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RESEARCH ARTICLE

Genetic diversity of provitamin-A cassava (*Manihot esculenta* Crantz) in Sierra Leone

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Abstract Understanding the genetic diversity among accessions and germplasm is an important requirement for crop development as it allows for the selection of diverse parental combinations for enhancing genetic gain in varietal selection, advancement and release. The study aimed to characterize 183 provitamin A cassava (*Manihot esculenta* Crantz) accessions and five Sierra Leonean varieties using morphological traits, total carotenoid content and SNP markers to develop a collection for conservation and further use in the cassava breeding program. Both morphological parameters and 5634 SNP markers were used to assess the diversity among the provitamin-A cassava accessions and varieties. Significant differences were observed among the accessions for most of the traits measured. The first five PCs together accounted for 70.44% of the total phenotypic variation based on yield and yield components among the 183 provitamin-A cassava accessions and five Sierra Leonean varieties. The present study showed that provitamin-A cassava accessions in Sierra Leone have moderate to high diversity based on morphological and molecular assessment studies. The similarity index among the 187 and 185 cassava accessions grouped them into 6 and 9 distinct clusters based on morphological and molecular analyses, respectively. A significant positive, but low correlation ($r = 0.104$; $p < 0.034$), was observed between the two dendrograms. The results obtained will serve as a guide and basis of germplasm management and improvement for total carotenoid content, yield and African cassava mosaic disease resistance in Sierra Leone.

Keywords *Manihot esculenta* accession · Morphological traits · Total carotenoid content · Collection SNP markers

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Introduction

Genetic diversity provides species with the ability to adapt to changing environments. Several studies have been reported on the use of morphological descriptors

to determine the genetic diversity among cassava genotypes (Rimoldi et al. 2010; Asare et al. 2011; Thompson 2013). Recent advances in molecular biology techniques have led to the development of important tools for genetic diversity study in several plant species. The accuracy in accession characterization may therefore, be enhanced/achieved with the use of molecular markers associated with morphological traits.

Previous studies in plant genetic diversity used DNA molecular markers for beta carotene improvement in cassava (Ferreira et al. 2008; Rimoldi et al. 2010), and included amplified fragment length polymorphism (Benesi et al. 2010), simple sequence repeats (Alves et al. 2011; Parkes 2009; Oliveria et al. 2012; Costa et al. 2013) and single nucleotide polymorphism (Kizito et al. 2005; Tangphatsornruang et al. 2008; Ferguson et al. 2011; Thompson 2013; Rabbi et al. 2015). With recent advances in high throughput genotyping technologies, single nucleotide polymorphism markers (SNPs) are increasingly becoming markers of preference for plant genetic studies and breeding.

SNPs are the most common types of genetic variation among species, involving just a change in a single nucleotide. Expressed Sequence Tags (ESTs) have been exploited to explain and detect SNPs in maize (*Zea mays* L.) (Ching et al. 2002) and soybean (*Glycine max* L. Merr.) (Zhu et al. 2003). Lopez et al. (2005) and Rabbi et al. (2014, 2015) have also reported SNPs detection from ESTs in cassava. Cassava being an outbreeding and highly heterogeneous crop, possesses an extreme level of phenotypic plasticity, and thereby, lacks the potential for unified classification system for cultivars (Kawano 1978). Consequently, characterization of agronomic traits becomes a challenge. To conduct a successful genetic diversity study on cassava germplasm in Sierra Leone, there is a need to unravel the genetic potential existing among Sierra Leone's cassava breeding program, which consists of fourteen released varieties and provitamin-A cassava accessions induction from Institute of International Tropical Agriculture, Nigeria. Thus, the need for assessing and understanding the genetic diversity among the provitamin-A cassava accessions and identifying gaps to be filled within the breeding program in Sierra Leone is required.

The objectives of the study, therefore, were to characterize, quantify and exploit the diversity of 183 provitamin-A cassava accessions and five Sierra Leonean varieties using morphological traits, SNP markers and total carotene content and to develop a collection for conservation and future use in the breeding programmes.

Materials and methods

Germplasm sources and experimental design

The plant materials used in the study consisted of 183 provitamin-A cassava accessions known for their varying levels of provitamin- A properties, obtained from the International Institute of Tropical Agriculture (IITA, Ibadan, Nigeria) and established at the Taiama experimental site in Sierra Leone, in 2014 (Table 1) and five Sierra Leonean cassava varieties. The trial was established and evaluated during the cropping season of 2015–2016 at the Njala Agricultural Research Institute (NARC), Foya crop site, Njala, representing the transitional rain forest agro-climatic zone (Van Vuure et al. 1972; Odell et al. 1974). The trial was laid out in an Alpha lattice design with two replications, and each replication had four blocks with 47 entries per block. The blocks were separated by 1 m and 2 m alleys between and within blocks to reduce intra and inter block plant competition, respectively. Each entry was grown on 10 m row ridge at a spacing of 1 m × 1 m between and within ridges, respectively. Cassava cuttings of 20–25 cm length were obtained from healthy stem cuttings and horizontally planted.

Morphological traits

Agro-morphological data was collected at 1, 3, 6 and 9 months after planting (MAP) on the parameters listed below using the IITA cassava descriptor (Fukuda et al. 2010) (Table 2).

Harvesting was done at 12 MAP (August–September). The following parameters were taken at harvest: number of marketable roots (expressed as count numbers), number of non-marketable roots (expressed as count numbers), total number of storage roots (expressed as count numbers), roots weight/tuber (kg), inner skin color, and outer skin color, ease of peel, root shape, marketable weight (kg), and non- marketable weight (kg). Dry matter content, expressed in

Table 1 Provitamin–A studied accessions and sierra leonean varieties and their pedigrees

