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ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) affects development, photosynthesis, and hormonal homeostasis in hybrid aspen (*Populus tremula* L. × *P. tremuloides*)

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ABSTRACT

ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) was first described as a protein involved in salicylic acid (SA)-, ethylene-, and reactive oxygen species (ROS)-dependent defense and acclimation responses. It is a molecular regulator of biotic and abiotic stress-induced programmed cell death. Its role is relatively well known in annual plants, such as *Arabidopsis thaliana* or *Nicotiana benthamiana*. However, little is known about its functions in woody plants. Therefore, in this study, we aimed to characterize the function of EDS1 in the *Populus tremula* L. × *P. tremuloides* hybrid grown for several seasons in the natural environment. We used two transgenic lines, *eds1–7* and *eds1–12*, with decreased *EDS1* expression levels in this study. The observed changes in physiological and biochemical parameters corresponded with the *EDS1* silencing level. Both transgenic lines produced more lateral shoots in comparison to the wild-type (WT) plants, which resulted in the modification of tree morphology. Photosynthetic parameters, such as quantum yield of photosystem II (ϕPSII), photochemical and non-photochemical quenching (qP and NPQ, respectively), as well as chlorophyll content were found to be increased in both transgenic lines, which resulted in changes in photosynthetic efficiency. Our data also revealed lower foliar concentrations of SA and ROS, the latter resulting most probably from more efficient antioxidant system in both transgenic lines. In addition, our data indicated significantly decreased rate of leaf senescence during several autumn seasons. Transcriptomic analysis revealed deregulation of 2215 and 376 genes in *eds1-12* and *eds1-7*, respectively, and also revealed 207 genes that were commonly deregulated in both transgenic lines. The deregulation was primarily observed in the genes involved in photosynthesis, signaling, hormonal metabolism, and development, which was found to agree with the results of biochemical and physiological tests. In general, our data proved that poplar EDS1 affects tree morphology, photosynthetic efficiency, ROS and SA metabolism, as well as leaf senescence.

1. Introduction

In the natural environment, plants are continuously exposed to different environmental stresses, which are artificially divided into biotic and abiotic (Rietz et al., 2011). The former includes various pathogens, whereas the latter includes excess/deficiency of light, UV radiation, drought, chill, heat, and salinity. Previously, it has been proven that plants have evolved a molecular system to simultaneously and conditionally respond to a mixture of biotic and abiotic stress factors (Karpiński et al., 2013; Mühlenbock et al., 2008, 2007; Wituszyńska et al., 2013). In *Arabidopsis thaliana*, the function of regulatory genes has been shown to be different when grown in controlled laboratory conditions from that of field conditions (Weinig et al., 2002; Malmberg et al., 2005; Mishra et al., 2012; Wituszyńska et al., 2013). This indicates that studies focusing on elucidating the function of stress-responsive regulatory genes should be conducted not only under highly

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controlled laboratory conditions but also under natural field conditions, abounding with multiple stresses. Previous studies also indicate that the biology of woody plants should be studied in their natural environment (Ślesak et al., 2015; Witoń et al., 2016).

Populus trichocarpa is a useful research model in the field of molecular biology, genomics, physiology, and biotechnology with respect to trees (Bradshaw et al., 2000; Taylor, 2002; Bhalerao et al., 2003). This poplar species has relatively small genomic size (485 Mb), undergoes easy vegetative breeding, has rapid growth, and its genetic transformation is relatively efficient. It was also the first species of trees whose genome was fully sequenced, which provided different possibilities in genomic studies of woody plants (Tuskan et al., 2006). Furthermore, current genetic engineering techniques have enabled to create new poplar hybrid genotypes with increased resistance to biotic and abiotic stresses, improved growth, and better properties of wood that can be used in the production of bioenergy and paper (Herschbach and Kopriva, 2002; Confalonieri et al., 2003; Osakabe et al., 2011). *Populus tremula* × *P. tremuloides* is a hybrid of European and American aspen created by artificial hybridization. It has all the advantages of *P. trichocarpa*, except for the fully sequenced genome (Nilsson et al., 1992; Ślesak et al., 2015). However, this hybrid is more useful in paper production than *P. trichocarpa* (Jokipii et al., 2004) as it possesses better wood technological properties; therefore, we used this hybrid in this study.

ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) was first described as an important regulator of salicylic acid (SA)-dependent defense response against biotic stresses (Glazebrook et al., 1996; Parker et al., 1996). Later, it has been shown to be involved in hypersensitive response (HR), accumulation of SA, and SA-dependent signaling (Feys et al., 2001; Shah, 2003; Wiermer et al., 2005). Under biotic stress, EDS1 promotes cell death (Rustérucci et al., 2001) and limits plants growth (Ochsenbein et al., 2006). EDS1 along with its interacting partner, PHYTOALEXIN DEFICIENT 4 (PAD4), shows homology to eukaryotic acyl lipases (Falk et al., 1999; Jirage et al., 1999). EDS1–PAD4 hub has been described as a regulator of PAMP-triggered immunity (PTI) as well as Toll–interleukin-1 receptor–nucleotide binding-leucine-rich repeat (TIR-NB-LRR) protein-mediated signaling in response to *Pseudomonas syringe* (Parker et al., 1996; Aarts et al., 1998; Wiermer et al., 2005). In *A. thaliana*, EDS1 interacts with PAD4, SENESCENCE-ASSOCIATED GENE 101 (SAG101), LESION SIMULATING DISEASE 1 (LSD1), RESPONSE TO LOW SULFUR 1 (LSU1), RIBOSOMAL PROTEIN S6 (RPS6), and four disease resistance proteins from CC-NBS-LRR and TIR-NBS-LRR classes (Feys et al., 2001, 2005; Bhattacharjee et al., 2011; Rietz et al., 2011; Zhu et al., 2011; Czarnocka et al., 2017). EDS1–PAD4 complexes are required for the accumulation of SA and systemic acquired resistance (SAR) (Rietz et al., 2011). EDS1 dimers are present predominantly in the cytoplasm, whereas EDS1–PAD4 and EDS1–LSD1 complexes are found in the nucleus, which suggests dynamic interactions between EDS1 and its signaling partners in multiple cellular compartments (Feys et al., 2005; Rietz et al., 2011; Czarnocka et al., 2017). Nuclear accumulation of EDS1 is required in the EDS1-dependent regulation of defense-related genes. However, the presence of EDS1 in the cytoplasm is also needed for the restriction of pathogen-induced cell death at the site of infection. Thus, it has been proposed that a balanced activity of EDS1 in both cytoplasmic and nuclear compartments is needed for the appropriate response to stress (García et al., 2010).

