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Discriminating Nigerian 'Egusi' Melon Accessions Using Agro-Morphological and Molecular Techniques

Idehen, E. O.[1, 2], Kehinde, O. B. [1] , Wang, X. [2] and Oyelakin, O. O.[3]

[1] Department of Plant Breeding and Seed Technology, Federal University of Agriculture, Abeokuta, P.M.B. 2240 Abeokuta, Ogun State, Nigeria [2] Department of Biotechnology, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, 100081, China [3]Biotechnology Centre Federal University of Agriculture, Abeokuta, P.M.B. 2240 Abeokuta, Ogun State, Nigeria

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Abstract

'Egusi' melon is an important vegetable crop in the tropics and subtropics that is rich in protein, oils and vitamins. Agro-morphological traits, sequence related amplified polymorphism (SRAP) and simple sequence repeats (SSRs) were used to evaluate 50 accessions of 'egusi' melon collected from various parts of the country. The FASTCLUS procedure grouped the accessions into ten distinct groups based on agro-morphological data, with accessions NG/TO/APR/09/029 and A23 characterized by long vines and high seed weight per fruit. A total of 197 bands were scored from 26 SRAP primers, while 49 bands were scored for 25 SSR primers. UPGMA cluster analysis based on dice genetic similarity revealed five and six distinct groups for the SRAP and SSR primers, respectively. A higher level of polymorphism was observed for the SSR primers (93.60%) as compared with the SRAP primers (64.65%), though the discriminating power of the SSR primer (2.28) was lower than that of the SRAP primer (3.71). The relatively higher frequency of SSR polymorphism should be helpful in phylogenetic analysis to better understand these relationships in the melon accessions.

Key words: 'Egusi' melon, Agro-morphological traits, SRAP, SSR, Discrimination

Correspondence: *emmaidehen@yahoo.com, ideheneo@funaab.edu.ng*

Introduction

Discrimination of germplasm using appropriate techniques assists breeders in the development or improvement of existing varieties. 'Egusi' melon is one of the most important vegetable crops in the tropical, subtropical and Mediterranean zones of the world (Schippers, 2000). The plant is an annual herb belonging to the family Cucurbitaceae and production is enhanced under dried environmental conditions with abundant sunshine (Kehinde and Idehen, 2008). The fruits vary much in size and seeds can be removed and roasted as an edible commodity (Soliman *et al*., 1985). The seeds are an important source of vitamin E, proteins and oils extracted for cooking purposes. Seeds can be ground into powder and used as soup thickener for cooking purposes (Badifu and Ogunsua, 1991). Many techniques have been employed by researchers to determine the extent of variability in germplasm collection of Egusi (melon). The principal component analysis (PCA) has been used to measure genetic divergence among genotypes and the relative discriminating power of the axes and their associated characters are measured by eigen values and factor scores, respectively (Hayman, 1967). Single linkage cluster analysis (SCLA) has also proven to be useful in determining relationship between germplasm based on similarity coefficients (Aliyu and Fawole, 2000). The FASTCLUS procedure identifies characters of materials clustered into various groups and this has assisted breeders in the choice of parents for hybridization. Agro-morphological characters of crops and their interaction with the environment may affect the accurate determination of genetic diversity. Molecular markers have advantages in that they are not affected by environmental factors (Roder *et al*., 1998). The sequence related amplified polymorphism (SRAP) marker system is a

novel, simple and reliable PCR-based system that has been successfully utilized in many crops such as potato, rice lettuce and celery genetic diversity analysis (Li and Quiros, 2001, Liu *et al*., 2004; Ruiz and Garcia-a-martinez, 2005). Many reports have shown that SRAP is an effective tool in genetic diversity analysis, cultivar identification and phylogenetic studies (Budak *et al*., 2004). Feriol *et al*., (2003) reported that information given by SRAP markers was more in agreement with morphological variability and evolutionary history of the morphotypes than that of AFLP markers. Single sequence Repeat (SSR) or Microsatellite is a short region of hypervariable DNA and are numerous and dispersed throughout the entire genome and do not require characterization of individual loci (Weising *et al*., 2005). This technique is highly reproducible and offers high levels of polymorphism. Comparative studies have shown the relative merits of molecular approaches over morphological markers for diversity and discriminating analysis (Fufa *et al*., 2005). In this study, 50 'egusi' melon accessions collected from different parts of Nigeria were discriminated using agro-morphological traits, SRAP and SSR markers as well as comparison between the marker systems.