No.	Accession name	Pedigree	Origin	No.	Accession name	Pedigree	Origin
1	TR 1563	IBA 082708	Ubiaja	101	TR 1073	IBA 102429	Ubiaja
2	TR 1337	IBA 011368	Ubiaja	102	TR 0890	IBA 070749	Ubiaja
3	TR 0421	IBA 051652	Ubiaja	103	TR 0316	IBA051740	Ubiaja
4	TR 1207	SM 3374	Ubiaja	104	TR 1199	IBA051740	Ubiaja
5	TR 0267	IBA 961439	Ubiaja	105	TR 1144	IBA 100224	Ubiaja
6	TR 0626	MM 050626	Ubiaja	106	TR 0982	IBA 102480	Ubiaja
7	TR 0431	IBA 011735	Ubiaja	107	TR 1244	IBA 050099	Ubiaja
8	TR 0085	IBA 050311	Ubiaja	108	TR 1279	SM 3374	Ubiaja
9	TR 1295	IBA 011412	Ubiaja	109	TR 1008	IBA 100198	Ubiaja
10	TR 1627	TMEB 693	Ubiaja	110	TR 0861	GM 3594	Ubiaja
11	TR 0224	IBA 000351	Ubiaja	111	TR 0983	IBA 070738	Ubiaja
12	TR 1578	BA 011371	Ubiaja	112	TR 1031	IBA 070593	Ubiaja
13	TR 0222	IBA 020134	Ubiaja	113	TR 0683	SM 3666	Ubiaja
14	TR 1755	IBA 070749	Ubiaja	114	TR 0772	KIBAHA	Ubiaja
15	TR 0854	KIBAHA	Ubiaja	115	TR 1229	GM 3594	Ubiaja
16	TR 1051	IBA961089A	Ubiaja	116	TR 0118	IBA 100403	Ubiaja
17	TR 0261	IBA 961439	Ubiaja	117	TR 0840	IBA 102286	Ubiaja
18	TR 1201	SM 3374	Ubiaja	118	TR 0396	IBA I011086	Ubiaja
19	TR 0894	IBA 102710	Ubiaja	119	TR 1788	SM 3374	Ubiaja
20	TR 0232	BA 010169	Ubiaja	120	TR 0485	IBA 970219	Ubiaja
21	TR 1302	IBA 070520	Ubiaja	121	TR 1152	SM 3444	Ubiaja
22	TR 1128	IBA 100198	Ubiaja	122	TR 0990	KIBAHA	Ubiaja
23	TR 1808	IBA 070539	Ubiaja	123	TR 1004	IBA 070520	Ubiaja
24	TR 0172	IBA 011404	Ubiaja	124	TR 0679	SM 3434	Ubiaja
25	TR 0382	IBA 010732	Ubiaja	125	TR 1515	IBA 980505	Ubiaja
26	TR 0384	IBA 011404	Ubiaja	126	TR 1735	IBA 071393	Ubiaja
27	TR 1688	TME B2026	Ubiaja	127	TR 0700	IBA 102286	Ubiaja
28	TR 1437	TME B2026	Ubiaja	128	TR 1463	IBA 980581	Ubiaja
29	TR 0696	IBA 102612	Ubiaja	129	TR 0365	IBA 011663	Ubiaja
30	TR 0033	IBA 102286	Ubiaja	130	TR 1620	TMEB 693	Ubiaja
31	TR 1034	IBA 050327	Ubiaja	131	TR 0289	IBA 961632	Ubiaja
32	O334	SM 3444- 2	Ubiaja	132	TR 1603	IBA 30572	Ubiaja
33	TR 1610	IBA 070525	Ubiaja	133	TR 1505	IBA 102429	Ubiaja
34	TR 0631	IBA 101040	Ubiaja	134	TR 1849	TME B778	Ubiaja
35	TR 1233	IBA 070675	Ubiaja	135	TR 0031	IBA 050311	Ubiaja
36	TR 0998	IBA 30572	Ubiaja	136	TR 0319	IBA 050099	Ubiaja
37	TR 1744	MM 090564	Ubiaja	137	TR 1198	SM 3374	Ubiaja
38	TR 1153	SM 3374	Ubiaja	138	TR 1256	IBA 070738	Ubiaja
39	TR 0886	SM 3666	Ubiaja	139	TR 1557	IBA 082708	Ubiaja
40	TR 0446	IBA 070749	Ubiaja	140	TR 0535	IBA 020091	Ubiaja
41	TR 0974	IBA 101438	Ubiaja	141	TR 0856	KIBAHA	Ubiaja
42	TR 1565	IBA 102480	Ubiaja	142	TR 1359	IBA 070520	Ubiaja
43	TR 0785	IBA 070620	Ubiaja	143	TR 0881	IBA 102480	Ubiaja
44	TR 1569	GM 3594	Ubiaja	144	TR 1405	IBA 083724	Ubiaja
45	TR 0713	IBA 082708	Ubiaja	145	TR 0385	SM 3374	Ubiaja

