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Forestry seedlings production by biotechnological methods, the forestry of 21th century in Albania.

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Abstract

The forest biotechnology on the Faculty of Forestry Sciences is an interdisciplinary research dedicated to the development and application of advanced technology for the enhancement of forest regeneration and adaptation. Technical capabilities include Eco physiology, forest ecology, tissue culture and adaptation to climate change mitigation. These techniques are also being used to improve nursery culture regimes, pest's management by biological fighting, planting regimes and new product development for a variety of broadleaf species (Oaks under ecological stress). Successful regeneration relies on the application of work from many forestry disciplines toward the common goal. At the center of any successful program is the production of high-quality seedlings that have god performance on reforestation site. Performance of an reforestation site depends on seedlings growth potential to be expressed. Seedlings growth potential is influenced by the inherent genetic make-up of source material and the culture used during nursery development. If these attributes can be directed toward improving seedling growth on a reforestation site, then the potential productivity of reforestation/afforestation will be increased. Disciplines that are oriented toward improving these faces of producing high-quality seedlings are the main focus of Forest biotechnology, the main objectives of which have been: (i) defining forest species through ecophysiological parameters; (ii) developing advanced propagation systems through somatic embryogenesis tissue culture technology; (iii) applying ecophysiological assessment techniques in supporting seedling production, improved quality and reforestation site performance. The first results, presented in this paper, have been optimistic, but still the profound studies are needed. The main benefit impacts using biotechnology methods in forestry are: (i) a model of sustainable development, (ii) maintaining forest biodiversity, (iii) extending to new areas. As the conclusion the Commercialization of biological technologies for forest tree species promise to dramatically lower raw material costs, maximize processing efficiencies, minimize environmental impacts, and improve product performances

Key words: tissue culture, ecophysiology, forest oak species, cost effectiveness.

1. Introduction

Genetic engineering is the physical isolation, modification, and asexual transfer of genes. It allows new, qualitative changes to trees to improve productivity and pest management traits [4]. As a new method for improving the productivity and adaptability of planted forests, it could be important to meet future challenges for preserving biodiversity via protection of wild forests from exploitation; carbon sequestration, replacing fossil energy and materials with renewable generated biomaterials; providing new means for bioremediation of lands and waters polluted; and producing trees that are able to cope with a variety of biotic and abiotic anthropogenic stresses [9].

Biotechnology may augment traditional tree improvement activities by providing valuable information to tree breeders and supplementing the production of high quality seed. Somatic embryogenesis tissue culture technology is the most recent vegetative propagation system to be implemented on an operational scale. The term somatic refers to embryos developing asexually from vegetative (or somatic) tissue. Somatic embryogenesis technology was developed for conifer tree species in the late 1980s, originally on spruce species (*Picea* spp.) [3]. Since then, somatic embryogenesis of tree species has expanded to encompass both conifer and hardwood species.

The benefits from the introduction of biotechnology to forestry are potentially large. The economic benefits resulting from the introduction of forest biotechnology are lower costs and increased availability of wood and wood products. Additionally, innovations in forest biotechnology have the potential to address important environmental issues, including the rehabilitation of habitats altered by disease, or invasive exotics. Biotechnological innovation in forestry falls into three main areas: the use of vegetative reproduction methods; the use of genetic markers; and the production of genetically modified organisms (GMOs), or transgenic trees. Most of the biotechnologies used in forestry today involve vegetative reproduction through tissue culture and molecular marker applications.

The advantages of using those methods are: (i) fast, effective and ever-seasonal propagation of elite clones, selected for practical interests, as well as autochthonous plant species; (ii) production of clones free from diseases, especially viruses; (iii) the genetic improvement of the cultivated plants, also using wild species and genetic resources in the gene introgression; (iv) the establishment of *in vitro* collections for germplasm preservation of wild, endemic, rare and threatened plants.

Our forests are under pressure from global trade, population growth, invasive threats, and increased demand on natural resources. Biotechnology is being used as a tool to grow trees with special characteristics.

