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Contribution of RAPD markers for the accumulation of total silicon in various plant parts of aerobic rice (*Oryza sativa* L.)

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Silicon is a major constituent of plant tissues, although not considered to be an essential nutrient, present in consistently high amounts in the terrestrial plants. Silicon has been found to be associated with various abiotic and biotic stress resistance mechanisms especially drought and blast disease in rice. A significant amount of variation was observed in the accumulation of silicon among 85 diverse rice genotypes under study in leaves at flowering and maturity stage as well as in rice grains. A set of 74 RAPD markers that were polymorphic across the 85 diverse genotypes were scored. Single marker analysis (SMA) and stepwise multiple regression analysis (SMRA) was used to determine the extent of markers associated with silicon accumulation in rice. Among the various RAPD markers, SMA established association of four RAPD markers among which $OPC14_{1900}$ alone contributes to 3.89% while in SMRA, $OPD8_{1000}$ and $OPC14_{1900}$ showed more than 11% contribution for silicon accumulation in leaves at flowering stage. For the accumulation of silicon in leaves at maturity stage, SMA established association of five RAPD among which $OPD9_{1400}$ contributing 5.71% and SMRA revealed $OPD9_{1400}$ contributing 6.74%. In SMA, $OPE7_{1900}$, $OPE1_{700}$ and $OPB8_{1250}$ contributed more than 18% towards silicon accumulation in rice grains. Taken together, our result suggests the existence of genotypic variation in silicon accumulation in rice genotypes and is also developmentally regulated and the markers identified here could be validated and used to determine their linkages with these traits on a segregating populations.

Key words : Silicon, Aerobic rice, RAPD markers.

INTRODUCTION

Rice is a symbol of cultural identity and global unity and is the world's most popular food. One of the major challenges for agriculture is to produce more food with less water. Rice is being mainly grown in submerged conditions. Moisture stress is the single most important factor limiting rice productivity in the rain fed habitats. However, there is a need to develop strategies for growing rice under aerobic conditions, which limit the use of water in rice production and to enhance the production of rice per unit volume of water consumed (Bouman and Tuong, 2001). However, to make the concept of "aerobic rice" successful, the existing genotypes needs to evaluated as well as demands the development of new varieties that are responsive to inputs (water, nutrients) to reach high yields under non-flooded conditions (Bouman *et al*., 2002).

Silicon a micronutrient, is the second most abundant element in the earth's crust after oxygen (Bond and McAuliffe, 2003) It is found to enhance photosynthesis, positive effect on growth and yield, impart resistance to water stress, lodging, diseases and abiotic stress like salinity, drought, and protection against temperature extremes (Epstein, 2001). It was believed that silicon accumulation in the cell wall prevents the pathogen entry, however recent studies suggest that silicon stimulates the expression of disease resistance genes in dicots (Fawe *et al.*, 2001). Silicon reduces the severity levels of diseases including blast, brown spot, sheath blight, leaf scald and grain discoloration (Seebold *et al*., 2000). Silicon stimulates the production of phenolic compounds and/or phytoalexins, which play a primary role in the defense response against rice blast infection (Rodrigues *et al*., 2003).

Molecular markers commonly referred to as DNA markers, show Mendelian inheritance, stably inherited, without pleiotropic effect and are unaffected by the environment or developmental stages. Marker assisted selection, gene pyramiding, QTL (Quantitative trait loci) mapping, targeted map based cloning of important genes, introgression of exotic germplasm, DNA fingerprinting of crops for detecting the markers associated with various traits are being commonly used in crop improvement program. Ma *et al.* (2004) by using microsatellites and expressed sequence tag (EST)-based PCR markers showed that silicon transporter gene is localized on chromosome 2 flanked by microsatellite marker RM5303 and expressed sequence tag-based PCR marker, E60168. In the present study, we evaluated 85 diverse rice

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genotypes grown under aerobic conditions, to understand the accumulation pattern of silicon and to find out the RAPD markers associated with silicon accumulation in rice.

MATERIALS AND METHODS

The plant material includes 85 diverse rice genotypes obtained from International Rice Research Institute, Philippines. The details of the genotypes are presented in Table 1.

