



The 1st International Congress on Biotech Solutions for Sustainability

ISBN: 978-625-94974-0-2

May 7-10th, 2024
Congress Hall
Gebze Technical University
Kocaeli, TURKIYE

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Scope

We aim to bring together researchers and industrial leaders worldwide to share their latest findings and advancements in their respective fields. Our congress takes pride in being at the forefront of the latest technological advancements, enabling us to achieve a hybrid and international format that caters to our attendees, wherever they may be. We are enthusiastic to host our esteemed guests online and in person at multiple locations.

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Letter of Conference Chair

Dear Colleagues,

We are delighted to have hosted the 1st International Congress on Biotech Solutions to Sustainability, which Gebze Technical University (GTU) and Mohammed Nawas Shareef University of Agriculture Multan Pakistan have co-organized. We are proud to see that the Memorandum of Understanding that was signed in 2022 between our universities triggered so much interaction and gave its fruits in this congress.

I would also like to thank other partners and societies that have contributed to our congress by organizing specific sessions, such as UN Sustainable Development Solutions Network (SDSN) of Turkiye in collaboration with our Sustainability Office, as well as Cell Death Society of Turkiye.

Since it was founded in 2016, The Institute of Biotechnology in GTU focused on both interdisciplinary collaborations within GTU as well as other with universities. We believe in the importance of collaborations, university-industry partnerships and bioentrepreneurship. Some of the companies with which we carried out R&D projects are also here with us at this congress, and they will be presenting the fruits of our collaborative endeavors. It is always a proud moment to see the academic work conducted in our laboratories take its place in the market, making a change in the world. We believe that biotech startups will be a dynamo in generating innovative ideas towards sustainable solutions, and we wish our students and faculty members to be a driving force in this aspect.

We have also been successfully coordinating 2 TUBITAK University Industry PhD program projects, one of which is with our long-term partner and the main sponsor of this congress, Gubretas, one of the leading fertilizer companies in Turkiye.

We strongly believe in the power of like-minded people and institutions coming together and working on a shared goal. Coordinating such efforts requires different academic and nonacademic institutions, bioentrepreneurs, NGOs, local and regional policymakers and investors coming together and creating a sustainable bioeconomy and biotechnology community and biocluster. This is even more important for an increasingly critical and challenging issue that our world is facing today - sustainability. To that end, the esteemed speakers, presenters and audience of this congress discussed various innovative solutions in medical, food and agricultural biotechnology, integrating new concepts such as AI and discussing how they can be valuable for our joint R&D efforts for a better world.

The core scientific programme provided 6 plenary lectures, 16 oral presentations, 14 short presentations and 55 poster presentations from Turkiye, Pakistan, Netherlands, Turkish Republic of Northern Cyprus, Germany, and Bulgaria.



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On behalf of the congress organizing committee, I would like to thank our Rector, Prof. Dr. Hacı Ali Mantar and our Vice Rector Prof. Dr. Elif Damla Arisan for their support, the Vice Director Assist. Prof. Baris Kutman and all the faculty members research assistants and administrative personnel of the Institute of Biotechnology for their impressively superhuman efforts during the organization of this congress, the PR department of our university, our sponsors for making this congress possible, and last but not least our Technology Transfer Office for their cooperation and management. I would also like to thank higher administration and faculty members of Mohammed Nawaz Shareef University of Agriculture in bringing this congress to life with us.

We sincerely hope that this meeting was a memorable and enriching experience for all, and we hope to see all our speakers and participants in the future congress series in the beautiful town of Gebze.

Prof. Dr. Işıl AKSAN KURNAZ
Congress Chair,
On behalf of the Organizing Committee



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Scientific Committee

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Ummara WAHEED (MNS University of Agriculture, Multan, Pakistan)
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Umar AKRAM (MNS University of Agriculture, Multan, Pakistan)
Muhammad Abubakar SADDIQUE (MNS University of Agriculture, Multan, Pakistan)



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The Programme

Day 1: May 7, 2024

09:00-17:00	Registration (will continue May 8) & Sponsor Desk Setup
14:00-15:30	Prof. Dr. Eckhard George "How to write a thesis and how to use AI tools for scientific writing"

