Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7818

ISSN: 2582 – 2845

Ind. J. Pure App. Biosci. (2019) *7*(5), 30-35

Research Article

Indian Journal of Pure & Applied Biosciences

Genetic Diversity Analysis in White Yam (*Dioscorea rotundata* Poir.) using Random Amplified Polymorphic DNA (RAPD) Markers

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Received: 10.09.2019 | Revised: 14.10.2019 | Accepted: 21.10.2019

ABSTRACT

White yam (Dioscorea rotundata Poir) is grown as a major food crop in tropical countries. It is emerging as an important tuber crop in India especially in Kerala and it is well known for its yield potential. ICAR- Central Tuber Crops Research Institute holds a field gene bank of 1100 Dioscorea accessions including 158 white yam genotypes. Genetic variability in plants can be used for developing improved cultivars with desirable traits. Present study focused on identifying genetic variability in 30 white yam genotypes (Dwarf, Semi dwarf and Tall) using 19 random oligonucleotide primers for RAPD analysis. On 19 RAPD primers, only 8 primers were selected based on their polymorphism and repeatability. Among the eight RAPD primers studied in white yam, OPW -16 was found be the best that produced ten polymorphic bands. All the primers evaluated resulted in 100% polymorphism. All the primers were recorded with high PIC value of >0.6. Dendrogram based on RAPD marker showed nine clusters at 0.8 dissimilarity level. It also shows the high divergence of the genotype V20 (DR17) and the clustering pattern indicates the grouping of the dwarf genotypes together. More or less similar clusters were obtained based on certain morphological characteristics of the genotypes observed in the field study.

Keywords: *Yams, genetic variability, RAPD analysis, polymorphism, dwarf genotype and morphology*

INTRODUCTION

Yams are the fourth most important tuber crop grown in the world. They are consumed as staple food and are rich in starch and energy. The most important part of yams is tuber. It can be eaten as boiled yam, fufu or fried in oil. Vitamin C has been found in unpeeled yam slices. It consists of pharmacologically active substances including dioscorine, saponin and sapogenin. Dioscorine is an alkaloid, which is a heart stimulant. Yams are considered to be monocots. They belong to the family dioscoreacea within the order dioscoreales (Ayensu & Coursey, 1972). The genus dioscorea is the largest genus of the family. The family includes ten genera and 650 species and are mainly tropical and subtropical and semi temperate in distribution.

Cite this article: Harikumar, P., & Sheela M.N. (2019). Genetic Diversity Analysis in White Yam (*Dioscorea rotundata* Poir.) using Random Amplified Polymorphic DNA (RAPD) Markers, *Ind. J. Pure App. Biosci. 7*(5), 30-35. doi: http://dx.doi.org/10.18782/2320-7051.7818

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Cytologically yams have a basic chromosome number n=10. But various degree of polyploidy exists even within the same species. *D. rotundata* plants have 2*n* = 40 chromosomes (Raghavan, 1958). Cultivars of *D. rotundata* were classified into early and late maturing cultivars.

Morphology of yams shows that the stem of *Dioscorea* is unable to support the weight of the leaves and have to climb by twining, but there are no specialized organs such as tendrils. The direction of twining, anticlockwise or clockwise is a characteristic of each taxonomic section (Coursey, 1967). The leaves vary in size between species, between cultivars within species and between different parts of a single plant. Average area is in the range 50-200cm^2.The phyllotaxy is spiral, can be opposite or alternate depending on the species MvKey et al., (1998). The flowers are usually unisexual and many cultivars rarely flower and set fertile seeds. It tuber shows tremendous variation in size, form and number of tubers per plant within and between species (Lebot, 2009).

The lack of knowledge about the origin, diversity and genetics of these species has extremely limited the effectiveness of genetic improvement programmes (Arnau et al., 2010). The acquisition of knowledge about the genetic diversity of the species at both agronomic and cytogenetic levels is essential for the effective genetic improvement programme. Hence considering this background, present study aims at revealing genetic diversity in 30 different accessions (Dwarf, semi dwarf and Tall) of white yam using 8 RAPD primers and identifying highly variable variety from these accessions along with the morphological study using certain parameters.

