Innovat International Journal Of Medical & Pharmaceutical Sciences, 2016; 1(1)

www.innovatpublisher.com

INTRODUCTION ABOUT PINUS ROOT

Review Article

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Received 2016.10.27-Accepted 2016.11.18

Abstract

Pinus roxburghii is a large tree reaching 30–50 m (98–164 ft) with a trunk diameter of up to 2 m (6.6 ft), exceptionally 3 m (10 ft). The bark is red-brown, thick and deeply fissured at the base of the trunk, thinner and flaky in the upper crown. The leaves are needle-like, in fascicles of three, very slender, 20–35 cm (7.9–13.8 in) long, and distinctly yellowish green. The cones are ovoid conic, 12–24 cm (4.7–9.4 in) long and 5–8 cm (2.0–3.1 in) broad at the base when closed, green at first, ripening glossy chestnut-brown when 24 months old. They open slowly over the next year or so, or after being heated by a forest fire, to release the seeds, opening to 9–18 cm (3.5–7.1 in) broad. The seeds are 8–9 mm (0.31–0.35 in) long, with a 40 mm (1.6 in) wing, and are wind-dispersed The environmental and seasonal effects on anatomical traits of *Pinus taeda* L. seedling roots were studied in the laboratory in three contrasting root growth media and also in typical outdoor nursery culture. Growth media with lower water regimen and high penetration resistance caused a reduction in lengths of the white and condensed tannin (CT) zones and acceleration of development of suberin lamellae in the endodermis. As a possible counter to this reduction in zone lengths, second-order laterals were produced closer to the tips of first-order laterals. This suggested there may be an advantage to producing more shorter roots under stressful conditions. Under outdoor nursery conditions (June to mid-December) the white zone was always a rather small part of the root system surface area (4.5% in December), but it dominated as a provider of cortical plasmalemma surface area (CPSA) in contact with modified soil solution (65% in December) because of its live cortex and capacity to increase nearly three fold the amount of CPSA per unit root length. The CT zone always provided most of the total root surface area (80% in December).

Key word: Pinus, root, Fungi.

INTRODUCTION

Pinus roxburghii (known as **chir pine**) is a species of pine. It is native to the Himalayas, and was named after William Roxburgh.

The range extends from northern Pakistan (North-West Frontier Province, Margalla Hills, Islamabad Capital Territory, Murree), across northern India (Jammu and Kashmir, Punjab, Himachal Pradesh, Uttarakhand, Sikkim) and Nepal to Bhutan.

It generally occurs at lower altitudes than other pines in the Himalaya, from 500–2,000 m (1,600–6,600 ft), occasionally up to 2,300 m (7,500 ft). The other Himalayan pines are *Pinus wallichiana* (blue pine), *Pinus bhutanica* (Bhutan white pine), *Pinus armandii* (Chinese white pine), *Pinus gerardiana* (chilgoza pine) and *Pinus densata* (Sikang pine).

Mycorrhizas are symbiotic structures formed between plant roots and fungi that act as an extension of absorption system, where the fungal partner obtains photosynthetic sugars from the host plant while, in return, the plant receives mineral nutrients from the fungus (Smith and Read, 1997). Ectomycorrhizas (ECM) are the main absorption organs in conifers, and the exchange of nutrients occurs in a specific structure that is formed between the fungal hyphae and the outer root layers. This structure is known as the Hartig net, which is formed by the hyphae penetrating from the surrounding mantle into the root apoplast (Smith and Read, 1997; Brundrett et al., 1996). This mutualistic relationship with fungi grants conifers an ecological advantage to withstand the harsh living conditions.

Stone pine is an extremely appreciated edible nut producer and one of the major plantation species in the Iberian Peninsula. Achieving its clonal propagation is a major goal in the biotechnological development for this species, but has met overwhelming difficulties. The complex interactions between pines and their ECM partners suggest that they might be capable of overcoming such difficulties. The objective of our work was to ameliorate the in vitro adventitious rooting (which does not develop well in the agar cultures) by co-culturing with ECM fungi collected from Stone pine stands (Oliveira et al., 2003). It was also to characterize the fungi-root interactions that enable the development of roots in microshoots and to identify the signalling mediators between ectomycorrhizal fungi and stone pine roots. These potentially new insights into the interactions that take place in the pine rhizosphere could allow us to develop an axenic system that mimics the promoting effect of ECM fungi Brundrett and , Kendrick (1988) .

MATERIALS AND METHODS

Plant Material

Mature seeds of stone pine were obtained in March 2007 from selected 'plus' trees (Alcácer do Sal Region, Portugal) and were stored in a cold chamber at 4°C until used.

Shoot Organogenesis

For the description of shoot organogenesis, see Oliveira et al. (2003) and Ragonezi et al. (2008).

Root Organogenesis

Microshoots were placed in rooting medium. The media used were WPMRI and WPMRE (woody plant medium root induction and expression, respectively) (Ragonezi et al., 2008).