Day 2: May 8, 2024

08:30-09:30	Registration
09:00-10:30	Opening ceremony
10:30-11:10	Prof. Dr. Levent Kurnaz "The Interconnected Impact: Exploring How the Climate Crisis Affects Biodiversity" Plenary Lecture 1
11:10-11:30	<i>Coffee break</i>
Session 1	Sustainability Moderator: Prof. Dr. Melek Ozkan
11:30-11:55	OP1- Prof. Dr. Melek Ozkan "Sustainable and Renewable Energy from Biomass"
11:55-12:20	OP2- Prof. Dr. Nevin Gul Karaguler "Exploring novel proteins for sustainable industrial processes"
12:20-12:45	OP3- Prof. Dr. Ozge Karadag "Promoting Health Equity in the Age of Biotechnology"
12:45-13:30	<i>Lunch break and poster hall visit</i>
Session 2	Sustainable Food and Agriculture Moderator: Assist. Prof. Bahar YILDIZ KUTMAN
13:30-14:10	Prof. Dr. Hakan Ozkan "Modern Crop Breeding for Global Food & Nutrition Security" Plenary Lecture 2
14:10-14:25	OP4- Assoc. Prof. Ceyhun Kayihan "Single-cell transcriptional profiling in root tissues of Arabidopsis thaliana exposed to boron toxicity at seedling stages"



14:25-14:50	OP5- Dr. Nihal Oztolan Erol “Nanoparticle-mediated gene editing: A path towards tailored crop improvement”
14:50-15:00	SP1- Assoc. Prof. Birsen Cevher Keskin “Comparative proteomic analyses revealed the response mechanism of drought-tolerant and susceptible olive cultivars (<i>Olea europaea</i> L.) under drought stress and recovery”
15:00-15:10	SP2- Abdelhameed Hamdan “Exploring aquaporins and dehydrins in barley under drought and salt stresses during early seedling development”
15:10-15:20	SP3- Rifat Bugra Bildiri “Valorization of steel slag as a safe soil amendment for sustainable crop production”
15:20-15:30	SP4- Aysenur Bayrak “Lettuce biofortification with selenium and its interactions with sulfur nutrition in an indoor vertical NFT system”
15:30-15:40	SP5-Halis Batuhan Unal "Cloning the SARS-CoV-2 structural proteins for transient expression of a VLP-based vaccine candidate in <i>Nicotiana benthamiana</i> "
15:40-16:05	Sponsored Talk: TBA
16:05-16:30	OP6- Elif Ozlem Guncaldi “Evaluation of plant growth promoting Rhizobacteria isolated from soils of Türkiye for microbial biofertilizer production”
16.30-17:00	<i>Coffee break and poster hall visit</i>
Session 3	HÖAD Session “Exosome: New Biotechnological Tool” Moderator: Prof. Dr. Seda VATANSEVER
17:00-17:25	Prof. Dr. Seda Vatansever “Exosomes: the rising star in medicine”
17:25-17:50	Prof. Dr. Ozge Cevik "New advances in exosome-based targeted drug delivery systems"
17:50-18:15	Dr. A. Caglar Ozketen “Exosomes as smart delivery systems for nanotheranostics: opportunities and challenges”



Day 3: May 9, 2024

Session 4	MNS & GTU Integrated Session (Online): Biotechnological Advances in Crop Improvement Moderators: Dr. Ummara Waheed and Dr. M. Abubakar Saddique
09:00-09:25	OP7- Dr. Allah Bakhsh "Potential of dsRNA technology for better crop improvement"
09:25-09:50	OP8- Dr. Awais Rasheed "Genomics-enabled wheat breeding for improved productivity and nutritional quality"
09:50-10:15	OP9- Dr. Zulqurnain Khan "Genome editing in plants: challenges and opportunities"
10:15-10:40	OP10- Dr. M. Mahmood Ahmad "Cotton genomics and transformation for insect resistance"
10:40-10:50	SP6- Prof. Dr. Muhammad Baber "Molecular markers and their applications in plant breeding"
10:50-11:00	SP7 - Dr. Kamran Qureshi "Hydrogen peroxide-induced stress acclimation in plants"
11:00-11:40	<i>Coffee break and online poster session</i>
Session 5	Bio-driven Solutions: Industrial Biotechnology Moderator: Assoc. Prof. Muge Isleten Hosoglu
11:40-12:20	Dr. Cem Albayrak "Development of Industrial Enzymes in Türkiye" Plenary Lecture 3
12:20-12:45	OP11- Deniz Kom "Utilization of bioreactors for morphine production in the opium poppy"
12:45-12:55	SP8- Bilgen Ozsoy "Alternative proteins as functional solutions: their sources and applications"
12:55-13:05	SP9- Esra Mese Erdogan "Bio-mimicking with membrane proteins for biotechnological applications"



