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Unraveling the genetics of calcium content in finger millet grains through association mapping

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Abstract

Combating mineral deficiencies has been a major challenge for agricultural scientists world over. Lack of calcium in diets could be overcome through biofortifying food crops especially cereals. Finger millet (*Eleusine coracana*) grains are highly rich in calcium, therefore in the present study this trait was explored using association study on a natural population of finger millet. A total of 238 accessions of finger millet were analysed using 85 simple sequence repeats (SSR) markers. SSR profiles generated 160 alleles and 20 primer pairs were polymorphic across the population with 0.14 to 0.78 polymorphic information content. Grain Calcium content (GCC) varied from 72 to 452 mg/100g seed. Two accessions, GPHCPB45 (452.8 mg) IE 2957 (447 mg) were found with highest GCC. Association analysis through the general linear model in TASSEL software detected two markers UGEP78 and UGEP60 in significant association (P < 0.001) to calcium content. The molecular marker, UGEP60 can be a potential marker with phenotypic variance amounting to 13.8%. However, the mixed linear model did not detect any significant associations probably because of low polymorphism within the finger millet collection considered in this study. Identification of two accessions with higher calcium content may be useful in finger millet improvement.

Key words: Association mapping, calcium-QTL, finger millet, population structure

Introduction

It was revealed in the 2018 Global Nutrition Report that almost every country in the world is affected with malnutrition, which itself indicates that we are still quite far from achieving food security to all (Development Initiatives 2018). There is a great need to grow diverse crops, which are nutritionally rich and can counter the malnutrition ensuring availability of nutrient-rich foods to human population. Among major nutrients required by human body, calcium (Ca) is one that has a major role in building human body structure and participate in regulatory functions as well (Beto 2015). Common staple cereals such as rice, wheat, and maize are quite low in calcium hence biofortification of such crops with calcium would have highly beneficial in eliminating calcium deficiency prevalent in under-developed and developing countries. *Eleusine coracana*, commonly known as finger millet, is a nutritionally rich millet crop with high concentrations of calcium, iron and zinc as well as quality proteins and carbohydrates (Kumar et al. 2016; Sood et al. 2016). Genetic variation with respect to seed calcium exists in finger millet germplasm and grain calcium concentrations as high as 450 mg (Panwar et al. 2010a) and 489 mg (Upadhyaya et al. 2011) per 100 g of seed has been reported in some of the finger millet genotypes. It is a well-established fact that variation across a germplasm is essential for identification of important/major alleles for achieving significant changes in a trait (Conn *et al.* 2012). Sharma et al. (2017), emphasized on molecular marker applications and omics based analyses in finger millet, which can be utilized in order to raise biofortified crops enriched with calcium. Approaches directed towards identification of genetic markers linked to or responsible for transport and accumulation of calcium in grains are essential to furnish details about mechanism of calcium transport. In this regard, linkage and association mapping methods have proved to be very versatile in genetic dissection of complex quantitative traits such as mineral accumulation. Subsequently, quantitative trait loci (QTLs) have been identified and reported for mineral uptake and

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accumulation in several cereals, legumes as well as other crop species (Ghandilyan et al. 2006; Waters and Grusak, 2008; Descalsota-Empleo et al. 2019). Simple sequence repeats (SSRs) are widely used marker system in studies pertaining to estimation of genetic structure, diversity and trait associations. The associated alleles/markers can be utilized further to fine tune the genetics of complex traits. Several, initiatives have been taken towards improvement of finger millet for key phenotypic traits. Babu et al. (2014a) identified genic and genomic SSR markers associated to *opaque2* modifier genes influencing tryptophan concentrations in finger millet grains. These associated markers showed phenotypic variance in the range of 11% and the key agro-morphological characters were also found to be associated with SSR markers in finger millet (Babu et al 2014b). GWAS for agronomic characters in Ethiopian and some exotic finger millet genotypes using 20 SSR markers also identified significant marker-trait associations (Lule et al. 2018). A global germplasm collection of finger millet was genotyped and SSR markers were associated to phosphorus (P) starvation responses and led to identification of four QTLs responsible for the trait (Ramakrishnan et al. 2017). Various such association studies utilizing the polymorphic properties of SSR markers have been reported in different species *viz., Zea mays* (Kim et al. 2017), *Helianthus annuus* (Darvishzadeh 2016); *Medicago sativa* (Jia et al. 2017); *Camellia sinensis* (Liu et al. 2018) and many more. Finding linked and associated molecular markers for seed calcium content through association mapping will help accelerate the selection process and significantly improve the breeding efficiency in finger millet. The present study was therefore aimed at identification of non-genic or genomic SSR markers associated to calcium content through an association mapping approach in a global finger millet collection.

Materials and methods

Plant material and phenotyping for grain calcium content (GCC)

A natural collection of 238 accessions of finger millet was procured from G.B.P.U.A&T. (Ranichauri Hill Campus) Pantnagar, India, International Crops Research Institute for the Semi-Arid Tropics, Patancheru and University of Agricultural Sciences Bangalore. Most of the studied finger millet accessions have their origin in India, South Asian regions, East and West Africa, one accession each from North America and Europe and two from unknown region. The accessions were grown at ICAR-VPKAS experimental farm in 10 blocks under augmented block design with two checks, VR708 and RAU8. Recommended agronomic practices (N:P:K 40:20:0) were followed for raising a good crop. The panicles were harvested, dried and threshed manually and stored in brown seed packets for biochemical analysis. The protocol for calcium concentration estimation was used as described by Barbeau and Hilu (1983). Moisture free 0.5g seed sample was acid digested in 5ml of 2:1 mixture of nitric acid and perchloric acid at 200°C followed by drying. The digests were re-suspended in 2% nitric acid. The acids used were trace metal grade and the water used was deionised via a MilliQ system (Millipore, Billerica, MA, USA). Samples were analyzed for Ca using atomic absorption spectrophotometer (AAS) (SensAA GBC Scientific Equipment). GCC was expressed in 'mg/100g seed' and calculated as an average of three replicates.

SSR genotyping

Genomic DNA from leaves was isolated following Murray and Thompson (1980) standard method, quantified and quality checked via electrophoresis in 0.8% agarose gel (Maniatis et al. 1989). A set of 85 SSRs reported by Dida et al. (2007, 2008) and Naga et al. (2012) were further used for screening of polymorphism in the 238 accessions (Table 1). PCR was performed in Thermal Cycler and the amplified products were electrophoresed on 3.5% Super Fine Resolution Agarose gels and clear fragments of size 100-400bp were scored.

The polymorphic information content (PIC) was obtained using PowerMarker version 3.25 (Liu and Muse 2005) and cluster analysis was performed using the UPGMA (unweighted pair group method with arithmetic mean) method and the phylogenetic tree was generated using the software MEGA version 4 (Tamura et al. 2007). Population structure was estimated using software program STRUCTURE version 2.3.4 which utilizes model based clustering (Pritchard et al. 2000; Falush et al. 2003). The program was run in default mode with admixture and correlated allele frequencies included between populations. The number of subgroups (K) was estimated by running different K values in the program (with K varying from 1 to 10). A burning period of 100,000 and 100,000 MCMC repetitions were used. The average estimated log probability of the data Pr ($x|k$) ideally should plateau at the most appropriate level of K. The hypothesis of association of molecular markers with phenotypic values and estimated population structure was inferred through software program TASSEL version 3.0.1 (Yu et al. 2006; Bradbury et al. 2007) with Q GLM (General Linear Model) and mixed linear model (MLM) functions with significant threshold for the association set at P < 0.01. The kinship matrix along with the genotypic and phenotypic data was used in the MLM approach.

