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Reassessment and Revalidation of DUS Characters in *Eucalyptus camaldulensis* *Dehnh. clones*

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ABSTRACT: The study was conducted to assess the Distinctiveness, Uniformity and Stability of the DUS descriptors of 22 *Eucalyptus camaldulensis* clones planted at four locations across Tamil Nadu and Andhra Pradesh with the objective of reassessment and revalidation of the leaf descriptors. Nine mature leaf traits *viz*., leaf shape, base symmetry, base shape, Hello apex shape, blade area, leaf length, leaf breadth, petiole length and blade ratio were studied. The study indicated that 18 out of 22 clones could be discriminated based on a combination of 5-7 leaf characters. The clones expressed 74±7.8 to 100% uniformity for leaf traits across all four locations. All leaf characters observed were near ideal similarity of 100% with chi-square values non-significant. The study indicated the reliability of these leaf traits for establishing distinctness, uniformity and stability in clonally propagated material.

Keywords : *Eucalyptus camaldulensis*, DUS descriptors, PPVFRA.

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INTRODUCTION

Eucalyptus camaldulensis Dehnh., commonly called as red gum is one of the earliest introduced and the most widely planed eucalypt species in India. The genus was first introduced to the Mysore hills from where it spread to different parts of the country (Ginwal, 2014). Over 600 species have been introduced to India of which a few *viz., Eucalyptus camalulensis, E. citriodora* (*Corymbia* sp.), *E. globulus, E. grandis, E. tereticornis,* and *E. urophyla,* their hybrids and clones are widely adopted by the forest department, farmers and paper companies (Dhiman and Gandhi 2014). There are several approaches to clonal propagation and tree improvement which is a long-term process. A standard approach includes identification and selection of superior individuals; vegetative propagation of the superior germplasm, provenance trials, selection of plus trees, clonal evaluation and progeny testing, formation of breeding population, genetic evaluation, mass propagation, field testing of improved material, release and deployment of the selected material. In spite of the long-term protocols and procedures the programme cannot have the desired results unless genotypes with best performance and desired characteristics are adopted and popularised among farmers, planters and organisations (Varghese *et al.*, 2017). With diverse germplasm available, it is very important that the right clone reaches the farmer. This warrants the exact identification and proper description of the clones.

Clones are described or identified with the help of descriptors (Ahmed *et al.*, 2013). Descriptors are characters specific to each species and are used to characterise a germplasm (https://www.upov.int/test guidelines/en). In simplest terms, they help to differentiate the clones used in planting. It was in the UPOV (UPOV stands for International Union for the Protection of New Varieties of Plants) Convention, 1961, measures were adopted to standardise how new plant varieties are examined to be examined or Distinctness, Uniformity and Stability (DUS) before granting protection. Test guidelines were developed by UPOV members to provide specific descriptors and testing procedures for each crop group. Initial attempts were for food crops, several arboreal crops were gradually included and tested later on. In India, the Protection of Plant Varieties and Farmers' Rights Act, 2001 (PPV&FR Act) adopted a *sui generis* system for implementing the DUS testing for food crops and forestry crops. Protection of Plant Varieties & Farmers' Rights Authority (PPV&FRA) was established in 2005 to operationalise DUS testing and variety registration making Distinctiveness, Uniformity and Stability (DUS) testing a statutory requirement for the registration of clones under Protection of Plant Varieties and Farmers' Rights Act, 2001 (PPVFRA).

The test guidelines and the DUS descriptors for two important forestry crops viz. Casuarina and Eucalyptus was developed by IGFBT. The DUS test descriptors developed for characterisation of clones often include morphological traits of leaves, bark, branches, flowers and fruits. The characters used as descriptors for clones should comply with distinctiveness, uniformity and stability (DUS) and are often used as tools to evaluate the clonal materials (Ahmed *et al.*, 2013). A database of easily distinguishable characters and reference varieties is available with PPVFA for *Eucalyptus camaldulensis* since 2010(https://plantauthority.gov.in/). However, validation of these characters at definite intervals of time ascertains the reliability of the morphological characters used as descriptors. Validation also helps to add on or remove the characters notified as descriptors based on the relevance and should be a part of long-term tree improvement programmes (Gopal *et al.*, 2008; Jamali *et al*., 2018). With the objective of revalidation of notified DUS characters for *E. camaldulensis*, across different locations data collection was done from 2017 to 2018.

MATERIALS AND METHODOLOGY

Four clonal trials established across Tamil Nadu and Andhra Pradesh were selected for the study. All the trials were laid out in Randomized Block Design (RBD) with four replications. Each replication had 16 trees that were planted in 4 rows at 3 meters spacing with 4 trees in each row at 2 meters spacing. A total of 22 commonly occurring genotypes across the four locations were selected for the study.

