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# The salt-responsive transcriptome of *Populus simonii*×*Populus nigra* via DGE

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ABSTRACT

In this study, the dynamic transcriptome of poplar (*Populus simonii*×*Populus nigra*) was investigated under salt stress using Solexa/illumine digital gene expression (DGE) technique. A total of 5453, 2372, and 1770 genes were shown to be differentially expressed after exposure to NaCl for 3 days, 6 days and 9 days, respectively. Differential expression patterns throughout salt stress were identified for 572 genes. Gene ontology classification analysis of these differentially expressed genes revealed that numerous genes mapped to "transporter activity" and "response to stress". The dynamic transcriptome expression profiles of poplar under salt stress obtained in this study may provide useful insights for further analysis of the mechanism of high salinity tolerance in plants. Furthermore, these differentially expressed genes under salt stress may allow identification of potential genes as suitable targets for biotechnological manipulation with the aim of improving poplar salt tolerance.



## 1. Introduction

Poplars are the most widely planted and most adaptable broad-leaved deciduous trees in the Northern Hemisphere. Poplar wood is commonly used for building materials, furniture and the manufacture of paper (Taylor, 2002). Poplar growth is decreasing rapidly due to the negative impact of various environmental stresses, particularly salt stress, which results from excessive salts in soil solution and causes inhibition of plant growth or plant death (Cuartero et al., 2006; Lopez-Hoffman et al., 2007; Redondo-Gomez et al., 2007).

*Populus simonii*×*Populus nigra* widely distributes in northern China and have been well used for afforestation and commercial forest. Though transcriptome responses of *Populus euphratica*, a salt-tolerant poplar species, have been well measured and many salt-related genes have been identified, the whole transcriptome responses of *P. simonii*×*P. nigra* have been reported poorly (Brinker et al., 2010; Ding et al., 2010; Gu et al., 2004; Janz et al., 2011; Qiu et al., 2011). The geographic conditions of *P. simonii*×*P. nigra* are different from *P. euphratica*. So the dynamic transcriptome and the differentially expressed genes identification of *P. simonii*×*P. nigra* under salt stress are important for further analysis of the mechanism of high salinity tolerance in poplar and biotechnological manipulation with the aim of improving poplar salt tolerance.

While saline soils contain numerous salts at elevated concentrations, NaCl is the most common, the harmful effects of which are principally due to a combination of osmotic stress and ionic stress (Gong et al., 2005). Plants respond to salt stress both as individual cells and synergistically as a whole system. The salt stress signal is first perceived at the membrane level by receptors, which results in generation of many secondary signal molecules, such as $Ca^{2+}$, inositol phosphates, reactive oxygen species (ROS) and abscisic acid. The stress signal is then transduced into the nucleus resulting in the induction of numerous stress responsive genes, the products of which ultimately lead to plant adaptation to salt stress tolerance. The mechanism of salinity tolerance is a highly complex process and many genes and pathways are involved (Huang et al., 2003; Magnan et al., 2008; Wang et al., 2009; Xu et al., 2009).

Plants are stressed by soil salinity via two mechanisms. High concentrations of salts in the soil decrease the uptake of water by the roots and are toxic within the plant. The responses to salt stress occur in two distinct phases over time. A rapid response is mounted to increased external osmotic pressure, while the response to the accumulation of $Na^+$ in leaves is slower. During the osmotic phase, the rate of shoot growth falls significantly when the salt concentration around the roots increases above a threshold level. The second, ion-specific, phase of the plant response to salinity starts when salt accumulates to toxic concentrations in old leaves, resulting in death.

High salinity stress causes an imbalance in $Na^+$ homeostasis, which is maintained by the coordinated action of various pumps, ions, $Ca^{2+}$ sensors and its downstream interacting partners, and ultimately results in the efflux of excess $Na^+$. Calcium plays an important role in this process. Accumulation of cytosolic $Ca^+$ induced by salt stress initiates the stress signal transduction pathways for stress tolerance (Anil et al., 2008; Hamamoto et al., 2008; Mahajan et al., 2008). In *Arabidopsis*

*Abbreviations:* DGE, digital gene expression; ROS, reactive oxygen species; MAPK, Mitogen-activated protein kinases; PSII, photosystem II; NGS, Next Generation sequencing; $F_o$, Basic chlorophyll fluorescence yield; $F_m$, maximal chlorophyll fluorescence yield; SBS, sequencing by synthesis; TPM, transcripts per million clean tags; KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, False Discovery Rate.

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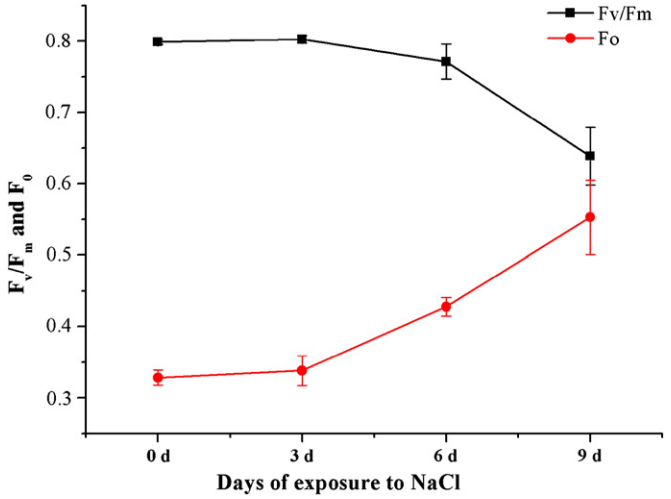


**Fig. 1.** Effect of NaCl on the chlorophyll fluorescence parameters $F_v/F_m$, and $F_0$. Results are presented as the mean ± SD of three independent experiments.