Table 1 continued

No.	Accession name	Pedigree	Origin	No.	Accession name	Pedigree	Origin
46	TR 0423	IBA 011206	Ubiaja	146	TR 1223	SM 3374	Ubiaja
47	TR 0887	IBA 082708	Ubiaja	147	TR 0680	KIBAHA	Ubiaja
48	TR 1785	SM 3434	Ubiaja	148	TR 1313	IBA 070520	Ubiaja
49	TR 0025	IBA 071393	Ubiaja	149	TR 0480	IBA 015654	Ubiaja
50	TR 1374	IBA 102480	Ubiaja	150	TR 1266	IBA 070738	Ubiaja
51	TR 1562	IBA 980505	Ubiaja	151	TR 1071	IBA 100649	Ubiaja
52	TR 1236	Z 960012	Ubiaja	152	TR 0703	SM 3434	Ubiaja
53	TR 0838	IBA 070557	Ubiaja	153	TR 0893	IBA 102480	Ubiaja
54	TR 1480	IBA 082708	Ubiaja	154	TR 1689	TMEB 2026	Ubiaja
55	TR 0937	IBA 082708	Ubiaja	155	TR 0707	SM 3434	Ubiaja
56	TR 0743	IBA 101094	Ubiaja	156	TR 1556	IBA 070749	Ubiaja
57	TR 1540	BA 011371	Ubiaja	157	TR 0927	IBA 070337	Ubiaja
58	TR 0747	IBA 102286	Ubiaja	158	TR 0688	SM 3374	Ubiaja
59	TR 1348	IBA 980501	Ubiaja	159	TR 1007	IBA 100645	Ubiaja
60	TR 1438	SM 3434	Ubiaja	160	TR 0299	IBA 051625	Ubiaja
61	TR 1477	IBA 070520	Ubiaja	161	TR 1289	IBA 011412	Ubiaja
62	TR 1243	IBA 070738	Ubiaja	162	TR 0851	SM 3444	Ubiaja
63	TR 0807	BA 011206	Ubiaja	163	TR 0295	IBA 051625	Ubiaja
64	TR 1389	IBA 083849	Ubiaja	164	TR 1590	IBA 30572	Ubiaja
65	TR 1259	IBA 070738	Ubiaja	165	TR 0918	IBA 101803	Ubiaja
66	TR 1182	IBA 100649	Ubiaja	166	TR 1133	IBA 100198	Ubiaja
67	TR 1543	KIBAHA	Ubiaja	167	TR 1331	IBA 070520	Ubiaja
68	TR 0975	IBA 083724	Ubiaja	168	TR 0461	IBA 051654	Ubiaja
69	TR 1155	IBA 070738	Ubiaja	169	TR 1419	IBA 102612	Ubiaja
70	TR 1404	SM 3374	Ubiaja	170	TR 0368	IBA 011663	Ubiaja
71	TR 1202	IBA 102429	Ubiaja	171	TR 0299	IBA 071393	Ubiaja
72	TR 0955	GM3594- 12	Ubiaja	172	TR 1448	IBA 083724	Ubiaja
73	TR 0520	IBA 101438	Ubiaja	173	TR 1322	IBA 070520	Ubiaja
74	TR 1208	IBA 083724	Ubiaja	174	TR 0399	IBA 071393	Ubiaja
75	TR 0843	SM 3374	Ubiaja	175	TR 1525	BA 011371	Ubiaja
76	TR 1113	IBA 101645	Ubiaja	176	TR 1753	IBA 070749	Ubiaja
77	TR 1316	IBA 071313	Ubiaja	177	TR 1501	IBA 102429	Ubiaja
78	TR 0693	SM 3374	Ubiaja	178	TR 0019	IBA 990313	Ubiaja
79	TR 1593	SM 3444	Ubiaja	179	TR 0296	IBA 961551	Ubiaja
80	TR 1598	IBA 982101	Ubiaja	180	TR 1360	IBA 070557	Ubiaja
81	TR 0282	IBA 070520	Ubiaja	181	TR 1527	IBA 102429	Ubiaja
82	TR 1350	IBA 102286	Ubiaja	182	TR 0560	MM 980747	Ubiaja
83	TR 0957	IBA 30572	Ubiaja	183	TR 1502	IBA 070557	Ubiaja
84	TR 1422	IBA 30572	Ubiaja	184	SLICASS 11	IBA 070749	Sierra Leone
85	TR 0932	IBA 050303	Ubiaja	185	SLICASS 4	Can't be traced	Sierra Leone
86	TR 1349	IBA 083849	Ubiaja	186	SLICASS 6	Can't be traced	Sierra Leone
87	TR 0810	IBA 101645	Ubiaja	187	SLICASS 7	Can't be traced	Sierra Leone
88	TR 0718	IBA 102612	Ubiaja	188	COCOA	Local Cultivar	Sierra Leone
89	TR 0907	IBA 030007	Ubiaja				
90	TR 335	IBA 030007	Ubiaja				

Table 1 continued

No.	Accession name	Pedigree	Origin	No.	Accession name	Pedigree	Origin
91	TR 1327	IBA 070520	Ubiaja				
92	TR 1666	IBA 070703	Ubiaja				
93	TR 1748	IBA 070749	Ubiaja				
94	TR 1361	IBA 070557	Ubiaja				
95	TR 0189	IBA 011206	Ubiaja				
96	TR 1269	IBA 070738	Ubiaja				
97	TR 1533	SM 3434	Ubiaja				
98	TR 1762	IBA 070557	Ubiaja				
99	TR 0015	IBA 990313	Ubiaja				
100	TR 0018	IBA 070593	Ubiaja				

Table 2 Parameters evaluated at 1, 3, 6 and 9 month after planting

Traits	Traits
Leaf color	Color of stem epidermis
Number of leaf lobes	Color of stem cortex
Length of leaf lobe	Growth habit of stem
Width of leaf lobe	Prominence of foliar scars
Lobe margin	Leaf retention
Pubescence of apical leaves	Level of branching
Color of apical leaves	Height at 1st branching
Orientation of petiole	Height at 2nd branching
Petiole color	Height at 3rd branching
Leaf area	Color of end branches of adult plant
Length of stipule	Percentage sprout
Stipule margin	African cassava mosaic disease
Stem color	Cassava green mite
Stem diameter base	Cassava anthracnose disease
Stem diameter-1foot below	

percentage was determined by selecting three representative storage roots. Slices of the fresh root were randomly selected and weighed to obtain a 100 g fresh mass sample per genotype, before being dried for 48 h in an oven at 80 °C. The dried samples were then re-weighed to obtain the dry mass. Disease occurrence and intensity were mostly measured in the 1st, 3rd, 6th and 9th month after planting.

Molecular characterization

The Dellaporta method of DNA extraction (Dellaporta et al. 1983) was carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. For genotyping-by-sequencing library preparation, the ApekI restriction enzyme (recognition site: G|CWCG) that produces less variable distributions of read depth, and therefore, a larger number of scorable SNPs in cassava (Hamblin and Rabbi 2014) was used. Two 96-plex GBS libraries were constructed as

described by Elshire et al. (2011) and sequenced at the Institute of Genomic Diversity at Cornell University, using the Illumina HiSeq 2500. Raw read sequences were processed through cassava GBS production pipelines developed using TASSEL 5.0V2. The GBS-derived SNPs were further filtered using the TASSEL software (Bradbury et al. 2007) to retain only polymorphic SNPs. Initially, filtered for minor allele frequency (MAF < 0.05), the generated 5634 SNPs were processed under the Next Generation Cassava project. The resulting SNP dataset was used for the diversity analysis study among the 188 cassava accessions already phenotyped and analyzed. Results from both the phenotype and genotype analyses were compared to check the correspondence between the two.

Data analysis

Agro-morphological data sets from this study were subjected to selected statistical packages for analysis. Analytical procedures comprised the following softwares and statistical procedures: descriptive statistics using XLSTAT (2010), MINITAB 15 and STATA 13. Principal Component Analysis (PCA) were performed using Princomp software to examine the structure of the correlations between the variables using SAS 9.3. Cluster analyses, based on Agro-morphological and SNP markers data sets, were performed to group observations together using the method of Ward's minimum variance distance using SAS 9.4. A dendrogram was plotted from the computed similarity values for each Agro-morphological traits and SNP markers to show the relationship among the accessions. The provitamin-A studied accessions and varieties were grouped based on the varying levels of total carotenoid content.

