

Original Research Article

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## Morphological and Molecular Diversity of *Cordia dichotoma* Frost F. Populations from Nanded District in Maharashtra, India

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### ABSTRACT

The Present study reveals the morphological and molecular variations in different accessions of *Cordia dichotoma* Frost F. a medicinally important plant collected from different locations of the Nanded district in Maharashtra, India. Morphological characterization of 10 year-old trees for 10 traits indicated wide variations among the accessions tested. Higher number of fruits per cyme and higher pulp: stone ratio was found in accession NCD7 collected from Pawdewadi. Overall, NCD7 was found to be a superior germplasm line for most of the horticulturally useful traits among the accessions tested as it had higher percent of fruit set, pulp:stone ratio and fruit weight. High significant positive correlation was obtained between leaf, fruit characters and pulp:stone ratio. Molecular analysis was carried out using RAPD markers. Out of the five primers screened, a total of 45 scorable polymorphic markers were generated. Average polymorphism resolved by these markers among these accessions was 67.86 %. Genetic diversity revealed by Jaccard's co-efficient was between 0.48–0.86. Study shows the existence of high genetic diversity among these accessions.

### Keywords

*Cordia dichotoma*,  
Evolution,  
Genetic  
diversity,  
Morphology,  
RAPD.

### Article Info

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### Introduction

*Cordia dochotoma* Forst is a medium sized tree with a short, usually crooked trunk (90-100 cm girth) and bearing globose fruits. It grows in India, Srilanka and other warmer countries (Kuppast, 2006; Rastogi, 1993; Kapoor, 1994) It has alternate corinaceous, obtuse, ovate, elliptic, or orbicular leaves, flowers white, polygamous, in axillary and terminal,

cymose panicles. The inflorescence carries numerous white flowers. Fruits are rounded to ovoid drupe, about 1.0 to 1.9cm in length, 0.9-1.74cm in width, arranged in clusters. (Cappers, 2006; Kislev, 2008) Their white-yellow color turns blackish when dry. The fruit pulp is very mucilaginous, is edible and has a sweetish flavor. It can be consumed fresh, dry and pickled. The fruits are cooling, astringent emollient, expectorant,

anthelmintic, purgative and diuretic (Yoganarsimhan, 2000). Fruit pulp has antimicrobial activity (Mulani *et al.*, 2013) and pharmacological properties such as analgesic, anti-inflammatory and the hepatoprotective activity. (Wassel *et al.*, 1987, Rapisarda *et al.*, 1992) A literature review shows that though *Cordia dichotoma* has been reported to have a number of beneficial effects on diverse experimental parameters. Its possible role in experimental cerebral ischemia has not yet been explored. (Samadia, 2007)

## **Materials and Methods**

### **Plant Material**

Leaves, fruits, and seeds were collected from different selected germplasms from farmers fields of 10 different villages of four talukas in Nanded district of Maharashtra. (Table 1) in June-July. The germplasms were named as NCd1 to NCd10.

### **Morphological Characters and Data Analysis**

Ten accessions of 10 years old tree were selected for morphological characterization. Eleven morphological characters (Table 2) including leaf, immature and mature fruit, pulp and stone (seed) were recorded (Vashishtha *et al.*, 1985; Nagar and Fageria, 2006; Samadia, 2007).

### **Genomic DNA Extraction**

0.1g of leaf tissue was weighed and incubated with extraction buffer (2% Cetyl trimethyl ammonium bromide (CTAB), 0.5%  $\beta$ -mercaptoethanol etc.) to lyse the cell wall and membrane. The lysate was then treated with Phenol:Chloroform:Iso-amyl alcohol mixture to eliminate complex organic intracellular contents. Finally, DNA was precipitated using chilled 70% Alcohol and eluted in 40 $\mu$ l of TE-RNase, which was

visualized using Agarose gel Electrophoresis, stained with Ethidium bromide. (Doyle, 1990)

