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Variation in growth traits and survival of landraces of *Eucalyptus globulus* Labill. in the Ethiopian highlands

P. Guzman[1], L. Gil[1] and W. Tadesse[2]*

[1] *Grupo de Investigación de Genética y Fisiología Forestal. ETSI Montes. Universidad Politécnica de Madrid. Ciudad Universitaria, s/n. 28040 Madrid, Spain*
[2] *Ethiopian Institute of Agricultural Research. Woreda 17 Kifle Ketema. Kebele 12/13. 2003 Addis Abeba, Ethiopia*

Abstract

Aim of study: Eucalyptus globulus Labill. is the most planted tree species in the Ethiopian highlands due to its potential to satisfy the increasing wood demand of the population. The objectives of this study are to assess the variation in growth traits and survival among *E. globulus* landraces and clones and to evaluate correlations between growth traits on saplings established in the field.

Area of study: The study was performed in the central highlands of Ethiopia.

Material and Methods: A landrace trial with Ethiopian seedlings and Spanish cuttings (Sancho and Tinto clones) of *E. globulus* was carried out. Root collar diameter, height and survival were evaluated on saplings one year after outplanted in the field.

Main results: The Spanish landrace showed an improved field performance with 35% higher root collar diameter, 35% taller saplings and 27% higher survival rates as compared to the Ethiopian plants. Sancho clone attained 10% higher root collar diameter than Tinto clone. Strong positive correlation between root collar diameter and height for all saplings was observed (r = 0.8785).

Research highlights: The present study can be considered as a starting point to implement a breeding program for *E. globulus*, which is nowadays of great importance in Ethiopia. The introduction of a new Spanish landrace may contribute to broadening the genetic base of this species in Ethiopia. Further tree breeding efforts with *E. globulus* should be undertaken to raise such genetic base and to increase the productivity of eucalypt plantations in the Ethiopian highlands.

Key words: clone; rural development; tree breeding.

Introduction

Ethiopia's remaining forest cover is estimated to be diminishing at a rate of 141,000 hectares per year (FAO, 2006). This fact together with the high demand for woody biomass from an ever-growing population makes critical the need to increase biomass significantly these days and in the coming future. Scarcity of fuel wood in rural areas compels population to burn dung and crop residues for household energy, thus implying a decrease in agricultural yields, while also causing soil degradation and accelerated erosion (Tadesse *et al*., 2003; Tizale, 2007). Furthermore, approximately 95% of the total demand for wood and woody biomass in rural Ethiopia is used for fuel wood, leaving little woody material for construction and other purposes (MONREP, 1994; EARO, 2000). The establishment of plantations on suitable lands becomes necessary not only to meet wood demand and reduce deforestation by decreasing pressure on natural forests, but also to restore degraded lands and enhance biodiversity (Parrotta, 1992; Tiarks *et al*., 1998; Jagger and Pender, 2003; Yirdaw and Luukkanen, 2003).

In order to satisfy the biomass energy demand of the country, 6% of the total usable land area would have to be put under tree plantations by 2014; thus requiring a major change of land use (Böjo and Cassels, 1995). In this sense, the choice of the tree species having the best potential for conversion of soil nutrients and water into biomass together with the selection of the planting sites constitutes an important decision-making process. Use of short rotation, high-yield plantations to grow an adequate supply of the renewable resource has

* Corresponding author: wubalem16@gmail.com
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several advantages (Marsh, 1962; Sedjo and Botkin, 1997) and appears to offer an efficient and cost effective solution to Ethiopia's woody biomass crisis (Pohjonen and Pukkala, 1990; Jagger and Pender, 2003).

Results on both indigenous and exotic species trials conducted in the Ethiopian highlands showed that, under most conditions, the genus *Eucalyptus* is the most efficient converter of energy into biomass (*e.g.* Newcombe, 1989; Stiles *et al*., 1991). *Eucalyptus globulus* Labill. is the best-performing species, with mean annual productivity of plantations between 10 and 30 m^3 ha^{-1} in poor site classes (Pohjonen and Pukkala, 1990).

