University of Tsukuba
筑波大学

稲への影響/飯舘村での実験を通して

RIM

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Organization for Educational Initiatives
SEINO A 205 / GGEC Program Office

20131117

ITF/ITM-Fukushima

Rice In
Iitate
Mura

3 R's
5 I`s
1 M

IBARAKI UNIVERSITY (School of Agriculture, Ami-machi)
[Tokyo University of Agriculture and Technology]

1993-2013

NIAS
NIES
AIST
SHOWA UNIVERSITY SCHOOL OF MEDICINE
TOHO UNIVERSITY FACULTY OF SCIENCE

UNIVERSITY OF TSUKUBA

筑波大学
University of Tsukuba

2013年 筑波大学大学院共通科目

Graduate General Education Courses (GGEC) Program 2013
University of Tsukuba

高等師範学校
Higher Normal School

東京文理科大学・東京高等師範学校
Tokyo Higher Normal School
Tokyo Liberal Arts and Science University

東京教育大学
Tokyo University of Education

初代学長 三輪 知雄
First President, University of Tsukuba
TOMOO MIWA

筑波大学の目指す大学院教育 ―大学院共通科目の開設―

Graduate General Education Courses Program – Graduate Education that University of Tsukuba aspires to

筑波大学長 永田 恭介 — NAGATA Kyosuke, President, University of Tsukuba

大学院共通科目への期待

The Role of Graduate General Education Courses Program

「人間力」と「リーダーシップ」の涵養

To Foster Rich Human Quality and Leadership

http://www.tsukuba.ac.jp/education/g-courses/kyoutsuukamoku.php

Aim and Need for Graduate General Education Courses

– Prof. Yoshihiro Shiraiwa,

Provost, Faculty of Life and Environmental Sciences, University of Tsukuba

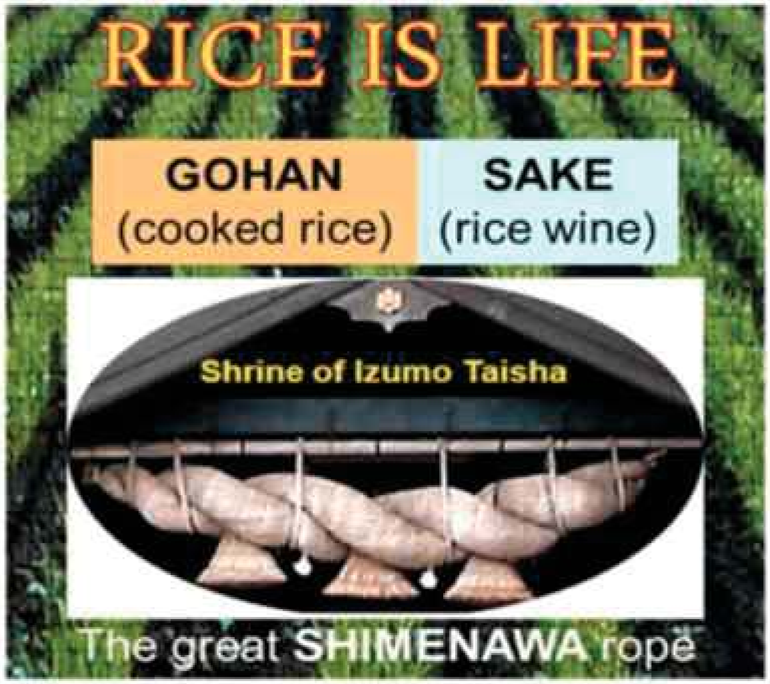
The aim of offering Graduate General Education courses is to enhance the quality and develop the ability of graduates at University of Tsukuba as well as to nurture rich human qualities. The current society needs to have academic researchers to acknowledge responsibilities and contribute to the society. We hope to have graduates who are aware of the necessity of satisfying the professional and social needs.

GGEC program offers courses to prospective candidates fostering cultural accomplishments and integrity coupled with professional skills, leading toward well-balanced researchers. Based on the basic skills and knowledge students have acquired during their undergraduates days, candidates are requested to go to the next step to fulfill his/her potential. Candidates should be flexible and sensible to respond to the social needs and to enhance one's career development.

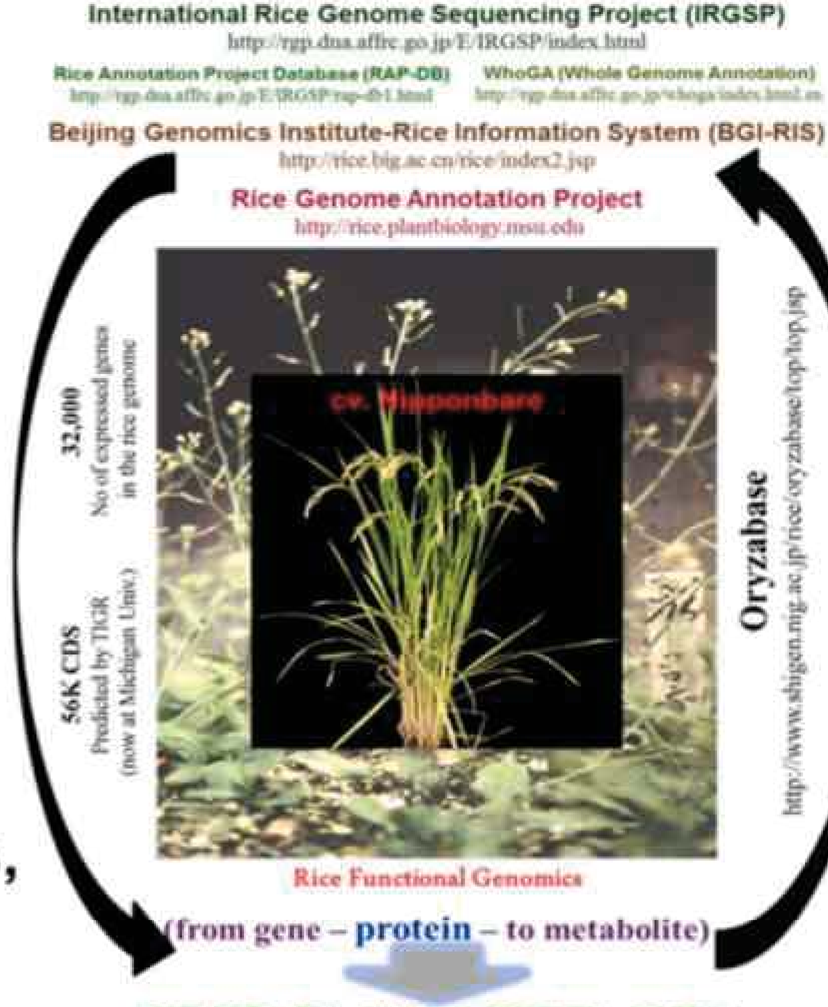
RICE : GREEN GOLD...

1. Rice is Life
2. Rice is Experimental Model

"Rice is not only FOOD for Billions



from Food Security to Bio-Fuels, RICE is in the NEWS



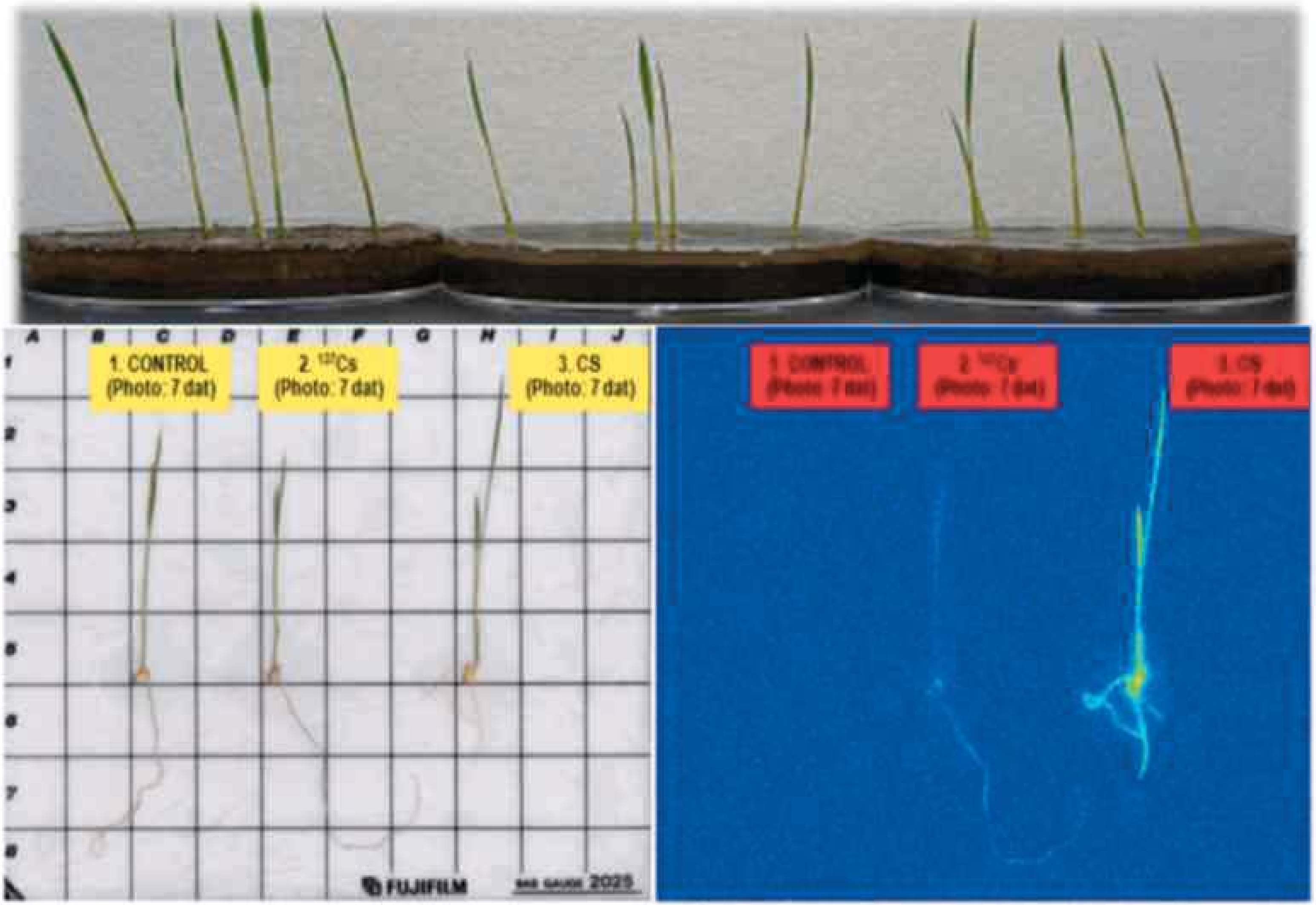
RICE PLANT BIOLOGY

but also a GENOME MODEL Plant"

RADIATION : Decade of Work

1. Contaminated Soil/Gamma radiation

2. Two-week-old model system for stress

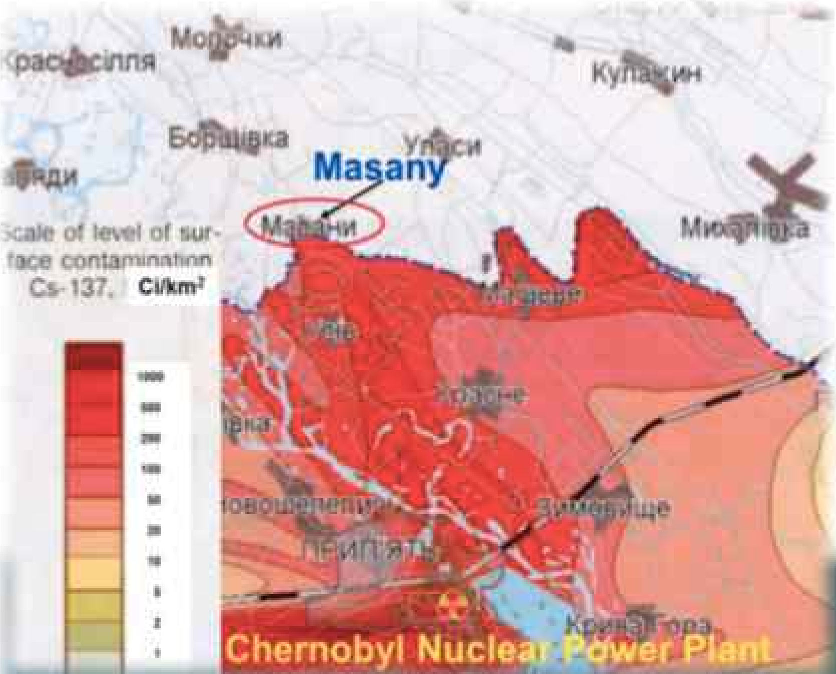


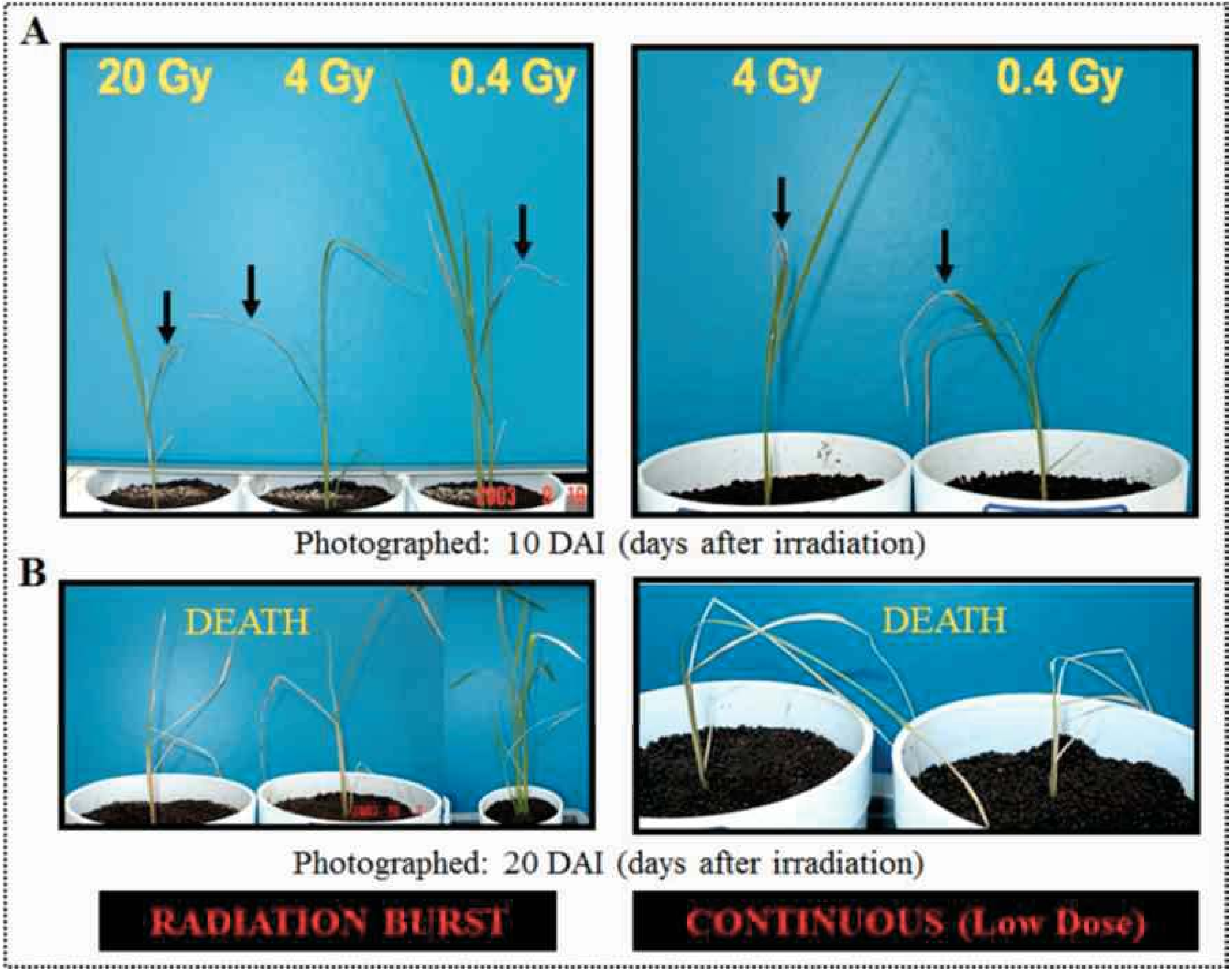
“Today, Chernobyl's soil, water, and air are among the most highly contaminated on Earth.”

