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تنوع هاپلوتایپی QTL مرتبط با تحمل به خشکی روی کروموزوم شماره ۲ برنج

Haplotype Diversity for QTL Associated with Drought tolerance on Chromosome 2 of Rice

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چکیده

به منظور بررسی تنوع هاپلوتایپی QTLهای کنترل‌کننده تحمل به خشکی در گیاهچه‌های ۲۲ ژنوتیپ برنج، آزمایشی به صورت فاکتوریل در قالب طرح بلوک کامل تصادفی با سه تکرار و در محیط کنترل شده تحت شرایط نرمال (بدون تنش) و تنش خشکی انجام گرفت. ١٦ جفت نشانگر ریزماهواره مرتبط با تحمل به خشکی استفاده گردید. صفات مورد مطالعه شامل قطر ریشه، وزن خشک ریشه، تعداد ریشه، وزن خشک ساقه، طول ساقه، طول ریشه، کد ژنوتیپی و بیوماس کل بود. تجزیه واریانس صفات مورد بررسی نشان داد تفاوت بسیار معنی‌داری بین ژنوتیپ‌ها از نظر صفات مورد مطالعه در دو شرایط نرمال رشد و تنش خشکی وجود داشت که بیانگر وجود تنوع ژنتیکی بین ژنوتیپ‌های مورد مطالعه بود. ١٦ جفت نشانگر ریزماهواره در مجموع ٤٩ آلل نشان دادند. تعداد آلل تولید شده به وسیله هر جفت آغازگر از ۲ تا ٦ آلل متغیر بود که میانگین آن ۳ آلل در هر لوکوس بود. محتوای اطلاعات چندشکلی (PIC) دارای دامنه‌ای از ۰/۲۰۷ تا ۰/٦۷۸ با میانگین ۰/٤٦۲ بود. آغازگرهای RM8030 و RM3302 مناسب‌ترین آغازگرها برای تشخیص ژنوتیپ‌های متحمل و حساس به تنش خشکی بودند و ممکن است در انتخاب به کمک نشانگر مفید باشند. ژنوتیپ‌ها در ۱۵ گروه هاپلوتایپی جای گرفتند و از رقم Bala به عنوان مرجع جهت بررسی تنوع هاپلوتایپی استفاده شد. گروه هاپلوتایپی شماره ۱ شامل ٤ ژنوتیپ سالاری، CT6516-24-3-2، IR77298-14-1-2 و IR50 می‌تواند جهت برنامه‌های اصلاحی تحمل به تنش خشکی مفید باشد.

واژه‌های کلیدی

برنج،
تنوع هاپلوتایپی،
محتوای اطلاعات چندشکل،
نشانگر ریزماهواره.

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Introduction

Rice is the most important crop and staple food for more than half of the world's population (Todaka et al. 2012). Drought is a major constraint to rice production in water-limited environments (Bernier et al. 2008). Drought is a more complex phenomenon than most other stresses, such as salinity, submergence, pests and diseases. These complexities, along with the uncertainty in drought timing, intensity and duration, have posed a major challenge for agricultural scientists (Lanng and Buu. 2008). Root system plays an important role under drought conditions (Bassuony and Anis. 2016). A deep root system could improve the adaptation of rice during drought through greater capacity for water extraction (Cleber et al. 2013). The deeper and thicker can enhance the tolerance of rice to water deficits (Gowda et al. 2011).

Molecular marker technology promises to improve the speed of advancement by allowing for the identification of QTLs that contribute to drought resistance, thereby leading the way to marker-assisted breeding (Price and Courtois. 2000). SSR markers are able to detect the high level of allelic diversity and they have been extensively used to identify genetic variation among rice subspecies (Ni et al. 2002). SSR markers are efficient in detecting genetic polymorphism and discriminating among genotypes from germplasms of various sources, even they can detect finer level of variation among closely related breeding lines within the same variety (Lapitan et al. 2007). Many SSR markers have been reported to be linked to drought tolerance traits or QTLs in rice such as yield under drought stress (Venuprasad et al. 2012), maximum root length (Courtois et al. 2000; Steele et al. 2006), relative spikelet fertility (Yue et al. 2006), basal root thickness (Qu et al. 2008) and root dry weight (Kanbar et al. 2011). On chromosome 2 at marker C601, a QTL was detected for leaf drying in the Bala×Azucena population (Price et al. 2002). Norton et al (2008) confirmed the presence of multiple QTLs for root growth on chromosome 2 of the Bala×Azucena rice mapping population. These QTLs have been detected for root mass at depth, root-to-shoot ratio, root thickness, penetration ability of a wax layer, and root length in hydroponic and soil cultures (MacMillan et al. 2006; Price et al. 2000; Price et al. 2002). A haplotype is a group of alleles in an organism that are inherited together from a single parent. The haplotype of SSR markers that flank QTL can help to predict whether an accession carries known or different QTL (Yue et al. 2006). The objectives of this study were to compare the SSR marker haplotypes of drought tolerant rice varieties/lines on chromosome 2 and to identify drought-tolerance rice varieties/lines.

