Gene expression in Scots pine populations from the Chernobyl exclusion zone: transcriptomic approach

P.Yu. Volkova[1]*, G.T. Duarte[2], S.A. Geras'kin[1]

[1] *Institute of Radiology and Agroecology, Obninsk, Russia*
[2] *Institute Jean-Pierre Bourgin, Versailles, France*

Abstract: Radioactive contamination of natural areas is one of the most long-lasting anthropogenic impacts on the environment. Scots pine (*Pinus sylvestris* L.) is an important species for radiation protection of biota because of its high radiosensitivity. We conducted the *de novo* transcriptome analysis of Scots pine populations growing in the Chernobyl-affected zone, which is still today contaminated with radionuclides. The transcriptional response involved modulation of the cellular redox system and ion balance, and enhanced expression of chaperones and histones. Our data suggest that the modulation of ROS level occurs mainly through the control of glutathione- and thioredoxin-related responses and most likely involves a fine-tuning of ROS-generating processes, which might be related to the control of gas exchanges via stomata. It is interesting to note that the stress response profile identified does not rely on the modulation of ABA biosynthesis or catabolism genes. These adaptive responses are triggered by radiation doses 30 times lower than the one accepted as safe for biota species by international regulations. These findings suggest that the environmental management in radiation protection should be reviewed.
Key words: radiation exposure; scot pine; *Pinus sylvestris* L.; transcriptome.

1. Introduction

The Chernobyl exclusion zone is a unique area for accessing the effects of ionizing radiation on the abundance, distribution, life history, and molecular changes caused on different plant species. Scots pine (*Pinus sylvestris* L.) is one of the most widespread tree species across Europe and Northern Asia, being a promising organism for radiation-related studies because of its high radiosensitivity (ICRP, 2008). Intending to gain insight into the ability of plants to withstand chronic radiation exposure, we evaluated the transcriptome profiles of Scots pine trees from four populations growing at clean and radioactively contaminated territories. This work has been recently accepted for publication (Duarte et al., 2019).

2. Materials and methods

The sample plots are located in the Bryansk region of Russian Federation and in the Gomel region of Belarus Republic. The reference plot (Ref) and the low-contaminated Zabor'e plot (Zab) are both located in the Bryansk region. The highly contaminated Kulazhin (Kul) and Masany (Mas) plots are situated in the Chernobyl exclusion zone. To perform the transcriptome analysis, we sampled pine needles from nine individual trees at each experimental plot. RNA of three pooled samples from a plot was sequenced using the Illumina HiSeq 2500 technology. The *de novo* transcriptome assembly was performed using BinPacker (Liu et al., 2016), SOAPdenovo-Trans (Xie et al., 2014) and Trinity (Grabherr et al., 2011). The transcriptome annotation was conducted with Trinotate (Bryant et al., 2017) and InterProScan (Jones et al., 2014). The detailed description of the RNA sequencing procedures and bioinformatics analyses are available (Duarte et al., 2019; Duarte et al., forthcoming).

3. Results and discussion

The gene expression profile of the four studied populations correlated to the radiation levels at the sample plots. The clustering analysis of the expression profiles grouped the two highly contaminated populations (Mas and Kul); the low contaminated population (Zab) did not differ significantly from the reference. Nevertheless, the transcriptome profile of Zab population revealed the same regulation pattern of stress-related genes that were also identified for the high-dose irradiated populations, Mas and Kul. Only seven transcripts were commonly regulated among the three contaminated sample plots. Four of them were related to stress responses: a transcript for an anionic peroxidase related to cell death responses (WUN1_SOLTU), and three abscisic acid (ABA)-responsive transcripts (CIPKK_ARATH, CIPKA_ARATH, and SLAC1_ARATH). Among the ABA-related genes, it is interesting to single out the down-regulation of the F-box protein PP2-B11 (P2B11_ARATH), which has been described as an attenuator of ABA responses (Cheng et al., 2017).

The functional analysis of the differentially expressed transcripts did not evidence a classical stress response signature (e. g. heat, hypoxia, cold, etc.). The response to chronic radiation exposure also did not resemble one to acute radiation, suggesting that the adaptation process to the former is rather unique. Those few genes that overlap between acute and chronic irradiation conditions showed inverse expression patterns. Short-term experiments also showed that the response pattern changes according to the radiation exposure duration (Kovalchuk et al., 2007).

The transcriptional profiles evaluated in our work suggest that the adaptation to chronic radiation exposure involves: (1) modulation of ROS accumulation through balancing of

ROS-generating processes and antioxidant molecules; (2) control of cellular damage by enhanced expression of chaperones and histones, along with the modulation of ions balance, to counteract the damaging effects of a higher ROS basal level on proteins activity, DNA, and membranes; (3) control of the activity of transposable elements (TEs).

The production of ROS is an inherent consequence of the exposure to radiation (Szumiel, 2015). During oxidative stress, plants activate antioxidant mechanisms including enzymes (superoxide dismutase, catalases, peroxidases, and enzymes of ascorbate-glutathione cycle) and accumulate low molecular weight antioxidants for minimizing the damage (Sewelam et al., 2016). On the other hand, ROS are also central signalling molecules during stress responses (Sewelam et al., 2016). Apparently, organisms under chronic radiation exposure have to adapt to a different ROS threshold, which would allow them to balance the buffering of ROS without blocking their signalling function. Our data suggest that the ROS level modulation occurs mainly through the control of glutathione- and thioredoxin-related responses and most likely involves a fine-tuning of ROS-generating processes.

