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Sequence Related Amplified Polymorphism (SRAP) Based Genetic Analysis of Nigerian 'Egusi' Melon Accessions

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ABSTRACT

Variability is germane in crop improvement. The ability of molecular markers to reveal polymorphism can assist in identifying the specific loci of a particular trait in crops. Sequence related amplified polymorphism (SRAP) is a useful technique for determining the genetic diversity of plants and was used to discriminate fifty 'egusi' melon accessions from different parts of Nigeria. The fifty accessions were subjected to SRAP analysis and 65.48% of the bands were found to be polymorphic with similarity coefficients of the accessions ranging from 0.51 – 0.96. Accessions DD98/3, NG/AU/MAR/09/012, NG/OE/MAR/09/015, NG/AO/APR/09/032 and A17 were found to be distinct from all other accessions. The high level of polymorphism exhibited by the 'egusi' melon accessions show they would be useful for maintaining genetic diversity in future breeding programs.

Keywords: Dendrogram, 'egusi' melon, genetic diversity, SRAP

INTRODUCTION

Egusi' melon [*Citrulus lanatus* (Thunb.) Matsum. & Nakai] belongs to the family cucurbitacea and it is an annual herb with hairy, trailing and angular stem, and dark green alternate leaves. The mesocarp of the fruit is extremely bitter, but the seeds can be removed, roasted, and eaten, in addition to being made into a soup thickener or flavouring agent (Badifu & Ogunsua, 1991). Conventional methods to characterize melon accessions based on phenotypic observations have been reported (Idehen et al., 2007, Kehinde & Idehen, 2008), but the use of molecular approach has become imperative when considering their diversity. Sequence related amplified polymorphism (SRAP) is a novel Polymerase Chain Reaction (PCR) based marker system as

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described by Li and Quiros, (2001) was to determine genetic diversity in peach and nectarine (Ahmad et al, 2004) and tomato (Ruiz and Garcia-Martinez, 2005). The aim of this study is to determine the relationship among Nigerian 'egusi' melon accessions using the SRAP marker technology.

MATERIALS AND METHODS

Plant Materials

Fifty accessions of 'egusi' melon were collected from Research Institutes and different parts of Nigeria (Table 1).

Genomic DNA Extraction

Genomic DNA was extracted from young leaves according to the modified CTAB method (Liu et al., 2003). DNA was then dissolved in 50 µl of double-distilled water, diluted to a final concentration of 40 ng/ µl-1 with 1×TBE buffer and stored at 4°C. Measurement of the DNA concentration and purity was determined using a NanoDrop ND 1000 spectrophotometer.

Primers and PCR Amplification

In this assay 156 primer combinations were screened and twenty-six different combinations which generated amplicons were used (Table 2). The SRAP-PCR reaction mixture totalling 20 µl consisted of 8.2 µl of double distilled water, forward and reverse primers of 1.5 µl each, dNTPs at 1.6 µl, 10x buffer at 2.0 µl, Taq DNA polymerase at 0.2 µl, and 5 µl of genomic DNA (40 ng/ µl). The amplification consisted of a denaturing step of 4 min at 94°C, followed by 35 cycles at 94°C for 1 min, 50°C for 1 min, 72°C for 1 min, and a final extension of 72°C for 10 min, followed by cooling to 4°C. The PCR products were electrophoresed on 6% non-denatured polyacrylamide gels in 1× TBE buffer, running at 120 V constant voltage for 3 h, and then silver stained (Bassam et al., 1991; Liu et al., 2007). The band patterns on the gels were photographed over white fluorescent light.

Statistical Analysis

The gel photographs were binary coded, with '1' indicating the presence of bands and '0' absence of bands. The assay efficiency index, also referred to as the polymorphism information content (PIC), was calculated using the algorithm:

$$PIC = 1 - \sum_{i=1} fi^2$$

Where, fi^2 is the frequency of the i^{th} allele.

A dendrogram was constructed using the Unweighted Pair-Group Method of the Arithmetic Mean (UPGMA). Principal component analysis (PCA) was performed to reveal the genetic similarity and diversity between the accessions.