Results and discussion

Genetic variation is essential to achieve significant increases in seed calcium concentrations through molecular breeding. Genetic variation has been reported in finger millet germplasm with respect to seed calcium (Vadivoo et al. 1998) in 36 finger millet genotypes, with maximum value of 487 mg/100g and a minimum of 162 mg/100g seed. Variations in calcium content have been recorded ranging from 50 to 390mg/100g seed (Admassu et al. 2009; Babu et al. 1987). In other studies, a maximum 450 mg/100 g and 489 mg/100 g, calcium contents in seed have been earlier reported (Panwar et al. 2910a; Upadhyaya et al. 2011). In the present study, data for GCC varied among finger millet genotypes from 72 mg to 452 mg at an average value of 267 mg per 100g seed and showed a continuous distribution pattern (Table 1). The highest concentration of calcium was observed in accessions, GPHCPB45, GPHCPB44, IE2957 and IE6537, which represent both the Indian subcontinent and the other

Table 1. List of genotypes with grain calcium contents used in the association mapping population

S. No.	Accession name	Ca content (mg/100g seeds)	S. No.	Accession name	Ca content (mg/100g seeds)	S. No.	Accession name	Ca content (mg/100g seeds)	S. No.	Accession name	Ca content (mg/100g seeds)
1.	GPHCPB45	452.80	29.	GE3138	330.60	57.	L5	104.73	85.	GPHCPB5	248.19
2.	GPHCPB44	439.76	30.	GPHCPB21	328.69	58.	HR911	110.63	86.	GPHCPB6	237.31
3.	GPHCPB31	417.46	31.	GE1163	327.38	59.	MR6	111.58	87.	GPHCPB8	224.18
4.	GPHCPB40	415.43	32.	GPHCPB37	326.05	60.	PR202	112.51	88.	GPHCPB11	226.89
5.	GE4711	393.63	33.	GPHCPB42	325.94	61.	GPHCPB1	117.56	89.	GPHCPB12	201.23
6.	GPHCPB35	392.60	34.	GE4449	325.38	62.	MR1	119.05	90.	GPHCPB15	242.40
7.	GPHCPB26	384.88	35.	GE4440	324.88	63.	VL315	133.32	91.	GPHCPB19	288.18
8.	GE1583	380.50	36.	GE3764	323.53	64.	PRM801	136.04	92.	GPHCPB22	219.55
9.	GE4404	377.50	37.	GE4547	322.00	65.	GPHCPB2	136.11	93.	GPHCPB23	248.97
10.	GE619	374.63	38.	GE469	320.38	66.	OUAT26	144.15	94.	GPHCPB24	294.84
11.	GE4471	341.17	39.	GE3019	316.67	67.	GPHCPB13	146.16	95.	GPHCPB25	248.21
12.	GE86	387.00	40.	GE1221	313.38	68.	GPHCPB27	150.50	96.	GPHCPB29	201.93
13.	GE1146	370.00	41.	GE1200	313.38	69.	GPHCPB9	163.94	97.	GPHCPB30	278.35
14.	GE2136	366.88	42.	GE3219	313.00	70.	VL149	165.15	98.	GPHCPB32	227.92
15.	GE4668	359.67	43.	PRM1	310.54	71.	GPHCPB28	165.45	99.	GPHCPB34	274.00
16.	GE2246	359.00	44.	GE778	309.63	72.	INDAF8	166.49	100.	GPHCPB38	269.10
17.	GE1680	355.63	45.	GPHCPB20	309.45	73.	GPHCPB18	168.24	101.	GPHCPB39	289.14
18.	GE4601	347.33	46.	GE591	308.13	74.	INDAF9	170.06	102.	GPHCPB41	286.27
19.	GE4596	345.82	47.	GE4798	302.12	75.	GPHCPB43	170.55	103.	GPHCPB46	270.59
20.	GE104	345.20	48.	GE3767	300.71	76.	PRM701	172.54	104.	GPHCPB48	294.07
21.	GE3885	344.73	49.	PES400	72.10	77.	GPHCPB7	174.13	105.	GPHCPB49	257.33
22.	GE225	343.33	50.	GPU28	72.65	78.	GPHCPB16	175.22	106.	GPHCPB51	249.25
23.	GE2447	343.25	51.	RAU8	76.52	79.	GPHCPB10	177.77	107.	GE4	272.50
24.	GE1376	333.87	52.	GPU26	80.58	80.	GPHCPB3	183.90	108.	GE106	283.88
25.	GE4806	333.13	53.	PES110	87.64	81.	GPHCPB4	184.13	109.	GE116	224.38
26.	GE3149	332.40	54.	GPU48	89.87	82.	GPHCPB50	192.33	110.	GE125	285.88
27.	GE123	332.38	55.	VR708	90.38	83.	GPHCPB47	197.57	111.	GE128	210.13
28.	GPHCPB52	330.70	56.	GPU45	97.75	84.	GE1936	196.00	112	GE149	274.50

113.	GE218	259.88	145.	GE3218	276.88	177.	IE7079	261.00	209.	IE3952	235.00
114.	GE224	260.65	146.	GE3248	300.00	178.	IE2957	447.00	210.	IE2430	294.00
115.	GE258	292.13	147.	GE3321	247.88	179.	IE6473	228.00	211.	IE4734	303.00
116.	GE278	288.25	148.	GE3147	270.25	180.	IE7018	229.00	212.	IE4028	237.00
117.	GE356	237.17	149.	GE4004	234.75	181.	IE3045	386.00	213.	IE6165	292.00
118.	GE384	269.00	150.	GE4678	261.25	182.	IE6350	224.00	214.	IE6221	251.00
119.	GE390	218.50	151.	GE4600	296.00	183.	IE2790	345.00	215.	IE4709	386.00
120.	GE482	251.00	152.	GE4692	227.63	184.	IE4797	226.00	216.	IE6240	258.00
121.	GE489	292.00	153.	GE4732	241.40	185.	IE3077	356.00	217.	IE6421	232.00
122.	GE554	224.00	154.	GE5194	295.88	186.	IE4121	246.00	218.	IE4497	298.00
123.	GE909	253.13	155.	IE2312	345.00	187.	IE4073	249.00	219.	IE4816	304.00
124.	GE1045	238.50	156.	IE3945	255.00	188.	IE2710	351.00	220.	IE5306	225.00
125.	GE1064	283.13	157.	IE4671	207.00	189.	IE2872	313.00	221.	IE2871	351.00
126.	GE1093	245.88	158.	IE5870	201.00	190.	IE5066	231.00	222.	IE3392	367.00
127.	GE1235	212.13	159.	IE2296	221.00	191.	IE7320	292.00	223.	IE4646	295.00
128.	GE1240	234.25	160.	IE6294	262.00	192.	IE4491	256.00	224.	IE5106	313.00
129.	GE1267	253.00	161.	IE5201	318.00	193.	IE2606	332.00	225.	IE3104	242.00
130.	GE1280	278.13	162.	IE6326	232.00	194.	IE2572	421.00	226.	IE6537	439.00
131.	GE1437	265.20	163.	IE3721	343.00	195.	IE2619	291.00	227.	IE5367	223.00
132.	GE1537	228.88	164.	IE2457	239.00	196.	IE2911	351.00	228.	IE6059	255.00
133.	GE1486	251.00	165.	IE6337	277.00	197.	IE501	324.00	229.	IE2821	233.00
134.	GE1621	277.33	166.	IE5537	281.00	198.	IE2437	273.00	230.	IE4329	224.00
135.	.GE1781	260.00	167.	IE4570	302.00	199.	IE6082	239.00	231.	IE4565	244.00
136.	GE1871	245.50	168.	IE518	287.00	200.	IE6154	248.00	232.	IE4795	268.00
137.	GE1899	233.88	169.	IE3391	333.00	201.	IE4757	307.00	233.	IE4622	273.00
138.	GE2063	218.63	170.	IE3317	334.00	202.	IE6514	217.00	234.	IE4545	312.00
139.	GE2154	227.25	171.	IE2034	319.00	203.	IE2042	265.00	235.	GPHCPB14	238.26
140.	GE2238	291.63	172.	IE2589	309.00	204.	IE5817	234.00	236.	GPHCPB33	366.75
141.	GE2547	239.83	173.	IE3470	279.00	205.	IE1055	293.00	237.	GPHCPB17	357.31
142.	GE2471	210.38	174.	IE3475	348.00	206.	IE5091	237.00	238.	GPHCPB36	352.73
143.	GE2624	283.00	175.	IE3614	326.00	207.	IE3973	196.00			
144.	GE3235	259.88	176.	IE4057	283.00	208	IE2217	231.00			

exotic areas. Selection of such accessions as potential parents or donors would be beneficial in crop improvement. Polymorphic alleles were generated by 20 SSR loci ranging from 2 to 7 with a mean of 3.1 alleles per microsatellite locus in the population. The average PIC was 0.36, varying from 0.14 to 0.78 (Table 2) which is in accordance with earlier reports of diversity estimation in finger millet germplasm using SSR markers (Babu et al. 2014a, b and c).