E. globulus was introduced to Ethiopia in 1895 and initially established around major towns in order to supply fuel wood for urban population (von Breitenbach, 1961; Bristow, 1995). Nevertheless, it was not until the mid-eighties that the establishment of large-scale plantations started (Stiles *et al*., 1991; Pandey, 1995). Nowadays, *E. globulus* is the prevailing feature of the rural landscape in the Ethiopian highlands and it is a very important part of smallholder livelihood (*e.g.* FAO, 2009; Gil *et al*., 2010). The species is almost perceived as 'indigenous' among local communities (Hunde *et al*., 2007) who have exhibited a strong preference for it (*e.g.* Zerfu, 2002; Mekonnen *et al*., 2007). In this regard, Pojhonen and Pukkala (1990) stated that *E. globulus* is the most important plantation species in Ethiopia.

Nevertheless, the information on the genetic status of *E. globulus* is limited in Ethiopia. The provenances and genetic base are unknown and probably sub-optimal. Likewise, there could also be a high degree of inbreeding in the developed landraces (MONREP, 1994; Davidson, 1995). Based on Zobel and Talbert (1984), Ethiopia would need to develop a tree breeding program, specifically for *Eucalyptus*.

The use of genetically based geographic variation is the first step in tree breeding when assembling a suitable base population for future generations of selection, crossing and genetic improvement (Elridge *et al*., 1994). Provenance (landrace) research should be given the highest priority at the outset of any program of forest tree improvement (Callaham, 1963).

This study describes the preliminary results of a *E. globulus* landrace trial consisting of two landraces from Ethiopia and Spain and two clones belonging to the Spanish landrace established in the central highlands of Ethiopia. The objectives are: (a) to assess the variation in growth traits and survival among the landraces and clones and (b) to evaluate correlations between growth traits. The present study can be considered as a starting point to perform a *E. globulus* long-term breeding program in Ethiopia.

Material and methods

Seedlings of *E. globulus* from Dire landrace (Ethiopia) and cuttings of two clones (234 –Sancho–, 231 –Tinto–) from Huelva landrace (Spain) were selected for establishing a landrace trial. Geographic and climatic characteristics of the location of the landraces are given in Table 1.

Clones were obtained within the *E. globulus* breeding program developed by the forest company ENCE since early 1990s. Previous provenance trials showed that the diameter increment of the local landrace, over which the process of selection took place, was within the range of the natural variation of the species (López, 2010). Huelva landrace grows under Mediterranean climate with severe summer drought on both sandy and shale soils (Toval, 2004). Almost 2,000 adult trees were selected in field plantations and fell to rejuvenate. A rooting test was undertaken on sprouts of 700 genotypes and about 100 clones were tested in field trials across the target area for planting. The result was the generation of 16 clones delivered for operational scale propagation (Cañas *et al*., 1994). Sancho and Tinto resulted from a crossing program carried out between selected individuals of these first-generation clones and subsequent rooting and field tests (López, personal communication). After production of coppice material in hedges or mother trees, macro-cuttings were prepared (8-10 cm lenght, with two nodes and leaf area reduced 50%), set to root in containers with a coconut fiber and peat medium in

Table 1. Geographic and climatic data of sites of *Eucalyptus globulus* landraces tested in the field trial established at Gefarsa, central highlands of Ethiopia

