

ABSTRACT

AGRICULTURAL BIOTECHNOLOGY CHALLENGES AND OPPORTUNITIES



MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT
VIETNAM NATIONAL UNIVERSITY OF AGRICULTURE



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HANOI, 12/2021

PREFACE

The World is facing many difficulties due to the COVID-19 epidemic, the general economic downturn, Vietnam also has overcome many difficulties and achieved the "dual goal" directed by the Vietnamese Government and the Prime Minister: Drastically prevent and combat the epidemic with the spirit of "fighting the epidemic like fighting the enemy", while focusing on socio-economic recovery and development, ensuring people's lives. In this success, science and technology made important contributions when Vietnam's economy grew positively by 2.91% in 2020, being one of the fastest growing countries in the region and in over the World.

On November 20, 2021, President Nguyen Xuan Phuc attended the celebration of Vietnam Teacher's Day and the opening of the school year 2021-2022 of VNUA, affirming that "VNUA is also very active in activities, such as scientific research, technology transfer with many valuable research achievements applied to daily life production, contributing to improving the productivity, quality and value of agricultural products, gradually forming the depicting farmers of the digital transformation era, creating a mark and brand for Vietnamese agricultural products on the World". President Nguyen Xuan Phuc also emphasized "What starts here (VNUA) will change agriculture and rural areas of Vietnam".

The Fourth Industrial Revolution, 4IR, or Industry 4.0 has created many breakthroughs in new technologies in fields such as AI artificial intelligence production, robot manufacturing, internet network development, 3D printing technology, nanotechnology, biotechnology, science, materials science, energy storage and informatics. Biotechnology in general, and agricultural biotechnology in the fields of veterinary medicine, plants, microorganisms, environmental protection and human health care are facing great challenges, especially in the impacts of the Covid 19 pandemic, such as the vaccine race, at the same time, there are opportunities to affirm and develop a key role in the development of sustainable agro-ecosystems./.

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ORGANIZATION BOARD

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**THE 03rd SYMPOSIUM
AGRICULTURAL BIOTECHNOLOGY: CHALLENGES AND OPPORTUNITIES**

1. Date: 18th December 2021 (Saturday, from 7:30-17:00, GMT +7)

2. Place: Offline at PH-1 meeting room (VNUA) and Online via Zoom Meeting

3. Agenda

Time	Subject	Person-in-charge
07:30-07:50	Join Zoom meeting	Organization and participants
07:50-08:00	Introduction of participants	Dr. Nguyen Thi Thuy Hanh Dr. Pham Thi Dung MC of symposium Faculty of Biotechnology, VNUA
08:00-08:10	Opening speech	Prof. Dr. Nguyen Thi Lan President Vietnam National University of Agriculture (VNUA)
MAIN SESSION		
Session 1: Policies on development of biotechnology and research trends in agricultural biotechnology Chairperson: Dr. Nguyen Thi Thuy Hanh Dr. Pham Thi Dung		
08:10-08:20	Summary report on Agricultural Biotechnology	Dr. Nguyen Xuan Cuong Former Minister Ministry of Agriculture and Rural Development (MARD) Senior advisor of VNUA
08:20-08:40	Program to develop bio-industry in agriculture to 2030	Dr. Pham Hong Hien Deputy Director, Department of Science and International Cooperation (DOSI) Vietnam Academy of Agricultural Sciences (VAAS)
08:40-09:00	Characterization of CRISPR/Cas9-induced knockout mutations in G β subunit gene in tomato	Dr. Ninh Thi Thao Lecturer, Researcher Excellent research group: Applied Biotechnology Faculty of Biotechnology Vietnam National University of Agriculture
09:00-09:20	Analysis of mitochondrial DNA and Y chromosome in Vietnamese population	Dr. Nguyen Thuy Duong Leader Human Genomics Laboratory Institute of Genomic Research
09:20-09:40	Genetic diversity of underutilized leafy <i>Amaranthus</i> genetic resource	Dr. Ken Hoshikawa Scientist, Researcher Biological Resources and Post-harvest Division Japan International Research Center for Agricultural Sciences (JIRCAS) Genetic Resources Headquarters, The World Vegetable Center

Symposium “**Agricultural Biotechnology: Challenges and Opportunities**”

09:40-10:00	Membrane phospholipids as internal phosphate reserve of plant cells to cope with phosphate starvation	Dr. Ngo Hai Anh Research Scholar, Institute of Plant and Microbial Biology, Academia Sinica, Taiwan
10:00-10:20	Plant breeding at the speed of light: the power of Genome Editing Technology	Dr. Yao Luo Manager of breeding technology Lark Seeds International
10:20-10:40	Relieve stress for better crop production	Dr. Yueh Cho Postdoctoral Fellowship, Institute of Plant and Microbial Biology, Academia Sinica, Taiwan
10:40-11:00	Application of barcoded DNA in identification of fruit varieties in Vietnam	Dr. Do Tan Khang Head of Department, Molecular Biotechnology Biotechnology Research and Development Institute Can Tho University
11:00-11:20	Application of biotechnology on the treatment of agricultural solid waste	Dr. Tran Thi Ngoc Bich Vice Director, Institute of Environmental Science and Technology Tra Vinh University
11:20-11:40	Application of biotechnology in plant breeding	Dr. Trinh Ngoc Ai Vice Dean, School of Agriculture and Aquaculture Tra Vinh University
11:40 -12:00	General discussion	Dr. Nguyen Thi Thuy Hanh Dr. Pham Thi Dung
12:00-13:00	Lunch Time	
13:10-13:30	Join Zoom meeting	Organization and participants
Session 2: Application of biotechnology in agriculture		
Chairperson: Assoc. Prof. Dr. Dong Huy Gioi Dr. Dinh Truong Son		
13:30-13:45	Improved bacterial leaf blight disease resistance in the major elite Vietnamese rice cultivar TBR225 using CRISPR/Cas9 system	Dr. Nguyen Duy Phuong Head of Department Molecular Pathology Department Agriculture Genetics Institute
13:45-14:00	Establishment of the digital tools for precious agriculture by machine learning	Dr. Chu Duc Ha Research, Lecturer Faculty of Agricultural Technology (FAT) University of Engineering and Technology (UET) Vietnam National University Hanoi (VNU)
14:00-14:15	Study on in vitro culture of triploid hemerocallis (<i>Hemerocallis spp</i>) like K1	Dr. Nguyen Xuan Truong Director Institute of Agro-biology Vietnam National University of Agriculture
14:15-14:30	The Influence of Conditions on the Antibacterial Properties of	Dr. Le Thanh Huyen Head of Department