Two major clusters A and B (Fig. 1) were identified. Cluster A consisted of sub-clusters A1 and A2 with A2 further dividing into two sub-clusters as A2a and A2b. Cluster A1 comprised of 3 genotypes each from South Asia and East Africa and a genotype from Western Africa. Cluster A2 was however larger with predominance of genotypes from Eastern Africa followed by genotypes from South Asia, and one genotype each from West Africa, North America and Europe in sub-cluster A2a. Clustering in A2b consisted of multiple sub clusters and these as a whole represented most of the genotypes from South Asia (representing northern and southern India), a few from East Africa, one accession from West Africa and rest

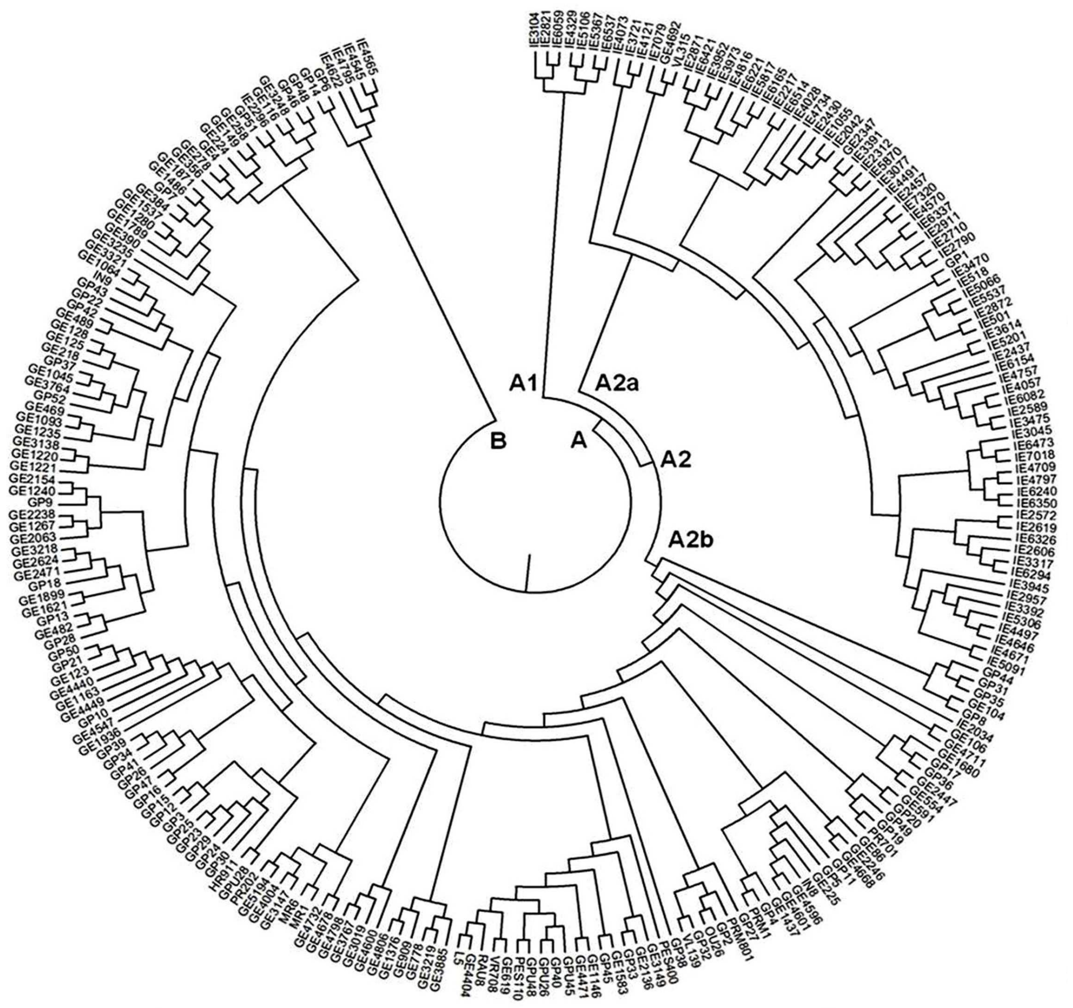

Fig. 1. Cluster analysis (UPGMA) showing two major clusters A and B among the 238 finger millet genotypes used in the study

from unknown locations. Cluster B was quite small with mainly East African genotypes. Analysis of Structure results through STRCTURE Harvester, which calculates the second order statistics, identified maximum ”K at K=2 (Fig. 2a). The Q-matrix generated in evaluating population structure explains per cent sub-population parentage for each accession under study. The two populations (Sub-population 1 and Sub-population 2) along with the admixed group as shown in Fig. 2b, represented 28.9%, 55.0%, and 15.9% accessions based on the *q*-value of 90%, respectively.

Sub-population 1 (69 accessions) had the estimated ancestry membership (*q*) of greater than 0.90 and had a mixture of East African and South Asian genotypes with a predominance of the former. Sub-population 2 was similar to cluster A2b in the phylogenetic analysis and constituted approximately half of the collection (55.0%) with predominance of genotypes from South Asian regions, including India, Nepal and Maldives. The admixed group, which had *q* values of less than 0.90 ranging from 0.16 to 0.90, contained 15.9% accessions of the finger millet collection. Among these, most of the accessions (22) had admixture of alleles from sub-population 2. Some of the genotypes from phylogenetic clusters A1 and B also fell into this category of admixed group.

The results of the present study indicated complex genetic relationship among entire set of 238 finger millet accessions. Dida et al. (2008) performed population-structure analysis of 79 accessions from domesticated and wild *E. coracana* lines and identified three sub-populations comprising *coracana* sub-population each of African and Asian region and *africana* sub-population. In present work also, the African and the Asian finger millet genotypes remain largely grouped into two separate sub-populations.