### **Random Amplified Polymorphic DNA using Polymerase Chain Reaction (RAPD)**

The genomic DNA obtained was amplified using RAPD primers of the BE series: OPBE-01, 02, 03, 08, 09. The PCR cocktail containing 10pM of primers, 25 $\mu$ M of each dNTP, 1.5mM MgCl<sub>2</sub> and 1IU of *Taq* polymerase, along with ~100ng of template, was found to be optimal for the required reaction. The amplification was carried out using 30 cycles of a thermal profile containing 94°C for 30s, 32°C for 1 minute to allow the annealing of the primers, and 72°C for 5min, followed by final extension for 2min 50s. The resultant amplicons were resolved using 1% agarose gel electrophoresis and visualized with ethidium bromide staining. (Lamboy, 1994; Lamboy, 1994; Garcia-Vallvé, 1999).

### **Data Analysis**

Based on the primary data (presence or absence of bands), pair wise genetic distance between samples were calculated using UPGMA. All the amplified bands were counted manually along with their size. The presence of bands were scored as '1' and absence as '0'. A pair-wise matrix of genetic distances between genotypes was determined using the Jaccard's similarity coefficient and a phylogenetic tree was constructed using NTSYSpc-2.02e version 2.0. (Nagy, *et al.*, 2012)

## **Results and Discussion**

### **Morphological Variation**

Wide variation was observed among the ten accessions of *Cordia dichotoma* germplasm

on various morphological parameters of leaf, fruits and stone(seed) (Table 2)The leaf shape of most of the accessions is round or ovate as they have length and width almost equal except for NCd3collected from Barad and and NCd7 collected from Pawdewadi which has oval leaves. Number of fruit set per cyme was greatly reduced and average percent fruit set to number of flowers per cyme was only 12.24 In most of the germplasms fruits are oval or round shape. While in germplasm NCd5 which was collected from S.R.T.M. University campus Vishnupuri the fruits are oval elongated shape (Table.2).

Fruit weight at pickle stage and ripe stage was found maximum in the accession NCd7 collected from Pawdewadi and the fruit weight was 3.65 g and 4.95 g respectively followed by NCd8 collected from Limbgaon (4.54 g). Average pulp:stone ratio per 10 fruits was 4.20 and the maximum being 6.34 (NCd9) collected from patnoor followed by 6.13 (NCd8) collected from Limbgaon (Table 2).

### **Molecular Diversity**

Among 45 bands amplified by 5 primers, 37 were polymorphic, and average number of bands per primer was 9.0 and average number of polymorphic bands per primer was 7.4,. The band size observed was 180-2000bp.The average polymorphism was 67.86.(Table.3) Maximum number of bands given by OPBE-08(10), followed by OPBE-01(9).Percent polymorphism varied between 57.77 and 81.25%.

Maximum polymorphism (81.25%) (Table.2.) by OPBE-09. Jaccard's similarity co-efficient among these accessions was 0.48–0.86. (Table.3)Highest similarity was found between accessions of NCd2 collected from Mudhkhed with NCd6, collected from

Bhokar NCd8 collected from Limbgaon. Phylogenetic analysis showed that there were three groups found among the accessions (Fig. 1). group I had highest similarity co-efficient of 0.86 and the lowest was group (0.62).

Phylogenetic tree constructed based on ten accessions of *Cordia dichotoma* germplasm analysed by RAPD primers by UPGMA method and Jaccard's similarity coefficient using NTSYSpc-2.02e version 2.0.1.5 software. Vertical distance is arbitrary and horizontal distance indicates genetic variations among different germplasms.

The *Cordia dichotoma* is one of the underutilized fruit trees, escaping from orchard cultivation. To bring under systematic cultivation the superior genotype of *Cordia dichotoma* needs to be identified, which requires an ideal genotype having oval-round shaped fruit, green to dark green unripe mature stage and big size (9–12 g), high pulp: stone ratio with high yielding and longer harvesting period (Samadia, 2005).

This study has identified a superior genotype, NCd7, which fulfils most of the criteria and in addition it has higher fruit set per cyme. The yield potential of this accession has to be tested in multiple locations to allow this accession to be released as a variety for commercial cultivation. This genotype can be utilized to harness the potential usage in liquor and processed food industries.

