RELATIONSHIP AMONG SIX SPECIES OF *CURCUMA* ASSESSED BY RAPD MARKERS

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ABSTRACT

Effective management and preservation of the plant species is depending on accurate assessment of genetic diversity. RAPD markers were used to assess the genetic diversity among six species of Curcuma (wild and cultivated) from different regions of Vidarbha. PCR amplification of genomic DNA produced 432 bands from 69 primers; maximum polymorphism percentage is 100%. Total number of bands range from 1 to 23. The data is used to calculate GS value among six species of Curcuma and to construct dendrogram. On the basis of the genetic variation observed within the six species of Curcuma, our analysis confirms the occurrence of two major clusters. Dendrogram depicts close relations between cultivated species C. longa and wild Curcuma species except C. neilgherrensis which forms a separate cluster.

Keywords : Genetic Diversity, *Curcuma,* UPGMA, hybridization, polyploidization

Introduction

Curcuma L., the third-largest genus of the family Zingiberaceae, containing many taxa of economic, medicinal, ornamental and cultural importance. The importance of *Curcuma* in health and nutrition has greatly been recognized since the discovery of antioxidant properties of curcuminoids found in the dried rhizome of turmeric (Srinivasan, 1953; Lechtenberg *et al.,* 2004). Currently India is the largest producer, exporter, and consumer in the world supplying 94% of the world's demand.

Despite the economic potential of the genus, its phylogeny and taxonomy is poorly understood, mainly due to polyploidization (2*x*–15*x*) and hybridization (Škorničková *et al.,* 2007 and Záveská *et al.,* 2011) which leads to genetic and morphological variations among species and made species boundaries blurred. Identifying characters used by various authors to define *Curcuma* (e.g. bract colour, inflorescence position, flower colour) are not unique for all members of the genus, thus the genus was in need of revision. Thus, it is necessary to adopt various methods for identification of different *Curcuma* species and evaluate their genetic relationship.

Molecular markers are the important tools for evaluation of genetic diversity within and between species and population. Among different markers random amplified polymorphic DNA (RAPD) technique (Williams *et al.,* 1990) is a popular tool for investigation of genetic variation and it has been used to detect diversity in some *Curcuma* species (Chen *et al.,* 1999; Xiao *et al.,* 2000; Syamkumar and Sasikumar, 2006). RAPD is used to analyse population genetic consequences to displays a large number of polymorphic (di-allelic) loci that can be obtained relatively easily even for species for which no prior genetic information is available. Sasikumar *et al.* (2004) developed a PCR based method for

detection of adulteration of marketed *C. longa* powder with that of a wild species, *C. zedoaria*. Similar study was conducted by Joshi *et al.*(2010), Hikmat *et al.*(2012), Sigrist *et al.* (2011) and Senan *et al.*(2013).

Material and Methods

Sample collection:

A leaf material from the representative of six *Curcuma* species was collected as a source of genomic DNA from the different areas of the Vidarbha region. CTAB DNA extraction protocols with some modifications as described by Doyle *et. al.* in 1990 was used to extract the genomic DNA from all these leaf samples. DNA obtained from these samples was purified with RNAase treatment (Sambrook *et al*., 1989). The quality of extracted DNA was analysed by means of agarose gel electrophoresis (0.8%), followed by ethidium bromide staining. Measures of DNA purity was determined by OD_{260}:OD_{280} ratios.

RAPD assay:

Good quality of genomic DNA isolated from the plants leaf was used as a template to amplify the RAPD markers as per the procedure reported by Williams *et al*. in 1990. Random decamer primers of OPA, OPB, OPC and OPD series supplied by 'Operon Technologies' USA with good resolving power were used for amplification of DNA.

A mastermix of reagents for the required number of reactions was prepared (0.4 mM of dNTPs mixture, 1x *Taq* buffer, 0.2 µM of decanucleotide primer and 1 unit of *Taq* DNA polymerase) and aliquots were dispended into PCR tubes followed by addition of template DNA in each reaction tube to make final volume of 25 µl, and one tube without template DNA used as control. PCR reactions were carried out by using 80 primers in an Eppendorf Master Cycler. The PCR conditions for the successful amplification of template was optimised on the Initial denaturation at 94°C for 6 min followed by denaturation at 94 °C for 45 min, annealing at 36°C for 1 min with total 38 cycles, Extension at 72° C for 1 min with Final extension 72° C for 10 min.