MATERIALS AND METHODS

In this study 22 rice genotypes (Bala as tolerant and Azucena as sensitive varieties) were used (Table 1) to evaluate the haplotype and allelic diversity under normal (nonstress) and drought stress (-5 bar) conditions at the seedling stage in hydroponic culture medium in the genetic laboratory of gonbad-e-kavous university of agricultural sciences. All seeds were surface sterilized with 5% (v/w) sodium hypochlorite for 5 min, then rinsed thoroughly with water, and then germinated on moist tissue paper for 5 days. Five-day-old seedlings were transplanted in hydroponic culture tanks (50 cm×30 cm×2.5 cm) and were grown normally for seven days. Under normal conditions, nutrient solution (Yoshida et al. 1976) was added to the tanks for four weeks. Under drought stress conditions, however, nutrient solution was added into the tanks for one week. then, drought stress was applied with mannitol (30 g per each tank) for three weeks. The solution pH (=5.5) was adjusted by adding NaOH or HCl. The studied characteristics included root diameter (mm), root dry weight (gr), root number, root length (cm), stem dry weight (gr), stem length (cm), genotypic score (the genotypic score of drought tolerance based on IRRI 1996) and total biomass (gr). Genomic DNA was extracted from young leaves tissue of 33-day-old plants according to the CTAB method (Saghi Maroof et al. 1994). The quantity and quality of the extracted DNA were determined on 0.8% agarose gel comparing to known concentrations of uncut λ genomic DNA. 16 SSR markers related to drought tolerance on chromosomes 2 (Table 2), were obtained from McCouch et al (2002) and Norton et al (2008). The polymerase chain reaction (PCR) was conducted in 15 µL volume using the ICYCLER-BIO-Rad thermocycler. Each 15 µL reaction contained 20 ng of genomic DNA, 1.5 µL of 10 × PCR buffer, 0.5 µL of 50 mM MgCl2, 0.18 µL of 10 mM dNTP, 0.4 µL of each SSR primer (5 µM) and 0.15 µL of 5 U/µL Taq DNA polymerase.

Table1. Name of rice genotypes

No.	Name	Origin	No.	Name	Origin
1	Bala	India	12	IR50	IRRI
2	Azucena	Philippine	13	Salari	Iran
3	IR30	IRRI	14	IR77298-14-1-2	IRRI
4	IR82589-B-B-84-3	IRRI	15	IR60080-48A	IRRI
5	IR55423-01	IRRI	16	Usen	Egypt
6	Gharib siah reihani	Iran	17	Line 229	Iran
7	IR344197	IRRI	18	IR82589-B-B-114-3	IRRI
8	Hashemi	Iran	19	Dom zard	Iran
9	IR81024-B-B-254-1	IRRI	20	Champa budar	Iran
10	IR83747-B-B-81-1	IRRI	21	CT6516-24-3-2	IRRI
11	CT6510-24-1-2	IRRI	22	B6144F-MR-6-0-0	IRRI

Table 2. Forward and Reverse sequences of microsatellite markers (McCouch et al. 2002; Norton et al. 2008).

Primer	Sequences	Annealing Temperature	Expected PCR product size
RM530	F: TTCTTTATTCCCTCGCACTGACC R: CAATGATGCCACAAACCGTAACC	55	161
RM3316	F: CGCATTTGAAACTGGAAACTCG R: GGACGAATACTGATATGGATGACTCC	55	207
RM8024	F: TTTCACTCAAGACCAGACCTGTACG R: GCACGTCATTGTAGTGACTAGTGAGG	55	125
RM6535	F: GAGCTTCCGGCCGTAGTTGTGC R: AGACCTTCATCCGGCGGTTCG	55	132
RM8255	F: ATCCATTCTTGCTCCCAACAAGC R: AGGAGGTGGAGGCTAGGGTTAGG	55	188
RM425	F: ACCACAGCAGGTGGAACAGG R: GCTAGCTAAGCCAACACCAACG	55	126
RM6481	F: TCAAGCATCTCAGTCAGCACAGG R: CTACAAGCTGAAGCGGCTCAAGG	60	105
RM14001	F: TGTGGCTGGGCTCCGATACC R: ACCCTGCAGGATCATCAGAACG	60	138
RM14002	F: TTGCCGTATCAACTTCTCTTCTCTCC R: TAGCCTTGCAGCTGGATTAGTACGG	60	140
RM8030	F: CAAGCATTATCAGTTGGCTTCC R: GTGCTAGACGACGTTCTCAAACC	55	131
RM8029	F: CAAGCATTATCAGTTGGCTTCC R: GTGCTAGACGACGTTCTCAAACC	55	138
RM3302	F: GAGATCGGGATCTAACACTGTAATGC R: TCGGACGGAGGGAGTATGTAGC	50	215
RM5460	F: ACAACCACAGCTGCTTGAATTGC R: AGAGGAACCCACTGCCCTTGC	50	160
RM6519	F: CCACACCACTACAAAGCTTTCTTCC R: ACAGCATCTGGTCGAAGAAGTCG	50	126
RM112	F: TGCCCTGTTATTTTCTTCTCTC R: GGTGATCCTTTCCCATTTCA	55	128
RM250	F: TCTGCAAGCCTTGTCTGATG R: TAAGTCGATCATTGTGTGGACC	55	153