Materials and Methods

Field evaluation and data collection: A total of 50 'egusi' melon accessions collected from different agro-ecological zones in Nigeria were used for this study (Table 1). The accessions were evaluated over two seasons in a randomized complete block design with three replicates. Each accession was grown in single row plots, with inter and intra-row spacing of 1 m each. Each row had 12 plants and data was collected on 10 plants per row.

Genomic DNA extraction: Genomic DNA was extracted from approximately 0.2g leaves of 5 – 7 days old seedlings according to the modified CTAB method (Liu *et al*., 2003). DNA concentration and quantity was determined using the NanoDrop 1000 spectrophotometer.

SRAP and SSR Analyses: For the SRAP assay, a total of 156 primer combinations were screened. Twenty-six primers that showed good banding patterns were selected for further analysis. While, for the SSR analysis a total of 25 informative SSR primers were chosen after the initial screening of 56 primers. PCR reaction for both SRAP and SSR were performed in a final volume of 20 µl consisting of 8.2 µl of double distilled H_20, 1.5 µl each of forward and reverse primers, 1.6 µl dNTPs, 2.0 µl of 10x buffer, 0.2 µl Taq DNA polymerase (Tiangen Biotech., Beijing, China) and 5.0 µl genomic DNA (40ng/ µl). The amplification consisted of a denaturing step of 4 min at 90^0C, followed by 35 cycles at 94^0C for 1 min, 50 ^{0}C for 1 min, 72^0C for 1 min and a final extension of 72 ^{0}C for 10 min, followed by cooling at 4 ^{0}C. The PCR products were electrophoresed on 6% non-denatured polyacrylamide gels in 1xTBE buffer, running at 120V constant voltage for 3 hours, and then silver stained (Basam *et al*., 1991, Liu *et al*., 2007). The band patterns on the gels were then photographed over white fluorescent light.

Data Analyses: The agronomic data for both seasons were pooled and single linkage clustering analysis was used to determine the relationship between the accessions and FASTCLUS procedure of SAS sorted the accessions into 10 groups using SAS (1999) package. For the molecular analysis, gel photographs were scored. The bands were binary coded with '1' indicating presence of bands and '0' absence of bands. The assay efficiency index, also referred to as the polymorphism information content (PIC) was calculated by the algorithm:

$PIC = 1 - \Sigma f_i^2 \quad i = 1$ where, f_i^2 is the frequency of the i^{th} allele.

For each primer pair, number of polymorphic bands, monomorphic bands, polymorphism percentage and PIC were calculated. To evaluate the efficiency of molecular markers, primers were rated according to their discriminating power. Cluster analysis was performed and dendrograms drawn using the Unweighted Pair Group Method with Arithmetic Means (UPGMA) based on Dice's similarity coefficient (Nei and Li, 1979) using NTSYS-PC (Rolph, 2000).

Results

Agro-morphological traits analysis: A dendrogram resulting from the single linkage clustering analysis based on the agronomic characters is presented in Figure 1. At a minimum distance of 9.69 level of similarity, all 50 accessions were distinct from each other, while at a coefficient of 530.18 all had

formed a single cluster. The accessions were clustered into 10 distinct groups, with group I and III having two accessions each. The highest number of accessions was clustered in group VII with eleven accessions; closely followed by group X with nine accessions. The FASTCLUS procedure which performs a disjoint cluster analysis showing the characters mean of each group, also sorted the accessions into 10 groups. Accessions in group 1(A23 and NG/TO/APR/09/027) were characterized by high yield and 100-seed weight, long vines and were late flowering. Group II was characterized by early fruiting plants with moderate yield. Accessions in group V were early flowering and high yielding, while those in group VIII were low yielding.

The agronomic characters evaluated of ten clusters of the 50 accessions of 'egusi' melon with means and standard deviation in parenthesis is presented in Table 2. The cluster groups were characterized by seed yield, number of days to germination, number of days to flowering, number of branches per plant, vine length, number of days to first fruiting, number of fruits per plants, fruit circumference, fruit weight per plant, seed weight per fruit and 100-seed weight. Group VII had the largest number of accessions (11); follow by group X with nine accessions. Group IX had seven accessions, while groups I and III had two accessions each. Accessions in group I (Table 2) were characterized by longer number of days to flowering, long vines, highest seed weight per fruit and 100-seed weight.