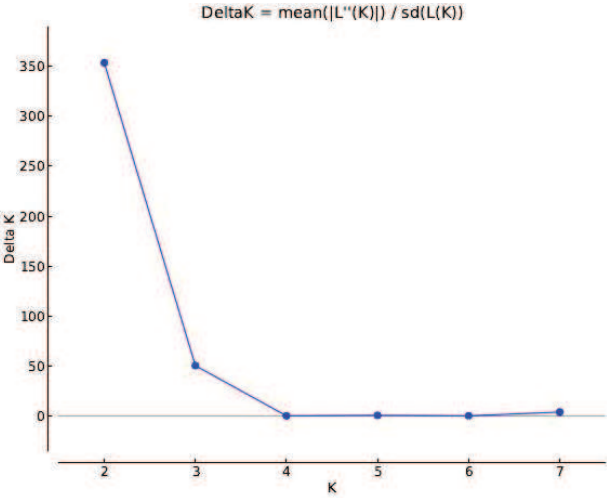

Fig. 2a. Identification of the appropriate sub-population number (*K*): Sub population number (*K*) against delta *K* and the maximum *K* value observed at *K*=2

Panwar et al. (2010b) carried out diversity analysis of finger millet genotypes from southern and northern India and grouped into distinct clusters indicating diversity among the genotypes. Grouping of accessions from different regions also suggest a possibility of targeted or accidental inter-crossing among the genotypes, gene flow or exchange of germplasm during crop-breeding programs. Babu et al. (2014b) analyzed genetic structure in a population comprising of finger millet minicore collection and few Indian genotypes and reported two sub-populations which was also in correspondence with the geographical origin of accessions. Ramakrishnan et al. (2016) reported existence of four sub-populations in their study on 128 finger millet genotypes. However, the analysis involved RAPD markers and all sub-populations showed an admixture of alleles and only one pure line was observed. Thus, population structure determination through molecular markers in finger millet gene pool mostly indicate 2-4 sub-populations with Indian and exotic genotypes separated in different sub-populations with a few exceptions. The present study also confirm the existence of genetic differentiation within the finger millet natural population. The study by Kumar et al (2014) using 14 polymorphic anchoring SSR markers reported four sub-populations and SSRs

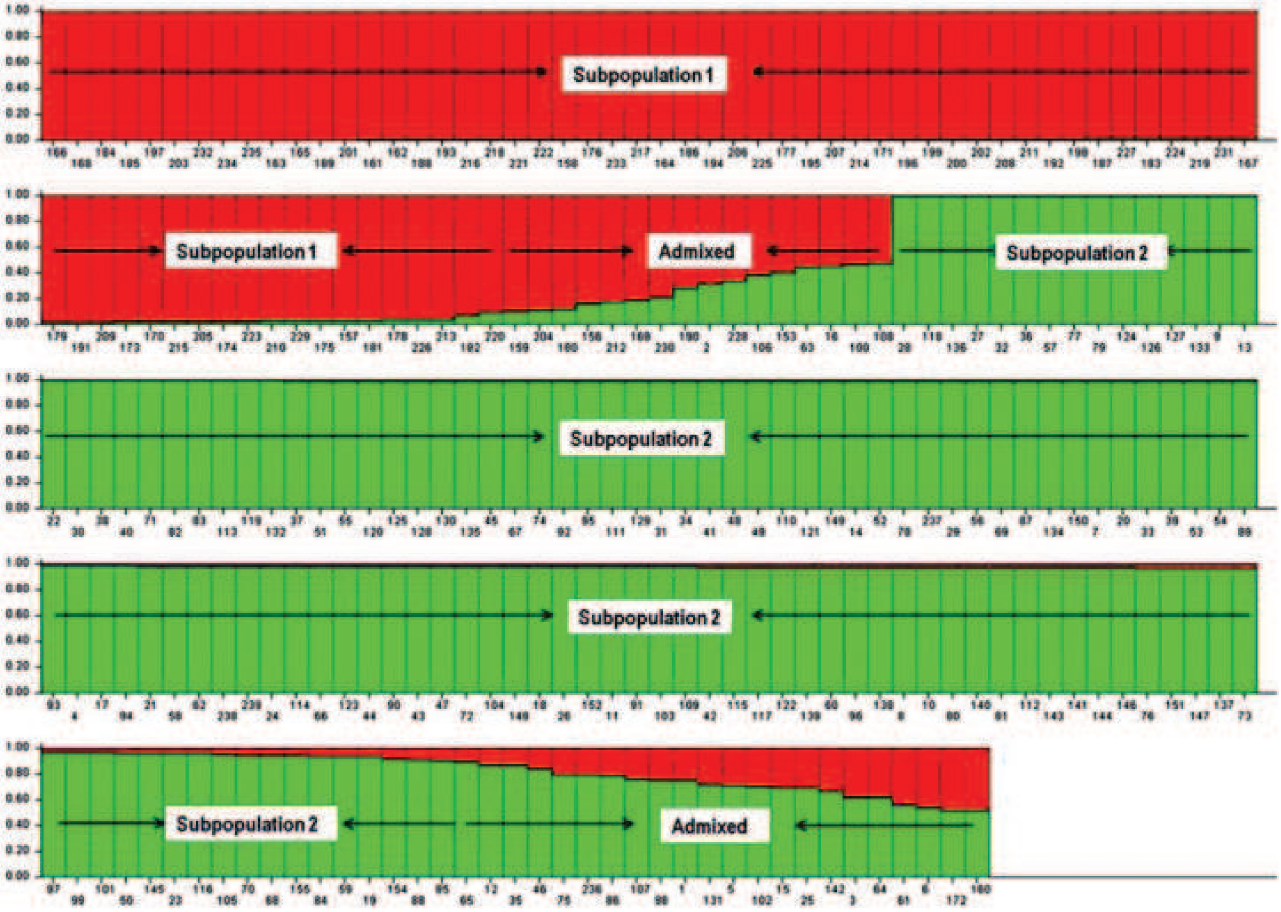

Fig. 2b. Population structure of finger millet genotypes derived using genomic SSR markers

Table 2. Number of alleles and polymorphic information content (PIC) detected in the finger millet association mapping population using microsatellites markers

Primer code	Product size	Allele number	PIC	Linkage group
UGEP8	297	2	0.233	NA*
UGEP10	400	5	0.658	8A
UGEP12	230	7	0.781	8B
UGEP15	180	5	0.456	3Aa
UGEP24	183	5	0.438	3B
UGEP53	226	3	0.357	2A
UGEP77	245	2	0.337	4B
UGEP78	244	2	0.368	2B
UGEP22	227	2	0.372	NA
UGEP27	247	2	0.148	NA
UGEP33	216	2	0.315	NA
UGEP57	445	2	0.345	NA
UGEP66	219	4	0.154	NA
UGEP67	229	2	0.372	NA
UGEP101	234	2	0.292	NA
UGEP111	160	2	0.332	NA
UGEP60	240	6	0.599	1B
FM20	200	3	0.312	NA
FM15	200	2	0.164	NA
FM3	200	2	0.143	NA

F = Forword, R = Reverse, NA = Not available

designed from calcium transporters and sensors were found to be associated with the grain calcium trait. However, the size of population and number of markers used were low in this case.

Marker trait associations, in present work, determined by GLM method of TASSEL software revealed that three SSR markers were linked to the calcium content QTLs at a significant threshold (P) level of <0.01 and <0.001. Markers UGEP78 and UGEP60 were found associated at a significant threshold (P) level of <0.001 and accounted for 6.4% and 13.8% phenotypic variance (R^2), respectively. Marker UGEP33 was found associated at P level <0.01 with a R^2 value of 2.9% (Table 3). Two additional markers (UGEP27 and UGEP57) were also found at the threshold (P) level <0.05 but these could only account for a mere 1% of phenotypic variation. Mixed linear model, that also utilizes the kinship values, did not detect any significant association between the genotypic and phenotypic data in this study. Low number of polymorphic markers available for genotyping most possibly hinders the true genetic structure from being deciphered and hence strong marker trait associations are not found. The loci UGEP78 and UGEP60 have been mapped on linkage groups 2B and 1B of finger millet (Dida et al. 2007). In rice also loci governing calcium content have been detected and QTL, *qCa1-1*, on chromosome 1 of rice explained about 9-14% of phenotypic variation for calcium concentrations in rice (Garcia-Oliveira et al. 2009). Strikingly, results in present analysis find marker UGEP60 linked to Calcium QTL which belongs to a linkage-group already reported colinear with rice chromosome 1 harboring QTL for calcium in grains. This particular linkage group in finger millet could be fine mapped using more advanced genetic or genomic tools with a possibility of locating similar grain calcium accumulation regulatory regions in finger millet genetic map. With calcium being a major need in our mineral nutrition regimes, the accociated SSRs revealed in this study can be a start towards unraveling genetic background for high calcium content in finger millet. As compared to major cereal crops *viz*., rice, wheat and maize, a scarce availability of genomic resources and a low intraspecific variation found in finger millet germplasm hampers the efficacy of studies conducted in this crop. The results of present study indicate that advanced whole genome based searches like genotyping-by-sequencing which generate high density markers are required to gain knowledge about control and regulation of complex trait such as grain mineral content.