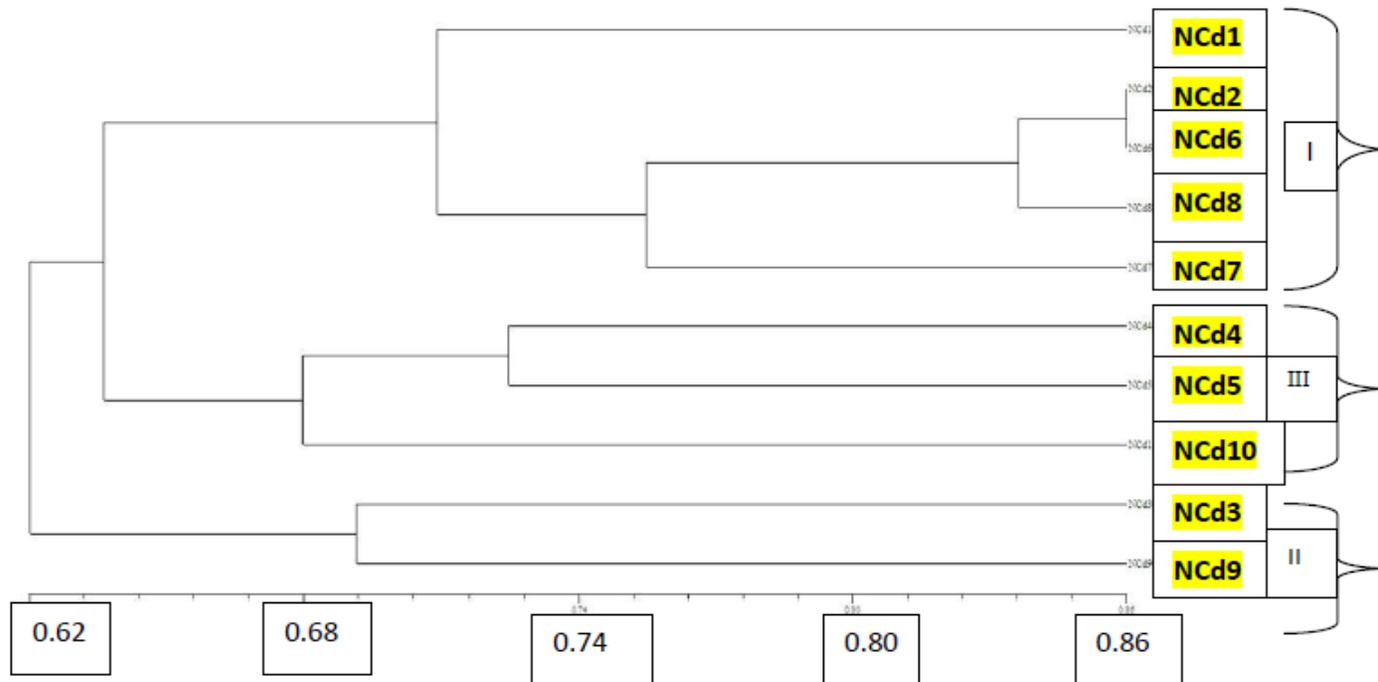
Antioxidant activity of methanolic extract of fruit of *C.dichotoma* populations collected from different geographical regions in Nanded district were done by Nandedkar and Mulani (2013)and observed that there is variation in phytochemical constituents and antioxidant activity among the different populations.

**Table.1** Details of different genotypes of *Cordia dichotoma* populations and collection sites in Nanded district of Maharashtra

Sr. No.	Accession No.	Village/district	Collection site	Latitude 'N'	Longitude 'S'
1	NCd1	Ardhapur/Nanded	Farmers' field	19.28	77.37
2	NCd2	Mudkhed/Nanded	Farmers' field	19.14	77.49
3	NCd3	Barad/Nanded	Farmers' field	19.18	77.42
4	NCd4	Tamsa/Nanded	Forest area	17.40	78.48
5	NCd5	Vishnupuri/Nanded	University campus	19.10	77.28
6	NCd6	Bhokar/Nanded	Farmers' field	19.22	77.67
7	NCd7	Pawadiwadi/Nanded	Farmers' field	19.15	77.33
8	NCd8	Limbgaon/Nanded	Farmers' field	19.17	77.20
9	NCd9	Patnoor/Nanded	Farmers' field	17.22	78.32
10	NCd10	Vishnupuri /Nanded	University campus	19.10	77.28

