Evaluation of genetic diversity of Chironji (*Buchnania Lanzan*) in Chhattisgarh

Chaitali Niratker[1] & D.Sailaja[2]
[1]Yash Biotech, Raipur, Chhattisgarh.
[2]Gokaraju Rangaraju Institute of Engineering & Technology, Hyderabad, Telngana.

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ABSTRACT *Buchnania lanzan (Chironji) Chironji is a life support and medicinally important tropical tree species and a significant source of livelihood for local tribal, it holds good proportions of antioxidants, essential nutrients and bioactive molecules. In present study, genetic variability of wild collection of Chironji (Buchnania lanzan) was evaluated using RAPDs. Total 18 wild genotypes were collected from all the three agrocliamtic zones of Chhattisgarh. Genomic DNA, extracted from leaf material using a modified CTAB method, was subjected to PCR with designed 20 decamer. Fifty-five polymorphic loci were identified, with mean of 9.16 ± 3.31 bands per primer and 100% polymorphism. The data was analyzed with GGT 2.0 software for diversity analysis. Among all eighteen genotypes Bastar Genotype (Km) showed 90.0% variability compared to other genotypes. The Chironji genotypes were found to have considerable genetic divergence. This is the very first report on genetic diversity analysis of Chironji using RAPD markers.*

Keywords: *Chironji, Genetic diversity, Agrocliamtic zones, RAPD.*

Introduction:

India possesses a distinct identity, not only because of its geography, history and culture but also because of the great diversity of its natural ecosystems. India is one of the mega diversity tropical countries that comprise rich vegetation and biodiversity and ranked seventh among 17 mega diversity countries of the world. Chhattisgarh the Herbal state and Genome state of India. Lying in the Vindhyan hill regions and Deccan plateau between 17-23.70N latitude and 80.40-83.380E longitude in Central Eastern India. It is one such hotspot which is home to many wild, unutilized, underutilized and Minor Forest Produce, otherwise known as Non-Timber Forest Produce, which includes most of the medicinal plants comprise broad-spectrum essential nutrients, vitamins and secondary metabolites they can be considered for cultivation, consumption and utilization and the Collection of Non Timber Forest Product (NTFP), is a way of life with tribal and rural communities in an around the forest.

About 31.08% population of Chhattisgarh is tribal population and is dependent on the Non Timber Forest Produce (NTFP). *Buchnania Lazan* (Chironji) is one of the major non-wood forest products of Chhattisgarh. Chironji is a multipurpose tree, provides food, fodder, timber, medicines and gives monitory reward to tribal community of India as a mean of living by collection of Chironji fruits and selling it in the local markets. Chironji plantation is natural in the state and therefore very diverse in different places across the state." (Posey, 1999 and Shiva, 1995). Chironji is a life support and medicinally important tropical tree species and a significant source of livelihood for local tribal, it holds good proportions of antioxidants, essential nutrients and bioactive molecules. (Das and Agrawal, 1991).

The seeds are the major source of regeneration of Chironji so highly heterozygous plant, which is cross pollinated, contributes genetic variation. Still there are no evidences for identified variety of this important minor fruit, Chironji throughout the country especially in the state of Chhattisgarh. From last few years, Due to lack of suitable and efficient harvesting techniques and unawareness in tribes regarding its own unique properties, such as nutritional and therapeutic values may leads increased in deforestation has resulted in extinction of this important forest produce. Though these crops grow in wild and have been neglected, conservation, cultivation and promotion of these are very crucial for nutritional, medicinal and economic purposes.