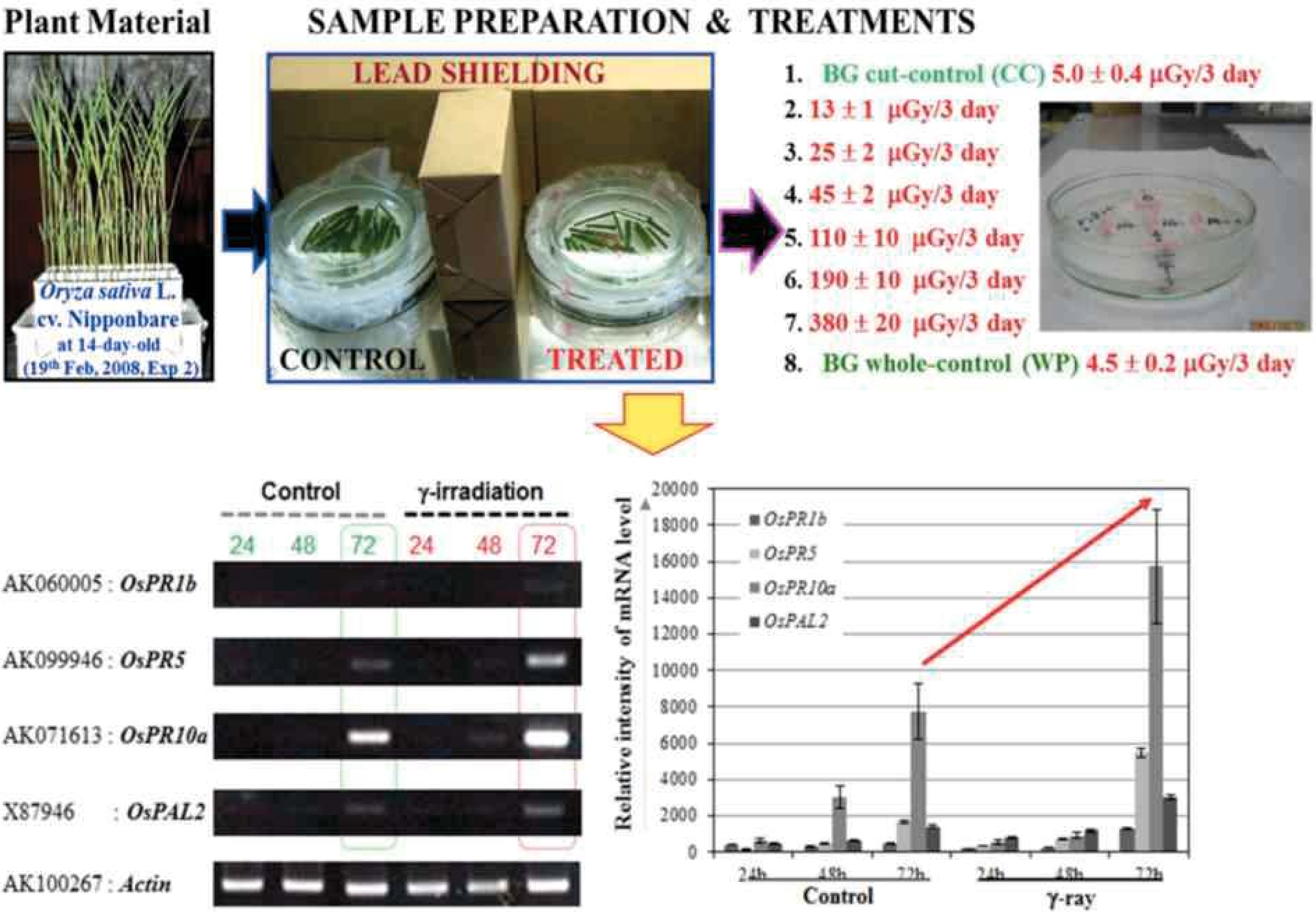
After Chernobyl, they refused to leave

By **Holly Morris,** Special to CNN / November 8, 2013 -- Updated 0148 GMT (0948 HKT)

http://edition.cnn.com/2013/11/07/opinion/morris-ted-chernobyl/index.html?iid=article_sidebar


Кулакин
Уласи
Masany
Scale of level of surface contamination Cs-137, Ci/km²
Зимовище
Chernobyl Nuclear Power Plant


A
20 Gy
4 Gy
0.4 Gy
4 Gy
0.4 Gy
Photographed: 10 DAI (days after irradiation)
B
DEATH
DEATH
Photographed: 20 DAI (days after irradiation)
RADIATION BURST
CONTINUOUS (Low Dose)



Plant Material
SAMPLE PREPARATION & TREATMENTS
Oryza sativa L. cv. Nipponbare at 14-day-old (19th Feb, 2008, Exp 2)
LEAD SHIELDING
CONTROL
TREATED
1. BG cut-control (CC) 5.0 ± 0.4 µGy/3 day
2. 13 ± 1 µGy/3 day
3. 25 ± 2 µGy/3 day
4. 45 ± 2 µGy/3 day
5. 110 ± 10 µGy/3 day
6. 190 ± 10 µGy/3 day
7. 380 ± 20 µGy/3 day
8. BG whole-control (WP) 4.5 ± 0.2 µGy/3 day
Control
γ-irradiation
24
48
72
24
48
72
AK060005 : OsPR1b
AK099946 : OsPR5
AK071613 : OsPR10a
X87946 : OsPAL2
AK100267 : Actin
Relative intensity of mRNA level
OsPR1b
OsPR5
OsPR10a
OsPAL2
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
24h
48h
72h
24h
48h
72h
Control
γ-ray

In the Laboratory - Not in the Field

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

S. KIMURA[1], J. SHIBATO[2], G.K. AGRAWAL[3], YK. KIM[4], BH. NAHM[4], NS. JWA[5], H. IWAHASHI[2] and R. RAKWAL[2,3]

1) Hazard Assessment and Epidemiology Research Group, National Institute of Occupational Safety and Health (NIOSH), 6-21-1 Nagao, Kawasaki, 214-8585 Japan
2) Human Stress Signal Research Center (HSS), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba West, 16-1 Onogawa, Tsukuba, 305-8569 Japan
3) Research Laboratory for Agricultural Biotechnology and Biochemistry, Kathmandu Nepal
4) Division of Bioscience and Bioinformatics, Myongji University, GreenGene BioTech Inc., Kyonggido, 449-728 Korea
5) Department of Molecular Biology, College of Natural Science, Sejong University, Seoul, 143-747 Korea
Correspondence: Dr. Randeep Rakwal
Tel & Fax: +81-29-861-8508; E-mail: rakwal-68@aist.go.jp (R. Rakwal)

Molecules and Cells

Growth Retardation and Death of Rice Plants Irradiated with Carbon Ion Beams Is Preceded by Very Early Dose- and Time-dependent Gene Expression Changes

Int. J. Mol. Sci. 2009, 10, 1215-1225; doi:10.3390/ijms10031215

OPEN ACCESS
International Journal of
Molecular Sciences

Ultra Low-Dose Radiation: Stress Responses and Impacts Using Rice as a Grass Model

Randeep Rakwal [1,2,*], Ganesh Kumar Agrawal [2], Junko Shibato [1], Tetsuji Imanaka [3], Satoshi Fukutani [3], Shigeru Tamogami [4], Satoru Endo [5], Sarata Kumar Sahoo [6], Yoshinori Masuo [1] and Shinzo Kimura [7]

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2 Research Laboratory for Biotechnology and Biochemistry (RLABB), GPO Box 8207, Kathmandu, Nepal; E-Mails: plantproteomics@gmail.com (R.R.); gkagrawal123@gmail.com (G.K.A.)
3 Research Reactor Institute, Kyoto University (KURRI), Osaka, Japan; E-Mails: imanaka@rri.kyoto-u.ac.jp (T.I.); fukutani@rri.kyoto-u.ac.jp (S.F.)
4 Laboratory of Growth Regulation Chemistry, Akita Prefectural University, Akita 010-0195, Japan; E-Mail: tamo_chem@akita-pu.ac.jp
5 Graduate School of Engineering, Hiroshima University (HU), Hiroshima, Japan; E-Mail: endos@hiroshima-u.ac.jp
6 Research Center for Radiation Protection, National Institute of Radiological Sciences (NIRS), Chiba 263-8555, Japan; E-Mail: sahoo@nirs.go.jp
7 Department of Research Planning and Coordination, Japan NIOSH, Kawasaki, Japan; E-Mail: kimura@h.jniosh.go.jp

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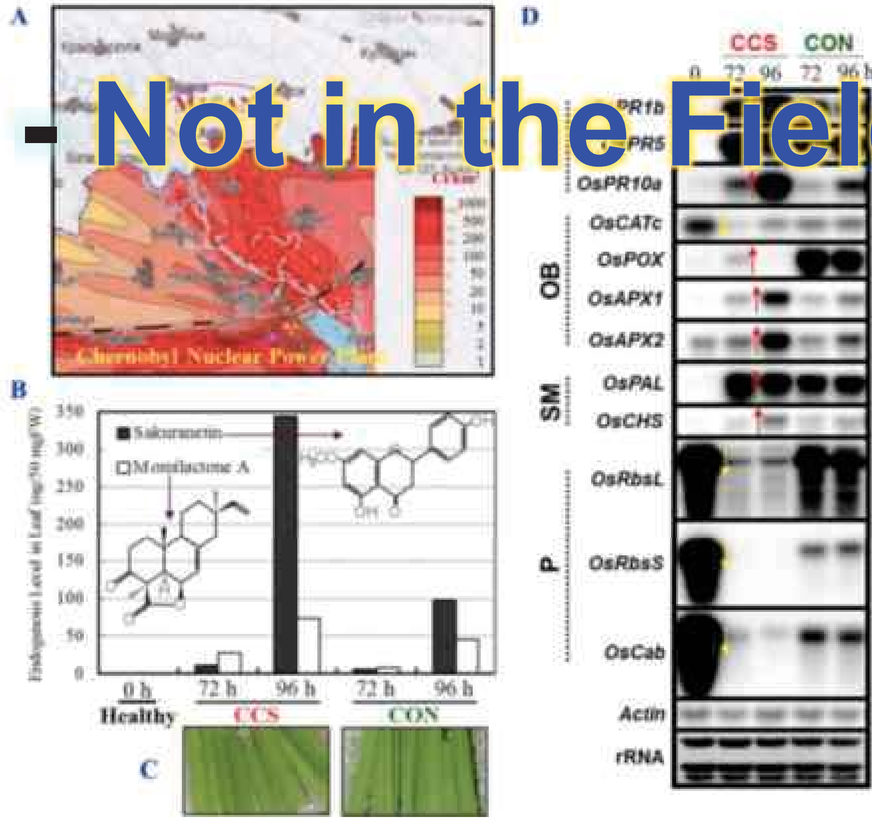
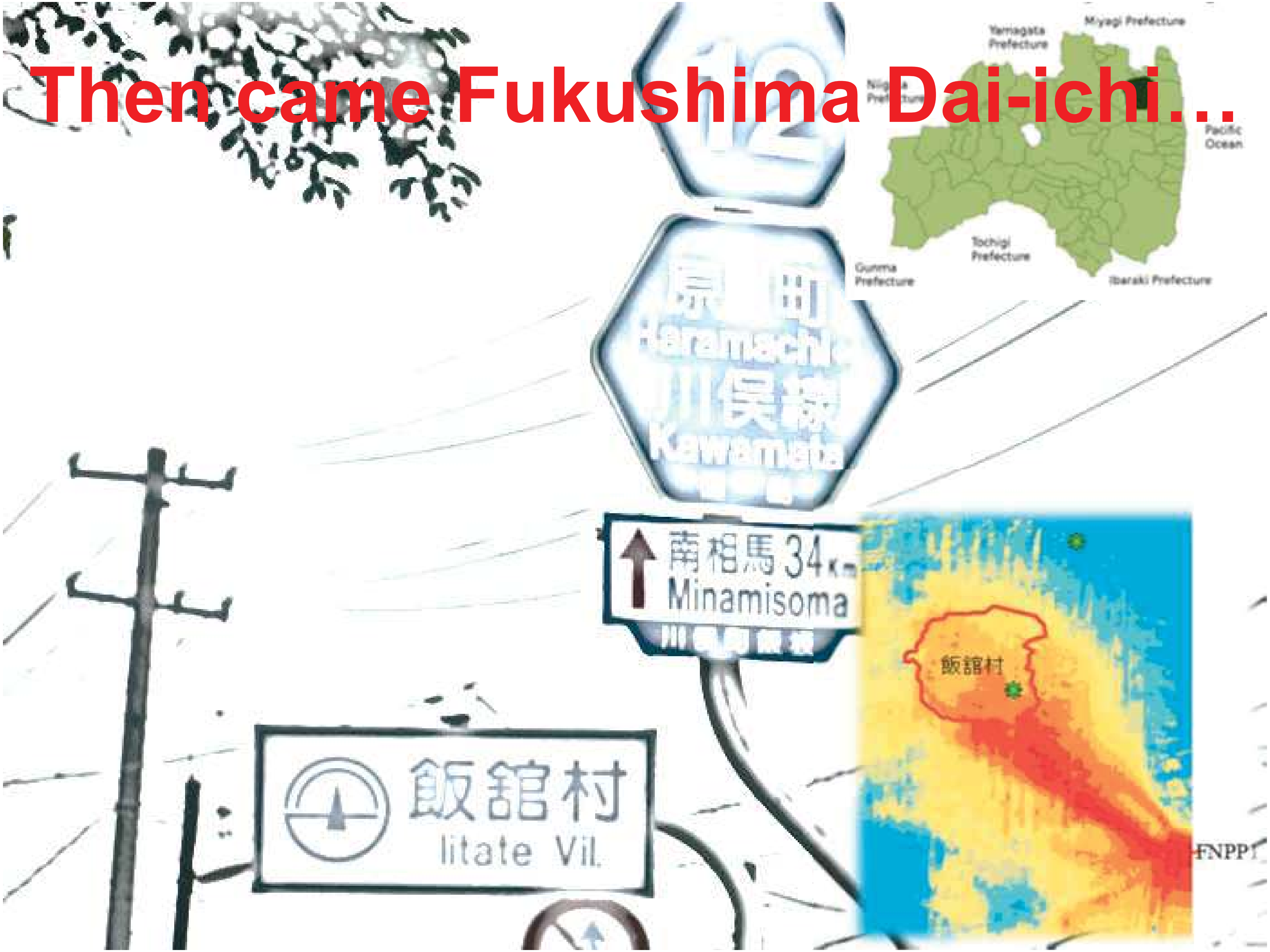
Received: 5 February 2009; in revised form: 11 March 2009 / Accepted: 13 March 2009 / Published: 16 March 2009