MATERIALS AND METHODS

The plant material comprises thirty accessions of tall, dwarf and semi dwarf varieties of white yam collected from germplasam maintained in the field gene bank of ICAR- Central Tuber Crops Research Institute, Kerala, India and given in Table 1. Fresh tender young leaves of *Dioscorea rotundata* Poir accessions were collected including 14 dwarf, 1 semi dwarf and 15 tall varieties. DNA was extracted from fresh and tender young leaves using modified protocol of Doyle and Doyle, (1987). Isolated DNA quantified using spectrophotometer (Systronics, India) at 260 and 280nm to ensure its yield and purity. The primers were selected from collected literatures given in table 2 that showing high polymorphic values in *Dioscorea* species, ordered and shipped from Integrated DNA Technologies, Inc., as lyophilized form.

The samples were amplified in thermal cycler using 8 different RAPD primers. The optimum amplifying conditions were standardized as shown in table 3. The amplified products were separated on 2% agarose gel along with 1Kb and 100bp ladders to identify molecular weight of obtained bands. The results were photographed on a digital gel documentation and image analysis system. Hence reproducible bands were scored visually and validated to detect polymorphism using PIC calculator.

A binary matrix of presence/absence was obtained from gels for each primer. The data matrix created in excel format was used as the input for cluster analysis. Estimation of genetic diversity parameters results in an overview of the genetic variability. Jaccard's similarity coefficient was calculated for use in clustering analysis by Unweighted Pair-group Method with Arithmetic Average (UPGMA). Jaccard's similarity coefficient was calculated for use in clustering analysis by Unweighted Pair-group Method with Arithmetic Average (UPGMA). Codes written in the R statistical language (http://www.rproject. org) used for analysis and the GLIMMIX procedure from SAS (2007) and DARwin5.5 (Perrier & Jacquemoud- Collet, 2006) were also used. The R statistical package was used for hierarchal cluster analysis based on Euclidean distance. Dendrogram grouping the 30 accessions based on RAPD marker,was constructed based on complete linkage method using Jaccard’s distance as well as Dice

coefficient on employing bootstraps using DarWin6.0 package. For morphological analysis three characters were considered including Plant height, Plant spread and Tuber yield.

RESULTS AND DISCUSSION

The amplicons obtained after the RAPD analysis of 30 accessions using the selected primers were initially resolved in two per cent agarose gel. Among the eight RAPD primers studied in white yam, OPW -16 was found be the best that produced ten polymorphic bands followed by OPG-02 and OPG-13 with six polymorphic bands. All the primers evaluated resulted in 100% polymorphism. The Hobs values ranged between 0.7377 (OPW1) to 0.8937 (OPW-16). OPG-13 and OPG-16 recorded high observed heterozygosity (>0.8). The polymorphism information content ranged from 0.6997 (OPW-1) to 0.8838 (OPW-16). All the primers recorded high PIC value of >0.6.

The genetic characteristics of the RAPD primers evaluated are given in fig 1. More clusters were resulted in dendrogram formed based on RAPD markers as in figure 2. At 0.8 dissimilarity level, it formed nine clusters. Cluster 1, 2 and 3 has only one accession *viz.* V23, V18 and V22. The fourth cluster has four genotypes and was again subdivided in to two sub clusters. The fifth cluster has three genotypes and also subdivided into two sub clusters 5a and 5b. The two genotypes included in 5b i.e. V19 and V24 were found to be similar. Sixth cluster has one genotype V20. The seventh cluster is the largest one with 16 genotypes which consist of mostly dwarf genotypes (11) and is divided into several sub clusters. The eighth cluster has one genotype (V15) and ninth cluster has two genotypes (V27 and V29). It also showed the high divergence of the genotype V20 (DR17) and the clustering pattern indicates the grouping of the dwarf genotypes together. Morphological study showed similar clustering pattern as compared with genetic analysis. DR 17 variety gives high tuber yield as compared to other genotypes which also showed variance on marker analysis.

On literature search different molecular markers were studied to identify genetic diversity in yams. The bulked segregate analysis approach was successfully used for the identification of RAPD markers linked to YMV and anthracnose resistance genes. Two RAPD markers, OPW18850 and OPX15850, closely linked in coupling phase with the dominant YMV- resistance locus *Ymv-1*, were identified (Mignouna et al., 2002c). Similarly, two RAPD markers, OPI171700 and OPE6950, closely linked in coupling phase with the anthracnose resistance locus, *Dcg-1*, were identified (Mignouna et al., 2002d). These RAPD markers will be easier to use for indirect selection once converted into co- dominant PCR-based sequence characterized amplified regions (SCARs).

