USING ON BASED MOLECULAR BIOLOGY APPROACHES TO ASSESS PLANT ELUSIVE RESPONSES MECHANISMS TO CHRONIC IONIZING RADIATION IN RADIONUCLIDE CONTAMINATED CHERNOBYL ZONE

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It is well known that the Chernobyl accident released large amounts radioactive isotopes into the environment. As a consequence, the vicinity of Chernobyl Nuclear Power Plant remains contaminated with long-lived radionuclide such as ^{137}Cs, ^{90}Sr, and several transuranic isotopes. The area will remain heavily contaminated by both micro- and nano-sized "hot particles" of long-lived radioactive isotopes for the foreseeable future [Rashydov N. 2012]. There have been a few molecular analyses of plants grown in the radio-contaminated Chernobyl area, but there is as yet no broad understanding of the mechanisms that underlie their survival or success.

Plants respond to biotic and abiotic factors not only with changes in their growth, developmental and physiology state but ultimately with altered phenotypes expressed by their progeny [Herman J.J., Sultan S.E. 2011]. Much research has addressed the nature of mutation sites in DNA, changes in signaling networks, epigenetic changes, changes at the transcription level, protein level, or levels of posttranslational modifications. All of these must be considered when designing a global strategy for understanding the thus far discovering elusive of resistance and adaptive mechanisms of plants to chronic ionizing radiation.

We previously reported that plant growth in the radioactive environment led to alteration in the proteome of developing soybean seeds along with decreased levels of oil and seed storage proteins (SSP). The results confirmed previous data indicating that alterations in the proteome including adaptation to heavy metal stress and mobilization of SSP. The results also suggest that there have been adjustments in carbon metabolism in the cytoplasm and plastids, increased activity of the Krebs cycle, and decreased condensation of malonyl-acyl carrier protein during fatty acid biosynthesis [Klubicova K., et al 2012]. Similar abundance profiles were detected for 2-DE spots, which are the dihydrolipoamide dehydrogenase (E3) component of the pyruvate dehydrogenase complex (PDC) [Hirani T.A. et al. 2011]. The mitochondrial PDC catalyzes the conversion of pyruvate to acetyl-CoA, which enters TCA cycle [Budde R.J., et al 1991]. The mitochondrial PDC (mtPDC) occupies a critical interface between glycolysis and the Krebs cycle and as a result, activity is extensively regulated [Ahsan N. et al 2012]. The most spectacular mechanism of regulation is via reversible phosphorylation. High levels of E3 at 4 weeks after flowering corresponds to increased production of acetyl-CoA but also to increased photorespiration during the cell division phase of soybean seed development in radioactive areas. Despite failing to note any changes in citrate synthase (CS), it is tempting to speculate that increased production of oxalacetate by PEPC and acetyl-CoA by PDC resulted in an increased production of citrate via the Krebs cycle. The connection between amount of citrate in cells and stress tolerance has been proposed previously [Koyama H. et al 2000]. These data imply that increased production of citrate in mitochondria might be important for successful plant seeds development in the radioactive Chernobyl areas.

It is perplexing that both the plants and the seeds they have produced are so little affected by the ionizing radiation in the environment near the Chernobyl NPP, and thus far the apparent adjustments we have seen to this environment have been subtle. These include: genome hypermethylation, up regulation of antioxidant system, signal transduction system, adjustment of repair enzymes, etc. Genomic and proteomic techniques provide the opportunity to investigate plant response to ionizing radiation in unprecedented detail. Soybean and flax seeds harvested from the contaminated environment jointly exhibited changes in abundance of lipoxygenase, enzymes related to glycolysis, and enzymes involved in biosynthesis of betaine (Danchenko et al., 2009; Klubicova et al., 2010).

The main goal of the proposed research is using on based molecular biology approaches to assess plant elusive responses mechanisms to chronic ionizing radiation in radionuclide contaminated Chernobyl zone. Seeds from plants which grown some generation under influence chronic ionizing radiation will be screened using both course (gross plant phenotype, seed germination, etc.) and fine (specific protein expression and abundance, DNA mutations, etc.) methodical criteria [Klubicova et al., 2013].