Table 1
Names and sources of 50 'egusi' melon accessions used for the study

S/N	ACCESSION NAME	SOURCE	S/N	ACCESSION NAME	SOURCE
1	DD98/550	NIHORT, IBADAN	26	NG/TO/ APR/09/029	NACGRAB, IBADAN
2	DD99/75	NIHORT, IBADAN	27	NG/AO/ APR/09/032	NACGRAB, IBADAN
3	DD/98/506	NIHORT, IBADAN	28	A1	BENIN, EDO
4	DD/98/4	NIHORT, IBADAN	29	A2	KADUNA
5	DD/98/3	NIHORT, IBADAN	30	A3	ZAMFARA
6	DL/91/71	NIHORT, IBADAN	31	A4	KOGI
7	DD/98/511	NIHORT, IBADAN	32	A5	OYO, NIGERIA
8	NG/SA/ DEC/07/0361	NACGRAB, IBADAN	33	A6	OYO, NIGERIA
9	NG/SA/JAN/09/027	NACGRAB, IBADAN	34	A7	KOGI, NIGERIA
10	NG/SA/JAN/09/028	NACGRAB, IBADAN,	35	A8 (ITO)	ABEOKUTA, OGUN
11	NG/SA/JAN/09/029	NACGRAB, IBADAN,	36	A9 (SEREWE)	OWO, ONDO
12	NG/SA/JAN/09/030	NACGRAB, IBADAN	37	A10	TARABA
13	NG/SA/JAN/09/031	NACGRAB, IBADAN	38	A11	GUSAU, SOKOTO
14	NG/SA/JAN/09/032	NACGRAB, IBADAN	39	A12	ZAMFARA
15	NG/AU/ MAR/09/012	NACGRAB, IBADAN	40	A13	ZAMFARA
16	NG/OE/ MAR/09/015	NACGRAB, IBADAN	41	A14 (EGBIRA)	KOGI,
17	NG/OE/ MAR/09/016	NACGRAB, IBADAN	42	A15	EDO
18	NG/AT/APR/09/001	NACGRAB, IBADAN	43	A16 (PAPA)	OYO
19	NG/AT/APR/09/002	NACGRAB, IBADAN	44	A17 (ITO)	IBADAN, OYO
20	NG/AT/APR/09/003	NACGRAB, IBADAN	45	A18 (SOFIN II)	OYO
21	NG/AT/APR/09/004	NACGRAB, IBADAN	46	A19	ILARO, ABEOKUTA
22	NG/TO/ APR/09/030	NACGRAB, IBADAN	47	A20 (PAPA)	SAKI, OYO
23	NG/OE/ MAR/09/014	NACGRAB, IBADAN	48	A21 (PAPA)	EPE, LAGOS
24	NG/TO/ APR/09/027	NACGRAB, IBADAN	49	A22 (SEREWE)	ABEOKUTA, OGUN
25	NG/TO/ APR/09/028	NACGRAB, IBADAN	50	A23 (IGBA)	ABEOKUTA, OGUN

Table 2
Base sequences of the Sequence Related Amplified Polymorphism (SRAP) primer combinations used for DNA amplification

Forward primer (5' → 3')	Reverse primer (5' → 3')
Me16: ACTACTTTGATGGACACTTGCCT	Em16: GCTCTGAAGTTGATTAGTCGGTC
Me18: TAGGGTTTAGAGTTAAGGGGTGG	Em18: TCTCCAAGAAAGAAAATGACCAA
Me20: GGGAAGACACTTTGGAGGAGTAC	Em21: GGATAATGTTTATGGTAGCTCGA
Me21: GTTTACGGTTCAGGGTTTAGGGT	Em22: GAAATCCTTTCCATCAGCTTTCT
Me22: ACCTGTCTCCATCTCCACCTTGT	Em23: GCAAGACATTGAGCCTTCTACTT
Me23: TAAAGAGCCAACAAACTCGAAAG	Em24: GTAAAACCGAACCGTACCGAACC
Me24: ATACCAAACCATATCCAAATCCT	Em25: AAACTAGATCTTGACCGCACATC
Me26: ATGGTTAGATATCAAATTGGAAAC	Em26: ACTAGTATTGACCCCATGCTAGG
Me27: TTCCTCCACCACCGCTGACACTA	Em27: GTTTAGCAAAATCTCCAACAACC