The forest biotechnology on the Faculty of Forestry Sciences is an interdisciplinary research dedicated to the development and application of advanced technology for the enhancement of forest regeneration and adaptation. Technical capabilities include ecophysiology, forest ecology, tissue culture and adaptation to climate change. These techniques are also being used to improve nursery culture regimes, pest's management by biological fighting, planting regimes and new product development for a variety of broadleaf species (Oaks under ecological stress).

Successful regeneration relies on the application of work from many forestry disciplines toward the common goal. The aim of the program was the production of high-quality seedlings that have god performance on reforestation site. Performance of an reforestation site depends on seedlings growth potential to be expressed. Disciplines that are oriented toward improving these faces of producing high-quality seedlings are the main focus of forest biotechnology, the main objectives of which have been: (i) Defining species through ecophysiological parameters; (ii) Developing advanced propagation systems through somatic embryogenesis tissue culture technology,

Applying ecophysiological assessment techniques in support of seedling production, improved quality and reforestation site performance. The first results, presented in this paper, have been optimistic, but still the profound studies are needed. The main benefit impacts using biotechnology methods in forestry were: (i) a model of sustainable development; (ii) maintaining forest biodiversity; (iii) Extending to new areas. As the conclusion the Commercialization of biological technologies for forest tree species promise to dramatically lower raw material costs, maximize processing efficiencies, minimize environmental impacts, and improve product performances.

2. Material and methods

To successfully realize the objectives of this study, first there is build up an efficient infrastructure on the Faculty of Forestry Sciences, the establishment of the trinomial laboratory of forest Botany (good equipped) capable to undertake such studies), Greenhouse for the plantation of plantlets (prepared to the laboratory) and nursery (AUT territory). For the establishment of tissue culture techniques, with Institute of the Biological Research are cooperated [5]. Financial support is provided by NATO for Stability Program, in cooperation of Agriculture University of Athens.

The vision of the study was: To develop low cost methodologies for improving vegetation which will result in functional ecosystems in far degraded Mediterranean areas by means of physiological studies and application.

Among the organizational work, much effort was directed in the establishing of the nursery and greenhouse at the Agricultural University of Tirana, Faculty of Forestry Sciences.

The main objectives to be done were:

1. Seed germination physiology and seedling stress selection, under which the following tasks were determined: (i) to study the seed germination dependency on temperature and humidity; (ii) to develop methods releasing germination beanies in species candidate for improving vegetation; (iii) to study, in the some species, the sensitivity of the germinating seeds and the seedlings on extreme temperatures; (iv) to select drought and cold resistant seedling among populations of different geographical origin; (v) to study the seeds germination and seedlings development on the different lighting and darkness conditions.

2. Nursery and greenhouse organization within the Agricultural University of Tirana, in order to product plant materials, under which the main tasks

were: (i) to provide GR-Revegetation (Albania) program with plant materials needed for out or indoors experiments; (ii) to provide with reforestation materials, governmental, municipal or private activities for upgrading Mediterranean ecosystems; (iii) to apply and if is necessary to develop modern micropropogation techniques in order to possibly lower the production and cost of these materials, proper for rehabilitation of forest vegetation; (iv) to propagate the knowledge and experience gained, among governmental and municipal, as well as private enterprises.

3. Seed germination seedling or plantlet survival under ecosystem condition; under which the main tasks were: (i) to organize the main experimental field within Agricultural University territory, adequately large, to allow the performance of experiments for a number of years; (ii) to provide with the scientific instruments for the measuring of the climatologic parameters, as well as the water potential status of the growing plants; (iii) the organization of the experimental fields at selected geographical regions of Southern Albania; (iv) to find out among the species of the local flora, the ones most proper for upgrading Mediterranean ecosystems. Also to develop methodologies and determine the intervention times for obtaining cost flowering and high survival rates for seedlings or pantalets. (v) to accommodate experiments for testing plant materials derived through colonel and stress selection.

The species and ecosystems selection is done based on strict criteria and indicators, identified on the preparatory phase: (i) large amplitude of the natural distribution; (ii) international principles of the conservation of wild life; (iii) rarity, (iv) vulnerability; (v) naturalness; (vi) presence of endemic species; (vii) typicality; (viii) particular interest; (ix) diversity; (x) stability; (xi) position of the ecosystem on the ecological structure of the region.

