Science of the Total Environment 496 (2014) 317–327

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Genetic diversity in Scots pine populations along a radiation exposure gradient

Stanislav A. Geras'kin *, Polina Yu. Volkova

Russian Institute of Agricultural Radiology and Agroecology, Kievskoe shosse, 109 km, 249032 Obninsk, Russia

HIGHLIGHTS

- Polymorphism of antioxidant enzymes was studied in affected Scots pine populations.
- Genetic processes in affected Scots pine populations increase genetic diversity.
- Chronic exposure at dose rates from 0.8 μGy/h lead to increasing of mutation rates.
- Changes in population genetic structure were observed at dose rates from 10.4 μGy/h.
- The higher rate of mutations had no effect on antioxidant enzymes activities.

ARTICLE INFO

Article history:
Received 28 March 2014
Received in revised form 5 July 2014
Accepted 5 July 2014
Available online xxxx

Editor: J. P. Bennett

Keywords:
Scots pine populations
Enzymatic loci mutations
Genetic diversity
Antioxidant enzymes
Chernobyl accident
Chronic radiation exposure

ABSTRACT

Polymorphisms of antioxidant enzymes were studied in the endosperm and embryos of seeds from Scots pine populations inhabiting sites in the Bryansk region of Russia radioactively contaminated as a result of the Chernobyl accident. Chronic radiation exposure at dose rates from 0.8 μGy/h led to a significant increase in the rate of enzymatic loci mutations. The main parameters of genetic variability of the affected Scots pine populations had considerably higher values than those from the reference site. Changes in the genetic makeup of Scots pine populations were observed at dose rates greater than 10.4 μGy/h. However, the higher mutation rate had no effect on the activities of antioxidant enzymes.

1. Introduction

As a result of radiation accidents in the South Urals, Russia in 1957 and at the Chernobyl NPP in 1986, large forested areas were severely affected because atmospherically dispersed radionuclides were absorbed through leaf stomata, directly influencing the plants (Tikhomirov and Shcheglov, 1994). Conifers are particularly vulnerable to radiation exposure, due to their large chromosome size (Sparrow et al., 1968). After the Chernobyl accident, the area of lethal destruction of pine forests was 500–600 ha, with strong and moderate damage across 3000 and 12 000 ha, respectively (Kozubov and Taskaev, 2002). As a result of the accident in the South Urals, pines were completely lost in an area of 2000 ha (Alexakhin et al., 2004). Much larger forest areas were polluted with levels of radioactive contamination that were insufficient for the mass death of trees. Currently, little is known regarding the long-term consequences of the chronic radiation exposure of these forest ecosystems.

Chronic radiation exposure can affect natural populations in many ways; genetic change is one of the more subtle effects with potentially large long-term consequences. Genetic variation is one of the three pillars of biodiversity recognized in the Rio Convention of 1993, because it contributes to phenotypic diversity and may facilitate adaptation to environmental change. This is especially important in long-lived trees, where adaptation to rapid changes in the environment must rely on existing variation within populations. Chronic radiation exposure can alter the structure of intra-population variability (Shevchenko et al., 1992; Theodorakis, 2001). However, to date, there is no complete understanding of the effects of increased frequencies of genetic and cytogenetic damage in somatic and germ cells on reproductive capacity, adaptive differentiation and the general fate of the populations (Geras'kin et al., 2013). Radiation is a form of stress that elicits community responses

* Corresponding author. Tel.: +7 48439 96964; fax: +7 48439 68066.
E-mail address: stgeraskin@gmail.com (S.A. Geras'kin).

http://dx.doi.org/10.1016/j.scitotenv.2014.07.020
0048-9697/

often similar to those resulting from other forms of stress (Woodwell, 1967). Therefore, considerable insight into the basic nature of plant communities and their ability to withstand or to recover from stress can be obtained from observations of irradiated forests.

Electrophoresis of isozymes can reveal more than 40% of the point mutations related to amino acid substitutions in isozymes as well as all mutations that affect isozyme functions (Altukhov, 2003). Therefore, this method can be used to quantify the genetic diversity and differentiation of populations in ecologically contrasting areas. Mutations in isozyme loci have co-dominant inheritance and manifest themselves in the seeds of the first generation (Verta et al., 2013). This is especially important in long-lived trees, where it is unfeasible to generate the inbred lines or crossed progeny that facilitate traditional analysis.

Traditionally, reactive oxygen species (ROS) were considered toxic by-products of aerobic metabolism. Under normal growth conditions, the production of ROS in cells is low, whereas many stresses, including ionizing radiation, increase their production (Limon-Pacheco and Gonsebatt, 2009). Radiation can cause ROS production through radiolysis of water or as by-products of a hampered metabolism. Enhanced production of ROS during stress can harm cells through membrane lipid peroxidation, protein oxidation, enzyme inhibition, and DNA and RNA damage. ROS are also thought to act as signals for the activation of stress-response and defense pathways (Mittler, 2002; Foyer and Noctor, 2005). Thus, ROS can be considered both the cellular indicators of stress and the secondary messengers involved in the stress-response signal transduction pathway.