RESULTS AND DISCUSSION

Polymorphism in 'egusi' melon accessions detected by SRAP primers

A total of 156 different combinations of primers, nine forward and reverse primers, were screened. A total of 26 combinations revealed polymorphism between the 'egusi' melon accessions (Table 3), from which one hundred and ninety-seven bands were obtained for analysis.

High number of bands were observed for primer combinations; Me18 - Em18 (15 bands), Me27 - Em27 (13 bands), Me18 - Em24 (12 bands) and Me21 - Em26 (12 bands). While, lower number of bands were recorded for primer combinations; Me26 - Em26, Me22 - Em18, Me22 - Em21), with three bands each. Polymorphic bands ranged between 1 to11 for primer combinations Me22 - Em21 and Me18 - Em18, respectively. The least polymorphism of 27.20% was observed for Me23 – Em26), while the highest polymorphism of 85.70% for Me16 – Em24 (Table 3).

Polymorphism information content (PIC) of the SRAP primers ranged from 0.14 – 0.72 for Me24-Em24 and Me20-Em26, respectively with a mean of 0.51. Representative banding pattern generated using primer Me20-Em26 are shown in Figure 1. Polymorphism information content of the SRAP loci and their ability to detect differences based on genetic diversity showed primer combination Me20 – Em26 is important in discriminating melon accessions. Higher number of polymorphic bands generated per primer pair combinations when compared to the previous study with melon using SSR primers (Idehen et al., 2012), which is an indication of the ability of SRAP primers to effectively identify multiple loci.

Analysis of phylogenetic dendrogram based on SRAP analysis

Dendrogram resulting from SRAP analysis with similarity coefficients for the 50 'egusi'

Table 3
Polymorphism and polymorphism information content (PIC) of 26 Sequence Related Amplified Polymorphism (SRAP) primer combinations

Primer Combinations	Total number of bands	Polymorphic bands	Polymorphism (%)	PIC
Me16 - Em16	6	3	50.00	0.58
Me18 - Em18	15	11	73.00	0.52
Me24 - Em24	9	5	55.00	0.14
Me26 - Em26	3	2	66.60	0.66
Me27 - Em27	13	10	76.92	0.22
Me20 - Em18	8	6	75.00	0.51
Me22 - Em18	3	2	66.60	0.39
Me23 - Em18	4	2	50.00	0.55
Me27 - Em18	7	4	57.10	0.43
Me22 - Em21	3	1	33.30	0.49
Me20 - Em22	6	4	66.60	0.54
Me21 - Em22	8	4	50.00	0.47
Me22 - Em23	10	8	80.00	0.66
Me23 - Em22	8	3	37.50	0.45
Me26 -Em22	9	5	55.50	0.36
Me27 - Em22	5	4	80.00	0.52
Me16 - Em24	7	6	85.70	0.60
Me18 - Em24	12	9	75.00	0.48
Me22 - Em24	9	7	77.70	0.49
Me27 - Em24	7	5	71.40	0.43
Me21 - Em25	4	3	75.00	0.54
Me27 - Em25	6	5	83.30	0.68
Me20 - Em26	8	5	62.50	0.72
Me21 - Em26	12	9	75.00	0.51
Me22 - Em26	4	3	75.00	0.68
Me23 - Em26	11	3	27.20	0.67
Mean	7.57	4.96	64.65	0.51
Total	197	129	-	-

melon accessions used in this study (Figure 2), clearly distinguished the accessions. Similarity coefficients for the six groups as revealed by SRAP analysis ranged from 0.51 – 0.96. The 50 'egusi' melon accessions were divided into 6 groups (I, II, III, IV, V and VI) (Figure 2), with group V having the highest number of accessions clustered together (19) and group III, the least (3 accessions). The dendrogram from SRAP analysis was able to distinguish all accessions except two (2) at a similarity coefficient of 0.96, whereas an

