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Radioactive contamination in Chernobyl and (epi)genetic stability of plants – A review

Rádioaktívna kontaminácia v Černobyle a (epi)genetická stabilita rastlín - Prehľad

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ABSTRACT

Rapid industrial and agricultural development brought, besides the indisputable advances, also risks related to the environmental pollution. Widespread soil deterioration represents a global problem. Chernobyl area contaminated by radionuclides after nuclear accident in 1986 provides the opportunity to analyze *in situ* the impact of radiation on plant systems. Unlike animals and humans, plants are not able to move to another place with better living conditions. Therefore, plants are an ideal object to study adaptation to the conditions of environmental stress. Long- term exposure to the ionizing radiation causes widespread changes in plant genome and epigenome. Also, these alterations may result in changed phenotype. In particular, this review discusses the effect of ionizing radiation on genetic and epigenetic stability of plant genome.

Keywords: Chernobyl, epigenome, genome, radioactive contamination

ABSTRAKT

Rozvoj priemyslu a poľnohospodárstva priniesol okrem nesporných výhod aj riziká súvisiace so znečistením životného prostredia. Rádioaktívnymi prvkami kontaminovaná oblasť Černobyľu po jadrovej havárii v roku 1986 poskytuje možnosť analyzovať vplyv radiácie na rastliny v podmienkach *in situ*. Rastliny na rozdiel od živočíchov a človeka nie sú schopné presídliť sa na iné miesto s vyhovujúcejšími životnými podmienkami. Z tohto dôvodu sú ideálnymi objektom pre štúdium adaptácie na podmienky prostredia zmenené pôsobením stresu. Dlhodobé pôsobenie ionizujúceho žiarenia môže mať za následok rozsiahle zmeny v genóme a epigenóme rastlín. Tieto zmeny môžu mať za následok zmenený fenotyp. Prehľad diskutuje najmä vplyv ionizujúceho žiarenia na genetickú a epigenetickú stabilitu rastlín.

Kľúčové slová: Černobyľ, epigenóm, genóm, rádioaktívna kontaminácia

INTRODUCTION

Nowadays, extensive agricultural areas lost their original function because of the contamination by substances coming from various natural and anthropogenic sources. Therefore, the question how to effectively manage and re-use the contaminated lands becomes of a great importance. Although, nuclear accidents are rare, they represent a serious threat for environment. Radionuclides released into the environment cause the degradation of land and persist in the environment for a long time. After the Chernobyl Nuclear Power Plant (CNPP) accident, radioactive cesium, plutonium, strontium and many other radioactive isotopes have been released into the environment, approximately 200 000 km^2 of land has been contaminated within Europe (Møller and Mousseau, 2016). Chernobyl accident is unique in the extent of radioactive contamination when comparing radiation doses that have been absorbed by living organisms as well as the wide spectrum of radionuclides in the soil to other nuclear accident such as the southern part of Ural Mountains in 1957 (Geras'kin et al., 2003).

The most exposed to the radiation were natural and agricultural ecosystems within the 30 km Chernobyl exclusion zone. In this area, the radioactive contamination reached several thousands of MBq/m^2 in 1986. The maximum rate of radiation was absorbed by living systems during the 10-20 days after the Chernobyl accident when a significant portion of the absorbed dose were short-lived radioisotopes. In the autumn 1986, the radioecological situation had been stabilized within the exclusion zone and the acute high-dose radiation was replaced by chronic low-dose radiation. The low-dose radiation persists until nowadays. Long-term exposure to the chronic irradiation can cause hereditary changes of genetic structures manifested by increased cytogenetic damage and chromosomal variability in the progeny of irradiated organisms (Geras'kin et al., 2003). Shortly after the Chernobyl nuclear accident, many studies have been conducted to identify mutations in the plant genome caused by irradiation. Despite the occurrence of genome alterations and mutations, a vast majority of the studies has indicated that the plants are able to adapt to the environment with increased level of radiation (Kovalchuk et al., 2004; Klubicová et al., 2010; Klubicová et al., 2012). The level of radionuclide accumulation by plants in the Chernobyl region depends mainly on the characteristics of soil, climate, biosphere, plant species and populations (Yablokov et al., 2009).