Landrace	Dire (Ethiopia)	Huelva (Spain)
Latitude	38° 54' E	6° 54' W
Longitude	9° 11' N	37° 16' N
Altitude	2,756 m	19 m
Mean annual precipitation	1,150 mm	500 mm
Mean annual temperature	13.8°C	17.5°C

a shade house and finally maintained in outdoor nursery (ENCE, Huelva). Cuttings were sent to Ethiopia and transplanted to 300 cm^3 plastic containers (15 cm height, 5 cm diameter) filled with a substrate made of forest soil, sand and compost (2:1:1) at Forestry Research Center nursery (Addis Ababa). Seeds of Dire landrace were directly sown at 0.5 cm depth in containers with the mentioned characteristics and filled with the same substrate at Forestry Research Center nursery. Both cuttings and seedlings were covered with roofs prepared with grasses to protect them from intensive sun radiation. Saplings were watered once a day and weeding was done at regular intervals until June 2009, when planted in the field. At this moment, plants were 10 (cuttings) and 12 months old (seedlings) and had mean values of root collar diameter (RCD) and height of 0.78 cm and 0.84 m, respectively, with no significant differences among landraces or clones.

The trial was carried out at Gefarsa, 18 km west of Addis Ababa (latitude 38° 38' E, longitude 9° 4' N, altitude 2,545 m). The mean annual rainfall in the area varies, but the 5 decades mean average 1931-1979 was 1,225 mm, 95% of which was received from June to September (FAO, 1982, reference therein Hunde, 1999). The site is almost flat with reddish brown clay-loam soil (Murphy, 1968, reference therein Hunde, 1999).

Saplings were planted in the field at 2 × 2 m spacing in a completely randomised design. Fifty-six plots (8 × 8 m), 28 for Huelva landrace (14 for each clone) and 28 for Dire landrace, were established. Each plot comprised 25 trees. The distance among plots was 5 m. One row of eucalypt (Dire landrace) was planted around the perimeter of the trial, spaced at the same interval as trees within the trial.

Traits assessed were RCD (cm), height (m) and survival (%) at age 22 and 24 months for cuttings and seedlings, respectively, one year after outplanted in the field. All measurements were restricted to the inner 4 × 4 m plot (nine seedlings if all alive) to avoid possible edge effects.

The following mixed model was fitted to the data and used for a two-level analysis: (A) analysis comprised data of Dire and Huelva landraces, with the last one broken down to two clones, and (B) analysis only included data of clones in order to accurately evaluate differences between their performance:

$$Y_{jkl} = \mu + Z_j + P_k(Z_j) + \varepsilon_{jkl}$$

Where Y_{jkl} is the observed trait value of individual saplings, μ is the grand mean, Z_j is the fixed effect of landrace/clone j [(A) analysis] or clone j [(B) analysis], $P_k(Z_j)$ is the random effect of plot k within landrace/clone j [(A) analysis] or clone j [(B) analysis] and ε_{jkl} is the residual variation; $j = 1 \ldots J$ (J is the number of landraces/clones), $k = 1 \ldots K$ (K is the number of plots), $l = 1 \ldots L$ (L is the number of trees per plot).

All data were tested for normality with Shapiro-Wilk test and with plots of studentized residuals *versus* predicted values. Independence and homoscedasticity were verified using lag-1 autocorrelation function and Bartlett's test, respectively. The assumptions about normally distributed data could not be met regarding seedling survival. Thus, Kruskal-Wallis test was performed. The analysis of variance for growth traits and survival was carried out at individual tree level and on plot means, respectively. Significant differences were further analysed using Tukey's honestly significant difference (HSD) or Kruskal-Wallis tests.

Correlation between growth traits was measured by Spearman rank correlation coefficient.

STATGRAPHICS Centurion XV Version 15.2.05 was used for all the analyses applied (StatPoint, Inc. 1982-2007).

Results

Significant differences between landraces were observed for RCD, height and survival percentage of saplings after one year of establishment in the field ($p < 0.05$; Table 2). Significant differences were also registered between clones for RCD ($p = 0.0095$), but not for height or survival (Table 2). Both clones of Huelva landrace differed from Dire landrace for all measured trait means, for which the lowest mean values (1.27 cm, 1.17 m and 53.8%) were registered. Sancho clone attained maximum RCD, height and survival percentage means (2.07 cm, 1.87 m and 73.7%, respectively). Tukey's HSD and Kruskal-Wallis tests did not reveal significant differences from Tinto clone for any of the trait means in (A) analysis, but Tukey's HSD did for RCD in (B) analysis (Table 3). When comparing the general landrace performance, the Spanish landrace showed saplings with about 35% higher both RCD and height and 27% higher survival rate as compared to the Ethiopian seedlings. For clones, Sancho had about 10% higher RCD than Tinto clone. Landrace/clone or clone nested into plot was

