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Phenotypic markers in early selection for tolerance to dieback in *Eucalyptus*

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ABSTRACT

Eucalyptus dieback is a physiological disorder characterized by lesions on the apex of the branches and the death of the shoot apex. Some clones are considered tolerant to this disorder, although, up to now, there are no reports on the use of phenotypic markers for selecting tolerant material. The use of phenotypic markers may contribute to an efficient selection, where the estimation of genetic parameters of the candidate markers are essential to knowledge of the population structure, the genetic potential and effectiveness of markers as selection criteria. In this study, we estimated the genetic parameters of morphological and nutritional markers in 13 commercial eucalyptus clones aiming early selection of tolerant genotypes to eucalyptus dieback. Estimates of the genetic parameters of all characteristics were obtained by the mixed model methodology, REML (Restricted Maximum Likelihood)/BLUP (Best Linear Unbiased Prediction) procedure. Water deficit, one of the factors that promote this disorder, was simulated in eucalyptus cuttings conducted in a greenhouse. Simulated water stress was carried out for 12 weeks by applying polyethylene glycol at different concentrations and by limiting water applied to seedlings. In order to select the most promising markers, 34 morphological and nutritional variables were evaluated for early selection and discrimination of genotypes tolerant to eucalyptus dieback and water deficit. Among the 34 evaluated characteristics, plant height, stem diameter, height increment, leaf area, N, K and B, exhibited significant heritability and high accuracy (greater than 70%). Promising phenotypic markers were identified for early selection of genotypes tolerant to water deficit and eucalyptus dieback. Tolerant clones were pooled and correctly discriminated from the susceptible ones under stressful conditions by the use of seven phenotypic markers in REML/BLUP analysis, selection index ranking and graphic dispersion analysis.

1. Introduction

Eucalyptus plantations are widely used for bioenergy production around the world. In places like Australia, North America, Europe (Jurskis, 2005) and Brazil (Mattiello et al., 2009), they have been affected by a so-called physiological disorder "dieback", which is characterized by a gradual deterioration in healthy trees over months or years, leading to premature death (Ferreira, 1989; Ross and Brack, 2015). In Australia, eucalyptus dieback have been reported in all states (Ross and Brack, 2015) and it is believed that it has caused problems in native and planted forests since 1920 (Ciesla and Donaubauer, 1994). In Brazil, eucalyptus dieback was first reported as SPEVRD (Seca de Ponteiros do Eucalipto do Vale do Rio Doce) in the 70's in the state of Minas Gerais (Ferreira, 1989). Latter in the 80's, in the state of Paraná (Maschio et al., 1996) eucalyptus dieback was also reported and since then has affected the eucalyptus cultivation (Fig. 1A, B and C) in several Brazilian states, causing significant losses (Mattiello et al., 2009).

Eucalyptus dieback is often referred to as a disease or "complex etiology" disorder caused by the combination of biotic and abiotic factors (Ferreira, 1989; Jurskis, 2005; Ross and Brack, 2015). Among the factors associated with dieback, insect attacks stand out (Ross and Brack, 2015), pathogens like *Phytophthora*, nutritional deficiency (Mattiello et al., 2009) and climate change (Jurskis, 2005). In addition, the eucalyptus dieback is also related to water deficit, as in the drier months of the year or in regions more favorable to scarce rainfall, eucalyptus trees can reach death due to low water potential (Jurskis, 2005).

However, currently some clones are more affected than others, and to date, the lack of markers associated with the tolerant phenotype hampers the process of selection of promising genetic materials. Despite