The touchdown PCR amplification was performed according to the cycle profile: an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing temperatures at 60°C-50°C for 30 seconds (decreasing by 1°C/cycle) and primer elongation at 72°C for 1 min and then a final extension at 72°C for 5 min. Amplified products were stored at -20°C until further use. PCR amplified products were separated in a 6% polyacrylamide gel at 60 Volts for 2 hours and stained with silver nitrate. The analysis of variance (ANOVA) and Mean comparison were calculated using the SAS 9.2 (SAS. 2008). Polymorphism information content (PIC) values were calculated based on the following formula (Anderson et al. 1993):

$$PIC_i = 1 - \sum_{j=1}^{n} p_{ij}^2$$

Where n is the number of marker alleles for marker *i* and P_{ij} is the frequency of the *j*th allele for marker *i*. PIC value was analyzed using Power Marker version 3.25 (Liu and Muse. 2005). Number of alleles per locus, gene diversity and Shannon's Information index were calculated using POPGENE version 1.31 (Yeh et al., 1999). Dendrogram of cluster analysis (Ward method) was performed using SPSS version 20.0. All the genotypes were scored for DNA bands using NTSYS pc 2.1 (Rohlf. 2002). Alleles were alphabetically coded (e.g., a, b, c, etc.) in decreasing size order.

RESULTS AND DISCUSSION

The results of ANOVA showed that there were significant differences ($p<0.01$) among the genotypes for all the studied traits. Genotype × Environment interactions were significant ($p<0.01$) for all the studied traits except for stem weight trait. These results indicated that the studied traits were highly affected by drought stress. Since the interaction effect was significant for most of studied traits, interaction effect sliced to normal and drought stress conditions. Analysis of variance under normal and drought stress conditions, separately, showed there were significant differences ($p<0.01$) among the genotypes except for stem weight trait (Table 3). Mean comparison of studied traits for all genotypes showed that there were significant differences between genotypes under both normal and drought stress treatments (Table 4). Results showed that drought stress reduced the mean of all traits. Under drought stress condition, Usen, Bala and Salari cultivars had the highest root weight, root number and root length respectively. Superior root phenotypes are considered as key components for improving drought tolerance characteristics which offer better performance under drought by efficient uptake and utilization of water in crops (Lopes et al. 2011). Root characteristics, particularly root depth, are likely to increase plant water uptake, dehydration avoidance mechanisms, and rice resistance to drought effects (Serraj et al. 2009). The maximum root length was found in Salari (4.36 cm) and Line229 (4.08 cm) genotypes respectively, while it was the lowest in IR60080-48A (1.23 cm) genotype. Bala and Hashemi genotypes had the maximum number of root, 7 for Bala and 5 for Hashemi, whereas it was minimized in Line229. The highest root dry weight was found in Usen (0.0037 gr), Bala (0.0036 gr) and CT6516-24-3-2 (0.0029 gr) respectively under drought stress. These results showed that genotypes used different ways against drought stress such as increasing root length, root dry weight and number of root. For total biomass, values ranged from 0.0067g for Azucena to 0.0135g for Salari under drought stress. The seedling stage drought tolerance evaluation demonstrated that 4 genotypes, included Bala, Salari, Usen and IR60080-48A, were rated as highly tolerant to tolerant (score 1 and 3), 6 genotypes were moderately tolerant (score 5) and 12 genotypes were susceptible (score 7 and 9). Bala had the lowest genotypic score. Azucena, IR30 and IR81024-B-B-254-1 had the highest genotypic score.

Cluster analysis among rice genotypes are presented in Fig 1 (Normal conditions) and Fig 2 (Drought stress conditions) based on studied traits using Ward method. Cluster analysis classified the genotypes into two groups in both normal and drought stress conditions. In drought stress conditions, genotypes that classified with Bala (tolerant cultivar to drought stress) named tolerant group and genotypes that classified with Azucena (sensitive cultivar to drought stress) named sensitive group. Tolerant group had higher root diameter, root dry weight, root number, stem dry weight and stem length than sensitive group in drought stress conditions.

Table 3. Analysis of variance for studied traits

Sources of variation	df	Mean Squares							
		RD	RDW	RN	SDW	SL	RL	GS	TB
Block	2	0.0088**	0.000001 ns	9.81*	0.000017 ns	27.76**	4.37*	0.84ns	0.000009 ns
Genotype(G)	21	0.036**	0.000002**	19.22**	0.000029**	32.89**	4.61**	6.42**	0.000037**
Environment(E)	1	2.10**	0.00004**	238.41**	0.0025**	17.91**	391.3**	8.30**	0.0032**
G*E	21	0.0091**	0.000003**	5.7**	0.000018 ns	19.07**	3.17**	6.42**	0.000027**
Error	86	0.0014	0.0000005	2.22	0.000011	3.95	1.03	0.36	0.000013
C.V (%)		8.94	27.02	27.90	26.04	21.78	23.60	17.29	23.17

Analysis of variance for studied traits under normal conditions

Block	2	0.012**	0.000009 ns	3.50 ns	0.00001 ns	35.65*	8.39*	0	0.0000057 ns
Genotype	21	0.03**	0.000004**	21.48**	0.00004*	45.30**	5.59**	0	0.0000058**
Condition	42	0.0019	0.0000009	3.40	0.00002	7.17	1.63	0	0.00002
C.V (%)		8.04	30.19	27.57	25.77	20.90	21.21	0	23.60