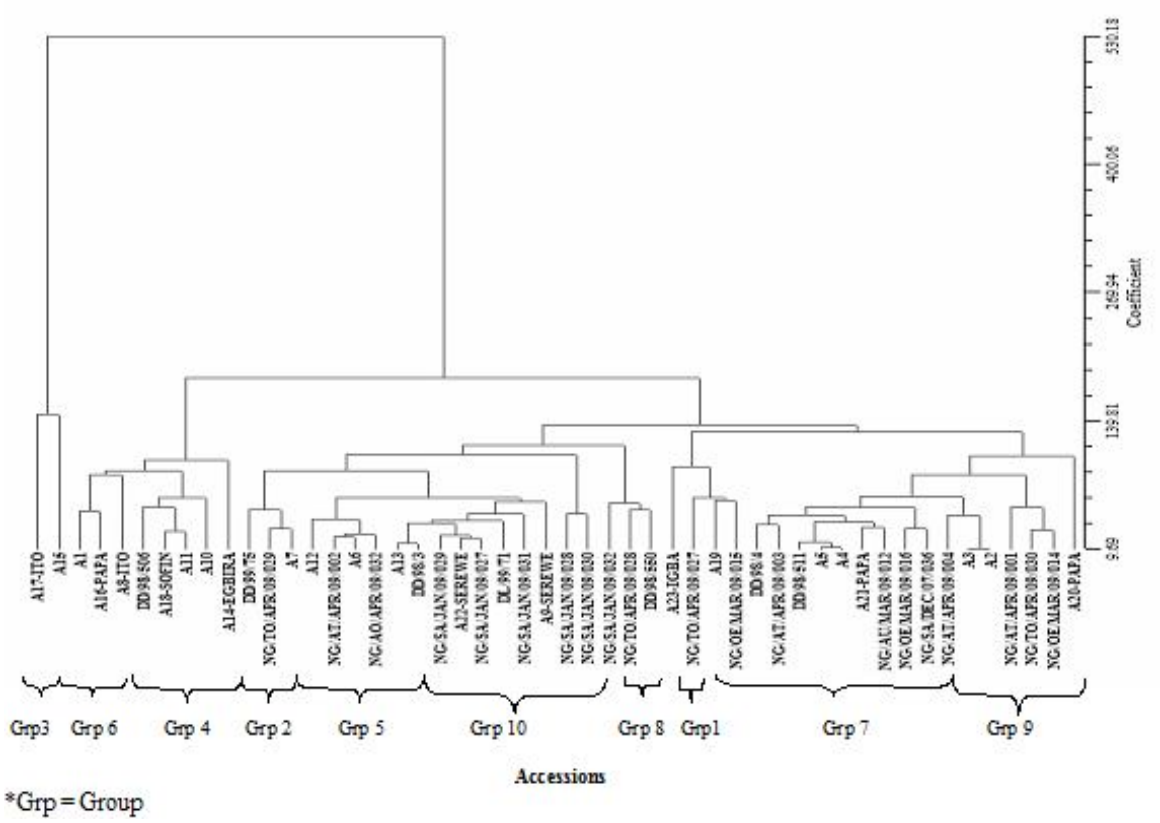

Figure 1. Dendrogram resulting from Single Linkage Cluster Analysis (SLCA) of 50 accessions of 'egusi' melon based onb Agro-morphological traits.

SRAP Analysis: A total of 156 SRAP primer combinations were screened and only twenty six gave a clear and consistent result and, thus, were used for the analysis of the 50 'egusi' melon accessions. A total of 197 bands were observed of which 129 (64.65%) were polymorphic (Table 3). The assay efficiency index of primer ranged from 0.14- 0.72, with an average of 0.51. The SRAP marker also revealed a high level of genetic distance, with similarity coefficient ranging between 0.51- 0.96. UPGMA grouped the accessions into six groups (figure 2). With group V recording the highest number of

accessions (19) and group III the least (3 accessions). The discriminating power as revealed by eigen values of the SRAP marker system was 3.71.

SSR Analysis: Twenty five primer pairs that gave the best amplification and high polymorphism were selected from the 56 primer pairs that were screened. A total of 49 bands were recorded out of which 42 (93.60%) were polymorphic (Table 3). The PIC ranged from 0.36- 0.80, with an average of 0.61. Similarity coefficient of the dendrogram ranged from 0.67- 1.00 and accessions were grouped into five groups (figure 3) with group V having the highest number of accessions (29) and only one accession in group 1 (NG/OE/MAR/09/014). The eigen value of this marker system is 2.28.