In recent years, there is an increasing tendency to remediate and re-cycle the areas destroyed by radio-contamination for agricultural purposes. Therefore, the molecular characterization of crops grown in areas around Chernobyl may be important for the future agricultural exploitation of these lands (Klubicová et al., 2011). Spectrum of biological approaches such as genomics, proteomics, cytogenetics or mutagenesis have been employed to investigate the effect of long-term chronic irradiation on organisms (Danchenko et al., 2016).

The effect of irradiation on the destabilization of plant genome

Various types of ionizing radiation cause multiple damage in living organisms ranging from changes at the molecular to ecosystem level (Ichikawa, 1981). Several structural and functional alterations occur in the DNA molecule as a consequence of ionizing radiation, these are responsible for most of the damage at both cellular and systemic level. Macroscopic differences in phenotype often result from changes in gene expression. The nature of DNA modifications that can lead to alterations in gene expression may vary from substitution or deletion of nitrogen base to the chromosomal aberrations and epigenetic modifications (Tanaka et al., 2010).

Plants have to deal with the consequences of radiation stress through the adequate defense mechanism (Lefebvre et al., 2009). For instance, the analysis of rice grown in the close proximity of Chernobyl showed numerous chromosomal abnormalities in leaf and root meristem. During the years after nuclear accident, the increased number of chromosomal aberrations in cereals also increased (Geraskin et al., 2003). The incidence of genetic abnormalities in radiation exposed pines was

7 times higher in 1986 and 8 times higher in 1993 in comparison to the pines grown in non- radioactive environment (Geraskin et al., 2005).

Up to this date, several cytogenetic and mutation studies have been conducted to address the plant exposure to the ionizing radiation. Cytogenetic studies have been carried out in various plant species (tobacco, peas, rice, wheat, salad). However, the comparison of individual cytogenetic studies is complicated due to the specific characteristics of each plant species and different level of radiosensitivity (De Micco et al., 2011). Cytogenetic analysis of primary root meristem of winter wheat grown in Chernobyl region confirmed that even 25 years after the nuclear accident, the frequency of chromosomal abberations was 2 to 7-fold higher than in control samples. The number of aberrant cells was 1.8 to 2.7-fold higher in comparison to the controls (Yakymchuk, 2013).

The soil in Chernobyl region is mainly contaminated by long-term radioisotopes ^{90}Sr and ^{137}Cs. The effect of ^{137}Cs on alterations in gene expression in Arabidopsis roots was studied by Sahr et al. (2005). The most of identified target sequences were genes encoding three types of proteins: a) proteins involved in cell growth, division and plant development, b) proteins providing control of translation, metabolism and stress control, and c) proteins involved in DNA repair. For instance, Danchenko et al. (2009) investigated mechanisms of soybean adaptation using the quantitative proteomic methods. The results of this study indicated that the mechanism of plant adaptation on the radioactive environment includes the activation of defense mechanisms such as heavy metal stress tolerance, protection against radiation induced damage and mobilization of soy proteins. Moreover, Klubicová et al. (2012) revealed the developmental changes in soybean proteome manifested by lower seed oil content.

Kovalchuk et al. (2004) analyzed the adaptability of Arabidopsis plants harvested in areas with various levels of radiation during the 1986-1992. Molecular mechanisms of mutagen resistance had been evaluated and revealed more than 10-fold lower frequencies of extrachromosomal homologous recombinations, differences in expression of genes associated with DNA repair and DNA hypermethylation. In particular, the main conclusion of the study was a low level of recombination in Chernobyl plants. This may serve as an indicator of plant adaptation since the low frequency of recombination may prevent an excessive genome rearrangement. The hypermethylation is considered to be a general defense mechanism which prevents a genome remodeling. The obtained data suggested the complexity of adaptation process which includes the epigenetic regulation and genome stabilization. All together increases the plant resistance to mutagens in the environment.