Table 2. Effect of landrace/clone (clone) and plot within landrace/clone (clone) on variance of root collar diameter (RCD), height and survival for Gefarsa *Eucalyptus globulus* landrace trial

Trait	(A) analysis			(B) analysis		
	Source of variation	F or H statistics	P value	Effect	F statistics	P value
RCD	Landrace/clone	54.90	<0.0001	Clone	7.84	0.0095
	Plot(landrace/clone)	2.86	<0.0001	Plot(clone)	1.61	0.0351
Height	Landrace/clone	36.11	<0.0001	Clone	3.71	0.0652
	Plot(landrace/clone)	3.09	<0.0001	Plot(clone)	2.19	0.0039
Survival	Landrace/clone	7.85	0.0198	Clone	0.00	0.9449
	Plot(landrace/clone)	—	—	Plot(clone)	—	—

Table 3. Least square means/medians (standard error) from ANOVA and Kruskal-Wallis tests (95% confidence interval) for measured attributes of *Eucalyptus globulus* landraces and clones at Gefarsa landrace trial

Landrace/clone	Growth traits		Survival (%)
	RCD (cm)	Height (m)	
Dire	1.27 a (0.05)	1.17 a (0.05)	53.78/44.00 a (4.62)
Tinto	1.86 b (0.06)	1.71 b (0.07)	73.14/70.00 b (6.42)
Sancho	2.07 b/c (0.06)	1.87 b (0.07)	73.71/86.00 b (6.42)

Means/medians with different letters were significantly different; (A) analysis/(B) analysis.

associated with significant variation of both RCD and height in (A) ($p < 0.0001$) and (B) analysis ($p < 0.05$) (Table 2), indicating within landrace/clone variation among different plots for these traits. Estimates of Spearman correlation coefficient between all data revealed a significant correlation between RCD and height ($r = 0.8785$; $p < 0.0001$).

Discussion

The results obtained in this study may indicate that genetic variation exists between Dire and Huelva *E. globulus* landraces growing in Gefarsa in growth traits (RCD and height) and survival and between Sancho and Tinto clones for RCD.

Results reported in the literature regarding to comparative performance of *Eucalyptus* seedlings and cuttings have been contradictory, describing a relative superiority of either seedlings (Kageyama and Kikuti, 1989; Cotterill and Brindbergs, 1997) or cuttings (Menk and Kageyama, 1988; Lambeth *et al*., 1994). Gaspar *et al*. (2005) stated that unfavourable propagation effects were associated with neither cuttings nor seedlings and highlighted the importance of plant quality of cuttings as a critical factor, at least during initial stages of development. Thus it has been considered that propagation effects were not likely to have affected the plant performance significantly, though the superiority of clones could be influenced by their degree of genetic improvement.

Lack of accurate data about maternal environments did not allow establishing a correlation among measured traits and climatic, edaphic or geographic characteristics of that environment. However, it is known to be a significant determinant of progeny variation, particularly for early growth traits, in several plant genera and species, including *Eucalyptus globulus* (López *et al*., 2003). Thus, further analysis including these data should be carried out.