SRAP and SSR comparison: Both marker systems showed high level of polymorphisms and were effective in discriminating among the 50 accessions analyzed (Table 3). The PIC values for SSR markers (0.61) was higher than that of the SRAP maker (0.51), although, the corresponding eigen value of the SRAP marker (3.71) was higher than that of the SSR marker (2.28). The dendrograms generated from SRAP markers and SSR data were similar with few exceptions. The SRAP separated the accessions into six groups, one more than that in the SSR derived analysis. The SSR analysis clearly distinguished accession NG/OE/MAR/09/014 in group 1 from all other accessions, whereas the SRAP analysis had this accession clustered with six other accessions also in group 1.

Discussion

Agro-morphological Analysis: Knowledge of genetic variation is important in the optimal design of plant breeding programmes, influencing the combination of parental genotypes used for the development of new cultivars. Morphological dendrogram grouped the accessions into ten distinct groups with accessions A23 and NG/TO/APR/09/027 (group I) characterized by high yield, though they were late flowering. Those in group V were high yielding and early flowering; as such materials from both groups could be selected in breeding for early flowering and high yielding lines. The usefulness of cluster characteristics gives ample room for breeders to make selection between groups for improvement programmes. In this study, the use of two genetic markers; SRAP and SSR to determine the levels of genetic similarity of the 50 accessions of melon, showed that accession NG/OE/MAR/09/014 was clustered in group I for both SRAP and SSR analyses, however, in the SSR analysis it was the only member of that group. FASTCLUS procedure revealed that the accessions were characterized by low fruit weight despite fruiting early; this could probably be as a result of poor accumulation of assimilates during the reproductive stage and this implies that days to fruiting will not be reliable as selection criteria for improvement of this accession.

SRAP and SSR Analysis: The similarity coefficients of 0.51 – 0.96 and 0.67 – 1.00 as measured by SRAP and SSR analyses, respectively indicates sufficient diversity in the accessions as this will provide information on the choice of parents in future breeding programmes. Accessions A10, A11 and A14 from the Montainic, Sudan and Sahel savannahs, respectively were all clustered into group IV as shown by the morphological dendrogram, but SRAP analysis grouped them into different groups, while SSR had A11 and A14 in group V and A10 into group II. These accessions were characterized by average seed yield (1348.61 kg/ha) which suggests that they could be from a common origin with greater diversity. This result also suggests the high discriminating power of SRAP, which is not influenced by environmental factors unlike the morphological characters. All accessions in group VI in SRAP analysis were also found in group V of the SSR analysis suggesting that they all have certain levels of similarity, thus, either of these (SRAP or SSR) could be used in discriminating analysis of melon to reduce cost. Most of the accessions in group V of SSR were from the rainforest agro-ecological zone. Group IV had the highest diversity in terms of the accessions as the cluster showed the materials from the rainforest, derived savannah and savannah zones.

The importance of different cluster groups based on similarity coefficients for morphological, SRAP and SSR analyses is that selection of parents could be made between groups for improvement programmes. The high polymorphism of SSR markers shows the ability to identify short nucleotides which is a function of unique slippage mechanism responsible for generating SSR allelic diversity (Pejic *et al*., 1998). The presence of many unique alleles may be explained by the relatively high rate of mutation in SSR loci as this was also observed by Henderson and Petes, (1992). Such alleles are important, because they may be diagnostic of particular regions of the genome specific to a particular type of melon. Both

SSR and SRAP markers have unprecedented utilities for the analysis of population genetics and phylogenetic diversity in melon, because they neither require radioactive isotopes and these methods can be used efficiently by researchers in developing countries (Udupa *et al*. 1998).