IITATE

Then came Fukushima Dai-ichi…



To go to Iitate Mura is to Believe




Contamination level of the experiment field by Cs-137 (August 2012)

700 kBq/m^2

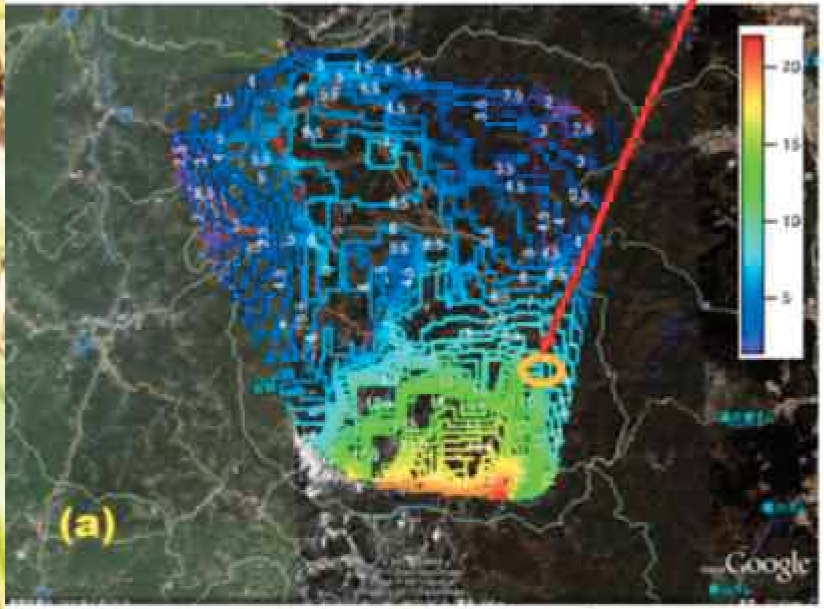
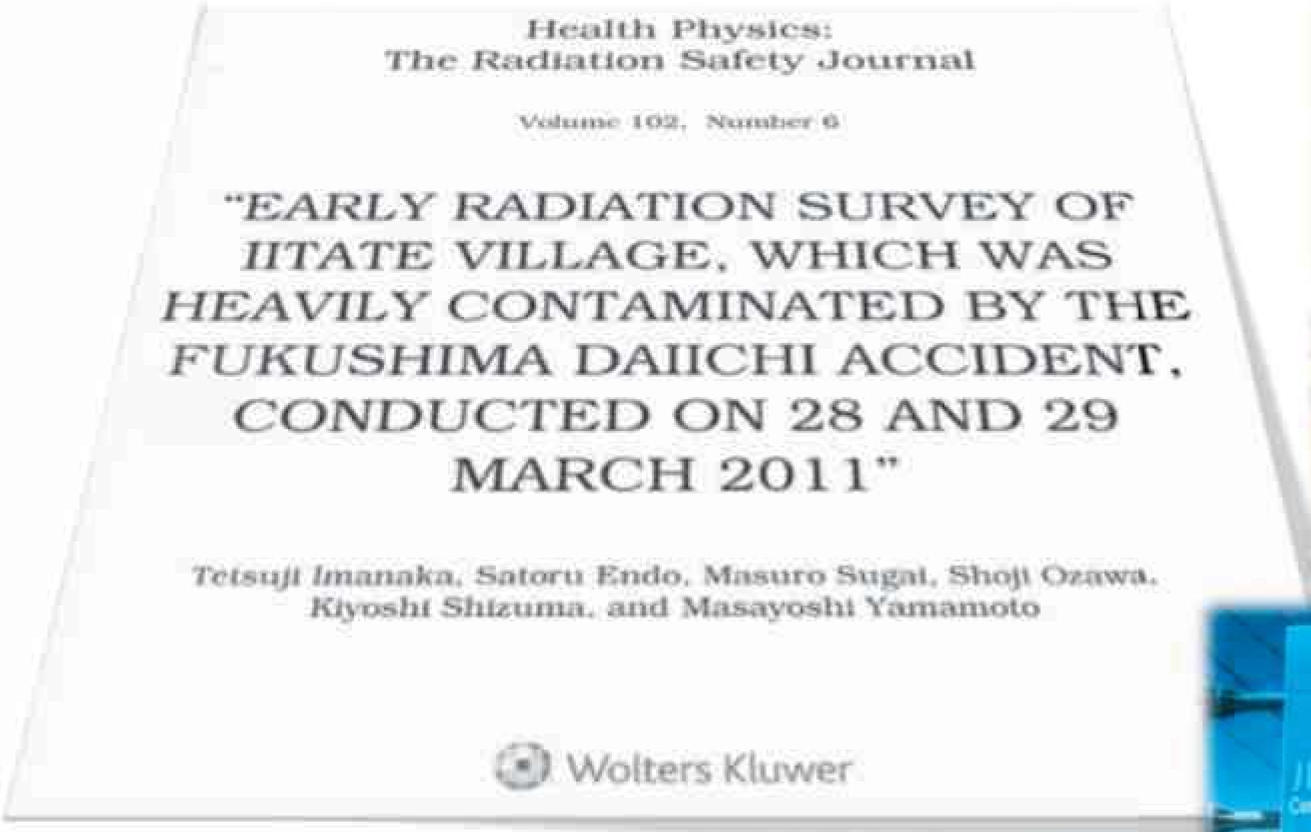


Table1. Measurement of soil samples (φ5 cm × 10 cm).

Capacity of the core : φ5 cm × 10 cm = 196 cm^3
Ground area of the core : 19.6cm^2 (=0.00196m^2)
[kBq/m^2] =([Bq/g] × [g] /0.00196m^2) × 0.001
Sampling date 2012/08/16
Measurement date 2012/11/02

Measurement at KURI by Germanium Semiconductor Detector

	Cs-134 [Bq/g]	Cs-137 [Bq/g]	[g]	Cs-134 [kBq/m2]	Cs-137 [kBq/m2]
I-1	8.7	16	109	483	862
I-2	3.1	5.4	194	307	537
I-3	3.2	5.5	234	376	660



Health Physics:
The Radiation Safety Journal

Volume 102, Number 6

"EARLY RADIATION SURVEY OF IITATE VILLAGE, WHICH WAS HEAVILY CONTAMINATED BY THE FUKUSHIMA DAIICHI ACCIDENT, CONDUCTED ON 28 AND 29 MARCH 2011"

Tetsuji Imanaka, Satoru Endo, Masuro Sugai, Shoji Ozawa, Kiyoshi Shizuma, and Masayoshi Yamamoto

Wolters Kluwer

MEGURO san

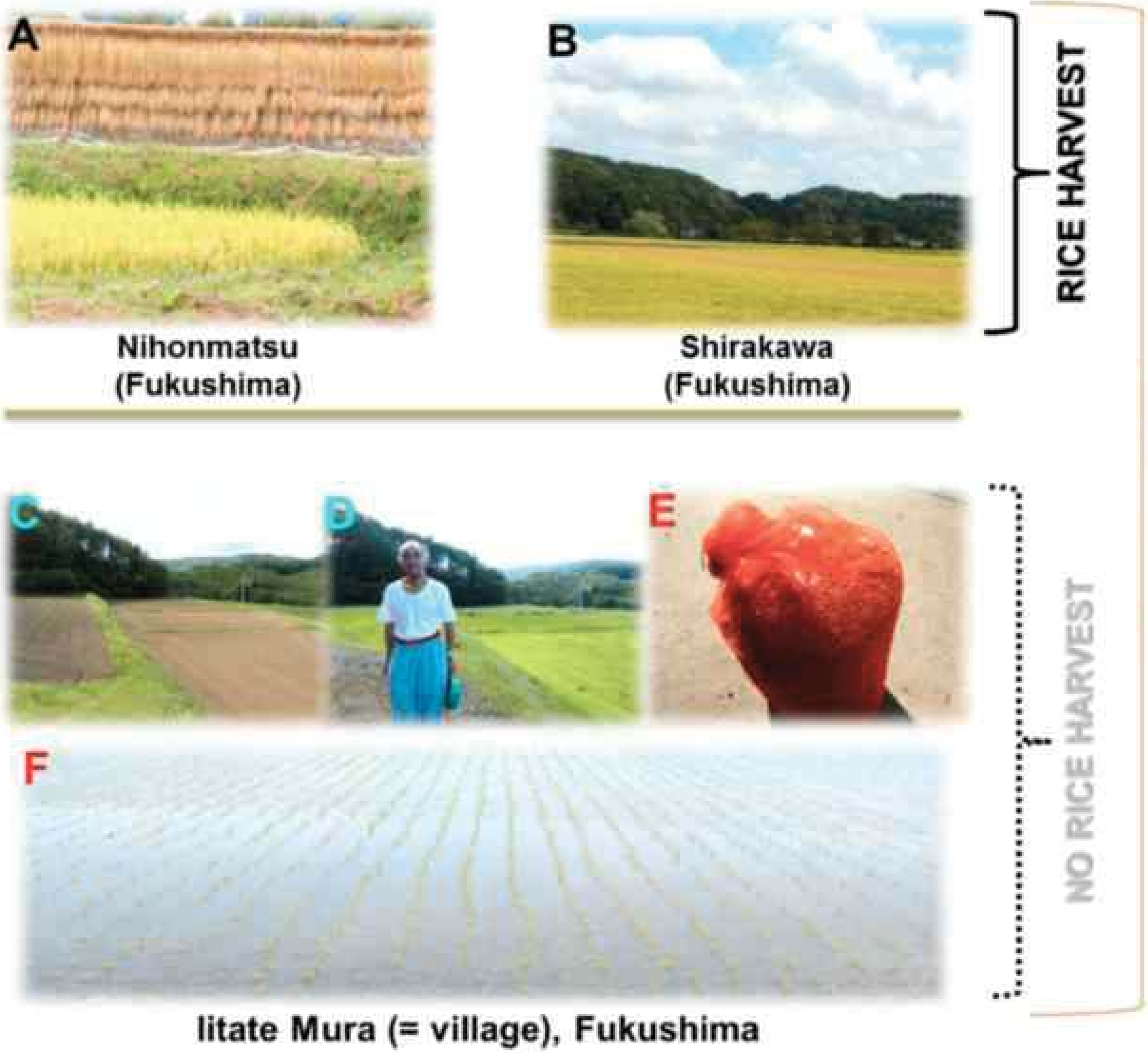
Meguro san wants to Grow Rice

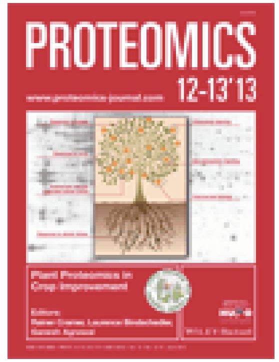
PROTEOMICS Annual Reviews Issue 2014

Rice proteomics: a model system for crop improvement and food security

Sun Tae Kim[1], Sang Gon Kim[2], Ganesh Kumar Agrawal[3,4], Shoshi Kikuchi[5], and Randeep Rakwal[3,4,6,7]

FIGURE 1





07-Nov-2013 Dear Prof. Dr. Rakwal, I am pleased to inform you that your manuscript, "Rice proteomics: a model system for crop improvement and food security", is now acceptable for publication in PROTEOMICS….

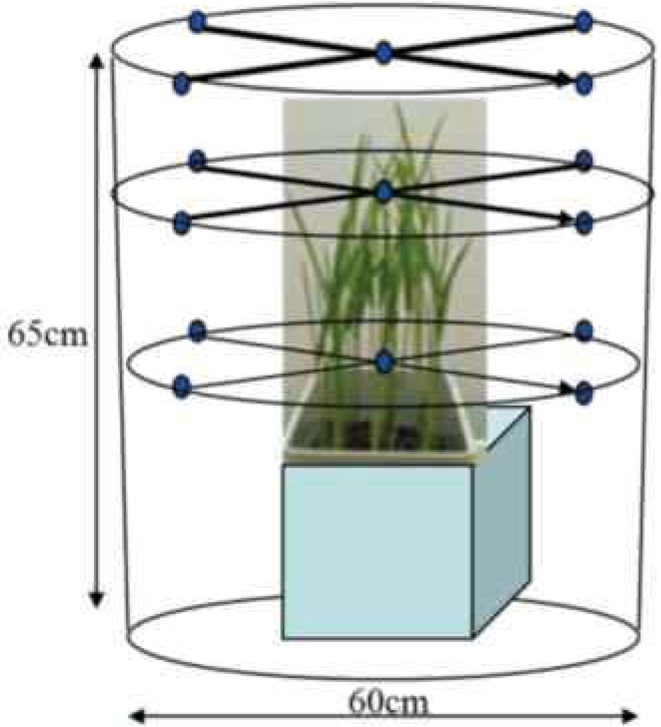
Experimental


A long road ahead
30 years....


