for qLFL-4 that decreased LFL (Table 2). At second of experiment, three QTLs were identified for LFL. One QTL of out of three QTLs was same with first experiment (qLFL-4). In F<sub>2:3</sub> mapping population, qLFL-4 with an LR score of 15.72 showed the large effects on the LFL and explained 18.98% of the total phenotypic variance and had additive effects of 2.57 cm for decreased LFL. QTLs alleles for decreased were from KHZ (Table 3).

### *QTL for width of flag leaf (WFL)*

One QTL and three QTLs for WFL were mapped in 2006 (on chromosome 7) and 2008 (on chromosomes 1, 8 and 11), respectively. qWFL-7 with a LR score 13.22, showed the low effect on the WFL, whereas qWFL-1, qWFL-8 and qWFL-11 explained 20.40, 18.94 and 22.58% of the total phenotypic variance. Two QTLs alleles out of all detected QTLs were from TAM and increased WFL. QTLs detected for WFL not overlapped in two places of experiments (Table 2 and 4).



**Fig 2.** Histogram related to plant height (PH), Harvest index (HI), weight of grain panicle (GWP), spikelet number of panicle (SNP), length of flag leaf (LFL), width of flag leaf (WFL) and biomass (BI) in the Tammahalli /Khazar  $F_{2:3}$  population.

**Table 4.** Correlation coefficients among traits studied

	BI	PH	LFL	WFL	SNP	GWP	HI
BI	1						
PH	0.293** -0.652**	1					
LFL	0.212** 0.311**	0.108 0.101	1				
WFL	0.153* 0.210**	-0.038 0.098	0.219** -0.105	1			
SNP	0.160* 0.451**	0.222** 0.137	0.230** 0.151*	-0.001 0.043	1		
GWP	0.204** 0.241**	0.267** 0.321**	0.229** 0.198*	0.016 0.753**	0.947** 0.874**	1	
HI	-0.084 0.121	0.154* 0.111	0.179* 0.193*	0.017 0.301**	0.718** 0.654**	0.715** 0.549**	1

\* and \*\* represented significant level at 5 and 1%, respectively. Above correlations relate to 2006 experiment and below correlations related to 2008 experiment.

#### ***QTLs for grain weight per panicle (GWP)***

Five QTLs were identified using F<sub>2</sub> mapping population. Two QTLs out of five QTLs located on chromosome 3 (Table 2 and 3). The QTLs qGWP-2, qGWP-3a, qGWP-3b, qGWP-5 and qGWP-12 with a LR score of 11.56 to 24.17 showed the large effects on the GWP and explaining 26.41 to 33.84% of the total phenotypic variance. In all QTLs alleles from TAM (except qGWP-2) decreased GWP by 0.42 gr on averages. These QTLs, exhibited partial dominance for decreased (qGWP-12) and increased (qGWP-3a, qGWP-3b, qGWP-2 and qGWP-5) GWP. In second experiment, seven QTLs were mapped on chromosomes 1, 2, 3, 5, 7, 11 and 12. All QTLs showed the large effects on the GWP and explaining 19.42 to 27.40% of the total phenotypic variance. QTLs alleles for three QTLs (qGWP-1, qGWP-2 and qGWP-7) from KHZ increased GWP.

#### ***QTLs for number of spikelet per panicle (SNP)***

Detected QTLs (Seven QTLs) of first experiment for SNP showed the large effect on SNP and explained from 18.22 to 26.39% of the total phenotypic variance. These QTLs located on chromosome 2 (two QTLs), 3 (two QTLs), 4, 5 and 12. The additive effect

of qSNP-4, qSNP-2a and qSNP-2b were positive whereas other QTLs had negative effects for SNP and these alleles were from TAM. qSNP-4 exhibited overdominance for decreased SNP. Three QTLs (qSNP-3a, qSNP-3b and qSNP-5) exhibited partial dominance for increased SNP and one QTL (qSNP-12) exhibited partial dominance for decreased SNP. qSNP-2a and qSNP-2b exhibited overdominance for increased SNP (Table 2). In second experiment, six QTLs were identified for SNP. These QTLs were showed the large effect on SNP and explained from 18.22 to 29.28% of the total phenotypic variance. In all QTLs (except qSNP-11) alleles from KHZ increased GWP. qSNP-2, qSNP-3 and qSNP-12 overlapped in two experiments and different mapping populations (Table 2 and 3).

