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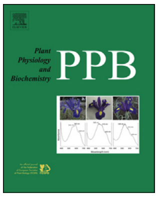

Research article

Water availability influences morphology, mycorrhizal associations, PSII efficiency and polyamine metabolism at early growth phase of Scots pine seedlings

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ABSTRACT

Scots pine (*Pinus sylvestris* L.) is adapted to various soil types with diverse water availabilities. However, Scots pine seedlings are vulnerable to abiotic stress during the early growth, when they may be exposed to both dry and wet conditions. Here, we focused on the above and below ground coping strategies of Scots pine seedlings under controlled wet, optimal and dry soil conditions by investigating morphological traits including seedling biomass, number of root tips, proportion of mycorrhizal root tips and brown needles. In addition, we studied metabolic and physiological responses including gene expression involved in biosynthesis and catabolism of polyamines (PA), PSII efficiency and the expression of the catalase (*CAT*) late-embryogenesis abundant protein (*LEA*), pyruvate decarboxylase (*PDC*), glutamate-cysteine ligase (*GCL*) and glutathione synthetase (*GS*) genes. We found that seedlings invested in shoots by maintaining stable shoot water content and high PSII efficiency under drought stress. Free and soluble conjugated putrescine (Put) accumulated in needles under drought stress, suggesting the role of Put in protection of photosynthesizing tissues. However, the expression of the PA biosynthesis genes, arginine decarboxylase (*ADC*), spermidine synthase (*SPDS*) and thermospermine synthase (*ACL5*) was not affected under drought stress whereas catabolizing genes diamino oxidase (*DAO*) and polyamine oxidase (*PAO*) were down-regulated in shoots. The morphology of the roots was affected by peat water content. Furthermore, both drought stress and water excess restricted the seedling ability to sustain a symbiotic relationship. The consistent pattern of endogenous PAs seems to be advantageous to the Scots pine seedlings also under stress conditions.

1. Introduction

Scots pine (*Pinus sylvestris* L.) is the most widely distributed Eurasian conifer and an economically important source of timber. Scots pine inhabits large variety of soil types, coping with diverse water availabilities. However, pine seedlings are most vulnerable to abiotic stress factors during their early growth, when they may be exposed to both dry and wet conditions. Especially on organic soils water level and peat water retention capacity has unique impact on pine (*Pinus* sp.) forest regeneration success (Saarinen, 2013).

Imbalance in root water uptake and leaf transpiration leads to dehydration of plant tissues (Aroca et al., 2012). Pines are considered as water-savers. They prevent transpiration and oxidative

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Abbreviations: ADC, arginine decarboxylase; CAT, catalase; CI, confidence interval; Cp, crossing point; DAO, diamine oxidase; DW, dry weight; FW, fresh weight; F_0, minimum fluorescence; F_v, variable fluorescence; Fm, maximum fluorescence; GAPDH, glyceraldehyde-tri-phosphate dehydrogenase; GS, glutathione synthetase; LEA, late embryogenesis abundant protein; MD, mean difference; ODC, ornithine decarboxylase; PA, polyamine; PAO, polyamine oxidase; PDC, pyruvate decarboxylase; PSII, photosystem II; Put, putrescine; SAMDC, S-adenosyl methionine decarboxylase; Spd, spermidine; SPDS, spermidine synthase; Spm, spermine; SPMS, spermine synthase; Tspm, thermospermine; TSPMS, thermospermine synthase; TUBA, α-tubulin; UBQ, ubiquitin.

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damage in tissues by strong stomatal control (Poyatos et al., 2008). Although, Scots pine is a xerophyte, the limited plasticity of xylem and needle properties has been suggested to explain its vulnerability to severe decline of water (Martinez-Vilalta et al., 2009). Moreover, some forest trees increase water uptake by extending their root capacity under arid conditions (Hamanishi and Campbell, 2011). Under water excess, instead, transport of oxygen and carbon dioxide can be hindered, which e.g. reduces photosynthesis (Aroca et al., 2012). The lack of oxygen (O_2) in the rhizosphere in flooding conditions switches energy production from mitochondrial respiration to fermentation in plant cells. Up-regulation of pyruvate decarboxylase (*PDC*) gene expression was used to indicate alcohol fermentation in flooding tolerant grey poplar (*Populus* × *canescence*) tissues (Kreuzwieser et al., 2009) under hypoxia.

In pines, dehydration induces many genes such as late-embryogenesis abundant proteins (*LEA*), dehydrins (*DHN*), catalase (*CAT*), water-stress-inducible proteins (*Ip2-3*) and early response to drought protein 3 (*ERD3*) (Lorenz et al., 2005, 2011). LEA proteins, the well-known osmoprotectors (Battaglia et al., 2008), are reported to accumulate under extreme desiccation conditions in higher plants (Yang et al., 2012) including pines (Lorenz et al., 2011; Velasco-Conde et al., 2012). LEA proteins have been grouped to various families on the basis of sequence similarity (Battaglia et al., 2008). Dehydrins belong to group II of LEA proteins and are important during acclimation of pines (Velasco-Conde et al., 2012). Abiotic stresses cause oxidative stress in plant cells, where one of the first responses is accumulation of reactive oxygen species (ROS), like hydrogen peroxide (H_2O_2). ROS are important signalling molecules, but in high concentrations they are toxic. CAT is an antioxidant enzyme, which dissociates H_2O_2 to water and O_2 (Mhamdi et al., 2012). In plant cells the CAT is mainly localized in peroxisomes and has an important role in ROS homoeostasis regulation (Mhamdi et al., 2012). Increased activity of *CAT* has been detected in drought-stressed pines (Lorenz et al., 2005). Furthermore, the oxidative stress induces plants to produce glutathione. The balance between reduced and oxidized forms of glutathione is a central component in maintaining redox state in plant cells (Galant et al., 2011). Glutathione is produced by two step reaction where first GCL catalyses the formation of γ-glutamylcysteine from cysteine and glutamate and then GS catalyses the addition of glycine to γ-glutamylcysteine to yield glutathione.

