Rooting and survival of cork oak cuttings

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Resumo

O sobreiro (*Quercus suber* L.) tem um valor ecológico, social e económico notável na região do Mediterrâneo. Devido ao interesse económico crescente da cortiça, a intervenção humana na produção de plantas e na renovação desta espécie é crucial. Assim, a otimização das metodologias de propagação para produzir árvores selecionadas e melhoradas para a produção de cortiça de alta qualidade é um fator chave no programa de melhoramento da espécie. O enraizamento e a sobrevivência das estacas são muito afetados por vários fatores externos e internos. Para contornar este problema, foram feitos ensaios usando plantas jovens como fonte de estacas de sobreiro, durante dois anos consecutivos. Estudamos a influência da aplicação de auxinas sintéticas, ácido indol-3-butírico (AIB) e ácido α-naftaleno acético (ANA), a concentração de AIB e remoção da casca da parte basal da estaca no enraizamento e sobrevivência das estacas de sobreiro. O AIB melhorou o enraizamento, a sobrevivência e o comprimento médio da raiz mais longa por estaca enraizada, mas não o número médio de raízes primárias produzidas. A remoção basal da casca associada a 0,5% de AIB promoveu a percentagem mais elevada de enraizamento e sobrevivência das estacas (60% e 54%, respetivamente). A aplicação de 0,1% de ANA não teve influência significativa no enraizamento e sobrevivência. Assim, é possível concluir que a remoção basal da casca juntamente com 0,5% de IBA tenha produzido a maior percentagem de enraizamento e sobrevivência com estacas plantadas em abril, porém é necessário efetuar estudos complementares envolvendo diferentes condições físicas e químicas.

Palavras-chave: *Quercus suber* L., remoção de casca, realização de ferida, AIB, ANA, propagação vegetativa.

Abstract

The cork oak (*Quercus suber* L.) has remarkable ecological, social and economic value in the Mediterranean region. Due to the growing economic interest in cork, human intervention in the plant production and renewal of this species is crucial. Thus, the optimization of the propagation methodologies to produce selected and improved trees for high quality cork production is a key factor in the species improvement program. Rooting and survival of cuttings are greatly affected by several external and internal factors. To circumvent this problem, experiments were made using young seedlings as a source of cork oak cuttings for two consecutive years. We studied the influence of the application of synthetic auxins, indole-3-butyric acid (IBA) and α-naphthalene acetic acid (NAA), the IBA concentration and the basal bark removal in rooting and survival of cork oak cuttings. The IBA improved the rooting, the survival and the mean length of the longest root per rooted cutting, but not the mean number of primary roots produced. The basal bark removal associated with 0.5% of IBA gave the highest percent of rooting and plant survival (60% and 54%, respectively). The application of 0.1% of NAA had no significant influence on the rooting and survival. Thus, it is possible to draw the conclusion that wounding along with 0.5 % IBA produced the highest percentage of rooting and survival with cuttings planted in April, however complementary studies involving different physical and chemical conditions are required.

Keywords: *Quercus suber* L., bark removal, wounding, IBA, NAA, vegetative propagation.

Introduction

The species *Quercus suber* L. appears mainly in the Northern hemisphere being characteristic of Mediterranean and subtropical woodlands (Costa et al. 2016) and associated to ecologically fragile areas (Maltez-Mouro et al. 2005). In Portugal the area covered by cork oak is 737 million ha, which represents 23% of the Portuguese forest (ICNF 2013). This area, although representing 50% of the world cork oak area, has been subject to several processes of agricultural land afforestation (18,000 ha) and deforestation of scrub and pasture area (28,000 ha), from 1995 to 2010 (ICNF 2013).

The cork oak is a very polymorphic evergreen tree, extremely long-lived, whose bark, composed by high amount of suberin in the cellular walls, favour water conduction and mechanisms of drought adaptation (Toribio et al. 2005; Zucca 2012). The cork extraction is made from the outer layer of the bark and starts when the tree is adult, approximately 25-30 years (Pereira 2007). This natural and renewable product is intensively used in wine bottling, in tiles and carpets with plastic and sound absorbing properties, and in the textile and the pharmaceutical industry (Moiteiro et al. 2001).