Genetic linkage maps based on AFLP markers have been constructed for *Dioscorea tokoro*, a wild yam (Terauchi & Kahl, 1999) and for the cultivated species, *D. rotundata* (Mignouna et al., 2002a) and *D. alata* (Mignouna et al., 2002b). Mignouna et al., (2005) studied the efficiency of different molecular markers in yams. The efficiency of RAPD, AFLP and SSR markers for the assessment of genetic relationships, and for cultivar identification and discrimination among 45 West and Central African white yam cultivars belonging to 22 morphotypes/cultivar groups was investigated The higher PIC and Hobs values obtained in the present study for the RAPD markers indicate the high variability of the population studied. It also indicated the usefulness of the RAPD markers identified, they can be used in elucidating genetic diversity among yams in future. From the study identified a high yielding (8 kg/plant), highly divergent white yam genotype named DR17 and that could be used for the genetic improvement of white yam in future. Morphological characterization supported the data analyzed by the molecular marker based analysis.

Table 1: Thirty accessions of tall, dwarf and semi dwarf varieties of white yam (*D. rotundata)* used in the study

SL. No.	Accessions	Plant height (cm)	Plant Spread (cm)	Tuber Yield (Kg/Plant)
1.	SREE DHANYA	62.50	71.2	3.5
2.	DRD 1110	83.00	84.0	4.5
3.	DRD 495	50.80	66.04	4.0
4.	DRD 1118	74.32	68.21	3 .0
5.	DRD 1835	68.28	75.20	3 .0
6.	DRD 1060	66.04	67.31	2 .0
7.	DRD 1033	53.34	81.28	3.0
8.	DRD 920	76.20	63.54	3.0
9.	DRD 1142	45.72	83.82	4.0
10.	DRD 949	55.88	63.51	4..0
11.	DRD 1157	83.82	78.74	3.0
12.	DRD 1068	58.42	68.54	2.0
13.	DRD 835	55.88	96.52	3.0
14.	SD 15	71.12	76.2	4.0
15.	DR 2	76.21	71.12	3.0
16.	DR 29	60.96	96.52	6.0
17.	DRS 47	420.0	120.3	5.0
18.	DRS 45	386..0	115.0	3.8
19.	DR 130	510.8	114.0	5.0
20.	DR 17	420.0	120.0	8.0
21.	DR 73	375.8	115.0	5.0
22.	DRS 652	425.0	125.0	4.2
23.	DR VIOLET	378.5	136.0	4.5
24.	DRS 36	560.3	112.0	5.5
25.	DRS 1155	490.0	120.0	4.8
26.	DRH 1047	525.5	116.0	6.2
27.	DRH 657	480.0	132.0	5.5
28.	DRH 657 A	466.0	125.0	4.0
29.	SREEPRIYA	515.0	120.0	4.5
30.	SREESUBHRA	525.0	135.0	5.2

Table 2: List of RAPD primers used in the present study

Sl. No	Primer name
1.	OPW-1
2.	OPG-02
3.	OPG-03
4.	OPG-05
5.	OPG-08
6.	OPG-13
7.	OPW-16
8.	OPW-18

Table 3: Protocol of RAPD PCR used in the present study

Cycling step	Temperature (ºC)	Time	Ramping rate	Cycles
Enzyme activation	94	5 min		1
Denaturation	94	1 min	~ 2 ºC/S	35
Annealing	36	1 min		
Extension	72	2 min		1
Final Extension	72	1 min		1
Hold	4	Infinite		1