Electrophoresis of PCR products

The amplified products were separated on 2 % agarose gel using 1X TAE buffer stained with ethidium bromide. The profile was visualized under UV transilluminator; the gel was photographed and documented using gel documentation (Alpha Innotech, AlphaImager HP) system. The documented RAPD profiles were carefully examined for polymorphism and number of bands produced by each primer was scored manually and tabulated.

Data scoring and analysis

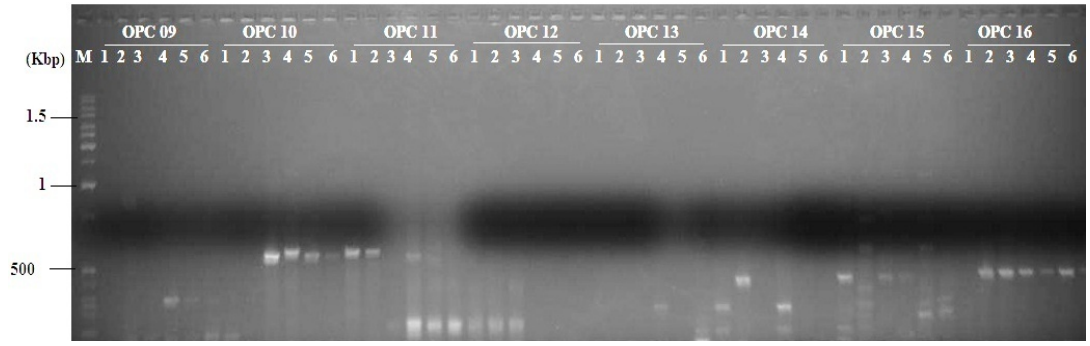
Amplification profiles of 6 species were compared with each other and bands of DNA fragments scored manually in binary data format in the form of (1) or (0) depending on the presence or absence of a particular band, respectively. The data was analysed using Numerical Taxonomy System of Multivariate Statistical Programme (NTSYS) software package (Rohlf, 2002). The dendrogram was constructed using Unweighted Pair Group Method of Arithmetic Averages (UPGMA) as per Sneath and Sokal (1973).

Results And Discussion

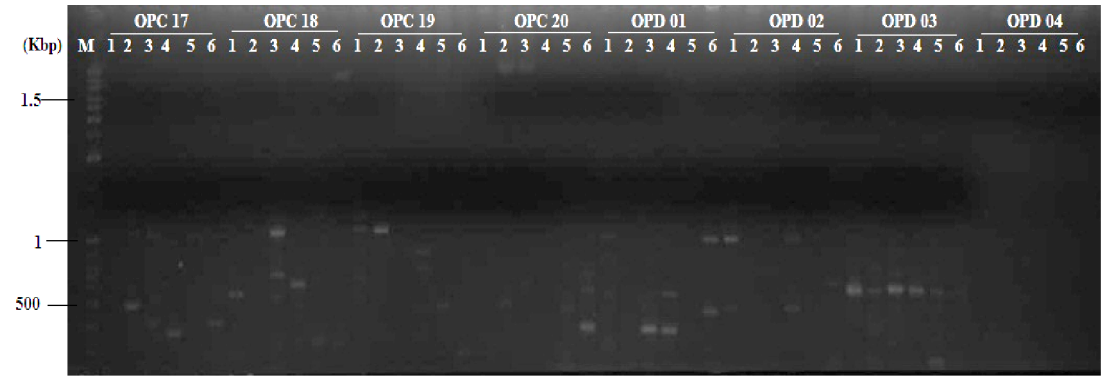
Genetic diversity was evaluated by 80 random decamer primers, out of which 69 primers resulted in reproducible and scorable bands. A total of 432 bands were scored from PCR amplification of genomic

DNA from six *Curcuma* species, all the bands were polymorphic. The percentage of polymorphism was a maximum of 100%, obtained with 69 primer.