In *A. thaliana*, EDS1 has been proven to be an important reactive oxygen species (ROS)-, SA-, and ethylene-dependent conditional regulator of programmed cell death (PCD) in response to various abiotic stresses, such as limitation of CO_2 assimilation, drought, root hypoxia, cold, excess light and UV-C radiation (Mühlenbock et al., 2008; Wituszyńska et al., 2013, 2015, Chen et al., 2015) and in the acclimation to naturally changing field conditions (Wituszyńska et al., 2013). Under UV-C stress, EDS1 regulates PCD, antioxidant system, and the expression of UV-responsive genes antagonistically to LSD1 (Wituszyńska et al., 2015). In *Nicotiana benthamiana*, EDS1 is involved in response to UV by regulating the content of SA (Catinot et al., 2008). It was further found that under non-stress conditions, EDS1 influences the level of photosynthetic pigments, such as chlorophyll, lutein, and carotene (Wituszyńska et al., 2015). Moreover, *A. thaliana eds1* mutant demonstrates differences in the xanthophyll cycle (Wituszyńska et al., 2015). This, combined with the deregulated antioxidant system (Mühlenbock et al., 2007, 2008, Wituszyńska et al., 2013, 2015) might affect the photosynthetic efficiency. Taken together, EDS1, LSD1, and PAD4 have been suggested to constitute a molecular hub that conditionally regulates signal transduction pathways, hormonal homeostasis, PCD, and defense responses in *A. thaliana* (Gilroy et al., 2016; Oracz and Karpiński, 2016).

As previously described, EDS1 has been broadly studied in the context of plants' response to biotic/abiotic stresses, photosynthesis, and SA/ROS homeostasis in *A. thaliana* and *N. Benthamiana*. *A. thaliana* orthologs of EDS1 have been functionally identified in other plant species, such as *Vitis vinifera*, *Lycopersicon esculentum*, and *Gossypium barbadense* (Gao et al., 2010; Hu et al., 2005; Su et al., 2014). However, the role of EDS1 in arborescent plants has not been studied yet. Therefore, in this study, we aimed to analyze the role of EDS1 in *Populus tremula* × *P. tremuloides* hybrid grown under the natural field conditions. Our results prove that EDS1 affects tree morphology, foliar SA and ROS content, ROS metabolism, photosynthetic pigments' concentration, photosynthetic efficiency, and leaf senescence.

2. Materials and methods

2.1. Generation of transgenic poplar lines and growing conditions

Genetic transformation of *Populus tremula* × *P. tremuloides* (background T89) hybrid was performed at the Umeå Plant Science Center according to the transformation protocol described by Nilsson et al. (1992). Silencing construct for *EDS1* (XM_002318590.2) was created based on the RNA interference using binary vector pH7GWIWG2(I) under the control of 35S promoter and "Gateway" technology (Karimi et al., 2002). Two independent lines, *eds1–7* and *eds1–12*, were selected for analysis. Propagation of transgenic lines was performed under sterile *in vitro* conditions. Well-rooted plants from *in vitro* conditions were transferred to pots filled with soil. After acclimatization in growth chamber, they were transferred to the greenhouse. After 9 months, transgenic and control plants were transferred to the field as previously described (Wituszyńska et al., 2013; Ślesak et al., 2015; Witoń et al., 2016). Morphological characterization was performed on 4-year-old trees during late spring–summer season: four *eds1–7*, two *eds1–12*, and three wild-type (WT) control trees. Pictures of a representative tree were taken for each specific genotype. For the counting of lateral shoots, each tree's main stem was divided into three parts: upper, middle and bottom. Each of these parts constituted one-third of the total height of the specific tree. The number of lateral shoots on the main stem and the main stem's height, weight, and diameter were measured for all the trees under study. Data were statistically analyzed using Tukey honest significant difference (HSD) test. All analyses, except from quantitative real-time PCR (qPCR) and senescence-related study, were performed on field-grown trees during late spring–summer season. The senescence-related study was performed at the turn of summer and autumn.

2.2. RNA isolation and quantitative real-time PCR

To examine the level of *EDS1* gene silencing, qPCR was performed. After *in vitro* establishment of shooting and rooting, young fully expanded leaves were collected from two individual plants per genotype. RNA isolation was performed using TRIsol reagent (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA was purified from DNA residues with DNA-free DNA Removal Kit (Thermo Fisher Scientific)