Vegetative propagation is important in horticulture, because the genotype of valuable tree is usually highly heterozygote, and the characteristics which distinguish them are often lost by seed propagation (Hartmann et al. 2011; White et al. 2007). The cork oak forested area increase using improved plant material requires vegetative propagation techniques to enable the use of mass phenotypic selection and clonal propagation of improved material (to estimate heritability and genetic gain and improvement program start), due to the referred species polymorphism (García Valdecantos 1992; Ribeiro 1995).

Some biotechnological approaches have been tested, such as micropropagation via organogenesis (e. g. Manzanera and Pardos 1990; Romano et al. 1992) and plant regeneration via somatic embryogenesis from different plant fragments, however this *in vitro* techniques are limited due to the recalcitrant feature of oak trees and need to be optimized (Álvarez et al. 2007; Jimenez et al. 2013; Jimenez et al. 2011; Manzanera and Pardos 1990). A protocol was developed for the large-scale production of rooted cuttings in some species of the genus *Quercus* L. (Gocke et al. 2008), but, in the case of *Q. suber*, the rooting conditions are inconclusive (Freitas 2002; Romano et al. 1992). Cuttings are one of the most expeditious processes to obtain vegetative copies, although it is necessary to optimize the physiological and environmental conditions of rooting to assure economically viable plants production for the nurserymen (Hartmann et al. 2011). The use of the adequate auxin concentration is a key factor for rooting success in many species, and the ability of auxins to promote adventitious root development in stem cuttings is well known, and has been attributed to enhance transport of carbohydrates to the base of the cutting (Hartmann et al. 2011). Wounding was reported to improve rooting in difficult-to-root species both in quality and quality, with or without auxin application (Hartmann et al. 2011), and in some species a synergistic effect with auxin was observed (e.g. de Silva et al. 2005).

In the current study, we tested the influence of the application of synthetic auxins, indole-3-butyric acid (IBA) and α-naphthalene acetic acid (NAA) and the bark removal from the base of leafy stem cuttings in the percentage of rooting and survival of rooted cuttings with the objective of achieving maximum cork oak propagation success through cuttings. All the experiments were made with leafy stem cuttings taken from young seedlings, due to the need by cloning young plants such as seedlings obtained after controlled pollination and to the difficulty of using adult plant material.

Material and methods

Plant material, leafy stem cuttings preparation and rooting condition

The plants used in this study were produced from acorns harvested in two adult trees located in the Talefa farm, Alter do Chão, Portugal (latitude 39°16'30" and longitude 7°54'00") and in the Lentiscais village, Castelo Branco, Portugal (latitude 39°44'40" and longitude 7°26'00"). After seed germination, the plants with about 14-15 cm long were selected for their uniformity and good physiological condition.

Leaf trimming or removal of some leaves from cuttings has been a common practice to balance the positive effect of photosynthesis and the negative effect of transpiration (Kamaluddin and Ali 1996). Thus, the leafy stem cuttings, about 7 cm long, were left with 4-5 leaves, at least. Afterwards, they were treated with a 6% fungicide solution (Benlate from Sigma-Aldrich, U.S.A.).

The rooting medium, a perlite and peat mixture (3:1 v/v), with a slow-releasing fertilizer (Osmocote Exact from Liscampo, Lisbon, Portugal), was placed in a Melfert bag (AFOCEL - Association Fôret Cellulose, Nangis, France). The rooting experiments were conducted inside a greenhouse with temperature and moisture control, and watering system (Estufas Sol, Lisbon, Portugal). The planted leafy stem cuttings were placed in rooting beds on a bench kept at 28ºC ± 1ºC, under intermittent mist delivered for six seconds every 20 min during natural photoperiod, the first two weeks, afterwards the mist duration and frequency was adjusted to prevent fungal attack and to keep the leaves moist. The leafy stem cuttings (hereafter cuttings) were sprayed weekly with the referred fungicide solution to prevent fungus attack. The greenhouse had sensors for both the air temperature and the relative humidity (RH). The cooling system was activated at 25ºC, and the RH was kept over 80%, with both intermittent mist and cooling system.

Treatments

In the treatments with IBA or NAA, the basal end of the cuttings was dipped into talc powder with the growth regulator at the specific concentration, just before wrapping them up in the Melfert bag. In experiment 1, which started in the beginning of April of the year one, two cuttings were prepared from each donor plant, one from the top and the other one from the base. In one set of cuttings the bark was removed from their basal centimetre with a very sharp knife avoiding cambium damage. The IBA was applied to the base of the cuttings at a concentration of 0.5, 1 and 2%, except for the control group. In experiment 2, performed in the following April, half of cuttings had the bark removed, as described above. Just before planting the cuttings for rooting, 0.5% IBA or 0.1% NAA was applied, except to the control treatment.