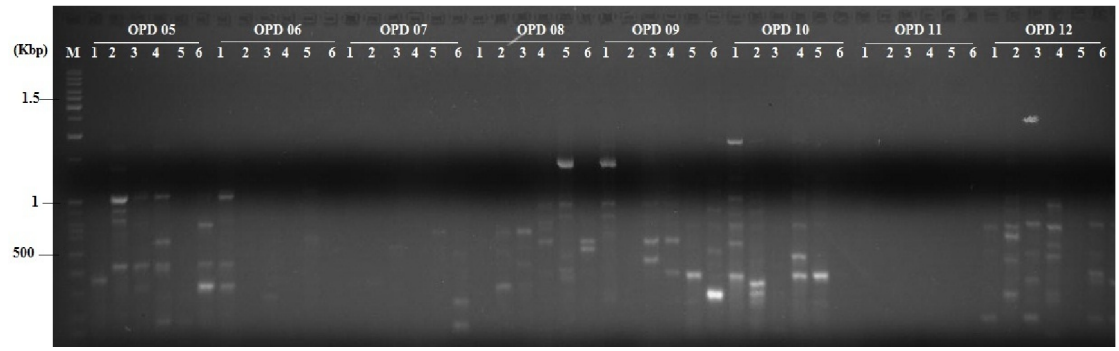
Total number of bands varied in different species of *Curcuma* with a maximum of 23 bands for primer OPD 19 and a minimum of 1 band. Fragment size ranges from 100 bp to 1500 bp. Similar banding pattern was observed in five species of *Curcuma* i.e. *C. zedoaria*, *C. pseudomontana*, *C. longa*, *C. inodora* and *C. decipiens* with OPC 16 having fragment size of 450 bp, which could differentiate them from *C. neilgherrensis*. Whereas, primer OPD 12 produced similar bands for *C. neilgherrensis, C. zedoaria*, *C. pseudomontana*, *C. longa* and *C. decipiens* at 900 bp to differentiate *C. inodora*.OPD 03 produced bands for *C. neilgherensis*, *C. zedoaria*, *C. pseudomontana*, *C. longa* and *C. inodora* with fragment size 600 bp to differentiate *C. decipiens***(Fig: E to H)**



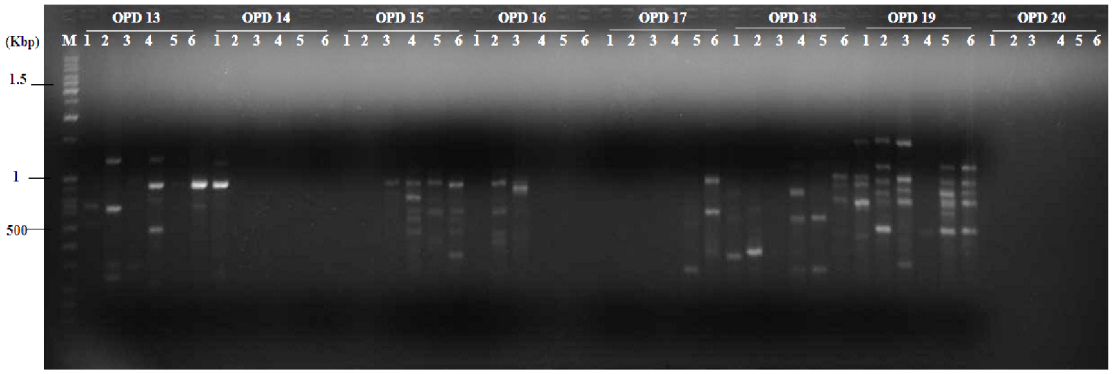
E. OPC 09 – OPC 16



F. Primer OPC 17 – OPD 04



G. Primer OPD 05 – OPD 12



H. Primer OPD 13 – OPD 20

Fig E – H : RAPD polymorphic profile for six *Curcuma* species from Vidarbha. M-DNA ladder; 1.*C. neilgherrensis*; 2.*C. zedoaria*; 3. *C. pseudomontana*; 4. *C. longa*; 5.*C. inodora*; 6.*C. decipiens.*

All the 432 bands were used to calculate the GS value among six species of *Curcuma*. The variations from 0.55 to 0.66 of GS value indicated great genetic differentiation between the species. As seen from the dendrogram at the GS value of 0.55 the six species were divided into two main clusters. The cluster I is further divided into three groups, the first group included single species *C. pseudomontana* (CP) second group consists of *C. inodora* (CI) and *C. decipiens* (CD) while *C. longa* (CL) and *C. zedoaria*(CZ) form group third with maximum similarity. The cluster II composed of only one species *C. neilgherrensis* (CN).