Analysis of variance for studied traits under drought stress conditions

Block	2	0.0005 ns	0.0000004*	6.62**	0.000006 ns	2.19*	0.008 ns	1.69 ns	0.000003 ns
Genotype	21	0.0074**	0.0000014**	3.44**	0.0000061 ns	6.66**	2.19**	12.84**	0.000006*
Drought	42	0.0007	0.00000008	1.13	0.0000036	0.44	0.29	0.71	0.000003
C.V (%)		9.52	14.21	26.66	21.70	12.20	20.82	14.03	17.14

ns Not significant; * Significant at $p < 0.05$, ** Significant at $p<0.01$

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Table 4. Mean comparison for studied traits of genotypes under drought stress and normal conditions, separately

	RD		RDW		RN		SDW	
Genotype	**Stress**	**normal**	**Stress**	**normal**	**Stress**	**normal**	**Stress**	**normal**
Bala	0.36^{a}	0.85^{a}	0.0036^{b}	0.0047^{ab}	7.20^{a}	15.2^{a}	0.0095^{abc}	0.024^{ab}
Azucena	0.27^{f}	0.22^{g}	0.0027^{c}	0.0029^{cdef}	3.44^{bcde}	5.13^{degh}	0.004^{d}	0.008^{e}
IR30	0.30^{bcde}	0.53^{defgh}	0.0012^{ij}	0.0015^{f}	4.0^{bcd}	7.0^{cdef}	0.007^{bcd}	0.015^{cde}
IR82589-B-B-84-3	0.28^{def}	0.56^{cdefg}	0.0020^{cde}	0.0029^{cdef}	3.33^{bcd}	4.0^{fgh}	0.008^{bc}	0.015^{cde}
IR55423-01	0.26^{ef}	0.54^{defg}	0.0022^{cd}	0.0030^{cdef}	4.66^{bc}	6.33^{cdefg}	0.008^{bc}	0.013^{de}
GharibSiahReihani	0.29^{cdef}	0.51^{fgh}	0.0013^{hij}	0.0021^{def}	4.66^{bc}	4.66^{efgh}	0.010^{ab}	0.016^{cd}
IR344197	0.28^{def}	0.57^{cdef}	0.0020^{cde}	0.0040^{bc}	4.66^{bc}	7.33^{bcde}	0.009^{abc}	0.019^{abcd}
Hashemi	0.32^{abcd}	0.54^{defg}	0.0020^{cde}	0.0025^{cdef}	5.0^{b}	7.66^{bcde}	0.009^{abc}	0.018^{abcd}
IR81024-B-B-254-1	0.28^{def}	0.51^{fgh}	0.0018^{defg}	0.0036^{bcd}	3.66^{bcd}	7.66^{bcde}	0.008^{bc}	0.018^{abcd}
IR83747-B-B-81-1	0.33^{abc}	0.51^{fgh}	0.0019^{cdef}	0.0039^{bc}	4.0^{bcd}	4.0^{fgh}	0.008^{bc}	0.018^{abcd}
CT6510-24-1-2	0.35^{ab}	0.56^{cdefg}	0.0023^{c}	0.0025^{cdef}	4.66^{bc}	7.0^{cdef}	0.009^{abc}	0.019^{abcd}
IR50	0.31^{bcd}	0.62^{bc}	0.0008^{j}	0.0036^{bcd}	3.33^{bcde}	6.33^{cdefg}	0.007^{cd}	0.022^{abc}
Salari	0.26^{f}	0.46^{h}	0.0015^{fghi}	0.0028^{cdef}	4.0^{bcd}	5.33^{defgh}	0.012^{a}	0.015^{cde}
IR77298-14-1-2	0.32^{abcd}	0.50^{gh}	0.0023^{c}	0.0022^{def}	4.0^{bcd}	8.0^{bcd}	0.008^{bc}	0.021^{abc}
IR60080-48A	0.30^{cdef}	0.57^{cdef}	0.0016^{efgh}	0.0062^{a}	4.0^{bcd}	7.33^{bcde}	0.009^{abc}	0.013^{de}
Usen	0.32^{abcd}	0.66^{b}	0.0037^{a}	0.0018^{ef}	3.33^{bcde}	3.33^{gh}	0.008^{bc}	0.013^{de}
Line229	0.30^{bcde}	0.58^{cde}	0.0016^{efgh}	0.0029^{cdef}	2.33^{de}	4.66^{efgh}	0.007^{bcd}	0.015^{cde}
IR825-B-B-114-3	0.28^{def}	0.57^{cdef}	0.0019^{cdef}	0.0026^{cdef}	4.0^{bcd}	6.66^{cdef}	0.009^{abc}	0.015^{cde}
Dom Zard	0.29^{cdef}	0.52^{efgh}	0.0014^{ghi}	0.0025^{cdef}	3.0^{cde}	$5.0d^{efgh}$	0.009^{abc}	0.018^{abcd}
ChampaBudar	0.31^{bcd}	0.60^{bcd}	0.0021^{cde}	0.0039^{bc}	4.33^{bc}	10.33^{b}	0.008^{bc}	0.017^{bcd}
CT6516-24-3-2	0.31^{bcd}	0.53^{defg}	0.0029^{b}	0.0033^{bcde}	3.33^{bcde}	7.0^{cdef}	0.008^{bc}	0.017^{bcd}
B6144F-MR-6-0-0	0.31^{bcd}	0.51^{fgh}	0.0014^{ghi}	0.0058^{a}	4.66^{bc}	9.33^{bc}	0.010^{abc}	0.025^{a}

