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Research Article

Simple Sequence Repeat (SSR) Marker Based Genetic Diversity Analysis in White Yam (*Dioscorea rotundata* Poir.)

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ABSTRACT

Diversity analysis of the genotypes helps to develop accurate breeding program along with the identification of elite genotypes from the cultivars. ICAR-Central Tuber Crops Research Institute, Kerala holds a field gene bank of 1100 Dioscorea accessions including 158 white yam genotypes. Present study focused on identifying genetic variability in 30 white yam genotypes (Dwarf, Semi dwarf and Tall) using 14 SSR primers. From 14 SSR primers, only 10 primers were selected based on their polymorphism and repeatability. Among the 10 SSR primers studied in white yam, YM15 recorded the maximum number of polymorphic alleles of five followed by Dab2D06 and YM26 with four alleles. The percentage of polymorphism ranged from 50 to 100. The observed heterozygosity values ranged from 0.4339 (Dab2C05) to 0.775 (YM 15). The polymorphism information content ranged from 0.3398 (Dab2C05) to 0.7388 (Dab2D06). YM15 also recorded high PIC value of 0.7377. Dendrogram based on SSR marker showed that white yam genotypes were grouped in to three clusters. It also shows the high divergence of the genotype V20 (DR17) and the clustering pattern indicates the grouping of the dwarf genotypes together along with a semi dwarf variety.

Keywords: *Yams, Genetic variability, SSR markers, Dendrogram, Dwarf genotype and Heterozygosity.*

INTRODUCTION

Genetic diversity among different crop plants plays a vital role in development of agricultural resources. Most of the genetic variability among crop plants including tuber crops are not yet discovered. This implies that thousands of valuable allelic variations of traits of economic significance remain unutilized (Hossain et al., 2007). Thus, identification of genotypes and its variability study is crucial. 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.

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Hence in yams, being an essential tuber crop research studies based on molecular markers were vital for the elucidation of its genetic diversity. The most important yam on worldwide basis is *Dioscorea rotundata* Poir (White yam or white guinea yam) grown on larger area compared to other yam species. The *Dioscorea rotundata-cayenensis* complex accounts for the ninety five percent of yam production worldwide (Waitt, 1961).

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). Development of new biotechnological techniques provides increased support to evaluate genetic variation in both phenotypic and genotypic levels. For developing new varieties in plants, by conventional method it takes lot of time, almost 25 years. It is time consuming due to lot of factors including long growth cycle. Due to the advancement of biotechnology, we can go for less, time consuming procedures like Marker assisted selection (MAS), that is identification of DNA markers linked to key traits, helpful for easy gene transfer. This method can overcome the shortcomings in traditional breeding, thus increasing the accuracy and efficiency of selection. It is especially valuable for traits with low to moderate heritability, which are difficult to be improved by traditional selection. Marker Assisted Selection (MAS) has proven to be useful in speeding up genetic improvement in agronomic plant species. Molecular markers, linkage maps, and QTL mapped on the whole genome are essential for MAS.

In many important agronomic plant species, a large number of DNA markers and linkage maps have been developed. Many QTLs for important traits have been mapped on the whole genomes, setting up the basis for rapid genetic improvement through MAS (Lee et al., 2015). Several DNA- based marker systems are available for genetic finger printing of plants like RAPD, ISSR, SSR etc. The development of genetic maps allows the use of marker-assisted selection (MAS).

Molecular markers are powerful tools in the assessment of genetic variation, in the elucidation of genetic relationships within and among species and have demonstrated the potential to detect genetic diversity and to aid in the management of plant genetic resources Virk et al., (2000). Simple sequence repeat is an important tool for genetic variation identification of germplasm. SSR marker have some merits such a quickness, simplicity, rich polymorphism and stability, thus being widely applied in genetic diversity analysis, molecular map construction and gene mapping Ma et al. (2011), construction of fingerprints Xiao et al., (2006) genetic purity test Peng et al. (2003) analysis of germplasm diversity Jin et al. (2010) utilization of heterosis, especially in identification of species with closer genetic relationship.

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. 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 & 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 1 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 10 different SSR primers. The optimum amplifying conditions were standardized as shown in table 2. 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 and 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 SSR marker, was constructed based on complete linkage method using Jaccard's distance as well as Dice coefficient on employing bootstraps using DarWin6.0 package.

RESULTS AND DISCUSSION

The amplicons obtained after the SSR analysis of 30 accessions using the selected primers were initially resolved in two per cent agarose gel. Among the ten SSR markers studied, the number of alleles per marker ranged from 2 to 5, while the number of polymorphic alleles ranged from 1 to 5. YM15 recorded the maximum number of polymorphic alleles (5) followed by Dab2D06 and YM26 with 4 alleles. The percentage of polymorphism ranged from 50 (Dab2C05, Dab2E07). The observed heterozygosity values ranged from 0.4339 (Dab2C05) to 0.775 (YM 15). The SSR makers *viz.* Dab2D06, YM15 and YM 26 recorded high Hobs values (>0.7).