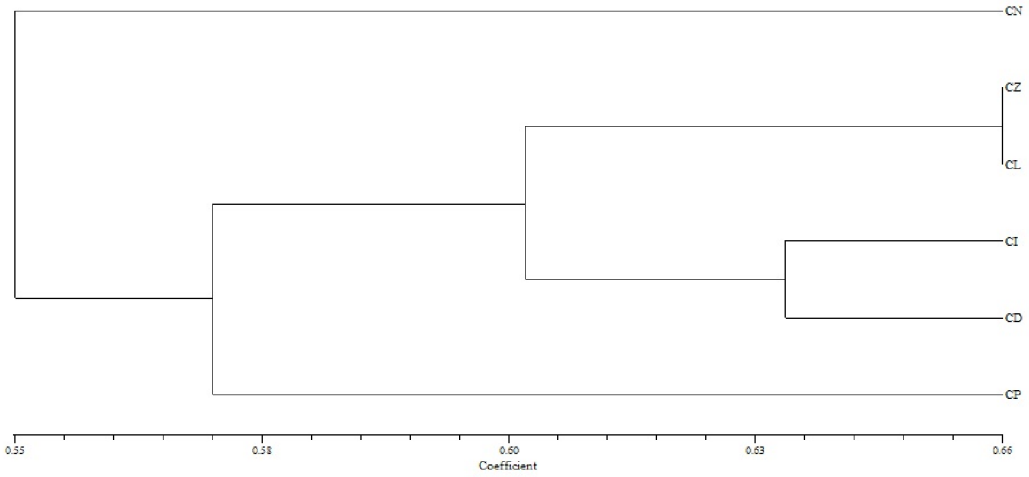


Fig : Dendrogram representing the relationship (genetic diversity) among six *Curcuma* species.

The first group of cluster I is represented by a lone species *C. pseudomontana* which showed 0.575 GS with *C. inodora* and *C. decipiens* of group II as well as *C. zedoaria* and *C. longa* of the group III. The relationship of *C. pseudomontana* with *C. inodora* and *C. decipiens* of group II may be due to certain common characters in these three species such as presence of both lateral and central spike, and absence of sessile tubers. These reports are in close correspondence with earlier report of Santapau (1952) that the two species *C.*

pseudomontana and *C, inodora* are endemic to the Western Ghats and restricted to several districts of Maharashtra. These two hexaploid species are similar morphologically but they differ in flower colour i.e. bright yellow vs. reddish flesh like colour. These species may have evolved from common ancestor and hybridization may have take place among them (Zaveska *et al.*, 2011).

In group II of cluster one two species are included i.e. *C. decipiens* which form a group with *C. inodora* at approximately 0.635 GS which revealed that the genetic relationship of these species were very close and the morphological differences occurred in them might be caused by environment in which they were growing. *C. inodora* differs from *C. decipiens* in flowers being longer than bracts, 2-3 flowered cincinni and labellum purple with deep yellow band.

Similarly from the dendrogram it revealed that the two species falling in group third of cluster I viz. *C. zedoaria* and *C. longa* showed maximum similarities irrespective of their morphological differences, they shared a common node at 0.66 GS. Both species are triploid (2n=3) (Ramchandran, 1961; Joseph *et al*., 1999), bears sessile tubers and reproduce vegetatively. In cluster I group second had 0.602 GS with group third, as these two groups are differed in rhizome, spike and floral characters.

C. neilgherrensis, distinct from all other species, maintained a distinct identity being a lone species in cluster II and showed 0.55 GS with other species. *C. neilgherrensis* is characterized by absence of sessile tubers, presence of stipitate tubers, presence of lateral and central inflorescence, light yellow coloured flowers. *C. zedoaria* and *C. longa* showed maximum similarity between them (0.66 GS) whereas maximum dissimilarity belonged to *C. neilgherrensis* showing to other *Curcuma* species.

RAPD markers were applied to detect genetic relationships and diversity among six species of *Curcuma* in China by Zou *et al.* (2011), dendrogram from their study revealed that genetic relationships of cultivated and wild populations of *Curcuma* species were not related to their geographical distribution and there was no separation between them. Such type of significant genetic variations were also reported in other species at cultivar level (Colombo *et al*., 1998; Das *et al*., 1998; Huang *et al*., 2003).

Aknowledgement

The present work is dedicated to my guide Late Dr. Shrikant B. Jain. I am also thankful to Dr. Waghmare, Senior Scientist, CICR Nagpur for providing laboratory facilities during the work.

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