Experimental design and layout :

Rice genotypes were raised during summer (dry) season of 2005. The experiment was laid out in randomized complete block design (RCBD) in an experimental field. The genotypes were grown under aerobic conditions, the irrigation was given at an interval of 5 days. Direct line sowing was done with a spacing of 40cm between lines and 20cm spacing between plants. Every genotype was replicated twice.

Total silicon estimation :

Total leaf silicon content was estimated in leaves at flowering and maturity stage and in grains in triplicate. The rice samples were dried at 65 ^{0}C for 3 days and powdered. Three hundred milligrams of sample was microwave-digested in acid mixture containing 3mL of Nitric acid (70%), 3mL of Hydrogen peroxide (30%), and 2mL of Hydroflouric acid (48%). The digested samples were diluted to 50mL with 4% boric acid. Si estimation was carried out according to the method described by Ma *et al*., (2002). To 0.05mL of digested sample, 6mL of 0.5N Hydrochloric acid, 0.8mL of 10% Ammonium molybdate, 0.8mL of 20% tartaric acid, 0.8mL of reducing agent (0.5g sodium sulphite + 0.25 g 1-amino-2-napthol-4-sulfonic acid, 15g sodium bisulphate in 100mL of distilled water) was added and diluted to 20.0 mL. Absorbance was measured at 600 nm after incubation at room temperature for two hour.

DNA Extraction:

DNA extraction was carried as per the method described by Porebski *et al.,* (1997) with certain modifications. 100mg of leaf powder in 2.0ml of pre-warmed extraction buffer (100mM Tris pH 8.0 containing 20mM EDTA, 1.4M NaCl, 1% b-ME, 3% CTAB) was incubated in water bath at 65^{0}C for 30 min with periodic shaking. Equal volume of chloroform: iso-amylalcohol (24:1v/v) was added and vortexed gently, centrifuged at 12,000 rpm for 20 min at 4^{0}C. Aqueous phase was repeatedly washed with equal volume of chloroform: iso-amylalcohol (24:1 v/v). To the aqueous phase, equal volume of chilled iso-propanol and one tenth of the volume of 5M NaCl was added, mixed gently, kept at –40^{0}C for overnight to accentuate the precipitation of DNA. Centrifugation was done at 12,000 rpm for 20 min at 4^{0}C to recover DNA pellet. The pellet was washed with 70% aqueous ethyl alcohol and air-dried. The pellet was dissolved in 100µl of TE buffer and incubated with 3µl (10mg/ml) of RNase overnight at 37^{0}C. Washed with equal volume of phenol: chloroform: iso-amylalcohol (25:24:1v/v) and chloroform: iso-amylalcohol (24:1v/v). DNA was precipitated by adding equal volume of chilled iso-propanol and kept at –40^{0}C for 2 hour's, centrifuged at 12,000 rpm for 20 min to pellet the DNA. DNA pellet was dissolved in 300 µl of TE buffer and stored at –40^{0}C. DNA quantification was done at OD_{260} nm and was diluted to a final concentration of 12.5çg ìl^{-1} and 2ìl of this DNA was used for the PCR amplification.

Selection of RAPD Primers:

120 random primers of arbitrary sequence (Operon Technologies Inc.) were screened by PCR analysis. Of the 120 primers 14 primers produced strong, intense and unambiguous bands, and were selected for analyzing the DNA sample of diverse rice genotypes. PCR products were separated on a 1.5 % agarose gel (Table 2).

PCR (polymerase chain reaction) was performed at the Plant Molecular Biology Laboratory, Department of Biotechnology, University of Agricultural Sciences, Bangalore India, in 0.2mL, thin walled PCR tubes, using MJ-Research Thermocycler (Model PTC-100). The RAPD protocol described by Williams *et al*. (1993) was used with the modifications (standardization of $MgCl_2$, deoxynucleoside triphosphates (dNTPs), template DNA and primer concentration) to amplify rice DNA. RAPD analysis was carried out in a total volume of 20 ìl containing 25-30 çg of template DNA, 1X *Taq* polymerase assay buffer, 200 ìmol/L each of the dNTPs, 3mmol/L $MgCl_2$, 10 pmol random decamer primers (Operon Technologies Inc., Huntsville, Ala.), and 1 U *Taq* polymerase. PCR amplification was carried out with an initial denaturation at 95 ^{0}C for 5 min followed by 45 cycles each step consists of denaturation at 95 ^{0}C for 1 min, primer anneling at 36 ^{0}C for 1 min, and extension at 72 ^{0}C for 2 min followed by a final extension of 10 min at 72 ^{0}C.

