Indian J. Genet., 76(3): 356-360 (2016)
DOI: 10.5958/0975-6906.2016.00053.5

Short Communication

Microsatellite based association studies for grain mineral content in local winter (*Sali*) rice of Assam

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(Received: April 2016; Revised: July 2016; Accepted: August 2016)

Abstract

In present investigation, grains of one hundred *indica* rice genotypes were analyzed for nine mineral contents namely, phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), calcium (Ca), zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) and genotyped using 112 SSR markers. STRUCTURE analysis identified k=4, indicating at least four distinct groups in a set of 100 rice accessions. Genome-wide scanning detected a total of 10 significant marker-trait associations (P<0.01), with the R^2 values ranging from 8.85% to 24.25%. Three markers, RM231 on chromosome (Chr.) 3, RM234 (chr. 7) and RM162 (chr. 6), were found to be linked for Ca. Three SSR markers, RM3 (chr. 6), RM400 (chr. 6) and RM286 (chr. 11) were found to be associated with Cu content. RM 311 on chr. 10 was found to be associated with Fe content. P was found to be associated with three SSR markers, namely, RM536, RM234 and RM600 on chromosomes 11, 7 and 1, respectively. Some novel QTLs were also detected and implications of these results are discussed.

Key words: Winter rice, minerals, association mapping, SSR markers

Low micronutrient densities in staple foods like rice are generally the major reasons for human micronutrient malnutrition in developing countries (Cakmak, 2009). Breeders have given attention to the improvement of nutritional quality, apart from grain yield by exploiting wide genetic variation for mineral elements in rice (Zhang et al. 2004). However, proper utilization of genetic variations requires a better understanding of underlying genetics of quality related traits. Such genetic analysis is greatly facilitated by DNA markers using Quantitative Trait Loci (QTLs) mapping approach (Norton et al. 2009). Association mapping (AM) is an another approach for QTL mapping which exploit the recombination events occurred in many generations in the naturally existing variations like landraces for mapping of the genes of interest. This has facilitated the effective utilization of conserved natural genetic diversity of crop germplasm resources to QTLs by examining the marker-trait associations, more particularly in rice (Jin et al. 2010). Since efforts made for identification of QTLs for grain mineral contents in indigenous rice of Assam are limited, the present investigation was therefore, conducted to identify markers linked to QTLs for mineral contents using a set of winter (*Sali*) rice germplasm following AM approach.

Grains of one hundred *indica* rice (*sali*) genotypes were obtained from two separate locations of Assam, i.e. Regional Agricultural Research Stations, Titabar (Upper Brahmaputra Valley Zone, UBVZ) and Karimganj (Barak Valley Zone, BVZ). Sample preparation was done as per the method described by AOAC (1970). The nine minerals *viz*., phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), calcium (Ca), zink (Zn), iron (Fe), manganese (Mn) and copper (Cu) were estimated using atomic absorption spectrophotometer (Chemito, AA203D, Double beam atomic absorption spectrophotometer).DNA extraction and PCR analysis was done as described earlier from this laboratory (Rathi et al. 2014).

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Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
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Table 1. Association (R2) of SSR markers with mineral parameters studied using MLM (Mixed Linear Model) approach

Trait	Marker	Chromosome no.	Map position	p	Marker R^2
Ca%	RM231	3	15.7	0.00664	0.15649
Ca%	RM234	7	88.2	0.02111	0.14437
Ca%	RM162	6	108.3	0.03537	0.09201
Cu(mg/100g)	RM3	6	74.9	0.03199	0.11847
Cu(mg/100g)	RM400	6	134.5	0.00101	0.24205
Cu(mg/100g)	RM286	11	0.0	0.0232	0.14736
Fe(mg/100g)	RM311	10	25.2	0.04184	0.16336
P%	RM536	11	55.1	0.02252	0.12825
P%	RM234	7	88.2	0.0465	0.08857
P%	RM600	1	67.3	0.04616	0.16114

