KAAB International Symposium 2023

Frontiers in Ecologically Friendly Crop Production

March 27, 2023 (Mon) 10:00-20:40
Main Lecture Room C110 & Web hybrid meeting, Niigata University Faculty of Agriculture

Poster Release

10:00-

Opening Remarks

14:00-14:10 **Toshiaki MITSUI**

Poster Session (Short talk)

Chair: **Norikuni OHTAKE**, **Naoki KANO**

14:10-17:00

Symposium

Chair: **Kimiko ITOH**

17:00-17:20 **Welcome speech**

Kazushige KAWABATA, Vice President of Niigata University

Hiroo SHINADA, Mayor of Kariwa Village

Chair: **Masanori YAMASAKI**, Niigata University

17:20-17:50 **Motoyuki ASHIKARI**, Nagoya University, Japan

"How does plant stem grow? Discovering of antagonistic regulation of stem growth in rice"

17:50-18:20 **Murat AYCAN**, Niigata University, Japan

"Employing the plant memory for ecologically friendly crop production: Transgenerational salt plasticity in rice"

Chair: **Hideo HASEGAWA**, Niigata University

18:25-18:55 **Kazunori MINAMIKAWA**, JIRCAS, Japan

"Dual approaches to disseminate paddy water management as a climate solution in Monsoon Asia"

18:55-19:25 **Hirohiko NAGANO**, Niigata University, Japan

"Dry soil rewetting for elucidating soil organic matter decomposition and carbon dynamics"

Chair: **Toshiaki MITSUI**, Niigata University

19:30-20:00 **Asiloglu RASIT**, Niigata University, Japan

"Applied protistology: A pioneering approach in ecologically friendly rice production"

20:00-20:30 **Iker Aranjuelo MICHELENA**, CSIC/UPNA Agrobiotechnology Institute, Spain

"Relevance of promoting a resource efficient and a low greenhouse gas emission agriculture model in current and near future agriculture"

Closing Remarks

20:30-20:40 **Naoki HARADA**

Poster Award Announcement **Masaru NAKANO**

Sponsored by Niigata University & CSIC

European Commission Marie Skłodowska-Curie Actions CropYQualT-CEC

新潟大學 KAAB

KAAB International Symposium 2023 Poster session Program

Session 1: Biochemistry Molecular Biology & Biotechnology

PS-1-1 **Effects of volatile compounds emitted from edible mushrooms shiitake fungi-beds as a Biostimulant on growth and biomass in rice seedlings**

[1]Kanga Clever Nkhokwe, [1]Okisaka Yui, [1]Hanamata Shigeru, [1]Mitsui Toshiaki, [1]Itoh Kimiko

[1]Graduate School of Science and Technology, Niigata Univ., Niigata

PS-1-2 **The Screening Rice (Oryza sativa L.) Genotypes Under Effect of Drought and High Temperature Stress**

[1,2] Ermelinda Maria LOPES HORNAI, [3] Murat AYCAN, [1,3] Toshiaki Mitsui

[1] Graduate School of Science and Technology, Niigata Univ., Niigata, Japan.

[2] National Division of Research and Statistics, Timor-Leste Ministry of Agriculture and Fisheries, Dili, Timor-Leste.

[3] Dept. of Appl. Biol. Chem., Fac. of Agric., Niigata Univ., Niigata, Japan.

PS-1-3 **Expression study of Type A response regulator genes under different abiotic stress conditions**

[1]Setu Rani Saha, [2]Kimiko Itoh

[1] Graduate School of Science and Technology, Niigata University, Niigata, Japan

[2] Institute of Science and Technology, Niigata University, Niigata, Japan

PS-1-4 **Characterization of Starch Granules Formed in Endosperm of Rice with Ectopic Overexpression of α-Amylase**

[1]Kaku Cho, [2]Sumiko Nakamura, [2]Ken-ichi Ohtsubo, [3]Yasunori Nakamura, [3]Naoko Fujita, [4]Hiromoto Yamakawa, [1]Toshiaki Mitsui

[1] Graduate School of Science and Technology, Niigata Univ., Niigata, Japan;

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[3] Faculty of Bioresource Science, Akita Prefectural University, Akita, Japan;

[4] Institute of Crop Science, National Agriculture and Food Research Organization (NARO), Tsukuba, Japan

PS-1-5 **Generation of transformants overexpressing Lotus japonicus CLE genes for their functional analysis.**

Kayano Ishige, Satoru Okamoto

affiliation Graduate School of Science and Technology, Niigata University

PS-1-6 **Chemo-morphological Characterization Activities of Pelargonium sidoides DC (Geraniaceae)**

[1]Mthiyane P., [2]Mavengahama S., [2]Opoku A. & [3]Kleynhans R.

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[2]Department of Agriculture and Department of Biochemistry, University of Zululand, Dlangezwa, 3886, RSA

[3]Agricultural Research Council- Roodeplaat, Vegetable and Ornamental Plant Institute, Pretoria, 0001, RSA

PS-1-7 **Molecular Weight Control of Lignopolyphenol by Neighboring Group Participation for Improved Biological Activity**

[1]Minami MASAKI, [2]Keigo MIKAME

[1] Graduate School of Science and Technology, Niigata Univ., Niigata, Japan

[2] Faculty of Agriculture Niigata Univ., Niigata, Japan

PS-1-8 **Analysis of the physiologic and metabolic response of wheat plants to over-expression of ferredoxin in the chloroplast under changing [CO2] conditions.**

[1]Dorra Fakhet, [2]Jon González, [2]Alicia Fernández-San Millán, [2]Luis Larraya, [1]Angie L. Gámez, 1Fermín Morales, 1Iker Aranjuelo, 2Jon Veramendi and 2Immaculada Farrán.

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Session 2: Agricultural & Food Science

PS-2-1 **Effects of Nitrogen Fertilizers on Top-Down Predatory Protist in Paddy Field Soils**

[1]Seda Bodur Ozer, [2]Rasit Asiloglu, [3]Solomon Oloruntoba Samuel, [4]Kazuki Suzuki, [2]Naoki Harada

[1] Graduate School of Science and Technology, Niigata University, Niigata, Japan

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[3] Department of Plant and Microbial Biology, North Carolina State University, Relaigh, NC 27696

[4] Institute of Research Promotion, Niigata University, Niigata, Japan

PS-2-2 **Low input of top-dressing nitrogen fertilizer to rice plants positively affects alcohol fermentation**
in sake brewing using the grains

[1]Takuji Miyamoto, [1]Ikuhisa Nishida, [2]Norikuni Ohtake, [1,2,3]Dai Hirata

[1] Sakeology Center, Niigata University, Niigata, Japan

[2] Graduate School of Science and Technology, Niigata University, Niigata, Japan

[3] Niigata Sake Brewers Association, Niigata, Japan

PS-2-3 **Effect of poultry manure on crop growth**

[1]Shinya Yamada, [1]Sharula, [1] Iffet Cakirsoy, [2]Takuji Miyamoto, [3]Saki Shimamoto, [3]Shinobu Fujimura, [3]Yoshitaka Motonaga, [3]Kuni Sueyoshi, [3]Norikuni Ohtake

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[2] Sakeology Center, Niigata Univ., Niigata, Japan

[3] Dept. of Appl. Biol. Chem., Fac. of Agric., Niigata Univ., Niigata, Japan

PS-2-4 **Anthocyanins and virus accumulation in BYDV-PAV-infected Brachypodium distachyon**

Kaname Iizuka, Nami Minato

Graduate school of Science and Technology, Niigata Univ., Niigata, JAPAN

PS-2-5 **Single infection of barley yellow dwarf virus-PAV (BYDV-PAV) has negative effects on preference and performance of insect vector compared to co-infection with the relative virus species.**

[1]Kai Nakagawa, [1]Shuichi Hatori, [1,2]Nami Minato

[1] Graduate School of Science and Technology, Niigata Univ., Niigata, JAPAN

[2] Institute of Science and Technology, Niigata Univ., Niigata, JAPA**N**

PS-2-6 **Evolution of plantago asiatica mosaic virus through long-term passages in Nicotiana plants and its impact on host adaptation**

[1]Daisuke Nakamura, [1]Nami Minato, [2]Minako Furuya, [2]Ken Komatsu, [3]Shin-Ichi Fuji

[1]Graduate School of Science and Technology, Niigata Univ., Niigata, JAPAN;

[2]Graduate School of Agriculture, Tokyo University of Agriculture and Technology (TUAT), Tokyo, JAPAN;

[3]Faculty of Bioresource Science, Akita Prefectural Univ., Akita, JAPAN

PS-2-7 **The exposure of maize seedlings to weed volatiles affects their growth and seed quality**