Data Analysis:

Single Marker Analysis (SMA) :

Association of RAPD markers with the means of the silicon content in rice was done independently. The R^2

Table 1: Diverse rice genotypes analyzed in the present study

Genotype No.	Designation	Source	Genotype No.	Designation	Source
1	AUS 196	A03WS-09	64	IR 77298-5-6	A03DS-03
3	B 6144F-MR-6	A03WS-10	65	PR 27699-B-D808-4-4	A03DS-03
5	CT 13370-12-2-M	A03WS-09	66	PSBRC 9	A03WS-01
7	CT 13382-8-3-M	A03WS-10	67	UPL RI 7	A03DS-03
8	CT 6510-24-1-2	A03WS-09	68	Yunlu 29	A03DS-03
9	CT 6516-24-3-2	A03WS-09	71	IR 76558-156-4-1-3	A04DS-02
13	IR 47686-30-3-2	A03WS-09	73	IR 76569-259-1-1-3	A04DS-02
14	IR 55419-04	A03WS-09	74	IR 76569-243-2-1-4	A04DS-02
15	IR 55423-01	A03WS-10	77	IR 76569-166-4-2-2	A04DS-02
18	IR 65907-116-1-B	A03WS-09	78	DGI-196	Binam
20	IR 66421-062-1-1-2	A03WS-09	79	DSL-89-3	BG300
21	IR 66424-1-2-1-5	A03WS-09	80	DSL-104-1	Jhna349
22	IR 68702-072-1-4-B	A03WS-09	81	DGI-195	Binam
23	IR 70358-84-1-1	A03WS-09	83	IR64-e7	
25	IR 71524-44-1-1	A03WS-09	85	DSU-16-3	Lemont
26	IR 71525-19-1-1	A03WS-09	88	RF-53-20	Binam
28	IRAT 170	A03WS-10	89	DSU-4-4	Feng-Ai-Zan
29	IRAT 177	A03WS-09	90	DSU-4-18	Feng-Ai-Zan
30	IRAT 212	A03WS-10	91	DSU-8-1	Babaomi
31	IRAT216	A03WS-09	92	DSL-109-3	MR 106
32	MARAVILHA	A03WS-09	93	DGI-296	FR 13A
35	PSBRC 82	A03WS-09	94	DSL-101-3	Ptb33
36	UPL RI 5	A03WS-09	95	DSL-89-10	BG300
37	UPL RI 7	A03WS-09	97	DGI 21	STYH
38	VANDANA	A03WS-09	98	DSU-4-11	Feng-Ai-Zan
41	WAB 638-1	A03WS-09	100	DGI-143	Type3
42	WAB 96-1-1	A03WS-09	101	DGI-138	Type3
43	WAY REREM	A03WS-09	102	DGI 32	STYH
45	IR 55419-04	A03DS-03	103	DSU-4-7	Feng-Ai-Zan
46	IR 64	A03WS-10	104	DGI 8	STYH
47	IR 70210-39-CPA-7-1-1-4-2	A03WS-01	105	DSL-81-1	Cisedane
48	IR 71525-19-1-1	A03DS-03	106	DSU-18-6	OM 1706
50	IR 72875-94-3-3-2	A03DS-03	107	DSL-78-10	IR72
51	IR 74371-3-1-1	A03DS-03	108	DSU-10-5	Basmati
52	IR 74371-46-1-1	A03DS-03	109	DGI-155	Type3
53	IR 74371-54-1-1	A03DS-03	110	DSL-111-4	MR 167
55	IR 74371-78-1-1	A03WS-01	112	DSL-69-6	Hua-Gen_Xian74
56	IR 74963-262-5-1-3-3	A03WS-01	114	IR 64	
57	IR 75003-95-5-1-3	A03WS-01	115	DGI 81	BR24
59	IR 77076-B-21-1-2	A03WS-01	118	IR 79907-B-3	A04DS-03
60	IR 77078-B-17-3-2	A03WS-01	260	IR 79907-B-145	A04DS-03
61	IR 77080-B-6-2-2	A03WS-01	292	IR 79907-B-177	A04DS-03
62	IR 77298-12-7	A03DS-03			

* The genotypes are represented by their number everywhere in the script.