NCd--- N= Nanded, Cd=*Cordia dichotoma*

**Fig.1** Phylogenetic Tree of selected germplasm of *Cordia dichotoma* populations from Nanded district in Maharashtra



**Table.2** Morphological variation of selected germplasm of *Cordia dichotoma* from Nanded district of Maharashtra

AC. No.	Leaf length (cm)	leaf width (cm)	Fruit length (cm)	Fruit width (cm)	Mature fruit weight (gm)	Ripen fruit weight (gm)	No. of Fruits /cyme	Seed length (cm)	Seed width (cm)	Seed weight (gm)	Pulp-Stone ratio (/10gm)
NCd1	6.79 ± 0.10	5.40 ± 0.10	1.07 ± 0.02	1.02 ± 0.01	1.35 ± 0.02	2.02 ± 0.02	7.40 ± 1.10	0.84 ± 0.02	0.81 ± 0.01	0.54 ± 0.02	3.26 ± 0.02
NCd2	7.42 ± 0.09	5.92 ± 0.18	1.13 ± 0.01	0.93 ± 0.02	1.98 ± 0.02	2.44 ± 0.03	7.05 ± 1.19	0.69 ± 0.04	0.66 ± 0.04	0.25 ± 0.00	2.91 ± 0.20
NCd3	10.42 ± 0.14	7.90 ± 0.18	1.24 ± 0.01	0.96 ± 0.02	1.19 ± 0.02	3.02 ± 0.03	6.10 ± 0.91	1.00 ± 0.02	0.71 ± 0.11	0.97 ± 0.01	2.54 ± 0.02
NCd4	8.11 ± 0.21	6.45 ± 0.20	1.06 ± 0.02	0.81 ± 0.02	0.98 ± 0.02	2.43 ± 0.05	8.00 ± 1.30	0.82 ± 0.01	0.80 ± 0.02	1.04 ± 0.01	2.12 ± 0.01
NCd5	6.30 ± 0.18	5.39 ± 0.15	1.83 ± 0.01	1.05 ± 0.02	2.34 ± 0.02	3.67 ± 0.02	6.20 ± 0.95	1.29 ± 0.01	0.87 ± 0.06	1.21 ± 0.01	4.12 ± 0.01
NCd6	8.56 ± 0.12	7.29 ± 0.15	1.22 ± 0.02	0.63 ± 0.01	1.57 ± 0.02	3.98 ± 0.02	8.05 ± 0.89	0.97 ± 0.01	0.39 ± 0.09	0.72 ± 0.01	2.85 ± 0.02
NCd7	10.37 ± 0.16	9.39 ± 0.17	1.94 ± 0.02	1.56 ± 0.02	3.66 ± 0.03	4.95 ± 0.03	10.30 ± 1.22	1.55 ± 0.02	1.53 ± 0.01	0.52 ± 0.01	6.71 ± 0.02
NCd8	6.49 ± 0.18	5.58 ± 0.19	1.34 ± 0.01	1.24 ± 0.02	3.12 ± 0.01	4.65 ± 0.02	8.00 ± 1.17	1.00 ± 0.01	0.99 ± 0.02	0.43 ± 0.01	6.14 ± 0.02
NCd9	4.51 ± 0.17	3.34 ± 0.18	1.04 ± 0.02	0.91 ± 0.02	1.47 ± 0.02	3.44 ± 0.02	10.10 ± 1.29	0.82 ± 0.01	0.80 ± 0.02	0.35 ± 0.01	6.35 ± 0.01
NCd10	3.10 ± 0.20	2.94 ± 0.16	1.83 ± 0.02	1.75 ± 0.03	2.78 ± 0.02	4.01 ± 0.02	6.10 ± 0.91	1.23 ± 0.01	1.20 ± 0.02	0.92 ± 0.01	5.10 ± 0.01
Mean ± SD	7.20 ± 2.22	5.96 ± 1.87	1.37 ± 0.34	1.09 ± 0.33	2.04 ± 0.86	3.46 ± 0.93	7.73 ± 1.80	1.02 ± 0.25	0.87 ± 0.30	0.70 ± 0.31	4.21 ± 1.65

Ac.No—Accession No.

