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Research Outline and Interests

Plants are my first love. Why? They are green, full of color that fascinates and inspires, and they give us food. To put it more simply, plants are beautiful, especially the green leaves on a rainy day – the water rolling over the leaves and down the branches is a pure delight.

The quote from Hal Borland, 1969; Our Natural World: says it all - “A root, a stem, a leaf, some means of capturing sunlight and air and making food—in sum, a plant. The green substance of this earth, the chlorophyll, is all summed up in the plants. Without them we perish, all of us who are flesh and blood.”

I have been working with plants since my College days, and starting with Botany, at the University of Delhi, India, and thereafter I progressed my way toward studying plant pathology at the Master’s program in a beautiful and large campus (just below towering Himalayas) of G.B. Pant University of Agriculture & Technology (Pantnagar, India), and finally Ph.D. (Biochem. & Biotech.) from Tokyo University of Agriculture & Technology (Japan); I did my research at School of Agriculture Campus at Ami-machi, of Ibaraki University. Thereafter, I worked at NIAS, NIES, AIST (mental stress using animal models), Showa University School of Medicine (neuroscience, anti-aging medicine, and DOHaD project), and finally University of Tsukuba (GGECh program) currently.

Plants are indeed life, sustaining human civilization, and essentially maintain biodiversity balance and mitigate environmental problems. Scientists studying plants are indeed lucky to be able to work with these distant cousins of ours. More importantly we have now, in the 21st century, at our disposal high-throughput genomics-based technologies (OMICS), namely transcriptomics and proteomics (Bradshaw, 2008) along with metabolomics that are helping to comprehensively understand plant biology. Proteomics of plants is now a discipline in its own right (Agrawal and Rakwal, 2008; Thiellement et al., 2007). Established the International Plant Proteomics Organization (INPPO; http://www.inppo.com/); see – Agrawal et al., 2011 and 2012.

I describe below in brief three of my research interests in plants. One thing I wish to emphasize, I do not always work alone, I work together with close colleagues who are the best of friends, and my life partner who is also my research partner; we discuss and interact face to face or online constantly with an aim to Go on Doing what we started to do a long time back. Our motto: “Good friends, good science”.
1. Jasmonates – Perfume to Plant Communicator

The oxylipin, jasmonic acid (JA; generally *trans*-JA) has a long history as a natural organic compound, and is well recognized as an important plant hormone mediating biotic and abiotic stress responses as well as aspects of growth and development. JA has invoked interest for its biology and function(s) among scientists involved in different disciplines from organic chemistry to plant functional genomics. Mounting evidence on JA functionality reveals its ever-increasing biological roles, which appears to be unique among other plant hormones. JA is a well-known regulator of secondary metabolites production as a signaling compound, growth and defensive reactions in plants. This knowledge on JA functionality did not transpire with one or few discoveries but evolved over almost 50 years since its discovery as a perfume component of the jasmine oil – *Jasminum grandiflorum* (*trans*-methyl jasmonate, MeJA). We have been investigating how JA is biosynthesized, metabolically and how it functions biologically, producing defensive secondary metabolites, including volatile organic compounds (VOCs). Rice (*Oryza sativa* L. cv. Nipponbare) and *Achyranthes bidentata* are the model plants we use.

Among jasmonates, amino acid conjugates of jasmonic acid (JA) such as jasmonoyl isoleucine/leucine (JA-Ile/Leu) and methyl jasmonate (MeJA) have been received greater attention due to their possible role as intra- and inter-cellular signaling compounds. Tremendous progress has been seen on MeJA signaling and pathways, yet the signaling is quite confusing because JA biosynthesis is regulated by a positive feedback MeJA might only serve as an inactive inter-cellular signaling compound, but once inside the plant cell it is converted into JA-Ile, which acts potentially as an active intra-cellular signaling compound. Moreover, endogenous JA-Ile induced by the feedback will also work as an active intra-cellular signaling compound. And the induced endogenous MeJA diffusing to neighbour cells possibly work as an inter-cellular signaling compound. Then conversion of inter-cellular MeJA and *de novo* biosynthesis of jasmonates will together play an important role in plant responses to environmental stimuli. Here, we emphasize the importance of quantitative analysis of JA including *cis* and *trans*-stereochemistry for understanding practical aspects of jasmonate signaling. We now are in position to reasonably state that jasmonate conversion (foreign jasmonates) and *de novo* biosynthesis of jasmonates will together play an important role in plant responses to environmental stimuli. In the current literature, a general lack of jasmonates quantification can be seen. This is surprising considering the important conclusions being derived on their behalf. If the chemistry of jasmonates is more important than associated gene expression (which is increasingly documented in studies on jasmonate signaling and biosynthesis) this would support a move away from molecular genetic studies towards metabolomic based ones. However, it seems more likely that each area is important and thus complimentary. Thus the integration of the absolute quantification of jasmonates with associated genetic and biochemical information will be important in solving the puzzle on the dualism which seems to be inherent in the role of jasmonates *in planta*.

