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Gene expression profiling in response to UV-B radiation in different *Populus alba* clones

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Background

Long-term depletion of the stratospheric ozone layer contributes to the increase in terrestrial solar ultraviolet-B radiation (UV-B), an environmental change with potentially deleterious consequences for plants. When exposed to elevated UV-B (280-315 nm), plants display a wide variety of physiological and morphological responses. Consequently, determining the molecular bases for acclimation to normal fluence and tolerance of high UV-B are important factors in sustaining plant yield.

To better understand the processes of UV-B acclimation, which result in altered plant morphology and physiology, we investigated gene expression in leaves of 5 different white poplar genotypes-clones at high UV-B fluence rate and exposure times.

Methods

Three years old plants were acclimated in two chambers identical for temperature and photoperiod but differing for the UV light. An UV-B lamp array and an ozone breakdown system is present in the UV chamber. The following parameters can be controlled inside the chambers: temperature, relative humidity, CO_2 concentration, O_3 concentration, visible (photosynthetic active radiation, PAR) and UV (UV-A and UV-B) radiation. The environmental light is developed to guarantee a good PAR-UVA-UVB ratio; in each chamber eighteen 36W fluorescent lamps are placed: 9 Osram Biolux 965 (day light), 9 Osram Fluora 77 (red blue light). Furthermore, 4 halogen Osram Power Star HQI-TS 400W/D ensure the presence of blue and UV-A radiation. Seven Sankyo Denki G40T10 (UVB radiation) lamps were used for UV-B treatment. UV-B lamps were wrapped in cellulose acetate film to cut radiation with wavelengths below 280 nm (UV-C).). Plants of 5 different clones of white poplar were grown in a nursery for 1 year and then acclimated in the chambers for 2 weeks before the start of the UV-B treatment. In order to simulate an environmental condition, plants were treated for 12 hours with an UV-B_{BE} (UVB biologically effective radiation) supplementary dose of 6 $KJ/m^2/day$ (0.15 W/m^2 at 100 cm from the lamps) and allowed to recover during the night (12 hours). Growing conditions were as follow: i) 25°C temperature during the day and 20°C during the night, ii) 60% of relative humidity, iii) CO_2 concentration (450 ppm), iv) photoperiod of 12 hours of light and 12 hours of dark with a simulation of sunrise and sunset, v) 6 $KJ/m^2/day$ supplementary dose of UV-B_{BE} during the daylight photoperiod. Four genes were selected for transcription analyses on the basis of the results of eco-physiological observation. Transcription analyses were used to analyze gene expression of plants treated for 3, 6, 12 and 36 hours with elevated levels of UV-B. These

genes resulted significantly down- or up-regulated (P value < 0.05 and fold change < 0.5 or >2) during the treatment. We analyzed the response of each genotype comparing (unpaired t test) the transcription levels between the treatment within and between the genotypes, to find out when the transcription levels were high, in the first case and which genotype responded to the treatment differently, in the second case. Before the start of UV-B treatment (0 hours), all genes transcription levels variation between control and treated samples, was not statistically significant for all the genotypes. Pooled leaf samples of the 5 clones were sampled from control and UV-B treated plants (3, 6, 12 and 36 hours); chlorophyll fluorescence of PSII was measured and RNA was extracted for qRT-PCR analysis of genes putatively related to UV-B response such as Chalcone Synthase (CHS), Caffeic Acid 3-O-Methyl Transferase (COMT2), Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase large subunit (RuBisCo), and CPD-photolyase class II

Results and Conclusions

The eco-physiological observations were focused on the photosynthetic efficiency, as estimated by chlorophyll fluorescence measurements. The photosynthetic efficiency values decreased for all the genotypes already after the first day, ranging from values around 0.83, up to values around 0.7/0.6 after 72 hours of analyses (the end of the treatment). This decrease in chlorophyll efficiency is not significant to suppose damage or photoinhibition to PSII, but rather a decrease of efficiency in the treated samples due to the presence of an elevated UV-B radiation. Therefore, analyses regarding this parameter did not show a permanent damage to the leaves for all the different genotypes.

Eco-physiological data indicated that the different genotypes were not able to recover in the same way after 36 hours of UV-Bb radiation. For this reason, the transcriptional levels of genes (*chs*, *comt*, *rubisco* and *cpd photolyase*) putatively related to UV-B response, were investigated through a time-course qRT-PCR analyses. We analyzed the response of each clones comparing (unpaired t test) the transcription levels between the treatment within and between the clones, to find out when the transcription levels were high, in the first case and which clone responded to the treatment differently, in the second case. Regarding to the *chs* gene, Policoro showed high levels of transcription already in the early hours and increased its levels during the treatment. Val Bormida as well, increased its transcription levels after 12 and 36 hours of treatment. The same two clones that present high transcriptional levels of *chs* gene showed a higher recovery during the fluorescence analysis, indicating an early activation of defense mechanisms with constitutive production of protection pigments. Similar trend was observed for *comt* gene that showed a different pattern of expression during the hours of treatment for all the clones. Differences were noticed in particular after 12 and 36 hours, where Val Bormida and Policoro genotypes showed higher levels of transcription compared to the others. This is explained with the fact that at the beginning we have first the increase of the *chs* levels, the first gene in the phenylpropanoids pathway, related with the production of anthocyanins, and then arrive to the synthesis of lignin.

We therefore hypothesize that Policoro and Val Bormida are the 2 clones that better tolerated to UV-B radiation, Policoro having the highest transcription of *chs* and *comt* gene leading to the hypothetical production of both anthocyanins and lignin, and Val Bormida having higher transcriptional levels than the other clones for *comt* with the production of lignin. From our analyses emerged that the transcription of CPD-photolyase gene is not influenced by the treatment. Regarding RuBisCo large subunit (*rbcl*) the trend of transcription levels for the different clones was similar.

In the first hours, the transcription levels did not seem to be influenced by the treatment. Instead in the following hours, after 12 and 36 hours of treatment, the transcriptional levels resulted down-regulated. This result, together with the results obtained from the

physiological measures which did not include damage to the leaves, photoinhibition damage to PSII, confirm the hypothesis that is RuBisCo, and not PSII, the primary target involved in inhibiting photosynthesis after exposure to UV-B radiation. The decrease of the photosynthetic efficiency found in the eco-physiological experiments is probably due to a decrease of the transcriptional levels of RuBisCo for all the clones.
In conclusion, the results evidenced that white poplar clones respond to high UV-B fluence with a differential transcription of analyzed genes. An important next step is understanding correlation between the individual response of clone and UV-B tolerance.

Competing interests
The author declares that they have no competing interests.