Symposium “**Agricultural Biotechnology: Challenges and Opportunities**”

	<i>Ganoderma aff. brownii</i> , <i>Ganoderma sinense</i> , and <i>Lentinus sajour-caju</i>	Dept. Toxicology and Environmental Monitoring, Faculty of Environment, Hanoi University of Natural Resources and Environment Collaboration with Strong research group: Edible and Medical Mushrooms
14:30-14:45	The role of biotechnology in plant protection in ensuring food security and sustainable agriculture in Vietnam	Dr. Trinh Xuan Hoat Deputy Director General Plant Protection Research Institute (PPRI)
14: 45-15:00	Tea-break (Video introduction of VNUA)	
15:00-15:15	Genetic Analysis of Rice Blast Disease in the North of Vietnam	Dr. Nguyen Thi Thuy Hanh Vice Dean Faculty of Biotechnology Vietnam National University of Agriculture
15:15-15:30	Agriculture in climate change scenarios: The dawn of microalgae biotechnology in Vietnam	Assoc. Prof. Nguyen Duc Bach Leader of research team: Application of microalgae biotechnology and exploitation of biologically active natural compounds Faculty of Biotechnology Vietnam National University of Agriculture
15:30-15:45	Application of Nanobiotechnology on plant tissue culture	Dr. Bui Thi Thu Huong Lecturer, researcher Member of strong research group: Nano Biotechnology and Recombinant Gene- Protein Technology Faculty of Biotechnology Vietnam National University of Agriculture
15:45-16:00	Engineering bacterial leaf blight resistant rice using genome editing	Dr. Luu Thi Van Rice team group leader Institute for Molecular Physiology Heinrich-Heine University of Düsseldorf Universitätsstraße 1, Germany
16:00-16: 15	Greenhouse and field cassava yield can be altered by different isolates of an agriculturally-relevant fungal symbiont	Dr. Erica McGale Postdoc Fellowship Department of Ecology and Evolution, University of Lausanne
16:15-16:45	General discussion	Assoc. Prof. Dr. Dong Huy Gioi Dr. Dinh Truong Son
16:45-17:00	Closing	Associate. Prof. Nguyen Xuan Canh Dean Faculty of Biotechnology Vietnam National University of Agriculture

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SUMMARY REPORT ON AGRICULTURAL BIOTECHNOLOGY

Nguyen Xuan Cuong



Dr. NGUYEN XUAN CUONG

Former Minister

Ministry of Agriculture and Rural Development (MARD)

Senior advisor

Vietnam National University of Agriculture

THE ROLE OF BIOTECHNOLOGY IN PLANT PROTECTION IN ENSURING FOOD SECURITY AND SUSTAINABLE AGRICULTURE IN VIETNAM

Trinh Xuan Hoat

Plant Protection Research Institute of Vietnam

Abstract

Food security and sustainable agriculture have become one of the most important issues worldwide in general and in Vietnam in particular especially for a changing global climate. Application modern agricultural biotechnology is considered to be one of the most promising developments in modern science and as the outstanding scientific achievements as well as economic impacts, positive society, and environment that technology brings to farmers, consumers, and communities in Vietnam in the last many years. Agricultural biotechnology cannot address all the emerging problems related to agricultural production but it has a promising potential to solve specific issues including (i) producing rapid detection kit for plant pathogens, (ii) developing biofertilizers for improvement of soil fertility, (iii) producing bio-pesticides for environmentally friendly approaches for effective and sustainable management of plant disease and insect pests, (iv) development of crop varieties having good quality and resistant/tolerant to pests, and environmental stresses, (iv) increasing crop productivity, diversifying crops, improving the nutritional value of food, reducing environmental impacts on agricultural production and enhance the durability of products during harvesting, shipping and promoting market competitiveness. To increase the competitiveness in the international economic integration, it would ensure that Vietnamese farmers should be encouraged to have access to high-quality training and education in agricultural production in general and in the field of plant protection, in particular, supporting farmers continue to produce safe food for domestic consumptions and export.