Based on the identities of plant seed proteins that change in abundance in response to low level chronic irradiation it was postulated that adaptation involves: A, signal system transduction activation; B, a non-specific heavy metal response; C, direct protection against radiation damage; and D, alteration in SSP mobilization. Consequently, for cultured plants we suggested that seeds produced in the radio-contaminated soil conditions, tolerate chronic irradiation through changes in the abundance of proteins from several signaling cascades, along with metabolic and protein-folding adjustment.

References

1. Rashydov N., Kliuchnikov O., Seniuk O., et al. Radiobiological Characterization Environment Around Object "Shelter" In book: Nuclear Power Plant, by edit. Soon Heung Chang, 2012, 342 p. Chapter 7, p. 231- 279.
2. Herman J.J., Sultan S.E. (2011) Adaptive transgenerational plasticity in plants: case studies, mechanisms, and implications for natural populations. //Frontiers in Plant Science. doi: 10.3389/fpls.2011.00102.
3. Katarma Klubicovb, Maksym Danchenko, Ludovit Skultety, Valentyna V. Berezhna, Uvackova L., Namik M. Rashydov, Martin Hajduch. Soybeans grown in the Chernobyl area produce fertile seeds that have increased heavy metal resistance and modified carbon metabolism. //PLoS ONE (2012). (research manuscript PONE-D-12-15150R2) (accepted in print 2012)
4. Hirani T.A., Tovar-Mendez A., Miernyk J.A., Randall D.D. Asp295 Stabilizes the Active-Site Loop Structure of Pyruvate Dehydrogenase, Facilitating Phosphorylation of Ser292 by Pyruvate Dehydrogenase-Kinase. //Enzyme Res. 2011; 2011: 939068. Published online 2011 January 17.
5. Budde R.J., Fang T.K., Randall D.D., Miernyk J.A. (1991) Acetyl-coenzyme a can regulate activity of the mitochondrial pyruvate dehydrogenase complex in situ. Plant Physiol 95:131-136.
6. Ahsan N., Swatek K.N., Zhang J., Miernyk J.A., Xu D., Thelen J.J. (2012) "Scanning mutagenesis" of the amino acid sequences flanking phosphorylation site 1 of the mitochondrial pyruvate dehydrogenase complex. //Front Plant Sci. 2012; 3: 153. Published online 2012 July 16. Prepublished online 2012 March 7. doi: 10.3389/fpls.2012.00153
7. Koyama H, Kawamura A, Kihara T, Hara T, Takita E, et al. (2000) Overexpression of mitochondrial citrate synthase in Arabidopsis thaliana improved growth on a phosphorus- limited soil. Plant Cell Physiol 41:1030-1037.
8. Danchenko M, Skultety L, Rashydov NM, Berezhna VV, Mbtel L, Salaj T, Pret'ovb A, Hajduch M (2009) Proteomic analysis of mature soybean seeds from the Chernobyl area suggests plant adaptation to the contaminated environment. J Proteome Res. 8: 2915-2922
9. Klubicova K., Danchenko, M., Škultyty, L., Miernyk J.A., Rashydov, N.M., Berezhna, V.V. , Preťovb, A. , Hajduch, M. Proteomics analysis of flax grown in Chernobyl area suggests limited effect of contaminated environment on seed proteome. Environmental Science & Technology. (2010) V. 44, Issue 18 p. 6940-6946.
10. Rashydov N, Hajduch M. (2013) Sustainable Remediation and Agricultural Recovery of Radioactively Contaminated Areas in the Chernobyl Zone. In book abstracts ICOBTE-2013, 0220-000209, Athens USA. P. 182
11. Katarina Klubicova, Namik M. Rashydov, and Martin Hajduch. Proteomics of Field Samples, the Case of the Chernobyl Area (part 37). In Book Plant Proteomics. Methods and Protocols, 2nd Edition,

Eds., Jesus V. Jorrin-Novo, Setsuko Komatsu, Wolfram Weckwerth, Stefanie Wienkoop, Humana Press Inc. 2013, 750 p. Series: Methods in Molecular Biology 1072. Springer Protocols.