Bayesian clustering analysis was carried the program STRUCTURE (Pitchard et al. 2000). Analysis parameters included an admixture model, correlated allele frequencies and assuming no population prior information. Each analysis with this program consisted of 10 replicate runs for K = 1 to 10, each with a burn-in of 10,000 replications, and a run length of 1,00,000 Markov chain Monte Carlo (MCMC) iterations. The most likely value for *K* was estimated using Evanno's ∆K method (Evanno et al. 2005; Earl and VonHoldt 2012). Finally STRUCTURE was run with 50 000 burn-in and 500 000 iterations, at the determined K. The hypothesis of association of SSR markers with mineral content in the presence of population structure was tested using a mixed linear model (MLM), as described by Yu et al. (2006), in the program TASSEL 2.0.1 (http://www.maizegenetics.net/).

The structure analysis was performed by setting the range of possible number of sub-populations (k)

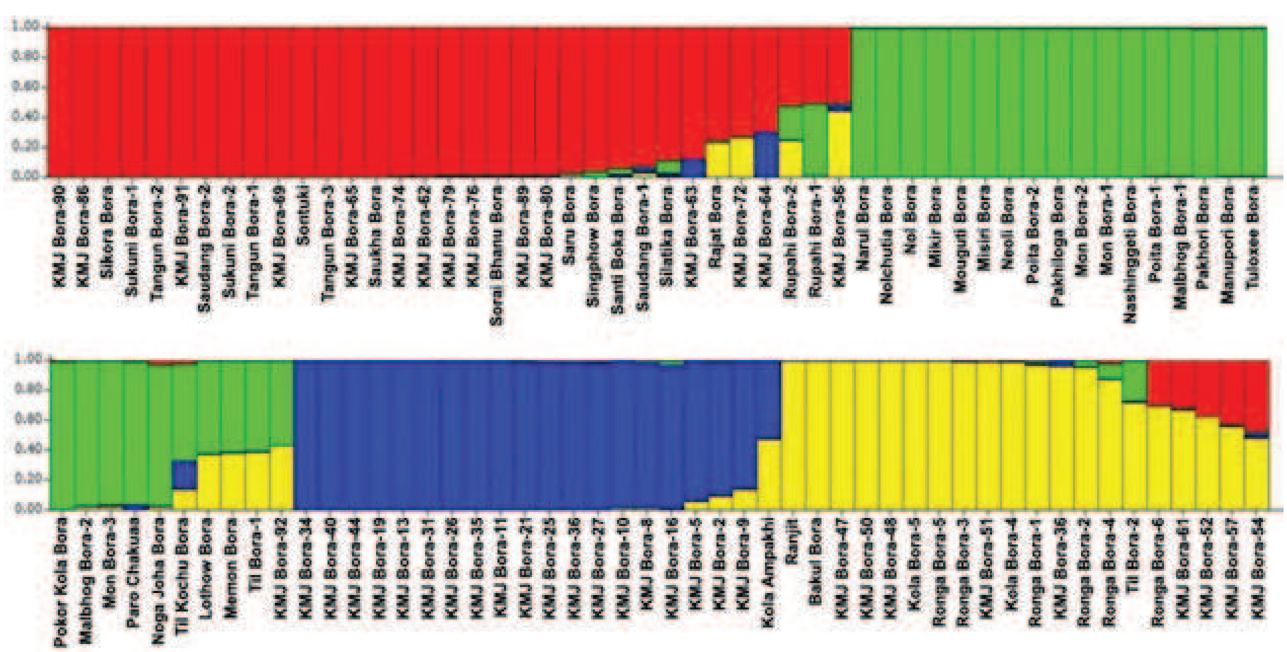

Fig. 1. Bar plot showing genetic diversity for 100 genotypes using the program STRUCTURE. Each accession is divided into a number of h0ypothetical subpopulations based on the proportional membership (a vertical bar expressed as %) from K = 1 to K = 10, with the most divergent subpopulations were obtained at K = 4. Each group is represented by a different color as listed: red, green, blue and yellow

as 1 to 10. Based on an Evanno test, the result showed that 4 was the optimum k number of sub-populations indicating at least four distinct groups in a set of 100 rice accessions panel (Fig. 1) (Table 2). The abundance of admixtures indicate that the accessions used here were rich in genetic variation as followed by Garris et al. (2005). The model-based approach using STRUCTURE is a better estimate of population