Basic diversity indices for the 183 provitamin-A studied accessions and varieties were calculated using Power marker (Liu and Muse 2005) and GenAlex version 6.41 (Peakall and Mouse 2006). The Power maker software was used to generate the following statistics: number of alleles per locus, major allele frequency, observed heterozygosity (Ho), expected heterozygosity (He) and polymorphic information content (PIC) (Bostein et al. 1980). PIC values were calculated with the equation:

$$PIC = 1 - \Sigma P^2 i - \Sigma 2P^2 i$$

where: $\Sigma P^2 i$ = sum of each squared ith haplotype frequency.

A Mantel matrix test (Mantel 1967) was carried out to compare the extent of agreement between dendrograms derived from morphological and molecular data using the distance matrices. The pairwise genetic distance (identity-by-state, IBS) matrix was calculated among all individuals using PLINK (Purcell et al. 2007). A Ward's minimum variance hierarchical cluster dendrogram was built from the IBS matrix, using the analyses of phylogenetic and evolution (ape) package in R.

Results and discussion

Summary statistics of morpho-agronomic traits of 183 provitamin-A studied accession and varieties

Table 3 shows summary statistics of some morpho-agronomic traits of 183 provitamin-A studied accessions and varieties. Sprouting was only recorded in the first month after planting (MAP) and ranged from 65 to 100% among the 183 provitamin-A studied accessions and varieties with an average of 9.56 seeds sprouted in the first month. Severity scores for African Cassava Mosaic Disease Cassava Bacterial Blight and Cassava Green Mite variably ranged from 0 to nine in the studied collection consisting of the 183 provitamin-A cassava collection and the five varieties. Percent incidence for African Cassava Mosaic Disease, Cassava Bacterial Blight and Cassava Green Mite variably ranged from 0 to 9. Most of the morphological characters both quantitative and qualitative were taken in the 3rd, 6th, 9th and 12th MAP. Color of apical lobe ranged from 3 to 9 about a mean of 6.8 ± 1.61 3 MAP; whereas the same traits scored ranged from 0 to 9 about a mean of 6.71 ± 1.74 9 MAP. Plant height ranged from 65.5 to 284.5 cm at 6 MAP about a mean of 155.69 ± 26.12 cm. Leaf area ranged from 10.24 to 73.93 cm^2 at 6 MAP; whereas leaf retention ranged from 1.75 to 4.5 at the same time. All yield related traits were recorded at 12 MAP. Yield per hectare ranged from 0.2 to 42.5 t/ha; while dry matter content ranged from 4.0 to 44.5% (Table 3). These parameters which were good indicators of growth showed considerable variation for the morpho-

Table 3 Summary statistics of some morpho-agronomic traits of the studied accessions and varieties

Trait	Descriptive statistics				
	Time of data collection (MAP)	Minimum	Maximum	Mean	Standard deviation
Sprouting (%)	1	6.5	10	9.56	0.6
ACMD Incidence (%)	1, 3, 6 and 9	0	4.25	0.08	0.42
ACMD Severity (score)	1, 3, 6 and 9	0.75	2	1.04	0.13
CAD Incidence (%)	1, 3, 6 and 9	0	2.75	0.11	0.41
CAD Severity (score)	1, 3, 6 and 9	0.5	2.75	1.05	0.23
CBB Incidence (%)	1, 3, 6 and 9	0	4	0.41	0.6
CBB Severity (score)	1, 3, 6 and 9	0.5	4.5	1.15	0.34
Mealybug incidence (%)	9	0	9	3.22	2.17
Mealybug severity (score)	9	1	6.5	2.54	0.84
CGM Incidence (%)	9	2	8	5.27	1.66
CGM Severity (score)	9	2	9	3.31	0.72
Colour of apical lobe (score)	3	3	9	6.8	1.61
Colour of apical lobe (score)	6	0	5	2.90	0.99
Colour of apical lobe (score)	9	0	9	6.71	1.74
Plant height (cm)	6	65.5	284.5	155.69	26.12
Height of branching (cm)	6	37	196.5	85.83	29.38
Stem diameter base (cm)	6	1.07	3.94	1.51	0.26
Stem diameter (mid height) (cm)	6	1.03	2.25	1.53	0.2
Leaf area (cm^2)	6	10.24	73.93	34.13	11.04
Leaf retention (score)	6	1.75	4.5	2.87	0.5
Shape of central leaflet (score)	6	1.75	6.25	3.13	0.94
Petiole colour (score)	6	0.5	7	1.94	1.48
Petiole colour (score)	9	1	8	3.2	1.54
Leaf colour (score)	6	1.5	5	3.69	0.87
Leaf colour (score)	9	3	6	3.94	0.77
Colour of leave vein (score)	6	3	18.75	3.85	1.73
Petiole length (cm)	6	3	32.95	14.79	6.09
Orientation of petiole (score)	6	0.5	7	2.55	1.13
Number of leaf lobes (no)	6	3.75	8	6.18	0.89
Length of leaf lobe (cm)	6	3.13	15.15	11.15	1.61
Width of leaf lobe (cm)	6	1.08	7.05	3.05	0.81
Lobe margin (score)	6	1.5	8	4.38	1.87
Length of stipules (cm)	9	1	4	2.97	0.22
Stipule margin (score)	9	1	5	1.31	0.59
Prominence of foliar scars colour (score)	9	3	6	4.93	0.39
Stem colour (score)	6	4	8	6.47	0.79
Colour of stem exterior (score)	6	1	7	2.55	0.71
Colour of stem epidermis (score)	9	4	8.5	6.52	1.1
Colour of end branches of adult plants (score)	9	1	32.5	4.62	2.47
Mean number of storage root (no)	12	7.5	88	44.83	14.21
Yield (t/ha)	12	0.24	42.5	12.09	5.69
Mean weight per storage root (kg)	12	0.09	28	0.47	2.62

Table 3 continued

Trait	Descriptive statistics				
	Time of data collection (MAP)	Minimum	Maximum	Mean	Standard deviation
Dry matter content (%)	12	4	44.5	29.56	6
Root size (score)	12	2	7	4.93	1.07
Root shape (score)	12	1	5	2.76	0.62
Outer root colour (score)	12	1	4	3.4	0.72
Inner root colour (score)	12	1	3	1.9	0.36
Pulp colour (score)	12	1	3	2.01	0.19
Ease of peeling (score)	12	2	7	2.83	0.53
Biomass (kg)	12	2.5	13.5	9.99	1.91