It should also be mentioned the existence of external factors which could influence the expression of growth traits and survival of landraces and clones. Such factors might be frost, drought, waterlogging, occasional presence of livestock and competition of weeds and recruitment of *Acacia decurrens* Willd. It was observed that many plants of the trial suffered from dieback and resprouted, which was confirmed by data of RCD and height of some saplings. *E. globulus* is sensitive to frost (Potts and Sava, 1989; Battaglia *et al*., 1996), especially one- or two-year-old seedlings and resprouts (FAO, 1979; Skolmen and Ledig, 1990), with critical temperatures of –6°C or –7°C (Turnbull and Pryor, 1978; Elridge *et al*., 1994). Frost related mortality of the species has been reported in Ethiopia when established in regions with mean annual rainfall up to 1,200 mm but affected by unusual dry years, particularly on shallow soils (FAO, 1979). Water stress is also known to be one of the main factors that restrict the productivity of eucalypts in natural and managed stands, even in areas considered favourable for their growth (Beadle *et al*., 1995). For instance, after a 20-year period of good performance, *E. globulus* trees planted in Nairobi arboretum (mean annual rainfall 838 mm), Kenya, failed due to four consecutive years of low rainfall (1928-1931) (Streets, 1962, reference therein FAO, 1979). Adverse climatic conditions (extreme minimum temperatures, lack of rains) affecting the area, at least since the establishment of the trial (National Meteorological Agency, 2009, 2010a,b), could then be the cause of the mortality and growth inhibition of the aerial part of the saplings. On the other hand, unfavourable heavy rains over the trial site were also reported by National Meteorological Agency (2009, 2010a). Sapling clusters were detected within the trial area. The presence of microsites of relatively longer duration of waterlogging, as observed during the first rainy season after trial establishment, may be a factor that explains the variation among different plots for growth traits. Although *E. globulus* grows well on a wide range of soils, it requires good drainage (Skolmen and Ledig, 1990; Bell, 1999). Furthermore, effects of adverse climatic conditions on plants, as those referred, are multiplied by negative effects of the soil where they occur (FAO, 1979). Otherwise, although it is said that grazing animals do not eat *E. globulus* trees (Pohjonen and Pukkala 1990), trampling has a negative effect on sapling survival. Damages on saplings due to both browsing and trampling were observed in the trial. Finally, presence of recruitment of *A. decurrens* and weeds could influenced the performance of the saplings due to competition for nutrients, water and/or sunlight, since *E. globulus* tolerance to interference by weeds and other plants during establishment is low (Fremlin and Misic, 1999; Ruiz *et al*., 2008).

The strong positive correlation between RCD and height demonstrates the possibility of indirect selection for one trait based on direct selection for another.

To sum up, significant differences between saplings of Dire and Huelva landraces for RCD, height and survival percentage and between Sancho and Tinto clones for RCD were found, with Huelva landrace and Sancho clone performing better than Dire landrace and Tinto clone, respectively. However, caution in interpretation of results is needed since both degree of genetic improvement of clones and external factors (climatic and edaphic factors, lack of management) may have influenced the performance of the saplings. Climatic factors affecting the study can not be considered representative of the Ethiopian highlands environmental conditions. Thus, establishing other trials under different environmental conditions might reveal accurately significant differences between both landraces and clones. On top of it, age of trees should also be considered, since early selection for breeding and deployment usually occurs between four and six years of age (Borralho *et al*., 1992; McRae *et al*., 2003) and traits were evaluated at about two years of age. Further evaluation should provide more robust results, but the trial was completely invaded by *A. decurrens* and hyenas were living in the trial site in August 2011, which makes difficult or prevent further that evaluation. Yet, the genetic resources of *E. globulus* established in this trial if managed to maturity can contribute to broaden the genetic base of the species in Ethiopia.

Conclusions

Significant differences between *E. globulus* landraces from Spain and Ethiopia were observed for RCD, height and survival percentage after growing for one year in the field, with the highest values corresponding to the Spanish one. Within the two clones belonging to the Spanish landrace, Sancho clone attained greater RCD than Tinto clone. Caution should be taken in interpretation of results since the landrace trial has been affected by a number of external abiotic and biotic stress factors. Even so, *E. globulus* genetic resources provided with this trial can contribute to widen the genetic base of the species in the Ethiopian highlands by providing high-quality forest reproductive material. This aspect is of great importance due to both the possible high degree of inbreeding in provenances developed in the country and the high demand of forest products.

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