#### ***QTLs for harvest index per plant (HI)***

In first year, five QTLs were mapped for HI. These QTLs showed the large effects on the HI and explained 21.35 to 31.28% of the total phenotypic variance (Table 2). Three QTLs had positive additive effects of 1.31 (qHI-4a), 4.23 (qHI-4b) and 2.36 (qHI-2) for increased HI. These putative QTLs alleles for HI were from KHZ. Other QTLs had negative additive effects of -7.21 (qHI-3) and -5.30 (qHI-5) for



decreased HI. These putative QTLs alleles for HI were from TAM. The dominant effects for qHI-3 and qHI-2 were positive and the dominant effects for other QTLs were negative. qHI-3 and qHI-5 showed partial dominance for increased and decreased HI, respectively whereas other QTLs (except qHI-2) showed overdominance for decreased HI. In second year, two QTLs were detected on chromosome 2 and 4. These QTLs showed the large effects on the HI and had positive additive effects of 6.48 (qHI-2) and 4.23 (qHI-4) for increased HI. Detected QTLs for HI on chromosome 2 and 4 overlapped and were mapped in the same regions (Table 3).

## Discussion

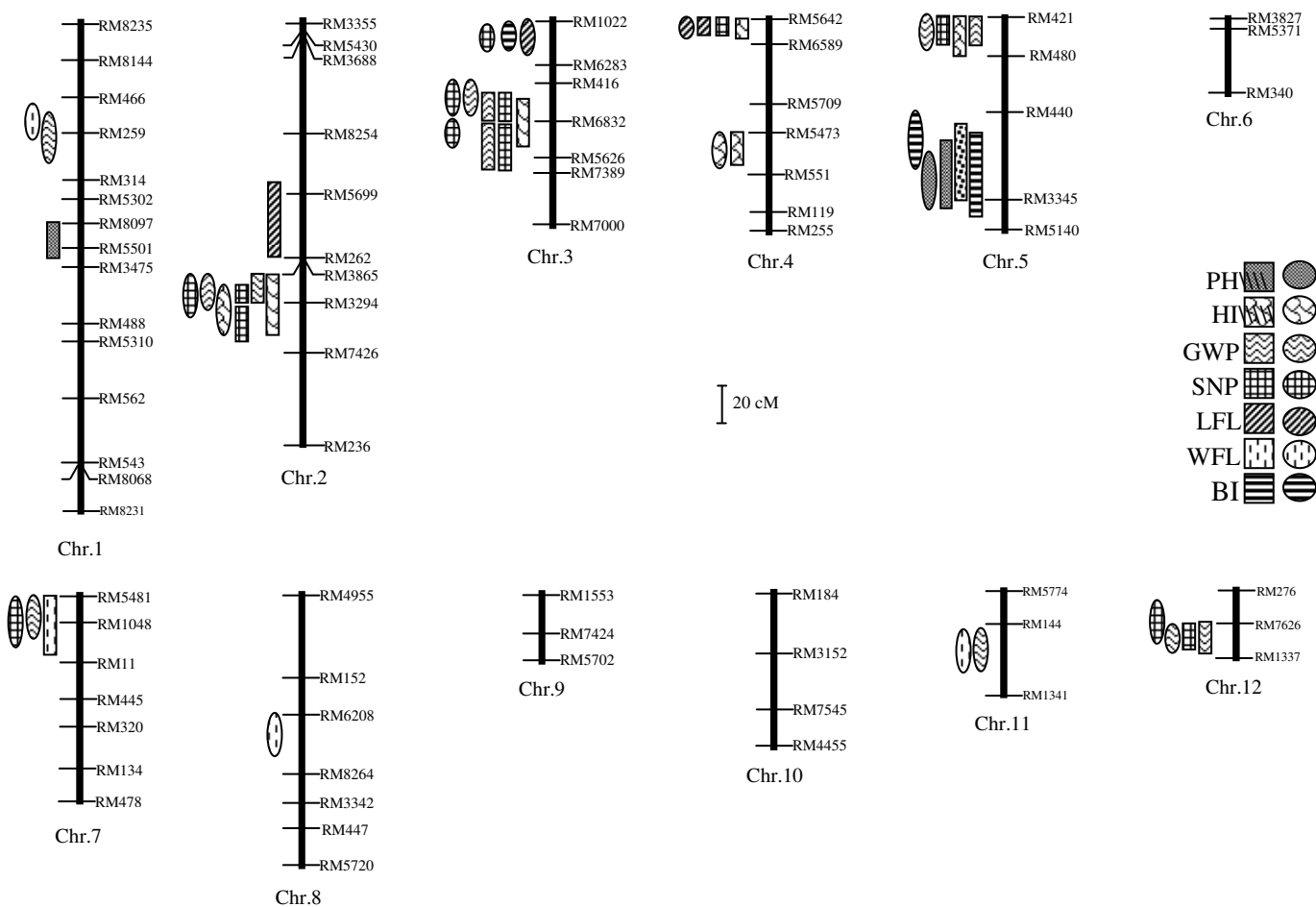
Transgressive segregation was found in  $F_2$  and  $F_{2:3}$  populations (Fig 1 and Fig 2) from the cross between TAM and KHZ. These results indicated that several genes are involved in the genetic control of the studied traits. Transgressive segregation might be attributed to the gathering of some QTLs associated with grain yield and yield related traits in Iranian population.