Table 3. SSR markers identified as significantly associated to *GCC* in finger millet through association mapping approach (cM= centi morgan distance)

Trait	SSR marker	Probability of marker	Phenotypic variance (R^2) (%)	Chromosome/ linkage group	Distance (cM)
Grain calcium content	UGEP78	0.00006***	6.4	2B	61.5
	UGEP60	0.0004***	13.8	1B	43.2
	UGEP33	0.007**	2.9	NA	NA
	UGEP27	0.037*	1.7	NA	NA
	UGEP57	0.039*	1.7	NA	NA

Significance at (P) value < 0.05, 0.01, 0.001are indicated by *, ** and *** respectively. NA: not available

Authors' contribution

Conceptualization of research (AK, SS); Designing of the experiments (AK, SY, SS); Contribution of experimental materials (SY, SS); Execution of experiments and data collection (SY, SS); Analysis of data and interpretation (SY, SS); Preparation of the manuscript (SY, SS).

Declaration

The authors declare no conflict of interest.

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Supplementary Table 1. List of finger millet SSR primers (from Dida et al. 2007, 2008; Naga et al. 2012 and anonymous sources). The primer codes, sequence, SSR motif, product size and respective linkage groups (*in Bold*) on finger millet genetic map are given.

Primer code	Sequence (5'-3')	SSR motif	Product size	Linkage Group
UGEP8F	ATTTCCGCCATCACTCCAC	(GA)13	297	NA
UGEP8R	AGACGCAAATGGGTAAATGTC			
UGEP10F	AAACGCGATGAATTTTAAGCTC	(GA)19	400	**8A**
UGEP10R	CTATGTCGTGTCCCATGTCG			
UGEP12F	ATCCCCACCTACGAGATGC	(CT)22	230	**8B**
UGEP12R	TCAAAGTGATGCGTCAGGTC			
UGEP15F	AAGGCAATCTCGAATGCAAC	(CT)22	180	**3Aa**
UGEP15R	AAGCCATGGATCCTTCCTTC			
UGEP24F	GCCTTTTGATTGTTCAACTCG	(GA)26	183	**3B**
UGEP24R	CGTGATCCCTCTCCTCTCTG			
UGEP53F	TGCCACAACTGTCAACAAAAG	(AG)26	226	**2A**
UGEP53R	CCTCGATGGCCATTATCAAG			
UGEP77F	TTCGCGCGAAATATAGGC	(CT)19	245	**4B**
UGEP77R	CTCGTAAGCACCCACCTTTC			
UGEP78F	AAGCAATCAACAAAGCCTTTTC	(GA)14	244	**2B**
UGEP78R	TACAACGTCCAGGCAACAAG			
UGEP22F	ACAGAGACACGGTGGAGGTG	(TC)29	227	NA
UGEP22R	CACCATGTCATCGTCACCAG			
UGEP27F	TTGCTCTGAGGTTGTGTTGC	(GA)19	247	NA
UGEP27R	TCAAGCATAGTGCCCTCCTC			
UGEP33F	TAGCCCGTTTGCTTGTTTTG	(TC)18	216	NA
UGEP33R	AAGGCCCTAGAACGTCAAGC			
UGEP57F	CCATGGGTTCATCAAACACC	(AG)16	445	NA
UGEP57R	ACATGAGCTCGCGTATTGC			
UGEP66F	CAGATCTGGGTAGGGCTGTC	(AG)29	219	NA
UGEP66R	GATGGTGGTTCATGCCAAC			
UGEP67F	CTCCTGATGCAAGCAAGGAC	(TC)22TT(GT)5	229	NA
UGEP67R	AGGTGCCGTAGTTTGTGCTC			
UGEP101F	GCTCACTTACCCATGGCTTC	(GA)7AA(GA)19	234	NA
UGEP101R	GAAATGTGGGGCACATAAGG			
UGEP111F	CTCTCCCTGAGTTGGTCTGC	(GAT)11	160	NA
UGEP111R	TTGGTCAGAAGCATCAGGTG			
UGEP60F	AGCTCTGCTTGGTGGAGAAG	(GA)37	240	**1B**
UGEP60R	TTTTCTACTGGTGGGCGAAG			
UGEP1F	TTCAGTGGTGACGGAAGTTCT	(TC)11	233	**5Ab**
UGEP1R	GGCTCCATGAAGAGCTTGAC			
UGEP3F	CCACGAGGCCATACTGAATAG	(CA)7N12(GA)15	206	**3Ab/3B**
UGEP3R	GATGGCCACTAGGGATGTTG			
UGEP5F	TGTACACAACACCACACTGATG	(TC)12AC(TC)4	215	**9B**

UGEP5R	TTGTTTGGACGTTGGATGTG			
UGEP6F	AGCTGCAGTTTCAGTGGATTC	(GA)3TA(GA)9	229	**5B**
UGEP6R	TCAACAAGGTGAAGCAGAGC			
UGEP11F	CCTCGAGTGGGGATCCAG	(CT)12	153	**5Ab**
UGEP11R	AAGACGCTGGTGGAAATAGC			
UGEP18F	TTGCATGTGTTGCTTTTTGC	(CT)12	318	**1B**
UGEP18R	TGTTCTTGATTGCAAACTGATG			
UGEP21F	CAATTGATGTCATTGGGACAAC	(GA)16	225	**1B**
UGEP21R	GTATCCACCTGCATGCCAAC			
UGEP26F	ATGGGGTTAGGGTTCGAGTC	(CGG)7	227	**5B**
UGEP26R	TGTCCCTCACTCGTCTCCTC			
UGEP31F	ATGTTGATAGCCGGAAATGG	(GA)12	241	**3Aa**
UGEP31R	CCGTGAGCCTCGAGTTTTAG			
UGEP52F	TCATGCTAGCTTCAACACAACC	(GA)16	215	**2A**
UGEP52R	TGCTGGGTGAAACCCTAGAC			
UGEP56F	CTCCGATACAGGCGTAAAGG	(GT)12	162	**9A**
UGEP56R	ACCATAATAGGGCCGCTTG			
UGEP65F	AGTGCTAGCTTCCCATCAGC	(CT)19	226	**8A**
UGEP65R	ACCGAAACCCTTGTCAGTTC			
UGEP68F	CGGTCAGCATATAACGAATGG	(CT)14	232	**9B**
UGEP68R	TCATTGATGAATCCGACGTG			
UGEP76F	GCACGTACGGATTCACATTG	(CAG)7	168	**3B**
UGEP76R	GGTACGGAGACATCGACACC			
UGEP81F	AAGGGCCATACCAACACTCC	(GT)12	192	**6B**
UGEP81R	CACTCGAGAACCGACCTTTG			
UGEP90F	GGCCTTTGCAGTCATGTGAG	(CT)11/(CT)8	232	**6B**
UGEP90R	CGACTCCAGGTGTTGTTGG			
UGEP102F	ATGCAGCCTTTGTCATCTCC	(TG)17	184	**10**
UGEP102R	GATGCCTTCCTTCCCTTCTC			
UGEP104F	TCAGCACCACCTGAATAGG	(CT)11	189	**3B**
UGEP104R	AATAGGGAGGGCGAAGACTC			
UGEP106F	AATTCCATTCTCTCGCATCG	(AC)12	175	**9B**
UGEP106R	TGCTGTGCTCCTCTGTTGAC			
UGEP107F	TCATGCTCCATGAAGAGTGTG	(GA)15	224	**1Ab**
UGEP107R	TGTCAAAAACCGGATCCAAG			
UGEP108F	GTTGGCTGCTCTGCTTATCC	(CTG)6(CAG)2	150	**8A**
UGEP108R	TATCTGCTTGTGCAGCTTCG			
UGEP110F	AAATTCGCATCCTTGCTGAC	(CT)12	192	**7A/B**
UGEP110R	TGACAAGAGCACACCGACTC			
UGEP7F	GCCAACAGTTCGCTCTGC	(GA)14	500	NA
UGEP7R	CTGTCTCCCGTTCTCTCCTG			
UGEP9F	GGTGGAGAAGGGAGAAGGAG	(GGA)7	239	NA
UGEP9R	TTGAAGGCCCAATAGCTACG			

