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Research article

Testing of genetic homogeneity of elite eucalyptus clones using DNA marker

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Abstract: Under 'Hariyali Prasar Yojna' Chhattisgarh State Forest Department has procured and planted elite eucalyptus clones on large scale in different forest circles/divisions of the state during the monsoon of 2015–16 and 2016–17. To assess the genetic homogeneity of the supplied clones, genetic fidelity testing of the procured clones was carried out using ISSR marker. The monomorphic pattern of ISSR profiles observed for the ramets of the respective clones in comparison with their mother plant confirmed the genetic purity. This also demonstrates the application of molecular marker technology for quality control in social forestry plantation.

Keywords: Eucalyptus - Clonal fidelity - ISSR - Molecular marker.

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INTRODUCTION

Due to short rotation age, faster growth, multipurpose utility, fire hardiness, good coppicing vigour, capability to over top weeds, browse resistance, wider adaptability and ease of mass propagation, eucalyptus became one of the world's leading industrial plantation species (Palanna 2017, Sumathi & Yasodha 2014). Tipu Sultan, the ruler of Mysore introduced eucalyptus in India and planted in his palace garden on Nandi hills near Bangalore around 1790 (ShyamSunder 1984). Afterwards, it was introduced in Niilgiri hills, Tamil Nadu, in 1843 and by 1856; regular plantations of *E. globulus* were raised for firewood (Wilson 1973). Wider adaptability of the eucalyptus to degraded and wastelands made it one of the prime species for social forestry plantations. Presently, India is one of the largest eucalypt growing countries in the tropics with an estimated area of over 20 million hectares (Varghese *et al.* 2009). Thus eucalyptus has helped to reduce pressure on natural forests by meeting requirements of people and industries.

In order to meet the increasing demand for wood and wood industry mass multiplication is being carried out clonally. Cloning of mature trees is generally preferred over seedling because it fixes genetic gain, interim or permanent within breeding program, whereas, through seedlings it is often not possible to determine whether these seedlings have the desired qualities as reflected by its mother plant (Nanda *et al.* 2004, Venkataramanan *et al.* 2015). Vegetative propagation technique is the handiest way to multiply eucalyptus quickly in industrial forestry.

To realise the advantages of clonal-propagation technique, it is very much necessary to maintain the genetic purity of the regenerants. Several strategies have been adopted for genetic purity testing. In past, morphological descriptions, physiological traits, cytological studies, isozymes (Gupta & Varshney 1999, Devarumath *et al.* 2002, Agnihotri *et al.* 2009, Singh *et al.* 2012a, 2012b) and many other techniques have been deployed to assess the genetic purity of clonally mass propagated plants. However, expression of the morphological and physiological traits may changes in response to the prevalent environmental conditions (Singh *et al.* 2013). Due to cytological aberrations, there are always possibility of phenotypically homogeneous looking plants may behave differently during flowering/fruiting and later stages, making conclusion about genetic purity invalid.

Both hybridization and PCR based DNA markers have become useful tools for confirming the genetic uniformity of clonally propagated plants and screening out the off-types. The inherent characteristics of DNA markers such as abundantness and insensitivity to environmental conditions makes them more useful than morphological and physiological traits in establishing the identity of particular tree/clone or testing the genetic

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purity or tracing its genetic relationship. Earlier, random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), sequence characterized amplified region (SCAR), DNA amplification fingerprinting (DAF), Arbitrarily primed polymerase chain reaction (AP-PCR) have been successfully employed for fidelity testing in various plant species (Rani & Raina 2000, Archana *et al.* 2013, Singh *et al*. 2013, Mohammad *et al.* 2016). Among these, ISSR is a very simple, quick, cost-effective, highly discriminative and reliable method that combines most of the advantages of SSRs and AFLP with the universality of RAPD (Reddy *et al.* 2002). They are more useful and reproducible than isozymes, RAPD and less cumbersome and cost effective for routine application than RFLP (Fang *et al.* 1997, Reddampalli *et al.* 2007).

Table 1. Details of the eucalyptus clones procured & planted under 'Hariyali Prasar Yojna' by Chhattisgarh State Forest Department.