LL- Leaf length (cm)

LW-leaf width (cm)

FL-Fruit length (cm)

FW-Fruit width(cm)

M.Fr.w-Mature fruit weight (gm)

NOF-Number of Fruits per cyme

SL-Seed length(cm)

SW-Seed width(cm)

SWt-Seed weight (gm)

Pulp-Stone ratio./10gm)

R.Fr.W.Ripen fruit weight(gm)

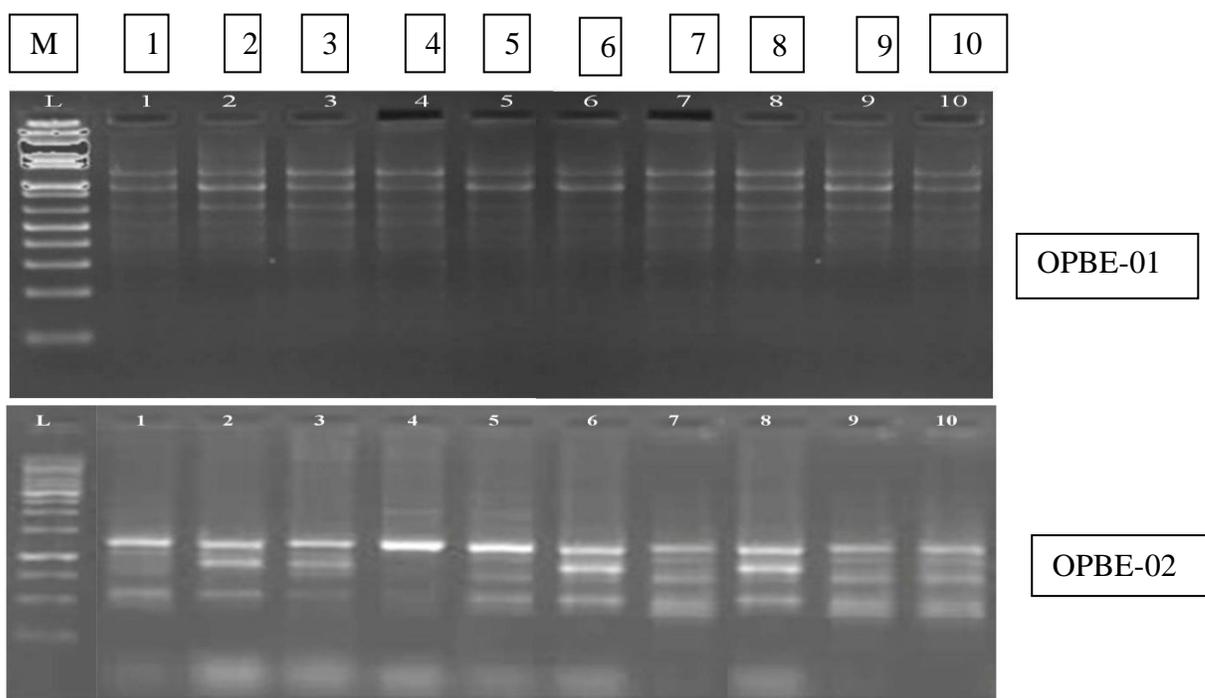
Cluster-I	NCd1,NCd2,NCd6,NCd7,NCd8
Cluster-III	NCd4,NCd5,NCd10
Cluster-II	NCd3,NCd9