There was a weak positive correlation between the HI, PH, LFL and WFL for  $F_2$  populations (Table 4). In this population, a significant positive correlation was observed between HI and GWP. Positive and significant correlation was observed between GWP and BI ( $r=0.204^{**}$ ) and HI ( $r=0.715^{**}$ ), but the correlation between GWP and SNP was much stronger than between GWP and HI (Table 4). This agreed to some extent with the results of QTL mapping for the traits concerned. Four QTLs were detected for HI on chromosome 2, 3, 4, and 5. These QTLs shared locations with the QTLs for SNP. Also, three shared locations with the QTLs for GWP, in the region RM3865-RM7424, RM416-RM5626, RM5642-RM6589 and RM440-RM5140. These results implied that HI, might partly share a common genetic basis with GWP and SNP. The close association of HI with grain yield could be established through both GWP and SNP. For  $F_{2:3}$  families, there was a significant and positive correlation between the BI with LFL, WFL, SNP and GWP (Table 4). Trait correlations and clustering of QTLs for traits correlated were often mapped in the same chromosomal regions. For example, qSNP-2, qGWP-2 and qHI-2 were found at approximately the same map locations in chromosome 2. These traits (SNP, GWP and qHI) showed a high correlation (Table 4). In these cases, the directions of the correlations were consistent with that of the effects of the QTLs on the traits, but, the directions of the correlations were

different with that of the effects of the QTLs on the traits in RM1022-RM6283 region.

Septiningsih et al. (2003) found one and two QTLs for plant height and grain weight, respectively and reported that plant height was mostly controlled by locus on chromosome 1 that coincided with the most important QTLs identified for grain weight. In this study, only one QTL on chromosome 1 for plant height in 2006 and two QTLs with large effects were mapped for WFL and WGP in 2008 that probably it is due to the low density of SSR linkage map. QTLs related to harvest index, were also identified by Thomson et al. (2003) and Brondani et al. (2002) on chromosome 1. In  $F_2$  mapping population, QTL controlling HI (qHI-4a) overlapped with QTL for LFL (qLFL-5) and the QTL for number of SNP (qSNP-5). Three QTLs bracketed by RM5642-RM6589 on chromosome 5. In  $F_{2:3}$  mapping population, QTL controlling HI (qHI-2) overlapped with QTL for SNP (qSNP-2) and the QTL for GWP (qGWP-2) on chromosome 2. Result of mapping on  $F_2$  population showed that QTLs controlling GWP (qGWP-3a and qGWP-3b) overlapped with the QTLs for number of SNP (qSNP-3a and qSNP-3b) and the QTL for HI (qHI-3). These QTLs, bracketed by RM416-RM5626, have decrease effects on GWP, HI and SNP; While QTLs controlling HI, GWP and SNP that bracketed by RM3865-RM7426 have increased effects. These QTLs, markers was also identified by Brondani et al. (2002) (for SNP), Xu et al. (2004) (for GWP). Result of second experiment about  $F_{2:3}$  population showed that QTLs controlling GWP (qGWP-2, qGWP-7 and qGWP-12) overlapped with the QTLs for number of SNP (qSNP-2, qSNP-7 and qSNP-12). We found a strong and positive correlation between GWP and SNP in this genetic background. Our results were evident the observations of Xiao et al. (1996), Zhang et al. (2004), Xu et al. (2004), Septiningsih et al. (2003) and Brondani et al. (2002) that correlated traits often have QTL mapping to the same chromosomal locations (Fig 3 and Table 2,3). Clustering of the QTLs for different traits at the same markers interval was observed for GWP, HI and SNP (Fig 2 and Table 2, 3). These suggest the pleiotropism and or tight linkage of different polygene (Falconer and Mackay 1996) or overlapping QTLs could be the possible reason for the congruence of several QTL. In these locations, the directions of the correlations were consistent with that of the effects of the QTLs on the traits (Fig 3 and Table 2,3).