つくば500
34-38

2012

1



Low: ~ 100 μSv/3 days

~ 180 μSv/3 days

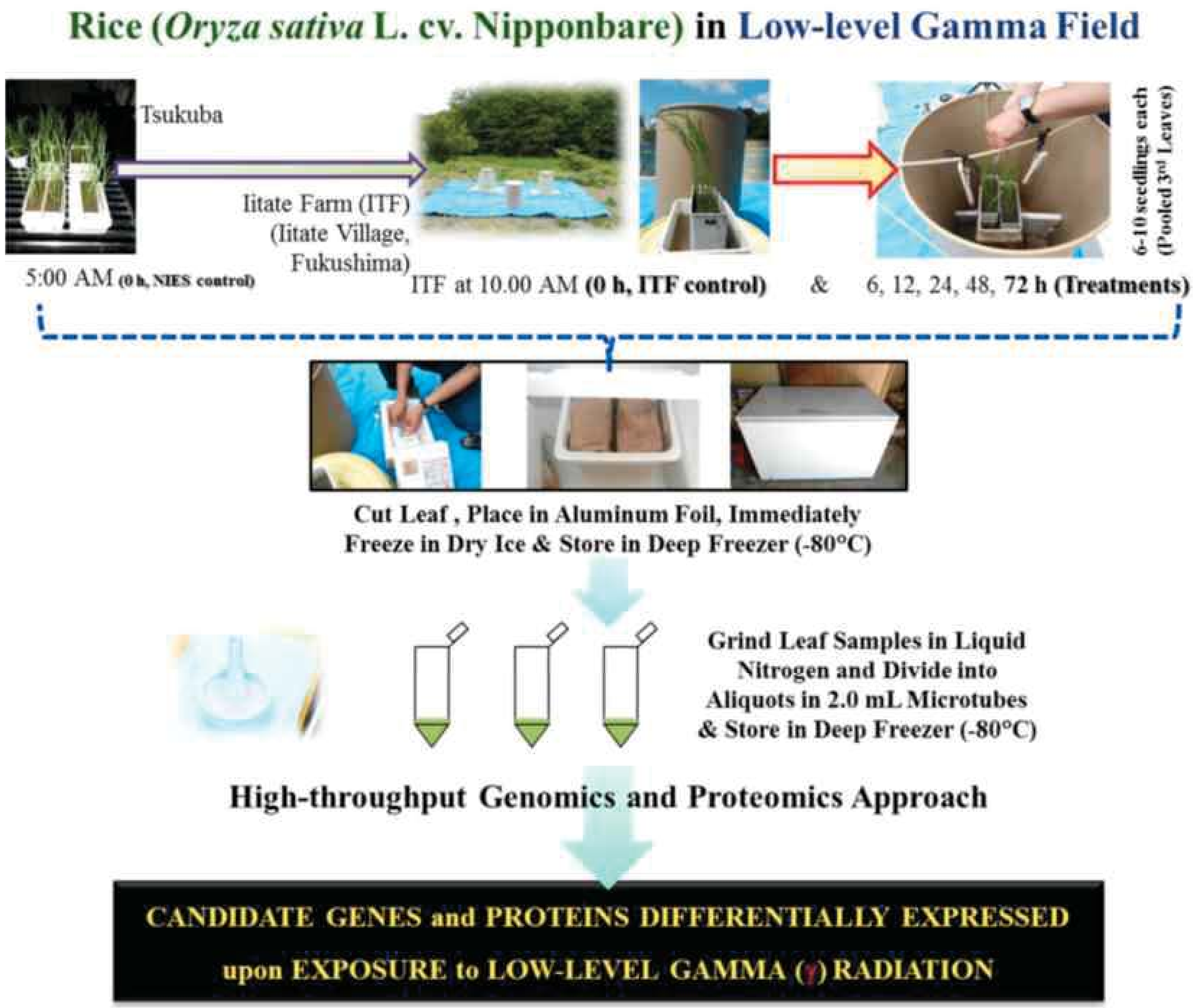
		S	N	E	W	C
++	B	1.5	1.5	1.5	1.5	1.9
	C	1.6	1.6	1.5	1.6	1.9
	T	1.7	1.7	1.7	1.7	2.0
+	B	2.5	2.5	2.5	2.5	2.8
	C	2.4	2.4	2.5	2.5	2.8
	T	2.6	2.5	2.5	2.5	2.9
-	B	4.2	4.3	4.3	4.2	4.3
	C	4.2	4.3	4.3	4.3	4.3
	T	4.2	4.2	4.3	4.2	4.3

[μSv/h]

B:bottom
C:center
T:top

High: ~ 280 μSv/3 days

2



Rice (Oryza sativa L. cv. Nipponbare) in Low-level Gamma Field
Tsukuba
Iitate Farm (ITF)
(Iitate Village,
Fukushima)
6-10 seedlings each
(Pooled 3rd Leaves)
5:00 AM (0 h, NIES control)
ITF at 10.00 AM (0 h, ITF control)
&
6, 12, 24, 48, 72 h (Treatments)
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

Blind Sampling

STEP 5

Next Day Sampling at 10 AM... 24 h

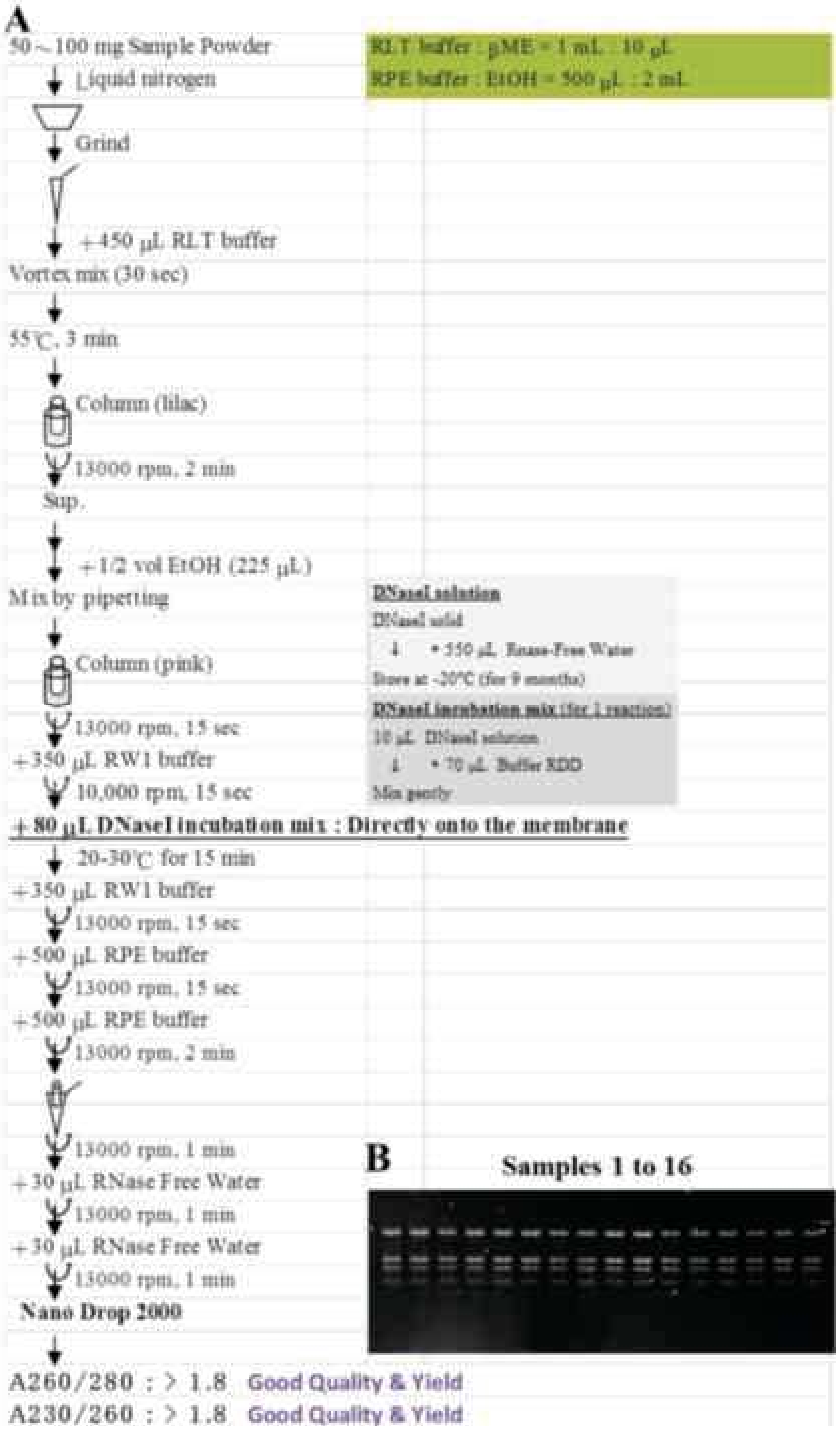
GRINDING

3.2. Preparation of Tissue Powders for Protein Extraction

1. Grind 100 mg of plant materials to a fine powder with liquid N_2 in a mortar and pestle. (*see* Note 23).

Note 23- Pre-chill the mortar and pestle, microfuge tubes and spatulas for transferring the sample tissue powders in liquid nitrogen, at least 15 min before starting the grinding of the sample tissues.

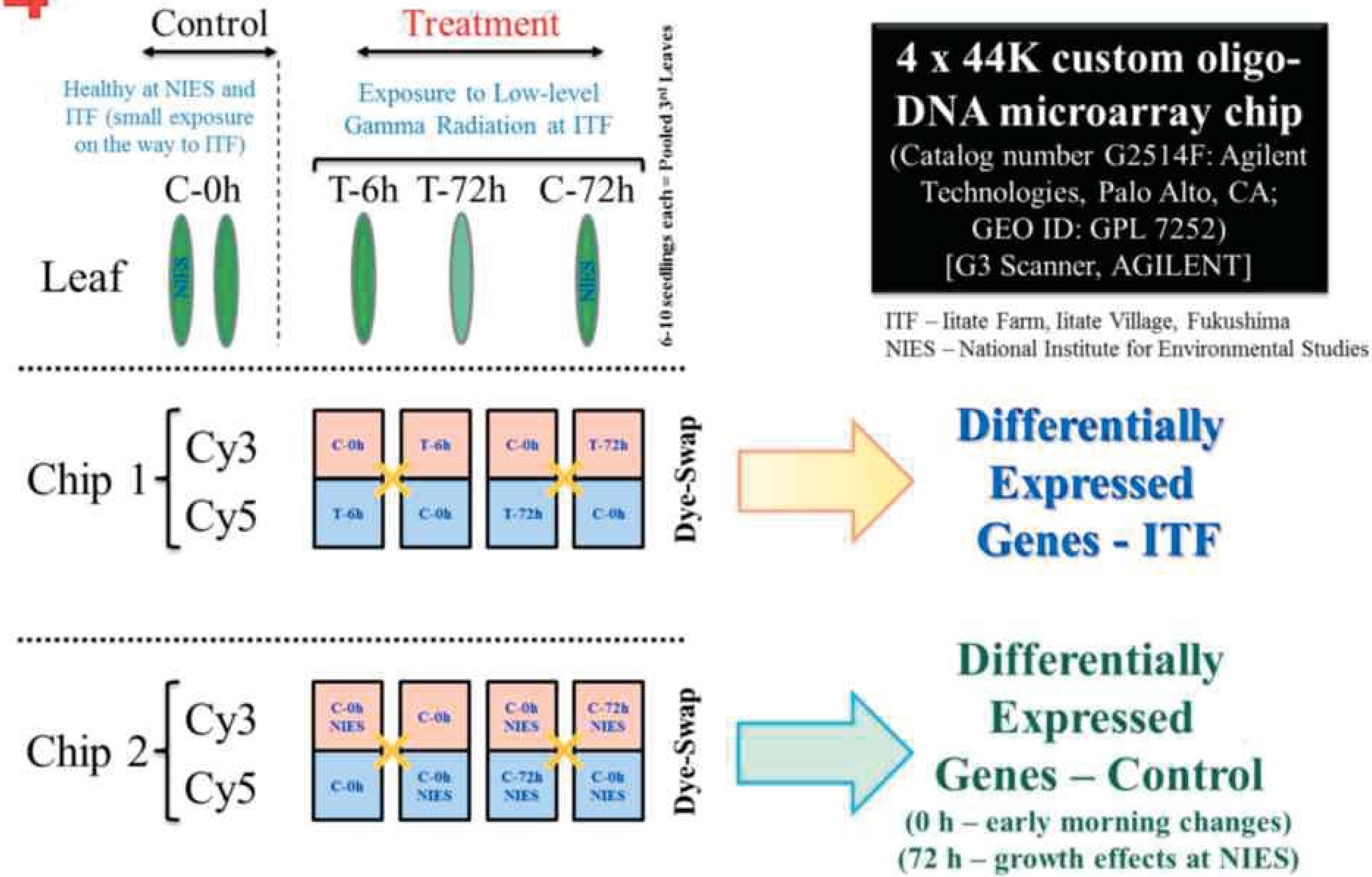
3



A
50～100 mg Sample Powder
Liquid nitrogen
Grind
+450 μL RLT buffer
Vortex mix (30 sec)
55℃, 3 min
Column (lilac)
13000 rpm, 2 min
Sup.
+1/2 vol EtOH (225 μL)
Mix by pipetting
Column (pink)
13000 rpm, 15 sec
+350 μL RW1 buffer
10,000 rpm, 15 sec
+80 μL DNaseI incubation mix : Directly onto the membrane
20-30℃ for 15 min
+350 μL RW1 buffer
13000 rpm, 15 sec
+500 μL RPE buffer
13000 rpm, 15 sec
+500 μL RPE buffer
13000 rpm, 2 min
13000 rpm, 1 min
+30 μL RNase Free Water
13000 rpm, 1 min
+30 μL RNase Free Water
13000 rpm, 1 min
Nano Drop 2000
A260/280 : > 1.8 Good Quality & Yield
A230/260 : > 1.8 Good Quality & Yield
B
Samples 1 to 16

