Growth Data from a Field Trial of *Quercus suber* Plants Regenerated from Selected Trees and from Their Half-Sib Progenies by Somatic Embryogenesis

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Abstract

The development of reliable clonal propagation technologies is a requisite for performing Multi-Varietal Forestry (MVF). Somatic embryogenesis is considered the tissue culture based method more suitable for operational breeding of forest trees. Vegetative propagation is very difficult when tissues are taken from mature donors, making clonal propagation of selected trees almost impossible. We have been able to induce somatic embryogenesis in leaves taken from mature oak trees, including cork oak (*Quercus suber*). This important species of the Mediterranean ecosystem produces cork regularly, conferring to this species a significant economic value. In a previous paper we reported the establishment of a field trial to compare the growth of plants of somatic origin vs zygotic origin, and somatic plants from mature trees vs somatic plants from juvenile seedlings. For that purpose somatic seedlings were regenerated from five selected cork oak trees and from young plants of their half-sib progenies by somatic embryogenesis. They were planted in the field together with acorn-derived plants of the same families. After the first growth period, seedlings of zygotic origin doubled the height of somatic seedlings, showing somatic plants of adult and juvenile origin similar growth. Here we provide data on height and diameter increases after two additional growth periods. In the second one, growth parameters of zygotic seedlings were also significantly higher than those of somatic ones, but there were not significant differences in height increase between seedlings and somatic plants of mature origin. In the third growth period, height and diameter increases of somatic seedlings cloned from the selected trees did not differ from those of zygotic seedlings, which were still higher than data from plants obtained from somatic embryos from the sexual progeny. Therefore, somatic seedlings from mature origin seem not to be influenced by a possible ageing effect, and plants from somatic embryos tend to minimize the initial advantage of plants from acorns.

INTRODUCTION

Genetic improvement programs of forest species have much more constraints than horticultural and crop species have. Very high variability, long life cycles, size of trees and usually inaccessible locations, make breeding of those species too long and expensive. Vegetative propagation has played for centuries a substantial role in the genetic improvement of many horticultural and woody crops, such as olive and vine: the development of these productive systems has been largely based on the clonal option. Cloning can also be one of the most powerful tools in the conservation and genetic improvement programmes of forest species: clonal plants serve not only to obtain precise estimations of genetic parameters and genotype × environment interactions, but also to conserve desired genotypes and to perform clonal forestry in plantations (Zobel and Talbert, 1984). To accomplish vegetative propagation, reliable and mass-producing

methods of somatic regeneration are required. Vegetative propagation has been extensively used in Forestry with different species that are easy to root, belonging to genus *Populus*, *Salix*, *Cryptomeria* and *Eucalyptus*. Forest biotechnology is beginning to provide different tools for performing Multi-Varietal Forestry (MVF) with previously recalcitrant species (Park, 2007).

Somatic embryogenesis is widely considered as the best tissue culture-based method to regenerate plants in forestry (Merkle and Nairn, 2005). During the last 15 years the development of somatic embryogenesis has been impressive, particularly in conifers. At present, private companies are producing in various countries thousands of somatic seedlings of several species of the genus *Picea*, *Pinus* and *Pseudotsuga*, to establish clonal tests in different locations that, coupled with cryopreservation of the embryogenic lines, will serve to develop the high-value clonal forestry (Nehra et al., 2005). Due to the weak ability of differentiated tissues of woody species for morphogenesis, most of the current protocols for plant regeneration are based on juvenile material. This fact precludes vegetative propagation of desired phenotypes at the age in which they can be reliably selected. However, we have been able to develop methods to clone adult oak trees by somatic embryogenesis (Toribio et al., 2000, 2004).

The cork oak (*Quercus suber*) is one of the most important tree species of the Mediterranean ecosystem. Cork is produced by this species for vine bottling and other industrial applications, justifying their improvement programs. We have developed regeneration protocols by somatic embryogenesis that can be applied to clone almost all adult cork oak trees (Hernández et al., 2003), therefore avoiding the risk of genetic erosion. The availability of these protocols has also allowed for the cryopreservation and transformation of selected phenotypes of this species (Valladares et al., 2004; Álvarez et al., 2004).

At present, the protocols developed for this species are able to produce the few hundreds of plants required for genetic tests, and for in situ or ex situ conservation purposes (Hernández et al., 2007). Although somatic embryos resemble true seeds without coats, the performance of somatic seedlings (emblings) in relation to sexual seedlings has to be tested. On the other hand, one of the main concerns when vegetative propagules are obtained from adult donors is that they may retain mature features due to the ageing process. In fact, many clonal propagation programs based on cuttings from hedged mother trees were affected by undesired long-term growth reductions due to precocious ageing (Smith, 1999). Therefore, we have established a field trial to compare clonal plants from selected adult trees with their half-sib progenies. In the previous paper (Celestino et al., 2004) we reported on the survival of an early growth of the plants implanted in this parcel. Somatic seedlings in the trial exhibited morphological development that was comparable to normal seedlings. However, survival and height after the first growing period were much better for seedling than emblings. This paper deals with growth data in this experimental plot after two additional growing periods.

