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Research article

Physiological, vascular and nanomechanical assessment of hybrid poplar leaf traits in micropropagated plants and plants propagated from root cuttings: A contribution to breeding programs

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ABSTRACT

Micropropagated plants experience significant stress from rapid water loss when they are transferred from an in vitro culture to either greenhouse or field conditions. This is caused both by inefficient stomatal control of transpiration and the change to a higher light intensity and lower humidity. Understanding the physiological, vascular and biomechanical processes that allow micropropagated plants to modify their phenotype in response to environmental conditions can help to improve both field performance and plant survival. To identify changes between the hybrid poplar [*Populus tremula* × (*Populus* × *canescens*)] plants propagated from in vitro tissue culture and those from root cuttings, we assessed leaf performance for any differences in leaf growth, photosynthetic and vascular traits, and also nanomechanical properties of the tracheary element cell walls. The micropropagated plants showed significantly higher values for leaf area, leaf length, leaf width and leaf dry mass. The greater leaf area and leaf size dimensions resulted from the higher transpiration rate recorded for this stock type. Also, the micropropagated plants reached higher values for chlorophyll *a* fluorescence parameters and for the nanomechanical dissipation energy of tracheary element cell walls which may indicate a higher damping capacity within the primary xylem tissue under abiotic stress conditions. The performance of the plants propagated from root cuttings was superior for instantaneous water-use efficiency which signifies a higher acclimation capacity to stressful conditions during a severe drought particularly for this stock type. Similarities were found among the majority of the examined leaf traits for both vegetative plant origins including leaf mass per area, stomatal conductance, net photosynthetic rate, hydraulic axial conductivity, indicators of leaf midrib vascular architecture, as well as for the majority of cell wall nanomechanical traits. This research revealed that there were no drawbacks in the leaf physiological performance which could be attributed to the micropropagated plants of fast growing hybrid poplar.

1. Introduction

The production of biomass crops, capable of fast growth, high yields and resilient to global warming, is a new challenge for the current plant breeding programs. Fast growing woody plants, such as poplars with a low lignin content, have been highlighted as a superior lignocellulosic biomass. By fermenting their carbohydrates they can be used successfully as a substrate in an integrated biorefinery for the production of second-generation biofuels and other chemicals (Sannigrahi and Ragauskas, 2010; Stolarski et al., 2015). The genus *Populus* comprise a host of species that are adaptable to multiple environmental stressors (Quinn et al., 2015). Interspecific hybrid poplars are rated among the most promising tree species for

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energy generation due to a minimal requirement for fertilizer and an ability to grow on marginal infertile lands. Grey poplar (*Populus* × *canescens*) originates from the spontaneous hybridization between white poplar (*P. alba*) and aspen (*P. tremula*). This taxon grows best in damp ground, near rivers and water meadows, but it is also tolerant to oscillations in underground water levels and is capable of growth in either acidic or heavy clay soil. All of these attributes make it a very promising tree not only for phytoremedation of metal-contaminated soils and for commercial planting under the current constraints of global warming, but also, as a parental species for artificial hybridization along with other poplar clones to create new genotypes with an altered content of cell wall components (Bojarczuk et al., 2015; Ďurkovič et al., 2013; Zemleduch-Barylska and Lorenc-Plucińska, 2015). Current breeding strategies, that involve grey poplar hybrids as a feedstock, are aimed at achieving both a higher saccharification performance associated with an increased bioethanol yield and the utilization of resilient and perspective clones in the pulp and paper industry (Kaňuchová and Ďurkovič, 2013; Kučerová et al., 2016).

Understanding the physiological, vascular and biomechanical processes that allow micropropagated plants to modify their phenotype in response to environmental conditions can help to improve both field performance and plant survival. Micropropagated plants experience significant stress from rapid water loss when they are transferred from an in vitro culture to either greenhouse or field conditions. This is caused both by inefficient stomatal control of transpiration and the change to a higher light intensity and lower humidity which thereby requires the rapid development of survival mechanisms based on environmentally-induced shifts in phenotype (Osório et al., 2013). Leaves must now produce a photoassimilate which they formerly imported from the culture medium. This requirement involves modifying the photosynthetic apparatus to maintain its efficiency under a varying light energy load and to alleviate the damaging effects of environmental extremes (Osório et al., 2012; Savitch et al., 2000). The success of in vitro propagation as a source of material for reforestation or for the establishment of commercial plantations and orchards therefore depends on efficient transplantation protocols that ensure high survival rates and allow the micropropagated plants to become established in their new environment (Hazarika, 2006). Field assessment of gas exchange will provide information about stomatal control of net photosynthetic rate, transpiration and water-use efficiency (Ďurkovič et al., 2016; Osório et al., 2012). The photosynthetic apparatus is a conservative element within the plant cell, and its reaction centres have low specific characterization, but the features (size, pigment composition) of the antenna complexes are highly variable and specific to different groups of plant species (Kirova et al., 2009). In recent studies, chlorophyll *a* fluorescence has been used as a tool for taxonomic classification and physiological segregation of plant species (Ďurkovič et al., 2014; Pollastrini et al., 2016). Salvatori et al. (2014) demonstrated that a multivariate statistical analysis of chlorophyll *a* fluorescence parameters allows the discrimination of plant species according to their respective functional groups. Differences among taxa derive from specific strategies for the use and conservation of energy, and from interactions between genotypes and their environment. As shown previously, differences in chlorophyll *a* fluorescence yields may also reflect intraclonal and intraspecific variation in relation to the plant origin (Ďurkovič et al., 2010, 2016).