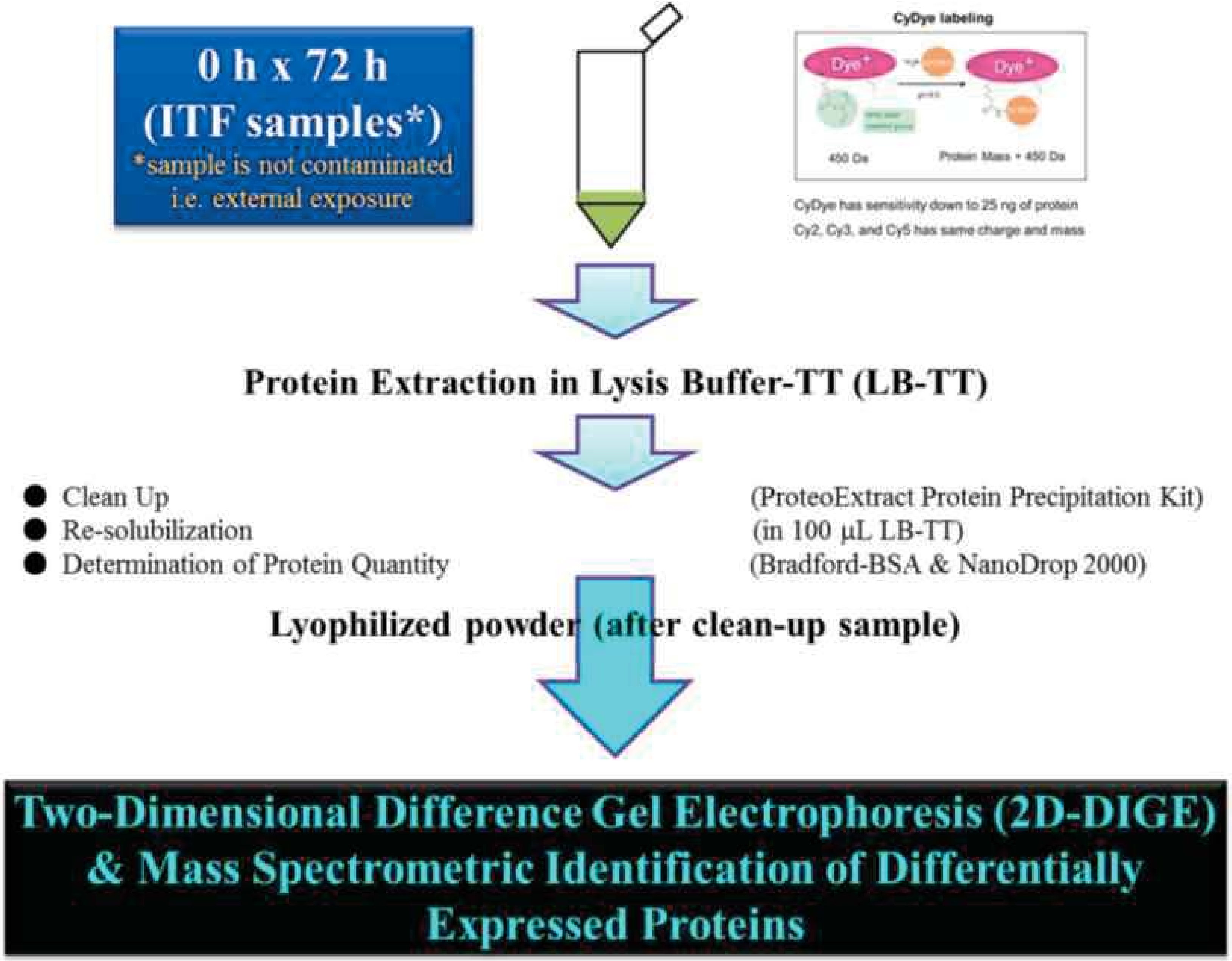
RNA

4


Control
Treatment
Healthy at NIES and ITF (small exposure on the way to ITF)
Exposure to Low-level Gamma Radiation at ITF
C-0h
T-6h T-72h C-72h
Leaf
NIES
6-10 seedlings each = Pooled 3rd Leaves
4 x 44K custom oligo-DNA microarray chip
(Catalog number G2514F: Agilent Technologies, Palo Alto, CA; GEO ID: GPL 7252)
[G3 Scanner, AGILENT]
ITF – Iitate Farm, Iitate Village, Fukushima
NIES – National Institute for Environmental Studies
Chip 1
Cy3
Cy5
C-0h
T-6h
T-72h
Dye-Swap
Differentially Expressed Genes - ITF
Chip 2
C-0h NIES
C-72h NIES
C-72h
Differentially Expressed Genes – Control
(0 h – early morning changes)
(72 h – growth effects at NIES)

GENE

5


0 h x 72 h
(ITF samples*)
*sample is not contaminated
i.e. external exposure
CyDye labeling
Dye+
Dye+
450 Da
Protein Mass + 450 Da
CyDye has sensitivity down to 25 ng of protein
Cy2, Cy3, and Cy5 has same charge and mass
Protein Extraction in Lysis Buffer-TT (LB-TT)
Clean Up
Re-solubilization
Determination of Protein Quantity
(ProteoExtract Protein Precipitation Kit)
(in 100 μL LB-TT)
(Bradford-BSA & NanoDrop 2000)
Lyophilized powder (after clean-up sample)
Two-Dimensional Difference Gel Electrophoresis (2D-DIGE)
& Mass Spectrometric Identification of Differentially
Expressed Proteins

PROTEIN

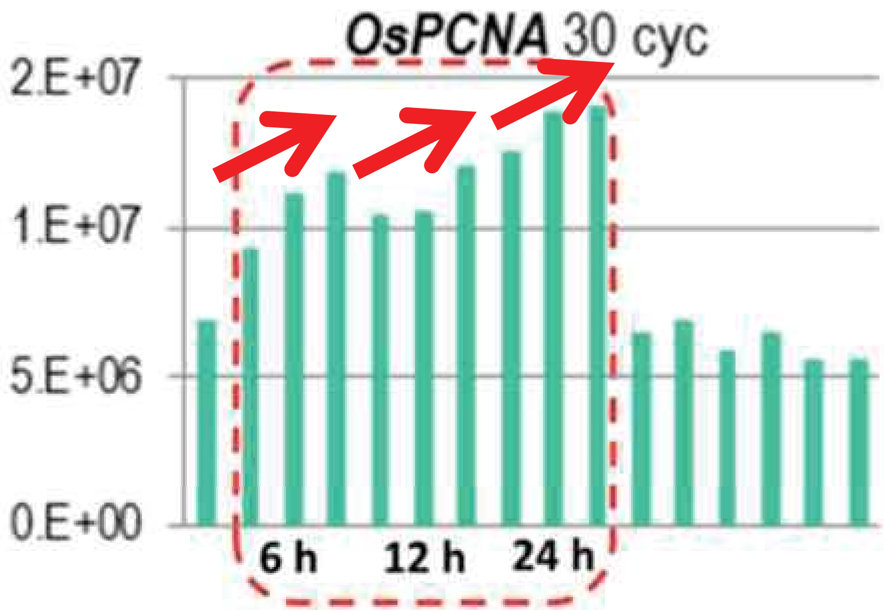
RESULTS - 1

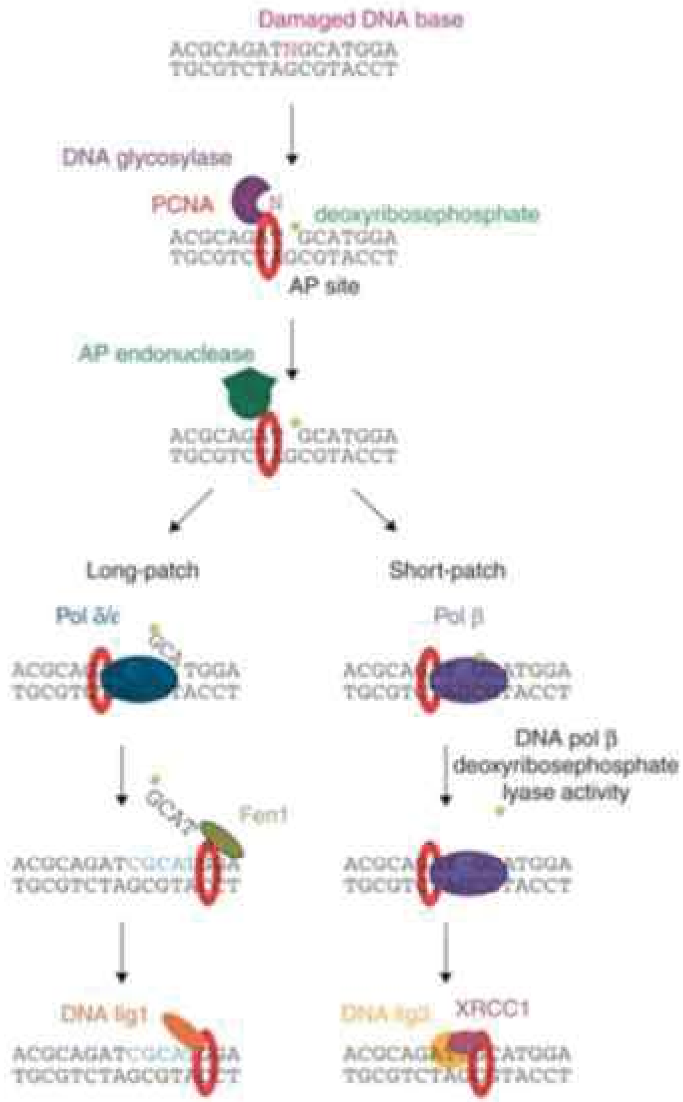

Damage to the Leaf
Dead tissue from tip
3rd leaf
3rd leaf
STEP 9-LAST
Recording the Growth of Rice seedlings 13 days after bringing back to Tokyo/Tsukuba from Iitate Farm/Iitate Mura

RESULTS - 2

Change in Gene Expressions

Proliferating Cell Nuclear Antigen is a key factor in DNA replication





Note - NOT INDUCED BY UV-IRRADIATION

Ann Bot. 2011 May;107(7):1127-1140.

Phenylalanine Ammonia-Lyase

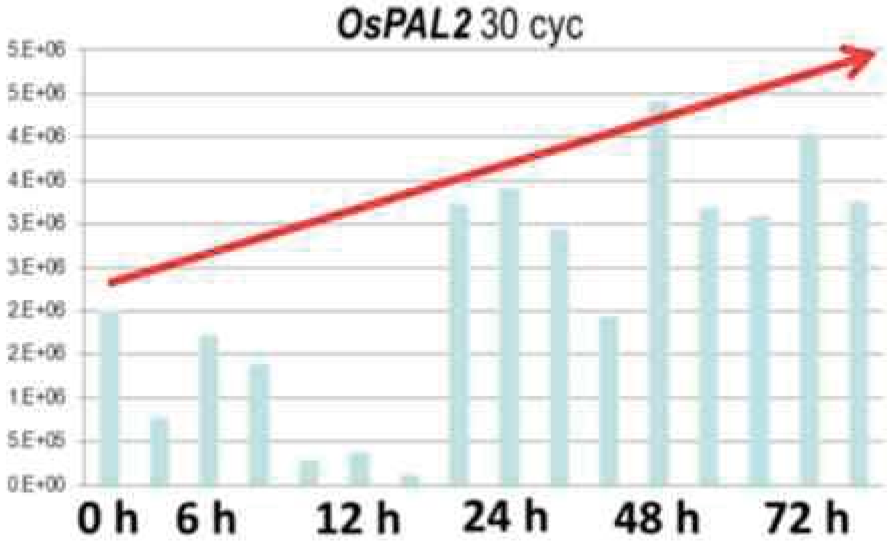
(Phenyl propanoid pathway of Plants)

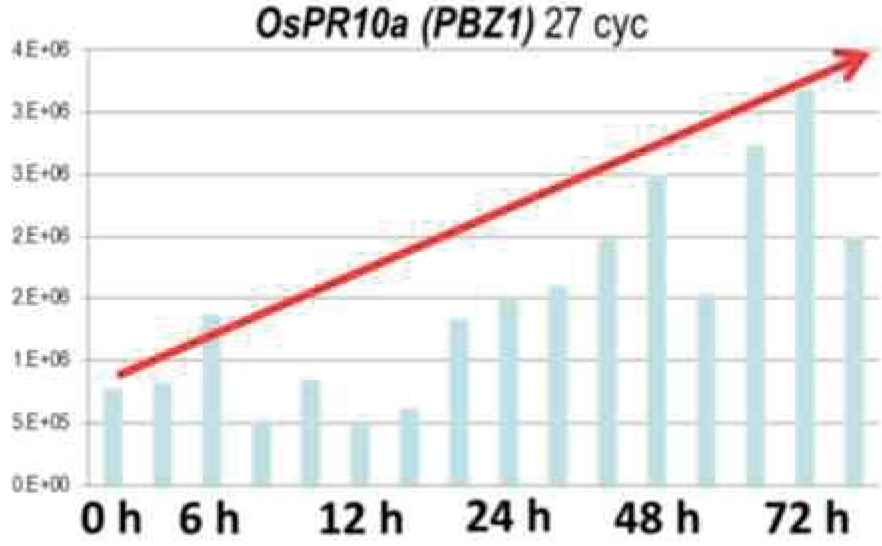
Contribute to all aspects of plant response to STRESS

Pathogenesis-Related 10a (PBZ1)

(Probenazole-InducedProtein1)

Plant DEFENSE/STRESS response related (RNAse activity) (Involved in Cell Death)