PERSONAL INFORMATION OF PRESENTER



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ANALYSIS OF MITOCHONDRIAL DNA AND Y CHROMOSOME IN VIETNAMESE POPULATION

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Abstract

Vietnam is an important crossroads within Mainland Southeast Asia (MSEA) and a gateway to Island Southeast Asia (ISEA), and as such exhibits high levels of ethnolinguistic diversity. However, comparatively few studies have been undertaken of the genetic diversity of Vietnamese populations. In order to gain comprehensive insights into Vietnamese populations, we analyzed complete mtDNA genomes and 2.3 Mb sequences of the male-specific portion of the Y chromosome from Kinh and 16 minority populations belonging to five language families (Austroasiatic, Tai-Kadai, Hmong-Mien, Sino-Tibetan and Austronesian). The study enabled us to carry out the first comprehensive analysis of the genetic diversity within Vietnam. Kinh, the majority group, exhibited low levels of differentiation with other groups while Mang and Sila have undergone recent, independent bottlenecks. The two Austronesian-speaking groups, Giarai and Ede, showed a potential impact of matrilocality on their patterns of variation. We found that isolation has been the major factor influencing the genetic structure of Vietnamese populations.

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PROGRAM TO DEVELOP BIO-INDUSTRY IN AGRICULTURE TO 2030

Pham Hong Hien

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GENETIC DIVERSITY OF UNDERUTILIZED LEAFY AMARANTHUS GENETIC RESOURCE

Ken Hoshikawa

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Abstract

Amaranthus tricolor is used as a traditional leafy vegetable in Asia. It has a remarkably valuable nutrient composition and a high tolerance to environmental stresses but has been neglected in modern breeding. Detecting DNA polymorphisms among accessions helps in understanding the genetic diversity and population structure of germplasm collections. In this presentation, I mention the genetic diversity and population structure among *A. tricolor* accessions held by the World Vegetable Center genebank with SNPs developed by the ddRAD seq.

Keywords: *Amaranthus tricolor*, ddRAD, genetic resource, SNP, genetic diversity

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MEMBRANE PHOSPHOLIPIDS: INTERNAL PHOSPHATE RESERVE OF PLANT CELLS TO COPE WITH PHOSPHATE STARVATION

Ngô Hải Anh

Research Scholar, Institute of Plant and Microbial Biology, Academia Sinica, Taiwan

Abstract

Phosphorus (P) represents a major and essential nutrient for plant growth that must be assimilated exclusively from the ground through the root system. As a sessile organism, wild plants often need to cope with the soil environment with scarce P. Among many mechanisms that provide plant internal P reserves, membrane phospholipids appear to be a reliable source for their abundance. Upon P starvation (P⁻), a straightforward metabolic conversion termed “membrane lipid remodeling” is stimulated and hydrolyzes a phosphate-containing headgroup of phospholipids to replace with non-P galactose, thereby presumably retrieving scarce P yet maintaining the integrity of cellular membranes. In this presentation, I will be summarizing our recent findings about metabolic pathways during lipid remodeling in plant cells in P-starved growth condition.

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PLANT BREEDING AT THE SPEED OF LIGHT: THE POWER OF GENOME EDITING TECHNOLOGY

Yao Luo

Manager of breeding technology, Lark Seeds International

Plant Breeding has successfully developed new varieties with high yields and better quality for all agriculture crops. Conventional breeding relies on the nature of recombination and undirected mutagenesis which is a lengthy and tedious process for introgression of beneficial traits into an elite germplasm. Moreover, the lack of natural or mutagen-induced diversity, linkage drag from a neighboring deleterious locus and species barrier of transferring loci limited further improvement of crops through conventional breeding. Genome editing technologies have progressed rapidly during the last ten years and become a powerful alternative to conventional breeding. Genome editing technologies enable immediate and precise introgression of multiple beneficial traits into an elite background within one generation without linkage drag and necessity to cross. Instead of the lengthy backcrossing process, edited crops can be evaluated in a commercially relevant trial within 2-4 years. Thus, the utilization of genome editing technologies will facilitate the breeding process at an unprecedented speed

PERSONAL INFORMATION OF PRESENTER



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ELIEVE STRESS FOR BETTER CROP PRODUCTION

Yueh Cho

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Abstract

Environmental fluctuation disrupts protein folding and causes unfolded protein accumulations in the endoplasmic reticulum (ER), which not only triggers ER stress, interferes cellular functions but also arrests plant development and reduces crop production. Plant and other eukaryotic cells have developed conserved mechanism-unfolded protein response (UPR) to sense and release ER stress. Recent studies in model plant like *Arabidopsis thaliana* show that the UPR machinery including ER sensors, molecular chaperones and transcription factors are required for plant development. The UPR contributes to hormone-regulated root growth during vegetative phase and also male gametogenesis. Noteworthy, UPR maintains ER protein homeostasis to support plant survival and reproduction under unfavorable conditions like high temperature and salinity. Better understanding of plant UPR benefits us to manage plant growth, improve stress tolerance and crop production for global food security.

Keywords: Environmental stress, endoplasmic reticulum (ER), unfolded protein response (UPR), crop production, food security

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APPLICATION OF DNA BARCODES IN IDENTIFICATION OF FRUIT VARIETIES IN VIETNAM

Do Tan Khang

Biotechnology Research and Development Institute, Can Tho University

Abstract

Identification of individual fruit varieties is really necessary in the free trade context. Application of barcoding DNA is regarded as one of the innovations in plant sciences. DNA barcoding involves the production of PCR amplicons from particular regions to sequence them and these sequence data are used to identify that organism to make a distinction from other species. In this study, some potential DNA barcodes of fruit cultivars including orange, pomelo, mandarin, dragon fruit, rambutan, durian, sour sop, star apple, mango and burmese grape in the Mekong Delta were sequenced and aligned to find SNPs for authorization. Six DNA barcodes including ITS, *matK*, *rbcL*, *atpF-atpH*, *psbI-psbK*, and *ycf1b* were utilized for their genetic analysis. DNA sequences were aligned and clustered using Mega software.