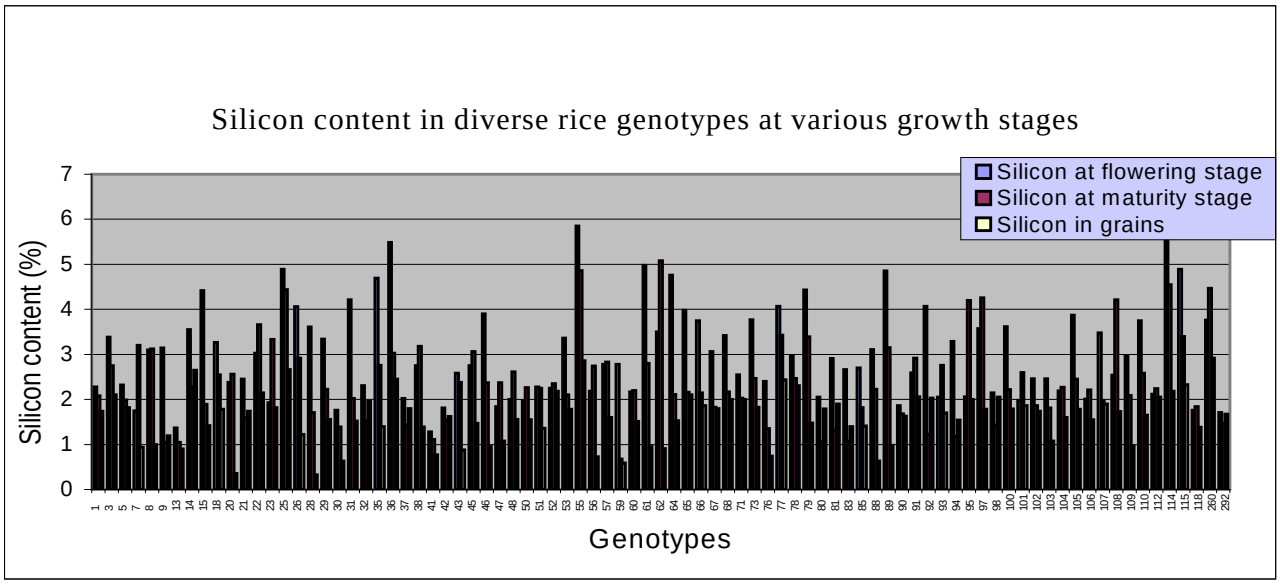

Fig. 1: Variation among diverse rice genotypes for silicon content in various plant parts at various growth stages.

values were determined to find the extend of variability explained by these markers; R^2 values assumed positive or negative values (parameter estimate) indicating the association of the markers (bands of appropriate size) with the increase or decrease in the silicon content.

Stepwise Multiple Regression Analysis (SMRA) :
Association between RAPD and means of silicon content of the genotypes were established using the multiple regression approach. Each trait (silicon content at various stages) was treated as a dependent variable and the various molecular marker genotypes (scored 1 for presence and 0 for absence) as independent variable. The analysis was based on the model (Virk *et al*., 1996):

$$Y = a+b_1m_1+b_2m_2+\text{————————}b_jm_j\text{————————}b_nm_n+d+e$$

Where,
Y = Genotype means for a silicon content in rice at various stages, m_j = determines the variation in the dependent variable, b_js = partial regression coefficient, d = residual effect between genotypes left after regression and e = random error of Y that includes environmental effect.

SMA (Single marker analysis) and SMRA (Stepwise multiple regression analysis) were carried out using SAS (Statistical analysis software) v6.12 program (SAS, 1989). Phylogenetic relationships among the genotypes were assessed by adopting cluster analysis using STATISTICA software. The dendogram (Fig. 2) was computed according to ward's method of clustering, using a minimum variance algorithm (Ward 1963).