The polymorphism information content ranged from 0.3398 (Dab2C05) to 0.7388 (Dab2D06). YM15 also recorded high PIC value of 0.7377. On average the SSR markers recorded Hobs value of 0.5715 and polymorphism information content of 0.4866 were given in fig 1. Norman et al. (2012) reported high polymorphic information content (PIC) of 0.8719 in loci Dab2C05. However, in the present study, Dab2C05 recorded lowest PIC value among the markers studied. This might be due to the difference in structure of the population studied. In the present study, the makers resulted in allele numbers of 2, 3, 4, 5 and 7 per locus, suggesting the presence of different ploidy levels of diploids, triploids, tetraploids, pentaploids in white yam studied. The results are in agreement with the research findings in tropical yams. 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. Siqueira et al. (2014) studied water yam (*Dioscorea alata* L.) diversity pattern with 12 SSR primers. Narina et al. (2011) had done work on generation and analysis of expressed sequence tags (ESTs) for marker development in yam (*Dioscorea alata* L.). Dendrogram based on SSR markers showed that white yam genotypes were grouped in to three clusters were given in fig 2. The cluster 1 with two genotypes (V29 and V30) is the smallest one. Cluster 2 comprise of 16 accessions of which V2 and V9 were found to be closely related. The dwarf genotypes (12) clustered together in Cluster 3. The semi dwarf genotype SD15 was grouped together with other dwarf genotypes. The present study could 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.

Table 1: SSR primers used in the present study

Sl. No	Primer name
1.	Dab2C05
2.	Dab2D06
3.	Dab2E07
4.	Da1A01
5.	Dpr3F04
6.	YM5
7.	YM15
8.	YM26
9.	MT 13
10.	MT 10

Table 2: Protocol of SSR PCR used in the present study

Cycling step	Temperature (ºC)	Time	Ramping rate	Cycles
Enzyme activation	94	5 min		1
Denaturation	94	30 s		35
Annealing	51	1 min	~ 2 ºC/S	
Extension	72	1 min		1
Final Extension	72	8 min		1
Hold	4	Infinite		1

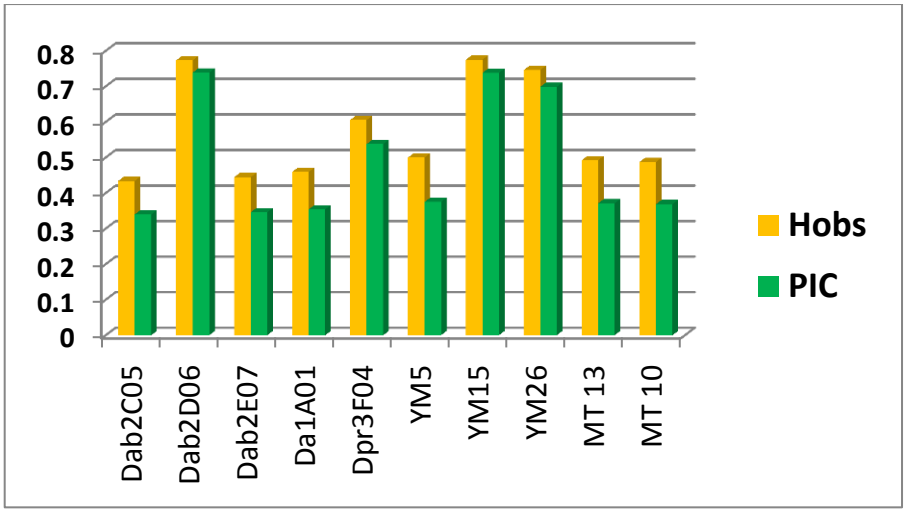

Fig. 1: The Hobs and PIC values of SSR markers evaluated in white yam

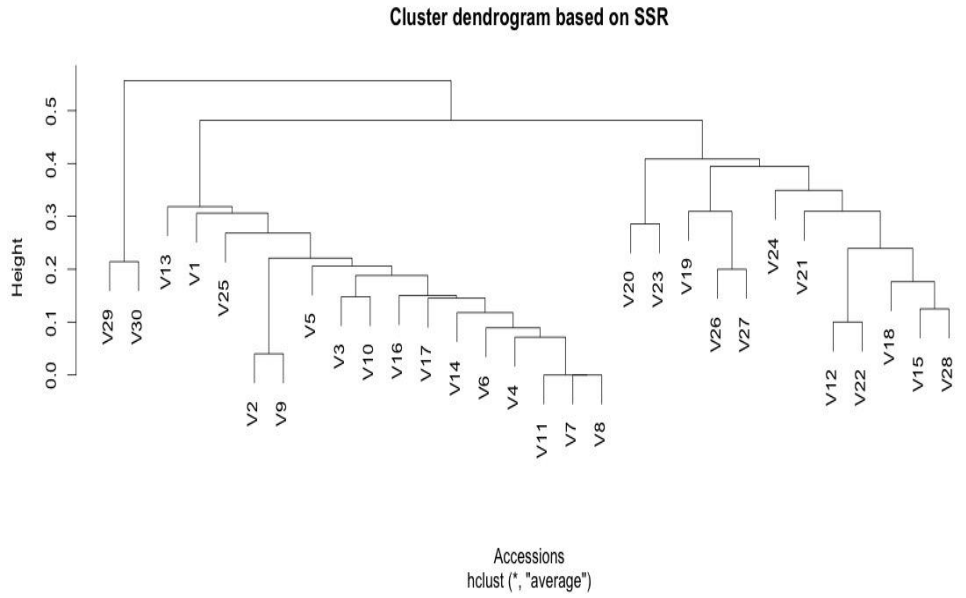

Fig. 2: Cluster dendrogram based on SSR marker in white yam

CONCLUSION

SSR 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 three different clusters on par with their morphological traits. SSR markers are excellent tool that can be used in elucidating genetic diversity among yams. The present study could 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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