International Journal of Farm Sciences **6(4):** 77-81, 2016

Characterization of pecan (*Carya illinoensis*) genotypes using RAPD markers

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ABSTRACT

Genetic characterization of 32 pecan genotypes was carried out using 40 RAPD primers of which 13 produced polymorphism. In total 75 polymorphic RAPD bands were observed with 22 unique bands. Dendrogram was divided into three broad clusters parallel to their origin. Similarity matrix ranged from 0.00 to 0.55. PIC value ranged from 0.62 to 0.84 with an average of 0.74.

Keywords: Characterization; genotypes; primers; RAPD

INTRODUCTION

Pecan, *Carya illinoensis* (Wang) K Koch is a valuable nut crop and belongs to the family Juglandaceae. Level of biodiversity prevalent in germplasm is vital to any breeding programme. Polymerase chain reaction (PCR)-based marker systems especially randomly amplified polymorphic DNA (RAPD) markers have proved quite useful in genetic studies (Sharma et al 2012) because of low technical input and unlimited marker number. Hence the present study was conducted for genetic characterization of pecan germplasm so as to use them as the well identified genetic stocks in future breeding programmes.

MATERIAL and METHODS

Source material and isolation of DNA

Eexotic as well as indigenous origin material of pecan germplasm was procured from Department of Fruit Science and Horticultural Research Station, Kandaghat, University of Horticulture and Forestry, Nauni, Solan, HP (Table 1).

Genomic DNA was isolated from freshly collected leaves following method given by Doyle (1990). Forty RAPD primers were initially screened out of which 13 primers were selected for further analysis on the basis of clear polymorphism (Table 2).

Table 1. Pecan accessions included in the present investigation

Accession	Place of origin	Accession	Place of origin
Indigenous seedling selections		$R_{12}P_7$	HP, India
		R_9P_4	HP, India
R_4P_4	HP, India	**Exotic introductions**	
R_6P_4	HP, India	Kanza (Major × Shoshoni)	USA, Texas
$R_{11}P_7$	HP, India	Chickasaw (Brooks × Evers)	USA, Texas
$R_{10}P_3$	HP, India	Cheyenne (Clark × Odom)	USA, Texas
$R_{12}P_5$	HP, India	Wichita (Halbert × Mahan)	USA, Texas
R_6P_1	HP, India	Kiowa (Mahan× Desirable)	USA, Texas
$R_{15}P_7$	HP, India	Mahan (a self of Schley)	USA, Florida
$R_{14}P_1$	HP, India	Major (native selection)	USA, Kentucky
R_4P_5	HP, India	Busseron (native selection)	USA, Indiana
$R_{11}P_5$	HP, India	Desirable (cross between	USA, Mississipi
R_9P_7	HP, India	unknown parents)	
R_6P_8	HP, India	Colby (native selection)	USA, Illinois
R_4P_7	HP, India	Giles (native selection)	USA, Kansas
R_5P_3	HP, India	Burkett (native selection)	USA, Texas
R_7P_5	HP, India	Winter Nelis (native selection)	USA, Florida
$R_{12}P_3$	HP, India	Western Schley (native selection)	USA, Texas

Table 2. Amplified profile generated by PCR using 13 random decamer primers

Primer	Sequence (5'- 3')	Total # amplified bands	Polymorphic bands	Unique bands	PIC value	# alleles
S073	CCAGATGCAC	7	7	1	0.79	28
S075	ACGGATCCTG	9	9	3	0.84	26
S077	CCGAATTCCC	6	6	3	0.78	15
S078	GGCTGCAGAA	5	5	2	0.74	16
S081	TCGCCAGCCA	3	3	0	0.62	13
S084	CAGACAAGCC	4	4	2	0.66	08
S088	GGTCCTCAGG	5	5	1	0.73	18
S089	CAGTTCGAGG	4	4	1	0.68	13
S091	TCGGAGTGGC	6	6	2	0.79	17
S092	ACTCAGGAGC	8	8	2	0.80	36
S093	CCACCGCCAG	5	5	1	0.70	20
S094	AGAGATGCCC	7	7	2	0.84	15
S095	CAGTTCTGGC	6	6	2	0.78	16
Total		75	75	22		

RAPD analysis

PCR reaction was performed with 13 selected primers. PCR reaction volume of 20 µl contained 1X Buffer B, 2.5 mM $MgCl_{2,}$ 250 µM dNTPs, 1 µM primer, 1 unit taq polymerase and 30 ng of genomic DNA with amplification as: initial denaturation at 94°C for 2 minutes followed by 35 cycles each of denaturation 1 minute at 94°C, annealing 1 minute at 36°C and extension 2 minutes at 72°C and finally extension at 72°C for 8 minutes followed by agarose gel electrophoresis in 1.4 per cent agarose gel and photographed by using gel documentation system.

The data were analysed with NTSYS-pc Ver 2.02 (Nei and Li 1979). Matrices were compared using Mantel test (Mantel 1967). Polymorphic information content (PIC) of primers was analysed using formula 1-(allele /number of genotypes^2) + (allele/number of genotype^2)... or 1-n pij^2 where p, i and j are frequencies of first, second and third allele.