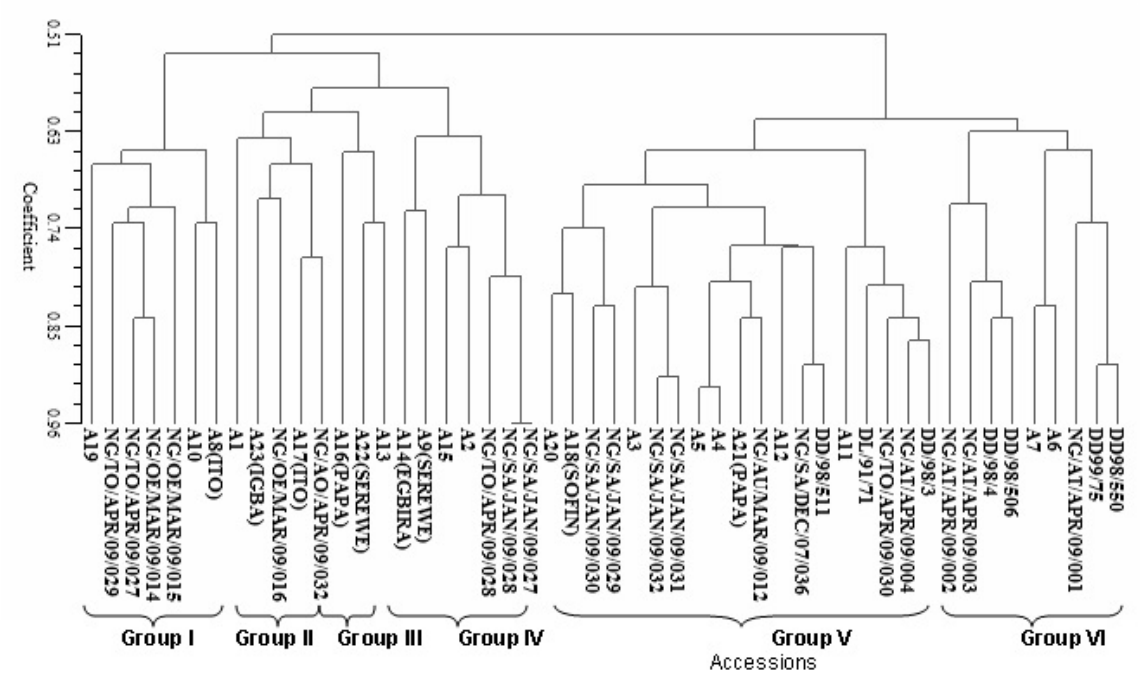

Figure 2. Dendrogram resulting from SRAP analysis showing similarity coefficients for the 50'egusi' melon accessions used in this study

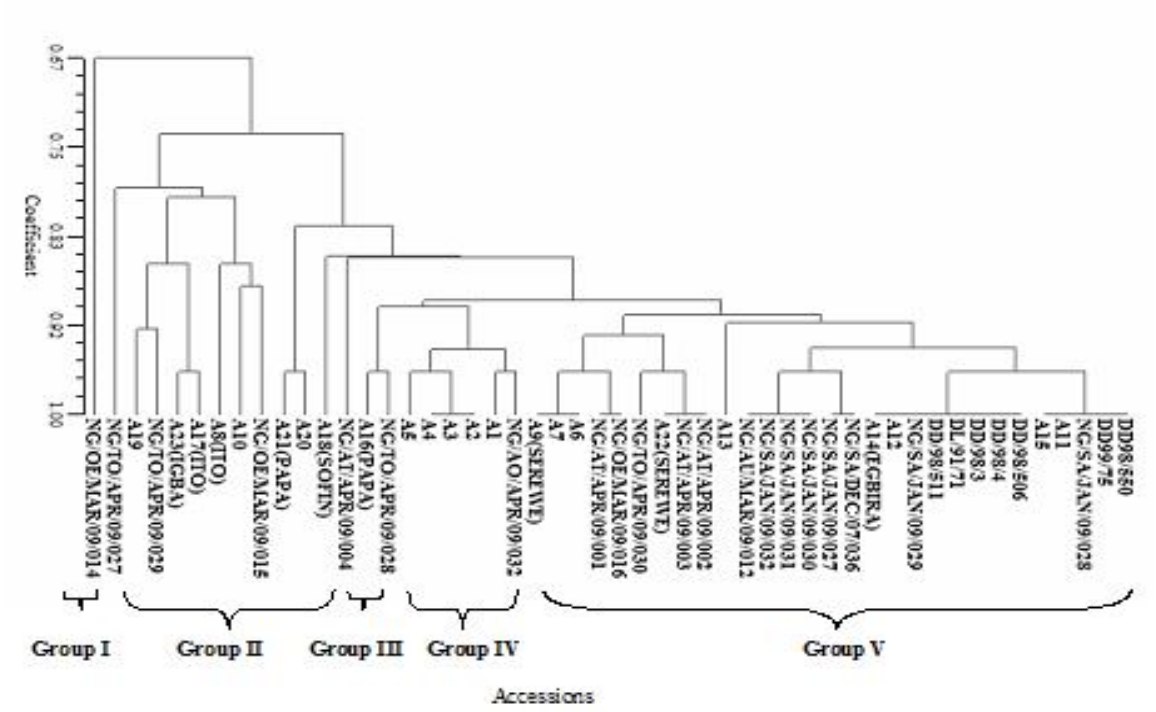

Figure 3. Dendrogram resulting from SSR analysis showing similarity coefficients for the 50 'egusi' melon accessions used in this study.