MATERIALS AND METHODS

The plant material established in the field trial and the way it was obtained was described elsewhere (Celestino et al., 2004). Briefly, acorns from open pollination and pieces of branches were collected from five cork oak trees growing in La Almoraima (Cádiz, south Spain). These trees were selected on the basis of their phytosanitary condition, growth, form, and quality and quantity of produced cork. Seedlings were raised from acorns forming five half-sib families. Emblings were obtained from somatic embryos induced in both leaves from adult trees (emblings of mature origin) and leaves from the half-sib seedlings (emblings of juvenile origin). The regeneration protocols are described elsewhere (Toribio et al., 2005). The experimental plantation was established in November 2003, following a design of five complete randomised blocks, in which 15 treatments (five genotypes and three progeny types, seedlings, emblings/mature, and emblings/juvenile) were replicated. Each experimental unit included three plants that were clonal in the case of emblings. Therefore, the whole trial comprised 225 plants in a

factorial design. Height and diameter measures of each plant were recorded annually in February. Differences 2006 minus 2005, and 2007 minus 2006, were considered the growth data for the periods 2005 and 2006 respectively. Data were analysed by ANOVA and multiple media comparisons were performed (SPSS 15.0).

RESULTS AND DISCUSSION

As described previously (Celestino et al., 2004), morphology and growth habit of all cork oak plants were similar, and no differences were noted between seedlings and emblings. Problems usually associated with other ways of vegetative propagation, such as plagiotropism, were not observed.

When considering cumulative growth along the two vegetative periods, genetic effects were no significant, but the type of progeny significantly affected both height and diameter increases. Means of seedlings were higher than means of emblings of juvenile origin. However, differences between height growth of seedlings and emblings of mature origin were not significant (Fig. 1).

There were important differences in each of the two consecutive growing periods. Although the mean increase in diameter was equal in both years (3 mm), during 2005 the mean increase in height was almost 60 cm, while it was 15 cm in 2006. When considered separately, genetic effects were not significant in 2005 but they were significant in 2006. Again, the type of progeny significantly affected growth parameters each year. During 2005 growth of seedlings was better than emblings, both in height and diameter, although differences in height increase between seedlings and emblings of mature origin were not significant (Fig. 2). These results were maintained during 2006, although the emblings of juvenile origin grew slightly better than in the precedent periods. In addition, growth of emblings cloned from adult trees equalled growth of seedlings, not only in height but also in diameter (Fig. 2). Both facts may indicate a general tendency of plants from somatic embryos to overcome the initial advantage of plants from acorns.

Unless great differences arose, significant effect of the genetic component is difficult to detect, because this term grouped clonal plants derived from mother trees and plants of their sexual progenies. However, in spite of this within group variability, a significant genetic effect was detected for both growth parameters in 2006. This fact would indicate that genetic differences were more clearly expressed in less favourable growing conditions than in favourable ones.

Most papers about field performance of vegetative propagated tree species refer to plants obtained from cuttings or by in vitro organogenic procedures, and usually show that plants from somatic or sexual origin have similar behaviour. Studies on silver birch reported that there were no differences in survival, height growth and pest resistance between seedlings and micropropagated plants (Viherä-Aarnio and Velling, 2001). Furthermore, plantlets cloned from adult silver birch trees may have been rejuvenated by tissue culture, because they showed similar stem elongation rates as seedlings (Jones et al., 1996). Regarding somatic embryogenesis, several studies carried out with conifers indicated that seedling and emblings show the same performance, including physiological parameters and growth patterns and rates (Benowicz et al., 2002). Similarly, studies in hardwoods revealed no differences related to the origin of plants. In the rubber tree the morphology of the root system was very similar (Carron et al., 2000). Moreover, the performance of emblings of oil palm cloned from selected trees was superior to that of improved seedlings from controlled crosses (Khaw and Ng, 1998).

The growth data reported here on cork oak are in agreement with other published work regarding micropropagation through somatic embryogenesis. As reported in most embryogenic systems (Pullman et al., 2003) somatic embryos do not mature completely, resulting in plants with reduced initial vigour, which may last for several years.

