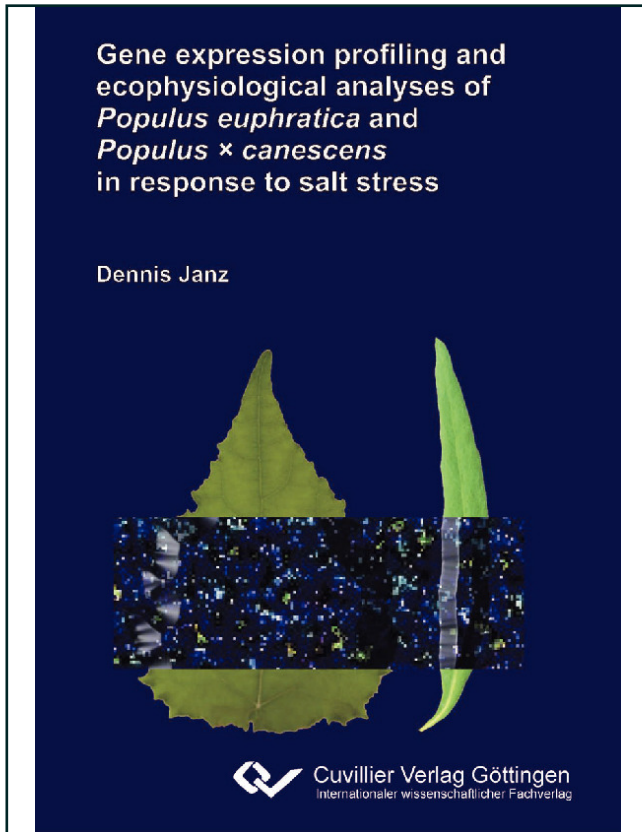




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Gene expression profiling and ecophysiological analyses of *Populus euphratica* and *Populus × canescens* in response to salt stress



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1. Introduction

Soil salinization is one of the major threats for agriculture worldwide. Currently, a total of 397 million ha, nearly 3 % of the total land mass, are covered by salt affected soils (FAO/AGL). Agricultural land accounts for 77 million ha thereof, a fraction that will increase in the coming years because of inappropriate irrigation regimes, increase in evapotranspiration, sea-level rise and decreased ground water recharge (Kundzewicz *et al.* 2007; Tester & Davenport 2003). To be able to cope with this situation in the future, new plant varieties with enhanced ability to grow on salinized soils must be available, generated either by efficient breeding or genetic engineering. Because of the increasing importance of trees as a renewable energy resource, this applies not only to agricultural crops, but also to woody plants.

An alternative approach to the problem of soil salinization is to counteract the underlying causes. In many cases, irrigation is used on agricultural land where drought-adapted, deep-rooted native vegetation has been replaced by annual crops. In these areas, surfacial irrigation and evapotranspiration will establish a capillary flow, by which often saline water from deep ground water tables is transported to the surface, leading to soil salinization (Salinity stress and its mitigation). To counter this problem, the establishment of a sustainable agricultural management has been proposed. This could include planting of deep-rooted, salt tolerant tree species to lower ground water tables (Pitman & Läubli 2002).

For the implementation of either of the two concepts, it is essential to increase our knowledge and understanding of mechanisms that are available to trees to tolerate high salinity. So far, research on salt tolerance mechanisms in plants has focused mainly on non-woody species, a circumstance that seems

inattentively, since tree species will play an important part both in the fields of genetic engineering and sustainable agricultural management (Flowers 2004; Turkan & Demiral 2009; Hasegawa *et al.* 2000).

Trees and herbaceous plants differ widely in respect to morphology and physiology. These differences are most apparent in form of the development of wood, *i.e.* secondary xylem. Secondary xylem is also formed in non-woody plants and thus can be investigated using classical model species like *Arabidopsis*. However, wood development is an immensely complex process. Since herbaceous and woody plants differ widely in their physiology and morphology (Demura & Fukuda 2007; Hertzberg *et al.* 2001; Taylor 2002), the influence of salt stress on secondary xylem formation will ultimately have to be studied in trees, because findings that have been made for herbaceous plants may only be transferred to a certain extent and with great care to tree species. In this work, I therefore focused on salt tolerance mechanisms in woody plants.

For this purpose, it is of practical interest to have one specific model organism to work on. Among herbaceous plants, *Arabidopsis thaliana* has been established as one of the most important model species to answer a multitude of questions. Reasons for this were amongst others a short life cycle, a wide ecological tolerance spectrum, availability of techniques for genetic manipulation, and a relatively small genome, making sequencing of the whole genome possible.