The main emphasis of tissue culture method is to use somatic embryogenesis to develop propagation systems of oak trees. These systems allow the mass propagation of elite families from tree breeding programs and the selection of superior clones that can be stored and propagated in a sustained manner [1].[2].

Valonia oak (*Quercus macrolepis* Kotchky) and Holm (*Quercus ilex* L.) oak have been studied as autochthonous forest species threatened to disappear. The intervention in degraded forests by way of reforestation will considerably improve the structure of that very important vegetation of the south coast of Albania [10].

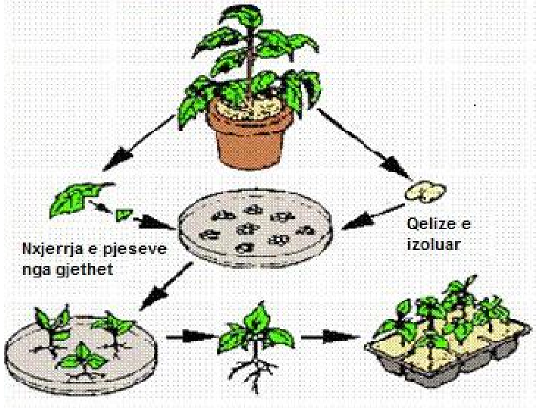

Figure 1. Methods of micro propagation

Our preliminary experiments aim to compare different micro propagation and embryogenesis methods for the production of oak trees seedlings. There are used two methods: (i) 2,4-D 0,1 mg l-1; (ii) 2,4-D 0,1, BAP and IAA 1. The embryonic axis are planted on MS medium diluted twice, adding by GA3 and BAP (1mgl-1 each).

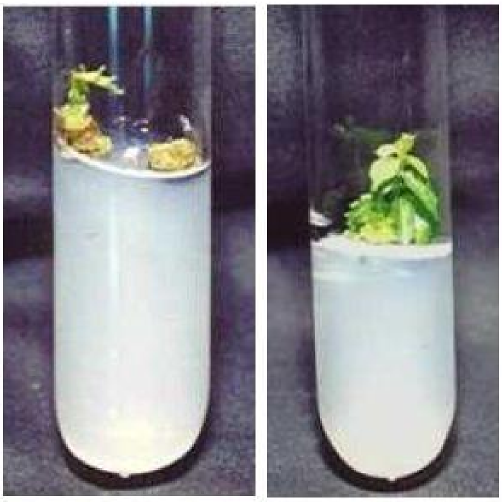

Figure 2. Oak's plantlets micropropagated

3. Results

From the investigation, studies and experiments provided results that: (i) Velipoja and Divjaka are the best location for the collections of Common Oak (*Quercus robur* L.) seeds; (ii) Butrinti, Kepi I Sillos and Ksamil are the best locations for the collection of Holm Oak (*Quercus* ilex L.) seeds; (iii) Dukat-Dhermi is the best location for the collection of Valonia Oak (*Quercus macrolepis* Kotchky) seeds; (iv) Liqenas and L. e Vjoses are the best locations for the collection of Macedonian Oak (Quercus trojana Webb.) seeds.

Table 1: Site selection for the collection of tree seeds

Location	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Velipoje	**	***	**	*	***	***	**	***	**	**
Kune-Vaine	***	**	*	*	**	***	**	***	*	**
Divjake	**	***	***	***	***	***	**	***	**	***
K. Libofshe	***	***	*	*	**	*	*	**	*	*
Butrint	*	***	***	**	***	***	*	***	***	***
Liqenas	**	***	*	**	***	***	***	**	**	***
L. e Vjoses	**	***	*	**	***	***	***	***	**	**
Ksamil	*	***	***	*	***	***	*	**	***	***
Kepi i Stilos	**	***	*	*	***	***	*	***	**	**
Dukat-Dher.	*	**	*	*	**	**	**	***	**	**