PA metabolic route is well known in plants (see Supplementary Fig. S1). PAs, Put, Spd, spermine (Spm) and thermospermine (Tspm), are small, positively charged nitrogenous compounds having an important but intricate role in stress and developmental pathways in plants, recently reviewed by Tiburcio et al. (2014). PA metabolism related genes of *Arabidopsis* have been divided to different categories with stress or developmentally related genes (Tiburcio et al., 2014). For instance, expression of *ADC2*, *SPMS* and most *PAO*s correlate positively with stress related genes in *Arabidopsis*, whereas expression of *ADC1*, *SPDSs* and *ACL5* co-express with other genes induced during development (Tiburcio et al., 2014). Thus, both PA biosynthesis and catabolism seem to play a role in stress and development of plants (Tiburcio et al., 2014).

PA levels are strictly regulated by biosynthesis, degradation, conjugation, back-conversion and transport and by interactions with other pathways in response to stress (Tiburcio et al., 2014). The PA metabolic route intermediates nitrogen metabolism, and has interactions with other metabolites, including stress protective compounds, hormones and signalling molecules (Moschou et al., 2012). For instance, accumulation of PAs by abscisic acid (ABA) treatment enhanced PA oxidation, which in turn launched protective effects such as stomatal closure in grapevine (*Vitis vinifera*) (Toumi et al., 2010). Furthermore, ACL5 and Tspm have a specific role in vascular development and stem elongation in *Arabidopsis* (Vera-Sirera et al., 2010) i.e. in PA mediated developmental processes in plants.

Here, we propose that water stress induced responses may be linked to PA metabolism especially during the vulnerable early growth phases of Scots pine. Therefore; we investigated young 6 to 17-week-old pine seedlings in controlled wet, optimal and dry conditions and monitored the changes in biomass, phenotype, and chlorophyll fluorescence (Fv/Fm). We measured the expression of *LEA* and *PDC* genes to describe the severity of the drought stress and water excess, respectively. The expression of *CAT*, *GCL* and *GS* genes was measured to indicate oxidative stress reactions under different treatments. The stress induced changes in the PA gene expressions and seedling morphology were compared to the optimal condition to elucidate the role of PA metabolism under different water levels (wet, optimal and dry) in needles, stems and roots. We found that the unfavourable water conditions changed the seedling morphology, disturbed symbiotic relationships and induced stress related gene expression. However, PA contents were mostly maintained at constant levels which underlines the importance of the strict regulation of PA metabolism for the early development of Scots pine seedlings.

2. Materials and methods

2.1. Plant material and growth conditions

Scots pine (*P. sylvestris* L.) seed orchard (Hiirola, Finland 61°49′N, 27°15′E) seeds were sterilized with 30% H_2O_2. Seeds, 80 seeds per pot, were sown in twelve 3 dm^{-3}, 16.5 cm tall plastic pots (i.e. altogether 960 seeds per 12 pots) (VG-Products, VG-potter, Billund, Denmark) filled with 690 g of 1:9 combination of fertilized (Kekkilä Oy, Vantaa, Finland) and non-fertilized (Biolan Oy, Kauttua, Finland) horticultural peat. Before sowing, the peat in the pots was watered properly with 1.5 dm^{-3} of H_2O and left on a grate until water no longer drained away. The pots were irrigated two to three times a week during the first six weeks. Nutrients [KH_2PO_4 25 mg/l, $(NH_4)_2HPO_4$ 12.5 mg/l, $CaCl_2$ 2.5 mg/l, NaCl 1.25 mg/g, $MgSO_4 \times 7H_2O$ 7.5 mg/l, $FeCl_3 \times 6H_2O$ 0.6 mg/l] were added to irrigation water. Within two weeks the seeds started to germinate and before the first sampling, the average length of the seedlings was 10 ± 4 cm. The photoperiod in the growth chamber was 6 h darkness and 18 h daylight with light intensity of ca. 200 $\mu mol\ m^{-2}\ s^{-1}$ and temperature 25 °C during the experiment. Water content of the peat in all pots was ca. 75%.

2.2. Watering treatments and harvesting

The watering treatments were based on the water retention characteristics of the peat (Heiskanen, 1993) determined with a pressure plate apparatus (soil moisture Equipment Corp., USA). The water retention curve indicates the level of suction required by the plant to get water from the soil as it dries (see Supplementary Fig. S2). The following watering treatments were planned to describe water availability to the pine seedlings: wet, optimal and dry.

In the wet treatment, the volumetric water content of the peat was kept as high as possible without reaching the minimum air-filled porosity level (20 vol.%) (Wall and Heiskanen, 2003). In the dry treatment, the water content was kept above the wilting point (Hillel, 1971), which here was 9.7 vol.%. The optimal water availability was targeted to be around field capacity (−10 kPa matric potential) (Hillel, 1971). It is noteworthy that both wet and optimal water treatments are within the limits where growth is unrestricted (see Supplementary Fig. S2). Thus, after first harvesting (week one), four pots were kept at a high water content (75% = wet