The mechanical properties of the cellular microenvironment, notably its rigidity and stiffness, possess a regulatory role for a variety of cellular responses including adhesion, migration, shape and division (Dufrêne et al., 2013; Janmey et al., 2009). For the cell walls of xylem tissue, quantifications of modulus of elasticity (MOE), adhesion and cell wall deformation play a key role in the assessment of the material stiffness, toughness and its adhesive properties (Ďurkovič et al., 2016). Nanoindentation is one method applied to the measurement of local mechanical properties of plant cell walls at the submicron level (Eder et al., 2013; Gindl et al., 2004). However, the spatial resolution of this technique is quite low. Atomic force microscopy (AFM), on the other hand, allows a much higher lateral spatial resolution than that of nanoindentation (Arnould and Arinero, 2015), as its scanning probes have more pointed angles than those provided by the tools of the indenters in nanoindentation. Nanoscale imaging by AFM provides valuable information concerning the cellulosic architectural structures (Ding et al., 2016; Zhou et al., 2014). Recent developments in PeakForce quantitative nanomechanical mapping (PeakForce QNM) have optimized this new AFM technique to a point where it enables high-resolution imaging of woody plant cell walls, so that the maps extracted from the arrays of force-distance curves provide crucial information on the nanomechanical properties of lignified cell walls (Ďurkovič et al., 2016; Ren et al., 2015).

In this study we focused on the verification of leaf physiological performance in micropropagated plants of hybrid poplar grown under field conditions when compared with plants propagated conventionally from root cuttings. The objectives for this study were: 1) to determine whether or not in vitro propagation technique compromises the performance of the micropropagated plants in leaf growth, gas exchange, chlorophyll *a* fluorescence yields, and vascular architecture; 2) to find possible differences between the examined stock types with respect to the nanomechanical properties of tracheary element cell walls, which could reveal any mechanical advantages for either stock type when using multiparametric quantitative imaging of force-distance curve-based PeakForce QNM; and 3) to identify correlations among the examined traits that could contribute to the leaf physiological performance of hybrid poplar plants in relation to the technique of vegetative propagation.

2. Materials and methods

2.1. Plant material, study site and sampling

The experiments were conducted on clonally micropropagated plants and plants propagated from root cuttings of the hybrid poplar clone T-14 [*Populus tremula* L. 70 × (*Populus* × *canescens* (Ait.) Sm. 23)]. This clone shows a decreased lignin content (17.37%) and a higher cellulose content (47.33%) compared to that of the reference clone *P.* × *euramericana* 'I-214′, predominantly planted in Slovak poplar plantations (Kačík et al., 2012). The procedures of in vitro micropropagation through axillary and adventitious shoot formation from the sprouting axillary buds and the acclimatization to an ex vitro environment have previously been described in detail (Kaňuchová and Ďurkovič, 2013). For leaf trait comparisons, the counterparts of the micropropagated plants were derived from root cuttings having a length of approximately 30 cm. Both the root cuttings and the axillary buds originated from identical donor plants, more than 30 years of age. One-year-old plants propagated either by in vitro tissue culture or from root cuttings of a uniform size were selected and then planted in the experimental field plot at the Arboretum Borová hora, Slovakia (lat. 48°35′N, long. 19°08′E, altitude 297 m). Proper care was taken during planting to avoid any damage to the root system. The planting holes were dug with a spacing of 3 × 3 m. No post-planting treatments such as irrigation or fertilization were applied. The climate of the area is characterized by a mean annual temperature of 6.4 °C, a mean annual precipitation of 532 mm, and a mean precipitation of 315 mm in the growing season. The main soil creative substrates are slope loams of tufa materials with an admixture of loess loam. The experiments