*thaliana*, the signal transduction components of the salt overly sensitive (SOS) pathway have been clearly identified (Liu et al., 2000; Qiu et al., 2002; Shi et al., 2000, 2002).

Mitogen-activated protein kinases (MAPK) are a specific class of serine/threonine protein kinases that play a central role in the transduction of various extracellular and intracellular signals, including stress signals. Many plant MAPKs have been reported to be induced by osmotic stress. These reports of salinity-induced MAPKs suggest that a MAPK signaling cascade is involved in mediating salinity stress tolerance in plants (Diedhiou et al., 2008; Jain et al., 2009).

When exposed to salt stress, plants are also subject to oxidative stress and ROS produced in response to oxidative stress can cause permanent cell damage. These scavengers can increase plant resistance to salinity stress. Overexpression of the aldehyde dehydrogenase gene in *Arabidopsis* has been reported to confer salinity tolerance (Sunkar et al., 2003). Aldehyde dehydrogenase catalyzes the oxidation of toxic aldehydes, which accumulate as a result of reactions of ROS with lipids and proteins.

Photosynthesis is a key metabolic pathway that is critically involved in the maintenance of growth under salt stress. High salt negatively affects photosynthetic efficiency at the electron donor side and/or the electron acceptor side of PSII (photosystem II). High salt stress is a major environmental factor that limits plant productivity due to reduced plant growth and is associated with a decrease in the rate of photosynthesis (Ahmad et al., 2008; Gao et al., 2008).

Plants can be exposed to multiple stress conditions simultaneously and have therefore developed mechanisms to cope with such situations. Furthermore, cross-talk between stress-signaling pathways can lead to cross-tolerance.

Next generation sequencing (NGS) methods have emerged as a cost-effective high-throughput approach to sequencing of a very large number of expressed genes even in small experiments. Taking advantage of this benefit, Solexa/illumine digital gene expression (DGE) analysis was used to characterize genome-wide poplar gene expression during NaCl treatment. Four DGE libraries were sequenced at 0, 3, 6 and 9 days after NaCl treatment and the gene expression profiles were analyzed to identify the genetic response to salt stress.

## 2. Materials and methods

### 2.1. Plant growth condition and NaCl treatment

Seedlings were planted in a mixture of turfy peat and sand (2:1 v/v) in a greenhouse with 75% relative humidity and a constant temperature of 24 °C. Salt stress conditions were applied by treatment with 1% NaCl solution (2 L) every two days. Healthy leaves of six biological replicates were harvested and snap frozen at 0, 3, 6 and 9 days after treatment for RNA isolation and further analysis.

### 2.2. Measurement of chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters were recorded at room temperature using a portable chlorophyll fluorimeter (PAM-2500). All samples were dark adapted for 30 min prior to fluorescence measurements. Basic chlorophyll fluorescence yield ($F_o$) and maximal chlorophyll fluorescence yield ($F_m$) were measured according to the instructions for operation provided by the manufacturer.

### 2.3. RNA isolation and library preparation for DGE

Total RNA was isolated from each sample using the CTAB method. All RNA samples were quantified and examined spectrophotometrically for protein contamination ($A_{260}/A_{280}$ ratios) and reagent contamination ($A_{260}/A_{230}$ ratios). Extracted RNA samples were selected based on 28S/18S rRNA band intensity (2:1) and spectroscopic $A_{260}/A_{280}$ readings between 1.8 and 2.0, $A_{260}/A_{230}$ readings greater than 1.5. Oligo(dT) magnetic beads were used to purify mRNA from the total RNA extract (6 μg) and used with oligo(dT) primers as a template for first and second-strand cDNA synthesis. The 5′ ends of tags were generated by *Nla*III digestion, which recognizes and cleaves the CATG sites. With the exception of 3′ cDNA fragments bound to

**Table 1**
Statistics of DGE sequencing.

| Summary | | Exposure to NaCl | | | |
|---|---|---|---|---|---|
| | | 0 d | 3 d | 6 d | 9 d |
| Raw data | Total | 6,046,597 | 5,767,998 | 6,058,881 | 5,936,713 |
| | Distinct Tag | 298,218 | 328,514 | 340,613 | 307,265 |
| Clean tag | Total number | 5,878,780 | 5,585,512 | 5,857,988 | 5,767,166 |
| | Distinct tag number | 131,785 | 146,514 | 140,427 | 138,792 |
| All tags mapping to gene | Total % of clean tag | 70.50% | 73.39% | 72.96% | 72.97% |
| | Distinct tag number | 68,052 | 80,006 | 74,217 | 73,250 |
| | Distinct tag % of clean tag | 51.64% | 54.61% | 52.85% | 52.78% |
| Unambiguous tags mapping to gene | Total % of clean tag | 69.98% | 72.87% | 72.51% | 72.50% |
| | Distinct tag number | 67,468 | 79,280 | 73,566 | 72,610 |
| | Distinct tag % of clean tag | 51.20% | 54.11% | 52.39% | 52.32% |
| All tag-mapped genes | Number | 19,937 | 21,941 | 21,178 | 20,720 |
| | % of ref genes | 44.27% | 48.72% | 47.03% | 46.01% |
| Unambiguous tag-mapped genes | Number | 19,779 | 21,750 | 20,995 | 20,546 |
| | % of ref genes | 43.92% | 48.30% | 46.62% | 45.62% |