15. Microarray analysis of rice leaf response to radioactivity from contaminated Chernobyl soil

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The present study attempts to examine the radioactivity-modulated change of global gene expression patterns in the leaf of rice [Oryza sativa L.; reference monocot genome model (Agrawal and Rakwal 2006)] seedlings upon exposure to radioactive Chernobyl soil (CS), which contains high levels of cesium-137 (^{137}Cs) and strontium-90 (^{90}Sr)- both fission products from the reactor core. An in vitro two-week-old rice seedling model system was used (Agrawal et al. 2003), and leaf segments from 3rd leaves were exposed to CS emitted radiation. The radioactivity in CS is measured to be 5.34 mGy/day; ca. 11-fold over the (shielded) control level. Leaves were sampled 96 h post-irradiation and total RNA was extracted and the quality checked before performing the Agilent 22K custom oligo DNA microarray [containing ~21,500 oligonucleotides (Kikuchi et al. 2003)] experiment. Dye-swap or reverse labeling with Cy3 and Cy5 dyes procedure was applied followed by hybridization and wash processes, and the hybridized microarrays were scanned using a GenePix microarray scanner (Molecular Devices, Sunnyvale, CA, USA) followed by the Gene Pix 4000 analysis application program for image analysis and data extraction processes. The GeneSpring Ver. 4 software (Silicon Genetics, California, USA) was used for normalization, and the fold changes (>2.0-fold and <0.5-fold) were calculated from the normalized log ratios. We detected radioactivity-modulated expression of 516 genes, among which 194 and 322 genes were either up- or down-regulated. To classify the rice genes according to their functional orthologous relationships, we used the database of eukaryotic orthologous groups, which consists of 4,852 clusters of orthologous groups of proteins, including 59,838 proteins from 7 genomes. Rice genes were classified into up-/down-regulated 3

main functional categories of information storage and processing (2%/7%), cellular processes and signaling (15%/7%), and metabolism (13%/19%). Interestingly, the first and last of these categories were suppressed, especially metabolism, whereas the cellular processes and signaling categories were slightly induced. Looking into these categories further, we could identify that the genes belonging to defense mechanisms, cell wall/membrane/envelope biogenesis, posttranslational modification, protein turnover, chaperones, and secondary metabolites biosynthesis, transport and catabolism were up-regulated, whereas genes concerned with translation, ribosomal structure and biogenesis, transcription, signal transduction mechanisms, energy production and conversion, general metabolism were strongly down-regulated (Fig. 1). A representative list of highly up- and down-regulated genes is shown in Table 1.

Among the genes of interest as defense/stress-related, the PBZ1 stands out as being the most highly induced, which is interesting because PBZ1 (also known as OsPR10a) is known to be induced in response to a variety of stresses, such as heavy metals, gaseous pollutants ozone and sulfur dioxide, etc. Induction of chitinase, GST20, another PBZ1 homolog, kin1, and subtilisin inhibitor genes reveal the pattern of stress-related gene modulation by radiation. On the other hand, GAPDH was highly down-regulated, along with POX22.3, RuBisCO small subunit, and ATPase epsilon chain mRNAs, suggesting a deleterious effect on the energy metabolism after radiation stress. The genome-wide transcriptional profiling of radiation-response genes in rice leaves provides us with the first report on how our radioactive environment affects a plant species, even when exposed to ultra-low level radiation dose for a short time-period. It would be useful to further examine the effect of the levels of radiation on plants with a goal to identify novel and specific biomarkers for radiation stress.
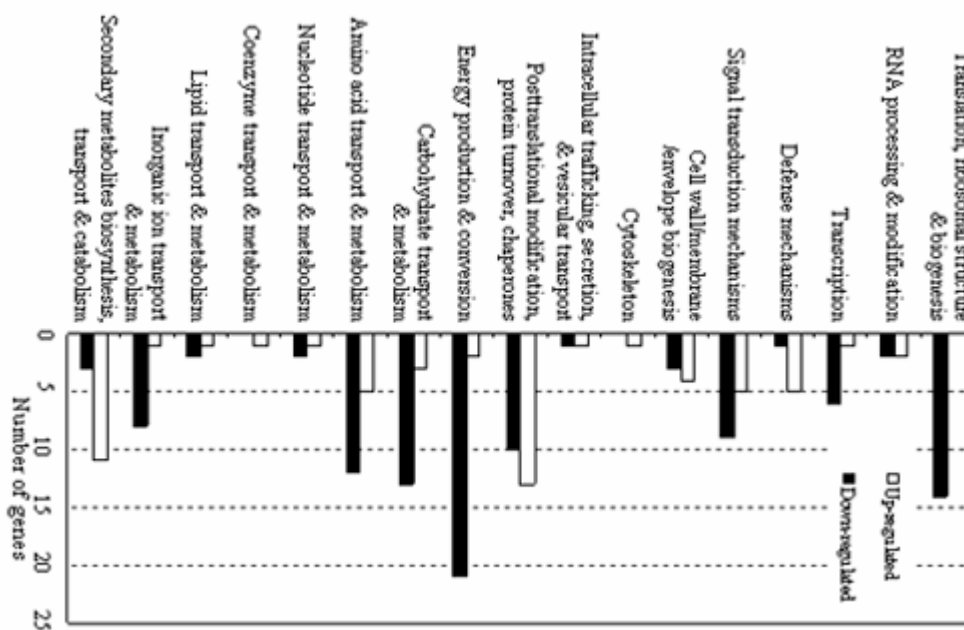