[1]Yusuke Sakurai, [1]Satomi Ishizaki, [2]Nami Minato, [1]Yasuko Hayashi, [3]Shota Izumi, [3]Takuma Yoshida, [3]Kaori Shiojiri and [4]Junji Takabayashi

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[3] Department of Agriculture, Ryukoku Univ., Otsu, Japan;

[4] Center for Ecological Research, Kyoto Univ., Otsu, Japan

PS-2-8 **Peanut photosynthesis response to drought can include diffusive and biochemical limitations depending on cultivar**

[1,2]David Soba, [3]Summer Parker, [3]Charles Chen, [4]Avat Shekhoofa, [3]Alvaro Sanz-Saez

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[2] Agrobiotechnology Institute (IdAB), Spanish National Research Council (CSIC) and Government of Navarre, Spain

[3] Department of Crop, Soil and Environmental Sciences, Auburn University, Auburn, AL, USA

[4] Plant science Department, University of Tennessee Knoxville, Jackson, TN, USA

ps-2-9 **Target role of stomatal aperture on leaf metabolism and growth of rice (Oryza sativa L.) under ambient and elevated [CO2]**

[1]María Ancín, [2]Koh Iba, [1]Iker Aranjuelo

[1] Instituto de Agrobiotecnología (IDAB), Consejo Superior de Investigaciones Científicas (CSIC)-Gobierno de Navarra, Avenida Pamplona 123, 31192 Mutilva, Spain;

[2] Faculty of Agriculture, Kyushu University, Fukuoka 812–8151, Japan

PS-2-10 **Impact of biostimulants on wheat plants grown under climate change conditions**

Picazo P.J., Morales F. and Aranjuelo I.

Instituto de Agrobiotecnología (IdAB), CSIC-Gobierno de Navarra, Avda. de Pamplona 123, 31192 Mutilva, Spain.

PS-2-11 **Hyperspectral devices applied to the determination of alfalfa nutritional quality traits**

[1,2]Angie Gámez, [3]Thomas Vatter, [3]Jose Luis Araus, [2]Iker Aranjuelo

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Session 3: Environmental Science and Technology

PS-3-1 **Development of Liquid Fertilizer Using Recovered Phosphorus from Sewage Sludge Ash and Application to Cultivation of Crop (Japanese mustard spinach)**

[1]Hikari Fukushima, [1]Sayano Hiyoshi, [1]Naoto Miyamoto, [1]Naoki Kano, [2]Norikuni Otake, [3]Hee-Joon Kim

[1] Chem. & Chem. Eng. Program, Fac. of Eng., Niigata Univ., Niigata, Japan;
[2] Appl. Lif. Sci. Program, Fac. of Agri., Niigata Univ., Niigata, Japan;
[3] Dep. of Environ. Chem., Fac. of Advanced Eng., Kogakuin Univ., Tokyo, Japan;

PS-3-2 **Effect of nitrogen fertilization on the percentage of soybean rhizobia carrying hupS and hupL genes.**

[1]Mana Ishikawa, [1]Ayaka Tanbo, [2]Masaya Yamada, [3]Takashi Miyamoto, [1]Kuni Sueyoshi, [2]Iffet Cakirsoy, [2]Soushi Takeda, [1]Norikuni Ohtake

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[2] Graduate School of Science and Technology, Niigata Univ., Niigata, Japan
[3] Sakeology Center, Niigata Univ., Niigata, Japan

PS-3-3 **Conservation Agriculture to preserve soils and to improve plant growth**

[1]Wiyao BANAKINAOU and [1,2]Tadao AODA

[1] Graduate School of Science and Technology, Niigata Univ. Niigata, Japan;
[2] Dept. of Watershed Environment, Faculty of Agriculture, Niigata Univ. Niigata, Japan

PS-3-4 **Microplastic Contamination on Turkish Arable Land due to Sewage Sludge Application**

[1]Serhat Murat Comert, [2]Muhittin Onur Akca, [2]Oguz Can Turgay, [3]Kazuki Suzuki, [4]Naoki Harada

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[3] Institute of Research Promotion, Niigata University, Niigata, Japan
[4] Institute of Science and Technology, Niigata University, Niigata, Japan

PS-3-5 **Determination of Atmospheric Greenhouse Gases Using UAV and Soil Fluxes Capturing with Ground Survey**

[1]Iaroslav Zakharevich, [2]Hideo Hasegawa, [2]Hirohiko Nagano

[1] Graduate School of Science and Technology, Niigata University, Niigata 950-2181, Japan
[2] Institute of Science and Technology, Niigata University, Niigata 950-2181, Japan

PS-3-6 **Synthesis of chitosan based new materials for adsorption of heavy metal**

[1]Enkhtuya Majigsuren, [1]Ulziidelger Byambasuren, [1,2]Munkhpurev Bat-Amgalan, [3]Naoki Kano, [1]Nasanjargal Shirendev, ○[1]Ganchimeg Yunden

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[2] Graduate School of Science and Technology, Niigata Univ., Niigata, Japan;
[3] Chem. & Chem. Eng. Program, Fac. of Eng., Niigata Univ., Niigata, Japan;

PS-3-7 **Fabrication and characterization of low-cost ceramic membrane developed with cross-linked chitosan for ultrafiltration of Cr(VI)**

[1,2]Munkhpurev Bat-Amgalan, [3]Naoto Miyamoto, [3]Naoki Kano, [2]Ganchimeg Yunden, [3,4]Hee-Joon Kim

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[4] Dep. of Environ. Chem., Fac. of Advanced Eng., Kogakuin Univ., Tokyo, Japan;

PS-3-8 **Effect of chelating agents (EDTA, NTA) on phytoremediation of Pb-contaminated soil by Helianthus Annuus**

[1]Muhammad Nabil Md Sari, [1]David Eva Vanessa Anak, [2]Naoto Miyamoto, [2]Naoki Kano

[1] Graduate School of Science and Technology, Niigata Univ., Niigata, Japan;

[2] Dept. of Chem. and Chem. Eng., Fac. of Eng., Niigata Univ., Niigata, Japan

PS-3-9 **Removal of Cadmium in Soil by Phytoremediation Using Helianthus Annuus & Marigold and by Washing Method Using Ferric Chloride**

[1]Saya Ito, [2]David Eva Vanessa Anak, [1]Naoto Miyamoto, [1]Naoki Kano

[1] Chem. & Chem. Eng. Program, Fac. of Eng., Niigata Univ., Niigata, Japan;

[2] Graduate School of Science and Technology, Niigata Univ., Niigata, Japan

Session 4: Other

PS-4-1 **Extremely Long Chains of Magnetic Particles via Large Plastic Beads Dispersed in Magnetic Elastomers**

Rio Urano, Shota Akama, Mika Kawai, Tetsu Mitsumata

Graduate School of Science and Technology, Niigata Univ., Niigata, Japan

PS-4-2 **Research on the Structure of Rice Brand Value**
- A Case Study of "Koshihikari" and "Koshihikari Niigata University NU1" -

Kazuki KATSUMI

Master's Program, Graduate School of Science and Technology, Niigata University

S-1

How does a plant grow its stem? Discovering of antagonistic regulation of stem growth in rice

Motoyuki Ashikari

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The stem is a primary structural axis of plants that supports the aerial organs such as leaves and flowers. Stem elongation enables plants to adapt and survive in their environment. For example, elongated stems keep the leaves exposed to light, thereby maximizing photosynthetic efficiency. In gramineous plants such as rice, barley and wheat, the stem, which is composed of nodes and internodes clearly, impacts plant height and productivity. The short stem (semi dwarf stature) is one of the most important agricultural traits, since it shows lodging resistance leading stable harvest. In fact, semi dwarf rice and wheat contributed to increasing grain production in the late of 20th Century, known as “Rice and Wheat green revolution”. Stem (Internode elongation) is stimulated by the plant hormone gibberellic acid (GA) through the activation of cell division and cell elongation. In a different aspect of internode elongation by GA, the internode requires activation of the intercalary meristem in internode to initiate the elongation. However, the regulatory factors and molecular mechanism of the GA response and intercalary meristem activity have been unknown. To fill the gaps between the GA response and acquisition of the ability for internode elongation, we identified two factors controlling internode elongation in response to GA in rice. In this symposium, I introduce the antagonistic regulation of rice stem (internode) elongation by an “accelerator” and a “decelerator” in concert with GA. The gene *ACCELERATOR OF INTERNODE ELONGATION1* (*ACE1*), which encodes a protein of unknown function, confers competence for cell division of intercalary meristematic region leading to internode elongation in the presence of GA. On the contrary, *DECELERATOR OF INTERNODE ELONGATION1* (*DEC1*), which encodes a zinc-finger transcription factor, suppresses internode elongation while downregulation of *DEC1* allows internode elongation. I also show that the mechanism of internode elongation mediated by *ACE1* and *DEC1* is conserved in Gramineae. Furthermore, genetic diversity analysis suggests that mutations in *ACE1* and *DEC1* had historically contributed to the selection of shorter plants for lodging resistance in domesticated populations and of taller plants for deepwater adaptation in wild species of rice. These antagonistic regulatory factors enhance our understanding of the GA response as an additional mechanism regulating internode elongation and environmental fitness beyond biosynthesis and GA signal transduction.