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13:05-14:00	Lunch break and poster hall visit
Session 6	Shaping Medical Biotechnology Moderator: Assist. Prof. Huseyin Cimen
14:00-14:40	Prof. Dr. Cem Bulent Ustundag "3D Printed Biofunctional Constructs for Biomedical Applications" Plenary Lecture 4
14:40-15:05	OP11- Dr. Mustafa Diken (online) "mRNA-based Cancer Vaccines"
15:05-15:30	OP12- Assoc. Prof. Sukru Anil Dogan "Therapies against mitochondrial diseases"
15:30-15:40	SP10- Basak Dalbayrak Demir "Characterization and investigation of biological effects of the microalgae-based microcarrier systems produced by electrospraying technique"
15:40-15:50	SP11- Mustafa Ginesar "The importance and emotional management of distant learning platforms for life sciences – Lessons from the Yes4Elearning Erasmus+ Project"
15:50-16:20	Coffee break and online poster session
Session 7	Innovative Solutions in Life Sciences Moderator: Assoc. Prof. Mehmet Ozbil
16:20-17:00	Prof. Dr. Yasar Murat Elcin "Regenerative Biomaterials" Plenary Lecture 5
17:00-17:25	OP13- Gencer Sancar (online) "The role of the FGF1/PDE4D pathway in the regulation of lipolysis, glucose levels, and insulin resistance"
17:25-17:35	SP12- Yunus Akkoc "Role of autophagy in carcinoma-associated fibroblasts and breast cancer metastasis"
17:35-17:45	SP13- Dilek Demircan Ceker "Development of a CAR-T cell therapy system targeting CD19 for the treatment of ALL and NHL"



17:45-18:10	OP14 - Yvor Ivanov "Antioxidant and antimicrobial activity of grape seed and skin extracts from four grape varieties"
18:45	<i>Gala Dinner</i>

Day 4: May 10, 2024

Session 8	AI in Biotech Moderator: Prof. Dr. Melih Bulut
09:30-09:55	OP15- Sedat Karakas "Artificial intelligence in food production"
09:55-10:35	OP16- Prof. Dr. Siddika Semahat Demir "Biotechnology, Artificial Intelligence, Sustainability: Managing Innovation"
10:35-10:45	SP14- Doga Nalci "Deep Learning Based Recognition System for Beekeeping"
10:45-11:30	<i>Coffee break</i>
11:30-12:10	Prof. Dr. Bedir Tekinerdogan "Digital Transformation and AI in Plant Biotech" Plenary Lecture 6
12:10-12:45	<i>Award ceremony and closing remarks</i>
12:45-13:30	<i>Lunch</i>

***Registered attendees**

13:30-17:30	Workshop-Discovery of Microbial and Cell Culture Bioreactor Characteristics (with contributions of IKA)
13:30-15:30	Site visit to the The Institute of Biotechnology, Gebze Technical University

OP: Oral Presentation

SP: Short Presentation

ST: Sponsored Talk

TBA: To Be Announced



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Plenary Speakers

Prof. Dr. Levent Kurnaz

"The Interconnected Impact: Exploring How the Climate Crisis Affects Biodiversity"

Plenary Lecture 1



In recent decades, the climate crisis has emerged as a pressing challenge, impacting both humanity and the planet. This presentation delves into the often-overlooked link between climate change and biodiversity decline. It explores how rising temperatures, shifting precipitation patterns, and other climate factors disrupt ecosystems, leading to habitat loss, changes in phenology, and heightened extinction risks.

Through scientific research and global case studies, we will examine how these changes affect species diversity and ecosystem adaptability. Additionally, we will analyze the cascading effects on ecosystem services, agricultural productivity, disease vulnerability,

and human well-being.

Ultimately, the talk emphasizes the urgent need for coordinated action to mitigate climate change effects and safeguard biodiversity. It aims to raise awareness and inspire individuals and communities to champion a sustainable, biodiverse future.

Prof. Dr. Hakan Ozkan

"Modern Crop Breeding for Global Food and Nutrition Security"

Plenary Lecture 2



The 21st century faces significant challenges in meeting the growing demand for high-quality food due to a rising global population and climate crises. It is essential to enhance traditional breeding techniques with modern tools. Modern plant breeding, enhanced by integrated genomic technologies such as molecular DNA markers and genome editing, can increase crop yield potential, stability, food quality, and resistance to abiotic and biotic stress. These advancements provide economic benefits and improve our understanding of crop genetics. Additionally, the social acceptance of genetically modified or genome-edited crops is vital for ensuring

global food and nutrition security. This presentation will explore both traditional and modern breeding techniques and highlight how genomic technologies can improve global food and nutrition security.



