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Short Communication

Genetic diversity of *Capsicum* using Random Amplified Polymorphic DNAs

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Pepper (*Capsicum* sp.) is one of the most popular and widely grown vegetables in Nigeria. A total of one hundred and fifty germplasm collections were characterised, evaluated and conserved in the National Horticultural Research Institute, Ibadan Nigeria. Random Amplified Polymorphic DNA (RAPD) analysis was performed on selected forty accessions of *Capsicum annum* and *Capsicum fructences* to estimate genetic diversity and taxonomic relationships. Cluster analysis using UPGMA seperated the accessions into four major groups. Based on the morphological and molecular data, remarkable difference was exhibited by the *Capsicum* accessions.

Key words: RAPD, genetic diversity, *Capsicum*.

INTRODUCTION

Capsicum is widely cultivated and consumed in all the states of Nigeria. Efforts have been made in National Horticultural Research Institute, Ibadan, to develop improved varieties using the traditional method. Morphological traits and phenotypic traits are important but unreliable when positive identification of an accession is desired. Frankel (1984) reported that to facilitate efficient germplasm collection and management there is a continual need for a greater understanding of the extent of genetic diversity within the germplasm and the nature of genetic relationships among the accessions. Information on genetic identity and relationships of genotype is crucial to the development of core collections. Molecular markers are important tool for breeding selection, genotype identification and studying the organization and evolution of plant genomes (Dettori et al., 2001). The objective of this study was to use Random amplified polymorphic DNAs (RAPDs) to identify genotypes of *Capsicum* germplasm collections and also to estimate genetic relationships among the accessions which will be useful in the improving the productivity and stability of the crops yield.

MATERIALS AND METHODS

Plant materials

A total of forty (40) accessions of *Capsicum* spp. (Table 1) were sown in pots containing top soil and placed on the floor in a screen house National Horticultural Research Institute Ibadan. Ten days later, young leaves were picked from one plant in each pot for DNA extraction and analysis. Additional leaves were collected and placed in bags stored in –80°C freezer for future use.

DNA extraction

Young leaves were collected in 1.5 ml eppendorf tube, quickly frozen in liquid nitrogen and ground with konte pestles into fine powder. DNA was extracted according to Dellarporta et al. (1983) minipreparation protocol. The purity of extracted DNA was tested on 1% agarose gel using 0.5X TBE (Tris borate EDTA) buffer and stained with 10 mg/ml ethidium bromide. The gel was exposed to UV-light and photographed.

Optimizations of the working dilutions were made using various dilution ratios. Finally, the dilution that produced amplification with the RAPD primer and three samples for screening was in ratio 1: 1000 after determining the concentration with a TD-700 Fluorometer.

PCR amplification

PCR reaction was carried out with a Perkin Elmer MJ cycler machine for amplification. A cocktail of 12.5 μl volume in a mixture containing: 1.25 μl of 10X buffer, 2.5 μl of 10 ng/ μl DNA, 1.00 μl $MgCl_2$, 1.00 μl mixture of 10 mM dNTPs (dATP, dCTP, dGTP and dTTP), 5.05 μl of ultra pure water, 0.5 μl of RAPD primers, 1.00 μl of 0.5% Tween 20 and 0.2 μl red hot Taq (promega). The RAPD profile consisted of 94°C for 3 min followed by 45 cycles consisting of 1 min at 94°C, 1 min at 35°C, and 2 min at 72°C. A final incubation for 7 min at 72°C was performed and the amplification products analyzed on 1.4% agarose gel in 0.5X Tris borate buffer at 120 V for 2 h.

Table 1. The forty accessions of *Capsicum* spp. used in this study.

S/ N	*Capsicum fructences* (Ata wewe)	S/N	*Capsicum annum* (Tatase)	S/N	*Capsicum annum* (Rodo)	S/N	*Capsicum fructences* (Sombo)
1	DA97/371-1	11	NHOP10	21	DT95/294	31	DA97/416
2	DA97/286	12	NHOpA1	22	DT95/301	32	DA95/409
3	DA97/153	13	NHOP	23	DT95/347	33	NH507
4	DA97/139	14	NHOP3	24	OL86/17	34	NHS05
5	DA97/442	15	NHOP4	25	AD6	35	NHSO6
6	DA97/192	16	NHOP5	26	DT97/429	36	NHSO1
7	DA97/448	17	NHOP8	27	AD7	37	NHSO2
8	DA97/440	18	NHOP9	28	DA97/469	38	NHSO3
9	DA97/313-10	19	NHOP6	29	AD8	39	NHSO4
10	DA97/270	20	NHOP7	30	DT95/276	40	DT95/175

Table 2. List of primers and the number of DNA bands amplified with the forty accessions of *Capsicum spp.*

Primer name	Nucleotide sequence 5' to 3'	No of bands	No polymorphic
OPERON – AE 07	CTCAAGCGCA	12	8
OPERON – AD 20	TGCGCTCCTC	10	6
OPERON - AF 20	CTCCGCACAG	14	9
OPERON – AC 20	ACGGAAGTGG	7	5
OPERON – AE 02	TGAGGGCCGT	8	4
OPERON – AE 03	CATAGAGCGG	10	7
OPERON - AC 13	TCTTGCCCTC	11	8
OPERON - AF 13	CACGAACCTC	14	10
OPERON – AC 07	GTGCCCGATC	13	10