For the validation of the DUS characters, field observations were made on leaf characteristics, primary branching, trunk and clear bole height, crown shape, bark texture and peeling as per the guide lines for DUS testing of Eucalyptus clones of which only leaf characters are reported here in this article

Distinctness among the 22 *Eucalyptus* clones was assessed using leaf characters. Morphological observations were recorded from four trees per replication, totalling sixteen trees per clone per trial, across four locations. Leaf characterisation was based on ten mature leaves sampled per tree (160 leaves per clone per trial). All clones were evaluated for nine mature leaf traits: leaf shape, base symmetry, base shape, apex shape, blade area, leaf length, leaf breadth, petiole length and blade ratio.

Uniformity was assessed within each replication and each location using leaf characters. For each clone at a site, 160 leaves were sampled and classified by shape or size. Leaves expressing the predominant type were recorded as major types, while deviations were scored as off-types. Uniformity (%) was calculated as the proportion of major types to the total number of leaves observed. Uniformity was computed separately for each trial and averaged across the four locations.

Character observations were recorded independently at each location using leaf samples; for each clone, the predominant character state was determined separately for each location. Trait stability across the four locations was expressed as percentage similarity: 100% for identical expression at all locations, 75% when observed at three locations, and 50% when two locations occurred. Complete stability (100% similarity) was considered the ideal condition. Departures from this ideal were tested using a chi-square goodness-of-fit test, comparing observed state frequencies with the expected proportion of complete similarity

RESULTS AND DISCUSSION

In the context of DUS testing, distinctness denotes the capability of a morphological descriptor to reliably distinguish a candidate genotype from all other officially registered or commonly cultivated genotypes.

Distinctiveness of leaf traits among clones. The 22 studied clones could be divided into two groups based on mature leaf shape; narrow lanceolate (2 clones) and lanceolate (20 clones). The two clones with narrow lanceolate shape of leaves could be discriminated based their leaf blade area as medium and large. 20 clones in the lanceolate group were further grouped based on leaf base symmetry, Asymmetric (5 clones) and Symmetric (15 clones). Subdivisions of the groups could be continued till all the clones could be discriminated. One clone among the clones studied required five characters *viz.*, lanceolate leaf shape, asymmetric leaf base and cuneate leaf base shape, subulate leaf apex and weak leaf waxiness for discrimination. 7 Clones required seven characters for discrimination. Majority of the clones required 5 characters for identification of the clones. While majority of the clones could be identified based on leaf morphometric traits, four could not be discriminated which necessitated the use of other descriptors.

Uniformity of the leaf traits studied. The results revealed that clones expressed 74±7.8 to 100% uniformity for leaf traits across all four locations. One clone exhibited 100% uniformity for mature leaf base symmetry (symmetric leaf base) and also for mature apex shape (acute apex shape). 2 clones exhibited 100% uniformity for mature leaf base shape. The average uniformity percentage was high for mature leaf base symmetry (93.0%), mature leaf apex shape (93.7%), mature leaf length (92.2%) and mature leaf blade ratio (92.8%). One clone expressed 94.3 % uniformity across all the leaf characters.

Stability of leaf traits across sites. Stable observation on the state of leaf character across all the four locations was taken as 100% similarity and any deviation in one location resulted in 75% similarity and 50% when two locations were observed one character state and other two location with another state for a particular character.

The observed percentages were analysed using chi square test against the ideal percentage of 100%. The non-significance reveals that the observed ratios are equal to the ideal value of 100% similarity. All leaf characters observed were near ideal similarity of 100% with chi-square values non-significant

Eucalyptus camaldulensis clones are described using 33 DUS characters including leaf, branch, bark, crown and reproductive characters by Protection of Plant Varieties and Farmers' Rights Authority (PPV&FRA). The leaf descriptors were validated under the present study using the same set of characters. Considering the leaf characters alone, 18 clones out of 22 were identified using a combination of leaf traits. Even when the use of just phenotype-based DUS traits are not highly preferred by few scientist (Janis and Smith, 2007; Deng and Hang, 2009) and highlights it as a possible weakness of the Plant Variety System the leaf traits serves to be the most useful descriptors with respect to uniformity, distinctiveness and stability.

CONCLUSIONS

The reassessment and revalidation of DUS leaf characters in *Eucalyptus camaldulensis* Dehnh. clones demonstrated that the evaluated descriptors are consistent, clearly distinguishable and stable across locations, thereby meeting the essential criteria prescribed under the PPV&FR Authority. The predominance of uniform character expression and high inter-site similarity confirms the reliability of these leaf traits for establishing distinctness, uniformity and stability in clonally propagated material. The validated descriptors strengthen the existing DUS framework for *Eucalyptus* and provide a standardized, field-applicable basis for clone identification, registration and protection under the PPV&FR Act, while also supporting effective deployment of improved planting material in operational forestry. DUS characterisation is important in view of the increasing adoption of clonal forestry and the economic importance of eucalyptus in pulpwood and timber. Future studies should focus on validating species- and clone-specific leaf morphological descriptors as leaf morphology varies widely.

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