Fig. 1

Table 1. Representative up-/down-regulated genes in rice leaves exposed to radiation from Chernobyl soil (CS).

Gene	Log	Fold	UP Description	Gene	Log	Fold	DOWN Description
AK099157	3.39	10.48	Rice mRNA for probenazole-inducible protein PBZ1, complete cds|PLN	AK071685	-4.08	0.06	Oryza sativa glyceraldehyde-3-phosphate dehydrogenase subunit (GAPDH) mRNA
AK065866	3.33	10.06	Oryza sativa (japonica cultivar) mRNA for chitinase, complete cds|PLN	AK073202	-3.93	0.07	Oryza sativa peroxidase (POX22.3) mRNA, complete cds|PLN
AK064650	3.23	9.38	Zea mays glutathione S-transferase GST 20 mRNA, complete cds|PLN	AK106037	-3.72	0.08	Arabidopsis thaliana clone 772 mRNA, complete sequence.|PLN
AK058438	3.22	9.34	Oryza sativa (japonica cultivar) Os-RelA2 mRNA for chloroplast RelA homologue 2	AK109409	-3.65	0.08	Arabidopsis thaliana clone 772 mRNA, complete sequence.|PLN
AK061045	3.13	8.78	Arabidopsis thaliana putative chloroplast nucleoid DNA-binding protein (At3g18490)	AK068816	-3.57	0.08	Arabidopsis thaliana putative receptor serine/threonine kinase (At1g29750) mRNA
AK071613	3.04	8.21	Rice mRNA for probenazole-inducible protein PBZ1, complete cds|PLN	AK066849	-3.56	0.08	Arabidopsis thaliana unknown protein (At2g46820) mRNA, complete cds|PLN
AK100429	3.03	8.17	Arabidopsis thaliana unknown protein (At4g01870) mRNA, partial cds|PLN	AK058875	-3.42	0.09	Oryza sativa (japonica cultivar-group) mRNA for the small subunit of ribulose-1,5-bisphosphate carboxylase, complete cds
AK059757	2.92	7.59	Zea mays S-like RNase (kin1) mRNA, complete cds|PLN	AK067717	-3.38	0.10	Arabidopsis thaliana clone 16944 mRNA, complete sequence.|PLN
AK067249	2.90	7.46	Arabidopsis thaliana putative beta-amylase (At3g23920) mRNA, complete cds|PLN	AK063906	-3.33	0.10	Lactuca sativa ATPase epsilon chain mRNA, partial cds; chloroplast gene
AK062495	2.89	7.40	H.vulgare mRNA for subtilisin-chymotrypsin inhibitor 2.|PLN	AK059299	-3.13	0.11	Arabidopsis thaliana unknown protein (At2g36145) mRNA, complete cds|PLN

Acknowledgements

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