To investigate the effect of low-dose irradiation on plants, species such as winter rye, barley, oat, pine have been exposed to various radioecological situations, for instance Chernobyl nuclear accident, soil contaminated by radionuclides due to the industrial processing of uranium and radium. Despite of many studies, the effect of chronic radiation exposure on living organisms has not been fully understood. Cytogenetic damage, genetic diversity, decreased reproductive capacity are the most common molecular consequences of irradiation (Geras'kin et al., 2013).

Plant epigenetic response to the radiation stress

Epigenetics is engaged in the study of mitotic hereditary changes; however these are not accompanied by changes in DNA sequence. Epigenetic modifications, particularly DNA methylation and post-translational histone modification, have an important role in the regulation of gene expression in response to the environmental stimuli (Garg et al., 2015).

Genomic alterations are often discussed in relation to the plant stress response (Kovalchuk and Baulch, 2008). Response of plants exposed to the radiation stress in environment may be also associated with epigenetic modifications (Chinnusamy and Zhu, 2009). Survival and adaptation of plants under the stress conditions can be epigenetically controlled at the level of DNA methylation. Genomic DNA can be rapidly and reversibly methylated by DNA methyltransferase enzymes. This modification underlines the genome adaptability under the harsh environmental conditions (Peng and Zhang, 2009; Tang et al., 2014).

DNA methylation means the addition of methyl group to the carbon-five (C^5) position of cytosines in CpG dinucleotides (Vaissiere et al., 2008). This type of epigenetic mechanism may cause modification of cytosines in plant genomic DNA, both in symmetric (mCpG and mCpHpG) and asymmetric (mCpHpH) context (Chinnusamy and Zhu, 2009; Peng and Zhang, 2009). The methylated cytosines are concentrated mostly in clusters called as CpG islands (Hernández et al., 2013). Two major classes of DNA methyltransferases are characterized in plants. Firstly, methyltransferases of the MET1 family methylate principally the cytosines in symmetric CpG context, and chromomethylases (CMTs) present exclusively in plants methylate the cytosines in CpNpG context (Gupta et al., 2010).

DNA methylation can decrease or completely inactivate the transposable elements and endogenous genes. Methylation in the gene promoter mostly results into inhibition of transcription unlike the methylation in coding gene sequence which has no significant effect on gene expression (Zhang et al., 2010). Kovalchuk et al. (2004) analyzed the DNA methylation level thorough the genome of Arabidopsis plants grown in the experimental area Tolsty Les in 1989 using the methylation sensitive restriction analysis. The Arabidopsis genome was significantly hypermethylated in comparison to the plants grown in the same experimental area during the 1991 and 1992. In general, epigenetic regulation can maintain the genome plasticity and allow relatively rapid adaptation to the changed environmental conditions without the alterations in DNA sequence (Causevic et al., 2005).

The opportunity to analyze the DNA methylation in the genome of plants growing in the real radioactive environment can contribute to the deeper understanding of the phenomena such as DNA methylations. Over the years, many techniques were developed for DNA methylation analyses, and the most common are as follows: bisulphite conversion, afinity purification of methylated DNA, and digestion using methylation-sensitive restriction enzymes (Zilberman and Henikoff, 2007). For the analyses of the presence or absence of the methyl group at the cytosine residues are mostly used the methods based on pretreatments of genomic DNA, and are suitable preferably for the analysis of the specific region in the genome (Laird, 2010).

Bisulphite sequencing is an effective method for determination of the exact position of 5-methylcytosine (m5C) on a single strand DNA after pretreatment with bisulphite ion. The principle of the bisulphite sequencing method is deamination of unmethylated cytosines by sodium bisulphite causing the alkaline desulfonation of all unmethylated cytosines in the analyzed DNA sample to the uracil, followed by PCR and direct sequencing of PCR products or sequencing of cloned DNA amplicons (Darst et al., 2010). Complete bisulphite conversion is crucial for successful distinguishing of unmethylated and methylated cytosines, therefore it can be difficult to distinguish between methylated cytosine and incomplete bisulphite conversion (Frommer et al., 1992; Grunau et al., 2001; Wang et al., 2011).