# For details see Tamogami et al., 2011 and 2012, and references therein.
## Figures, Adapted from Tamogami et al.
# Collaborators – Prof. Shigeru Tamogami, Akita Prefectural University, Japan, and Dr. Ganesh Kumar Agrawal, RLABB, Kathmandu, Nepal.
Method of MS/MS using Analysis

1. acetone extraction
2. CHCl₃ extraction (pH=3)
3. Rough purification Sep-Pak Light C18(Waters)
2. Ozone – Protector to Air Pollutant

Tropospheric ozone (O₃) is one of the notorious environmental gaseous pollutants, produced by photochemical reactions between volatile organic compounds and nitrogen oxides. Since late 1970s, the annual increase in nitrogen oxides due to combustion of fossil fuels for industrialization has been a major factor in global O₃ increment. Climate models forecast that average level of ground O₃ will reach phytotoxic range in the future with greater increase in its concentration in Asia, Africa and USA, where major crops such as rice, wheat, corn and soybean are cultivated. Currently, O₃ concentrations sometimes exceed a threshold level to cause visible foliar injury by diurnal fluctuations of O₃ concentration. For example, according to a database of NIES (Japan) maximum hourly oxidant concentrations exceeded 200 ppb at 29 monitoring sites in the Kanto region surrounding Tokyo in 2005. Acute O₃ exposure in plants causes foliar symptoms such as leaf necrosis and chlorosis (terms for foliar injury), accompanied with damage to cellular membranes and accumulation of pigments in injured/dead cells. Foliar injury results in reduction of photosynthesis leading to reduced plant growth rate and productivity in some cases. On the other hand, the ever-increasing concentration of atmospheric O₃ poses a great threat to yield and quality of the global socio-economic crops, chronically. Indeed, the yield of crops such as rice, wheat, maize, barley, soybean, bean and potato is remarkably reduced depending on elevating O₃ concentrations compared to the basal O₃ level. However, to understand mechanisms of O₃ effects on plants, it is necessary to clarify plant responses related to visible injury formation among various molecular responses caused by O₃. Furthermore, we need to evaluate the extent of O₃-induced visible foliar injury precisely in the well-controlled experimental conditions using an indoor growth cabinet, since symptoms similar to O₃-induced injury could appear without O₃ in the field. We have selected rice *japonica* cv. Nipponbare as a model system (*Agrawal and Rakwal, 2006*) with socio-economic importance. Two-week-old rice seedlings are exposed to O₃ (200 ppb) and the leaves collected at multi-time points are used for evaluating the severity of foliar injury and large-scale transcript profiling, proteomics and metabolomics. We also use mature rice plants, panicles and seeds for further detailed analyses using *Japonica* and *Indica* cultivars in combination with heat stress.
For details see Cho et al., 2008, 2011 and 2012, and references therein.

## Figures, Adapted from Cho et al.

### Collaborators
- Dr. Kyoungwon Cho, Korea Basic Science Institute (KBSI), South Korea,
- Dr. Akihiro Kubo, NIES, Japan,
- Dr. Shoshi Kikuchi, NIAS, Japan, and Dr. Ganesh Kumar Agrawal, RLABB, Kathmandu, Nepal. Part of work supported by Environment Research and Technology Development Fund (A-0806) of the Ministry of Environment, Japan, and CRIEPI.
3. **Radiation – To go to Iitate Mura is to Believe**

We have studied the molecular changes in leaves of rice plants exposed to ultra low-dose ionizing radiation, first using contaminated soil from the exclusion zone around Chernobyl reactor site. Results revealed induction of stress-related marker genes (Northern blot) and secondary metabolites (LC-MS/MS) in irradiated leaf segments over appropriate control. Second, employing the same *in vitro* model system, we replicated results of the first experiment using in-house fabricated sources of ultra low-dose gamma (\(\gamma\)) rays and selected marker genes by RT-PCR. Results suggest the usefulness of the rice (grass) model in studying ultra low-dose radiation response/s.