Results and Discussion

Nutrient management is an important factor for increasing yield of a crop. Silicon is a major constituent of plant tissues, considered to be an essential nutrient for horsetails (class Equisetaceae) but not for terrestrial plants in general (Epstein, 1994, Hoffman and Hillson, 1979). Silicon gives resistance against diseases, drought and metal toxicity (Hodson *et al*., 2005, Seebold *et al*., 2000). Silicon stimulates the production of some phenolic compounds and/or phytoalexins. These findings bring the new insights into the complex role played by silicon in imparting resistance to rice blast disease (Rodrigues *et al*., 2003). Fauteux and co-workers in 2005 reported that the unique properties of silicon make it bioactive and regulator of plant defense system. It acts as a secondary messenger. It binds to the –OH group of proteins, which are involved in signal transduction.

Evaluation of diverse rice genotypes for total silicon accumulation:
Total silicon estimation was done from the rice leaf at flowering, maturity stage and in grains. Significant difference in total leaf silicon content at flowering stage was observed. Genotype 55 had the highest silicon content (5.85%) followed by the genotypes 114, 36 and 61. Genotype 41 had the lowest (1.28%) followed by the genotypes 13, 292 and 7. Total leaf silicon content at maturity stage also showed significant variation among

Table 2 : Illustration of percent polymorphism generated from each marker.

Marker	DNA Band		% Polymorphism	PIC
	Monomorphic	Polymorphic		
OPE 13	1	3	75.00	0.35
OPE 4	2	6	75.00	0.21
OPE 2	1	6	85.70	0.26
OPE 7	5	4	44.44	0.01
OPE 1	1	4	80.00	0.31
OPD 5	0	4	100.00	0.35
OPD 8	0	5	100.00	0.35
OPD 9	0	6	100.00	0.36
OPC14	4	3	42.85	0.36
OPD 3	0	8	100.00	0.26
OPC 11	1	6	85.71	0.09
OPB 8	1	6	85.71	0.24
OPA 9	4	7	63.63	0.16
OPA 11	0	6	100.00	0.30