In this study, we attempted to answer the following questions: (i) Do Scots pine populations growing under relatively low levels of chronic radiation exposure (0.8–14.8 μGy/h) show an increased rate of enzymatic loci mutations? (ii) Can chronic radiation exposure modify the genetic makeup of Scots pine populations in the dose rate range studied? and (iii) Does chronic radiation exposure in the dose rate range studied significantly modify the activity of antioxidant enzymes?

2. Materials and methods

2.1. Test organism

Scots pine (*Pinus sylvestris* L.), the dominant tree species in North European and Asian boreal forest, was chosen as the test organism for an assessment of the possible effects of the radioactive contamination. Scots pines are widespread in the area affected by the Chernobyl accident, and samples can be obtained from trees growing in different contamination levels. The reproductive organs of conifers are particularly susceptible to radiation exposure because of their complex organization and long generative cycle (Cairney and Pullman, 2007). The availability of a haploid endosperm (megagametophyte) and a diploid embryo in each seed enables the investigation of mutagenesis on the gametic level, which allows the direct determination of the haplotype and recessive mutations (Verta et al., 2013). Such special properties cause conifers to be a unique model for the study of the mutation processes in populations developing under chronic radiation exposure.

2.2. Study area and data collection

In 1986, the Bryansk region was significantly contaminated by the fallout from Chernobyl, with an initial ^{137}Cs ground deposition level greater than 1 MBq/m^2 in some locations (Ramzaev et al., 2008). Currently, sites still exist in this area where radioactive contamination significantly exceeds background. Four radioactively contaminated sites, VIUA (52° 29′ N; 31° 50′ E), SB (52° 33′ N; 31° 44′ E), Z1 (53° 5′ N; 31° 42′ E), and Z2 (53° 5′ N; 31° 42′ E), were chosen approximately 200 km northeast of the Chernobyl nuclear power plant (Fig. 1). The reference site, Ref (53° 1′ N; 33° 55′ E), was selected based on its proximity to the impacted area and the similarity of its environmental properties. Latitude and longitude measurements were made using a geographical positioning system (GPS).

The γ-radiation dose rates were measured at the study sites using a DRG-01T dose rate meter ("Leninez", Russia) at a height of 1 m above the ground, 5–7 times under every tree from which cones were collected. All dose rate values were expressed in Roentgen units initially and were converted to Gray units (Gy) using a multiplication factor of 8.76×10^{-3} (Mashkovich and Kudriavtseva, 2013). Dose rates at the study sites ranged from 0.37 to 1.21 μGy/h, compared with 0.10 μGy/h at the reference site (Table 1). Insofar as we could determine, all other readily measurable aspects of the microenvironments at these study sites, except the radiation level, were identical.

Samples were collected at the same sites as in our previous study of the influence of chronic radiation exposure on cytogenetic effects and

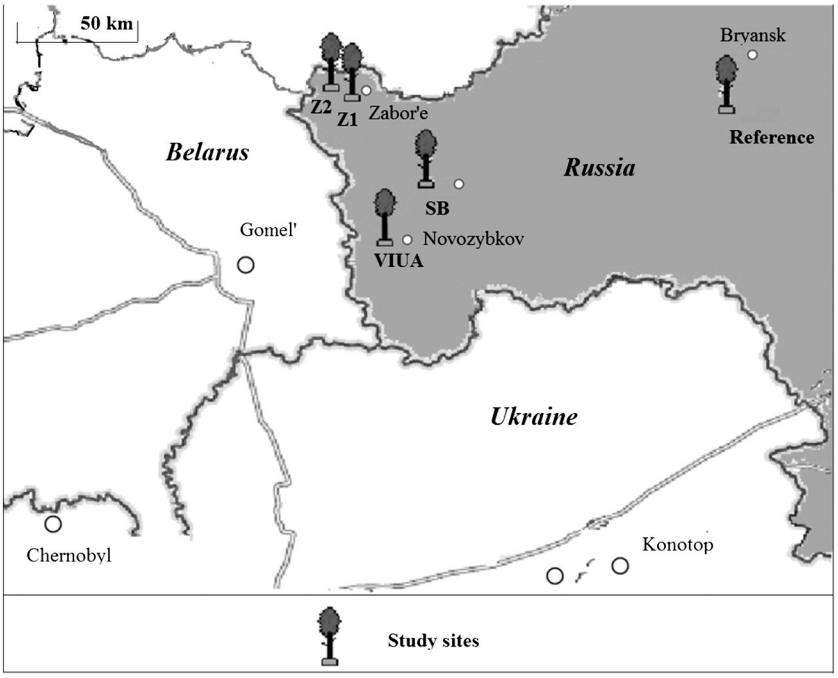

Fig. 1. Location of the study sites.