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S-2

Employing the plant memory for ecologically friendly crop production: Transgenerational salt plasticity in rice

Murat AYCAN[1*], Lutfun NAHAR[2], Marouane BASLAM[1], Toshiaki MITSUI[1]

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[2] Department of Life and Food Sciences, Graduate School of Science and Technology, Niigata University, Niigata, Japan.

Transgenerational plasticity effects in plants are thought to allow for rapid adaptation to environmental changes. Transgenerational plasticity, or phenotypic plasticity, enables organisms and their offspring to adapt to the environment without changing the underlying DNA. To test the transgenerational salinity tolerance plasticity on rice plants, we performed a multi-factorial reciprocal transplant experimental design, crossing the past environment experienced by ancestors with the present one experienced by progeny. We employed 99% identical genetic backgrounded salt-tolerant and salt-sensitive rice genotypes to test if non-genetic environmental induced phenotypic modifications and transgenerational salinity affect the phenomenon and to exclude nuclear genomic factors for two generations. We found that the parentally salt-stressed salt-sensitive genotype showed higher growth performance, photosynthetic activity, yield performance, and transcriptional responses compared to parentally non-stressed salt-sensitive plants under salt-stress conditions. Surprisingly, salt-tolerant genotypes did not show a transgenerational salt plasticity effect. Phenotypes of offspring plants differed based on the environment of their ancestors. The impacts of ancestral environment on progeny resulted in heritable phenotypic modifications in salt-sensitive genotypes.

Keywords: Salt-stress, parental effect, maternal effect, transcriptional memory, stress memory

S-3

Dual approaches to disseminate paddy water management as a climate solution in Monsoon Asia

Kazunori Minamikawa, Kenichi Uno

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Monsoon Asia is the world's rice basket but more production is still needed to feed the growing population in the future. At the same time, rice paddies are one of the major anthropogenic sources of methane (CH_4), a potent and short-lived greenhouse gas, and also the largest consumer of irrigation water in agriculture. Solution options are therefore required for each of these problems.

Water management in rice paddies has a potential to simultaneously solve the above problems. Multiple drainage practices, such as alternate wetting and drying (AWD) and midseason drainage followed by intermittent irrigation (MiDi), which is the conventional practice in Japan, save irrigation water and reduce soil CH_4 emission compared to those in continuous flooding (CF) practice. In addition, recent field studies in Vietnam reported that multiple drainage practices significantly increased rice grain yield. This is worth digging deeper because the yield increase enables the voluntary dissemination of water management to farmers in Monsoon Asia. However, there have been also many field studies reporting yield non-increase by multiple drainage practices. Therefore, it is necessary to investigate the determinants of yield increase and non-increase through field studies under various environmental and agronomic settings.

Another approach to disseminate paddy water management is to utilize the institutions in public and private sector, such as carbon crediting schemes. This approach can cover the rice areas with yield non-increase by multiple drainage practices and provide some incentive to the farmers. To implement such mitigation actions accurately and transparently, a low-cost methodology for monitoring, reporting and verification (MRV) is required. Comparing with just aggregating many fields that managed by different farmers, an idea to reduce the cost of MRV implementation is to simultaneously manage water level in a large paddy area (e.g., >10 ha) that shares the same irrigation and drainage canals/facilities.

Creating some incentive for farmers is a promising way to disseminate paddy water management as a climate solution in Monsoon Asia. In any sector, applied scientists for climate change mitigation should draw various scenarios for the dissemination of climate solutions as well as developing promising technologies.

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S-4

Dry soil rewetting for elucidating soil organic matter decomposition and carbon dynamics

Hirohiko Nagano

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Rewetting of dry soil is a crucial topic in investigating soil organic matter (SOM) decomposition and carbon dynamics from both viewpoints to elucidate the climate change impacts on them and the soil microbial utilization of substrate organic matter. Here, we present the substantial potential of dry soil rewetting in SOM research, introducing two studies. One is the evaluation of the effects of dry-wet cycles on soil carbon dioxide (CO_2) release using the soil incubation experiment. Dry-wet cycles substantially stimulated soil CO_2 release up to more than ten-fold of CO_2 release under the continuously constant moisture condition. Thus, predicted changes in precipitation patterns, increasing intensity, and decreasing frequency of rainfall likely cause an unexpected increase in soil CO_2 release. Another study is the application of dry soil rewetting to elucidate microbial utilization of soil organic substrate under different substrate availability. Here, water-extractable organic matter from rewetted dry soils likely reflected soil microbial utilization of carbon and nitrogen substrate, exhibiting the contrast enrichments of stable isotope signature under different substrate availability. Thus, dry soil rewetting has substantial potential to evaluate SOM decomposition and soil carbon dynamics associated with climate change and soil microbial ecology. Accelerating research on dry soil rewetting can bring practical advantages to understanding and predicting features and the future of SOM decomposition and soil carbon dynamics.

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S-5

Applied Protistology: A Pioneering Approach in Ecologically Friendly Rice Production

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Rice is one of the most essential crops feeding more than half of the world population. The growth of rice plants depends on diverse microorganisms inhabiting the rhizosphere, which obligates us to better understand the plant-microbe interactions for sustainable rice production. Among the microbial groups in the rice rhizosphere, protists, single-celled eukaryotes, are the least studied group. In this presentation, a series of our previous studies focusing on the effect of protists on bacterial communities and rice plant growth will be introduced. Briefly, our research showed that top-down effect of protist predation is one of the most important factors controlling bacterial communities in paddy field soil. Their predation on rhizobacteria enhances nutrient turnover and bacterial activities. We also discovered that protist predation upon rhizobacteria alters endophytic bacterial community composition and enhanced their population, which may results in a better plant growth. In conclusion, we provide a novel insight into underground plant-microbe interactions. Additionally, this results significantly contributes to advancing plant microbiology by providing a new promising tool—protists—to manipulate plant microbiome needed for sustainable and high-yield plant production.

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S-6

Relevance of promoting a resource efficient and a low greenhouse gas emission agriculture model in current and near future agriculture

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Twenty-first century agriculture faces a multitude of challenges, emphasizing that of feeding an increasing population under conditions of global change. Therefore, agriculture must be more productive, while improving the safety and quality of food and reducing its environmental impact. In this context, it is mandatory to increase our efforts on the development/identification of the cultivars/biological markers that enable the required increase in sustainable (in economic and ecologic terms) crop production.

Anthropogenic activities such as land-use change, agriculture and waste management have altered terrestrial biogenic greenhouse gas fluxes, and the resulting increases in greenhouse gas (GHG) emissions in particular can contribute to climate change. In particular, agriculture is a significant source of GHGs emissions to the atmosphere, accounting for ~11% of total global anthropogenic emissions. Within this context, there is an urgent need to develop more sustainable and environmentally friendly crop management protocols.

In terms of promotion of ecofriendly agriculture protocols, optimized irrigation and fertilization management technologies ensure that crops are getting the right amount of water at the right time. Precision agriculture approaches, such as the integration of IoT in the irrigation management systems, are intended to provide the farmers tools for real-time observation, measurement, and responses to variability in crops. They help to reduce the environmental impacts of agriculture practices, but also to increase crop yields and reduce costs (including labour costs), optimizing process inputs and contributing to increase profitability.

In summary the current talk will focus, within a climate change context, on the revision of the novel technological applications of phenotyping, precision agriculture and development of modified plants aiming to increase crop yield in a resource efficient strategy context.

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PS-1-1

Effects of volatile compounds emitted from edible mushrooms shiitake fungi-beds as a Biostimulant on growth and biomass in rice seedlings

Kanga Clever Nkhokwe[1], Okisaka Yui[1], Hanamata Shigeru[1], Mitsui Toshiaki[1], Itoh Kimiko[1]
[1]Graduate School of Science and Technology, Niigata Univ., Niigata

Biostimulants are becoming the best option for achieving sustainable agriculture because of their ability to promote growth, enhance crop yield, quality and tolerance to abiotic stress. Recent studies have reported volatile compounds (VCs) emitted by fungi as having biostimulant effect on plants. However, methods and technologies to culture fungi and extract their VCs are currently complex, but fungi-beds used to cultivate edible mushrooms are readily available and their supply chain is steady and sure. The current study investigated the effect of VCs emitted from Shiitake fungi-beds as a potent novel Biostimulants. Rice seedlings were cultivated in closed containers with non-contact exposure to fungi-beds under *27ºC 13/hours light and 23ºC/11 hours dark* conditions. At 14 days old, rice seedlings exposed to VCs emitted by Shiitake fungi-beds had more pronounced growth and biomass accumulation as compared to control plants. Additionally, the higher the dose of Shiitake fungi-bed the more the increase in growth and biomass of rice seedlings. The positive interaction between the VCs emitted from Shiitake fungi-bed and rice seedling clearly show their potentiality as possible new Biostimulants.