Data collection

In each experiment, the data was collected two and three months after planting. The parameters collected were the number of rooted cuttings (R), number of primary roots (NR) and the length of the longest root (LLR), per rooted cutting. Three months after the beginning of every experiment, cuttings, along with the Melfert, were placed in a plastic bag filled with standard nursery soil. Five months after the beginning of the experiment 1, the percent survival (number of rooted cuttings surviving in relation to the initial number of cuttings planted per treatment) was recorded.

Experimental design and statistical analyses

The experimental design was completely randomized. In experiment 1, twelve cuttings were employed per treatment, with three replicates, and two factors, the IBA concentration (0, 0.5, 1 and 2%) and the basal bark status (intact and removed). In experiment 2, twenty-five cuttings were the sample size, with five replicates, and two factors, the synthetic auxin (IBA and NAA) and the basal bark status (intact and removed). The data distribution was verified with the Kolmogorov-Simirnov test and for the statistical analysis, the fix model of analysis of variance was performed in the STATGRAPHICS statistical package, version 6.0, with the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta_{ij}) + \varepsilon_{ijk}$$

Where Y_{ijk} = the observed value, μ= the population mean, α_i, β_j = the main effects of the two factors, $(\alpha\beta)_{ij}$ = the interactions of the two factors, and ε_{ijk} = the residual deviation (Sokal & Rholf, 1981). The Duncan's new multiple range test was applied in testing all pairs of means, with a significance level of 5% (Steel & Torrie, 2006).

Results and discussion

Our trials showed that for the IBA at a concentration of 0.5% and 1%, in experiment 1, the percentage of rooting after three months was 54 and 52% ($p<0,05$), respectively, while with IBA at 2% the result was 38%. The percentage of survival after five months was 48 and 50% ($p<0,05$) for 0.5% and 1% IBA concentration, respectively (fig. 1A). Therefore, the application of 0.5% and 1% IBA increased the percent of rooting and survival (fig. 1A) and supported the evidence that rooting cuttings in this species seems to be sensitive to exogenous auxin, when planted in April. The adventitious rooting is a multifactorial response that leads to the formation of new roots at the base of stem cuttings and to the formation of an autonomous plant, but requires a high concentration of auxin (da Costa et al. 2013). Thus, in difficult-to-root species, the use of an appropriate concentration of exogenous auxin may be a decisive factor for the rooting success, due to the increase in transport of carbohydrates to the base of the cutting (Hartmann et al. 2011). Nevertheless, Romano et al. (1992) with young cork oak cuttings obtained rooting percentages, after three months, lower than those presented here, ranging from 5 to 25%, with no significant differences among the hormonal treatments. These results may be due to the absence of responsiveness of the cuttings to exogenously applied auxin before bud break (Romano et al. 1992). Some authors found that the effect of auxin depends on the application timing, with or without interaction of other factors (sensitivity of tissues, auxin protectors, rooting promoters and others). Additionally, in the current study, the application of 0.1% NAA had neither influence on the percent of rooting nor in percent of survival (fig. 1C). Since we used only one

NAA concentration, it is not possible to say whether the lack of responsiveness was due to an inappropriate NAA concentration or to the lack of responsiveness of the cork-oak cuttings to NAA in general. Blazich et al. (1983) further suggested that IBA is more efficient than NAA in promoting rooting. Comparison of the two auxins is not easy because many factors can condition their action mechanism.