Table 4. continued

Genotype	SL		RL		GS		TB	
	Stress	normal	Stress	normal	Stress	normal	Stress	normal
Bala	6.62^{bcd}	18.96^{ab}	3.04^{cde}	9.12^{a}	1^{e}	0^{a}	0.0131^{ab}	0.029^{ab}
Azucena	2.61^{j}	6.36^{h}	1.25^{i}	4.69^{ghij}	9^{a}	0^{a}	0.0067^{e}	0.0108^{f}
IR30	4.33^{ghi}	90.3^{fgh}	2.37^{defg}	4.8f^{ghij}	9^{a}	0^{a}	0.0089^{de}	0.017^{def}
IR82589-B-B-84-3	4.09^{hi}	9.33^{fgh}	3.22^{bcd}	6.93^{bcde}	7^{b}	0^{a}	0.0101^{bcde}	0.018^{cdef}
IR55423-01	4.89^{fgh}	11.5^{defg}	2.77^{def}	7.80^{bc}	7^{b}	0^{a}	0.0107^{abcde}	0.016^{def}
GharibSiahReihani	6.72^{bcd}	17.83^{ab}	1.26^{hi}	4.2^{ij}	5^{c}	0^{a}	0.0121^{abc}	0.018^{cdef}
IR344197	5.19f^{g}	10.33^{fgh}	3.7^{abc}	6.1^{cdefghi}	7^{b}	0^{a}	0.0113^{abcd}	0.023^{abcd}
Hashemi	9.16^{a}	11.46^{defg}	1.66^{ghi}	4.7^{ghij}	7^{b}	0^{a}	0.0114^{abcd}	0.020^{cde}
IR81024-B-B-254-1	4.27^{ghi}	11.06^{efg}	2.29^{efg}	6.06^{cdefgh}	9^{a}	0^{a}	0.0098^{cde}	0.022^{bcde}
IR83747-B-B-81-1	4.35^{ghi}	10.56^{fgh}	2.73^{deg}	6.66^{bcdefg}	7^{b}	0^{a}	0.0105^{abcde}	0.022^{bcde}
CT6510-24-1-2	4.84^{fgh}	13.13^{cdef}	2.47^{defg}	6.53^{bcdefgh}	5^{c}	0^{a}	0.0115^{abcd}	0.022^{bcde}
IR50	5.77^{def}	15.43^{bcde}	2.32^{efg}	7.26^{abcd}	5^{c}	0^{a}	0.0079^{e}	0.025^{abc}
Salari	6.73^{bcd}	19.86^{a}	4.36^{a}	6.16^{cdefgh}	3^{d}	0^{a}	0.0135^{a}	0.018^{cdef}
IR77298-14-1-2	5.17^{fgh}	16.86^{abc}	2.14^{fgh}	5.9^{cdefgh}	5^{c}	0^{a}	0.0106^{abcde}	0.024^{abcd}
IR60080-48A	7.23^{bc}	7.26^{gh}	1.23i	5.5^{defghij}	3^{d}	0^{a}	0.0114^{abcd}	0.020^{cde}
Usen	6.48^{cde}	11.9^{def}	2.29^{efg}	4.43^{hij}	3^{d}	0^{a}	0.0119^{abcd}	0.015^{ef}
Line229	4.75^{fgh}	8.93^{fgh}	4.08^{ab}	6.13^{cdefgh}	7^{b}	0^{a}	0.0093^{cde}	0.018^{cdef}
IR825-B-B-114-3	4.89^{fgh}	11.8^{def}	3.7^{abc}	5.03^{efghij}	5^{c}	0^{a}	0.0119^{abcd}	0.018^{cdef}
Dom Zard	5.46^{ef}	11.1^{efg}	2.44^{defg}	5.76^{cdefghij}	7^{b}	0^{a}	0.0108^{abcde}	0.020^{cde}
ChampaBudar	7.68^{b}	18.16^{ab}	2.37^{defg}	3.76^{j}	5^{c}	0^{a}	0.0106^{abcde}	0.021^{bcde}
CT6516-24-3-2	3.67^{ij}	15.2^{bcde}	2.65^{def}	8.33^{ab}	7^{b}	0^{a}	0.0118^{abcd}	0.020^{cde}
B6144F-MR-6-0-0	4.81^{fgh}	15.83^{abcd}	2.61^{def}	6.86^{bcdef}	7^{b}	0^{a}	0.0114^{abcd}	0.031^{a}

Means with the same letter are not significantly different. ($LSD_{0.05}$).