Fungi

Fungi were isolated from single ectomycorrhizas as described by Oliveira et al. (2003) collected at the same location as the cones used for obtaining mature seeds for organogenesis experiments. The fungi were maintained in pure culture using standard procedures (Brundrett et al., 1996). Sixty-five independent isolates were obtained (unpublished results) and screened for their effect on root growth, many of which are included in the present study.

In Vitro Co-Culture

Following the rooting induction and expression phase, the individuals that showed root development were transferred to the double-layer medium and, after a brief period of adaptation to the medium, inoculated with selected fungi, with matching controls to monitor root growth in the absence of inoculation (Oliveira et al., 2003).

Acclimation

The plantlets were transferred to vermiculite for two weeks and then transferred two different substrate systems to observe the development of the root system: mixed substrate in containers, or peat in a rhizotron (Finlay and Read, 1986). During acclimation plants grew in a growth chamber at 25/19°C day/night temperatures, with 16 h photoperiod (270 µmol s-1m-2) for 10 weeks. The relative humidity of the growth chamber started at 80% and gradually decreased to 60%. Plants were watered as required with alternating sterile water and liquid WPM (macronutrients only) Wilcox 1968. .

Rhizotron

Rhizotrons allow the visualization of root development whenever desired without disturbing the normal functions of the plants. Basically, they are made of two acrylic plates, 20 x 20 cm each, with interval made by 5 mm spacers and filled with turf, in order to support and feed the plants, as suggested by Finlay and Read (1986) with some adaptations from Bending and Read (1996). The root growth was observed thereafter for 4 to 6 weeks, the time required for most of the plants to explore the available space in the rhizotron. Rhizotrons were very useful for the identification of target structures, as well as other symbiotic features that could then be extracted for further studies with minimal disturbance to the rest of the root architecture.

Axenic Root Cultures

Root segments of 2 cm long obtained from germinated seeds were excised and were cultured in liquid medium in an orbital shaker (125 rpm) according to Kaska et al. (1999) for 3 to 4 weeks. Afterward, the roots were photographed and used for histological comparison.

Fig 1: Sshow pinus in Iran

RESULTS AND DISCUSSION

Pinus roxburghii is a large tree reaching 30–50 m (98–164 ft) with a trunk diameter of up to 2 m (6.6 ft), exceptionally 3 m (10 ft). The bark is red-brown, thick and deeply fissured at the base of the trunk, thinner and flaky in the upper crown. The leaves are needle-like, in fascicles of three, very slender, 20–35 cm (7.9–13.8 in) long, and distinctly yellowish green.

The cones are ovoid conic, 12–24 cm (4.7–9.4 in) long and 5–8 cm (2.0–3.1 in) broad at the base when closed, green at first, ripening glossy chestnut-brown when 24 months old. They open slowly over the next year or so, or after being heated by a forest fire, to release the seeds, opening to 9–18 cm (3.5–7.1 in) broad. The seeds are 8–9 mm (0.31–0.35 in) long, with a 40 mm (1.6 in) wing, and are wind-dispersed.

Pinus roxburghii is closely related to *Pinus canariensis* (Canary Island pine), *Pinus brutia* (Turkish pine) and *Pinus pinaster* (maritime pine), which all share many features with it. It is a relatively non-variable species, with constant morphology over the entire range.

Adventitious roots regenerated by stone pine microshoots as well as axenic embryo root cultures developed mycorrhizal- like (dichotomous) root laterals even without fungal infection Kumar 2003 . This is not exceptional and was observed for intact plants as well as for excised roots. In the former, these structures appeared in all experimental settings tested, with the frequency of dichotomous branching increasing as a consequence of a reduction of macro-nutrients in the medium (compared with complete macronutrients) and also in cultures that spent more than one month on the same co-culture medium. These results suggested that without any fungus interaction, some stone pine genotypes responded to less favourable in vitro growing conditions (i.e., lower nutrient concentration and/or lower water availability) by producing mycorrhizal coralloid-like structures. Also coralloid structures appeared as a consequence of applied naphthalene acetic acid (NAA) in the induction medium Taylor and Peterson (2000)

On the other hand, under in vitro co-culture (pine-fungus) there was a great variability in the plant response. Some combinations of pine clones with specific fungi showed dichotomous branching while others did not .

After a few months in substrate, at the moment of transferring to larger containers, dichotomous ectomycorrhizal-like root tips were detected for several inocula, each producing a different rootlet type, indicative of specificity in the development of these structures as a function of the provided inoculum. Barrowclough et al. 2000.

Fig 2: Show Male cones in pinus

At least in these earlier stages, non-inoculated controls were devoid of such structures (Fig. 3). Rhizotron-contained plants did not show any such developments. A careful examination of all ECM- like morphotypes failed to reveal Hartig net anatomical features, thus not confirming the symbiotic nature of these structures. The mycorrhizal potential of the fungal inocula is still under investigation, thus not allowing to determine whether that is due to the taxonomical identity of the fungi or the mimicry of dichotomous development by ECM fungi which might start a symbiotic relationship but failed to persist in that condition . Ashford et al. 1988.