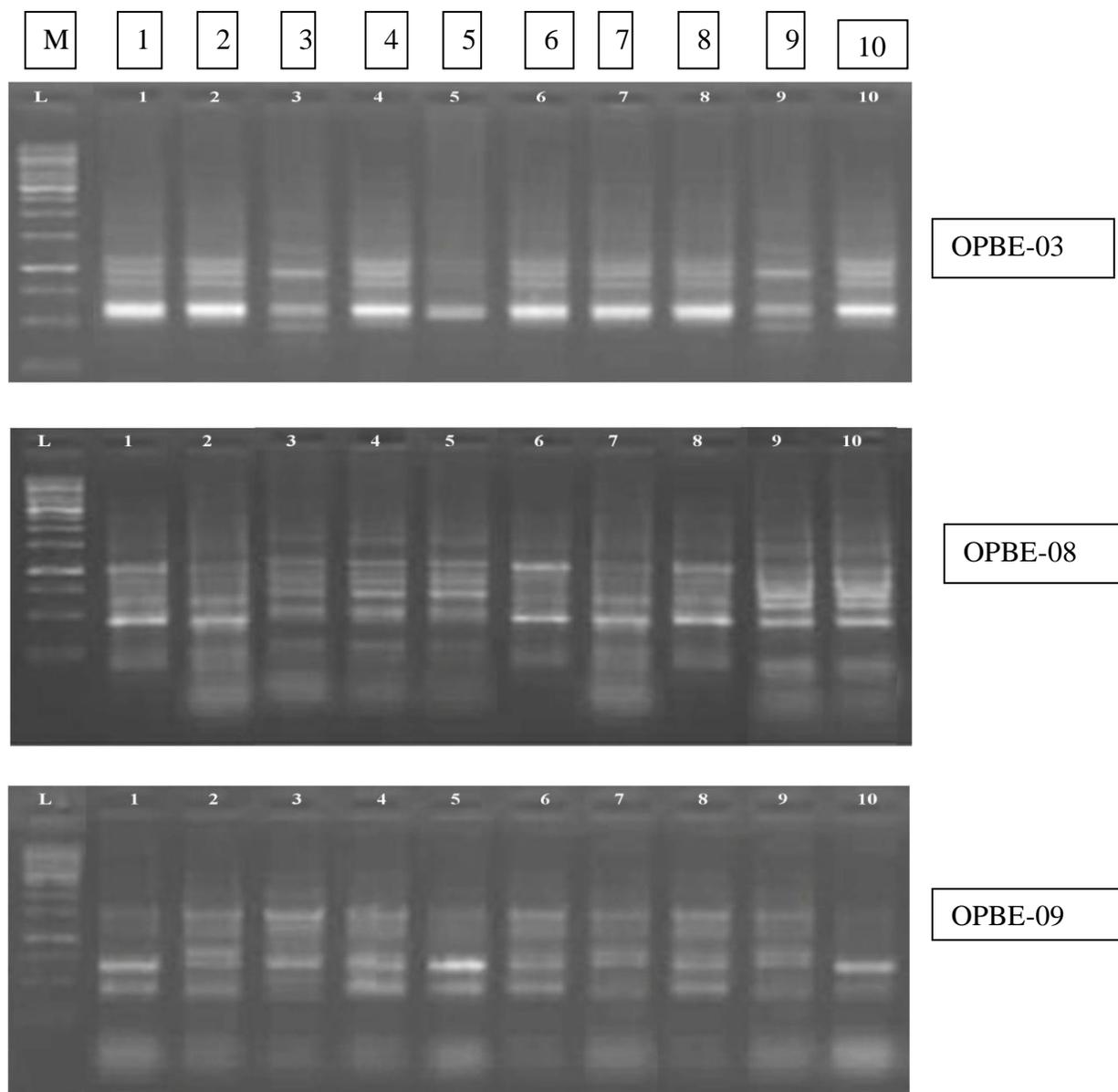
**Table.3** Details of primers selected for RAPD

Sr.No.	Primers	Sequences	Total no. bands	Percent polymorphism
1	OPBE-01	CACTCCTGGT	09	66.66
2	OPBE-02	ACGCCTGTAG	09	57.77
3	OPBE-03	TGGACTCGGT	09	66.66
4	OPBE-08	GGGAAGCGTC	10	67.00
5	OPBE09	CCCCTTTCC	08	<b>81.25</b>