RESULTS

Out of 40 primers tested 13 generated strong amplification (Table 2) with 75 bands giving an average of 5.76 per cent of polymorphic bands per primer. The individual primers generated bands ranging in number from three to nine. Primer S073 and S084 had given a unique band in Colby while two unique bands were generated with primer S084 in seedling selections $R_{14}P_1$ and $R_{12}P_7$ respectively. Similarity values varied from zero to 0.55 and the maximum similarity value of 0.55 was observed between Chickasaw and Kanza. This is rightly so as evident from the fact that both the accessions have Evers in their ancestory. In cluster analysis (Fig 1) three major clusters were obtained, cluster 'B' being largest including the 15 genotypes. Cluster 'A' was the second largest with 14 genotypes; 'C' the third cluster included two seedling selections. Lastly Wichita remained a singlet. Cophenetic correlation 'r' that measures the relatedness of two matrices was 0.91. PIC value ranged from 0.62 with primer S081 to as high as 0.84 with each of two primers viz S075 and S094 with an average of 0.74. Primer S073 amplified a total of 28 alleles whereas primers S075 and S094 amplified 26 and 15 alleles respectively.

DISCUSSION

DNA-based markers such as RAPD are practically unlimited in number, remain unaffected by environment and growth conditions and are simply inherited (Saxena et al 2011, Sharma et al 2012). Of 40 random decamer primers only 13 yielded clear polymorphic banding profiles on these accessions. Maximum similarity was between Chickasaw and Kanza ie 0.55, both have their origin in USA. Least similarity was observed between a number of accessions which were seedling selections as well as standard cultivars. A high level of

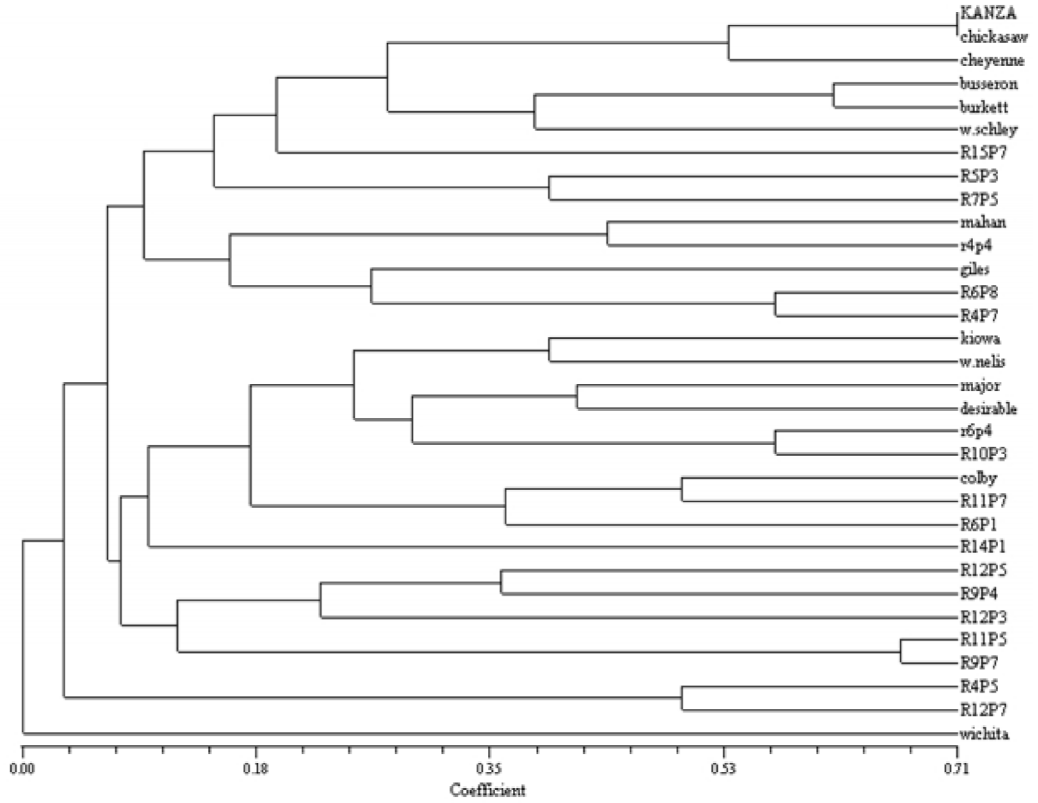

Fig 1. Dendrogram of 32 accessions of pecan based on RAPD analysis

polymorphism using RAPD markers in *Carya dabieshanesis* was observed which suggested a rich genetic variation and offered excellent prospectus for seedling selection using RAPD markers (Wang et al 2006). Cophenetic correlation 'r' was as high as 0.91 between two matrices viz similarity coefficient matrix and dendrogram data which showed a very good fit for cluster analysis with similarity matrix. It was concluded from the study that RAPD primers divided the accessions according to their origin. High level of polymorphism between different genotypes offers excellent prospects for characterization.

REFERENCES

Doyle JJ 1990. Isolation of plant DNA from fresh tissue. Focus **12:** 13-15.

Mantel N 1967. The detection of disease clustering and generalized regression approach. Cancer Research **27(2):** 209-220.

Nei M and Li WH 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the United States of America **76(10):** 5269-5273.

Saxena B, Kaur R and Bhardwaj SV 2011. Assessment of genetic diversity in cabbage cultivars using RAPD and SSR markers. Journal of Crop Science and Biotechnology 1**4(3):** 191-196.

Sharma D, Kaur R, Kumar K and Bhardwaj SV 2012. Genetic diversity among selected genotypes of almond *Prunus dulcis* Miller DA Webb assessed by random amplified polymorphic DNA (RAPD) markers. African Journal of Biotechnology **11(83):** 14877-14883.

Wang ZJ, Huang YJ, Guo CY, Qin HJ, Fang WH 2006. RAPD analysis on genetic diversity of *Carya dabieshanensis* populations. Journal of Plant Ecology **30(3):** 534-538.

Received: 12.1.2016 ***Accepted: 23.9.2016***