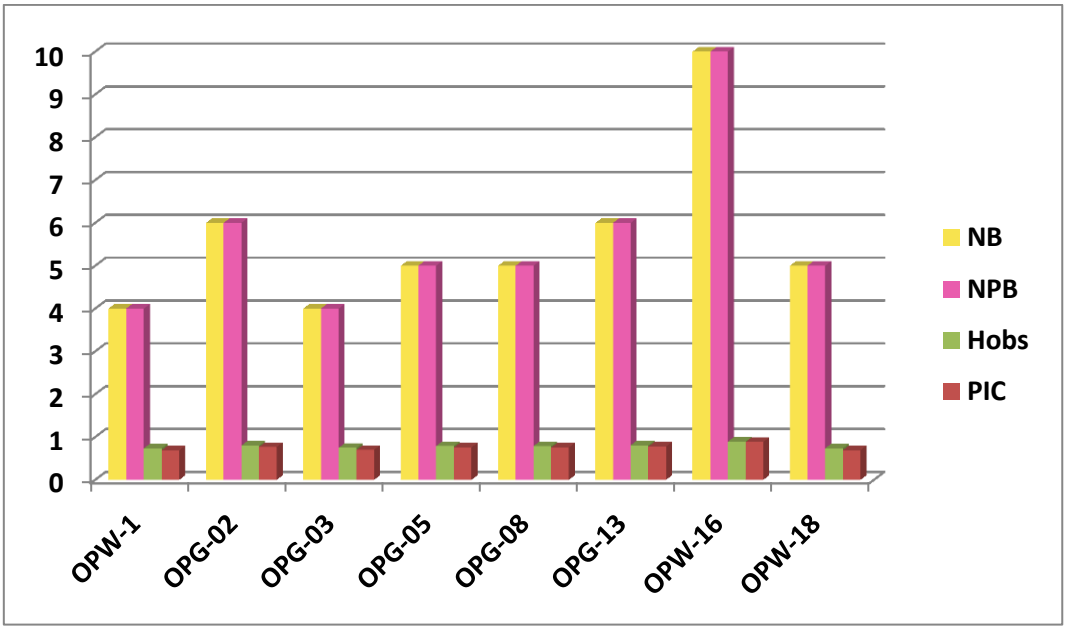

Fig. 1: Analysis of genetic characteristics of RAPD markers white yam

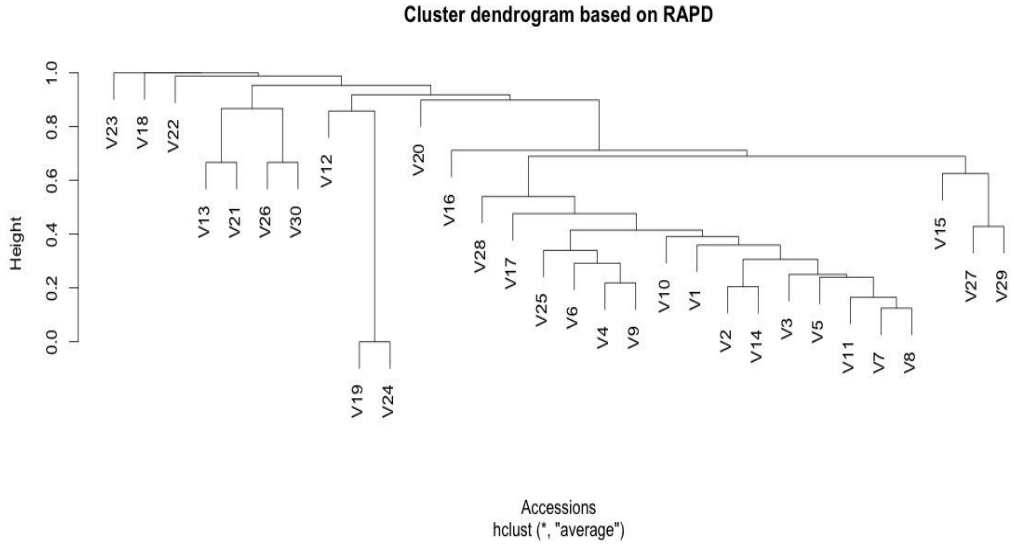

Fig. 2: Dendrogram formed based on RAPD markers analysis of white yam genotypes

CONCLUSION

RAPD markers indicate the high variability of the population studied by analyzing the PIC and Hobs value. Dendrogram obtained on Cluster analysis classified genotypes in to different clusters on par with their morphological traits. RAPD markers are excellent tool that can be used in elucidating genetic diversity among yams. The present study helped to identify a high yielding (8 kg/plant), highly divergent white yam genotype, DR17 that could be used for the genetic improvement of white yam in future.

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