There are used the upper parts of the apical buds of Holm and Valonia oaks, planted on the greenhouse of AUT, as well as buds or vegetative pieces of leaves taken directly on the natural ecosystems. The vegetative propagation of the selected lines was an effective method for the production of clones and the breeding of those trees. Below are shown some preliminary data on the micro propagation of explants of *Valonia* and *holm* oaks. One year or 6 month old *Valonia* and holm oak plants were used, produced by seeds germinated in screenhouse. Knots 20 mm long, with lateral buds and meristems were cut. MS medium was diluted twice by adding (in mg.l-1) 100 mio-inositol; 0.5 nicotinic acid; 0.5 pyridoxine HCl; 0.1 thiamine HCl; 2 glycine; 30 g l sugar, 0.6% agar. Two different concentrations of phyto-hormones were used: a) 2,4-D 0.1 mg l $^{-1}$ and b) 0.1 mg l of 2,4-D (acid 2,4- dichlorophenoxyacetic); combined with 5 mg l-1 of BAP and 1 mg l of IAA (indol-acetic acid) [11]. In all the above cases the explants produced calluses of different forms and size with color from beige to green. By comparing the elementary growth of the explants it was observed that one-year-old plants reacted better than the juvenile ones. Among the meristems, that appeared to be more developed than the knots, it was observed that they primarily produced calluses, and then appeared the embryos and finally the new buds. During the process of organogenesis, developed green plants with lateral twigs were formed.

While comparing the plants grown with different phyto-hormones the development was stimulated when two auxins (2,4-D and IAA) and cytokinin BAP were combined. Such a combination promoted not only the formation of callus, but also the fast formation of new embryos and buds.

Some of the preliminary results:

(i) it was showed callus-genesis of the explants on the presence of 2,4-D auxins, on the case of a unique node cutting. Calluses have been different in size and color (from green decelerated to green) The plantlets are good growth, particularly on the first variant, in which two auxines are combined (2,4-D and IAAA) and BAP. These combinations stimulate not only the development of calluses, but the quick buds creation. The growth tissues ate passed to organogenesis. The plantlets provided from the growth tissues were good developed and green color.

The comparison propagation indicator, for explants of both planted oak species, at the same medium, demonstrate that the explants of Valonia Oak (*Quercus macrolepis* Kotchky) are regenerated feeble compare with those of Holm Oak (*Quercus ilex* L.), because the high tannin continence. Brown hue, in the initial phase agar culture of Valonia Oak (*Quercus macrolepis Kotchky*) explants also dedicated high tannin's presence.

In embryonic axes culture, on the first stage, a callus and tissue mass production were observed. Somatic embryogenesis or shoot formation did not occur in this tissue.

We have faced difficulties in *in vitro* culture because of tannin production; others have managed to successfully use the propagation by explants or somatic embryogenesis by embryo axis [8].

The comparison of index for the new plants formed out of *Valonia* and *holm* oak explants indicated that the former reacted more effectively towards the "*in vitro*" culture. Embryos for the two forest trees were inoculated in the following experiments, which according to the literature [8]. were considered as the most effective elementary explants for the production of *Valonia* and *holm* oak plants.

4. Conclusion

The benefits of biotechnology in forestry go beyond economic advantages—including increased production, lower costs to consumers, and trees modified for easy processing or specific production values—to such environmental benefits as helping to preserve biodiversity and mitigate global warming [6].

Ecological viewpoint, biotechnological methods are efficient tools for the production of forest tree seedling, under ecological stress, necessary for the rehabilitation of damages and vulnerable ecosystems, as well as rare trees and those which have low seeds production.

Economic viewpoint, biotechnological methods can be successfully use on forest nurseries for low cost and high quality seedling productions [7].

From both Mediterranean oak trees, Holm Oak (*Quercus ilex* L.) and Valonia Oak (*Quercus macvrolepis Kotchky*), the best results are achieved on the first one. The weak results on the micro-propagation of Valonia Oak (*Quercus macrolepis Kotchky*), dues to the high countenance of tannins. In this context the experiment with the countenance of the medium (MS) must to be continued.

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Biotechnological innovations also raise concerns about biosafety and the effects of transgenic plants on the resistance of pathogens and on the natural ecosystem, particularly the question of genetic exchange between domestic and wild populations.

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