Table 1. Nigerian 'Egusi' melon accession code, name, collection source and agro-ecological zone used in this study

Accession code	Accession Name	Collection Source	Agro-ecological zone	Accession code	Accession Name	Collection Source	Agro-ecological zone
1	DD98/550	NIHORT, IBADAN	Rainforest	26	NG/TO/APR/09/029	NACGRAB, IBADAN	Rainforest
2	DD99/75	NIHORT, IBADAN	Rainforest	27	NG/AO/APR/09/032	NACGRAB, IBADAN	Rainforest
3	DD/98/506	NIHORT, IBADAN	Rainforest	32	A5	OYO	Rainforest
4	DD/98/4	NIHORT, IBADAN	Rainforest	33	A6	OYO	Rainforest
5	DD/98/3	NIHORT, IBADAN	Rainforest	43	A16 (PAPA)	OYO	Rainforest
6	DL/91/71	NIHORT, IBADAN	Rainforest	44	A17 (ITO)	IBADAN, OYO	Rainforest
7	DD/98/511	NIHORT, IBADAN	Rainforest	45	A18 (SOFIN II)	OYO	Rainforest
8	NG/SA/DEC/07/036	NACGRAB, IBADAN	Rainforest	47	A20 (PAPA)	SAKI, OYO	Rainforest
9	NG/SA/JAN/09/027	NACGRAB, IBADAN	Rainforest	35	A8 (ITO)	ABEOKUTA, OGUN	Rainforest
10	NG/SA/JAN/09/028	NACGRAB, IBADAN	Rainforest	46	A19	ILARO,ABEOKUTA	Rainforest
11	NG/SA/JAN/09/029	NACGRAB, IBADAN	Rainforest	49	A22 (SEREWE)	ABEOKUTA, OGUN	Rainforest
12	NG/SA/JAN/09/030	NACGRAB, IBADAN	Rainforest	50	A23 (IGBA)	ABEOKUTA, OGUN	Rainforest
13	NG/SA/JAN/09/031	NACGRAB, IBADAN	Rainforest	36	A9 (SEREWE)	OWO, ONDO	Rainforest
14	NG/SA/JAN/09/032	NACGRAB, IBADAN	Rainforest	48	A21 (PAPA)	EPE, LAGOS	Rainforest
15	NG/AU/MAR/09/012	NACGRAB, IBADAN	Rainforest	28	A1	BENIN, EDO	Rainforest
16	NG/OE/MAR/09/015	NACGRAB, IBADAN	Rainforest	42	A15	EDO	Rainforest
17	NG/OE/MAR/09/016	NACGRAB, IBADAN	Rainforest	31	A4	KOGI	Derived Savannah
18	NG/AT/APR/09/001	NACGRAB, IBADAN	Rainforest	34	A7	KOGI	Derived Savannah
19	NG/AT/APR/09/002	NACGRAB, IBADAN	Rainforest	41	A14 (EGBIRA)	KOGI	Derived Savannah
20	NG/AT/APR/09/003	NACGRAB, IBADAN	Rainforest	30	A3	ZAMFARA	Sudan Savannah
21	NG/AT/APR/09/004	NACGRAB, IBADAN	Rainforest	39	A12	ZAMFARA	Sudan Savannah
22	NG/TO/APR/09/030	NACGRAB, IBADAN	Rainforest	40	A13	ZAMFARA	Sudan Savannah
23	NG/OE/MAR/09/014	NACGRAB, IBADAN	Rainforest	38	A11	GUSAU, SOKOTO	Sudan Savannah
24	NG/TO/APR/09/027	NACGRAB, IBADAN	Rainforest	37	A10	TARABA	Montainic Savannah
25	NG/TO/APR/09/028	NACGRAB, IBADAN	Rainforest	29	A2	KADUNA	Derived Savannah

NACGRAB: National Centre for Genetic Resources and Biotechnology
NIHORT: National Horticultural Research Institute

Table 2. Agronomic characters evaluated of ten clusters of 50 'egusi' melon accessions with their means and the standard deviation in parenthesis

