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Chronic radiation exposure as an ecological factor: Hypermethylation and genetic differentiation in irradiated Scots pine populations[☆]

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ABSTRACT

Genetic and epigenetic changes were investigated in chronically irradiated Scots pine (Pinus sylvestris L.) populations from territories that were heavily contaminated by radionuclides as result of the Chernobyl Nuclear Power Plant accident. In comparison to the reference site, the genetic diversity revealed by electrophoretic mobility of AFLPs was found to be significantly higher at the radioactively contaminated areas. In addition, the genome of pine trees was significantly hypermethylated at 4 of the 7 affected sites.

1. Introduction

Natural and anthropogenic stress factors often determine the distribution of species and can even impose a selective evolutionary pressure on a given population (Boyko and Kovalchuk, 2008; Geras'kin, 2016). To accurately estimate the biological consequences of anthropogenic factors on natural populations, it is convenient to consider those stress factors that can be easily measured and for which mechanisms of biological action are well-known. Ionizing radiation meets both requirements: the influence of ionizing radiation on molecular processes is quite well understood (Reisz et al., 2014), and dosimetric models for a specific object allow an accurate estimation of the absorbed radiation dose (ICRP, 2008), especially in controlled experiments. Meanwhile, the precise dose determination can be a challenging task under field conditions. The maximum dose rate considered to be safe for terrestrial plants in natural populations is 400 μGy/h (UNSCEAR, 2008), meaning that lower doses should be safe for reproduction and the long-term destiny of plant populations. However, even lower doses of ionizing radiation can induce stress responses of an irradiated organism (Gálvan et al., 2014; Volkova et al., 2017). Nevertheless, the precise dose threshold for triggering the emergence of adaptation processes in natural populations in response to chronic low-dose irradiation still is the subject of discussions in the scientific literature (Geras'kin et al., 2013). At the same time, the detailed molecular mechanisms of plant adaptation to chronic radiation exposure still remain to be discovered (Kovalchuk et al., 2003, 2004; Boubriak et al., 2016; Møller & Mosseau, 2016).

Ionizing radiation is known to interact differently on plant growth and development, ranging from stimulatory effects at low doses, to increasingly harmful effects for vegetative growth at medium levels, and pronounced decreases in reproductive success and yield at high radiation levels (Jan et al., 2012). The degree of effects and the exposure dose at which the response occurs depend on the species, age, plant morphology, physiology and genome organization (Holst and Nagel, 1997). At the genetic level, ionizing radiation can induce diverse effects that vary from simple base substitutions to single- or double-strand breaks of the DNA (Grosovsky et al., 1988). Increased mutation rates can be observed all over the genome (Kuchma et al., 2011), while essential genes of

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the antioxidant and metabolic pathways may serve as read-outs (Volkova et al., 2017). High mutation rates found in plant species living in radioactively contaminated areas may participate in the possible induction of adaptive responses to chronic radiation exposure (Kalchenko et al., 1993; Kovalchuk et al., 2000; Fedotov et al., 2006; Pozolotina et al., 2012; Møller and Mosseau, 2015; Georgieva et al., 2017). However, epigenetic changes are more suitable for explaining the rapid adaptation of plants to environmental stress factors (Sahu et al., 2013).

There are not many places in the world where the influence of ionizing radiation on plant populations in natural conditions can be studied. The most prominent are the areas of Ukraine, Belarus and Russia that were contaminated by the Chernobyl Nuclear Power Plant accident in 1986. This disaster is considered to be the most severe radiation accident in the world. More than 200,000 km^2 (ARPA, 2009) were contaminated by radionuclides with a total released radioactivity of 5300 PBq (Steinhauser et al., 2014). The radioactive contamination remains a problem in this area even 30 years after the accident, and natural populations of plants and animals have been living there under radiation exposure conditions since 1986. The study of possible genetic changes in plant populations living under challenging environmental conditions can provide valuable information about the mechanisms and patterns of adaptation to chronic radiation exposure in a situation where the stress factor gradually decreases due to radioactive decay.

High level of genetic diversity, efficient stress responses and a long history of ecological genetic research make conifers a good model for studying the genetic effects on a changing environment (Neale and Savolainen, 2004; Guevara et al., 2005; Kuchma et al., 2011). In this context, Scots pine (*Pinus sylvestris* L.) became one of the main natural test objects for ecological monitoring (Micieta and Murin, 1998; Prus-Glowacki et al., 1999). Indeed, the International Commission on Radiation Protection has included pine in the list of Reference Animals and Plants used for the goals of radiation protection of non-human biota (ICRP, 2007). Therefore, any information about the biological effects of ionizing radiation on pine species in field conditions is especially valuable. Scots pine exhibits one of the highest sensitivities to radiation among various species investigated (ICRP, 2007). A widespread characteristic of a species' radiosensitivity is the median lethal dose (LD_{50}), which is 5–20 Gy for Scots pine, while for many angiosperm plants this value reaches hundreds of Gy. In addition, Scots pine is the main woody species of the Chernobyl zone, making it valuable for assessment of the ecological consequences of the Chernobyl accident.