Based on the degree of nucleotide difference, ITS is a potential sequence to identify the “Da xanh” pomelo variety. The *ycf1b* gene shows that there are five SNPs that can distinguish honey orange varieties from other varieties. The *matK* gene can be used as a barcoded DNA to identify the “yellow-fleshed” rambutan, “Java” rambutan and “Tien Cuong” hybrid rambutan. The *atpF-H* sequence showed that the sequence of “Ha Chau” burmese grape was completely different from the control samples at 11 nucleotide positions. The analysis results showed that the *rpoC1* gene, the research results gave 7 positions of nucleotide difference. In which, there are two positions specific to the species, four positions are capable of identifying the custard apple species. There are six SNPs detected among sequences of durian samples. There is a specific SNP for durian variety “Ri-6” at position 444, in durian “Ri-6” it is G while the rest is C. Red flesh dragon fruit variety was identified by three loci, *atpF-H* + *rbcL* + *matK*. Based on the DNA sequence of *atpF-atpH* region, there are differences between five varieties. Through the analysis of the *matK* sequence region, a variety of variable positions occurred in most of the 16 sequences, of which 27 positions could identify “Hoa Loc” mango with the rest of the samples. The findings could be considered for development identifying protocols for authorization of fruits related in commercial issues.

Keywords: DNA barcode, fruit, sequencing, alignment

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APPLICATION OF BIOTECHNOLOGY ON THE TREATMENT OF AGRICULTURAL SOLID WASTE

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Abstract:

Agricultural and food industries produce a huge amount of wastes globally, which further affects public health and the environment by their disposal. Agriculture is an important economic sector in Vietnam that has doubled in terms of value in the last decade. Intensification has been the sector's response to increasing rapidly food demand. Vietnam has also increased a large amount of agricultural waste. Especially, agricultural solid waste management has been a challenge for certain decades in Vietnam, where tons of agricultural solid wastes are generated from several sources and this pattern has given rise to environmental problems. In 2020, the total volume of agricultural waste by-products is more than 156.8 million tons. Each year solid waste sources from livestock farms about 84.5 million tons/year, 68% of livestock farms treat animal waste to biogas 30.2%, selling solid waste 25.6%, use bio-mat bedding 6.4%, compost 1.9%, and other treatment methods 3.9%. Another 32% of animal wastes release directly into the environment without any treatment. Rural areas yearly generate more than 14,000 tons of pesticide and fertilizer packaging, 76 million tons of rice straw, and about 47 million tons of waste. These wastes produced don't have proper disposal methods usually disposed of by dumping method which requires a large area and it contains toxic substances that may cause to environmental pollution and harmful effect on human and animal health. Most of the agricultural solid wastes are untreated and underutilized, therefore in maximum reports, it disposed of either by burning, dumping, or unplanned landfilling. There are several areas that agricultural residues can be exploited through the biotechnological route. The biotechnological process has opened up a new avenue by utilizing agricultural solid waste as a source of nutrients for microorganisms to produce valuable bio-products such as enzymes, organic acids, and other useful metabolites having diverse applications in various fields. Sustainable utilization of these seemingly waste materials as feedstock towards energy generation and production of chemicals and other value-added products. Thus, bio-techniques have become important avenues for green pretreatment and conversion of agricultural biomass wastes. Moreover, it is important to consider the overall cost optimization to make the process economically feasible, profitable and increase industrial adoption. This review brings the new aspects to provide a better understanding of the advancement of economic biotechnology on the treatment of agricultural solid waste. These are advancements in the disposal of agricultural solid waste using biotechnological methods.

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APPLIED BIOTECHNOLOGY IN PLANT BREEDING: AN OVERVIEW

Trinh Ngoc Ai, Nguyen Phuong Thuy, Nghi Khac Nhu, Tran Thi Ngoc Bich

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Abstract:

With the increasing world population, it is critical to have enough food grains and vegetables to feed them. The traditional methods to cultivate plant crops is not sufficient to produce enough food grains and vegetables, hence the use of modern technology is required. Plant biotechnology is a powerful tool for the development of new plant traits and varieties. One of the most important applications of advanced biotechnology in plant breeding include key topics such as biometry applied to molecular analysis of genetic diversity, genetically modified plants, plant tissue culture and more that bring desired changes, allow plant breeders or farmers to make precise genetic changes in plants to impart beneficial traits, which include size, yield, color, taste, and appearance. In this paper, we will concentrate on the recent developments and spin-off applications of biotechnology in plant breeding as well as consider their potential benefits and risks for the public, reviews the regulatory role and comments on biotechnology's implications for agriculture.