Table 2. Four sub-populations based on STRUCTURE

Sub pop.	Germplasm	Ash (%) and macronutrients (%)						Micronutrient content mg/100g			
		Ash	P	K	Na	Mg	Ca	Zn	Fe	Mn	Cu
1	KMJ Bora 90	1.4	0.407	0.209	0.058	0.041	0.024	3.556	3.21	2.245	0.74
	KMJ Bora-86	1.4	0.305	0.220	0.058	0.049	0.029	3.305	3.19	1.514	0.90
	Sikora Bora	1.6	0.348	0.213	0.064	0.044	0.029	3.075	2.74	2.618	1.24
	Sukoni Bora-1	1.6	0.347	0.220	0.059	0.045	0.025	3.109	3.12	2.238	0.86
	Tangun Bora-2	1.4	0.392	0.212	0.060	0.044	0.035	3.140	3.22	2.524	0.50
	KMJ Bora-91	1.4	0.367	0.220	0.064	0.046	0.031	3.444	2.37	2.400	0.52
	Saudang Bora-2	1.4	0.377	0.209	0.056	0.047	0.028	3.928	3.30	3.026	0.64
	Sukoni Bora-2	1.6	0.272	0.209	0.058	0.046	0.031	3.209	2.78	1.992	0.50
	Tangun Bora-1	1.6	0.403	0.220	0.063	0.049	0.031	4.31	3.37	1.584	1.22
	KMJ Bora-69	1.6	0.366	0.206	0.062	0.043	0.027	3.396	2.70	2.403	0.90
	Sontuki	1.6	0.322	0.201	0.064	0.049	0.023	4.012	3.94	2.368	1.14
	Tangun Bora-3	1.4	0.381	0.216	0.064	0.047	0.030	2.694	3.75	1.804	1.18
	KMJ Bora-86	1.4	0.305	0.220	0.058	0.049	0.029	3.305	3.19	1.514	0.90
	KMJ Bora-65	1.8	0.301	0.220	0.064	0.048	0.025	2.728	3.12	2.841	0.82
	Saukha-Bora	1.2	0.367	0.220	0.062	0.047	0.034	3.002	2.89	2.062	0.78
	KMJ Bora74	1.2	0.319	0.203	0.060	0.040	0.027	3.300	2.56	2.425	0.66
	KMJ Bora-62	1.8	0.328	0.212	0.056	0.047	0.024	4.661	3.23	2.306	0.56
	KMJ Boa-79	1.2	0.328	0.220	0.051	0.047	0.026	3.012	3.79	1.836	1.20
	KMJ Bora-76	1.6	0.299	0.216	0.064	0.049	0.031	3.278	3.91	2.494	0.78
	SoraiBhanu Bora	2.0	0.401	0.198	0.059	0.042	0.028	3.421	3.14	2.486	0.64
	KMJ Bora-89	1.0	0.321	0.207	0.051	0.045	0.032	4.630	3.45	2.371	0.64
	KMJ Bora-80	1.2	0.322	0.203	0.062	0.048	0.034	3.231	3.85	2.582	0.62
2	Narul Bora	1.2	0.334	0.220	0.059	0.047	0.024	3.143	3.76	1.807	0.82
	Nolchutia Bora	1.8	0.386	0.201	0.056	0.042	0.033	3.211	3.43	2.312	0.64
	Nol Bora	1.0	0.346	0.216	0.063	0.047	0.032	3.648	3.20	1.936	0.68
	Mikir Bora	1.6	0.367	0.220	0.059	0.046	0.032	3.401	2.88	1.984	0.42
	Mouguti Bora	2.0	0.412	0.220	0.063	0.044	0.026	3.532	3.40	2.486	0.84
	Misiri Bora,	1.4	0.356	0.207	0.065	0.043	0.028	3.330	3.59	2.556	0.82
	Neoli Bora	1.4	0.298	0.220	0.059	0.046	0.035	3.075	3.78	2.400	0.70
	Poita Bora-2	1.0	0.364	0.201	0.055	0.040	0.035	4.032	3.21	2.430	0.80
	Pakhiloga Bora	1.6	0.348	0.203	0.059	0.044	0.032	3.347	3.37	2.054	1.14
	Mon Bora-2	1.2	0.370	0.22	0.056	0.048	0.028	2.518	2.69	1.554	0.58
	Mon Bora-1	1.0	0.378	0.204	0.059	0.046	0.025	3.234	3.21	1.836	1.36
	Nashinggeti	1.4	0.318	0.207	0.058	0.045	0.029	3.278	3.56	2.054	0.44
	Poita Bor-1	1.6	0.345	0.204	0.059	0.043	0.034	4.121	3.65	2.242	0.42
	Malbhog Bora-1	1.8	0.388	0.220	0.058	0.045	0.031	2.932	2.77	2.744	0.76
	Pakhori Bora	1.4	0.345	0.206	0.058	0.045	0.033	3.907	3.80	1.952	1.12
	Manipuri Bora	1.4	0.314	0.209	0.056	0.046	0.023	3.762	3.48	1.866	0.80
	Tuloxee Bora	1.6	0.346	0.207	0.051	0.042	0.030	3.503	3.67	2.226	0.62
3	KMJ Bora 34	1.6	0.337	0.213	0.062	0.049	0.024	4.551	3.46	2.148	0.74
	KMJ Bora-40	1.0	0.298	0.203	0.056	0.044	0.023	3.278	3.55	1.084	1.64
	KMJ Bora-44	1.9	0.342	0.213	0.058	0.044	0.035	3.209	3.66	2.242	0.74
	KMJ Bora-19	1.6	0.353	0.204	0.063	0.042	0.024	3.291	3.24	1.890	1.02
	KMJ Bora-13	1.8	0.341	0.198	0.057	0.044	0.027	3.648	2.80	2.242	0.84
	KMJ Bora-31	1.6	0.294	0.213	0.065	0.049	0.034	4.430	3.11	2.336	0.56
	KMJ Bora-26,	1.2	0.314	0.207	0.059	0.042	0.028	3.449	3.57	2.454	0.68
	KMJ Bora-35	1.4	0.288	0.203	0.064	0.041	0.027	3.621	3.21	2.532	0.64