The inability to limit or escape stress exposure makes the survival of plants dependent on efficient short- and long-term adaptation strategies based on the control and expression of their genetic information. Therefore, the assumption that genetic diversity is of fundamental importance for the adaptation of any species to environmental changes is reasonable in the context of a population's survival. It is suggested that maintaining genetic diversity within natural populations can maximize their potential to withstand and adapt to biotic and abiotic environmental changes (Jump et al., 2008). Accordingly, the positive relationship between genetic diversity and plant fitness was confirmed as a general pattern (Leimu et al., 2006; Reed and Frankham, 2003).

One of the simplest and most informative ways for estimating the genetic diversity and mutation rate of a population is to use DNA markers. The amplified fragment length polymorphism (AFLP) technique (Vos et al., 1995) allows the scoring of a large number of loci that are assumed to be randomly distributed within the genome of the studied individuals. Previous studies estimated the mutation rates for AFLPs for some plant species as 10^{-5}-10^{-6} per locus per generation (Mariette et al., 2001; Kropf et al., 2009). In AFLPs, mutations can occur at the enzyme restriction sites or primer-elongation binding sites, or they can be due to insertion/deletions (indels) in the amplified region, resulting in the loss and/or appearance of a “new” AFLP band (Li et al., 2007; Kropf et al., 2009). Hence, AFLP markers are a sensitive and useful tool for investigating changes of mutation rates because of radiation exposure (Kuchma et al., 2011). In addition, AFLP-based markers data can also be used for the investigation of genetic structure of populations (Bonin et al., 2007).

A higher tolerance of some individuals or particular species to chronic radiation exposure was shown in a number of studies (Shevchenko et al., 1992; Fedotov et al., 2006; Rodgers and Holmes, 2008; Geras'kin et al., 2013). One of the mechanisms that may be related to such increased tolerance is the epigenetic modifications, which can directly influence the gene expression pattern and the genome stability. Epigenetic adaptability is an important but still poorly understood factor for the survival and reproductive success of tree species living in radioactively contaminated territories. Recent studies showed that epigenetic-related pathways (DNA methylation, histone variants and modifications, positioning of nucleosomes and small RNAs) are important components of plant growth and reproductive regulation (Feng et al., 2010). DNA methylation is the most studied and probably the best understood type of epigenetic modification, which could represent a way to allow phenotypic variability in a changing environment without having to rely on genetic variation (Angers et al., 2010). Indeed, epigenetic regulation of stress response of irradiated plants in field conditions can include genome hypermethylation and changes in gene expression (Kovalchuk et al., 2003, 2004). The knowledge of epigenetic modifications together with the information about changes in the genetic structure of the respective population may provide important insights about the formation of adaptive responses in chronically irradiated populations of plants. In this context, the main aim of this work was to analyse genetic and epigenetic changes in Scots pine populations from areas that are radioactively contaminated for more than 30 years.

2. Materials and methods

2.1. Experimental sites, soil sampling and dose assessment

Samples were collected from seven experimental sites at locations contaminated as a result of the Chernobyl accident and from one non-contaminated reference area. The reference site (Ref1) and the four affected sites named VIUA, Starye Bobovichi (SB), Zabor'e Pole (Z1) and Zabor'e Kladbishche (Z2) are located in the Bryansk region of Russia. The three other experimental sites, namely Kozhushki (Kozh), Masany (Mas) and Kulazhin (Kul), are located in the Gomel' region of Belarus (Supplementary Table 1). Soil samples and biological material (cones from Scots pine trees) were taken from each site for estimation of radionuclide (^{137}Cs and ^{90}Sr) and heavy metal content (Cd, Cu, Co, Cr, Mn, Ni, Pb, Zn). Physical and chemical properties of soils were also recorded (pH; % of humus, N, P_2O_5, K, Ca, Mg; cation exchange capacity; hydrolytic acidity). Detailed description of these estimations is given in (Geras'kin et al., 2011; Volkova et al., 2017). The radionuclide concentrations were then used for parameterization of the dosimetric model (Geras'kin et al., 2011).

2.2. Plant material and DNA isolation

Fresh needles were collected from 15 to 20 trees at each site. The trees were between 40 and 50 years old, meaning that they received a high dose of radiation during the first months after the Chernobyl accident. For each tree, five needles were collected from different cardinal directions of the crown from a height of 1.5–2 m

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