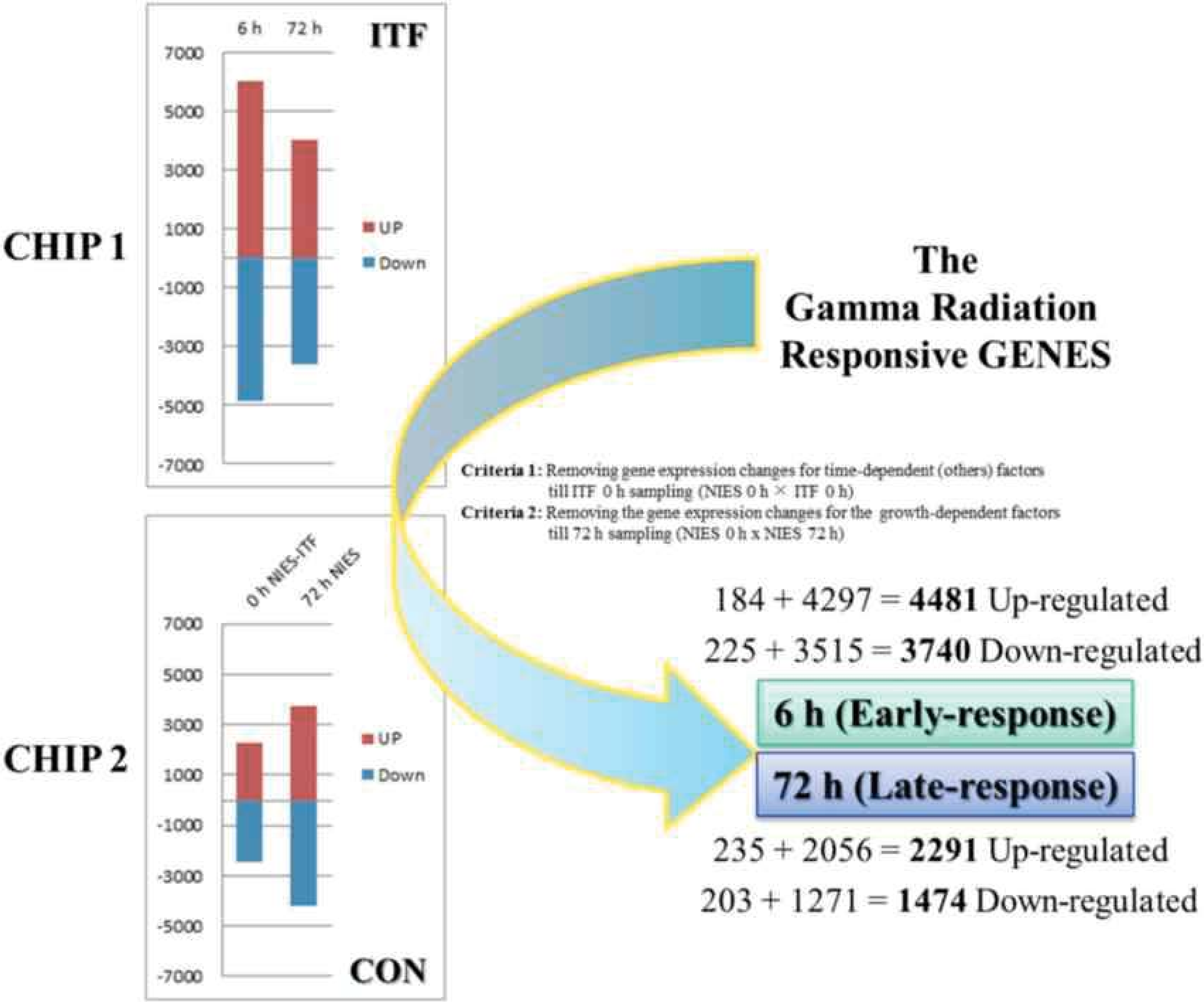




RESULTS - 3

Large DATASETS

						251784510080_1_1			251784510080_1_2					251784510080_1_3			251784510080_1_4				
						0h x 6h H			swap					0h x 72h H			swap				
ProbeN	Systemat	annota	descrip	AK	Seque	gProces	rProces	Fold	gProc	rProce	Fold	average Log	average Fold	gProces	rProces	Fold	gProc	rProce	Fold	average Log	average Fold
R5_c01_0	LOC_Os0	TBC dom	RabGAP/	AK242339	GGTAAT	2192	2643	1.21	1701	1486	0.87	0.23	1.18	885	933	1.05	767	867	1.13	-0.05	0.97
R5_c01_0	LOC_Os0	expressed	Conserve	AK059894	GTTACG	13	60	4.78	33	15	0.45	1.70	3.25	12	30	2.47	14	14	1.03	0.63	1.54
R5_c01_0	LOC_Os0	monocopp	Similar to	AK101455	CAACTT	302	564	1.87	547	272	0.50	0.95	1.94	188	318	1.69	292	160	0.55	0.81	1.76
R5_c01_0	LOC_Os0	expressed	Similar to	AK067316	GTTGGT	9185	6446	0.70	5788	7982	1.38	-0.49	0.71	5988	4335	0.72	3920	5030	1.28	-0.41	0.75
R5_c01_0	LOC_Os0	R3H dom	Single-stra	AK121362	TGTGTAC	10423	9756	0.94	9384	10472	1.12	-0.13	0.92	6921	6296	0.91	5649	6927	1.23	-0.22	0.86
R5_c01_0	LOC_Os0	40S ribos	Similar to	AK121523	CCCAAT	4467	34182	7.65	35564	4810	0.14	2.91	7.52	2457	11379	4.63	12536	2822	0.23	2.18	4.54
R5_c01_0	LOC_Os0	expressed	Protein of	AK122012	AGTTTGT	3407	2739	0.80	2848	2697	0.95	-0.12	0.92	2195	1619	0.74	1969	2269	1.15	-0.32	0.80
R5_c01_0	LOC_Os0	sphingosin	Pyridoxal-	AK243573	GTTGTAC	13463	13503	1.00	12248	12565	1.03	-0.02	0.99	8891	7515	0.85	6432	7939	1.23	-0.27	0.83
R5_c01_0	LOC_Os0	E-1 enzyn	2,3-diketo-	AK067866	TTCCATC	2407	608	0.25	672	2186	3.25	-1.84	0.28	1884	9598	5.10	9131	1721	0.19	2.38	5.20
R5_c01_0	LOC_Os0	snurportin	Conserve	LOC_Os0	TTGTGTG	791	287	0.36	319	658	2.06	-1.25	0.42	471	1514	3.22	1475	490	0.33	1.64	3.11
R5_c01_0	LOC_Os0	hypothetic	NONE	LOC_Os0	TAGTTGA	11	13	1.18	16	14	0.87	0.22	1.16	12	15	1.30	14	14	1.04	0.16	1.12
R5_c01_0	LOC_Os0	nucleic ac	RNA-bind	AK099952	CTGTGG	1045	939	0.90	1176	1156	0.98	-0.07	0.96	880	982	1.12	993	775	0.78	0.26	1.20
R5_c01_0	LOC_Os0	chaperone	Heat shoc	LOC_Os0	TGCGTTT	23431	5593	0.24	4543	20487	4.51	-2.12	0.23	16914	7561	0.45	6204	14611	2.36	-1.20	0.44
R5	LOC_Os0	expressed	Conserve	AK103498	TTGGAG	1873	208	[illegible]	192	[illegible]	[illegible]	[illegible]	[illegible]	1218	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.20
R5	LOC_Os0	expressed	NONE	LOC_Os0	TGGGGT	158	183	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	163	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
R5	LOC_Os0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	45	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	48	[illegible]	[illegible]	[illegible]	50	0.73	[illegible]	[illegible]
R5	LOC_Os0	[illegible]	[illegible]	AK	CC	[illegible]	10873	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	7552	0.74	[illegible]	[illegible]
R5	[illegible]	[illegible]	[illegible]	AK	[illegible]	[illegible]	22354	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	145	1.77	[illegible]	[illegible]
R5	[illegible]	[illegible]	[illegible]	AK	[illegible]	[illegible]	361	[illegible]	324	144	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	106	1.14	[illegible]	0.72
R5_c01_0	LOC_Os0	shikimate	Shikimate	AK	[illegible]	5939	13756	2.32	10541	4103	0.39	1.29	2.44	2727	12049	4.42	12211	2418	0.20	2.24	4.72
R5_c01_0	LOC_Os0	translocon	Transloco	AK063774	TGCCTG	6704	8352	1.25	7026	5335	0.76	0.36	1.28	3968	2773	0.70	2608	3893	1.49	-0.55	0.68
R5_c01_0	LOC_Os0	retrotransp	DEAD/DE	AK064456	ACAAAA	2620	1910	0.73	1769	2835	1.60	-0.57	0.67	1531	1176	0.61	1024	1870	1.83	-0.79	0.58
R5_c01_0	LOC_Os0	retrotransp		LOC_Os0	GATATTG	21	14	0.69	14	17	1.16	-0.38	0.77	14	15	1.11	31	15	0.49	0.58	1.50
R5_c01_0	LOC_Os0	retrotransp		LOC_Os0	TCAACG	41	13	0.32	12	45	3.83	-1.78	0.29	31	15	0.49	14	47	3.40	-1.40	0.38
R5_c01_0	LOC_Os0	light-induc	Light regul	AK067670	GGCGC	8098	5172	0.64	5849	7107	1.22	-0.45	0.72	5207	222	0.04	341	5031	14.77	-4.22	0.05
R5_c01_0	R01-TIGR		Non-protei	AK066123	TAACCA	32	28	0.88	61	21	0.35	0.68	1.60	32	16	0.51	49	23	0.47	0.06	1.04
R5_c06_2	LOC_Os0	protein LE	Late embr	AK063726	TAATCAC	17	2620	158.22	2385	46	0.02	6.49	90.17	13	14	1.11	15	15	1.03	0.05	1.04
R5_c01_0	LOC_Os0	peptide tra	TGF-beta	AK058723	CACCAC	1234	309	0.25	237	1204	5.08	-2.17	0.22	786	533	0.68	381	862	2.26	-0.87	0.55
R5_c01_0	R01-TIGR		Non-protei	AK109199	GTTAACT	7115	6952	0.98	7009	6983	1.00	-0.01	0.99	5088	3546	0.70	3675	5332	1.45	-0.53	0.69
R5_c01_0	LOC_Os0	3-beta-hyd	Emopamil	AK059848	TATCATT	3562	3442	0.97	3506	2936	0.84	0.10	1.07	2307	1241	0.54	1469	1912	1.30	-0.64	0.64
R5_c01_0	LOC_Os0	expressed	Conserve	AK070525	GCCTTA	510	805	1.58	808	459	0.57	0.74	1.67	278	463	1.66	400	258	0.65	0.68	1.61
R5_c01_0	LOC_Os0	expressed	Conserve	AK102782	AAGTGC	9162	8565	0.93	8279	8724	1.05	-0.09	0.94	6107	3544	0.58	3973	6545	1.65	-0.75	0.59
R5_c01_0	LOC_Os0	expressed	Polynucle	AK069975	CTACAG	65	25	0.39	30	26	0.89	-0.60	0.66	52	13	0.25	13	19	1.44	-1.27	0.41
R5_c01_0	LOC_Os0	leucine-ric	C-type lec	AK072087	GGCCTC	6234	4698	0.75	4361	5972	1.37	-0.43	0.74	4111	2839	0.69	2496	4005	1.60	-0.61	0.66
R5_c01_0	LOC_Os0	expressed	Zinc finger	AK072797	ACTATC	53471	45585	0.85	42383	47789	1.13	-0.20	0.87	36434	35200	0.97	25112	21785	0.87	0.08	1.06
R5_c01_0	LOC_Os0	NAC dom	No apical	LOC_Os0	TGTACTC	23	34	1.50	14	33	2.37	-0.33	0.79	29	15	0.51	16	50	3.13	-1.30	0.41
R5_c01_0	LOC_Os0	hypothetic	NONE	LOC_Os0	TTTCTCC	17	26	1.50	38	40	1.05	0.26	1.19	16	37	2.24	36	101	2.83	-0.17	0.89
R5_c01_0	LOC_Os0	stress res	Ricin B-re	AK060132	GCGGAC	8273	16404	1.98	13288	8507	0.64	0.82	1.76	5391	13095	2.43	11688	6357	0.54	1.08	2.11
R5_c01_0	LOC_Os0	NAC dom	No apical	AK242268	TATATGT	162	106	0.65	117	181	1.55	-0.63	0.65	171	104	0.61	122	180	1.47	-0.64	0.64
R5_c01_0	LOC_Os0	light-medi	Similar to	AK100613	GGATGT	9763	10359	1.06	9372	7992	0.85	0.16	1.12	6070	6099	1.00	5851	5267	0.90	0.08	1.06
R5_c01_0	LOC_Os0	ARR1 prot	Similar to	LOC_Os0	TCTTGCA	74	2939	39.55	2459	16	0.01	6.30	78.65	11	13	1.11	13	14	1.04	0.05	1.03



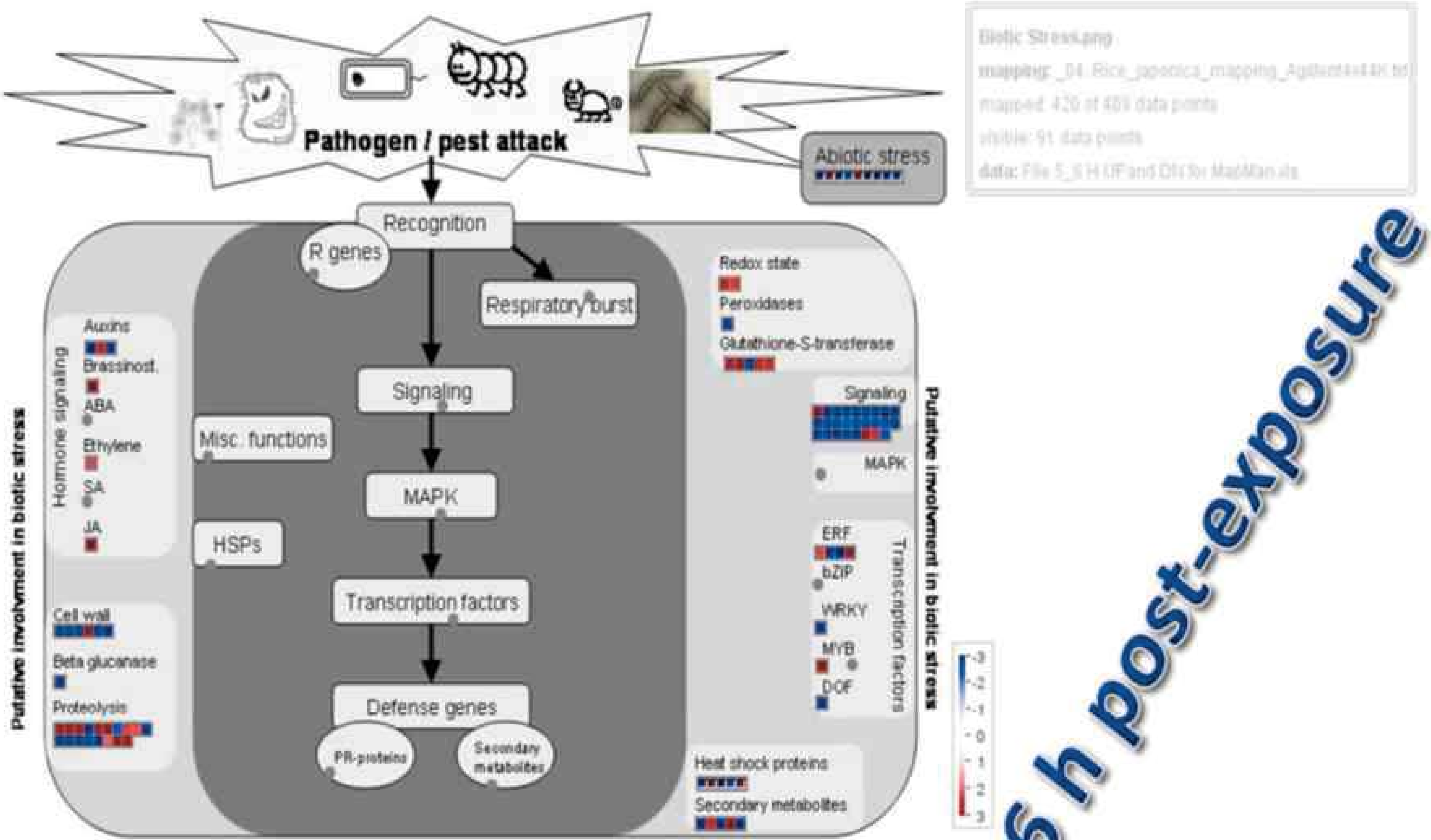
CHIP 1
ITF
6 h
72 h
7000
5000
3000
1000
-1000
-3000
-5000
-7000
UP
Down
CHIP 2
0 h NIES-ITF
72 h NIES
7000
5000
3000
1000
-1000
-3000
-5000
-7000
UP
Down
CON
The Gamma Radiation Responsive GENES
Criteria 1: Removing gene expression changes for time-dependent (others) factors till ITF 0 h sampling (NIES 0 h × ITF 0 h)
Criteria 2: Removing the gene expression changes for the growth-dependent factors till 72 h sampling (NIES 0 h x NIES 72 h)
184 + 4297 = 4481 Up-regulated
225 + 3515 = 3740 Down-regulated
6 h (Early-response)
72 h (Late-response)
235 + 2056 = 2291 Up-regulated
203 + 1271 = 1474 Down-regulated

MapMan is a user-driven tool that displays large datasets (e.g. gene expression data from Arabidopsis Affymetrix arrays) onto diagrams of metabolic pathways or other processes.