**Table.4** Jaccard's similarity co-efficient of selected germplasm of *Cordia dichotoma* populations from Nanded district in Maharashtra

	NCd1	NCd2	NCd3	NCd4	NCd5	NCd6	NCd7	NCd8	NCd9	NCd10
NCd1	<b>0.000</b>									
NCd2	<b>0.687</b>	<b>0.000</b>								
NCd3	<b>0.588</b>	<b>0.705</b>	<b>0.00</b>							
NCd4	<b>0.636</b>	<b>0.705</b>	<b>0.65</b>	<b>0.00</b>						
NCd5	<b>0.709</b>	<b>0.676</b>	<b>0.62</b>	<b>0.72</b>	<b>0.000</b>					
NCd6	<b>0.666</b>	<b>0.862</b>	<b>0.68</b>	<b>0.68</b>	<b>0.656</b>	<b>0.00</b>				
NCd7	<b>0.718</b>	<b>0.735</b>	<b>0.63</b>	<b>0.68</b>	<b>0.657</b>	<b>0.71</b>	<b>0.00</b>			
NCd8	<b>0.774</b>	<b>0.843</b>	<b>0.68</b>	<b>0.68</b>	<b>0.611</b>	<b>0.833</b>	<b>0.81</b>	<b>0.00</b>		
NCd9	<b>0.500</b>	<b>0.648</b>	<b>0.69</b>	<b>0.60</b>	<b>0.621</b>	<b>0.540</b>	<b>0.67</b>	<b>0.58</b>	<b>0.00</b>	
NCd10	<b>0.625</b>	<b>0.600</b>	<b>0.51</b>	<b>0.64</b>	<b>0.718</b>	<b>0.485</b>	<b>0.62</b>	<b>0.54</b>	<b>0.68</b>	<b>0.000</b>





**Fig.2** Gel image of (Polymorphism) the selected Ten accessions of *Cordia dichotoma* germplasm revealed by RAPD primers. Lane L—1 kb DNA ladder; lane 1—NCd1(Ardhapur) : lane 2—NCd2(Mudhkhed); lane 3—NCd3(Barad); lane 4—NCd4(Tamsa); lane 5—NCd5(Vishnupuri); lane 6—NCd6(Bhokar); lane 7—NCd7(Pawdewadi); lane 8—NCd8(Limbgaon); lane 9—NCd9(Patnoor); lane 10—NCd10(Vishnupuri).

Genetic diversity in *C.dichotoma* from arid region of Rajasthan was investigated by Sivlingam (2012) and reported that there is much variation in fruits morphological character as well as molecular observations. Importance of Randomly Amplified

Polymorphic DNA (RAPD) marker in plants have been increased which gives important clues about ecological and morphological variations in the plant species. (Kumar, 2011)

Even molecular analysis in other plant species were done by many workers in different plants like in *Rauvolfia tetraphylla* by Mahesh *et al.*, (2008); in *Jatropha curcus* by Subramanyam (2010); in sugarcane by Kanwar *et al.*, (2009); in *Azadirachta indica* by Dhillon *et al.*, (2007); in *Bunium persicum* populations by Hashemi *et al.*, (2010); in *Artemisia judaica* by Al-Rawashdeh (2011) and RAPD markers are used in authentication of medicinal plants like *Cuscuta reflexa* (Khan *et al.*, 2010) and this technique is also used in separation of biovers of *Ralstonia solaneaceae* genotypes in *Capsicum annum* and *Solanum tuberosum* from Nanded district of Maharashtra.

In conclusion, the genotypes of *Cordia dichotoma* has morphological variation. The molecular variation indicated by phylogenetic tree. The ten genotypes has divided in three clusters .Cluster-I includes genotypes NCd1,NCd2,NCd6,NCd7,NCd8, Cluster-II includes NCd3 and NCd9,Cluster-III has NCd4,NCd5,NCd10.

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