Groups/ Accession codes Characters	I 24, 50	II 2, 26, 34	III 42, 44	IV 5, 37, 38, 41, 45	V 19, 27, 33, 39, 40	VI 28,35, 43	VII 4, 7, 8, 15, 16, 17,20,31, 32, 46, 48	VIII 1, 14, 25	IX 18, 21,22, 23, 29, 30, 47	X 5, 6, 9, 10, 11, 12, 13, 36, 49
Seed yield(kg/ha)	2246.67 (0.00)	1213.33 (132.66)	940.80 (105.32)	1348.61 (61.41)	2184.99 (119.82)	1693.33 (0.00)	1659.16 (141.20)	762.38 (103.05)	893.05 (94.99)	2673.33 (84.85)
Number of days to germination	6.00 (0.00)	5.41 (0.32)	5.26 (0.63)	5.38 (0.25)	5.77 (0.69)	6.33 (0.00)	5.49 (0.57)	5.19 (0.57)	5.39 (0.87)	5.69 (0.94)
Number of days to flowering	36.00 (0.00)	32.16 (1.03)	35.93 (1.86)	33.89 (1.73)	31.89 (2.45)	34.67 (0.00)	32.50 (2.08)	32.23 (2.36)	33.16 (1.73)	31.67 (1.41)
Number of branches/plant	2.33 (0.00)	3.08 (0.49)	3.06 (0.36)	2.83 (0.75)	3.00 (0.33)	3.33 (0.00)	2.91 (0.31)	2.81 (0.32)	2.83 (0.34)	2.67 (0.00)
Vine length (cm)	838.33 (0.00)	359.83 (59.85)	602.86 (96.56)	267.11 (86.59)	347.77 (16.94)	677.33 (0.00)	379.75 (115.09)	284.33 (72.17)	329.61 (73.00)	251.33 38.65
Number of days to first fruiting	42.33 (0.00)	35.50 (1.75)	37.53 (0.96)	37.00 (1.90)	37.33 (0.33)	36.00 (0.00)	36.08 (1.34)	36.66 (1.69)	35.39 (1.45)	38.17 (0.70)
Number of fruits/plant	2.33 (0.00)	2.41 (0.32)	2.80 (0.18)	2.66 (0.36)	2.22 (0.19)	2.00 (0.00)	2.41 (0.17)	2.47 (0.46)	2.44 (0.34)	3.00 (0.46)
Fruit circumference (cm)	35.00 (0.00)	37.25 (4.93)	33.60 (2.75)	33.61 (2.94)	34.55 (0.83)	34.67 (0.00)	35.91 (2.36)	35.52 (2.44)	31.22 (2.94)	34.00 (1.14)
Fruit weight/plant (g)	705.00 (0.00)	739.16 (125.36)	645.20 (87.35)	520.27 (62.06)	693.33 (86.66)	800.00 (0.00)	619.16 (44.75)	697.85 (143.69)	423.05 (84.95)	542.00 (1.88)
Seed weight/fruit (g)	142.00 (0.00)	137.33 (20.92)	126.79 (22.12)	129.38 (11.34)	139.77 (27.98)	162.00 (0.00)	129.91 (5.67)	123.90 (21.82)	110.88 (25.61)	119.50 (15.31)
100-seed weight (g)	17.05 (0.00)	14.08 (1.08)	15.32 (1.36)	14.07 (1.01)	14.43 (1.37)	16.80 (0.00)	15.43 (1.17)	14.79 (1.45)	15.48 (1.70)	14.52 (0.91)

Table 3. Polymorphism and comparison of the discriminating capacity of SRAP and SSR markers used for the 50 accessions of 'egusi' melon

Indexes	Marker System	
	SRAP	SSR
Number of markers used	26	25
Total number of bands	197	49
Number of polymorphic bands	129	42
Number of monomorphic bands	1	16
Average number of polymorphic bands	4.96	1.68
Average number of bands	7.57	1.96
Mean polymorphism (%)	64.65	93.60
Polymorphism information content (Range)	0.14 – 0.72	0.36 – 0.80
Polymorphism information content (Average)	0.51	0.61
Dendrogram grouping	6	5
Similarity coefficient (Range)	0.51 – 0.96	0.67 – 1.00
Discriminating power (Eigen value)	3.71	2.28

Conclusion

The high level of allelic diversity of SSR and SRAP markers observed in this study probably were associated with the extensive range of genetic diversity present in the 'egusi' melon accessions. Differentiation among melon accessions was much higher for molecular markers than for morphological classification because molecular markers have the ability to identify specific loci for each trait whereas morphological classification is prone to environmental factors and experimental error. The SSR analysis was also able to reveal the accessions cluster across agro-ecological zones, thus revealing their diversity.