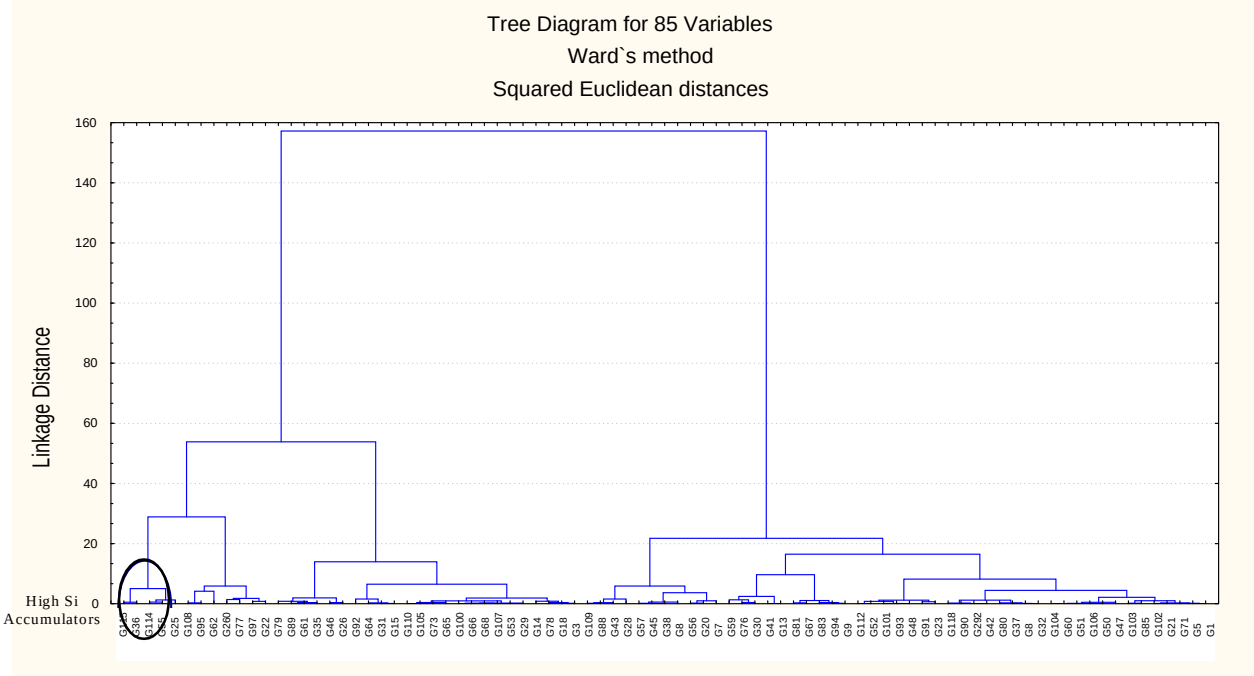

Fig. 2: Dendrogram of rice genotypes developed from combined silicon data

the rice genotypes. Genotype 62 showed highest silicon content in the leaves at maturity stage (5.08%) followed by genotypes 55, 114 and 260. Genotype 59 had the lowest leaf silicon content at maturity stage (0.68%), followed by the genotypes 13, 9 and 80. Significant difference among the genotypes for the grain silicon accumulation was observed. Genotype 260 had highest grain silicon content (2.92%) followed by the genotypes 55 and 25. Genotype 28 was found to have the lowest grain silicon accumulation (0.33%) followed by the genotypes 20, 59 and 30 (Fig. 1). It has been observed that the silicon content in monocots is by and large higher than dicots.

Especially in rice, it is reported to as high as 6.3% (Takahashi and Miyaki, 1976, Nishimura *et al.*,1989). However, the genotypic variation in rice is quite significant as compared to other cereal crops (Deren *et al.*, 1992; Winslow *et al.*, 1997).

Cluster analysis was done to group the genotypes on the basis of silicon accumulation. Two major clusters were formed, all the high silicon accumulators clustered together (Fig. 2).

Identification of RAPD markers associated with silicon accumulation in rice:

Of the 120 primers 14 primers produced strong, intense and unambiguous bands, and were selected for analyzing the DNA sample of diverse rice genotypes (Table 2). A total of 94 bands were amplified by the fourteen RAPD primers, out of which 74 (78.72%) were polymorphic. The band size ranged from 350bp to 2.4Kb. The total number of fragments amplified per primer ranged from 4 to 11 with an average number of amplified fragments 6.7. Among the primers employed for amplification, OPA9 amplified maximum fragments (11) and OPD5 amplified minimum fragments (4). 100% polymorphism was observed with the primers OPD3, OPD5, OPD8, OPD9 and OPA11 whereas polymorphism was the least (42.85%) in OPD11 (Table 2). The PIC (polymorphic

Table 3 : Single Marker Analysis and Stepwise Multiple Regression Analysis carried out for total silicon content in rice leaves at flowering stage and RAPD markers

Marker	Partial R^2	Total R^2	PE	Prob.>F
Single Marker Analysis				
$OPE13_{1900}$	0.0592	0.0592	-0.5571	0.0249*
$OPD3_{750}$	0.0672	0.0672	-0.5548	0.0166*
$OPC14_{1900}$	0.0389	0.0389	0.5547	0.0405*
$OPB8_{500}$	0.0482	0.0482	-0.4939	0.0435*
Stepwise Multiple Regression Analysis				
$OPD3_{750}$	0.0672	0.0672	-0.63161	0.0166*
$OPD8_{1000}$	0.0535	0.1206	0.50051	0.0284*
$OPC14_{1900}$	0.0577	0.1783	0.47666	0.0194*
$OPE7_{500}$	0.0396	0.2179	-2.6866	0.0475*
$OPA9_{650}$	0.0523	0.3373	-2.90064	0.0159*

Table 4: Single Marker Analysis and Stepwise Multiple Regression Analysis carried out for total silicon content in rice leaves at maturity stage and RAPD markers