Generally, abiotic stress factors such as cutting, chilling, planting density, and rubbing reduce the level of DNA methylation, and salt stress causes opposite response manifested as hypermethylation (Peng and Zhang, 2009). Changes in gene expression can be induced, depending on the type of stress, through hypermethylation or hypomethylation of DNA (Gupta et al., 2010). Reduction of gene expression is in many cases associated with hypermethylation in gene promoter, but hypomethylation may result to the inverse effect of gene over-expression (Antwih et al., 2013). Increasing doses of irradiation can induce the reduction in genomic DNA methylation. Previous results suggest that DNA hypomethylation occurs mostly at CHG or CHH sites (Kim et al., 2013). The spontaneous deamination of methylated cytosines frequently causes mutations of cytosines to thymines. Epigenetic mutations may be converted into the permanet epigenetic trait, resulting from the exposure to the long-term stress (Boyko and Kovalchuk, 2011). Kovalchuk et al. (2003) evaluated global methylation level in pine trees exposed to the irradiation in Chernobyl and observed significant DNA hypermethylation in genome. The level of hypermethylation was dose dependent. Thus, hypermethylation can be considered as plant defense strategy that allows survival in extreme environmental conditions and prevents genomic instability.

Stress factors and transposon activation

Plant genome consists of genes and intergenic DNA. Genes unlike the transposons have a relatively stable position in the genome. Transposons are mobile genetic elements able to move from one position to another, moreover retrotransposons are firstly transcribed into ribonucleic acid and then converted back into DNA sequences by reverse transcription (Maumus, 2009). Mobile genetic elements are abundantlty represented across the plants genome, for instance DNA transposons and retrotransposons represent approximately 10% of Arabidopsis genome, barley retrotransposon BARE1 represents up to the 50% of genome. Transposons are known for their important function in genome dynamics and development of species, therefore are extensively studied (Maumus, 2009).

The most abundant transposable elements in the plants genome are Long Terminal Repeat (LTR) retrotransposons and Miniature Inverted Transposable Elements (MITE) (Casacuberta and Santiago, 2003). Based on the presence of long terminal repeats, retrotransposons are divided into two subgroups: 1. retrotransposons flanked by long terminal repeat sequences, so-called LTR-retrotransposons and 2. retrotransposons that do not contain long terminal repeat sequences, non-LTR retrotransposons (Staton et al., 2009).

Retrotransposons are inactive during the plant development, however their activation is manifested under the various biotic and abiotic stress conditions. Retrotransposon activation due to the external stimuli is a common phenomenon for most eukarytes (Grandbastien, 1998). In particular, LTR-retrotransposons are characterized by a high level of variability under the stress conditions. Most of the plant LTR- retrotransposons produce large number of transcripts in response to the changed environmental stimuli. Moreover, the epigenetic activation of mobile elements can induce changes in expression of the neighboring gene. New retrotransposon insertion into the gene coding sequence may result in mutations, alterations in gene expression, structural and functional rearrangement of the genome. Activation of retrotransposons can play a crucial role in plant development and adaptation to the changed environmental conditions (Mansour, 2007; Woodrow et al., 2010).

It is known that plant retrotransposons may be activated by various stress factors, for instance activation of *Ty1-copia* in chickpea by drought (Rajput and Upadhyaya, 2010), wheat *Ttd1a* activation in salt and light stress (Woodrow et al., 2010), retrotransposon *OARE-1* activation in oat in response to various stresses such as jasmonic acid, salicylic acid, patogen infection (Kimura et al., 2001), patogen induced activation of *Tnt1* element in tobacco (Grandbastien, 1998; Grandbastien et al., 2005) or heat stress activated *ONSEN* element in Brassicaceae (Ito et al., 2013).