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Plenary Speakers

Dr. Cem Albayrak

"Development of Industrial Enzymes in Türkiye"

Plenary Lecture 3



This talk will summarize the role of precision fermentation in transforming industrial biotechnology and how this technology is deployed at Livzym to develop and manufacture enzymes.

Prof. Dr. Cem Bulent Ustundag

"3D Printed Biofunctional Constructs for Biomedical Applications"

Plenary Lecture 4



This talk delves into our research utilizing 3D printing for the fabrication of biocompatible constructs, addressing the critical need for advancements in personalized medicine and regenerative therapies. We will provide a detailed examination of how 3D printing facilitates the development of customized scaffolds for neural, skin, and cartilage tissue engineering, alongside tumor dressings designed for targeted drug delivery in cancer treatment. Furthermore, the creation of biocompatible wound dressings tailored for diabetic ulcers will be explored. The presentation will also encompass cutting-edge micro-needle-based scaffolds and drug

delivery systems. Through a rigorous analysis of the findings and implications of these innovations, the research underscores their potential to fundamentally transform biomedical practice. This interdisciplinary approach not only emphasizes the versatility of 3D printing but also solidifies its central role in propelling the field of biomedicine forward, offering promising avenues for the development of patient-specific treatments and regenerative therapies.



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Plenary Speakers

Prof. Dr. Yasar Murat Elcin
"Regenerative Biomaterials"
Plenary Lecture 5



Regenerative biomaterials have significant potential in replacing damaged tissues and organs and restoring normal body function. Developments in life, materials and engineering sciences offer broad opportunities in this context. This talk will mainly focus on our new studies and some of our prototypes on biological regenerative biomaterials that have the ability to activate the body's self-renewal potential.

Prof. Dr. Bedir Tekinerdogan
"Digital Transformation and AI in Plant Biotech"
Plenary Lecture 6



The rapid advancement of digitalization and artificial intelligence (AI) provides transformative opportunities across multiple sectors. This presentation will highlight the role of AI and digital technologies in enhancing precision agriculture, highlighting their potential to significantly boost food production efficiency and environmental sustainability. We will delve into various AI methodologies and digital solutions, showcasing their application within the precision agriculture domain. The presentation will further address the challenges of incorporating AI and digital innovations into agriculture and underscore the critical need for digital ecosystems specific to precision agriculture. These ecosystems, as vital collaborative networks, encourage innovation, facilitate the exchange of knowledge, and ensure broad access to technology. Additionally, we will present several case studies to provide valuable insights and lessons learned.



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Abstracts For Oral Presentations*

* The abstracts for presentations are listed according to the programme and submission order.

Sustainable and Renewable Energy from Biomass

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The increasing global population, environmental pollution, and global warming pose significant sustainability challenges. High energy demand due to the rise in population necessitates the utilization of renewable energy sources such as solar power, hydropower, biofuel and biogas based on biomass, which in turn will decrease CO₂ emissions.

There has been an increasing trend in the production and utilization of renewable energies over fossil fuels in last 20 years. However, ratio of renewable energy sources to total World energy utilization is still not very high. Biofuels based on biomass makes around 1% of total energy sources and 11% of total renewable energy sources of the World today. That ratio has to be increased since annual biomass production is very high, and it is regarded as the most important renewable source for energy production. The main biomass-based biofuel is bioethanol, the major renewable energy of transportation sector, is produced mainly from edible foods including corn, sugar cane or sugar beet. For a more sustainable way of bioethanol production, use of non-food materials such as lignocellulosic biomass should be considered. Advances in pretreatment of lignocellulosic waste have improved the conversion of this biomass into fermentable sugars, making biofuel production more viable. Increasing bioethanol utilization in the World can be achieved by progresses in the biotechnological research focusing on optimization of enzyme performance, improvement of microbial fermentation processes, and the development of integrated biorefineries.

In order to decrease CO₂ emissions to limit the global warming to below 2°C by the year 2030, widespread use of renewable energy sources especially biomass-based biofuels is a requirement. The last but not least, the energy policy of the country, which depends on its energy reserves, geography and climate, is the most important factor for deciding the establishment of infrastructure for renewable energy production and increasing its utilization.