PS-1-2

The Screening Rice (*Oryza sativa* L.) Genotypes Under Effect of Drought and High Temperature Stress

Ermelinda Maria LOPES HORNAI[1,2], Murat AYCAN[3], Toshiaki Mitsui[1,3]
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Future extreme events, like drought and high temperature/heat waves, are predicted to become more frequent and severe due to global climate change. Although high temperature and drought's individual effects on crop development and productivity have been researched, less is known about how these stresses will interact. To test single and multiple stress effects on four rice genotypes, we employed high temperature (35~40 ºC), drought (15% PEG600 and 30% soil moisture), and multiple stress (high-temperature and drought) on rice seed, seedling, and reproductive stages. we set up a hydroponic system in a growth chamber for seedling stage experiments, but we conducted a semi-controlled experiment for reproductive stage tests. Our results showed that among the tested genotypes Niigata University1 (NU1) genotype showed better growth performance, ascorbate peroxidase activity (APX), proline (PRO) content under high temperature, drought, and multiple stress. Especially, the perfect grain number was found almost 60% and yield performance increased by 75% in the NU1 genotype under high-temperature stress. It seems that NU1 genotype has a function to increase osmoprotectant PRO and antioxidant APX activity to overcome the damaging effect of high temperature and drought stress in rice plant.

KEYWORDS: Stress tolerance, NU1, perfect grain, antioxidant,

PS-1-3

Expression study of Type A response regulator genes under different abiotic stress conditions

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Cytokinins are phytohormones involved in adaptation to stress as well as growth in many plant species. A response regulator (RR) is regulatory component in cytokinin signal transduction pathway, and rice genome has 34 putative RR genes which are classified into four subfamilies: Type A, Type B, Type C, and pseudo-response regulator. We herein made a comparative study for gene expression changes in some type A response regulators genes (OsRR6, OsRR9, and OsRR10) under different abiotic stress conditions, namely, 250 mM NaCl (for salinity), 25% PEG (for drought), 0.5% Na_2CO_3 (for alkaline stress), and wounding by inflicting mechanical injury for 0 hr, 1 hr, 6 hr, and 12 hr. In our previous transcriptomics, those 3 genes showed upregulation in the leaves of rice adapted to high temperature and elevated CO_2 stress conditions and acquired tolerance to both stresses. Results show that three RR genes responded to different stresses and upregulated in different intensities. Especially OsRR9 and 10, are almost identical in their amino acid sequences but showed different manner in stress response. Our results provide hints for further functional analysis of the selected genes by making overexpression and loss of function mutants with respect to studied abiotic stresses.

PS-1-4

Characterization of Starch Granules Formed in Endosperm of Rice with Ectopic Overexpression of α-Amylase

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α-Amylase catalyzes the hydrolysis of internal α-1,4-glycosidic linkages of α-glucan polymers such as starch and is known as an enzyme that can directly degrade raw starch that has not been heat-glued. This study aimed to characterize rice endosperm starch in rice with ectopic overexpressing α-amylase. Transgenic rice plants transformed with 35S (cauliflower mosaic virus 35S promoter)::AmyI-1, 35S::AmyII-4, and P10 (10kDa prolamin promoter)::AmyI-1 were grown under normal temperature (23°C, 12 h in the dark/ 26°C12h in the light) and brown grains were harvested. Each brown rice grains exhibited characteristic chalkiness, and electron microanalyzer (EPMA)-SEM images revealed numerous small pits in the starch granules, which may be due to the action of α-amylase enzyme. Fluorescence labeling and capillary electrophoresis analysis of starch chain length distribution showed that there was no significant change in starches of 35S::AmyI-1 and 35S::AmyII-4 transgenic rice, while P10::AmyI-1 transgenic rice showed extremely short (GP2-8) of α-glucan chains in P10::AmyI-1-transgenic rice. Rapid viscoanalyzer analysis (RVA) also showed that the difference in chain length distribution was interpreted as a change in viscosity, indicating that the starch produced by P10::AmyI-1 has novel structural characteristics. Structural analysis of the starch by NMR is on-going in our laboratory.

PS-1-5

Generation of transformants overexpressing *Lotus japonicus CLE* genes for their functional analysis.

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CLE genes are conserved in a variety of plant species, and CLE peptides are known to mediate cell-to-cell or organ-to-organ communications in plants. In *Arabidopsis thaliana*, *AtCLE2* and *AtCLE3* are expressed mainly in roots, and their expression levels are upregulated under photoassimilates-deprived conditions and overexpression of *AtCLE2* or *AtCLE3* results in an increase of sucrose content in roots. These findings propose a model in which roots require photoassimilates to shoots via long-distance mobile CLE peptides (Okamoto et al., 2022). In *Lotus japonicus*, *LjCLE43* was recently found to respond to 24-hour dark treatment. On the other hand, *LjCLE-RS2* and *LjCLE-RS3* are known to be up-regulated in roots by rhizobial inoculation and act as long-distance signaling molecules from roots to shoots to control the number of nodules on roots. Considering that the amino acid sequences of these *Lotus* CLE peptides show high homology with those in AtCLE2 and AtCLE3, there is a possibility that *Lotus* CLE peptides have similar effect to that of AtCLE2 and AtCLE3. To explore this possibility, we generated *L. japonicus* transformants overexpressing theses *LjCLE* genes with using *Agrobacterium*-mediated transformation method. To evaluate the expression level of theses *LjCLE* genes, we conducted real-time PCR analysis with using the T1 lines. As a result, we obtained 2 *LjCLE-RS2*-overexpressing lines , 2 *LjCLE-RS3*-overexpressing lines, and 5 *LjCLE43*-overexpressing lines. Furthermore, recently, we evaluated T2 plants of those *LjCLE*-overexpressing lines by genomic PCR and obtained 1 *LiCLE-RS2*-overexpressing line, 1 *LjCLE-RS3*-overexpressing line and 2 *LjCLE43*-overexpressing lines that probably possess T-DNA constract homozygously.

PS-1-6

Chemo-morphological Characterization Activities of *Pelargonium sidoides* DC (Geraniaceae)

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Pelargonium sidoides is a medicinal plant indigenous to South Africa used for the treatment of tuberculosis and fevers. The aim of this research was to evaluate the variability in traits and correlate different morphotypes with coumarins. Principal Component Analysis and Cluster Analysis were used to evaluate the morphological variability. Morphological characterisation of accessions of *P. sidoides* was done using IPGRI/IITA/BAMBNET list for Bambara groundnut. The PCA revealed that the first three principal components exhibited Eigenvalues greater than 1 and explained 74.170% of the total variability, contributing the entire variable to the morphological variation of the accessions established. Nine groups of the morphotypes were selected and screened for coumarins using Thin Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC). Variation was observed in the coumarins content of morphotypes of P. sidoides. The content of umckalin varied between 13.90 mg/ml and 4.41 mg/ml in the roots of morphotypes and 0.15mg/ml and 3.90 mg/ml in the leaves of morphotypes. The high variation among quantitative characters measured in studied germplasms indicated that, a good possibility exists of finding desirable traits to meet the demands of both researchers and farmers interested in the development of promising cultivars of *P. sidoides.*

KEYWORDS: *Pelargonium sidoides,* morphological variation, TLC, HPLC and coumarin.

PS-1-7

Molecular Weight Control of Lignopolyphenol by Neighboring Group Participation for Improved Biological Activity

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Abstract

Functionalization of lignin requires control of molecular weight and increase of hydroxyl groups. This study showed that molecular weight control by degradation of lignin polymers and control of hydroxyl groups can improve the functionality of lignin. The unique feature of this study is the use of Neighboring Group Participation (NGP) to control the molecular weight. We obtained various types of phenolated lignin (Lignopolyphenol: LP) using the phase separation system. The LP was depolymerized under alkaline conditions, and their molecular weights were controlled intentionally based on the substituent pattern of the phenolic derivatives grafted (Fig. 1).

The introduction of polyhydric phenols resulted in much more hydroxyl groups compared to natural lignin. In addition, the degraded product was found to have much higher long-wavelength UV absorption properties than the original LP. The degradation products obtained by molecular weight control exhibited various biological activities (antioxidant activity, antityrosinase activity) comparable to polyphenols.