Epigenetic regulation is a key mechanism in plant adaptation to radiation stress (Kovalchuk et al., 2004). DNA methylation is one of the most frequent responses to the irradiation and is closely linked to the transposon activation. There are indications that transposons are activated in the response to the gamma irradiation (Alzohairy et al., 2014; Grandbastien, 2015; Orozco-Arias et al., 2019). Regarding the plants in Chernobyl, up to this date, majority of the published works was dealing with the global genome methylation analysis and the study of transposon activation was only marginally covered. However, the most recent research is showing the activation of specific LTR-retrotransposons in flax growing under the chronic irradiation in Chernobyl area (Lancikova et al., 2020).

Recent advances in genomics and phenomics are dealing with the question whether transposable elements can play a key role in plant adaptation and evolution (Lisch, 2013). Insertion polymorphism of transposons can cause mutations and genome destabilizaton, however on the other side may positively affect gene regulation and adaptation to the changed environmental conditions (Sinzelle et al., 2009; Lisch, 2013; Cavrak et al., 2014). Individual observations suggest that insertion polymorphism may increase the evolution potential of species. Periods of low and increased activity of transposable elements may be essential for development of genetic variability (Guerreiro, 2012).

In most cases, the transposon activation by acute stress factor is discussed. Based on these observations, long-term exposure to the chronic radiation stress may not lead to the transposon activation. Lower radiation doses may not lead to an immediate reaction and transposon activation.

Plant radioresistance

The most essential part of the plant adaptation mechanisms is the ability to repair direct or indirect damage caused by radiation. Reparation mechanisms are manifested on both, DNA and protein level (Møller and Mousseau, 2016).

Acute or chronic radiation stress induces plant adaptation mechanisms and allows the development of radioresistance. The effect of ionizing radiation on biological systems includes several steps from absorption of radiation energy to the manifestation of radiation damage at the biological level. Mostly, the primary site of radiation damage is DNA molecule. Ionizing radiation may induce DNA breaks resulting in both genomic and chromosomal abnormalities. The level of radiation damage depends on the radiation dose and the radiosensitivity of species (Esnault et al., 2010).

Plants are an ideal object to study adaptation mechanisms under the radiation stress since they are not able to move from one location to another. Plants inhabiting areas around the CNPP accumulate radionuclides from external sources, soil and also internally from plant surface (Hinton et al., 1995; Boubriak et al., 2016). Among mechanisms which the most likely trigger the adaptation process belong the upregulation of DNA repairs such single and double strand breaks (Boubriak et al., 2016). For instance, Georgieva et al. (2016) revealed higher level of DNA strand breaks in soybean seedlings growing in the Chernobyl area at the stage of primary root development. On the other side, the DNA breaks were efficiently repaired at the cotyledon stage. Moreover, increased morphogenetic and cytogenetic variability, epigenetic regulation and homologous recombination are signs of plant adaptation, for instance lower frequency of extrachromosomal homologous recombination observed in *Arabidopsis plants* (Kovalchuk et al., 2004; Boubriak et al., 2016).

It is generally known that hypermethylation is one of the most commonly observed genomic responses to irradiation, thus change in DNA methylation is considered as the crucial mechanism in building the stress tolerance (Beresford et al., 2019). However, there are indications that genome does not necessarily have to be hypermethylated, Horemans et al. (2018) observed decrease in global genome methylation level in *Arabidopsis* plants samples from Chernobyl exclusion zone. Clearly, it can summarized that there is an obvious link between changed environmental conditions and methylation status of the organism (Horemans et al., 2018).

Development of plant radioresistance is highly individual and depends on multiple factors while the most decisive are species, plant life cycle, age, and tolerance to other stresses. Therefore, there is still a lot of questions which need to be taken into account. The proper understanding of plant adaptation process may allow re-use of extensive land areas destroyed by radiation.