Keywords: *Sustainability, bioethanol, lignocellulosic biomass, global warming, renewable energy.*



Exploring Novel Proteins for Sustainable Industrial Processes

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In recent years, demand has grown for creating green processes due to the rising awareness of environmental risks from traditional chemical processes. This strong focus on environmentally friendly processes makes proteins an attractive alternative in synthetic biology with advantages such as non-toxicity, and biodegradability, as they perform nearly all biological processes in living beings. Despite their potential, the instability and often poor performance of proteins under industrial conditions have led to bottlenecks in the applications of proteins in biotechnology. Although several approaches have been applied to address these limitations, our group mainly concentrated on protein engineering and metagenomics strategies.

We aim to discover novel proteins (mostly enzymes) from extremophilic regions by metagenomics to be used in any industrial application and then to develop them by protein engineering approaches. Through these studies, many novel proteins were recombinantly produced on a lab scale and characterized. Today, hundreds of proteins are being used industrially, and over 500 commercial products including several pharmaceuticals are made using protein technology. As Frances Arnold emphasized the biological world is abundant with proteins whose capabilities extend well beyond those that may serve humanity including i) finding cures for disease, ii) solutions to energy crises iii) a warming world, iv) food and clean water for a growing population, and v) ways to arrest the miseries of aging. We should still keep seeking the functional proteins known to synthetic chemistry but perhaps not yet discovered in nature.

Parts of this study were supported by TÜBİTAK, DPT-Turkish State Planning Organization, and İstanbul Technical University.



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**Single-Cell Transcriptional Profiling in Root Tissues of *Arabidopsis thaliana*
Exposed to Boron Toxicity at Seedling Stages**

Hikmet YILMAZ¹, Ceyhun KAYIHAN¹, Halis Batuhan ÜNAL¹, Oğuzhan YAPRAK¹, Emre AKSOY², Buğra CAN¹

¹Department of Molecular Biology and Genetics, Faculty of Science and Letters, Başkent University, Ankara, Türkiye

²Department of Biological Sciences, Middle East Technical University, Ankara, Türkiye

Boron (B) toxicity negatively affects plant growth and development, resulting in yield losses in plant production. In order to prevent the damage and grasp the progress of tolerance pathways, it is important to reveal the molecular mechanisms of B toxicity at high resolution. However, cells of eukaryotic multicellular organisms are heterogeneous by nature. In other words, data obtained from transcriptomic studies with classical methods are cumulative from different cells. and the possibility of obtaining gene expression profiles of cells of different developmental stages and individual responses of cell types are limited by these methods. Today, with the advancement of omics technologies, single cell RNA sequencing technology stands out among other methods.

Cell-specific transcriptional responses to environmental stimuli are yet to be fully characterized in plants. In this study, for the first time in the literature, high throughput single cell RNA sequencing was conducted in root tissues of *Arabidopsis thaliana* exposed to B toxicity to analyze how the plant cell-specific response and differentiation processes were affected by B toxicity. After the protoplast was obtained from the roots, cell barcoding, library construction and sequencing was performed by using the droplet based, high throughput 10X Genomics Chromium platform. Single-cell transcriptomes from protoplasts of more than 2750 *Arabidopsis* root cells were obtained. Plotting the single-cell transcriptomes via t-SNE projections yielded six major cell clusters including quiescent cells (QC), endodermis, cortex, columella, trichoblast (root hair), and root cap. The maximum number of most significantly upregulated genes were determined in columella under 1 mM B and in endodermis under 2 mM B condition. We also showed that these upregulated genes were highly correlated with “glutathione metabolism” in columella and “carbon metabolism” in root cap. Our study provides a gene expression map at single-cell resolution and describes the extent of heterogeneity at the molecular level among populations of different cell types in *Arabidopsis* root under B toxicity conditions.

This study was supported by TÜBİTAK under the Grant Number 121Z029.