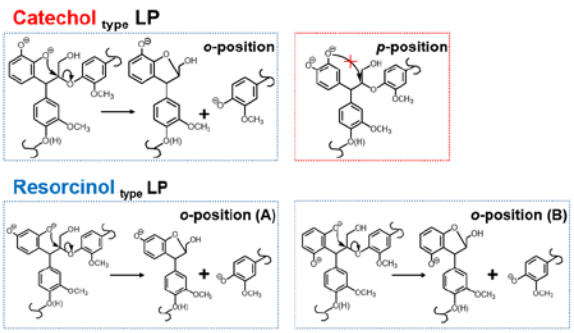

Fig. 1 Degradation of Catechol and Resorcinol type LP by NGP

PS-1-8

Analysis of the physiologic and metabolic response of wheat plants to over-expression of ferredoxin in the chloroplast under changing [CO_2] conditions.

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Ferredoxins (Fds) are a group of small, soluble electron carriers that distribute electrons from PSI to diverse acceptors involved in several metabolic processes. Previous studies pointed out the relevant role of Fds in plant performance under changing environmental conditions. In a context of climate change, in order to investigate the role of Fd on physiologic and metabolic functioning, a wheat wild type (WT) and three transgenic wheat genotypes that over-express Fd in chloroplasts (OexFd1, OexFd3 and OexFd10) were tested in the current study under ambient (415 ppm) and elevated (700 ppm) [CO_2] conditions. Several physiologic and metabolic processes in which Fd plays a crucial role were determined in flag leaf during early grain filling stage (anthesis). Our results provide clear evidence that, under ambient [CO_2] conditions, the overexpression of Fd in the chloroplast increased the photosynthetic rates in transgenic wheat leaves by improving electron transport rate (ETR), which was due to an enhanced actual PSII efficiency as a consequence of an increased photochemical quenching (qP) level. However, under elevated [CO_2] conditions, the increase in photosynthetic activity detected in transgenic leaves (OexFd3 and OexFd10) could not be attributed to the effect of overexpression of Fd on ETR. Under elevated [CO_2] conditions, Fd overexpression didn't induce a significant effect on ATP levels. However, over expression of Fd had a considerable effect on cellular redox metabolism of wheat leaves. Under elevated [CO_2] conditions, the overexpression of Fd increased the level of NADPH in the leaves of the three transgenic lines. Also, in two out of three transgenic plants (OexFd1 and OexFd3), $NADP^+$, malate and oxaloacetate levels increased under elevated [CO_2], putatively through the conversion of pyruvate to malate and oxaloacetate. Under elevated [CO_2] conditions, Fd overexpression might affect alternatively, or in addition, the reducing equivalent regulation reactions, where the malate/oxaloacetate shuttle is involved. Finally, this study showed that there was no considerable effect of Fd overexpression on nitrogen metabolism, reflected in similar amino acids concentrations in flag leaves under elevated [CO_2] conditions.

PS-2-1

Effects of Nitrogen Fertilizers on Top-Down Predatory Protist in Paddy Field Soils

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Protists are one of the most diverse and dominant microbial groups in the soil ecosystem. Protist community composition responds differently to biotic and abiotic factors such as geography, climate, soil water content, pH, effects of other microorganisms, root exudates, and inorganic or organic fertilizers. Among them, nitrogen fertilizer is one of the most important factors affecting protist diversity and community composition. Although various nitrogen fertilizer types are used in paddy fields, still less is known about the effects of different nitrogen fertilizers on protist community composition. In this study, we aimed to reveal the short-term effects of different nitrogen fertilizers (ammonium chloride, potassium nitrate, ammonium nitrate, ammonium sulfate, urea, diammonium hydrogen phosphate, and organic fertilizer) on the composition of protist communities in three soil types with distinct physicochemical properties. The protist community composition was analyzed with a high-throughput sequencing method. The majority of the protists in paddy field soils were identified as predators and decomposers, followed by photoautotrophs and parasites. Taxonomical classification showed that Stramenopiles, Rhizaria, Amoebozoa, and Archaeplastida were dominant in all soil types. N-fertilizers had distinctive effects on taxonomic and functional protist groups. Amoebozoa and Rhizaria were the most affected protist taxonomical groups, while consumers (predators and decomposers) were the main functional groups that were affected by the N-fertilizers. A detailed analysis of the top 20 N-affected protist sequences showed that 65% of them were predators of bacteria. Overall, our results showed that N-fertilizers affect protist communities and functionalities, especially predatory protists. Considering the top-down effect of predators on soil bacterial communities, the change in the predator community can indirectly affect the soil food-web. Our results provide a new perspective on the involvement of nitrogen in the trophic relationships between protists and bacteria.

PS-2-2

Low input of top-dressing nitrogen fertilizer to rice plants positively affects alcohol fermentation in sake brewing using the grains

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Application of nitrogen (N) fertilizers contributes to improved crop productivity. However, the manufacture of N fertilizers requires the burning of fossil fuels to fix atmospheric N_2. In addition, the intensive use of N fertilizers enriches reactive N compounds, leading to soil acidification, water eutrophication, and atmospheric pollution. Thus, to establish a sustainable agricultural system, reducing N fertilizer use is urgently required. In this study, we cultivated rice (*Oryza sativa*) plants with varying dosages of top-dressing N and evaluated the impacts of low-N input on sake fermentation and quality using the grains. Our results provide beneficial information for establishment of ecologically friendly production of rice grains and its-derived products.

Alcohol fermentation by sake yeast (*Saccharomyces cerevisiae*) was enhanced in the sake brewing test using rice grains produced under a low level of N applied as top-dressing fertilizer compared with that using rice grains produced under higher levels of N applied. The use of rice grains from the low-N treatment yielded a higher volume of sake with a higher alcohol concentration and decreased amino acid concentrations. Our analysis data also suggest that varying dosages of N fertilizer applied to rice plants affects the digestibility of grains, as well as the protein content. Mechanism(s) underlying the positive impacts on alcohol fermentation in sake brewing using the grains from the low-N treatment is(are) being further investigated.

PS-2-3

Effect of poultry manure on crop growth

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The effect of poultry manure compost fertilizer on crops was investigated. 28-day-old female chunky strain broilers were fed a diet with dried bonito by-product for 10 days and poultry manure were collected. Compost fertilizer was prepared by mixing poultry manure, rice husks, and rice bran at 30:5:1, respectively. The experiment was also conducted with three types of poultry manure: one with 3% dried bonito flakes mixed in the feed given to the chickens, one with 5% bonito flakes mixed in the feed, and one without bonito flakes mixed in the feed. The dried bonito flakes were added in the hope of increasing the nitrogen content in the poultry manure and changing the composition of the nitrogen component.

These poultry manure compost fertilizers were applied to Edamame and Chinese cabbage. The concentration of dried bonito residue in the diet had little effect on the effect of compost fertilizer on the crop. Compared to chemical fertilizer, the composted chicken manure tended not to secure initial growth. To ensure initial growth, these organic fertilizers are expected to be effective when combined with chemical fertilizers

PS-2-4

Title Anthocyanins and virus accumulation in BYDV-PAV-infected *Brachypodium distachyon*

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Plant viruses induce various disease symptoms on their host plants, thereby leading to yield loss in crops. Barley yellow dwarf virus-PAV (BYDV-PAV) has huge economic importance across continents by infecting cereal plants such as barley and wheat via aphids. BYDV-PAV induces symptoms such as dwarf, leaf yellowing, and reddening, although the mechanisms are largely unknown. In this study, we addressed how BYDV-PAV induced leaf reddening symptoms in *Brachypodium distachyon* by quantifying accumulation of anthocyanins and virus. First, the accumulation of anthocyanins in BYDV-PAV-infected *B. distachyon* was quantified at 14, 21, and 28 days post inoculation (dpi). Anthocyanin levels were significantly increased in red leaves of virus-infected plants compared to healthy leaves of non-infected plants. These results suggested that the significant accumulation of anthocyanins is associated with leaf reddening in BYDV-PAV-infected *B. distachyon*. Next, we quantified virus accumulation in BYDV-PAV-infected plants every 7 days from 14 dpi to 98 dpi in spring by RT-qPCR. In BYDV-PAV-infected plants virus accumulation was significantly increased from 42 to 49 dpi, and gradually decreased by 84 dpi. The viral accumulation peaked at 49 dpi in the second iteration in autumn, suggesting that the temporal dynamism of virus accumulation in *B. distachyon* may not depend on the environmental conditions.

PS-2-5

Single infection of barley yellow dwarf virus-PAV (BYDV-PAV) has negative effects on preference and performance of insect vector compared to co-infection with the relative virus species.