[DOR: 20.1001.1.25885073.1397.7.2.12.3]

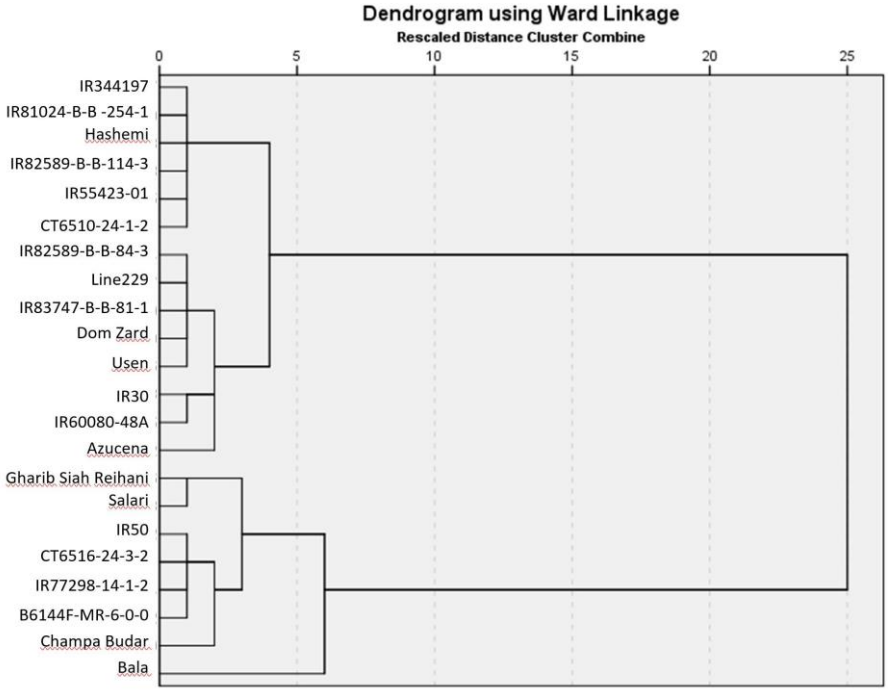

Fig 1. Cluster analysis for rice genotypes based on studied traits under normal (no stress) conditions using Ward method

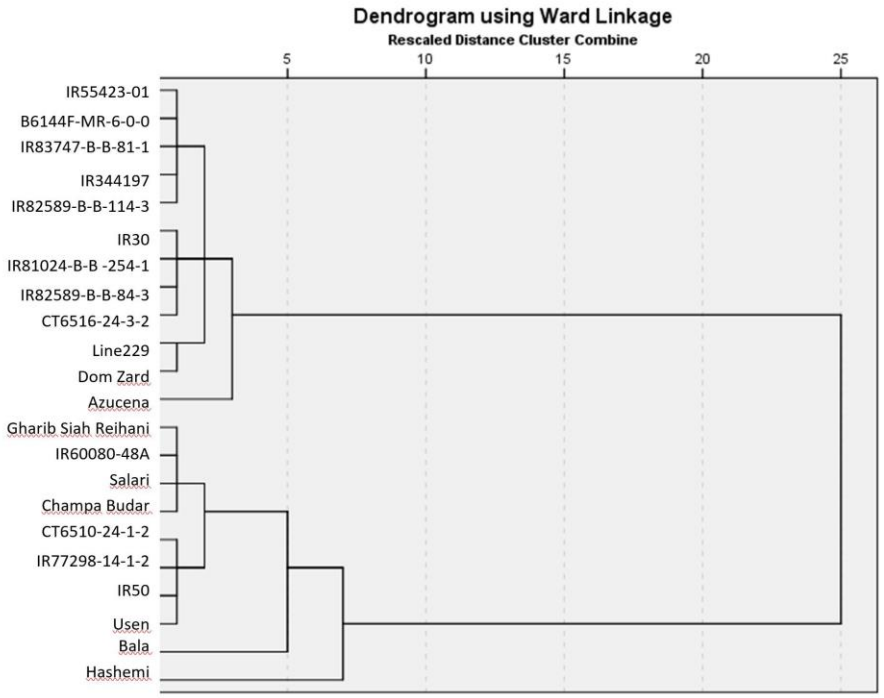

Fig 2. Cluster analysis for rice genotypes based on studied traits under drought stress conditions using Ward method

To overcome the drought stress problem, researchers have used molecular markers to identify germplasm

with traits related to drought tolerance in various breeding programs (Afiukwa et al. 2016). The SSR markers are efficient along with the system of choice for genetic analysis in rice because of their abundance in the rice genome and high level of polymorphism (Singh et al. 2010). QTL-linked markers are robust in estimating genetic diversity (Yadav et al. 2013). The 16 SSR markers revealed 49 alleles among the 22 rice genotypes (Table 5). The number of alleles per locus ranged from 2 to 6 alleles with an average of 3. The maximum number of alleles was found at RM3302 locus (six alleles) followed by RM8030, RM112, RM8029 and RM5460 (four alleles). The lowest number of alleles were observed in RM14001, RM14002, RM6519, RM8024, RM530 and RM6481(two alleles).