MAP, month after planting; ACMD, African cassava mosaic Disease; CAD, cassava anthracnose disease; CBB, cassava bacterial blight; CGM, cassava green mite

agronomic traits evaluated in the study, and the findings were in concordance with previous studies by Mbah et al. (2019) who reported that agro morphological parameters exert strong influence on cassava root yield. In the present study, descriptive analysis of the 183 provitamin-A studied accessions and varieties based on various traits showed high variability among the accessions. The significant variation observed among the 183 provitamin-A studied accessions and varieties studied for these economically important traits, such as African cassava mosaic disease, yield and dry matter content (DMC) offers a prospect for progress in cassava breeding program in Sierra Leone. Diversity studies of cassava germplasm has been widely undertaken worldwide (Bolanos 2001; Chavez et al. 2005; Morillo 2009; Fregene 2007; Parkes 2011; Njoku 2012; Thompson 2013) with little or no attention in Sierra Leone. These findings agree with the findings by Carvalho and Schaal (2001) who reported, in Brazil, a high degree of variability among 94 cassava accessions of Brazilian origin. Raghu et al. (2007) in a similar study, in India, also identified a high level of diversity among 58 cassava accessions from South Indian cassava germplasm based on 29 morphological traits. Lyimo et al. (2012) reported significant variability among 39 cassava accessions of Tanzanian origin using 14 morphological traits. Thompson (2013) observed a moderate to high diversity among 150 Ghanaian landraces and introduced accessions from IITA, Ibadan, Nigeria using 25 morphological traits in Ghana.

Summary statistics of the genetic variation among the 183 provitamin-A studied accessions and varieties using SNP markers

Summary statistics for number of alleles observed, expected heterozygosity and polymorphic information content are presented in Table 4. The number of observed alleles ranged from 1.30 to 1.47, with an average of 1.38 alleles per locus. The expected heterozygosity was the lowest for TR 1233 (0.15) and SLICASS 6 (0.15) and highest in TR 1525 (0.23), with a mean of 0.19. The observed heterozygosity per individual observation ranged from 0.30 (TR 1233) to 0.47 (TR 1525) with a mean of 0.38. The mean of

Table 4 Summary statistics for number of alleles observed, expected heterozygosity and polymorphic information content

stats	Maf[a]	no of allele	He[b]	Ho[c]	Pic[d]
Mean	0.81	1.38	0.19	0.38	0.14
Maximum	0.85	1.47	0.23	0.47	0.18
Minimum	0.77	1.30	0.15	0.30	0.11

[a]Maf, majority of allele frequency; [b]He, heterozygosity; [c]Ho, momozygosity; [d]Pic, polymorphic information

observed heterozygosity (0.38) was moderately higher than the expected heterozygosity (0.19). This substantiates the difference in the relatedness of most of the provitamin-A studied accessions which were developed from varieties of half sib families with different female know parental sources (Female plants) been pollinated by different sources. However, the major allele frequency (MAF) of all the 'markers used in the observations was generally, below 0.95, indicating that they were all polymorphic. PIC values ranged from 0.11 in TR 1233 to 0.18 in TR 1199 and TR 1525 with a PIC mean of 0.14. The higher the PIC value the more informative is the marker. Since morphological traits are influenced by the environment, molecular markers which are not influenced or controlled by the environment are preferable in genetic diversity studies (Kaemmer et al. 1992; Gepts 1993; Njoku 2012; Thompson 2013). The study carried out by Kawuki et al. (2009) was the first published report where SNPs were used for genetic diversity studies in cassava. They characterized and identified some SNP markers and assessed their utilization in cassava genetic diversity analysis assessment. The present study seems to be the first reported case in Sierra Leone, where SNP markers were used in cassava diversity study of provitamin-A cassava accessions. Using the 5634 SNP markers, 95% of them were polymorphic. The informativeness of a genetic marker is measured by the polymorphic information content (PIC). The mean PIC value observed in this study (0.14) is relatively lower than previously reported. Indeed, Kawuki et al. (2009) reported a PIC value of 0.29 in 74 cassava accessions using 26 SNP: while Thompson (2013) also reported PIC value of 0.29 using 150 cassava accessions. PIC values for SNP markers in cassava are generally lower than observed in genetic diversity studies in other crops. For instance, Yang et al. (2011) reported PIC value of 0.34 in maize genotypes using 884 SNP markers.

Principal component analysis among yield and yield related traits of 183 provitamin-A cassava studied accessions and varieties

The first five PCs together accounted for 70.44% of the total phenotypic variation among the 183 provitamin-A cassava studied accessions and five varieites (Table 5). PC1 axis had an eigenvalue of 4.44 and acounted for 27.74% of the total variation, whereas PC, PC3, PC4 and PC5 axes had eigenvalues of 3.1, 1.45, 1.11% and 1.09% acounted for 19.8%, 9.03%, 6.95% and 6.82% of the total variation, respectively. Marketable root, marketable weight and yield had positive loadings on PC1. Non-marketable weight, storage root weight and ease of peel had positive loadings on PC2. Unmarketable root and total number of storage roots had negative loadings in PC3. Root Size had a positive loading in PC4 and Inner color had a positive loading in PC5.