ROS-generating processes were repressed, while an enrichment of processes related to the control of gas exchanges via stomata was also observed. This observation suggests that the photosynthesis rate, which is a major ROS source, might be under a fine-tuning. It is interesting to note that the control of stomata movement seems not to involve the modulation of ABA levels. Conversely, the gene expression pattern suggests the control of the sensitivity of the pathway, for instance via the repression of PP2-B11, a SnRK2 negative regulator. A perspective for future work is the confirmation of ABA attenuation and of the control of photosynthesis rate on chronic irradiated plants.

Interestingly, the transcriptome analysis revealed the absence of activation of repair processes in the populations under chronic radiation exposure, responses that are known to occur during acute radiation stress condition (Culligan et al., 2006). This observation can be understood as another evidence of the adaptation of the evaluated populations to the chronic stress, being the expression of the repair machinery below the detection threshold. Nevertheless, the constant exposure to ionizing radiation imposes a permanent risk for the integrity of the molecules in the cells, including DNA single- and double-strand breaks (Caplin and Willey, 2018). In this context, our analyses indicate that the strategy adopted by the evaluated populations is to increase the maintenance of the integrity of the molecules in the cell, which could be explained by the enhanced expression of chaperones and histones.

Finally, the control of TEs activity, which is also a source of DNA damage, is also evident in all chronically irradiated populations. However, the activity of the TEs was inversely correlated to the radiation dose exposure. While more active TE families were identified in the low-dose exposed individuals from Zab, the opposite trend was shown for Kul individuals.

4. Conclusions

Radioactive contamination of the natural areas is one of the most long-lasting anthropogenic impacts on the environment. Scots pine (*Pinus sylvestris* L.) is a promising organism for radiation-related research because of its high radiosensiti-

vity, but the genome size of Pinacea species has imposed obstacles for high-throughput studies so far. In this work, we conducted the analysis of the de novo assembled transcriptome of Scots pine populations growing in the Chernobyl-affected zone, which is still today contaminated with radionuclides because of the accident at the nuclear power plant in 1986. The transcriptome profiles indicate a clear pattern of adaptive stress response, which seems to be dose-dependent. The transcriptional response indicates a continuous modulation of the cellular redox system, enhanced expression of chaperones and histones, along with the control of ion balance. Interestingly, the activity of transposable element families is inversely correlated to the exposure levels to radiation. These adaptive responses, which are triggered by radiation doses 30 times lower than the one accepted as safe for biota species by international regulations, suggest that the environmental management in radiation protection should be reviewed.

References

Bryant D.M., Johnson K., DiTommaso T. et al. A tissue-mapped axolotl *de novo* transcriptome rnables identification of limb regeneration factors. *Cell Rep*. 2017;18:762–776.

Caplin N., Willey N. Ionizing radiation, higher plants, and radioprotection: From acute high doses to chronic low doses. *Front. Plant Sci*. 2018;9:847.

Cheng C., Wang Z., Ren Z. et al. SCFAtPP2-B11 modulates ABA signaling by facilitating SnRK2.3 degradation in Arabidopsis thaliana. *PLoS Genet*. 2017;7:1006947.

Culligan K.M., Robertson C.E., Foreman J. et al. ATR and ATM play both distinct and additive roles in response to ionizing radiation. *Plant J*. 2006;48:947-961.

Duarte G.T., Volkova P.Yu., Geras'kin S.A. The response profile to chronic radiation exposure based on the transcriptome analysis of Scots pine from Chernobyl affected zone. *Environmental Pollution*. 2019;250:618–626. DOI 10.1016/j.envpol.2019.04.064.

Duarte G.T., Volkova P.Y., Geras'kin S.A. A Pipeline for Transcriptome Assembly, Annotation, and Gene Ontology Analysis for Non-model Organisms: a Case Study with Scots Pine. MethodsX (forthcoming).

Grabherr M.G., Haas B.J., Yassour M. et al. Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nat. Biotechnol*. 2011;29:644–652.

ICRP. 2008. Publication 108. Environmental protection - the concept and use of reference animals and plants. *Ann. ICRP* 38.

Jones P., Binns D., Chang H.Y. et al. . InterProScan 5: genome-scale protein function classification. *Bioinformatics*. 2014;30:1236–1240.

Kovalchuk I., Molinier J., Yao Y. et al. Transcriptome analysis reveals fundamental differences in plant response to acute and chronic exposure to ionizing radiation. *Mutat. Res*. 2007;624:101–113.

Liu J., Li G., Chang Z. et al. BinPacker: packing-based *de novo* transcriptome assembly from RNA-seq data. *PLoS Comput. Biol*. 2016; 12:e1004772.

Sewelam N., Kazan K., Schenk P.M. Global plant stress signaling: Reactive oxygen species at the cross-road. *Front Plant Sci*. 2016;7:187.

Szumiel I. Ionizing radiation-induced oxidative stress, epigenetic changes and genomic instability: the pivotal role of mitochondria. Int. *J. Radiat. Biol*. 2015;91:1–12.

UNSCEAR. 2008. Effects of ionizing radiation on non-human biota. In Sources and Effects of Ionizing Radiation, United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) Report, Volume II, Annex E.

Xie Y., Wu G., Tang J. et al. SOAPdenovo-Trans: *de novo* transcriptome assembly with short RNA-Seq reads. *Bioinformatics*. 2014;30: 1660–1666.

Conflict of interest. The authors declare no conflict of interest.