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Plants frequently interact with multiple viruses simultaneously. In recent years, increased attention has focused on the tripartite interactions of plant, insect-borne virus, and insect vector. Virus acquisition alters the host selection of its insect vectors for virus spread. We have shown that barley yellow dwarf virus-PAV (BYDV-PAV) manipulates the host selection behavior of its vector aphid *Rhopalosiphum padi*. BYDV-PAV single-infection on wheat reduces the attraction of viruliferous aphids compared to non-infection and co-infection with cereal yellow dwarf virus-RPS (CYDV-RPS). In this study, we explored the impacts of two virus species on aphid preferences to single- and co-infected host plants. Viruliferous aphids carrying CYDV-RPS significantly preferred co-infected plants compared to BYDV-PAV single-infected and non-infected plants. We next investigated how virus single- and co-infection on host plants affect physiological traits of the nonviruliferous vector insects. Aphids weighed less on the single-infected relative to non-infected plants, whereas body size had no significant difference between the treatments. These results indicate that the virus infection on host plants affected vector growth, thereby leading viruliferous aphids to prefer co-infected plants for spreading multiple viruses.

PS-2-6

Evolution of plantago asiatica mosaic virus through long-term passages in *Nicotiana* plants and its impact on host adaptation

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Many plant viruses that are considered as a major constraint for crop production frequently infect perennial wild plants. Generally, viruses in wild plant communities have high intraspecific genetic diversity compared to those isolated from cultivated crops. Plantago asiatica mosaic virus (PlAMV), a destructive pathogen causing necrosis symptoms in *Lilium* crops, naturally infects many wild plant species. PlAMV isolates infecting wild plants are genetically more diverse than those from ornamental lilies. A green fluorescent protein (GFP)-tagged full-length cDNA clone of PlAMV-Vi (PlAMV-Vi$_{GFP}$) infects systemically *Nicotiana benthamiana* without apparent symptoms. In this study, we investigated the evolution and host adaptation of PlAMV-Vi$_{GFP}$ in *N. benthamiana* through long-term serial passages. Serial passaging of the virus in *N. benthamiana* by mechanical inoculation resulted in the appearance of the new virus line inducing leaf crinkle symptoms and 2 weeks earlier accumulation in non-inoculated upper leaves. The 14th passaged line of asymptomatic PlAMV-Vi$_{GFP}$ accumulated to non-inoculated leaves 1 week earlier than the original virus. These results suggested that the new virus lines improved replication and/or movement for host adaptation. Compared with the original PlAMV-Vi$_{GFP}$, the passaged leaf crinkle line had mutational differences with 12 nucleotide changes, resulting in the 5 amino acid changes scattered through the viral genome. Consequently, long-term serial passaging of asymptomatic PlAMV in a single plant host species resulted in increased virulence and host adaptation. This long-term passaging experimental system can be applied to *Lilium* crops and wild plants for further virulence evolution analysis.

PS-2-7

The exposure of maize seedlings to weed volatiles affects their growth and seed quality

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Plants exposed to volatiles emitted from damaged conspecific or heterospecific plants exhibit increased resistance to herbivorous insects. We examined whether volatiles from artificially damaged weeds can be used in maize cultivation by affecting their resistance, growth and reproduction. Seven days after germination, maize seedlings were exposed to volatiles emitted by artificially damaged mugwort or tall goldenrod plants either separately, or as a mixture of the two, for seven days. Treated and control (unexposed) seedlings were cultivated in an experimental field without any insecticides applied. Plants exposed to either of the three volatile treatments sustained significantly less damage than controls. Seedlings exposed to either goldenrod or mixed volatiles produced more leaves and tillers than control plants. Furthermore, a significant increase in the number of ears was observed in plants exposed to the volatile mixture. In all treated plants, ear sugar content was significantly higher than that in the controls. Further, we cultivated seedlings that were either exposed to the volatile mixture or unexposed, under the conventional farming method using pesticides. Similar significant differences were observed for sugar content, number of tillers, leaves, damaged leaves, and ears. In the laboratory experiments, a significant reduction in the growth of common armyworm larvae was observed when maize plants were exposed to the volatile mixture. In addition, cucumber mosaic virus (CMV) infection was also suppressed. Exposure to the volatile mixture increased salicylic acid content in the seedlings, although the amount of jasmonic acid was not affected. Our results suggest that seedling exposure to the weed volatiles can increase the seed quality and resistance against herbivores and pathogens of maize plants.

PS-2-8

Peanut photosynthesis response to drought can include diffusive and biochemical limitations depending on cultivar

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Photosynthesis is one of the key processes to be affected by water deficit via decreased CO_2 diffusion and metabolic constraints. However, there is still no unified consensus of the weight of each mechanism on the drought response. This research assessed the underlying limitations to photosynthesis in nine peanut (*Arachis hypogaea* L.) genotypes grown in pots under progressive drought. In order to test performance of genotypes, growth, transpiration per plant, gas exchange measurements (leaf and canopy level), chlorophyll florescence and A-C_i response curves were analyzed under drought and well water conditions. In general, drought first affected photosynthesis (at leaf and canopy level) by stomatal closure and then by chlorophyll florescence in all genotypes but at different rates. Maximum rate of carboxylation (V_{cmax}) and the maximum rate of electron transport (J_{max}) were not affected during the onset of the drought but were at the end with the exception of some drought resistant cultivars. Nevertheless, V_{cmax} decreased more accutely than J_{max} on these cultivars, suggesting than Rubisco carboxylation was more affected than RuBP regeneration. When taken together, and with transpiration per plant data, different drought responses were observed differentiating water spender and water saver drought tolerant genotypes and drought sensitive ones. Findings presented here highlight the importance of genetic variation in photosynthetic peanut response to drought which should be considered when breeding for future climates.

PS-2-9

Target role of stomatal aperture on leaf metabolism and growth of rice (*Oryza sativa* L.) under ambient and elevated [CO_2]

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Stomatal opening (g_s) is a target factor regulating CO_2 uptake and water evaporation, and consequently crop performance in terrestrial ecosystems. Further, according to previous studies, among other factors, stomatal opening has been described to be involved in the photosynthetic acclimation process of plants under elevated [CO_2]. However, despite of its relevancy, little is known about the impact of different stomatal aperture ranges on rice leaf physiology and metabolism. In this study, we provide a physiological and proteomic characterization of leaves in rice (*Oryza sativa* L.) plants grown under ambient (415 ppm) and elevated (700 ppm) [CO_2]. For this purpose, rice cultivars with different stomatal opening such as IR64, Taichung 65 and SLAC1-deficient mutant (with limited capability to regulate stomatal opening) were characterized.

The current study showed that the plants with the lowest g_s values (IR64), were the ones in which the exposure to 700 ppm showed the higher photosynthetic down-regulation linked with an impaired N metabolism. In the other hand, Taichung 65 plants showed a higher responsiveness to [CO_2], being capable to double their biomass thanks to the up-regulation of several proteins linked with light capture/management and CO_2 diffusion. Finally, in case of SLAC1 mutants, regardless [CO_2], those were the plants that showed the highest g_s values, together with the highest transpiration rates. Moreover, those plants showed a remarkable diminishment of water transport aquaporins and the overexpression of stress related proteins, that revealed the deleterious impact of un-controlled stomatal opening on plant physiology and growth. In summary, the current study showed that, regulation of stomatal opening is a target process with a potential double effect (positive or negative) that has an important impact on leaf metabolism and responsiveness to changing [CO_2].

PS-2-10 Impact of biostimulants on wheat plants grown under climate change conditions

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The use of biostimulants in agriculture is a promising approach to promote crop growth in a sustainable way. Further, biostimulants have been described to enhance the productivity, resilience and development of crops in stressful growth conditions. In this study, 4 different high yielding bread wheat (*Triticum aestivum* L.) genotypes were grown under different [CO2] (415 *versus* 800 ppm) and water availability (fully irrigated *versus* irrigated at 50% of full irrigation) conditions. Inoculation with biostimulants, provided by Atens S.L. (Tarragona, Spain), was carried out in half of the plants prior to germination. For this purpose, seeds were immersed in solution containing a plant growth promoter microorganisms association including 3 different species such as *Bacillus megaterium*, *Rhizoglomus irregular* and *Trichoderma koningii*. Crop biomass, gas exchange, leaf protein and nitrogen concentration analyses were carried out when the plant reached heading stage. Obtained results showed significant genotypic differences on growth, physiologic and metabolic traits. Biostimulant application tended to increase photosynthetic activity and plant growth, such effect was especially remarkable under ambient [CO2] and waters stress conditions. Further, significant differences in crop biomass and CO2 assimilation were found mainly in the water availability and the [CO2] treatments. Also, significant differences in carbon-nitrogen ratio and protein concentration were found under drought and elevated [CO2] treatments. Leaf protein values differed among the genotypes and the different [CO2] and water availability conditions. These data show the relevance of a deeper study of the overall effects of the biostimulant applications, the mechanisms that promote plant-microorganism association and its effects in the plant alongside the factor of water stress and high [CO2].