Marker	Partial R^2	Total R^2	PE	Prob.>F
Single Marker Analysis				
$OPD5_{1500}$	0.0413	0.0413	-0.3785	0.0420*
$OPD9_{1400}$	0.0571	0.0571	0.4783	0.0276*
$OPD9_{900}$	0.0547	0.1118	-0.5723	0.0274*
$OPC14_{500}$	0.0727	0.0727	-0.5442	0.0126*
$OPA9_{650}$	0.0783	0.0783	-1.7180	0.0095**
Stepwise Multiple Regression Analysis				
$OPA9_{650}$	0.0783	0.0783	-4.7039	0.0095**
$OPC14_{500}$	0.0832	0.1616	-0.8791	0.0055**
$OPD9_{1400}$	0.0674	0.2290	0.39544	0.0094**
$OPD9_{900}$	0.0509	0.2799	-0.6183	0.0197*
$OPD3_{1650}$	0.0423	0.3222	---	0.0292*

information content) ranged from 0.01 (OPE7) to 0.36 (OPC14 and OPD9) with an average PIC 0.26 (Table 2).

The data obtained from RAPD profiles were used to find out RAPD markers contributing for the accumulation of silicon in various rice plant parts. Single marker analysis and Step wise multiple regression analysis for accumulation of silicon in various rice plant parts and at various stages showed the following results:

Single marker analysis and Step wise multiple regression analysis (SMA and SMRA):

SMA revealed the presence of four RAPD markers associated with total leaf silicon content at the flowering stage. Maximum association was shown by $OPD3_{750}$ (6.72%) followed by $OPE13_{1900}$ (5.92%), $OPB8_{500}$ (4.82%) and $OPC14_{1900}$ (3.89%). Only $OPC14_{1900}$ showed the positive PE (parameter estimate) value. SMRA revealed the association of five markers, which together showed 27.03% association towards total leaf silicon content at the flowering stage. The positive PE values were shown by $OPD8_{1000}$ and $OPC14_{1900}$ (Table 3). Five RAPD markers were found associated with the total leaf silicon content at the maturity stage by SMA. Maximum association was shown by $OPA9_{650}$ (7.83%) followed by $OPC14_{500}$ (7.27%), $OPD9_{1400}$ (5.71%), $OPD9_{900}$ (5.47%) and $OPD5_{1500}$ (4.13%). Only $OPD9_{1400}$ showed the positive PE value. SMRA revealed the association of five markers, which together showed 32.21% association towards total leaf silicon content at the maturity stage, out of the five markers identified by SMRA, maximum association was shown by $OPC14_{500}$ (8.32%) and the positive PE value was shown by only $OPD9_{1400}$ (Table 4). Seven RAPD markers were found associated with total grain silicon content in rice by SMA. Maximum association was shown by $OPD3_{750}$ (10.13%), followed by $OPA11_{1650}$ (8.17%), $OPA9_{1700}$ (7.21%), $OPE1_{700}$ (7.02%), $OPE7_{1900}$ (6.50%), $OPB8_{1250}$ (5.27%) and $OPA9_{775}$ (4.92%). The positive PE values were shown by $OPE7_{1900}$, $OPE1_{700}$ and $OPB8_{1250.}$ SMRA revealed the association of fifteen markers, which together showed 61.22% association towards total grain silicon content in rice. Out of the fifteen markers identified by SMRA maximum association was shown by $OPD3_{750}$ (10.13%). $OPE1_{700}$, $OPE7_{700}$, $OPE13_{900}$ and $OPE2_{1500}$ were found positively associated with the grain silicon accumulation and contributed 16.54% (Table 5).