UGEP13F	AGCACGATCGAAATCCATTC	(TC)18	206	NA
UGEP13R	AGATCTCGCAGCGGTTTATG			
UGEP16F	TCTCCAGCTACATATGTTTTACT	(CT)14(AT)16	197	NA
UGEP16R	ATATATGGGACAACCATCCA			
UGEP17F	CATTGGTGTCGTTCACTTGG	(GTT)7	218	NA
UGEP17R	TGCACCGTATGAGCAACAAG			
UGEP19F	GGCAAGGATACCTTCATTCG	(GA)18	230	NA
UGEP19R	TACCTCTGCTCTGCACATGG			
UGEP20F	GGGGAAGGCAATGATATGTG	(GA)20	153	NA
UGEP20R	TTGGGGAGTGCCAACAATAC			
UGEP25F	GTTCGGCCTCATCGTCAG	G8(GA)19	292	NA
UGEP25R	TTTATCCGACGTGACAGGTG			
UGEP28F	GAAGTTGCCATCCATGCTTC	(TC)17	228	NA
UGEP28R	AGGAACCGCCAGGAAATTAG			
UGEP29F	TACCATTCCATTGCATCCAC	(CT)12	234	NA
UGEP29R	TTAATTAGCACTTGGGCATGG			
UGEP34F	AAGATCCTCACACGCAAACAC	(CA)12	196	NA
UGEP34R	ACCCTCAACTGGTGCCATAG			
UGEP45F	GGTCTCTTCAAAGCCACTGC	(GA)13	216	NA
UGEP45R	AACCAACCAATCCCAATATCC			
UGEP46F	CAAGTCAAAACATTCAGATGG	(GA)14	163	NA
UGEP46R	CCACTCCATTGTAGCGAAAC			
UGEP47F	ACAGGCCCCATAAATTCCAC	(GA)18	198	NA
UGEP47R	CCCCAATCATTCTTGTATGC			
UGEP50F	CACCCAGACATGACCCTTTC	(CAA)7	249	NA
UGEP50R	ACTAGTGCAGGGTGGTCTCG			
UGEP51F	CAGCAGCACGACTGACCTG	(GT)11	299	NA
UGEP51R	TGCCACCTGACTACTCTCTGC			
UGEP54F	ACATTCCTGTTCGGGTTCTG	(GTT)8	222	NA
UGEP54R	TCGTGCACCATACCAATAGC			
UGEP58F	CATGGTGGTGGAGAGAAACC	(CA)15	194	NA
UGEP58R	GCAATGGTGACATTGAGCTG			
UGEP79F	CCACTTTGCCGCTTGATTAG	(CT)12	173	NA
UGEP79R	TGACATGAGAAGTGCCTTGC			
UGEP100F	TCAAGTGTTTCTGGCACTCG	(CT)12	223	NA
UGEP100R	TGCAAAAGCTGGGAGTAACC			
FMBhssr1 F	AAGATCGAAACAAGCAAAACA	(GA)10	100	NA
FMBhssr1 R	GAAAGAGTATGTGTTGCTTG			
FMBhssr5 F	AAGATCCATACAAGCAAAAG	(GA)12	50	NA
FMBhssr5R	TCTTTTGCTTGTTTCGATCT			
FMBhssr6 F	AGATGTGACACCGAAACTAG	(CT)14	180	NA
FMBhssr6R	GGCACCAAAATACAGACAAG			
FMBhssr8 F	TCATTCCGAGTTGTCACCAA	(CT)10	150	NA

FMBhssr8R	CTCAAGCTATGCATCCAACG			
FMBhssr9 F	CGGCCGCGAATACACTA	(CAC)6	200	NA
FMBhssr9 R	ATGTGCGTCAGACTCAATGG			
FMBhssr10 F	TAGCAGCAGTAGCAGCAGCA	(GA)11	150	NA
FMBhssr10R	CGAGCGGTACTGTGTTGTGT			
FM1F	GAAGTGTGGGGGAGTGAAAT	(GA)16	450	NA
FM1R	CCGCACATTACCCTCTCATT			
FM2F	ATCGAGGCGATGAGAGTTTG	(TTC)8	200	NA
FM2R	AAATGGCCAAACGAAACAAC			
FM4F	GGCGGCTGCTAGGGTTC	(GAGGC)5	300	NA
FM4R	CGCCTCAATCATGACAACAC			
FM5F	TCCCTCCTCTCATCCTCTGA	(CT)17	400	NA
FM5R	GGCAAATTCGATTGAGGCTA			
FM6F	GCTGAGTCGTACCGAGATTAGTT	(GA)12	400	NA
FM6R	CGACGACGAGTCGTACTTGA			
FM7F	GCCACTCGAAACGCAAG	(CCT)7	300	NA
FM7R	GAAACGGTGCAGCCTCTTAG			
FM8F	AGATCGGCAGCCACTACATC	(CGT)7	400	NA
FM8R	GAGACTGAGAAGCCGTGCAT			
FM9F	CGTCGATCAGTCAGTCATGC	(TCCC)5	400	NA
FM9R	CATGGGGTTGATCTTGGAGA			
FM10F	GAGGCATGCACGTACAACAC	(GCG)7	400	NA
FM10R	GGAGGGAGGGAATTCACAAT			
FM11F	CATCTCCATCTCCATCTCCA	(AAGAG)7	400	NA
FM11R	AAGGACGATCGCAACCAG			
FM12F	CATCTCCATCTCCATCTCCA	(AAGAG)7	200	NA
FM12R	GGACTTGAGGCAGTTGCAG			
FM13F	CGCTAGTAGTACATCACAGCTA	(AAGAG)7	200	NA
FM13R	CTGATGGCGTATGGGAGTCT			
FM14F	CACTACACCGCATCATCTCG	(AGA)18	200	NA
FM14R	AGCCGTGATGCCTACAACTC			
FM16F	ATGGACCAAGAAACCTCACG	(GCG)7	300	NA
FM16R	TCCTCGAACGGGAATCTCTA			
FM17F	TCCATCATCCATCTCCATCTC	(AAGAG)7	400	NA
FM17R	GGCGTTGAGGCTCCTGAC			
FM18F	CATCTCCATCTCCATCTCCA	(AAGAG)7	150	NA
FM18R	GGACTTGAGGCAGTTGCAG			
FM19F	AGGAGCCTAGGACGAACTCC	(GAG)7	200	NA
FM19R	CCTCCTCCTCCTCCTCATCT			
FM3F	GGGACTCTAGTTTCCGCTTTC	(TC)15	200	NA
FM3R	AAGCTCCAAATCCACACGTC			
FM15F	GCGAGTGAGAGAGGGAGCTT	(AG)16	200	NA
FM15R	GTCCAGCTGTTGCTGTTGAA			
FM20F	CCACCTGCTCCATCTACATCT	(AAGAG)7	200	NA
FM20R	AAGGACGATCGCAACCAG			

NA: not available

Supplementary Table 2: List of genotypes used in the Association mapping population. The Accession name, grain calcium contents, source and place of origin are given