Chernobyl and its biological consequences

Chernobyl accident affected various ecosystems which includes humans, animals and plants as well. To some extent, various environments, urban, agricultural, forest and aquatic, have been affected by radioactive contamination. Remedial actions, surface areas of buildings, roads had been washed off immediately after the explosion (Rashydov and Nesterenko et al., 2018) and weather conditions such as wind or rain ensured the cleanup of urban areas and the radiation level was significantly reduced. Even though, there was a notable decrease in radionuclide contamination of agricultural lands, the meat and milk from grazing animals or vegetables cultivated in the area still represent a substantial internal dose of radiation for humans. Although, humans living in the close proximity to the Chernobyl had been promptly evacuated, they still absorb contamination through the plant and animal consumption (Alexakhin et al., 2005; Burger and Lichtscheidl, 2019).

Particularly high uptake of ^{137}Cs has been observed for plants and animals inhabiting forest environment while it is assumed that the high level of contamination will persist for many years to come (Alexakhin et al., 2005). Nowadays, wildfires represent especially high risk of radioactivity release into environment (Ager et al., 2019). When analysing the water areas, only minor radioactive contamination has been observed. Contamination of aquatic systems dropped rapidly during the weeks after the accident due to the radionuclide dilution, decay and absorption to the soil (Alexakhin et al., 2005).

Period after the Chernobyl accident can be divided into two phases, acute and chronic irradiation. For comparison, chronic radiation dose is 40 - 80 fold lower than acute radiation dose (Rashydov and Nesterenko, 2018). Shortly after the CNPP, high doses of irradiation had detrimental effect on biota. Then, acute radiation has been replaced by chronic, low dose radiation and the adaptation mechanisms of organisms living around the Chernobyl exclusion zone have been manifested (Beaugelin-Seiller et al. 2018). Effect of released radionuclide contamination on the surrounding environment depends on multiple factors such as species, their age, radiosensitivity and absorbed radiation dose (Boubriak et al., 2016; Beresford et al., 2019).

In terms of Chernobyl impact on human health, several remedial measures have been implemented shortly after the accident. Nevertheless, Fesenko et al. (2013) reported that 20 years after the CNPP, radiation absorbed by humans from contaminated food still represents up to the 50%. Radionuclides accumulate in tissues and bones and the most often can result into cancer (Burger and Lichtscheidl, 2019). It is known that health conditions of about seven million people have been in some way affected by Chernobyl and they suffer from numerous diseases as a direct consequence of radioisotope exposure (Yablokov et al., 2009; Ager et al., 2019).

Since the Chernobyl nuclear accident, increased frequency of chromosomal aberrations, physical abnormalities and genomic instability have been manifested by non-human organisms (Omar-Nazir et al., 2018; Hancock et al., 2020). Several studies have been investigating the genetic diversity of plants and animals inhabiting the Chernobyl area. Multiple species among plants, birds or rodents have shown increased mutation rates on genetic level (Fuller et al. 2019). Moreover, changes in soil microbiome have been detected as well (Geras'kin et al., 2016). On the other side, Fuller et al. (2019) observed no changes in genetic diversity in aquatic biota, *Asellus aquaticus*. Three decades after the Chernobyl, the biological consequences of radio-contamination are still present and it will take a long time for ecosystems to return to the pre-accident stage, if ever. Despite the economic losses and environmental damage, plants and animals have managed to survive and adapt on the changed environmental conditions.

CONCLUSION

Long-term exposure to radiation allows the plant adaptation and the development of radiation resistance. In particular, chronic low-dose irradiation mostly affects the genetic structure of population. The plant adaptation process is often manifested by the reduction of genetic variability. DNA methylation is one of the major mechanisms of epigenetic control in response to the stress factors. Over the years, there has been an ongoing process involving the reduction of radioactive contamination and stabilization of life in the Chernobyl exclusion zone. Nowadays, the differences between plants grown on the contaminated and non-contaminated soils may be minimized. Identification of crop that are capable to adapt to the radioactive environment is crucial for further agricultural use of the contaminated lands.

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