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ESTABLISHMENT OF THE DIGITAL TOOLS FOR PRECISION AGRICULTURE BY MACHINE LEARNING

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Sciences

Abstract

Precision agriculture has been considered as one of the key components of the digital transformation in Vietnam. In the view of information and communications technology, five major cases, including smart-crop monitoring, drone farming, smart-livestock monitoring, autonomous farming machinery, and smart-building and -equipment management have the potential to radically transform many aspects of agriculture. However, the establishment of digital tools used in smart agriculture programs has been still lacking. Of our interest, we reported two out of many cases of Internet-of-Things- (IoT-) based tools for the research in agriculture. In the first case, we investigated an electronic trap for automated monitoring of fall armyworm (FAW). Briefly, FAW (*Spodoptera frugiperda*) has been reported as one of the most devastating pests that can attack maize (*Zea mays*) at all growth stages. Since the first occurrence of FAW in Vietnam has been reported in 2019, this lepidopteran pest had caused huge damage to maize production in the Northern provinces of Vietnam. Thus, monitoring, identification, and management of FAW in the fields become one critical task for sustainable agricultural production. As the result, we introduced an automated FAW (adult moths) counting system based on the traditional pheromone traps. The IoT sensors have been merged into the instrument to count the frequency of adult moths and together record the temperature and humidity data. The general data, including the real-time amount of trapped insects and environmental conditions, has been analyzed based on the machine-learning method, consequently, send to the Internet browser and applications. In the second case, we constructed a cost-effective phenotyping machine for automated seed imaging. Particularly, agronomists have an issue with the estimation of various typical characteristics of seeds, like length, width, mass, the color of the skin, and pubescence. By using the computer vision approach, we generated an easy-to-use tool for automatically measuring the general features of crop seeds. The construction of this tool can significantly replace labors with only simple operations. Taken together, our tools could significantly provide a collection of digital tools for supporting the digital transformation in research and development in the agriculture sector.

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**STUDY ON *IN VITRO* MICRO-PROPAGATION OF TRIPLOID DAYLILY
(*HEMEROCALLIS* SPP) CV. K1**

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ABSTRACT

Background:

Hoa Kim cham-Daylilies (*Hemerocallis* sp.) are used for many purposes such as beauty, medicinal, ornamental, nutritional value. Due to the great demand for ornamental needles, every year many newly varieties are supplied to the market with many shapes, flower colors and diversity in polyploidy (diploid: $2x = 22$), triploid: $3x = 33$ and tetraploid: $4x = 44$). The triploid plant cannot be propagated by seed, because of sterile pollen. So it can only be propagated by traditional methods such as growing by bulb or separating shoot. Despite its importance, *in vitro* propagation remains extremely challenging, especially in triploid multiplication. This study is carried out under room environmental conditions and concentrated on developing an efficient triploid daylily “K1 cultivar” *in vitro* propagation protocol that uses the petal tissue as the principal explant.

Results:

This is the first study on the development of *in vitro* propagation protocol of triploid daylily. Various samples of triploid plant such as leaf, petal, receptacle, and bulb were investigated for callus induction, shoot organogenesis and rooting formation after treating in different growth regulator on Murashige and Skoog (MS) medium. The results showed that the petals were induced highest callus formation rate (83.3%) on MS medium supplemented with 2 mg L^{-1} 2,4-D (2,4-Dichlorophenoxyacetic Acid) + 10 mg L^{-1} BA (Benzyl Adenine). The callus were incubated on MS medium containing 2 mg L^{-1} BA + 0.25 mg L^{-1} α -NAA (α -Naphthyl Acetic Acid) gave highest rate of shoot formation (12.9 shoots per callus). Shoots gave most root number (5.78 roots per shoot) on MS medium supplemented with 0.3 mg L^{-1} active charcoal. The regenerated plant grew normally in the greenhouse for field trait evaluation.

Conclusion:

Using petal tissue at young stage (two cm length of flower bud) of triploid daylily-K1 cultivar as main explant is an efficient way for mass shoot multiplication. This could lead to a broad and rapid propagation of the triploid daylily under *in vitro* environmental conditions to meet the market demand.

Keywords: Triploid daylily, petal culture, plant regeneration, shoot multiplication

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ENGINEERING BACTERIAL LEAF BLIGHT RESISTANT RICE USING GENOME EDITING

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GREENHOUSE AND FIELD CASSAVA YIELD CAN BE ALTERED BY DIFFERENT ISOLATES OF AN AGRICULTURALLY-RELEVANT FUNGAL SYMBIONT

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**THE INFLUENCE OF CONDITIONS ON THE ANTIBACTERIAL PROPERTIES OF
GANODERMA AFF. BROWNNI, *GANODERMA SINENSE***

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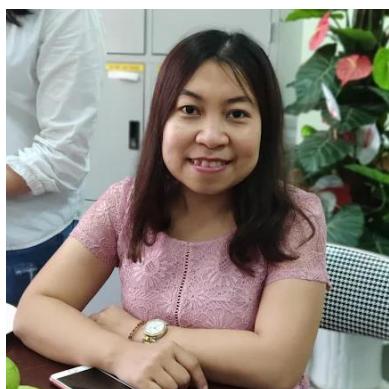
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ABSTRACT

This study will showed the results of the influences of temperature, pH and nutritional conditions on the mycelium grown of *Ganoderma* were investigated in laboratory experiment. The results of the experiment indicated that Potato Dextrose Agar (PDA), Meat Pepton Broth (MPB), and Glucose Yeast Extract Agar (YEA) were the most suitable media for the mycelium growth of *Ganoderma*. The optima temperature for mycelium growth of both *Garnoderma* was obtained at 29⁰C and pH 6-7. The colonies of *Ganoderma aff. brownni* and *Ganoderma sinense* in different condition include temperature, pH and media were able to resist to bacteria *E.coli*, *V. parahaemolyticus* and *S. aureus*. The optimal temperature, pH and media of fungal growth effected directly to the antibacterial ability of *Ganoderma aff. brownni* và *Ganoderma sinense*.