Sl. No.	Name of forest circles / divisions selected for Eucalyptus plantation	Clonal detail
1	Durg	Clone no. 03, Clone no. 07, Clone no. 316 & Clone no. 413
2	Bastar	Clone no. 06, Clone no. 07 & Clone no. 316
3	Bilaspur	Clone no. 07, Clone no. 316 & Clone no. 413
4	Kanker	Clone no. 07, Clone no. 288, Clone no. 316, Clone no. 413 & Clone no. 526
5	Raipur	Clone no. 07, Clone no. 288, Clone no. 316, Clone no. 413
6	Manendragarh	Clone no. 413
7	Koriya	Clone no. 413
8	Surguja	Clone no. 413
9	Gariyaband	Clone no. 316, Clone no. 413
10	Surajpur	Clone no. 413

In the year 2015–16 and 2016–17, Chhattisgarh State Forest Department under the 'Hariyali Prasar Yojna' procured more than two cores of plantlets of different eucalyptus clones for plantation in different forest divisions/circles of the state (Table 1). We have assessed the clonal purity of the plantlets forwarded by the CG forest department to the institute. Therefore, present study was carried out with a definite aim to ascertain the genetic homogeneity of the plantlets of different elite eucalyptus clones procured and planted under the scheme using inter simple sequence repeats (ISSR) markers.

MATERIALS AND METHODS

Plant material and DNA extraction

Figure 1. Samples of the eucalyptus clone number 7, 413 and 316 received from Balodabazor forest division (Raipur Circle), Chhattisgarh for clonal fideliy testing.

Three to five random samples (ramets) of the each clonal lot along with mother leaf samples were provided by the Chhattisgarh State Forest Department (Fig. 1). These ramets along with mother leaf samples were subjected to genomic DNA extraction following extraction method described by Deshmukh *et al.* (2007). Total DNA was quantified and its quality was verified by UV spectrophotometer (Cintra 404, Australia) and each sample was diluted to 40 ng per 3 ul with TE buffer and stored at 4°C. No further purification of DNA before amplification was found necessary.

PCR Amplification

Five ISSR primers (Tables 2) that were successfully used in our earlier study in *Litsea glutinosa* (Patel 2015) were screened on eucalyptus samples. Primer UBC-854 which produces well resolved and consistently reproducible fragments was selected for further testing.

Table 2. Details of the ISSR primers screened with eucalyptus clonal samples.

Sl. No.	Primer Code	Primer sequence (5'–3')	Tm	GC (%)
1	UBC-821	GTGTGTGTGTGTGTGTT	50.3°C	47
2	UBC-853	TCTCTCTCTCTCTCTCAT	47.6°C	50
3	UBC-854	TCTCTCTCTCTCTCTCGG	51.5°C	44.4
4	UBC-859	TGTGTGTGTGTGTGTGGC	56.1°C	55.5
5	UBC-880	GGAGAGGAGAGGAGA	47.9°C	60

ISSR amplifications were performed in a volume of 10 ul containing 40 ng of genomic DNA, 1X Taq polymerase buffer, 0.1 mM of each dNTPs, 2.5 mM $MgCl_2$, 1U Taq polymerase and 0.8 uM of ISSR primer. The amplification reaction consisted of an initial denaturation step at 94°C for 3 min, followed by 35 cycles of 30 seconds at 94°C (denaturation), 30 seconds at a 50°C annealing temperatures and 1 min at 72°C (extension) followed by a final extension step at 72°C for 10 min. DNA amplification fragments were separated in 2.0% agarose gel (SeaKem^R LE Agarose) using 0.5X TBE buffer and stained with ethidium bromide. Gels were visualized using a gel documentation system (Alfa Innotech, USA). The size of the amplification products was estimated from GeneRuler^{TM} 100-bp DNA ladder (Genetix, Biotech Asia Pvt. Ltd).

RESULTS AND DISCUSSION

Clonal propagation is serving as an important tool for increasing the competitiveness of the forestry based plantation industry (Sivarajan *et al.* 2014). However, for use of clonal propagation as continuous source of planting material for commercial utilization on large scale, periodic monitoring of the genetic purity is of utmost importance.