MapMan http://mapman.gabipd.org/web/guest/mapman

Bin	Functional Category	6 h UP		6 h DOWN		72 h UP		72 h DOWN	
		Count	%	Count	%	Count	%	Count	%
1	PS	2	1.1	1	0.4	1	0.4	0	0.0
2	major CHO metabolism	0	0.0	3	1.3	3	1.3	0	0.0
3	minor CHO metabolism	1	0.5	5	2.2	1	0.4	1	0.5
4	glycolysis	1	0.5	0	0.0	1	0.4	0	0.0
5	fermentation	1	0.5	0	0.0	0	0.0	1	0.5
7	OPP	0	0.0	1	0.4	0	0.0	0	0.0
8	TCA / org. transformation	1	0.5	1	0.4	0	0.0	3	1.5
10	cell wall	1	0.5	5	2.2	6	2.6	1	0.5
11	lipid metabolism	2	1.1	5	2.2	6	2.6	1	0.5
12	N-metabolism	1	0.5	0	0.0	0	0.0	0	0.0
13	amino acid metabolism	1	0.5	2	0.9	4	1.7	0	0.0
15	metal handling	0	0.0	1	0.4	1	0.4	2	1.0
16	secondary metabolism	2	1.1	3	1.3	11	4.7	4	2.0
17	hormone metabolism	4	2.2	2	0.9	10	4.3	12	5.9
18	Co-factor and vitamine metabolism	0	0.0	1	0.4	1	0.4	1	0.5
19	tetrapyrrole synthesis	0	0.0	0	0.0	2	0.9	0	0.0
20	stress	7	3.8	11	4.9	5	2.1	16	7.9
21	redox.regulation	2	1.1	0	0.0	3	1.3	1	0.5
22	polyamine metabolism	1	0.5	0	0.0	0	0.0	0	0.0
23	nucleotide metabolism	0	0.0	0	0.0	2	0.9	1	0.5
26	misc	11	6.0	14	6.2	23	9.8	22	10.8
27	RNA	17	9.2	16	7.1	15	6.4	20	9.9
28	DNA	3	1.6	2	0.9	2	0.9	0	0.0
29	protein	45	24.5	19	8.4	25	10.6	11	5.4
30	signalling	3	1.6	22	9.8	15	6.4	19	9.4
31	cell	1	0.5	6	2.7	5	2.1	2	1.0
33	development	3	1.6	2	0.9	1	0.4	4	2.0
34	transport	6	3.3	7	3.1	9	3.8	6	3.0
35	not assigned	69	37.5	101	44.9	89	37.9	79	38.9
	The number of non-redundant gene	184	100	225	100	235	100	203	100

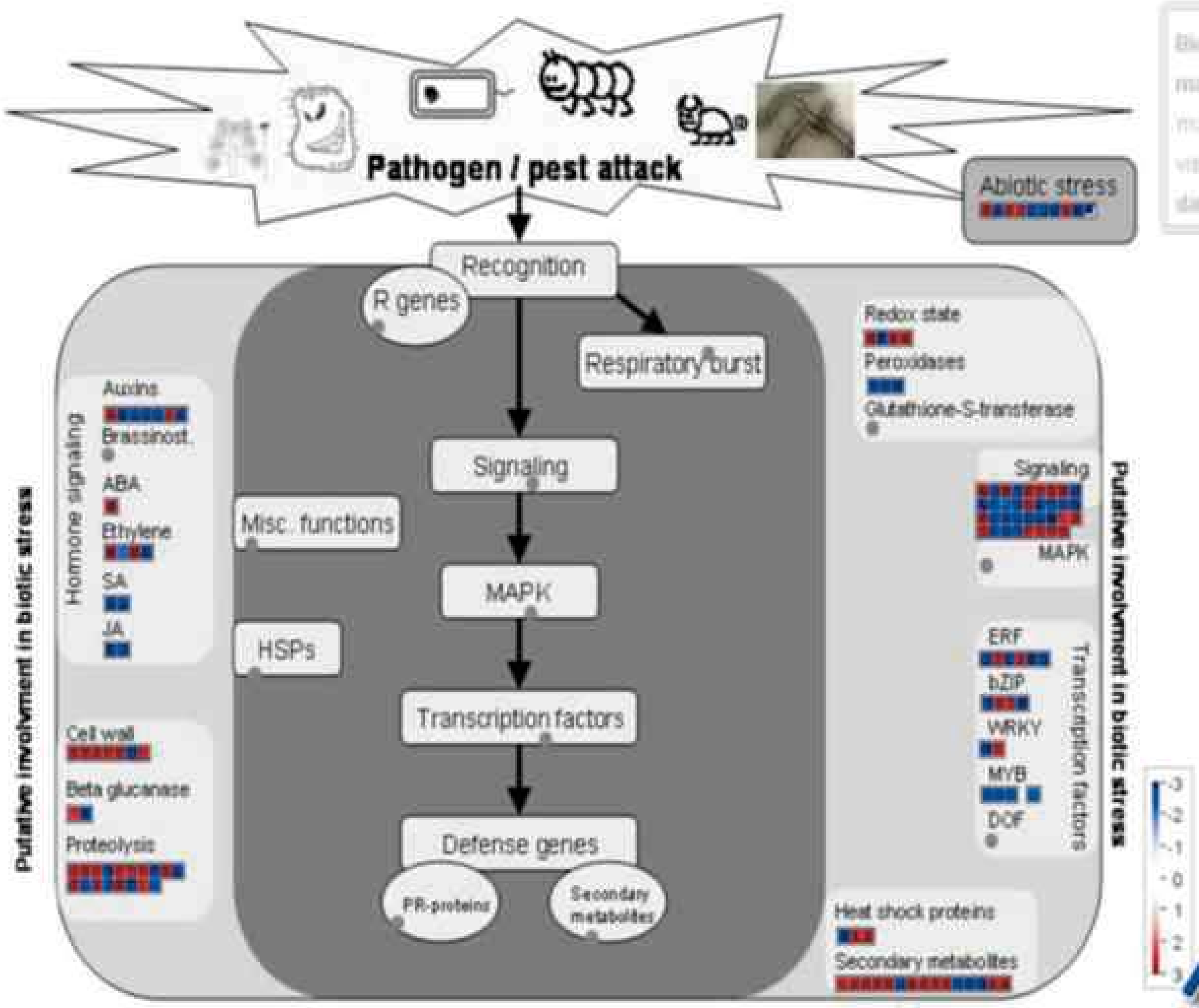
STRESS CHARACTERISTICS



6 h post-exposure

EARLY

STRESS CHARACTERISTICS



72 h post-exposure

LATE

Molecular Analyses – PROTEOMICS

Preparation of LB-TT

1. **Add 4 g CHAPS in a 300 (or 500) mL very clean/sterile Glass Beaker (calibrated if possible).**
2. Dissolve in **50 mL MQ** water by slowly shaking using wrist movement; or better use a stirrer to mix, not to strongly…
3. **42 g** of **Urea** was measured and added in parts, and mixed by gently turning the beaker .
4. Occasionally dip the beaker end in a 40-50°C water bath; for 15 sec or so, not too long at one time.
5. Add **15.2 g Thiourea** and mix gently again/stirrer.
6. Into the dissolved mixture solution add **1.8 mL of 1 M Tris-HCl** and mix/stirrer.
7. Add **169.5 mg Trizma base** and mix nicely/stirrer.
8. **Take 2 tablets of EDTA-free proteinase inhibitor** and dissolve (need to use stirrer for 10 min or so…).
9. To this mixture add slowly **0.2 mL of Triton X-100** and mix avoiding air bubbles.
10. To the almost completely dissolved buffer solution add **771.5 mg of DTT** and mix/stir.
11. Finally, add **1 mL of Ampholyte** and mix up to **100 mL with MQ water** (be careful to rinse any powders in the beaker walls by MQ)
12. Vacuum filter the solution with a **0.45 or 0.22 micron SteriCup filter** unit or Syringe filter unit.
13. Store **1 mL filtered LB-TT** in Eppendorf tubes at **-80°C**.
14. Thaw at room temperature (RT) before use.

LB-TT is a modified LYSIS BUFFER, which was originally developed by O'Farrell in 1975 [O'Farrell, P. H., J Biol Chem 1975, 250, 4007-4021].

1. **C-0h = RF1 x 2 tubes** (lyophilized)

2. **T-72h = RF3 x 2 tubes** (lyophilized)

Note – If each tube contents are solubilized in **100 µL LB-TT** we get a yield of 2.16 microgram per microliter protein.

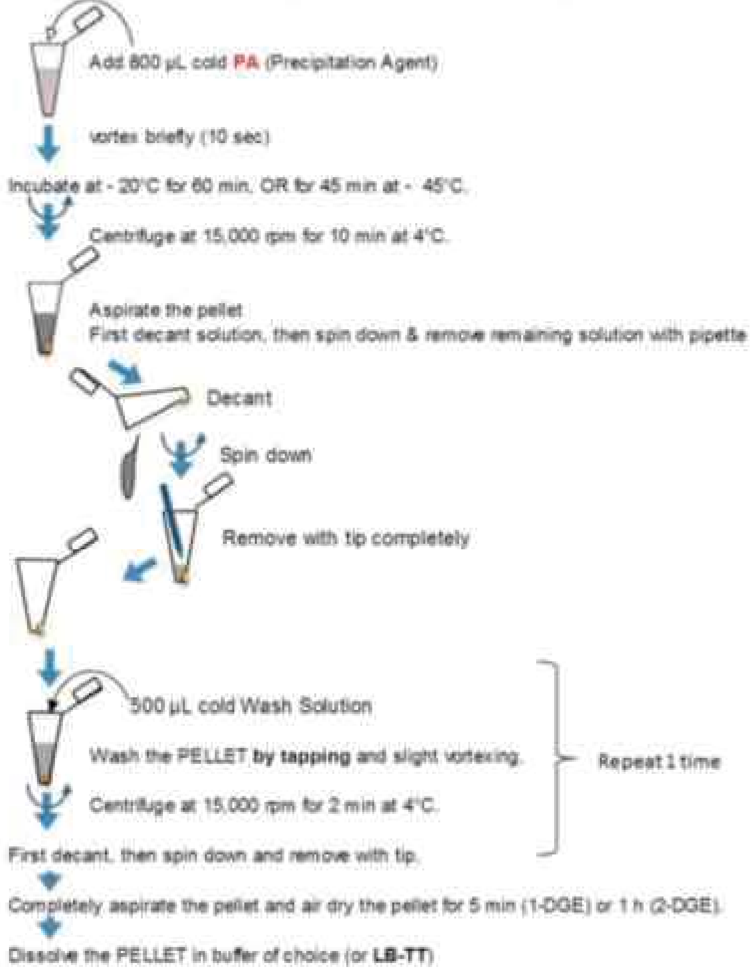
Protein Extraction PROTOCOL

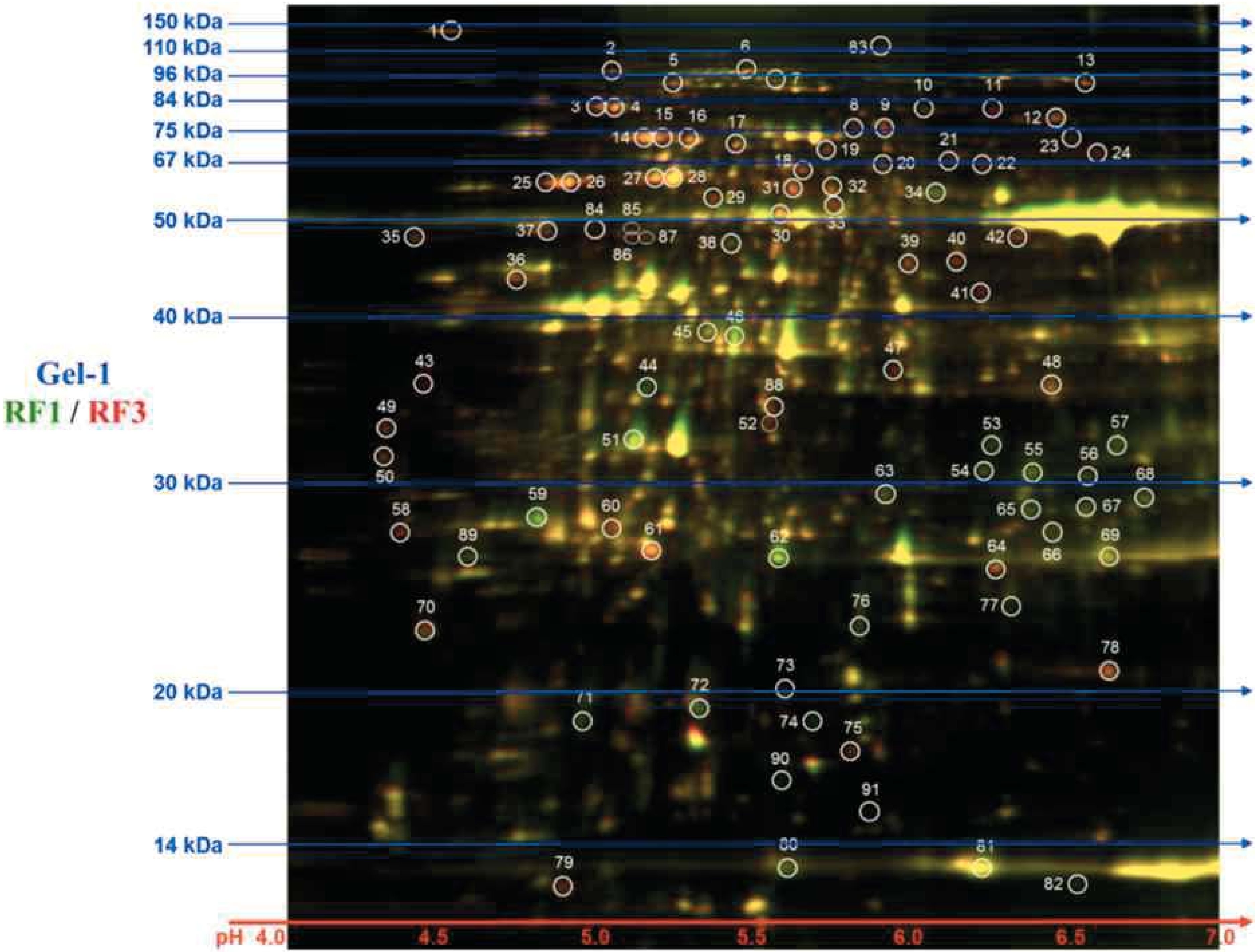
See also - Agrawal GK, Jwa NS, Jung YH, Kim ST, Kim DW, Cho K, Shibato J and Rakwal R: Rice proteomics: sample preparation to protein identification, In: Methods in Molecular Biology: Rice Protocols (Editor, Y. Yang), The Humana Press, New Jersey, USA, 956: 151-184. doi: 10.1007/978-1-62703-194-3_12. 2013.

ProteoExtract Protein Precipitation Kit Protocol (Illustrated)

Prepare Precipitation Agent (Kit components)
To one bottle of **Precipitant 1** (29 mL), add 1.7 mL of Precipitant 2, 3 and 4.
MIX WELL, and label **PRECIPITATION AGENT-PA** (34.1 mL solution; **store at - 20°C**)
Prepare Wash Solution
Add 150 mL ethanol to the provided Wash solution (Kit component).
Mix and reconstituted **WASH SOLUTION** can be stored for 1 year at **- 20°C**.