The comparisons of result related to two different population and places of experiment from same origin cross (TAM  $\times$  KHZ) showed that QTLs of some traits are stable. These QTLs were mapped on same



**Fig 3.** Genetic linkage map showing the location of QTLs for plant height (PH), Harvest index (HI), weight of grain panicle (GWP), spikelet number of panicle (SNP), length of flag leaf (LFL), width of flag leaf (WFL) and biomass (BI) in the Taromahalli / Khazar  $F_2$  (four-fold) and  $F_{2:3}$  (circle) population. The markers are signed on the right side of the chromosomes.

regions in two experiments. For example, QTLs related to HI, GWP and SNP in RM3865-RM7427 interval on chromosome 2, GWP and SNP in RM416-RM5626 interval on chromosome 3, LFL in RM5642-RM6589 on chromosome 4, HI in RM5473-RM551 on chromosome 4, GWP and SNP in RM421-RM480 interval on chromosome 5, PH and BI in RM440-RM3345 interval on chromosome 5, SNP in RM7626-RM1337 on chromosome 12, were mapped in the same of regions. So, we can use from these markers for QTL pyramiding and marker assistant breeding programs. QTL pyramiding is the processes assemble many genes the work well together and for a specific trait, assemble the alleles with similar effects from different loci. This process can create the superior genotypes to improve the variety.

In this study, the alleles of several QTLs from the high yield variety KHZ were pyramided in some plant. Our result indicated that breeding methods of QTLs pyramiding by using marker-assisted selection could be very useful for the development of new varieties with a high level of grain yield.

#### Acknowledgments

We thank Prof Abdolmajid Rezai, Dr. Ali Moumenni, M. Katouzi and A. A. Ebadi for their assistance. We also thank the Gonad High Education Center and Rice Research Institute of Iran for their support.

## References

- Brondani C, Rangel PHN, Brondani RPV, Ferreira ME (2002). QTL mapping and introgression of yield-related traits from *Oryza glumaepaltula* to cultivated rice (*Oryza sativa* L.) using microsatellite markers. *Theor Appl Genet.* 104:1192-1203
- Basten CJ, Weir BS, Zeng ZB (2001). QTL Cartographer: a reference manual and tutorial for QTL mapping. North Carolina State University, USA. pp. 163.
- Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR (1997). Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theor Appl Genet.* 95: 553–567.
- Hong EL, Balakrishnan R, Dong Q, Christie KR, Park J, Binkley G, Costanzo MC, Dwight SS, Engel SR, Fisk DG, Hirschman JE, Hitz BC, Krieger CJ, Livstone MS, Miyasato SR, Nash RS, Oughtred R, Skrzypek MS, Weng S, Wong ED, Zhu KK., Dolinski K, Botstein D, Cherry JM (2008) Gene Ontology annotations at SGD: new data sources and annotation methods. *Nucleic Acids Res.* 36: 577–581.
- Falconer DS, Mackay FC (1996). Introduction to quantitative genetics. Longman, England.
- Guo LB, Xing YZ, Mei HW, Xu CG, Xu CG, Shi CH, Wu P, Luo LJ (2005). Dissection of component QTL expression in yield formation in rice. *Plant Breeding.* 124: 127-132.
- Kosambi, DD (1944). The estimation of map distances from recombination values. *Ann. Eugen.* 12: 172–175.
- Liu BH (1998). Statistical Genomics, Linkage, Mapping and QTL Analysis, CRC Press, New York, USA.
- Lin HX, Qian HR, Zhuang JY, Lu J, Min SK, Xiong ZM, Huang N, Zheng KL (1996). RFLP mapping of QTLs for yield and related characters in rice (*Oryza Sativa* L.). *Theor Appl Genet.* 92: 920-927.
- Lu C, Shen L, Tan Z, Xu Y, He P, Chen Y, Zhu L (1996) Comparative mapping of QTL for agronomic traits of rice across environment using a doubled-haploid population. *Theor Appl Genet.* 93: 1211-1217.
- McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q, Kono L, Yano M, Fjellstrom R, DeClerck, G, Schneider D, Cartinhour S, Ware D, Stein L (2002). Development and mapping of 2240 new markers for rice (*Oryza Sativa* L.). *DNA Res.* 9: 199-207.
- Manly KF, Olson JM (1999). Overview of QTL mapping software and introduction to map manager QTL. *Mammalian Genome.* 10: 327–334.
- Mei HW, Luo LJ, Ying CS, Wang YP, Yu XQ, Guo LB, Paterson AH, Li ZK (2003). Gene actions of QTLs affecting several agronomic traits resolved in a recombinant inbred line population and two testcross populations. *Theor. Appl. Genet.* 107: 89-101.
- Mei HW, Li ZK, Shu QY, Guo LB, Wang YP, Yu XQ, Ying CS, Luo LJ (2005). Gene actions of QTLs affecting several agronomic traits resolved in a recombinant inbred line population and two backcross populations. *Theor Appl Genet.* 110: 649-659.
- Moncada P, Martinez CP, Borrero J, Chatel M, Gauch H, Guimaraes E, Tohme J, McCouch SR (2001). Quantitative trait loci for yield and yield component in an *Oryza sativa* × *Oryza rufipogon* BC<sub>2</sub>F<sub>2</sub> population evaluated in an upland environment. *Theor Appl Genet.* 102: 41-52.
- Redona ED, Mackill DJ (1998). Quantitative trait locus analysis for rice panicle and grain characteristics. *Theor Appl Genet.* 96:957-963.
- Septiningsih EM, Prasetyono J, Lubis E, Tai TH, Tjubaryat T, Moeljopawiro S, McCouch SR (2003). Identification of quantitative trait loci for yield and yield components in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon*. *Theor Appl Genet.* 107: 1419-1432
- Sasahara H, Fukuta Y, Fukuyama T (1999). Mapping of QTLs for vascular bundle system and spike morphology in rice, (*Oryza sativa* L.). *Breed Sci.* 49: 75-81.
- SaghiMaroof MA, Biyashev RM, Yang GP, Zhang Q, Allard RW (1994). Extraordinarily polymorphic microsatellite DNA in barely species diversity, chromosomal location, and population dynamics. Paper presented at the National Academy of Sciences, USA. 91. 5466-5570.
- Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T, McCouch SR (2000). Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theor Appl Genet.* 100: 697–712.
- Thomson MJ, Tai TH, McClung AM, Lai XH, Hinga ME, Lobos KB, Xu Y, Matinez CP, McCouch SR (2003). Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson. *Theor Appl Genet.* 107: 479-493.