Considering the need and scope of reorienting on-going crop improvement strategies in Chironji at Chhattisgarh. Studies with molecular markers have made significant contributions to our understanding of genetic diversity; when compared with other types of markers, they present a greater number of polymorphic loci, which allows distinguishing between accessions that may have similar morphological and agronomical traits (GONÇALVES *et aL.,* 2008).Various molecular techniques have been successfully applied in determining the genotypic profiles of individuals and/or populations of numerous wild and cultivated plant species, and in identifying traits of interest within germplasm banks. In this context, RAPD has been employed extensively not only for the determination of genetic variability within plant taxonomic groups

but also as an auxiliary tool in breeding programs and in obtaining genetic maps (Williams *et aL.,* 1990; Dunemann *et aL.,* 1994; Paillard *et aL.,* 1996). Additionally, the RAPD technique is fundamental in developing specific sequence-characterized amplified region (SCAR) markers for use in the assisted selection of crops (Marieschi *et aL.,* 2010; Wu *et aL.,* 2010). Keeping in view the above facts, the research was planned to analyze genetic diversity among the collected wild genotypes of chironji in all the three agro climatic zone of Chhattisgarh.

Method and Material:

The study Area:

The study was conducted in three major Agro climatic zones of Chhattisgarh. Total of 18 wild Chironji genotypes with two replicates of six trees with maximally 15km distance from each other were referred to as a population Malvolti *et al*., (1994), from Chhattisgarh plains zone, Bastar plateau Zone and Northern Hill Zones were marked for detailed study.

Plant Materials:

Around fifty seeds of each genotype were sown in seedling trays in a greenhouse under a temperature of 25℃. When plants were two weeks old, two leaves per plant were collected from a total of 18 individuals per nine locations Table 1. The leaves were then kept at -80°C until DNA extraction.

DNA Isolation and PCR amplification:

Fresh young leaves were surface sterilized with absolute alcohol followed by washing with autoclaved distilled water. DNA isolation was done by modified CTAB protocol given by Doyle and Doyle (1990). The RAPD reaction was performed according to the method developed by McClelland et al., 1995.

A set of 20 random decamer primers were synthesized using vast literature survey (Trupti Asolkar *et al*.,2011,Ayada Tagizad *et al*.,(2010) to be used as a single primer for the amplification of RAPD fragments. Primers were screened for the presence of consistent and distinct bands. The reaction was carried out in 25µl volume in a tube. Each reaction tube contained 3 µl of template DNA (50ng), 2 µl of PCR Buffer with 15 mM $MgCl_2$ (10X), 1µl of dNTP's (10mM) , 3 U/µl of Taq DNA polymerase (Sigma aldrich, USA). The amplification was performed in a DNA thermal cycler (Biorad) using the following conditions: complete denaturation (94°C for 5 min), 10 cycles of amplification (94℃ for 45 sec, 35°C for 1 min and 72°C for 1.5 min) followed by 30 cycles of amplification (94°C for 45 sec, 38°C for 1 min and 72°C for 1 min) and the final elongation step (72°C for 5min). (0.5 µg / ml) PCR products were resolved in 1.5 % agarose gel in 1X TBE buffer with 1kb molecular weight marker. The PCR amplified products were visualized and photographed under a U.V transilluminator. Electrophoretic profile was analyzed for polymorphism based on the presence and absence of DNA bands on agarose gel. The sizes of DNA fragments were estimated by comparison with standard ladder (100 bp, Sigma aldrich, USA).

Data analysis:

Comparison of genotype based on presence and absence of fragment produced by RAPD markers amplification.'1' was designated for presence of fragments and '0' was designated for absence of fragments. The cluster analysis was performed for molecular data using **UPGMA tree method and constructed by GGT2 software.**

RESULTS & DISCUSSION:

The data obtained in the present study regarding RAPD Molecular Marker studies in 18 Chironji Genotypes. The present study reveals that RAPD markers are good choice for assessing the genetic diversity and relationship in Chironji genotype. The information obtained could be of practical use for mapping the mulberry genome as well as for classical breeding. The study also provides a closer basis for Int.J.Curr.Microbiol.App.Sci (2016) 5(1): 778-787 782. For accuracy of the results, the high quality and purity of genomic DNA free from secondary metabolites was isolated from these genotypes by modified CTAB method for RAPD reaction, it was necessary to standardize the variables used for the successful amplification of PCR. Table 1. Shows paasport data of selected samples.