Keywords: *Antibacterial, nutritional conditions, mycelium, temperature, Ganoderma.*

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GENETIC ANALYSIS OF RICE BLAST DISEASE IN THE NORTH OF VIETNAM

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Abstract

Rice blast, caused by the ascomycetes fungus *Magnaporthe oryzae* (anamorph, *Pyricularia oryzae* Cav.), is the most devastating fungal disease of rice (*Oryza sativa*) in the two major rice production areas in Vietnam, the Red River and Mekong River Deltas. In this present study, 52 isolates from four provinces-Thai Binh, Nam Dinh, Ha Tay, Hai Phong-in the Red River Delta were collected then detected *AVR-Pik*, *AVR-Pii*, *AVR-Pita* and *AVR-Pizt* by using the Southern hybridization with *BamHI*, *Psp1406I*, *HindIII*, *Sall* enzymes and APik500, APii213, APita766, APizt3038 probes. The results showed that, isolates from Thai Binh had all these avirulence genes *AVR-Pik*, *AVR-Pii*, *AVR-Pita* and *AVR-Pizt*, isolates from Nam Dinh had *AVR-Pik*, *AVR-Pizt* and *AVR-Pita*, isolates from Ha Tay had *AVR-Pizt* and *AVR-Pita*, isolates from Hai Phong did not contain any of four *AVR* genes. This reveals the genetic differentiation and diversity among studied isolates collected from different provinces in the Red River delta. Moreover, the differences among studied isolates were also shown their virulent and avirulent on rice carrying resistant gene (*Pik*, *Pita*, *Pizt*, *Pii*, *Pia*). The obtained information might supply useful information for breeding sustainably rice blast resistant cultivars in the North of Vietnam.

Key words: *AVR* genes, Blast disease, *Magnaporthe oryzae*, *Pyricularia*, Rice (*Oryza sativa* L.)

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AGRICULTURE IN CLIMATE CHANGE SCENARIOS: THE DAWN OF MICROALGAE BIOTECHNOLOGY IN VIETNAM

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Abstract

Recently, climate change significantly influenced crop and livestock production and changed the agriculture structure. Located in an area strongly affected by climate change, Vietnam needs a specific strategy to maintain and develop sustainable agriculture to bring stable income for farmers and ensure socio-economic security. The unfavourable weather phenomena such as droughts, floods, sea-level rise, narrowing of cultivated areas, and increased saltwater intrusion have greatly affected many agricultural production areas of the country.

Microalgae are a large domain with photoautotrophic species characterized by small size, widely distributed in freshwater, brackish and saltwater bodies. To date, it is estimated that there are between 200,000 and 800,000 species of microalgae with 50,000 of these have been described. Microalgae adapt widely to different ecological habitats and are relatively easy to upscale to industrial production because they have a fast growth rate in relatively low-cost culture media, and effectively convert solar energy and CO₂ to biomass. Biochemically, microalgae are rich in proteins, unsaturated fatty acids, pigments, vitamins, minerals and secondary metabolites such as carotenoids, steroids, polysaccharides. Therefore, microalgae can be cultivated for a wide application range in many fields such as food supplements for humans, pharmaceuticals and cosmetics, feed in aquaculture, animal husbandry, bioplastics, biogas, biofuels and environmental pollution treatment. Currently, in Vietnam, microalgae species such as *Arthrospira* (Cyanobacteria), *Chlorella*, *Nannochloropsis*, *Chaetoceros*, *Isochrysis*, *Tetraselmis*, *Thalassiosira*, *Dunaliella*, *Haematococcus* and many other algae species have been cultivated at various scales mainly for dietary supplements, aquafeeds and pharmaceuticals and cosmetics. In the climate change scenarios, developing the microalgae biotechnology industry is one of the promising and sustainable directions for the agriculture of Vietnam.

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APPLICATION OF NANOBIO TECHNOLOGY ON PLANT TISSUE CULTURE

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Abstract

Nano silver (NS) has been known as a novel material which can not only destroy the structure of cell microorganisms but also have positive effects on *in vitro* plant morphology. In this review, we summarized the use of NS in several steps of plant tissue culture. The first use was to sterilize several samples such as lily, lotus, banana, etc.... which made up higher percentages of sample survival and clean than that of normal sterilizing solutions such as HgCl₂ 0,1 %, Sodium Hypochlorite. Moreover, NS could stimulate morphogenesis *in vitro* as forming lily bulblets, callus from bulb scales samples; banana callus, regenerating and multiplying shoots, inducing roots, and improving nursery phase of *in vitro* propagation. Therefore, the findings of using NS particles on tissue culture can be a potential idea which would provide valuable information in investigating the effect of NS on our samples as well as other varieties.

Keywords: nano biotechnology, nano silver, morphogenesis, sterilizing, tissue culture.

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CHARACTERIZATION OF CRISPR/CAS9-INDUCED KNOCKOUT MUTATIONS IN GB SUBUNIT GENE IN TOMATO