- Xiao, J, Li J, Yuan L, Tanksley SD. (1996). Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a subspecific rice cross. *Theor Appl Genet.* 92: 230–244.
- Xie X, Jin F, Song M, Suh J, Hwang H, Kim Y, McCouch SR, Ahn SN. (2008) Fine mapping of a yield-enhancing QTL cluster associated with transgressive variation in an *Oryza sativa* × *O. rufipogon* cross. *Theor Appl Genet.* 105:248-257.
- Xing YZ, Tan YF, Hua JP, Sun XL, Xu CG, Zhang Q (2002). Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. *Theor Appl Genet.* 116:613-622.
- Xiong LZ, Liu KD, Dai XK, Xu CG, Zhang Q (1999). Identification of genetic factors controlling domestication-related traits of rice using an F<sub>2</sub> population of a cross between *Oryza sativa* and *O. Rufipogon*. *Theor Appl Genet.* 98: 243-251.
- Xu JL, Yu SB, Luo LJ, Zhong DB, Mei HW, Li ZK (2004). Molecular dissection of the primary sink size and its related traits in rice. *Plant Breeding.* 123: 43-50
- Yagi T, Nagata K, Fukuta Y, Tamura K, Ashikawa I, Terao T (2001). QTL mapping of spikelet number in rice (*Oryza sativa* L.). *Breed. Sci.* 51:53-56.
- Yamagishi M, Takeuchi Y, Kono I, Yano M (2002). QTL mapping for panicle characteristics in temperate *japonica* rice. *Euphytica.* 128. 219-224.
- Yu SB, Li JX, Xu CG, Tan YF, Gao YJ, Li XH, Zhang Q, SaghaiMaroof MA (1997). Importance of epistatic as the genetic basis of heterosis in an elite rice hybrid. Paper presented at the National Academy of Sciences. USA 94: 9226-9231.
- Yoshida S (1981). Fundamentals of crop science. Intl. Rice Res. Inst., Los Banos, Philippines. pp.20-47.
- Zhang ZH, Li P, Wang LX, Hu ZL, Zhu LH, Zhu YG (2004). Genetic dissection of the relationship of biomass production and partitioning with yield and yield related traits in rice. *Plant Sci.* 167:1-8
- Zhao F, Liu G, Zhu H, Ding X, Zeng R, Zhang Z, Li W, Zhang G (2008). Unconditional and Conditional QTL Mapping for Tiller Numbers at Various Stages with Single Segment Substitution Lines in Rice (*Oryza sativa* L.). *Agr. Sci in China* 7: 257-265
- Zhuang JY, Lin HX, Lu J, Qian HR, Hittalmani S, Huang N, Zheng KL (1997). Analysis of QTLs × environment interaction for yield components and plant height in rice. *Theor Appl Genet.* 95:799-808.