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References

Aliyu, B. and Fawole, I. (2000) Inheritance of pubescence in crosses between *Vigna unguiculata* and *V. rhomboidea*. Nigerian Journal of Genetics 15: 9 - 14

Badifu, G. I. O. and Ogunsua, A. O. (1991). Chemical composition of kernels from some species of Cucurbitaceae grown in Nigeria. Plant foods Human Nutr. 41: 35-44

Bassam, B. J., Caetano-anolles, G., Gresshoff, P. M. (1991). Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem. 196, 80 – 83.

Budak, H., Shearman, R. C., Parmaksiz, I. and Dweikat, I., (2004). Comparative analysis of seeded and vegetative biotype buffalo grasses based on phylogenetic relationship using ISSRs, SSRs, RAPDs and SRAPs. Theor. Appl. Genet. 109, 280–288.

Ferriol, M., Pico, B. and Nuez, F., (2003). Genetic diversity of some accessions of *Cucurbita maxima* from Spain using RAPD and SBAP markers. Genet. Res. Crop. Evol. 50 (3), 227–238.

Fufa, H., Baenziger, P.S., Beecher, B.S., Dweikat, I., Graybosch, R.A., andEskridge, K.M., (2005). Comparison of phenotypic and molecular marker-based classifications of hard red winter wheat cultivars. Euphytica 145, 133–146.

Henderson, S. T. and Petes, T. D. (1992). Instability of simple sequence DNA in *Saccharomyces cerevisae*. Molecular Cell Biology 12: 2749 – 2757.

Kehinde, O. B. and Idehen, E. O. (2008). Genetic Variability and Correlation Studies in 'Egusi' Melon (*Citrullus lanatus* (Thunb.) Matsum & Nakai). Acta Agronomica Hungarica. 56 (2):213–221

Hayman, H. H. (1967). Modern factor analysis. Second edition. University of Chicago Press, Chicago pp 474

Li, G.,and Quiros, C. F. (2001). Sequence-related amplified polymorphisim (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. *Theor. Appl. Genet.* 107 (1):168 – 180.

Liu, L., Guo, W., Zhu, X., and Zhang, T. (2003). Inheritance and fine mapping of fertility-restoration for cytoplasmic male sterility in *Gossypium hirsutum* L. Theor. Appl. Genet. 106:461–469.

Liu, L. W., Gong, Y. Q., Huang, H. (2004). Novel molecular marker system— SRAP and TRAP and their application. Hereditas 26 (5):777–781.

Liu, G., Liu, L., Gong, Y., Wang, Y., Yu, F., Shen, H., and Gui, W. (2007). Seed genetic purity testing of F1 hybrid cabbage (*Brassica oleracea* var. *capitata*) with molecular marker analysis. *Seed Sci. Technol.* 35:476–485.

Nei, M., Li, W.H., (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci*. U.S.A. 76, 5269–5273.

Pejic, I., Ajmone-Marsan, P., Morgante, M., Kozumplick, V., Castiglioni, P., Taramino, G. and Motto, M. (1998). Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. *Theoretical Applied Genetics* 97: 1248-1255.

Roder, M.S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.H., Leory, P. and Ganal, M.W. (1998): A microsatellite map of wheat. Genetics 149, 2007—2023.

Rohlf, F. J. (2000). NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, version 2.1. Exeter Software, Setauket, New York.

Ruiz, J. J. and Garcı´a-Martı´nez, S. (2005). Genetic variability and relationship of closely related Spanish traditional cultivars of tomato as detected by SRAP and SSR markers. *J. Am. Soc. Hort. Sci.* 130 (1), 88–94.

Statistical Analysis System (1999). *Statistical Methods*. SAS Institute Inc. Cary North Carolina

Schippers, R. R. (2000). African indigenous vegetables. An overview of the cultivated species, Chathan, U. K. Natural Resources Institute ACP-EU Technial Center for Agricultural and Rural Cooperation. 57-58 pp.

Soliman, M. A., EL Sawy, A. A., Fadel, H. M., Osman, F. and Gad, A. M. (1985). Volatile components of roasted *Citrullus colocynthis* var *colocynthoides*. Agr. Biol Chem. Tokyo 49: 269 – 275.

Udupa, S. M., Weigand, F., Saxena, M. C. and Kahl, G. (1998). Genotyping with RAPD and microsatellite markers resolves pathotype diversity in the ascochyta blight pathogen of chickpea. *Theoretical Applied Genetics* 97: 299- 307.

Weising, K., H. Nybon, K. Wolff, and Gunter, K. (2005). DNA Fingerprinting in Plants, Principle Methods and Applications, 2nd edn. CRC Press, Boca Raton FL USA. 472 pp