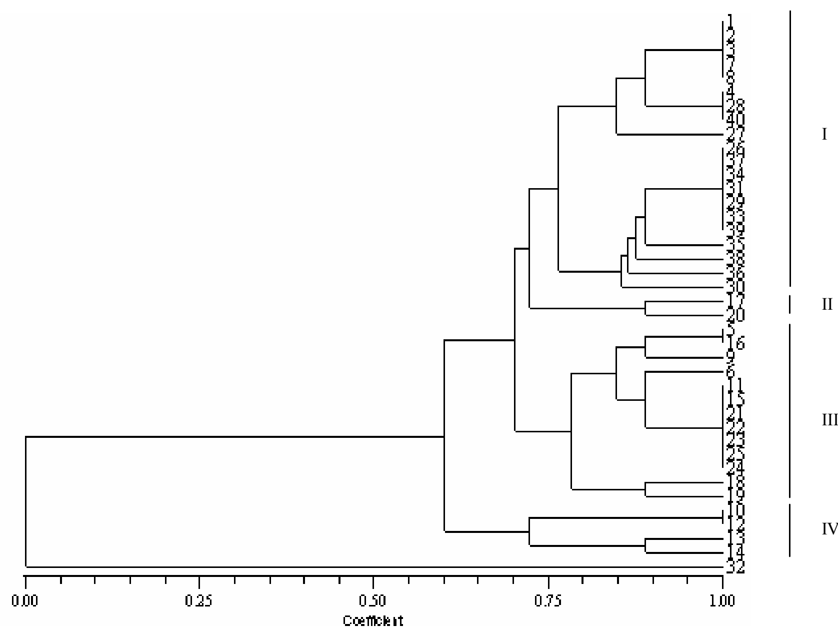

Figure 1. Dendrogram showing the distribution of the forty *Capsicum* accessions.

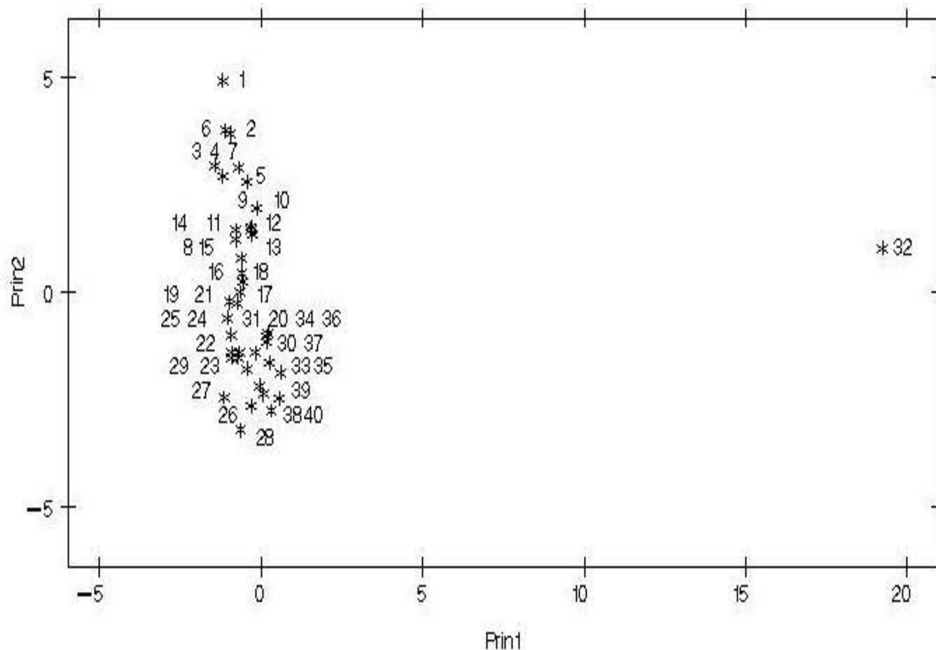

Figure 2. Principal component analysis showing the relationships among the forty *Capsicum* accessions.

The agarose gel was stained in ethidium bromide, visualised under UV light and photographed using a Polaroid MP4 camera. The 1 Kb ladder DNA from GIBCO BRL (New York, USA) was used as a standard molecular weight size marker. The random primers used for the DNA amplification were ten base sequences from AD, AC, AE, AF and AG (Operon Technologies Inc., Alameda CA. USA) (Table 2).

Data scoring

Fragments that were clearly resolved on the gels were scored as 1 or 0 (i.e., present or absent, respectively) across all the 40 accessions of *Capsicum*. Bands that could not be confidently scored were regarded as missing data.

RESULTS AND DISCUSSION

The total number of DNA bands amplified with the forty accessions of *Capsicum spp.* as well as the number of polymorphic bands among these is presented in Table 2. Pairwise distance (similarity) matrices were computed using sequential, hierarchical and nested (SAHN) clustering option of the NTSYS-pc version 2.02j software package (Rohlf, 1993). The program also generated a dendrogram (Figure 1), which grouped the accessions and species on the basis of Nei genetic distance (Nei, 1972) using unweighted pair group method with arithmetic average (UPGMA) cluster analysis (Sneath and Sokal, 1973). The population clustering exhibits four main clusters when truncated at 75% similarity level coefficient. Sample 32 distinguished itself from others, even at zero coefficients where others grouped into one clusters. Cluster I is the largest cluster consisting of twenty samples. There are two sub-clusters within this cluster showing great resemblance among some samples at 100% similarity level. Cluster II showed relationships between 2 samples (17 and 20). They are similar at 90% similarity level. There were thirteen samples in cluster III; eight of them cannot be distinguished as they show some genetic resemblance within themselves. In cluster IV, samples 13 and 14 were grouped together in this cluster at about 90% similarity level.

The linear relationships among the forty *Capsicum* accessions are shown in the principal component analysis (PCA, Figure 2) . All accessions separated into two groups; the major one consisting of thirty-nine samples and the other minor group having only one sample (32).

The PCA and dendrogram results showed that sample 32 is distinct; this is an indication that it has peculiar traits different from others. Generally, many samples were genetically similar and needs further studies to confirm their genetic diversity.

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