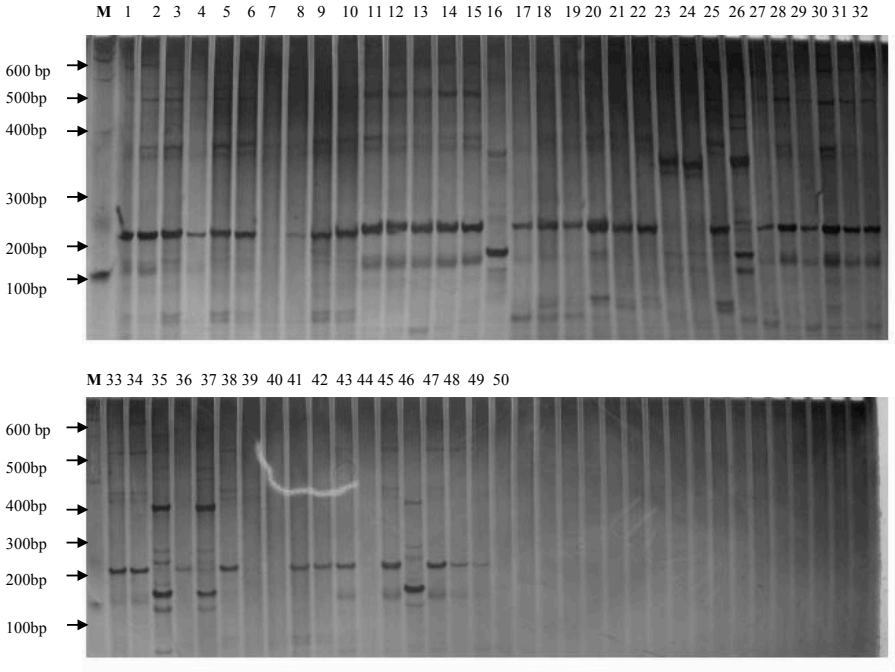

Figure 1. Representative banding pattern generated using SRAP primer combination Me20-Em26

*M- Marker
*Numbers above represents accessions

1=DD98/550	11=NG/SA/JAN/09/029	21=NG/AT/APR/09/004	31=A4	41=A14 (EGBIRA)
2= DD99/75	12=NG/SA/JAN/09/030	22=NG/TO/APR/09/030	32=A5	42=A15
3=DD/98/506	13=NG/SA/JAN/09/031	23=NG/OE/MAR/09/014	33=A6	43=A16 (PAPA)
4=DD/98/4	14=NG/SA/JAN/09/032	24=NG/TO/APR/09/027	34=A7	44=A17 (ITO)
5=DD/98/3	15=NG/AU/MAR/09/012	25=NG/TO/APR/09/028	35=A8 (ITO)	45=A18 (SOFIN II)
6=DL/91/71	16=NG/OE/MAR/09/015	26=NG/TO/APR/09/029	36=A9 (SEREWE)	46=A19
7=DD/98/511	17=NG/OE/MAR/09/016	27=NG/AO/APR/09/032	37=A10	47=A20 (PAPA)
8=NG/SA/DEC/07/0361	18=NG/AT/APR/09/001	28=A1	38=A11	48=A21 (PAPA)
9=NG/SA/JAN/09/027	19=NG/AT/APR/09/002	29=A2	39=A12	49=A22 (SEREWE)
10=NG/SA/JAN/09/028	20=NG/AT/APR/09/003	30=A3	40=A13	50=A23 (IGBA)

earlier study on melon by Idehen et al. 2012, the accessions were not distinguishable even at a similarity coefficient of 1.00 as this was evident in majority of the accessions especially those in group five (5). This might be attributable to an earlier report by Budak et al., 2004 who stated the effectiveness of SRAP analyses in genetic diversity analysis, cultivar identification and phylogenetic studies.