		Ash	P	K	Na	Mg	Ca	Zn	Fe	Mn	Cu
	KMJ Bora-11	1.4	0.338	0.190	0.063	0.042	0.028	2.811	3.11	2.156	1.16
	KMJ Bora-21	1.8	0.311	0.207	0.059	0.044	0.023	3.762	4.20	1.584	0.98
	KMJ Bora-25	1.4	0.348	0.201	0.060	0.046	0.028	4.638	3.22	1.742	1.30
	KMJ Bora-36	1.4	0.311	0.204	0.055	0.047	0.028	4.421	3.98	2.628	0.80
	KMJ Bora-27	1.4	0.338	0.220	0.055	0.047	0.033	3.621	3.12	1.020	1.16
4	Ranjit	1.4	0.393	0.212	0.059	0.044	0.028	3.120	2.94	2.684	1.08
	Bokul Bora	1.4	0.382	0.209	0.062	0.042	0.031	3.516	3.42	2.339	0.68
	KMJ Bora -47	1.8	0.393	0.207	0.065	0.047	0.032	3.711	3.27	2.784	0.58
	KMJ Bora-50	1.8	0.344	0.207	0.060	0.045	0.033	4.411	3.38	1.992	1.64
	KMJ Bora-48	1.4	0.365	0.206	0.062	0.042	0.031	3.266	3.7	2.902	0.70
	Kola Bora-5	1.6	0.328	0.190	0.059	0.040	0.023	3.933	3.78	2.312	0.64
	Ronga Bora-5	1.8	0.374	0.207	0.058	0.047	0.032	3.071	3.19	2.086	0.52
	Ronga Bora-3	1.0	0.382	0.220	0.051	0.045	0.026	4.120	3.87	2.808	0.94
	KMJ Bora-51	1.2	0.361	0.220	0.063	0.048	0.034	2.863	3.29	1.998	0.58
	Kola Bora-4	1.4	0.378	0.202	0.057	0.045	0.024	3.824	3.61	3.026	0.84
Admix	Saru Bora	1.5	0.368	0.216	0.051	0.047	0.024	3.122	2.88	2.556	0.56
	Singphow	1.6	0.292	0.203	0.065	0.045	0.032	3.444	3.28	2.996	0.66
	Santi Boka Bora	1.2	0.376	0.204	0.063	0.041	0.028	2.911	3.49	2.420	0.62
	Saudang Bora-1	1.6	0.362	0.207	0.063	0.044	0.029	4.157	3.82	1.710	0.96
	Silatika Bora	1.6	0.386	0.209	0.059	0.044	0.029	2.933	3.78	2.298	0.82
	KMJ Bora-63	1.6	0.279	0.203	0.055	0.045	0.034	2.936	2.96	2.234	0.54
	Rajat Bora	1.2	0.353	0.203	0.062	0.043	0.025	2.103	3.33	2.180	0.60
	KMJ Bora-72	1.6	0.348	0.220	0.064	0.042	0.028	3.211	2.89	2.438	0.58
	KMJ Bora-64	1.6	0.284	0.213	0.056	0.043	0.027	2.993	2.94	3.566	0.48
	Rupohi Bora-2	1.4	0.286	0.209	0.064	0.044	0.033	3.602	3.21	2.054	0.58
	Rupohi Bora-1	1.6	0.312	0.207	0.058	0.048	0.035	3.002	3.28	2.054	0.58
	KMJ Bora-56	1.0	0.324	0.204	0.062	0.043	0.033	4.305	3.69	3.394	0.68