Principal Component Analysis is a technique which identifies plant traits that contribute most to the observed variation within a group of 183 provitamin-

Table 5 Principal component analysis of yield and yield related traits

Variable	Eigenvectors				
	Comp1	Comp2	Comp3	Comp4	Comp5
mrot	**0.40**	0.05	− 0.15	− 0.02	0.02
unmrot	0.09	0.24	− **0.63**	− 0.04	0.06
tsr	0.37	0.00	− **0.47**	− 0.06	0.05
mwet	**0.44**	0.05	0.23	0.00	0.00
nmwet	0.06	**0.46**	0.12	0.00	0.15
twet	0.39	0.25	0.25	0.01	0.05
yld	**0.44**	0.05	0.23	− 0.03	0.06
wsrot	− 0.12	**0.50**	0.09	0.10	− 0.06
dmc	0.14	− 0.28	0.14	− 0.31	0.03
rz	0.30	− 0.08	0.02	0.26	− 0.24
rs	− 0.03	0.12	− 0.13	− 0.32	0.67
ocol	− 0.01	− 0.17	0.29	0.00	0.39
incol	− 0.06	− 0.01	0.01	**0.68**	**0.45**
pcol	− 0.09	0.10	0.16	− 0.45	0.13
epeel	− 0.09	**0.46**	0.10	0.10	− 0.07
biomas	0.11	− 0.25	− 0.06	0.20	0.28
Eigenvalue	**4.44**	**3.18**	**1.45**	**1.11**	**1.09**
Difference	**1.26**	**1.74**	**0.33**	**0.02**	**0.18**
Proportion	**27.74**	**19.89**	**9.03**	**6.95**	**6.82**
Cumulative	**27.74**	**47.64**	**56.67**	**63.62**	**70.44**

The bold column in tables signifies the traits that contributed higher negative or positive loadings to the percent variance explained

mrot, marketable roots; unmrot, non-marketable roots; tsr, total number of storage roots; mwet, marketable weight; nmwet, Non-marketable weight; twet, total weight; yld, yield; wsrot, storage root weight; dmc, dry matter content; rz, root size; rs, root shape; ocol, Outer color; epeel, ease of peel

A studied accessions and five varieties. The tool has a practical application in the selection of parent lines for breeding purposes and varietal development. The cumulative variance of 70.44% by the first five axes with eigen values > 1.0 indicates that the identified traits within these axes exhibited great influence on the phenotype of these accessions, and could effectively be used for selection among them. This study agrees with findings of Afuape and Nwachukwu (2005; Afuape et al. 2010), who reported a cumulative variance of 70.09% for the first three axes in the dry evaluation of nine sweetpotato genotypes, weight of total roots, weight of biomass, and dry matter as the important traits that distinguished the elite materials been researched on.

Cluster groupings of the studied accessions and varieties based on morpho-agronomic traits using ward's minimum variance and SNP markers

Agro-morphological traits diversity analysis: The dendrogram constructed based on the data generated from the agro-morphological traits divided the provitamin-A studied accessions and five varieties into six major clusters (A to F), and at a genetic distance of 0.30, and each had sub clusters apart from Cluster A (Table 6). Cluster A consisted of only two cassava accession germplasm with no sub clusters. Cluster B, had two sub cluster, Cluster D recorded the highest number of accessions, 57 in total, followed by Cluster E and F, grouping 53 and 34 accessions, respectively. In general, most of the accessions in this study were grouped according to their morpho-agronomic traits and geographical location. For example, the accessions in major Cluster E scored similar values for most of the morph-agronomic traits studied. Three out the five Sierra Leonean varieties developed in Sierra Leone were grouped into cluster F: while cluster B and D contained only provitamin-A studied accessions introduced to Sierra Leone in the form of seeds from IITA, Nigeria, and had a discrete pattern of clustering, which have been grouped more or less per their state, geographical distribution or country.

SNP markers diversity analysis: The 181 Provitamin-A cassava accession germplasm and 4 Sierra Leonean varieties were grouped into nine clusters based on the 5643 SNP markers (Fig. 1). Clusters A, B, C, D and E, had 21, 7, 11, 8, and 16 accessions, respectively; while cluster F, G, H and I consisted of 10, 47, 50 and 17 accessions, respectively (Table 7). Clusters A, B, C, E, G, H and I had 3, 1, 2, 4, 9, 10, and 1 accessions with varying levels of total carotenoid content. Cluster I consisted of only one provitamin-A studied accessions.

Correlation Analysis between Clusters from Agro-Morphological Traits and SNP Makers: A comparison of the two dendrogram based on Mantel matrix test showed a significant positive, but weak correlation between the morphological and molecular data sets ($r = 0.104, p < 0.034$). In a similar study, Raghu et al. (2007) mentioned that 24 morphological traits out of 28, contributed to the total variation observed. Here, our clustering study showed six and nine distinct clusters based on morphological and molecular analyses, respectively, indicating a large variability in the collection. In a similar study, Carvalho and Schaal (2001) identified 22 distinct clusters using 94 cassava accessions in Brazil, whereas Raghu et al. (2007) identified six distinct groups using 58 accessions. Our study is, therefore, in agreement with all these studies. Although the morphological and SNP data grouped the accessions into six and nine distinct clusters, respectively, some similarities were observed. Accessions TR 0747 and TR 0365 which were selected as provitamin-A studied accessions were found to be closely similar using both morphological and genetic markers. This could explain why the morphological and molecular analysis showed similar accessions between the two clusters. There are no reports on the genetic diversity of provitamin-A cassava accessions using morphological traits, molecular markers and total carotenoid content so far. This remains the first study using morphological, genetic diversity characterization and total carotenoid content levels of our provitamin-A cassava accessions in Sierra Leone.

The study reveals a moderate degree of diversity among the provitamin-A cassava accessions and varieties which can be further used for crop improvement. This may provide an opportunity to enhance and boost the breeding strategy.

Thirty provitamin-A studied accessions with varying levels of total carotenoid content, yield and dry matter content

The 30 accessions grouped in the different clusters were selected as provitamin-A studied accessions for formation of core collection, conservation and

Table 6 Cluster groupings of the 182 provitamin-a studied accessions and five sierra leonean varieties based on morpho-agronomic traits using ward's minimum variance