Some plants presented a great number of monopodial short roots with root hairs; others dichotomous branching without hairs and also dichotomy with hairs (Figs. 4a, b, c). Only through the histological observations it was possible to distinguish between true mycorrhizal symbioses and mycorrhizal-like structures as well as ectoendomycorrhizae, the latter not correlating with a particular co-culture inoculum, thence probably from contamination with E-type propagules (Figs. 5a, b).

Axenic root cultures showed profuse dichotomous branching similar to those of in vitro cultured plants (Fig. 6).

Many environmental or cultural conditions could have influenced the capacity of some stone pine clones to produce mycorrhizal-like structures. At the current stage of our study it is impossible to know if there is a correlation between pine clones and their propensity to form mycorrhizal-like structures. Responses to the inocula used in the co-culture (either the resumed growth of roots previously formed as a consequence of plant growth regulators treatment, or dichotomous branching) was not consistently correlated with the induction of mycorrhizal symbiosis in the acclimation phase. Nevertheless, the

characterization of biochemical signals that are likely mediating these effects will continue to be pursued, in order to understand the possible physiological implications for the plants from the development of these structures.

CONCLUSIONS

- This is the first report on the abundant mycorrhizal-like structures in stone pine roots that were produced by axenic cultures, in in vitro-cultures and in subsequent acclimation phase in mixed substrates.

- There was a strong similarity between extensive dichotomous and coralloid branching of lateral roots that grew spontaneously in stone pine with those derived from fungal inoculation.

- Due to this similarity it may be difficult to diagnose ectomycorrhizas without confirmation of the ECM status by histological analysis.

- Since this response appeared to be ‘genotype dependent’ more studies will be needed to establish correlation between stone pine clones and the root system morphology.

- The biochemical studies that are being carried out presently on the co-cultured roots could elucidate the nature of the compounds that cause a highly effective adventitious rooting in the presence of certain fungi and afterwards in the acclimation phase.

External Morphology of Pinus

Pinus is a large, perennial, evergreen plant.

Branches grow spirally and thus the plant gives he appearance of a conical or pyramidal structure.

Sporophytic plant body is differentiated into roots, stem and acicular (needle-like) leaves.

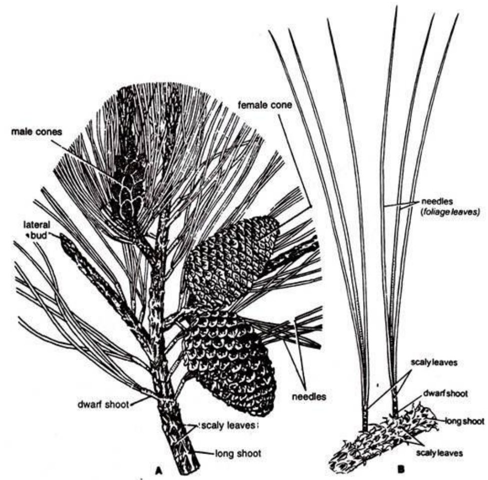

Fig. 26. Pinus gerardiana. A, Branch of a mature plant bearing male and female cones; B, A part of stem showing two types of shoots.

4. A tap root with few root hair is present but it disappears soon. Later on many lateral roots develop, which help in absorption and fixation.

5. The ultimate branches of these roots are covered by a covering of fungal hyphae called ectotrophic mycorrhiza.

6. The stem is cylindrical and erect, and remains covered with bark. Branching is monopodial.

7. Two types of branches are present: long shoots and dwarf shoots. These are also known as branches of unlimited and limited growth, respectively.

8. Long shoots contain apical bud and grow indefinitely. Many scaly leaves are present on the long shoot.

9. Dwarf shoots are devoid of any apical bud and thus are limited in their growth. They arise on the long shoot in the axil of scaly leaves.

10. A dwarf shoot has two scaly leaves called prophylls, followed by 5-13 cataphylls arranged in 2/5 phyllotaxy, and 1-5

***Fig 3: Pinus roxburghii* forest,**

Old trees which die from fire or drought, undergo some metamorphosis in their wood due to the crystallization of the resin inside the heart wood. This makes the wood become brightly coloured (various shades from translucent yellow to dark red) and very aromatic with a brittle, glassy feel. This form of wood known as *jhukti* by the locals is very easy to ignite. (It never gets wet or waterlogged.) They use it for starting fires and even for lighting, as a small piece of this burns for a long time (owing to the high resin content). Of all the conifer species in the area, only this one seems to be ideal for that purpose.

Every autumn, the dried needles of this tree forms a dense carpet on the forest floor, which the locals gather in large bundles to serve as bedding for their cattle, for the year round. The green needles are also used to make tiny hand brooms Addoms (1946) .

The locals of the Jhaunsar region of Uttarakhand have several uses for this tree which is known in the local dialect as *salli*.

It is also occasionally used as an ornamental tree, planted in parks and gardens in hot dry areas, where its heat and drought tolerance is valued

ACKNOWLEDGEMENTS

This work was supported by semnan university in Iran.

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