For similar reasons, *Populus* has now been firmly established as a model species for trees (Bradshaw *et al.* 2000; Brunner *et al.* 2004; Jansson & Douglas 2007; Taylor 2002; Tuskan *et al.* 2006). The genus *Populus* is formed by a group of closely related tree species. *Populus* has, compared to other tree species, a relatively small genome, and protocols for genetic manipulation are available. But all tree species have life cycles that require several years, which complicates their use as model organisms. Poplars however compensate this

disadvantage by a rapid growth rate and by being easily multipliable by vegetative propagation, thus allowing experiments to be conducted on genetically identical plants rather than inbred plant lines. Finally, while individual poplar species are not necessarily generalists, the many different, closely related species among the genus *Populus* cover a wide ecological range. In addition, poplars are of great commercial value, especially to the pulp and paper industry.

Thellungiella halophila, a salt tolerant plant closely related to *Arabidopsis*, is often used as a model to research salt tolerance in herbaceous plants (Bartels & Sunkar 2005; Taji *et al.* 2004; Wang *et al.* 2004). Among poplars, *Populus euphratica* OLIV. meets the claims for this task. *P. euphratica* grows naturally in areas with saline soil and ground water, ranging from western Europe to China (Feng *et al.* 2001). It is well known for tolerating higher salt concentrations than other poplar species (Watanabe *et al.* 2000; Bolu & Polle 2004; Sixto *et al.* 2005). Under saline conditions, *P. euphratica* is able to maintain higher growth rates and higher photosynthetic rates than salt-sensitive poplar species (Wang *et al.* 2007; Chen *et al.* 2003b). Many works have analyzed the physiological responses of *P. euphratica* to salt stress, and compared them to different salt sensitive poplar species (Wang *et al.* 2007; Chang *et al.* 2006; Hukin *et al.* 2005; Fayyaz 2007; Fung *et al.* 1998; Chen *et al.* 2003b). In single studies, the transcriptomic response of *P. euphratica* to salt and drought stress was even examined on microarrays, and typical genes have been found to be upregulated (Gu *et al.* 2004; Brosché *et al.* 2005; Bogeat-Triboulot *et al.* 2007). However, since no comparison of the salt stress response transcriptome of *P. euphratica* with a salt sensitive poplar exists, it is still unknown which genes or mechanisms are particularly important for the salt tolerance.

The lack of data in this field might be due to the fact that only spotted cDNA microarrays were available. These are made from species-specific cDNA libraries, and therefore should only be used for the respective species; comparing the microarrays' source-species with a different species was not possible. But the acceptance of *Populus* as a model tree now has led to a sound informational basis. Recently, the whole genome of *Populus trichocarpa* TORR. & GRAY has become available through a sequencing project, and annotation of genes has begun (Tuskan *et al.* 2006; JGI *Populus trichocarpa* genome release 1.1). This in turn allowed the development of a whole genome microarray by Affymetrix, the GeneChip Poplar Genome Array, for gene expression analyses of different poplar species (Affymetrix GeneChip Poplar Genome Array Data Sheet). Affymetrix GeneChips feature a new technology; probes are no longer spotted cDNA fragments as in earlier microarray generations, but are oligonucleotides that are synthesized *in situ* on the microarray (Thomas & Burke 1998). This leads to a high reproducibility between separate microarrays, rendering the use of two colors and technical reproductions obsolete (Hardiman 2004).

Thus, with an adequate model tree and new techniques for high-throughput analyses of gene expression, it is now possible to compare the transcriptome of the salt tolerant *P. euphratica* to that of a salt sensitive poplar. For this, I employed *P. × canescens* (AITON) SM., a salt sensitive poplar native to Europe whose natural range is in the temperate zone (Bolu & Polle 2004; Hawighorst 2007). In this study, the following topics were addressed:

In Chapter II, the effects of salt stress on the anatomy of trees were studied by comparing the transcriptomes and the morphology of *Populus × canescens* and *P. euphratica* xylem formed under long-term salt stress. The aim of this chapter is to gain more insight into mechanisms of stress adaption and stress tolerance in wood.

Chapter III comprises the work on roots of *P. euphratica*, which display a distinct thickening when formed under saline conditions. Here, the transcriptional changes that concur with these morphologic changes were analysed to investigate the molecular adaption of *P. euphratica* to salt stress.

In Chapter IV, the transcriptomes and metabolomes of *P. euphratica* and *P. × canescens* leaves formed in the absence of salt stress were compared. Hereby, I address the questions whether *P. euphratica* uses preventive strategies to anticipate salt stress, and which stress tolerance mechanisms are employed.