Cluster A	Cluster B	Cluster C	Cluster D	Cluster D	Cluster E	Cluster E	Cluster F
TR1808	TR0560	TR1590	TR0700	TR0033	TR1578	TR0396	TR1603
TR1259	TR1207	TR1133	TR1202	TR1313	TR1735	TR1233	TR1788
	TR1128	TR0703	TR1031	TR0019	TR0743	TR0631	TR0890
	TR1004	TR0421	TR1563	TR1744	TR0983	TR0535	TR1533
	TR1007	TR1144	TR1337	TR1208	TR0520	TR1610	TR0480
	TR0894	TR0707	TR1201	TR0810	TR1515	TR0446	TR1666
	TR0990	TR0918	TR0696	TR1501	TR1279	TR0974	TR0851
	TR1034	TR0688	TR1350	TR1289	TR0881	TR0998	TR1155
	TR0296	TR1269	TR1316	TR1244	TR0868	TR0385	TR1051
	TR9689	TR0365	TR1556	TR1071	TR0282	TR1405	TR0854
	TR1113	TR0368	TR0893	TR1557	TR1753	TR0085	TR0955
	TR0295	TR1748	TR0856	TR1525	TR1349	TR1565	TR0399
	TR1480	TR0316	TR1198	TR1569	TR1404	TR1562	TR1688
	TR1223	TR1477	TR0907	TR1502	TR1598	TR1302	TR0384
	TR0747	TR0838	TR1199	TR1448	TR1295	TR1153	TR1374
	TR1361	TR0172	TR0840	TR1419	TR0887	TR1540	TR1327
	TR0382	TR0679	TR0807	TR0289	TR0713	TR1755	TR1849
	TR0319	I1635/Slicass 11	TR0861	TR0118	TR1073	TR1543	TR1422
	TR0431		TR0772	TR335	TR0957	TR1182	TR1360
	TR0267		TR0693	TR1505	TR0222	TR0232	TMEB419/Slicass 7
	TR0927		TR0886	TR1322	TR1229	TR1256	TR1463
	TR1437		TR0718	TR1620	TR0785	Slicass4	TR1348
	TR0025		TR0461	TR1331	TR1762		TR1359
			TR1627	TR1008	TR0299		TR0485
			TR1438	TR1527	TR0031		TR1389
			TR1236	TR0018	TR0957		TR1785
			TR1243		TR0015		TR0937
			TR1152		TR1593		TR0224
			TR0932		TR0015		TR0423
			TR0982		TR1593		TR0189
			TR0683		TR0626		O334
							Slicass6
							TR0843
							Cocoa

improvement in the breeding program. These accessions were selected based on the higher levels of total carotene content after laboratory analysis using color chat and the i-check device. The core selected provitamin-A cassava accessions across different clusters revealed significant variation of total carotenoid content, yield and dry matter. These provitamin-A cassava accessions TR 0998, TR 0222, TR 1337 and TR 0461 contained higher levels of total carotenoid content with TR 0365 been the lowest. Dry matter content ranged from 12.5 (TR 0696) to 39.5 (TR 1208) with yield ranging from 2.0 (TR 0461) to 22.8 (TR 0232) in the study provitamin-A accessions. TR 0747, TR 1337, TR 0232, TR 0998 and TR1755 clustered

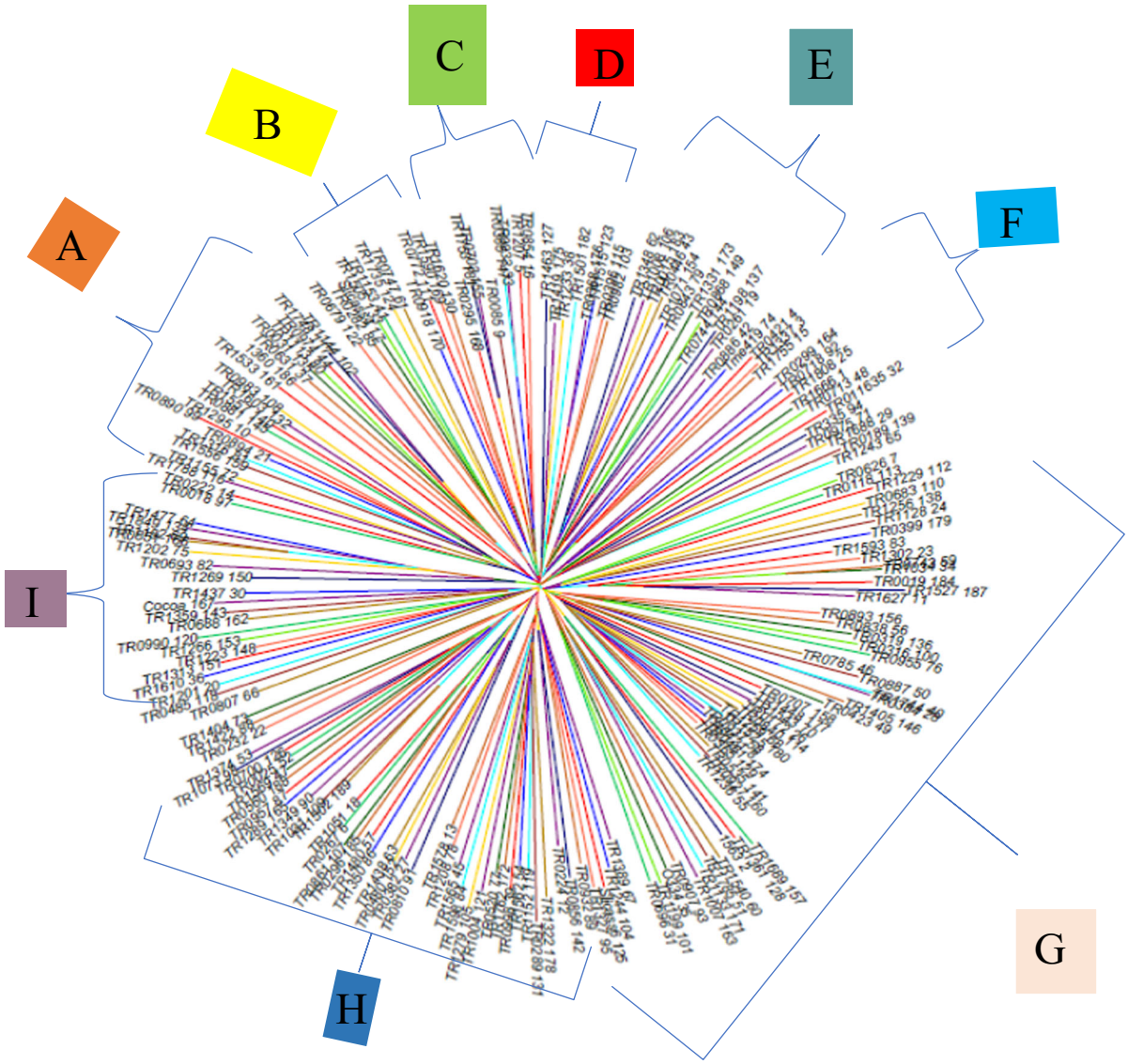

Fig. 1 Dendrogram of 182 Provitamin-A studied accessions and Sierra Leonean varieties based on SNP markers

similarly morphologically and genetically (B, D, E, E and E). The wide range of total carotenoid content, dry matter content, yield, and distribution of morphological variability revealed in the study might provide a broader scope for the crop's improvement through hybridization and selection. The higher dry matter content and significant variability observed in some provitamin-A cassava accessions in this study contradict findings reported by Esuma et al. (2012) who reported high DMC and low total carotenoid content for local white cassava root varieties using the Ugandan landraces.