S. No.	Accession name	Ca content(mg/100g seeds)	Source	Origin
1.	GPHCPB45	452.80	Ranichauri, GBPUAT	India
2.	GPHCPB44	439.76	Ranichauri, GBPUAT	India
3.	GPHCPB31	417.46	Ranichauri, GBPUAT	India
4.	GPHCPB40	415.43	Ranichauri, GBPUAT	India
5.	GE4711	393.63	UAS, Bangalore	East Africa
6.	GPHCPB35	392.60	Ranichauri, GBPUAT	India
7.	GPHCPB26	384.88	Ranichauri, GBPUAT	India
8.	GE1583	380.50	UAS, Bangalore	South Asia
9.	GE4404	377.50	UAS, Bangalore	South Asia
10.	GE619	374.63	UAS, Bangalore	South Asia
11.	GE4471	341.17	UAS, Bangalore	South Asia
12.	GE86	387.00	UAS, Bangalore	South Asia
13.	GE1146	370.00	UAS, Bangalore	South Asia
14.	GE2136	366.88	UAS, Bangalore	South Asia
15.	GE4668	359.67	UAS, Bangalore	East Africa
16.	GE2246	359.00	UAS, Bangalore	South Asia
17.	GE1680	355.63	UAS, Bangalore	South Asia
18.	GE4601	347.33	UAS, Bangalore	South Asia
19.	GE4596	345.82	UAS, Bangalore	South Asia
20.	GE104	345.20	UAS, Bangalore	South Asia
21.	GE3885	344.73	UAS, Bangalore	South Asia
22.	GE225	343.33	UAS, Bangalore	South Asia
23.	GE2447	343.25	UAS, Bangalore	South Asia
24.	GE1376	333.87	UAS, Bangalore	South Asia
25.	GE4806	333.13	UAS, Bangalore	East Africa
26.	GE3149	332.40	UAS, Bangalore	South Asia
27.	GE123	332.38	UAS, Bangalore	South Asia
28.	GPHCPB52	330.70	Ranichauri, GBPUAT	India
29.	GE3138	330.60	UAS, Bangalore	South Asia
30.	GPHCPB21	328.69	Ranichauri, GBPUAT	India
31.	GE1163	327.38	UAS, Bangalore	South Asia
32.	GPHCPB37	326.05	Ranichauri, GBPUAT	India
33.	GPHCPB42	325.94	Ranichauri, GBPUAT	India
34.	GE4449	325.38	UAS, Bangalore	South Asia
35.	GE4440	324.88	UAS, Bangalore	South Asia
36.	GE3764	323.53	UAS, Bangalore	South Asia
37.	GE4547	322.00	UAS, Bangalore	South Asia
38.	GE469	320.38	UAS, Bangalore	South Asia
39.	GE3019	316.67	UAS, Bangalore	East Africa
40.	GE1221	313.38	UAS, Bangalore	South Asia
41.	GE1200	313.38	UAS, Bangalore	South Asia

42.	GE3219	313.00	UAS, Bangalore	South Asia
43.	PRM1	310.54	Ranichauri, GBPUAT	India
44.	GE778	309.63	UAS, Bangalore	South Asia
45.	GPHCPB20	309.45	Ranichauri, GBPUAT	India
46.	GE591	308.13	UAS, Bangalore	South Asia
47.	GE4798	302.12	UAS, Bangalore	East Africa
48.	GE3767	300.71	UAS, Bangalore	South Asia
49.	PES400	72.10	UAS, Bangalore	India
50.	GPU28	72.65	UAS, Bangalore	India
51.	RAU8	76.52	UAS, Bangalore	India
52.	GPU26	80.58	UAS, Bangalore	India
53.	PES110	87.64	UAS, Bangalore	India
54.	GPU48	89.87	UAS, Bangalore	India
55.	VR708	90.38	UAS, Bangalore	India
56.	GPU45	97.75	UAS, Bangalore	India
57.	L5	104.73	UAS, Bangalore	India
58.	HR911	110.63	UAS, Bangalore	India
59.	MR6	111.58	UAS, Bangalore	India
60.	PR202	112.51	UAS, Bangalore	India
61.	GPHCPB1	117.56	Ranichauri, GBPUAT	India
62.	MR1	119.05	UAS, Bangalore	India
63.	VL315	133.32	VPKAS, Almora	India
64.	PRM801	136.04	Ranichauri, GBPUAT	India
65.	GPHCPB2	136.11	Ranichauri, GBPUAT	India
66.	OUAT26	144.15	Orissa, India	India
67.	GPHCPB13	146.16	Ranichauri, GBPUAT	India
68.	GPHCPB27	150.50	Ranichauri, GBPUAT	India
69.	GPHCPB9	163.94	Ranichauri, GBPUAT	India
70.	VL149	165.15	VPKAS Almora	India
71.	GPHCPB28	165.45	Ranichauri, GBPUAT	India
72.	INDAF8	166.49	UAS, Bangalore	India
73.	GPHCPB18	168.24	Ranichauri, GBPUAT	India
74.	INDAF9	170.06	UAS, Bangalore	India
75.	GPHCPB43	170.55	Ranichauri, GBPUAT	India
76.	PRM701	172.54	Ranichauri, GBPUAT	India
77.	GPHCPB7	174.13	Ranichauri, GBPUAT	India
78.	GPHCPB16	175.22	Ranichauri, GBPUAT	India
79.	GPHCPB10	177.77	Ranichauri, GBPUAT	India
80.	GPHCPB3	183.90	Ranichauri, GBPUAT	India
81.	GPHCPB4	184.13	Ranichauri, GBPUAT	India
82.	GPHCPB50	192.33	Ranichauri, GBPUAT	India
83.	GPHCPB47	197.57	Ranichauri, GBPUAT	India
84.	GE1936	196.00	UAS, Bangalore	South Asia

85.	GPHCPB5	248.19	Ranichauri, GBPUAT	India
86.	GPHCPB6	237.31	Ranichauri, GBPUAT	India
87.	GPHCPB8	224.18	Ranichauri, GBPUAT	India
88.	GPHCPB11	226.89	Ranichauri, GBPUAT	India
89.	GPHCPB12	201.23	Ranichauri, GBPUAT	India
90.	GPHCPB15	242.40	Ranichauri, GBPUAT	India
91.	GPHCPB19	288.18	Ranichauri, GBPUAT	India
92.	GPHCPB22	219.55	Ranichauri, GBPUAT	India
93.	GPHCPB23	248.97	Ranichauri, GBPUAT	India
94.	GPHCPB24	294.84	Ranichauri, GBPUAT	India
95.	GPHCPB25	248.21	Ranichauri, GBPUAT	India
96.	GPHCPB29	201.93	Ranichauri, GBPUAT	India
97.	GPHCPB30	278.35	Ranichauri, GBPUAT	India
98.	GPHCPB32	227.92	Ranichauri, GBPUAT	India
99.	GPHCPB34	274.00	Ranichauri, GBPUAT	India
100.	GPHCPB38	269.10	Ranichauri, GBPUAT	India
101.	GPHCPB39	289.14	Ranichauri, GBPUAT	India
102.	GPHCPB41	286.27	Ranichauri, GBPUAT	India
103.	GPHCPB46	270.59	Ranichauri, GBPUAT	India
104.	GPHCPB48	294.07	Ranichauri, GBPUAT	India
105.	GPHCPB49	257.33	Ranichauri, GBPUAT	India
106.	GPHCPB51	249.25	Ranichauri, GBPUAT	India
107.	GE4	272.50	UAS, Bangalore	South Asia
108.	GE106	283.88	UAS, Bangalore	South Asia
109.	GE116	224.38	UAS, Bangalore	South Asia
110.	GE125	285.88	UAS, Bangalore	South Asia
111.	GE128	210.13	UAS, Bangalore	South Asia
112.	GE149	274.50	UAS, Bangalore	South Asia
113.	GE218	259.88	UAS, Bangalore	South Asia
114.	GE224	260.65	UAS, Bangalore	South Asia
115.	GE258	292.13	UAS, Bangalore	South Asia
116.	GE278	288.25	UAS, Bangalore	South Asia
117.	GE356	237.17	UAS, Bangalore	South Asia
118.	GE384	269.00	UAS, Bangalore	South Asia
119.	GE390	218.50	UAS, Bangalore	South Asia
120.	GE482	251.00	UAS, Bangalore	South Asia
121.	GE489	292.00	UAS, Bangalore	South Asia
122.	GE554	224.00	UAS, Bangalore	South Asia
123.	GE909	253.13	UAS, Bangalore	South Asia
124.	GE1045	238.50	UAS, Bangalore	South Asia
125.	GE1064	283.13	UAS, Bangalore	South Asia
126.	GE1093	245.88	UAS, Bangalore	South Asia
127.	GE1235	212.13	UAS, Bangalore	South Asia