Due to continuous cycles of clonal propagation, there are possibilities of somaclonal variations, mutagenic changes, cytoplasm effects and even admixtures when propagation is on large scale. Due to these, desired characters may even lost that formed the basis of selection of the elite genotype, thereby resulting in considerable economic losses. True-to-type clonal fidelity is important for realising the advantages of clonal propagation. Therefore testing of genetic fidelity becomes very much essential especially in forest trees having long rotation cycles (Lakshmanan *et al.* 2007).

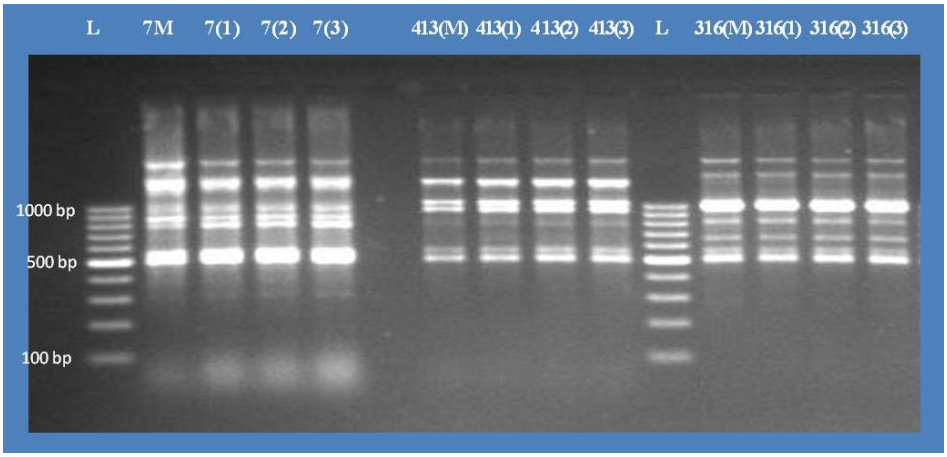

Figure 2. Amplification profile of the eucalyptus clones 7, 413 and 316 received from Balodabazor Forest Division (Raipur Circle), Chhattisgarh using ISSR primer. Where, M- mother plant, 1–3: ramets of the respective clone.

Many approaches ranging from morpho-metric-physiologic to biochemical features were tried to assess the genetic fidelity. However, these traits are found not reliable as it got affected by the environment and expression is stage dependent. DNA-based molecular markers have emerged as a powerful technique for this purpose and therefore are being used in many crops and trees (Cuesta *et al.* 2010, Negi & Saxena 2011, Pandey *et al.* 2012, Singh *et al.* 2013, Mohammad *et al.* 2016).

The amplification profiles of the clonally propagated plantlets of eucalyptus and their mother plant generated using the ISSR marker UBC-854 is shown in figure 2. Both, ramets of the clones and the mother plant of respective clones showed an identical banding pattern. These provide the representative example of monomorphic bands obtained with ISSR primers and has clearly shown the absence of genetic impurity/admixtures among the tested ramets of the respective eucalyptus clones. The scoring data of well-resolved bands were subjected to calculation of similarity matrix based on Jaccard's similarity coefficient. The pair-wise value of the *ramets* and the mother plant of respective clones was 1, indicating 100% similarity. This confirmed the true-to-type nature of the clonal lot supplied for testing. This also establishes the usefulness of ISSR marker system in ascertaining the genetic purity of clones. Earlier also, ISSR markers were successfully employed in bamboo species (Agnihotri *et al.* 2009, Singh *et al.* 2013), date palm (Kumar *et al.* 2010), apple (Gupta *et al*. 2009), jalamdasa (Chandrika & Rai 2009), acacia (Nanda *et al*. 2004), stevia (Lata *et al*. 2013), albizia (Mohammad *et al.* 2016) and by many others.

CONCLUSION

From the monomorphic pattern of ISSR amplification, we may conclude that ramets of the respective eucalyptus clones supplied for testing is genetically homogeneous and corresponds to mother sample provided for testing.

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