CONCLUSIONS

Three years after the establishment of the field trial, cork oak plants obtained from somatic embryos are growing like seedlings. No signs of abnormal growth have been

detected, and all plants seem to follow the same growth patterns. The initial vigour of seedling as regards emblings is being reduced, and the origin of the latter, mature *vs* juvenile is not affecting their growth. This is of particular importance, because equal vigour of plants vegetatively rose from juvenile seedling or from adult trees means that somatic embryogenesis is able to rejuvenate selected phenotypes.

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Figures

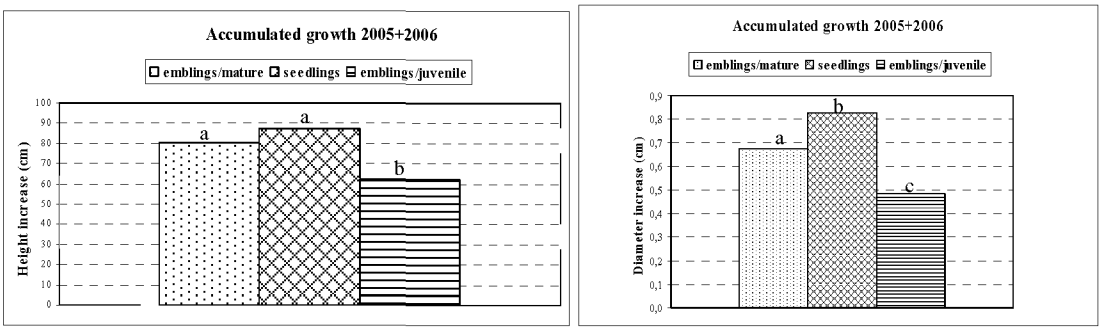

Fig. 1. Vigour of cork of cork oak zygotic and somatic seedlings from different origin, during a two-year growth period. Mean height increase (left) and diameter increase (right) of plants from (from left to right) somatic embryos induced in leaves from adult trees (mature origin, from acorns, and from somatic embryos induced in leaves from seedlings (juvenile origin). Bars with the same letter indicate that means are not significantly different (Duncan test, $p<0.05$).

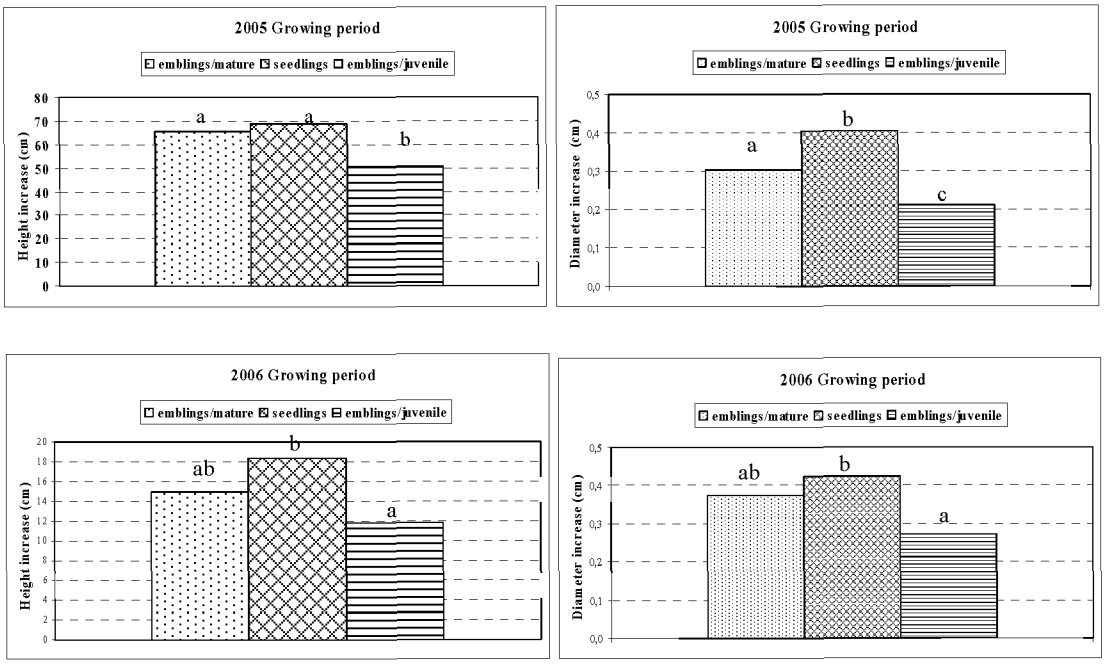

Fig. 2. Vigour of cork of cork oak zygotic and somatic seedlings from different origin, during two consecutive growth periods. Mean height increase (left) and diameter increase (right) of plants from (from left to right) somatic embryos induced in leaves from adult trees (mature origin, from acorns, and from somatic embryos induced in leaves from seedlings (juvenile origin). Bars with the same letter indicate that means are not significantly different (Duncan test, $p<0.05$).