128.	GE1240	234.25	UAS, Bangalore	South Asia
129.	GE1267	253.00	UAS, Bangalore	South Asia
130.	GE1280	278.13	UAS, Bangalore	South Asia
131.	GE1437	265.20	UAS, Bangalore	South Asia
132.	GE1537	228.88	UAS, Bangalore	South Asia
133.	GE1486	251.00	UAS, Bangalore	South Asia
134.	GE1621	277.33	UAS, Bangalore	South Asia
135.	GE1781	260.00	UAS, Bangalore	South Asia
136.	GE1871	245.50	UAS, Bangalore	South Asia
137.	GE1899	233.88	UAS, Bangalore	South Asia
138.	GE2063	218.63	UAS, Bangalore	South Asia
139.	GE2154	227.25	UAS, Bangalore	South Asia
140.	GE2238	291.63	UAS, Bangalore	South Asia
141.	GE2547	239.83	UAS, Bangalore	South Asia
142.	GE2471	210.38	UAS, Bangalore	South Asia
143.	GE2624	283.00	UAS, Bangalore	South Asia
144.	GE3235	259.88	UAS, Bangalore	South Asia
145.	GE3218	276.88	UAS, Bangalore	South Asia
146.	GE3248	300.00	UAS, Bangalore	South Asia
147.	GE3321	247.88	UAS, Bangalore	South Asia
148.	GE3147	270.25	UAS, Bangalore	South Asia
149.	GE4004	234.75	UAS, Bangalore	South Asia
150.	GE4678	261.25	UAS, Bangalore	East Africa
151.	GE4600	296.00	UAS, Bangalore	South Asia
152.	GE4692	227.63	UAS, Bangalore	East Africa
153.	GE4732	241.40	UAS, Bangalore	East Africa
154.	GE5194	295.88	UAS, Bangalore	East Africa
155.	IE2312	345.00	ICRISAT	South Asia
156.	IE3945	255.00	ICRISAT	East Africa
157.	IE4671	207.00	ICRISAT	South Asia
158.	IE5870	201.00	ICRISAT	South Asia
159.	IE2296	221.00	ICRISAT	South Asia
160.	IE6294	262.00	ICRISAT	East Africa
161.	IE5201	318.00	ICRISAT	South Asia
162.	IE6326	232.00	ICRISAT	East Africa
163.	IE3721	343.00	ICRISAT	East Africa
164.	IE2457	239.00	ICRISAT	East Africa
165.	IE6337	277.00	ICRISAT	East Africa
166.	IE5537	281.00	ICRISAT	South Asia
167.	IE4570	302.00	ICRISAT	East Africa
168.	IE518	287.00	ICRISAT	South Asia
169.	IE3391	333.00	ICRISAT	East Africa
170.	IE3317	334.00	ICRISAT	East Africa

171.	IE2034	319.00	ICRISAT	South Asia
172.	IE2589	309.00	ICRISAT	North America
173.	IE3470	279.00	ICRISAT	South Asia
174.	IE3475	348.00	ICRISAT	South Asia
175.	IE3614	326.00	ICRISAT	Unknown
176.	IE4057	283.00	ICRISAT	East Africa
177.	IE7079	261.00	ICRISAT	East Africa
178.	IE2957	447.00	ICRISAT	Europe
179.	IE6473	228.00	ICRISAT	East Africa
180.	IE7018	229.00	ICRISAT	East Africa
181.	IE3045	386.00	ICRISAT	South Asia
182.	IE6350	224.00	ICRISAT	East Africa
183.	IE2790	345.00	ICRISAT	East Africa
184.	IE4797	226.00	ICRISAT	South Asia
185.	IE3077	356.00	ICRISAT	South Asia
186.	IE4121	246.00	ICRISAT	East Africa
187.	IE4073	249.00	ICRISAT	East Africa
188.	IE2710	351.00	ICRISAT	East Africa
189.	IE2872	313.00	ICRISAT	East Africa
190.	IE5066	231.00	ICRISAT	West Africa
191.	IE7320	292.00	ICRISAT	East Africa
192.	IE4491	256.00	ICRISAT	East Africa
193.	IE2606	332.00	ICRISAT	East Africa
194.	IE2572	421.00	ICRISAT	East Africa
195.	IE2619	291.00	ICRISAT	East Africa
196.	IE2911	351.00	ICRISAT	East Africa
197.	IE501	324.00	ICRISAT	South Asia
198.	IE2437	273.00	ICRISAT	East Africa
199.	IE6082	239.00	ICRISAT	South Asia
200.	IE6154	248.00	ICRISAT	South Asia
201.	IE4757	307.00	ICRISAT	South Asia
202.	IE6514	217.00	ICRISAT	East Africa
203.	IE2042	265.00	ICRISAT	South Asia
204.	IE5817	234.00	ICRISAT	South Asia
205.	IE1055	293.00	ICRISAT	Unknown
206.	IE5091	237.00	ICRISAT	East Africa
207.	IE3973	196.00	ICRISAT	East Africa
208.	IE2217	231.00	ICRISAT	South Asia
209.	IE3952	235.00	ICRISAT	East Africa
210.	IE2430	294.00	ICRISAT	East Africa
211.	IE4734	303.00	ICRISAT	South Asia
212.	IE4028	237.00	ICRISAT	East Africa
213.	IE6165	292.00	ICRISAT	South Asia

214.	IE6221	251.00	ICRISAT	South Asia
215.	IE4709	386.00	ICRISAT	East Africa
216.	IE6240	258.00	ICRISAT	East Africa
217.	IE6421	232.00	ICRISAT	East Africa
218.	IE4497	298.00	ICRISAT	East Africa
219.	IE4816	304.00	ICRISAT	South Asia
220.	IE5306	225.00	ICRISAT	East Africa
221.	IE2871	351.00	ICRISAT	East Africa
222.	IE3392	367.00	ICRISAT	East Africa
223.	IE4646	295.00	ICRISAT	East Africa
224.	IE5106	313.00	ICRISAT	East Africa
225.	IE3104	242.00	ICRISAT	South Asia
226.	IE6537	439.00	ICRISAT	West Africa
227.	IE5367	223.00	ICRISAT	East Africa
228.	IE6059	255.00	ICRISAT	South Asia
229.	IE2821	233.00	ICRISAT	South Asia
230.	IE4329	224.00	ICRISAT	East Africa
231.	IE4565	244.00	ICRISAT	East Africa
232.	IE4795	268.00	ICRISAT	East Africa
233.	IE4622	273.00	ICRISAT	East Africa
234.	IE4545	312.00	ICRISAT	East Africa
235.	GPHCPB14	238.26	Ranichauri, GBPUAT	South Asia
236.	GPHCPB33	366.75	Ranichauri, GBPUAT	South Asia
237.	GPHCPB17	357.31	Ranichauri, GBPUAT	South Asia
238.	GPHCPB36	352.73	Ranichauri, GBPUAT	South Asia