Table 5. Number of alleles and PIC of sixteen SSR markers

Marker	Allele No	Gene Diversity	Shannon's Information index	PIC
RM14002	2	0.483	0.6890	0.366
RM250	3	0.623	1.0844	0.553
RM112	4	0.677	1.2945	0.623
RM6519	2	0.462	0.6765	0.355
RM8030	4	0.727	1.3513	0.678
RM14001	2	0.462	0.6255	0.355
RM8255	3	0.491	0.9562	0.407
RM8024	2	0.235	0.5360	0.207
RM8029	4	0.657	1.2232	0.593
RM425	3	0.376	0.6555	0.343
RM530	2	0.351	0.5360	0.289
RM6481	2	0.483	0.7595	0.366
RM3316	3	0.657	1.0844	0.583
RM6535	3	0.644	1.0901	0.572
RM3302	6	0.685	1.4658	0.651
RM5460	4	0.491	1.0836	0.450
Mean	3.0625	0.532	0.9445	0.462

The gene diversity of a locus is defined as the probability that an individual is heterozygous for the locus in the population (Liu. 1998). Based on Shannon's Information index, the highest gene diversity (0.727) was observed for RM8030 followed by RM3302 (0.685) and RM112 (0.677). Polymorphic information content (PIC) value refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency; thus, it provides an estimate of the discriminating power of the marker (Nagy et al. 2012; Freeg et al. 2016). PIC value is a principal factor in distinguishing the percentage of polymorphism of a marker at a specific locus; the higher PIC value is in SSR markers, the higher percentage of polymorphism is detected (Anupam et al. 2017). The highest PIC value was found for RM8030 (0.678), followed by RM3302 (0.651) and these markers were found to be superior for analysis of genetic diversity in this region for further studies. DNA bands pattern generated by RM14002 is shown in Fig 3.

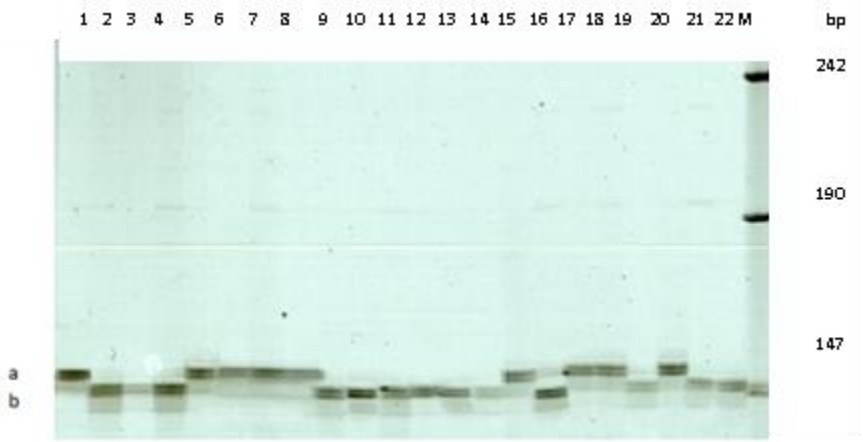

Fig 3. DNA bands pattern generated by RM14002. M: ladder
(Lane numbers correspond to genotypes numbers indicated in table 1)

Haplotypes are a combination of alleles at different markers along the same chromosome that are inherited as a unit. Assignment of alleles to the chromosome (haplotypes) can be powerful because it yields information about recombination, which is the physical exchange of DNA during meiosis. Haplotypes represent sequences along the chromosome that are either preserved intact or separated by recombination over time. (Dana and Deborah. 2005). Based on the allelic pattern of this region, genotypes arranged in various haplotype groups (Table 6). Rice genotypes showed different reaction to drought stress at seedling stage viz haplotype group 1 was ranked as tolerant genotypes. 15 haplotypes were identified among the 22 rice genotypes. Twelve genotypes allocated to 12 single haplotypes (haplotypes number 4 to number 15) and Azucena genotype did not have any common alleles with Bala pattern. None of the genotypes produced similar haplotype to Bala in this region but the comparison of these genotypes with Bala pattern showed that Salari, CT6516-24-3-2, IR77298-14-1-2 and IR50 genotypes (haplotype group 1) amplified the same SSR alleles for 11 primers that common allele combinations can be important to controlling drought tolerance.