Two-dimension (2D) plots from the principal component analysis (Figure 3) revealed accessions DD98/3, NG/AU/MAR/09/012, NG/OE/MAR/09/015, NG/

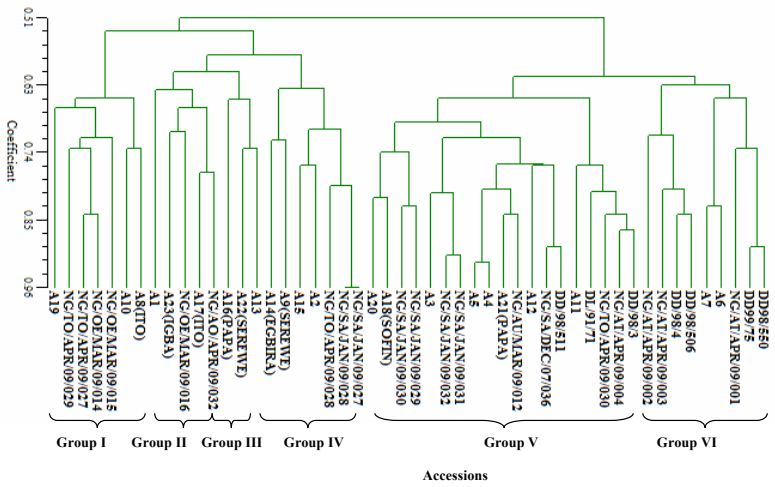

Figure 2. Dendrogram resulting from SRAP analysis showing similarity coefficients for the 50 'egusi' melon accessions used in this study

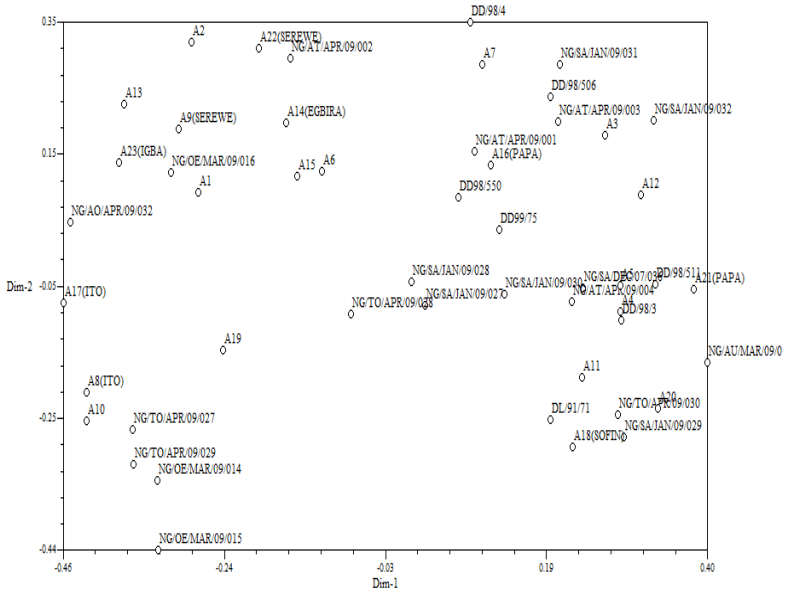

Figure 3. Two-dimension (2D) plot of 50 'egusi' melon accessions based on SRAP markers

AO/APR/09/032 and A19 collected from research Institutes, with the exception of A19 which was sourced locally were distinct from all other accessions. The two (2) dimension plot was also able to clearly discriminate and show the spatial distribution of the accessions.

UPGMA analysis grouped the accessions into six groups, with majority of the accessions clustered in group V. The

clusters observed between the accessions collected from the research Institutes and those from other agro-ecological zones in the country show that they possess one or more character in common and suggest that they may have been from the same origin.

CONCLUSION

This study revealed that the SRAP primers were highly polymorphic and thus able to distinguish the accessions effectively. The highest polymorphism was observed for primer combination Me16 - Em24 and the high PIC of primer combinations Me20 – Em26 shows its ability to effectively discriminate several loci. The highest number of accessions clustered was in group V thus indicating their level of genetic similarity. Hence, selection for hybridization should be made from accessions in distant groups in order to benefit from their genetic variability.

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