Sr.No	Agrocliamtic Zone	Collector Number	Biological Status	Location	Longitude	Latitude
1	Bastar Plateau	Bl104	Wild	Jagadalpur	19.07	82.03
2		Bl105	Wild	Kondagaon	19.60	81.67
3		Bl106	Wild	Keshkal	20.08	81.67
4.	Northen Hills	Bl107	Wild	Sarguja	23.11	83.20

5		Bl108	Wild	Koriya	23.51	82.50
6		Bl109	Wild	Jashpur	22.8	83.38
7	Raipur Plains	Bl101	Wild	Raipur	21.30	82.0
8		Bl102	Wild	Bilaspur	21.30	82.0
9		Bl103	Wild	Raigarh	21.87	83.38

Table 1: Passport data of the locations of selected genotype.

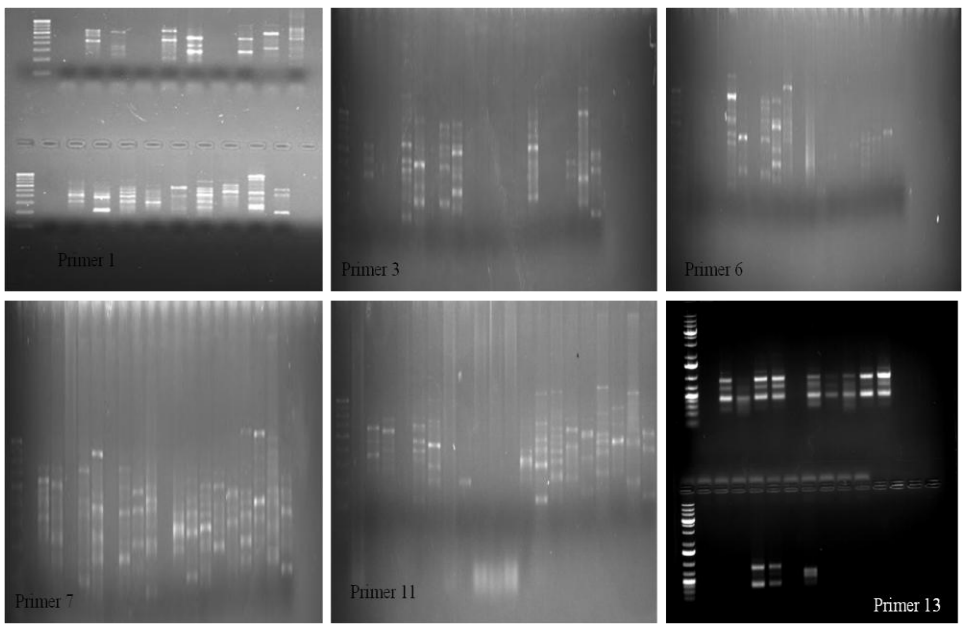

Fig 1: Plates showing RAPD marker amplification

	Bb	Ms	Gb	Kt	Rt	Pr	Md	Bh	Sp	Pp	Mg	Ck	Ap	Vp	Pb	Kg	Km	Mp
Bb																		
Ms	0.26																	
Gb	0.44	0.41																
Kt	0.41	0.59	0.41															
Rt	0.33	0.15	0.48	0.52														
Pr	0.19	0.44	0.26	0.30	0.52													
Md	0.30	0.48	0.44	0.33	0.56	0.26												
Bh	0.22	0.11	0.37	0.48	0.11	0.41	0.44											
Sp	0.22	0.26	0.44	0.41	0.26	0.41	0.30	0.15										
Pp	0.19	0.27	0.46	0.46	0.27	0.31	0.27	0.15	0.08									
Mg	0.37	0.41	0.59	0.33	0.41	0.56	0.37	0.30	0.15	0.23								
Ck	0.37	0.41	0.22	0.48	0.48	0.33	0.37	0.37	0.30	0.31	0.37							
Ap	0.19	0.30	0.48	0.44	0.37	0.37	0.33	0.26	0.19	0.23	0.26	0.33						
Vp	0.19	0.37	0.41	0.44	0.37	0.37	0.33	0.26	0.11	0.15	0.26	0.33	0.15					
Pb	0.26	0.44	0.41	0.30	0.52	0.22	0.26	0.41	0.26	0.31	0.33	0.33	0.15	0.22				
Kg	0.41	0.37	0.56	0.52	0.44	0.52	0.41	0.33	0.26	0.35	0.33	0.48	0.30	0.37	0.30			
Km	0.52	0.48	0.44	0.56	0.48	0.48	0.44	0.44	0.37	0.46	0.44	0.30	0.41	0.41	0.33	0.41		
Mp	0.33	0.44	0.33	0.37	0.37	0.37	0.48	0.33	0.33	0.35	0.41	0.33	0.37	0.37	0.52	0.52	0.56	