PROCEED FOR PRECIPITATION/CLEANUP
Take equal amounts (as close as possible) of each protein in 200 µL volume





150 kDa
110 kDa
96 kDa
84 kDa
75 kDa
67 kDa
50 kDa
40 kDa
30 kDa
20 kDa
14 kDa
Gel-1
RF1 / RF3
pH 4.0
4.5
5.0
5.5
6.0
6.5
7.0

Spot number	MALDI well number	Match Quality	Top Ranked Protein Name [Species]	Accession No.	Protein MW	Protein PI	Pep.Count	Protein Score	Protein Score C.I.%	Total Ion Score	Total Ion C.I.%	Comments or Other Possibilities
1	D1		Protein STAR1 OS=Oryza sativa subsp. Japonica GN=STAR1 PE	STAR1_ORYSJ	36,955	8.8	1	26		26	96	post-translational modification?
2	D2		Ribulose bisphosphate carboxylase small chain, chloroplastic OS	RBS1_ORYSJ	19,634	9.0	7	40	65			
3	D3		Heat shock protein 81-1 OS=Oryza sativa subsp. japonica GN=H	HSP81_ORYSJ	80,144	5.0	14	67	100	18	77	
4	D4		OSJNBa0091D06.15 [Oryza sativa Japonica Group]	gi|38567873	82,227	5.7	25	700	100	557	100	
5	D5		Cell division cycle protein 48, putative, expressed [Oryza sativa (	gi|110289141	89,755	5.1	24	205	100	81	100	
6	D6		chloroplast inner envelope protein, putative, expressed [Oryza s	gi|110289317	110,725	5.5	30	270	100	115	100	
7	D7		Pyruvate, phosphate dikinase 2 OS=Oryza sativa subsp. japonic	PPDK2_ORYSJ	96,491	5.4	11	123	100	105	100	
8	D8		Endoribonuclease Dicer homolog 1 OS=Oryza sativa subsp. japo	DCL1_ORYSJ	210,070	6.2	8	24		24	96	degradation product?
9	D9		Os02g0285800 [Oryza sativa Japonica Group]	gi|115445587	73,485	7.1	27	633	100	461	100	post-translational modification?
10	D10		Os02g0519900 [Oryza sativa Japonica Group]	gi|115446385	93,961	5.9	27	200	100	69	100	degradation product?
11	D11		DEAD-box ATP-dependent RNA helicase 3, chloroplastic OS=Ory	RH3_ORYSJ	81,566	6.1	27	472	100	293	100	
12	D12		5-methyltetrahydropteroyltriglutamate-homocysteine methyltransf	gi|108862992	84,582	5.9	19	218	100	136	100	
13	D13		Os04g0118400 [Oryza sativa Japonica Group]	gi|115456914	93,913	5.9	20	83	98	38		
14	D14		heat shock protein 70 [Oryza sativa Indica Group]	gi|21664287	70,953	5.2	26	786	100	605	100	
15	D15		RecName: Full=Heat shock cognate 70 kDa protein	gi|123650	71,182	5.1	21	546	100	425	100	
16	D16		ATP-dependent zinc metalloprotease FTSH 1, chloroplastic OS=C	FTSH1_ORYSJ	72,658	5.5	12	129	100	82	100	
17	D17		Putative heat shock 70 KD protein, mitochondrial precursor [Oryz	gi|27476086	70,403	5.5	18	247	100	150	100	
18	D18		Protein STAR1 OS=Oryza sativa subsp. Japonica GN=STAR1 PE	STAR1_ORYSJ	36,955	8.8	2	38	48	33	99	post-translational modification?
19	D19		Phosphoglucomutase, cytoplasmic 2, putative, expressed [Oryza	gi|108710732	54,499	4.9	16	196	100	113	100	post-translational modification?
20	D20		Os01g0372700 [Oryza sativa Japonica Group]	gi|115436816	62,549	5.7	21	413	100	271	100	
21	D21		putative thiamin biosynthesis protein [Oryza sativa Japonica Gro	gi|13435255	70,160	6.0	21	407	100	289	100	
22	D22		putative thiamin biosynthesis protein [Oryza sativa Japonica Gro	gi|13435255	70,160	6.0	26	523	100	329	100	
23	D23		Phenylalanine ammonia-lyase OS=Oryza sativa subsp. japonica (	PAL1_ORYSJ	75,451	6.1	12	58	99	18	58	
24	D24		Ribulose bisphosphate carboxylase small chain, chloroplastic OS	RBS1_ORYSJ	19,634	9.0	7	40	59			
25	E1		Protein STAR1 OS=Oryza sativa subsp. Japonica GN=STAR1 PE	STAR1_ORYSJ	36,955	8.8	3	32		23	94	
26	E2		RecName: Full=RuBisCO large subunit-binding protein subunit alp	gi|134102	57,485	4.8	16	231	100	151	100	
27	E3		Endoribonuclease Dicer homolog 3a OS=Oryza sativa subsp. jap	DCL3A_ORYSJ	184,774	6.4	20	48	95			degradation product?
28	E4		RecName: Full=RuBisCO large subunit-binding protein subunit bet	gi|2506277	62,945	5.9	13	508	100	405	100	
29	E5		ATP synthase subunit alpha, chloroplastic OS=Oryza sativa subs	ATPA_ORYSI	55,630	6.0	9	91	100	57	100	post-translational modification?
30	E6		ATP synthase subunit beta, chloroplastic OS=Oryza sativa subsp	ATPB_ORYSI	53,921	5.4	28	1,100	100	831	100	
31	E7		Ketol-acid reductoisomerase, chloroplastic OS=Oryza sativa sub	ILV5_ORYSJ	62,338	6.0	15	700	100	608	100	
32	E8		Ketol-acid reductoisomerase, chloroplastic OS=Oryza sativa sub	ILV5_ORYSJ	62,338	6.0	15	489	100	406	100	see hit #2
33	E9		Thioredoxin reductase NTRC OS=Oryza sativa subsp. japonica (	NTRC_ORYSJ	56,116	6.1	10	161	100	120	100	see hits #'s 2 and 6
34	E10		ATP synthase subunit alpha, chloroplastic OS=Oryza sativa subs	ATPA_ORYSI	55,630	6.0	30	878	100	605	100	
35	E11		Calreticulin OS=Oryza sativa subsp. Japonica GN=Os07g024620	CALR_ORYSJ	48,279	4.5	12	165	100	102	100	
36	E12		Fructose-1,6-bisphosphatase, chloroplastic OS=Oryza sativa su	F16P1_ORYSJ	43,577	5.0	13	181	100	105	100	
37	E13		Os08g0388000 [Oryza sativa Japonica Group]	gi|115467746	58,894	5.2	30	642	100	394	100	degradation product?
38	E14		Eukaryotic initiation factor 4A-1 OS=Oryza sativa subsp. japonic	IF4A1_ORYSJ	47,058	5.4	19	390	100	253	100	
39	E15		S-adenosylmethionine synthase 2 OS=Oryza sativa subsp. japon	METK2_ORYSJ	42,874	5.7	23	700	100	486	100	see hit #4
40	E16		S-adenosylmethionine synthase 1 OS=Oryza sativa subsp. japon	METK1_ORYSJ	43,193	5.7	23	792	100	568	100	
41	E17		Chalcone synthase 1 OS=Oryza sativa subsp. Japonica GN=CHS	CHS1_ORYSJ	43,237	5.9	14	365	100	269	100	
42	E18		Ribulose bisphosphate carboxylase large chain OS=Oryza sativa	RBL_ORYSI	52,847	6.2	14	66	100			
43	E19		Putative protein ABIL2 OS=Oryza sativa subsp. japonica GN=Os	ABIL2_ORYSJ	36,100	8.7	7	31				
44	E20		Os02g0240300 [Oryza sativa Japonica Group]	gi|115445243	34,154	5.3	11	282	100	221	100	
45	E21		Ribulose bisphosphate carboxylase/oxygenase activase, chloro	RCA_ORYSJ	51,421	5.4	18	601	100	497	100	degradation product?

11	D11		DEAD-box ATP-dependent RNA helicase 3, chloroplastic OS=Ory	RH3_ORYSJ

DEAD-box helicases. A diverse family of proteins involved in ATP-dependent RNA unwinding, needed in a variety of cellular processes including splicing, ribosome biogenesis and RNA degradation

Superfamily II DNA and RNA helicases [DNA replication, recombination, and repair / Transcription / Translation, ribosomal structure and biogenesis]

Molecular cloning and characterization of a salinity **stress**-induced gene encoding DEAD-**boxheli case** from the halophyte Apocynum venetum.
Liu HH, Liu J, Fan SL, Song MZ, Han XL, Liu F, Shen FF.
J Exp Bot. 2008;59(**3**):633-44. doi: 10.1093/jxb/erm355. Epub 2008 Feb 13.

21	D21		putative thiamin biosynthesis protein [Oryza sativa Japonica Grou	gi|13435255

Recent reports suggest that vitamin B1 (thiamine) participates in the processes underlying plant adaptations to certain types of abiotic and biotic stress, mainly oxidative stress.

The upregulation of **thiamine** (vitamin B1) **biosynthesis** in Arabidopsis thaliana seedlings under salt and osmotic **stress** conditions is mediated by abscisic acid at the early stages of this **stress response**.

Rapala-Kozik M, Wolak N, Kujda M, Banas AK.

BMC Plant Biol. 2012 Jan 3;12:2. doi: 10.1186/1471-2229-12-2.

41	E17		Chalcone synthase 1 OS=Oryza sativa subsp. Japonica GN=CHS	CHS1_ORYSJ

The primary product of this enzyme is 4,2',4',6'-tetrahydroxychalcone (also termed naringenin-chalcone or chalcone), which can under specific conditions spontaneously isomerize into naringenin.

Secondary metabolite biosynthesis; flavonoid biosynthesis.

Gene expression profiles deciphering **rice** phenotypic variation between Nipponbare (Japonica) and 93-1 1 (Indica) during oxidative **stress**.
Liu F, Xu W, Wei Q, Zhang Z, Xing Z, Tan L, Di C, Yao D, Wang C, Tan Y, Yan H, Ling Y, Sun C, Xue Y, Su Z.
PLoS One. 2010 Jan 8;5(**1**):e8632. doi: 10.1371/journal.pone.0008632.

Conclusion 1:

1. Gamma radiation effects rice plant growth under low-dose

2. Three days exposure to rice plant produces –

- ✓ Damage to leaf tips
- ✓ Change in expression of
  - DNA damage/repair genes
  - Defense/stress-related genes
  - Large number of genes genome-wide
- ✓ Change in protein abundance proteome-wide

Manuscript in Preparation – INVITATION Journal of Heredity

Observation of Rice Gene/Protein Expression by Low-level Gamma Ray Exposure in Iitate Village

G. Hayashi[1], T. Imanaka[2], J. Shibato[3], N. Itoh[4], A. Meguro[5], S. Kimura[6], A. Kubo[7], S. Ozawa[8], K. Ichikawa[9], S. Fukutani[2], S. Kikuchi[10], Kyoungwon Cho[11], Jai Singh Rohila[12], Ganesh Kumar Agrawal[13], Randeep Rakwal[3,13,14*]

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2013

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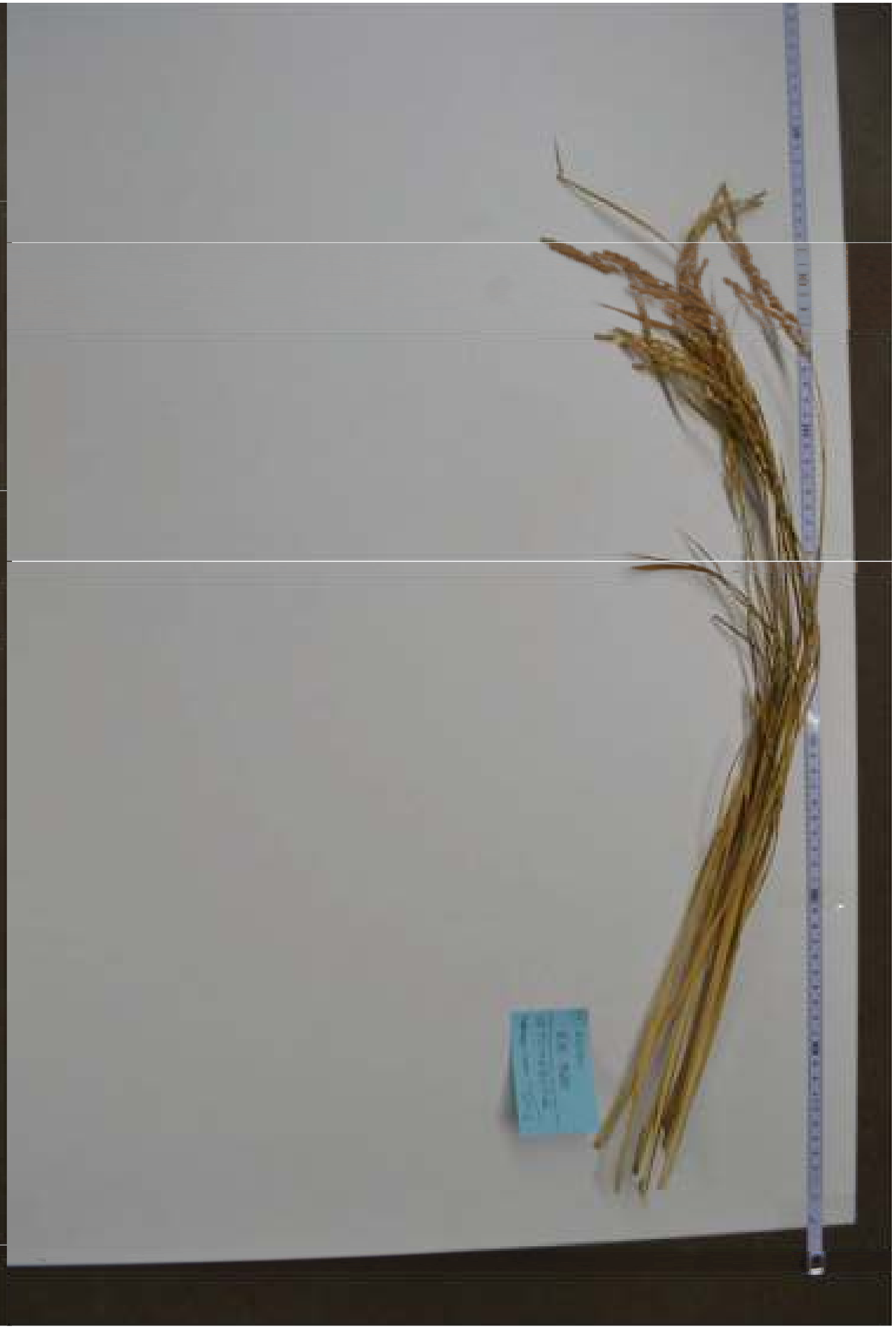
Koshihikari

Akitakomachi

Hitomebore




In Progress:
1. Radionuclides in Rice Stalk/Leaf and Seed
2. Gene, Protein, and Metabolite Expressions

Generation Next – Let's not Forget