Recently, the “opportunity” to study effect of radiation in a low-level gamma field presented itself at Iitate village in Fukushima. Rice plants respond to radiation exposure by activating self-defense mechanisms. High-throughput omics tools have enhanced our ability to observe and analyze these molecular changes genome-wide – namely at the level of gene, protein and metabolite. In particular, gene expression profiles can be cataloged using whole genome DNA microarray chip. In 2012, we examined the effects of radiation exposure in rice (*Oryza sativa* L. cv. Nipponbare), in the village of Iitate in Fukushima prefecture – a highly contaminated site due to the Fukushima Daichi Accident (*Imanaka et al. 2012, Health Physics: The Radiation Safety Journal*) following the Great Tohoku Earthquake. Healthy rice seedlings were transported from a controlled greenhouse in Tsukuba to Iitate Farm (hereafter ITF) and placed in a low-level gamma field. There was no direct contact between the rice seedlings and the contaminated soil, thus helping us observe primarily the effects of gamma radiation alone. Exposure times were set at 6, 12, 24, 48, and 72 h after arrival at ITF, and the rice leaves at the 3rd position (from the base) from 6 to 10 seedlings were sampled. Sampling was performed by immediately placing the cut leaves in an aluminum foil under dry ice followed by storing the samples in a deep freezer. As a control, rice leaves were sampled at the start in Tsukuba and immediately at arrival upon ITF; a sample set was also taken at 72 h from the healthy rice seedlings in the greenhouse at Tsukuba. For molecular-level analysis rice leaves were transported to Tsukuba and Tokyo and stored at \(-80^\circ\text{C}\). Prior to analysis of gene expression, the first part of the analyses, total RNA was extracted from the leaves, and whose quality and quantity were determined to be excellent for downstream analysis. As a first step, selected gene expression profiles of internal control, DNA repair/damage, oxidative stress, photosynthesis, and defense/stress functions were examined by semi-quantitative RT-PCR. Results revealed that low-level gamma radiation affects the expression of numerous genes, in particular showing the early (6 h) induction in DNA repair/damage-related genes and the late (72 h) induction of a previously described marker gene for defense/stress responses. Based on these results, which confirmed our data from preliminary experiments using detached rice leaves for exposure to radiation, we proceeded to the next step of DNA microarray analysis. Using the established dye-swap approach, we analyzed the differentially expressed genes at 6 and 72 h time points using a whole rice genome 4 x 44 K custom chip. Obtained results showed that exposure to low-level gamma radiation differentially regulated 4481 (induced) and 3740 (suppressed) and 2291 (induced) and 1474 (suppressed) rice leaf genes at 6 and 72 h post-exposure, respectively, by at least two-fold changes. Inventory of large number of gamma radiation-responsive genes in leaves provide new information and increased knowledge on novel regulatory processes in rice.
Rice (*Oryza sativa* L. cv. *Nipponbare*) in Low-level Gamma Field

- **Tsukuba**
- **Iitate Farm (ITF)** (Iitate Village, Fukushima)
- Cut Leaf, Place in Aluminum Foil, Immediately Freeze in Dry Ice & Store in Deep Freezer (−80°C)
- Grind Leaf Samples in Liquid Nitrogen and Divide into Aliquots in 2.0 mL Microtubes & Store in Deep Freezer (−80°C)

**High-throughput Genomics and Proteomics Approach**

- **CANDIDATE GENES and PROTEINS DIFFERENTIALLY EXPRESSED upon EXPOSURE to LOW-LEVEL GAMMA (γ) RADIATION**

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# For details see Rakwal et al., 2009, and references therein.

## Figures, Adapted from Rakwal et al. and Unpublished data.

## Collaborators – Dr. Shinzo Kimura, Dokkyo Medical University, Japan, and Dr. Tetsuji Imanaka, Research Reactor Institute, Kyoto University, Japan, and 2012年～Iitate-Mura Society for Radioecology (IISORA; 飯舘村放射能エコロジー研究会 http://iitate-sora.net/) Member/メンバー
References


Books

Plant Proteomics: Technologies, Strategies, and Applications


Seed Development: OMICS Technologies toward Improvement of Seed Quality and Crop Yield