PS-2-11 Hyperspectral devices applied to the determination of alfalfa nutritional quality traits

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Alfalfa (*Medicago sativa* L.) alfalfa is a key crop in cattle feed. The nutritional quality of this plant is usually analysed by time and cost demanding laboratory chemical methods. Within this context, near-infrared (NIRs) reflectance spectroscopy has been broadly applied in forage crop nutritional characterization. However, high resolution spectroradiometer (350 nm – 2500 nm) has been less explored in alfalfa, particularly when assessed directly in the field. The main objective of this study is to estimate quality parameters in commercial alfalfa plants (grown in different field trials and environmental conditions) using a full range spectroradiometer. Reflectance spectra were recorded with a portable spectroradiometer (FieldSpec 4) at canopy and leaf levels. Such spectra were used for the development of models aiming to predict biomass production, leaf pigments, sugars, protein and mineral content. Climatic conditions and phenology stages were included as predictors into the models. Our results showed that climatic variables and phenology increase the accuracy up to 14% in the models. The best prediction models applied in test sets, according the variance explained (R^2), were obtained for biomass (0.74), sucrose (0.68), flavonoids (0.62), protein (0.60), nitrogen (0.64), zinc (0.53) and phosphorus (0.52); with normalized root mean squared errors between 0.09 and 0.30. In conclusion, the use of high-resolution spectral methods at field conditions might be a base for a rapid screening to evaluate quality parameters in alfalfa.

PS-3-1

Development of Liquid Fertilizer Using Recovered Phosphorus from Sewage Sludge Ash and Application to Cultivation of Crop (Japanese mustard spinach)

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Recently, the demand of fertilizers is increasing due to increase of the food consumption by population growth in developing countries and the use of biofuel energy. However, phosphorus resources are distributed at limited regions in few countries. Therefore, securing domestic phosphorus resource in Japan is very important. We developed the new recovery process of phosphorus from sewage sludge ash, and the process is consisted of both acid and alkaline elution and precipitation steps, namely “Two-step Elution method”. In this study, we have performed the cultivation of crop by using the final precipitation from sewage sludge with adding acids (hydrochloric acid and sulfuric acid) as liquid fertilizer. The purpose of this work is to develop an efficient process for recovering phosphorus (P) from sewage sludge ash, and to investigate the usability of final precipitation as liquid fertilizer.

After recovering P as hydroxyapatite ($Ca_5(PO_4)_3(OH)$) from sewage sludge ash, the synthesis of liquid fertilizer was performed. In the synthesis of liquid fertilizer, the supply of nitrogen (N) and potassium (K) are conducted by adding reagents such as $NaNO_3$, $(NH_4)_2SO_4$ and KOH. Their molar ratio of the fertilizer is N:K:P=10:10:8. In addition, iron (Fe) was added with $FeSO_4$ along with EDTANa. After that, pH was adjusted to 6-7 by using NaOH. Furthermore, the essential trace element was also added. Then the liquid fertilizer was diluted based on the concentration of K in Hawkland nutrient medium, The crop used in this work is Japanese mustard spinach: “Komatsuna”. A plant environmental control system (LPH-220N, Nippon Medical & Chemical Instruments Co. LTD, Japan) was used to cultivate Japanese mustard spinach from the seed.

Consequently, the following results were mainly obtained: (1) The final precipitate recovered from sewage sludge ash by Two-step Elution method contains high contents of phosphorus (more than 80% recovery) and low contents of heavy metals. (2) The final precipitate mainly consists of hydroxyapatite ($Ca_5(PO_4)_3(OH)$) and can be effectively used as fertilizer. (3) From the application to cultivation of Japanese mustard spinach, the fertilization effect was clearly found. Particularly, the effect was remarkable when preparing liquid fertilizer with adding hydrochloric acid.

PS-3-2

Effect of nitrogen fertilization on the percentage of soybean rhizobia carrying *hupS* and *hupL* genes.

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Soybean root nodules are known to change their infection numbers, development, and nitrogen fixation in plant roots depending on the nitrogen concentration in the rhizosphere. There are two types of soybean rhizobacteria: Hup^+ strains, which can recover hydrogen generated during nitrogen fixation, and Hup^- strains, which cannot recover hydrogen. It has been reported that Hup^+ nodules can increase the growth and yield of soybean.

In this study, we investigated the effects of different amounts of nitrogen fertilizer on the total dry weight of soybean plants and the number of rhizoids, as well as the saprophytic ratio of Hup^+ and Hup^- strains when grown in the Igarashi sandy field at Niigata University. The figure of a plant changed when nitrogen fertilizer was increased in soybean cultivation. The proportion of nodules carrying both *hupS* and *hupL* genes decreased with increasing nitrogen fertilizer application. This suggests that the nitrogen nutrient status of the soil changes the strain of soybean nodules.

PS-3-3

Conservation Agriculture to preserve soils and to improve plant growth

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To preserve soil in various climate, Conservation Agriculture (CA) is spread widely in the world. CA has three main principles as follows; 1) Minimum or no tillage, 2) Organic multi to prevent erosion and evaporation, and 3) Variety crop rotation. In this study, we compared soil properties and yield on three plots, set respectively for conservation agriculture (CA), organic farming (OF), and conventional cultivation (CC). The experiment fields are in Japan (Ikarashi campus, Niigata Univ., sandy unripe soil) and Togo (Zio, Agbelouve, clay soil). In Japan, we set up three plots (CA, OF, CC). In Togo, we set up two plots (CA, CC). The prototypes were soybean (*Glycine max*), corn (*Zea mays*), and mini-tomato *(Solanum lycopersicum* var. *cerasiforme*). Only soybean and corn are planted in Togo. In Niigata, tomato yield at the CA plot has 8 times higher than OF. At the CA plot in Togo, the yield of corn was 1.4 times higher than OF plot. This yield was 2 times higher than the average of this area from 2015 to 2020 in Togo. Practicing conservation agriculture contributed to reducing soil hardness, decreasing hydraulic conductivity, and improving crop yield. However, the adoption of this practice is still low in Japan, and non-existent in Togo.

PS-3-4

Microplastic Contamination on Turkish Arable Land due to Sewage Sludge Application

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Traditionally, studies of microplastic contamination have focused on the aquatic environment; however, recent research has identified the significance of microplastics in terrestrial ecosystems. Sewage sludge is often used as an agricultural fertilizer, with approximately 50% of sludge being recycled in this way in Europe and North America. The application of sewage sludge to farmland has been theoretically estimated as one of the largest sources of microplastics in the environment. In this study, soil samples were taken from 0-5 cm depth in a field in the Konya region of Turkiye, where sewage sludge was used as a fertilizer. It was determined that the texture of the soil was clayey, the organic matter level was moderate, and the soil was non-saline and neutral. Sewage sludge was applied to the soil at 50 kg ha^{-1} for one year growing period. NaCl (1.2 g cm^{-3}) solution was used to extract microplastics in the soil sample. The number of microplastic particles in the soil where sewage sludge was applied was 347 particles kg-$soil^{-1}$. The two dominant forms of the sample were “fiber” and “fragment”. The mean size of fiber-type microplastics was 0.41±0.05 mm. The mean size of fragment-type microplastics was 0.64±0.17 mm. After this step, Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR) was used to investigate the substances of the microplastics in the sewage sludge applied, and it was found that the dominant polymer type was polyethylene (PE). As a result, these findings confirm that using sewage sludge in agriculture leads to a significant microplastic accumulation in the soil. In Japan, the national and prefectural governments have developed their own new varieties of rice, and there are more than 300 varieties. Despite the large number of varieties, several new varieties of rice are introduced to the market every year. In order for new brands of rice to survive market competition, it is necessary to further clarify the value of each brand more than ever before.

PS-3-5

Determination of Atmospheric Greenhouse Gases Using UAV and Soil Fluxes Capturing with Ground Survey

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The increasing concentration of CO_2 in the atmosphere, primarily due to human activities, has led to extreme weather conditions, posing a threat to food security and human needs. Despite many efforts and initiatives to understand and mitigate carbon emissions, inaccuracies in understanding the patterns of emission sources, accumulation, and distribution remain. Our project is targeted for understanding the carbon cycle and its implications for climate change, and for developing effective strategies to mitigate its impact on the environment. This study evaluates the feasibility of using unmanned aerial vehicles (UAVs) and ground-based gas chambers to measure the impact of agriculture on climate change. The first experiment involves measuring atmospheric carbon dioxide using a UAV equipped with a sensor, sample collection pump, and GPS module. The second experiment uses gas chambers to analyze carbon dioxide emissions from soil and assess the effects of different fertilization techniques on soil gas flux. The atmospheric carbon dioxide measurements using the UAV showed seasonal fluctuations, with the lowest CO_2 value recorded in August at 412.5 ppm and the highest level in May at 417.7 ppm. The variability in the content of carbon dioxide was found to be up to 5.2 ppm. The soil gas flux chamber-based system showed that the plot under compost fertilizer had the highest activity, with CO_2 emissions of 4.73 μmol $m^{-2}s^{-1}$, while the deep fertilizer placement test plot had the lowest CO_2 emissions of 2.02 μmol $m^{-2}s^{-1}$. Results show that vegetation coverage has a significant impact on atmospheric carbon dioxide levels, and various fertilization types have different effects on soil gas flux.