In our study, the bark removal seems to improve the sensibility of tissues to the exogenous IBA application, promoting the percent rooting, when the right concentration (0.5% IBA) and propagation date were used. In experiment 1, after two months, the bark removal favoured LLR with a mean length of 54.4 mm (fig. 2B), while in unwounded cuttings it reached only 21.5 mm (fig. 2B). Nonetheless, in experiment 2 there was no difference between both values, 78.8 mm and 73.3 mm (fig. 2D). In both experiments from our study, it was noted after three-month time, that bark removal improved the effect of IBA application on rooting, minimum 60% of rooting and 54% survival were obtained (table 1). The initial stages of adventitious rooting in various organs necessarily include wound response and water stress that can initiate the necessary hormonal changes that contribute to reprogramming the cells competent to respond to the rooting stimulus (da Costa et al. 2013). In the hours following the cut, there is an increase of phenolic compounds and auxin in the base of the cutting associated with a low transient peroxidase activity and with the establishment of a reservoir of carbohydrates in this area (da Costa et al. 2013 and references included). Nevertheless, with NAA application and bark removal, 37% of the cuttings rooted, a value not different from the control (table 2). Mackenzie et al. (1986) reported that mechanical wounding seems to improve auxin penetration besides rooting ability in apple cuttings. With peach cuttings, Testolin et al. (1988) observed an exponential increase of the rooting in wounded and IBA treated cuttings. The results we obtained with the *Q. suber* experiments are like those reported for other species and, thus, wounding, especially in some difficult-to-root species can induce a better rooting, both in quality and quantity, and act synergistically with auxin, through the physical alteration and chemical environment, which may be more efficient in tissue differentiation and induction of root primordial (e.g. Dirr and Heuser 1987). In the present study, cuttings were taken from very young plants, once the rooting ability generally depends on the age of the plant material and the ability to form adventitious roots is often lost during the change of stage from the juvenile to the adult tree (Abu-Abied et al. 2014). In addition, it has been reported that juvenile material need a lower IBA concentration (0.5%) than the older material (1%) (Freitas 2002).

Conclusion

Wounding along with 0.5% IBA produced the highest percentage of rooting and survival with cuttings planted in April (after bud break). Nevertheless, further studies including the relationship between propagation date and IBA application, the use of different auxins, especially NAA in other concentrations, different types of wounding, the age of the cutting, rejuvenation processes and other physical rooting conditions (rooting medium, fog vs. mist, etc.) are needed to have a better understanding of the rooting process on cork oak cuttings.

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Table 1 - IBA concentration effects (0, 0.5, 1 and 2%) and basal bark removal (bark and no bark), after two and three months on rooting (%), and after five months on survival (%), for Experiment 1. Values followed by different letters are significant by Duncan's test considering the different treatments (P<0.05).

Bark removal	AIB (%)	Time of observation		
		2 months	3 months	5 months
No bark	0	13 b	25 b	21 b
	0.5	42 a	67 a	54 a
	1	33 ab	50 ab	50 ab
	2	33 ab	46 ab	38 ab
Bark	0	8 ab	17 b	17 b
	0.5	38 a	42 ab	42 ab
	1	17 ab	54 a	50 a
	2	4 b	29 ab	21 ab
Sig.		*	*	*

Table 2 - The effects of auxin application (no auxin, 0.5% IBA and 0.1% NAA) and basal bark removal (*bark* and *no bark*), on rooting (%), after two and three months (A), and on survival (%), after five months (B), for Experiment 2. Values followed by different letters are significant by Duncan's test considering the different treatments (P<0.05).

Bark removal	Auxin	Time of observation		
		2 months	3 months	5 months
No bark	No auxin	36 b	44 b	44 b
	0.5% IBA	57 a	61 a	61 a
	0.1% NAA	23 b	37 b	37 b
Bark	No auxin	21 a	23 b	21 b
	0.5% IBA	27 a	41 a	40 a
	0.1% NAA	19 a	34 ab	33 ab
Sig.		*	*	*