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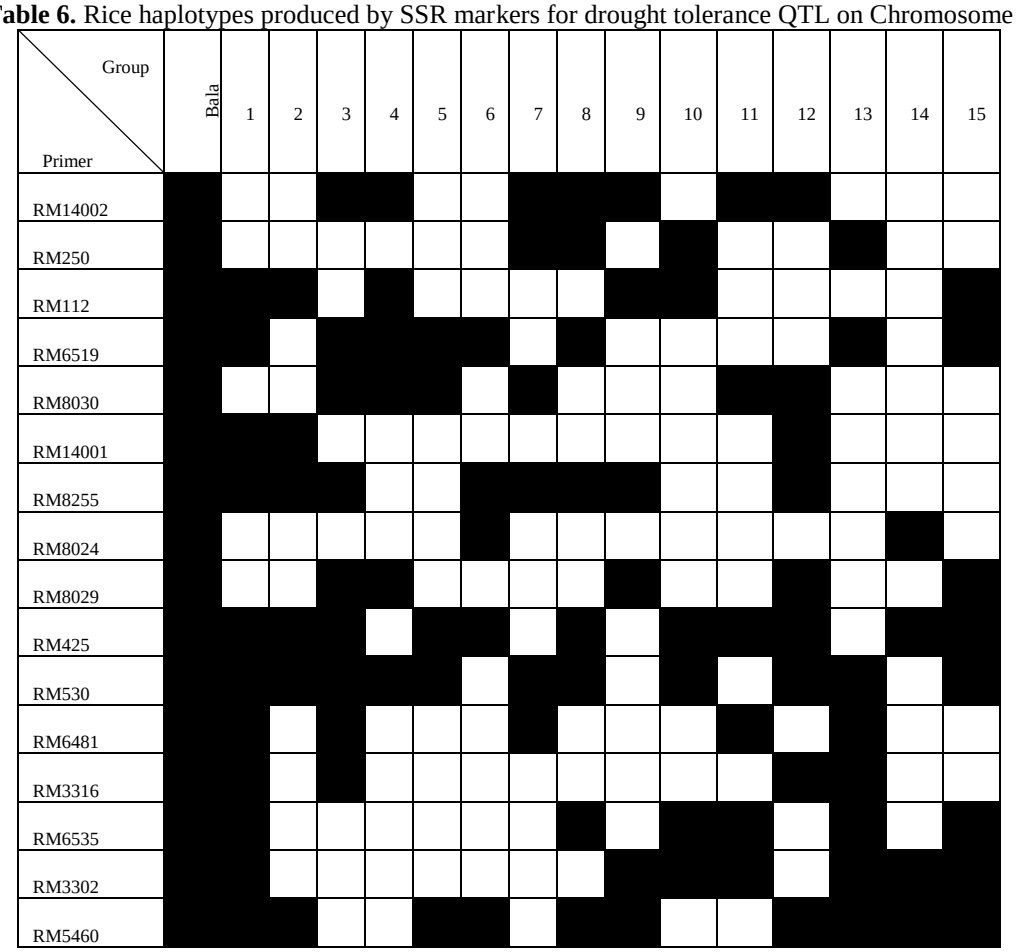
Table 6. Rice haplotypes produced by SSR markers for drought tolerance QTL on Chromosome 2



■ Presence of loci
□ Absence of loci

Haplotypic groups: 1: Salari, CT6516-24-3-2, IR77298-14-1-2, IR50 2: Dom zard, IR30 3: Hashemi, champa budar 4: IR60080-48A 5: CT6510-24-1-2 6: B6144F-MR-6-0-0 7: IR55423-01 8: IR344197 9: IR82589--B-B-114-3 10: Usen 11: Gharib siah reihani 12: Line 229 13: IR83747-B-B-81-1 14: IR82589-B-B-84-3 15: IR81024-B-B-254-1

This study illustrates the utility of microsatellite markers to identify the presence of the drought-tolerant QTL in rice genotypes. The underlying assumption is that if a line has the same allelic pattern for marker loci flanking the QTL as that in the known tolerant line, the two lines most likely have the same QTL (Bai et al. 2004; Sun et al. 2003; McCartney et al. 2004); on the other hand, if a line has a different allelic pattern from that in the known resistant line, the two lines most likely have different alleles of the QTL (Yu et al. 2006). From the comparison of the haplotypes with Bala pattern, it can be assumed that the rice genotypes possessing the Bala band type for RM8030 and RM3302 markers were tolerant to drought stress at the seedling stage. Therefore, these markers appear to have a strong and positive association with drought tolerance at seedling stage of rice and can be useful for rice breeding program for drought tolerance. Haplotype group 1, included Salari, CT6516-24-3-2, IR77298-14-1-2 and IR50 genotypes, had the most similarity allelic pattern to Bala. The results showed that there is genetic diversity for studied traits among the genotypes and genotypes of haplotype group 1 can be used to improve drought tolerance in high yield rice varieties.

RESULTS AND DISCUSSION

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Haplotype Diversity for QTL Associated with Drought tolerance on Chromosome 2 of Rice

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Abstract

In order to investigate the haplotype and allelic diversity of 22 rice (*Oryza sativa* L.) genotypes, a factorial experiment was conducted based on RCBD with three replications under normal (no stress) and drought stress (-5 bar) conditions at seedling stage. The 16 primer pairs of SSR markers related to drought tolerance were used for genotyping. The studied traits were included root diameter, root dry weight, root number, root length, stem dry weight, stem length, genotypic score and total biomass. Analysis of variance for studied traits showed that there was a significant genetic variation among genotypes. 16 used SSR loci produced 49 alleles. The number of alleles per locus generated by each primer pair varied from 2 to 6 alleles with an average of 3. The polymorphic information content (PIC) values ranged from 0.207 to 0.678 with an average of 0.462. RM8030 and RM3302 microsatellite markers were useful for discriminating between tolerant and susceptible genotypes and therefore may be useful for marker-assisted selection. 15 haplotypes were identified among these genotypes using Bala haplotype as reference for haplotype diversity. Genotypes of haplotype group 1 including Salari, CT6516-24-3-2, IR77298-14-1-2 and IR50 genotypes can be useful for breeding programs to drought tolerance.

Key words: Haplotype diversity, PIC, Rice, SSR marker.

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