PS-3-6

Synthesis of chitosan based new materials for adsorption of heavy metal

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In this research work, a new type of adsorbent bead material was obtained using chitosan and kaolin. The kaolin was produced from the clay of the "Tsogt-Ovoo" deposit in the Umnogovi province of Mongolia. The kaolin was treated with acid and heat to remove its mechanical impurities from the clay. The mixtures of chitosan and kaolin were taken with various ratios (8:1, 8:2, and 8:3) for chemical processing to obtain a chitosan-based adsorbent bead. It was determined that the adsorption capacity of the adsorbent bead was the highest for chromium ion (Cr(VI)) when the chitosan and kaolin mass ratio 8:2. The adsorption properties of Cr(VI) were studied depending on the pH of the solution, time, temperature, initial concentration of chromium ion solution, and mass of adsorbent material. The adsorption efficiency was the highest (80.25%) when the initial concentration of the chromium ion was 50 mg/l at the following condition; pH=3, temperature 25 ^oC, time 3 hours, and the mass of the adsorbent material 0.1 g. It shows that the chitosan-based adsorbent bead can be used for the removal of chromium (Cr(VI)) from aqueous solutions.

PS-3-7

Fabrication and characterization of low-cost ceramic membrane developed with cross-linked chitosan for ultrafiltration of Cr(VI)

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Water pollution has become one of the major problems in recent years. In particular, heavy metals in water bodies are causing concern due to their high toxicity even at low concentrations. Membrane filtration technologies for removal of heavy metals have attracted great attention. This work presents the preparation of a ceramic membrane from ultrafine raw starting materials and the development of a chitosan top layer. The ceramic membranes were sintered at temperatures ranging from 950 to 1150°C for 1 and 3 hours. Various factors including pure water flux, water absorption and compressive strength were examined to determine the membrane performance. Additionally, cross-linked chitosan is fabricated as an adsorptive layer for the removal of heavy metal. Thereby, the prepared composite membrane can be used both adsorbent and separator for heavy metals in the single step process with good removal efficiency, flux, uniform structure, excellent reusability, chemical and mechanical durability. Thus, this study promised that the ceramic membrane layered with chitosan can be an effective, environmentally friendly and broad application for the treatment of heavy metals and sewage purification.

PS-3-8

Effect of chelating agents (EDTA, NTA) on phytoremediation of Pb-contaminated soil by *Helianthus Annuus*

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Heavy metal contamination of soil is typically brought on by air pollution or by contamination of river systems, and it is difficult to remove heavy metals from polluted soil. Exhaust emissions of leaded gasoline are significant contributors to Pb soil contamination around highways. Nonferrous metals, fertilizers, chemical herbicides, insecticides, and sewage sludge are additional sources of Pb contamination. As the absorbance of heavy metals causes serious harm to humans, plants, and animals, there is an urgency to find the best solution to soil contamination. In this study, phytoremediation by *Helianthus Annuus* (*H. Annuus*) was adopted to remediate Pb-contaminated soil. However, successful phytoextraction requires metal hyperaccumulation to the above-ground portion of the plants for harvest, although this is limited by metal bioavailability. Hence, we propose the addition of chelating agents, specifically ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA) to increase the solubility of heavy metals in soil and to improve the efficiency of phytoremediation. The concentration of chelating agents was varied to search for the most optimal condition. The content of Pb was determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Translocation Factor (TF) value was calculated to determine the ability of plants to transport heavy metal from the roots to the shoots.

PS-3-9

Removal of Cadmium in Soil by Phytoremediation Using *Helianthus Annuus* & *Marigold* and by Washing Method Using Ferric Chloride

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In this study, two remediation methods such as phytoremediation and washing method using chemical solvent were used to investigate inexpensive and efficient methods for removing cadmium (Cd) from soil. In phytoremediation, *Helianthus Annuus* and *Marigold* were used as remediation plants. Chelating agents (EDTA and NTA) were also used to assist the absorption capacity of Cd by the plants. Cd solution and chelating agents were added to the soil and the plants were grown in an environmental control system. After harvest, the biomass was measured and the uptake of Cd in the shoots and roots was determined by ICP-AES. In the washing method, ferric chloride ($FeCl_3$) was used as a chemical solvent, and paddy soil was employed. After the soil and $FeCl_3$ were stirred at prescribed time, the concentration of Cd in liquid portion was determined by ICP-AES. Consequently, the following results were mainly obtained: (1) In *Helianthus Annuus*, the amount of Cd accumulation was higher in the roots than in the shoots. Also, NTA was more effective than EDTA. (2) In *Marigold,* the amount of Cd accumulation was higher in the shoots than in the roots. (3) Optimal conditions for removing Cd in paddy soil by $FeCl_3$ were as follows: the concentration of $FeCl_3$ was 1.2 M, solid-liquid ratio was 1.0 g :20 mL, shaking time was 1 hour, and shaking temperature was 298 K.

PS-4-1

Extremely Long Chains of Magnetic Particles via Large Plastic Beads Dispersed in Magnetic Elastomers

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Magnetic elastomer is one of stimuli-responsive soft materials, whose elasticity increases when a magnetic field is applied. We have studied thus far the methodologies for amplifying the magnetorheological effect of magnetic elastomers. It was found that bimodal magnetic elastomers consisting of magnetic and nonmagnetic particles showed a significant magnetorheological effect compared to monomodal magnetic elastomers. In this study, the relationship between the magnetorheology of bimodal magnetic elastomers with high concentrations (60 vol.%) of plastic beads with diameters of 8 μm or 200 μm and the meso-structure of the particles was investigated. Dynamic viscoelasticity measurements revealed that the change in storage modulus of the bimodal elastomer with 200 μm beads was 2.8×10^5 Pa at a magnetic field of 370 mT. The change in the storage modulus for monomodal elastomer without beads was 4.9×10^4 Pa. The bimodal elastomer with 8 μm beads hardly responded to the magnetic field. In-situ observation for the particle morphology was carried out using synchrotron X-ray computed tomography. For the bimodal elastomer with 200 μm beads, a highly aligned structure of magnetic particles was observed in the gaps between the beads when the magnetic field was applied. On the other hand, for the bimodal elastomer with 8 μm beads, no chain structure of magnetic particles was observed. It was found that the addition of beads with a diameter of 200 μm linked the chains of magnetic particles, while beads with a diameter of 8 μm prevented the chain formation of the magnetic particles.

PS-4-2

Research on the Structure of Rice Brand Value
- A Case Study of "Koshihikari" and "Koshihikari Niigata University NU1" -

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1. Background of the study

In Japan, the national and prefectural governments have developed their own new varieties of rice, and there are more than 300 varieties. Despite the large number of varieties, several new varieties of rice are introduced to the market every year. In order for new brands of rice to survive market competition, it is necessary to further clarify the value of each brand more than ever before.

2. Outline of this study

This study presents the structure of rice brand value. The subjects are (1) "Koshihikari," the most widely produced variety in Japan as a maturity brand, and (2) "Koshihikari Niigata University NU No. 1," a new variety with high temperature and high CO2 tolerance as a budding brand. This study was conducted through interviews with (1) suppliers, (2) consumers, and (3) BIT (brand incubation third parties). As a result, a total of 1136 value-related terms were extracted for Koshihikari and 433 for NU1. The extracted words were structured as brand values based on the definition of brand value given by Wada et al. (2021). In addition, values common to all three were extracted as core values. The brand values indicated by the results of this survey are as follows. Values common to both NU1 and Koshihikari include the ideational value "Niigata" and "best," and the sensory value "delicious," "sweet," and "luster". The characteristic values of Koshihikari are: ideational value "Niigata," "Uonuma," "high quality," "best," and relational value "famous," "standard" etc. The characteristic values of NU1 include the ideational value "Koshihikari" and "new," and the relational value "cooperation," "support," and "expectation" etc.

3.reference

Wada et al (2021), *Brand Incubation Strategy: Brand value co-creation by leveraging the power of the third*, Yuhikaku Publishing