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Abstract

Heterotrimeric G proteins, consisting of alpha (α), beta (β) and gamma (γ) subunits, are signal transducers that are essential for the regulation of development and stress responses in most eukaryotic organisms. Our current knowledge on the roles of G proteins in plants largely comes from studies on the dicot *Arabidopsis thaliana* or monocots including rice and maize. There are considerable differences in morphological phenotypes and defense responses in G protein mutants between *Arabidopsis* and monocot plants, that suggest evolutionary divergence of G protein functions in plants. However, it is unclear when this divergence has occurred due to the lack of G protein research in dicot plants other than *Arabidopsis*. Here, CRISPR/Cas9-induced knockout mutations in the $G\beta$ proteins in dicot species tomato, *Solanum lycopersicum* cv. Money maker, were generated and studied to provide a better insight into the evolution of G protein function in plants. We show that loss-of-function mutations in the tomato $G\beta$ gene (*SIGB1*) resulted in a seedling lethal phenotype, indicating the essential role of *SIGB1* in tomato plant growth and development. The *slgb1* seedlings displayed a normal germination rate, however, developed necrotic lesions at the top of the hypocotyl that progressively extended along the entire hypocotyl and into the cotyledons and roots. 100% of the *slgb1* seedlings died within four weeks of germination without ever producing true leaves. The *slgb1* seedlings exhibited elevated levels of ROS production, salicylic acid, cell death and increased expression of defense-related genes, indicating that the seedling lethality is due to induction of the autoimmune response. The seedling lethal phenotype of tomato $G\beta$ mutants is very similar to rice and maize $G\beta$ null mutants but distinct from *Arabidopsis* $G\beta$ knockout mutants that complete their life cycle without autoimmunity. Our study has contributed to insights into the divergent functions of G proteins in plants. The G proteins, at least for the β subunit, may have a conserved function in monocot and dicot plants, suggesting that the *Arabidopsis* G proteins could have evolved some different functions.

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IMPROVED BACTERIAL LEAF BLIGHT DISEASE RESISTANCE IN THE MAJOR ELITE VIETNAMESE RICE CULTIVAR TBR225 USING CRISPR/CAS9 SYSTEM

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¶These authors contributed equally to this work.

Abstract

TBR225 is one of the most popular commercial rice varieties in Northern Vietnam. However, this variety is highly susceptible to bacterial leaf blight (BLB), a disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) which can lead to important yield losses. *OsSWEET14* belongs to the *SWEET* (Sugars Will Eventually be Exported Transporters) gene family that encodes sugar transporters. Together with other Clade III members, it behaves as a susceptibility (*S*) gene whose induction by Asian *Xoo* Transcription-Activator-Like Effectors (TALEs) is absolutely necessary for disease. In this study, we sought to introduce BLB resistance in the TBR225 elite variety. First, two Vietnamese *Xoo* strains were shown to up-regulate *OsSWEET14* upon TBR225 infection. To investigate if this induction is connected with disease susceptibility, the sequence of *tal* genes of these *Xoo* strains and TBR225 *OsSWEET14* promoter were analyzed. In order to generate transgenic rice plants, *Agrobacterium*-mediated transformation protocol of TBR225 rice cultivar was optimized with an efficiency of about 6.5%. Then, nine TBR225 mutant lines with mutations in the AvrXa7, PthXo3 or TalF TALEs DNA target sequences of the *OsSWEET14* promoter were obtained using the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated 9) editing system. Genotyping analysis of T₀ and T₁ individuals showed that mutations were stably inherited. None of the examined agronomic traits of three transgene-free T₂ edited lines were significantly different from those of wild-type TBR225. Importantly, one of these T₂ lines, harboring the largest homozygous 6-bp deletion, displayed decreased *OsSWEET14* expression as well as a significantly reduced susceptibility to a Vietnamese *Xoo* strains and complete resistance to another one. Our findings indicate that CRISPR/Cas9 editing conferred an improved BLB resistance to a Vietnamese commercial elite rice variety.

Keywords: *Bacterial leaf blight; CRISPR/Cas9; Xanthomonas oryzae* pv. *oryzae; OsSWEET14; TBR225; transgene-free planDr.*

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NEWLY COLLECTED *Trametes versicolor* STRAINS

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Abstract

Trametes versicolor is widely utilized not only as a nutritional supplement but also in environmental protection. One of the major challenges currently for the commercial cultivation of *Trametes versicolor* is finding superior strains that can produce high yields. In an attempt to search for high yield potential *Trametes versicolor*, two wild strains, namely VNUA and BV, were isolated and optimized culture conditions for improving mycelial growth and yield performance. The results revealed that the ideal medium and temperature for favorable mycelial growth of *Trametes versicolor* were PGA and 30 °C, respectively. The optimal spawning material for upscaling of the mycelium was Treatment D (20% rice grain, 79% sawdust, 1% calcium carbonate). The strains were successfully cultivated in a substrate combination of sawdust and rice husk supplemented with wheat bran. Investigated strains responded differently to different substrates cultivation. Of note, compared with strain BV, strain VNUA showed a significantly higher biological efficiency (7.3%). Collectively, our results suggest that strain VNUA could be considered as a potential strain for commercial cultivation. This report may assist in the cultivation of *Trametes versicolor* at the industrial scale.

Keywords: *Trametes versicolor*, wild strain, mycelial growth, cultivation substrate

GENETIC DIVERSITY OF VIETNAMESE NATIVE CHICKEN BREEDS BASED ON MITOCHONDRIAL DNA D-LOOP SEQUENCE

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Abstract

Domestic chicken is the most widely distributed species of all livestock in the rural households in Southeast and East Asia. Diversity of the chicken genetic resource is important for the better conservation of indigenous chicken breeds. Mitochondrial DNA sequence is an useful tool to investigate the genetic background of both closely related species and individuals within species (Harpending et al. 1998). Previous studies used mtDNA showed the high genetic diversity of local chickens (Liu et al. 2006; Oka et al. 2007; Cuc et al. 2011) and multiple origins of domestic chicken in East Africa (Mwacharo et al. 2011), Bangladeshi (Bhuiyan et al. 2013), Indian Ocean Rim (Al-Qamashoui et al. 2014) and Swedish (Englund et al. 2014).