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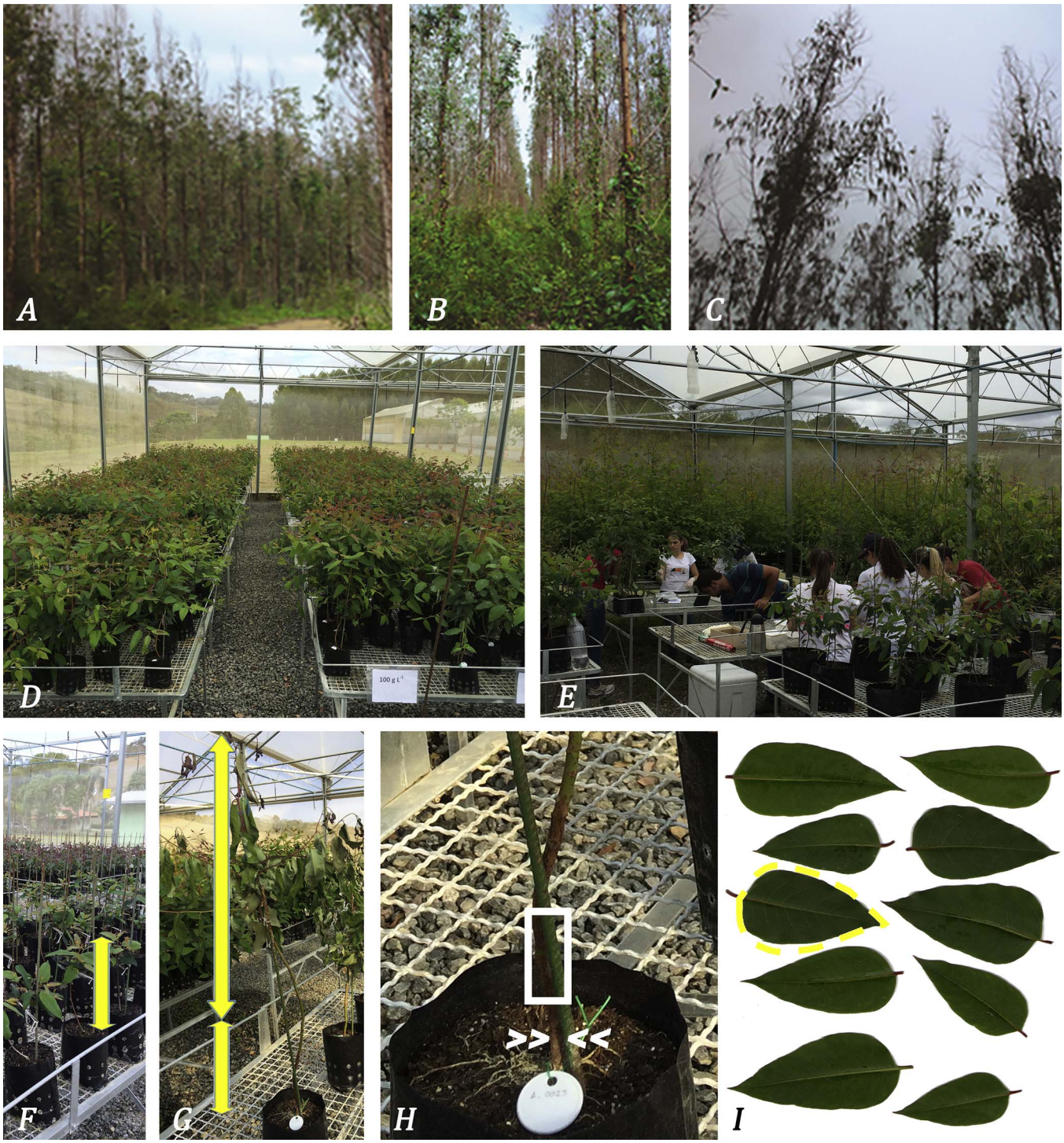

Fig. 1. Eucalyptus displaying dieback symptoms in field condition, experiment conduction and morphological variables used in the statistical analysis.
A, B – Commercial plantation with plants presenting the physiological disorder; C – detail of the shoot apex death (dieback); D – experiment setting at the greenhouse; E – evaluation of the experiment after 12 weeks conduction; F – plant height at the beginning of the experiment; G – clone displaying water deficit, the height of the plant and height increment at the end of the experiment; H – stem diameter (*D*) measured at 2.5 cm above the collar region and the region sampled for stem density estimation; and I – sampled leaves for nutrient quantification and for leaf area (LA) of a susceptible clone (Suz – 10). Yellow lines and arrows – schemes for plant height; white arrow heads local for *D* measurement; white rectangle – sample for stem density; doted yellow lines – area of one leaf.

several approaches of the possible causes of eucalyptus dieback, a methodology for early selection of tolerant genotypes has not been described, which has currently been carried out only based on empirical data and observations.

The breeding of a perennial species is a lengthy process. Procedures that can reduce the crop cycle or advance the selection process can contribute significantly to a eucalyptus breeding program success, where early selection is one of the alternatives among these procedures. The early selection has been efficient in the genetic improvement of eucalyptus (Pinto et al., 2014), where characteristics of younger plants are used as predictors of features that are presented in productive age, anticipating the genetic gains (Moraes et al., 2014). Early selection of genotypes tolerant to dieback, a characteristic manifested in adult plants, may be useful to establish a methodology that allows clones to