	Malbhog Bora-2	1.6	0.396	0.209	0.055	0.042	0.033	3.982	3.12	2.940	0.62
	Mon Bora-3	1.4	0.361	0.210	0.055	0.043	0.033	2.910	3.29	3.874	0.48
	Paro Chakuwa	1.2	0.378	0.220	0.060	0.049	0.034	4.221	3.36	2.494	0.64
	Pokor Kola Bora	1.6	0.321	0.210	0.062	0.041	0.023	3.878	3.89	1.150	0.52
	Nogajoha Bora	1.8	0.364	0.206	0.064	0.043	0.029	3.131	3.28	3.500	0.88
	Til Kochu Bora	1.4	0.346	0.213	0.055	0.046	0.024	3.702	3.49	2.194	0.52
	Lothow Bora	1.4	0.317	0.209	0.064	0.049	0.031	2.935	3.46	1.898	0.92
	Memon Bora	1.2	0.349	0.206	0.059	0.040	0.035	3.902	2.70	2.368	0.86
	Til Bora-1	1.0	0.376	0.213	0.063	0.048	0.032	2.903	3.79	1.984	0.58
	KMJ Bora-92	1.6	0.349	0.212	0.063	0.044	0.036	3.431	2.60	2.548	0.48
	KMJ Bora-10	1.4	0.312	0.213	0.062	0.043	0.033	3.002	3.21	1.930	0.52
	KMJ Bora-8	1.2	0.318	0.218	0.065	0.044	0.031	3.240	3.24	1.836	0.44
	KMJ Bora-5	1.8	0.364	0.232	0.051	0.045	0.034	3.140	2.34	1.960	0.60
	KMJ Bora-16	1.4	0.372	0.220	0.065	0.047	0.025	3.422	2.38	2.294	0.78
	KMJ Bora-2	1.6	0.323	0.208	0.060	0.042	0.031	3.891	4.21	1.858	0.64
	KMJ Bora-9	1.2	0.298	0.202	0.053	0.039	0.032	3.330	2.40	1.302	0.40
	Kola Ampakhi	1.4	0.389	0.213	0.056	0.043	0.027	3.590	3.94	2.121	0.58
	Ronga Bora-1	1.8	0.378	0.203	0.059	0.042	0.028	3.712	3.22	1.490	0.96
	KMJ Bora-49	1.6	0.378	0.202	0.058	0.044	0.035	3.203	2.58	2.556	0.48
	Ronga Bora-2	1.4	0.394	0.216	0.060	0.045	0.034	2.932	2.93	2.682	0.64
	Ronga Bora-4	1.8	0.362	0.204	0.055	0.042	0.029	3.876	3.52	2.328	0.9
	Til Bora-2	1.6	0.385	0.204	0.062	0.043	0.029	3.004	3.94	2.220	0.68
	Ronga Bora-6	1.4	0.359	0.220	0.060	0.046	0.033	3.794	3.44	2.430	0.50
	KMJ Bora-61	1.6	0.349	0.207	0.059	0.044	0.031	3.933	3.48	2.426	0.78
	KMJ Bora-52	1.0	0.334	0.213	0.051	0.041	0.031	4.312	3.44	2.650	0.98
	KMJ Bora-57	1.4	0.323	0.197	0.064	0.041	0.034	3.140	3.33	1.984	0.84
	KMJ Bora-54	1.0	0.392	0.209	0.051	0.039	0.031	4.120	3.55	3.236	0.56