Zargar and Prasad (2007) found out 3 RAPD markers contributing more than 18% for accumulation of silicon in rice grains and a SSR marker RM 10 contributing 4.15% for the accumulation of silicon in leaves at maturity stage in rice. The silicon was estimated from the whole grain but deposition of silicon is mainly in the husk. As the DNA was extracted from the leaf samples and silicon was estimated from the whole grain possessing endosperm which is triploid, so the markers we found out associated with grain silicon accumulation are mainly contributing for silicon accumulation in the grain husk. Various attempts have been made with fruitful results in establishing association between traits and markers across germplasm collection in oat , rice , maize , and barley . In oat, Beer *et al.* (1997) found association between markers and 13 quantitative traits in a set of 64 landraces and cultivars. Shalini *et al.* (2007) determined the combination of 6 SSRs showed 100% association with mite infestation and a combination of 3 RAPDs accounts for 83.86% of mite resistance in the selected materials using SMRA. Ivandic *et al.* (2003) found associations between markers and the traits of water –stress tolerance and the powdery mildew resistance in 52 wild barley lines. Benmoussa *et al.* (2003) evaluated 123 DH lines of a cross between IR64 and Azucena in the field in two different environments. They revealed that individual QTLs showed a range of sensibility to environment as some QTLs were detected only in a single environment while others were detected in two environments. Virk *et al.* (1996) used a set of 63 polymorphic RAPD markers generated from 8 RAPD primers to amplify 47 diverse Asian rice genotypes and carried out multiple regression analysis to determine association between the presence and absence of individual marker. Their results showed that 85% of the variation in the culm number and nearly all the variation in the flowering time could be explained by regression models using sets of RAPD markers. The association that have been detected by coupling of molecular markers (RAPDs) and with the multiple regression analysis could subsequently change the way that crop biodiversity will be used in the future (Kurata *et al.*, 1994). Under specialized assessment conditions (such as stress tolerance) these markers will provide an efficient means of predicting the value of additional germplasm for such characteristics and even will help to identify suitable material among the germplasm in situ.

Conclusion:

Silicon is a beneficial element for the growth of rice. It also gives resistance against various biotic and abiotic stresses. The 85 diverse rice genotypes of rice evaluated under low moisture conditions showed significant difference in the silicon content in various rice plant parts at various stages. Many RAPD markers were found contributing for the silicon accumulation in various rice

Table 5 : Single Marker Analysis and Stepwise Multiple Regression Analysis carried out for total grain silicon content and RAPD markers

Marker	Partial R^2	Total R^2	PE	Prob.>F
Single Marker Analysis				
$OPE7_{1900}$	0.0650	0.0650	1.9700	0.0185*
$OPE1_{700}$	0.0702	0.0702	0.2900	0.0142*
$OPD3_{750}$	0.1013	0.1013	-0.3550	0.0030**
$OPB8_{1250}$	0.0527	0.0527	0.2738	0.0345*
$OPA9_{1700}$	0.0721	0.0721	-1.0827	0.0129*
$OPA9_{775}$	0.0492	0.1214	-0.2138	0.0350*
$OPA11_{1650}$	0.0817	0.0817	-0.3687	0.0080**
Stepwise Multiple Regression Analysis				
$OPD3_{750}$	0.1013	0.1013	-0.1335	0.0030**
$OPE1_{700}$	0.0597	0.1611	0.3152	0.0179*
$OPA9_{1700}$	0.0480	0.2090	-0.4407	0.0295*
$OPE7_{700}$	0.0409	0.2499	2.5109	0.0400*
$OPE7_{500}$	0.0378	0.2877	-1.4778	0.0439*
$OPD5_{800}$	0.0398	0.3275	-0.5310	0.0347*
$OPE13_{900}$	0.0437	0.3712	0.4873	0.0234*
$OPA9_{2400}$	0.0441	0.4153	---	0.0191*
$OPD8_{1500}$	0.0584	0.4737	-0.4440	0.0051**
$OPB8_{500}$	0.0334	0.5071	-0.2043	0.0281*
$OPD9_{900}$	0.0249	0.6242	-0.4201	0.0374*
$OPA9_{1500}$	0.0224	0.6466	-0.3734	0.0432*
$OPA11_{1100}$	0.0218	0.6684	-0.5273	0.0413*
$OPE2_{1500}$	0.0211	0.6895	0.3951	0.0392*
$OPB8_{1400}$	0.0149	0.7771	-0.3763	0.0499*

Note: **Significant at 0.01 probability level *Significant at 0.05 probability level

plant parts. These markers can be used to identify the new genotypes for there silicon content which will indirectly tell us about the potential of a genotype in giving resistance against various biotic and abiotic stresses. As the diverse genotypes are used so large number of markers were detected that vary across the genotypes rather than between just 2 parents. The markers identified here could be validated and used to determine their linkages with these traits on a segregating populations.

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