Native Vietnamese chicken possesses various good characteristics such as: adaptability to harsh conditions, high disease resistance, adaptation to poor nutrition, the quality of meat and eggs, and many rare genetic resources. However, due to traditional poultry farming with small-scale production and to the imported chicken breeds, may have led to introgression of exotic chicken breeds into Vietnamese gene pool, so rare genetic resources are at risk of being hybridized. The GLM is one of native breeds contribution to the economic value of rural citizens of Lien Minh, Tran Chau, and Cat Hai district. Dr. G9C breed is a multi-toes chicken (5-9 toes) with decreasing quantity and have been conserved at the National Park of Xuan Son, Phu Tho Province. The GNH chickens are aggressive and brave so people wish to have them in chicken fighting games. GNH's legs are green colors (Su et al. 2004). In recent years, Vietnam has a program to conserve indigenous genetic diversity, in which GLM, G9C, and GNH chickens are on the preserved list.

Genetic diversity of three Vietnamese indigenous chicken breeds including GLM, GNH and G9C were evaluated using mitochondrial DNA (mtDNA) D-loop sequence polymorphism. 1,050 bp of D-loop region of 48 individuals was amplified, sequenced, and analyzed. Comparative alignment results showed that the three Vietnamese native chicken breeds were distributed over 16 haplotypes belonged to five haplogroups A, B, C, D and E, of which clade B was dominant. Individual of GLM and G9C breeds clustered mainly in clade B and GNH chicken belonged to clade C and D. This study shows the relatively high genetic diversity of native Vietnamese chicken breeds and contributes to the conservation and breeding strategy of indigenous chicken in Vietnam.

Keywords: DNA D-loop sequence, genetic diversity, Vietnamese indigenous chickens.

ENHANCING POWDERY MILDEW RESISTANCE IN SOYBEAN BY INDUCING TARGETED MUTATIONS OF *MLO* GENES USING CRISPR/CAS9 SYSTEM

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Abstract

Powdery mildew is the major disease causing great losses in soybean yield and quality. In Vietnam, soybean powdery mildew is caused by *Erisiphe diffusa* and thrives soybean cultivation in the North area during winter and spring seasons. Using disease-resistant varieties has been considered as the most effective method to prevent crops from powdery mildew. In this study, we utilized CRISPR/Cas9 system to induce targeted mutations of the *MLO* (Mildew Locus O) genes to increase powdery mildew resistance in soybean. The CRISPR/Cas9 construct was designed and transferred into the soybean cultivar ĐT26 through *A. tumefaciens* mediated method. Three T0 transgenic soybean lines showed targeted mutations of *MLO* genes at biallelic, chimeric and homozygous forms. The inheritance and segregation of induced mutations were confirmed and validated at T1 and T2 generations. Different soybean lines showing simultaneous loss-of-function mutations for 3 genes (*GmMLO02*, *GmMLO19*, *GmMLO23*) or for 4 genes (*GmMLO02*, *GmMLO19*, *GmMLO20*, *GmMLO23*) were identified at the T2 generation. Of which, mutant line 3.1-3-41 carrying quadruple mutations of *MLO* genes showed the enhanced resistance to soybean powdery mildew under artificial infection at greenhouse condition. The reduction in disease severity of mutant soybeans could reach up to 36.4% as compared to wild-type plant. The density of conidia per leaf area of the mutant lines decreased by 1.5-4 times compared with the control. In addition, fungal hyphae grew more slowly on the leaf surface of mutant lines. The less production and late germination of fungal conidia were also observed on mutant soybean leaves. Under the greenhouse condition, the mutant soybean plants did not show any difference in terms of morphology and yield compared with the control plant. The *MLO* homozygous mutant lines with enhanced resistance to powdery mildew will be potential materials for further soybean breeding in Vietnam.

Keywords: CRISPR/Cas9, *MLO*, powdery mildew resistance, soybean, targeted mutation

ACKNOWLEDGEMENT

The scientific symposium "**AGRICULTURE BIOTECHNOLOGY: CHALLENGES AND OPPORTUNITIES**" is financial supported by World Bank in the Development Projects: Support for Autonomous Higher Education Project (SAHEP) for Vietnam National University of Agriculture (VNUA).

This symposium is held on December 18, 2021 at Vietnam National University of Agriculture in conjunction with online on the Zoom platform has received attention and support. Nearly 200 delegates are scientists, lecturers, doctoral candidates, graduate students and students from universities and research institutes in Vietnam and abroad.

The symposium has 20 presentations from speakers from 7 countries/regions: Canada, Switzerland, Germany, Japan, Taiwan (China) and Vietnam. The speakers enthusiastically shared many opinions, preciously discussed the challenge and the opportunities, the results and the new research trends in the different fields of agricultural biotechnology.

The organizers would like to thank the delegates for participating, submitting reports, presenting, sharing and contributing valuable ideas to the symposium. Your contributions have made an important success of the symposium and are really meaningful for our cooperation in scientific research in the future.

Although the organizers have tried to prepare the symposium in the best way, it is inevitable that shortcomings cannot be avoided. The organizers are looking forward to receiving the sympathy and suggestions from scientists to make the next seminars more successful.

Once again, the organizers of the symposium "Agricultural Biotechnology: Challenges and Opportunities" would like to thank for the participation and highly appreciate the scientific presentations of speakers from research institutes, universities, businesses, domestic and international scientists. We would like to wish all delegates and guests good health, happiness and success. Thank you very much.

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