Similarity matrix, based on Jaccard Coefficient genetic distance, of 18 Genotypes of Chironji based on variation in RAPD markers.

Fig 2: similarity matrix for 18 chironji genotypes

According to similarity Matrix based on Jaccard coefficient and genetic distance of all the 18 genotypes of Chironji, least similarity ratio is about 0.11 and divergence ration is maximum from0.56-0.59, showed in fig 2.

Availability and assessment of genetic variation are central to the improvement of any crop species. Out of 20 decamer primers which were used for diversity analysis of Chironji, only 6 primers generated polymorphic bands and rest have produced only monomorphic bands. They showed 104 reproducible

bands among 18 genotypes. The number of bands obtained per primer ranged from 6-9 with exception in some of the lanes where no amplification and bands formation has not taken place was observed. A total of 90% polymorphism was observed. The complete amplification details are presented in the table 2. The size of the obtained amplicons ranged from 250-800 kb. Data analysis for Divergence and association among all genotypes studies by Unweighted Pair- group method with arithmetic mean (UPGMA) cluster analysis with GGT 2.0 software is presented in figure 3.

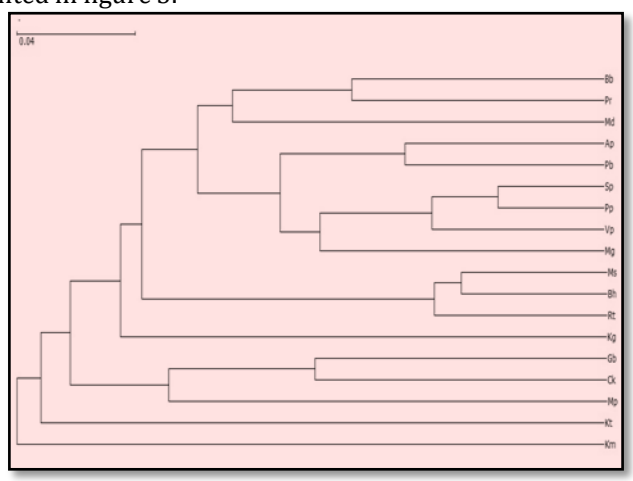

Fig 2: Dendrogram showing genetic variation among the 18 genotypes of Chironji

The dendogram was constructed with the similarity coefficient of RAPD data. It was based on RAPD markers reveled two distinct clusters, first consisting of two genotype one belonging to Raipur planes and other from Bastar the similarity coefficient between two was (0.19). The distributions of populations in different clusters indicates that even though the samples were selected from same geographical areas, the genetic drift, natural and unidirectional selection pressure and human intervention by transferring specimen from one generation to another may be the cause of high level of genetic diversity among different populations (Kiambi et al., 2005, Ward et al., 2005). The present studies and similar studies on molecular analysis for genetic diversity of Mango (Neetu Thakur et al., 2017). (Majid *et al*., 2008, Pruthvish *et al*., 2016 and Souza, 2011) in Mango , found RAPD-PCR markers best for analysis of genetic Divergence studies. The data present here reflects the Utility of RAPD in the analysis of genetic divergence of Chironji. The presence of high genetic Diversity indicates that the population has plenty of scopes for evolution to occur. On the basis of present Investigation, it could be concluded that there is tremendous genomic variability and scope of development of this multifarious species of Chironji *genotypes* collected from high biodiversity state like Chhattisgarh.

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