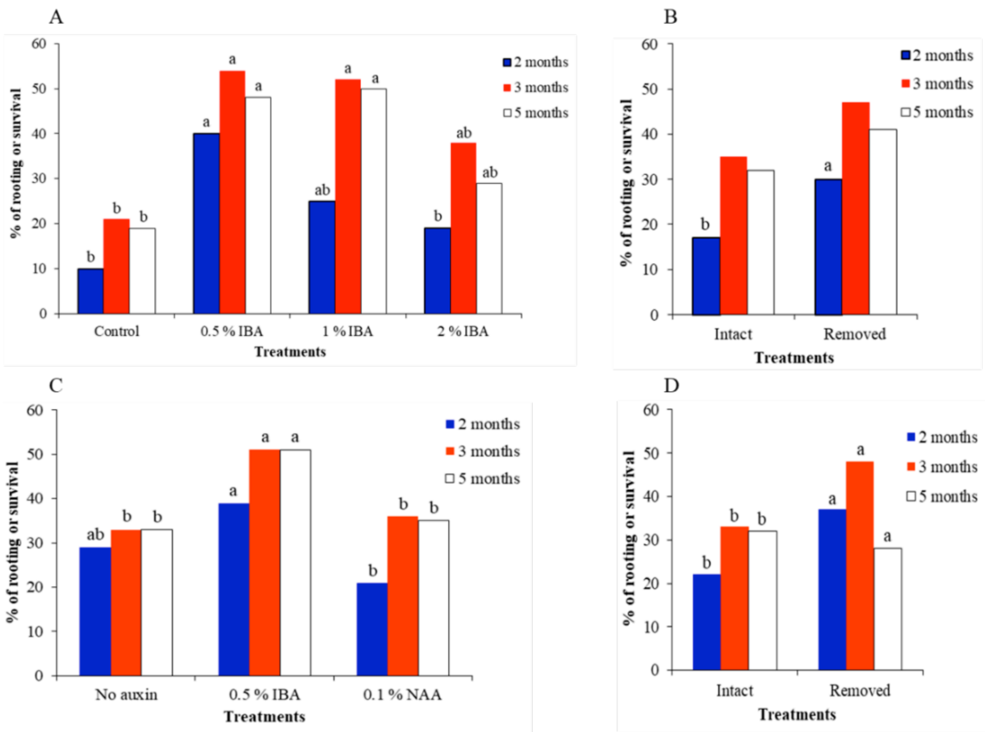

Figure 1 - Simple effects of IBA concentration, basal bark removal and two different growth regulators application (IBA or NAA) on the percentage of rooting, after two and three months and on the percentage of survival after five months, for Experiment 1 (A and B) and 2 (C and D). Values followed by different letters are significant by Duncan's test considering the different treatments (P<0.05).

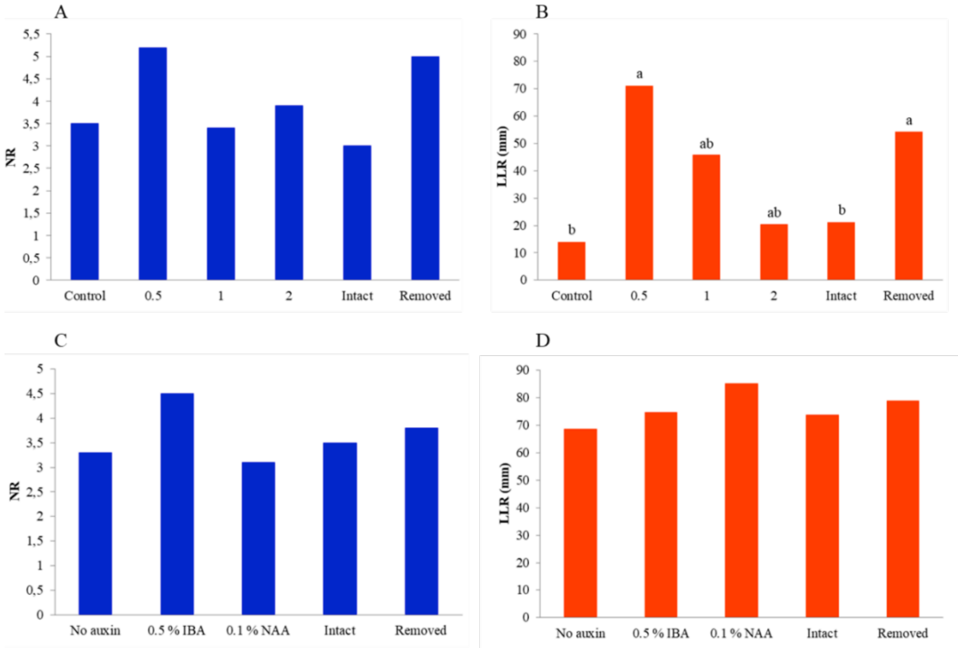

Figure 2 - Simple effects of IBA concentration, basal bark removal and different auxin application (NAA) on the mean number of primary roots (NR) and mean length of the longest root, in mm (LLR), after two months, for Experiment 1 (A and B) and 2 (C and D). Values followed by different letters are significant by Duncan's test considering the different treatments (P<0.05).