Conclusion

The present morphological and molecular assessment studies reported that provitamin-A cassava accessions in Sierra Leone have moderate to high diversity based on total carotenoid content, morphological, and molecular assessment (Table 8).

The inter-relationships of morpho- agronomic factors in determining cassava fresh root yield based on provitamin-A cassava accessions require additional research to fully understand concept of improving total carotenoid content and yield on provitamin-A

Table 7 Cluster groupings of the 181 Provitamin-A Studied Accessions and Sierra Leonean Cassava varieties based on SNP Markers

Cluster A	Cluster B	Cluster C	Cluster D	Cluster E	Cluster F	Cluster G	Cluster G	Cluster H	Cluster H	Cluster I
TR0018	TR0679	TR0918	TR1643	TR1348	TR0299	TR0626	TR0707	TR1389	TR0480	TR0801
TR0222	TR0282	TR0172	TR1419	TR1008	TR0718	TR0118	TR1448	TR1244	TR1438	TR0485
TR1788	Slicass 4	TR1590	TR1233	TR0015	TR1808	TR1229	TR1543	Slicass6	TR1350	TR1201
TR1155	TR1505	TR1620	TR1501	TR0446	TR1666	TR0683	TR0172	TR1327	TR1480	TR1610
TR1556	TR1534	TR0295	TR0368	TR1071	TR0713	TR1256	TR0840	TR0932	TR0269	TR1313
TR3168	TR1735	TR1753	TR1515	TR0843	1/1635/Slicass 11	TR1128	TR1259	TR0856	TR0681	TR1223
TR0894	TR0747	TR0703	TR0306	TR1313	TR335	TR0399	TR0461	TR0224	TR0267	TR1266
TR0890		TR0085	TR0982	TR0868	TR0975	TR1593	TR0189	TR1322	TR1051	TR0990
TR1295		TR0033		TR0744	TR1688	TR1302	TR1152	TR0025	TR1502	Cocoa
TR0881		TR1207		TR1198	TR1243	TR1034	TR0365	TR0700	TR1031	TR1437
TR1557		TR0854		TR0261		TR0019	TR0535	TR1562	TR1349	TR1269
TR1603				TR0886		TR1527	TR0927	TR0998	TR1289	TR0693
TR0983				TMEB419/ Slicass 7		TR1627	TR1236	TR1762	TR0957	TR1202
TR1533				TR0421		TR0893	TR1689	TR0520	TR0560	TR0851
TR1360				TR1337		TR0319	TR1361	TR1004	TR1569	TR1182
TR0631				TR1755		TR0316	TR1563	TR1279	TR0688	TR1840
TR0031						TR0955	TR1540	TR1004	TR1359	TR1477
TR1113						TR0785	TR1785	TR1279	TR1073	
TR0974						TR0887	TR1133	TR1598	TR1374	
TR1748						TR1744	TR1007	TR1565	TR0232	
TR1144						TR0384	TR0907	TR1208	TR1422	
						TR1405	O334	TR1578	TR1404	
						TR0423	TR1199	TR0810		
							TR0696	TR0382		

cassava accession germplasm. Even though the agro-morphological traits are generally employed to estimate genetic diversity in crop plants, such a method has its own limitations as the traits are heavily influenced by the environmental conditions and climate being the main factor influencing the growth and development of the species (Cadena Iniguez and Arevalo Galarza 2011). This also confirms the importance of molecular techniques and markers on Provitamin-A cassava accession germplasm to carry out successful research and improvement studies. The present study has revealed that during provitamin-A cassava variety development, high dry matter content (quality trait) is a priority trait that should be considered at both primary and advance (yield evaluation) stages with good root qualities to facilitate adoption after varietal release.

Finally, the genetic diversity revealed from this study would provide the cassava breeding program in Sierra Leone an opportunity to boost the breeding strategy on crop genetic improvement for Provitamin-A cassava varieties with end-use preferred traits (total carotenoid content, dry matter, yield and African cassava mosaic disease resistance).

Table 8 Thirty provitamin-A studied accessions with varying levels of total carotenoid content, dry matter content and yield

Accession	Total carotenoid content (μg/g fresh weight)	Phenotypic cluster name	Genotypic cluster name	Yield	Dry matter content
TR 0747	10.9	B	B	4.3	29.5
TR 0365	7.0	B	C	2.3	25.5
TR 0560	9.7	B	H	7.5	25.5
TR 1208	8.9	D	G	7.5	39.5
TR 0461	11.5	D	G	2.0	23.0
TR 1337	11.8	D	D	14.6	25.5
TR 1569	10.3	D	H	21.8	26.5
TR 0683	10.2	D	G	5.0	28.5
TR 1198	10.8	D	E	7.0	28.5
TR 1313	11.7	D	E	11.0	35.0
TR 0696	11.1	D	G	6.5	12.5
TR 1322	9.9	D	H	13.0	29.5
TR 1350	9.0	D	G	8.0	29.5
TR 0907	9.1	D	G	6.0	31.5
TR 1557	11.2	D	A	10.6	18.0
TR 1152	8.08	D	G	4.8	33.0
TR 0232	9.9	E	E	22.8	27.0
TR 1279	9.1	E	H	6.3	35.6
TR 0031	10.3	E	A	6.9	29.5
TR 0222	13.1	E	A	7.8	37.0
TR 0998	13.7	E	E	2.8	38.1
TR 1755	10.7	E	E	5.3	24.0
TR 1182	10.4	E	I	10.8	24.0
TR 1753	8.6	E	C	16.8	35.0
TR 0713	8.2	E	F	7.5	28.0
TR 0423	8.7	G	F	6.5	25.5
TR 0384	10.6	G	F	5.5	27.0
TR 1327	11.1	F	H	4.5	21.0
TR 0399	11.1	F	G	11.8	25.5

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Compliance with ethical standards

Conflict of interest We the authors of this manuscript declare that we have no conflict of interest.

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