Pop = Pupulation; Admix = Admixture

structure than the distance-based approach (Pritchard et al. 2000) as individual membership can be probabilistically assigned and allows modeling of admixture and assignment of individuals to more than one sub-population.

By carrying out genome-wide scanning, three markers, RM231 chromosome (Chr.) 3, RM234 (Chr.) 7 and RM162 (Chr.) 6, were found to be linked for Ca. Three SSR markers, RM3 (Chr.) 6, RM400 (Chr.) 6 and RM286 (Chr.) 11 were found to be associated with Cu content. RM 311 on chromosome 10 was found to be associated with Fe content. P was found to be associated with three SSR markers, namely, RM536, RM234 and RM600 on chromosomes 11, 7 and 1, respectively (Table 1). Garcia-Oliveira et al. (2008) identified only one QTL for Cu content near RM204 on Chr. 6. So this QTL might have some similarity with the QTL detected in present study. There are no available report suggesting the presence of QTL for Fe content on Chr. 11. Previous report suggested that the QTL detected in this study on Chr. 1 for P content by Nagesh et al. (2013) might be similar one. By identifying some novel QTL being not reported elsewhere, the present study highlighted the scope of use of AM in identifying QTLs in local rice germplasm warranting a better characterization of these resources.

Authors' contribution

Conceptualization of research (RNS, SB); Designing of the experiments (RNS, SB); Contribution of experimental materials (RNS, SB, HV); Execution of field/lab experiments and data collection (KPSR, HV); Analysis of data and interpretation (RNS, KP); Preparation of the manuscript (KP, SR).

Declaration

The authors declare no conflict of interest.

Acknowledgments

This work was supported by the Department of Biotechnology, Govt of India (No. BT/PR10812/AGR/02/586/2008).Helps form computing facility of Departmental Information Centre (DIC), Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat, India and Chief Scientists, Regional Agricultural Research Stations of Titabar and Karimganj.are acknowledged.

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