Nanoparticle-Mediated Gene Editing: A Path Towards Tailored Crop Improvement

Gülnur ŞENER¹, Ravzanur YAZICIOĞLU¹, Melike Çokol ÇAKMAK¹, Stuart J. LUCAS¹,
Nihal Öztolan EROL¹

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Changing climate conditions and dwindling resources pose significant challenges for agricultural production. However, advancements in agricultural technology, including gene editing, robotics, digitalization, and omics technologies, offer promising solutions to meet the growing food demand with precision and speed. The surge in biotechnology advancements has led to the development of transformative solutions for producing resilient and healthier plants. The CRISPR/Cas9 gene editing technique has revolutionized plant engineering, enabling the simultaneous editing of multiple genetic targets and facilitating transient gene expression. Previously, physical, chemical, and biological methods were used for vector transfer into plant cells. These methods are often invasive and cause cell damage or require plant tissue culture. Fortunately, the use of nanoparticles presents a more efficient and gentler approach. Nanoparticles smaller than 50 nm can bypass the plant cell wall entirely when directly applied to plant tissues, minimizing cellular damage during transformation. A study on *Cucurbita pepo* L. leaves demonstrates that gene delivery mediated by carbon nanotubes, carboxylated and functionalized with PEI, is a promising method to enhance the gene delivery methodologies on mature plants. This study has also opened the innovative design of bio-compatible nanoparticles that can be applied to many plant species. Further research on various nanoparticles is ongoing to optimize this approach for diverse applications in agriculture.

Keywords: *CRISPR/Cas9, single-walled carbon nanotubes, Cucurbita pepo L., gene delivery*



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Comparative Proteomic Analyses Revealed the Response Mechanism of Drought-Tolerant and Susceptible Olive Cultivars (*Olea europaea* L.) under Drought Stress and Recovery

Birsen CEVHER-KESKİN^{*1}, Bihter UÇAR^{1,2}, Merve ÖZTUĞ-KILINÇ³, A. Hediye SEKMEN-ÇETİNEL⁴, Nurcan ULUÇAY⁵, Ünal KAYA⁵

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Drought is one of the most important abiotic stresses that cause adverse effects worldwide and reduce crop yields. Investigating the molecular basis of the drought tolerance mechanism in plants is essential in creating a new perspective, especially in breeding strategically important crops, increasing agricultural yield, defining the stress resistance level of cultivars, and revealing their molecular mechanisms. Olive (*Olea europaea* L.) is one of the major crops in the Mediterranean region, traditionally grown under drought conditions. Global climate warming and drought significantly affect the production of this important strategic plants. The objective of this study is to characterize the drought stress tolerance mechanism of the olive plant (*Olea europaea* L.) at the proteome level. Liquid chromatography-mass spectrometry (LC-MS/MS) was used to identify protein differences caused by drought stress in drought-susceptible (Gemlik) and tolerant (Çekişte and Memecik) olive cultivars. LC-MS/MS analysis detected 905 protein groups and 1753 peptide groups after Sequest HT scanning using Proteome Discoverer Software. According to the results, 150 proteins were detected in the drought-sensitive cultivar, which increased at a high rate (2-fold) after drought stress application, and 257 and 191 proteins were detected in the drought-tolerant cultivars (Çekişte and Memecik), respectively. It was determined that 67 proteins in drought-sensitive cultivars and 34 and 51 proteins in drought-tolerant cultivars decreased at a high rate (0.5-fold). In the recovery groups, it was determined that the number of increasing proteins decreased while the number of decreasing proteins increased. It was determined that more protein was increased in the sensitive cultivar (88) than in the tolerant cultivars (69 and 57). Furthermore, tolerant cultivars have a significantly lower amount of protein (111 and 179) than sensitive cultivars (104).

According to the gene ontology analysis results obtained, an increase in protein was detected in all three main independent sub-ontologies as a result of drought stress application in all cultivars. At the same time, a decrease was determined in the recovery groups. This study we have conducted is essential in revealing the molecular basis of the drought tolerance mechanism in the olive plant, one of the crops of strategic importance, and creating a new perspective in defining the stress resistance level of the cultivars.

Keywords: Olive (*Olea europaea* L.), drought, proteomic, LCMS/MS, recovery



**Exploring Aquaporins and Dehydrins in Barley Under Drought and Salt Stresses
during Early Seedling Development**

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Abiotic stresses are considered key crop yield reduction factors in agriculture, and drought and salt stresses are primary abiotic factors causing crop losses. Various studies in different plant species have shown contribution of aquaporin and dehydrin genes to abiotic stress response, in particular water related stresses. Barley (*Hordeum vulgare* L.) has been long domesticated by humanity and is currently an agriculturally important grass that is used in the malting industry and as animal feed. However, changing environmental conditions are endangering the continuity of barley grain production. Therefore, in our research, roles of representatives of aquaporin and dehydrin gene families in barley during selected water stresses are enlightened. Stress answer potentials and aquaporin and dehydrin gene expression under stress conditions are studied with agriculturally important Turkish barley varieties during early seedling development. Thus, the investigation conducted in this study presents valuable findings into the behavioral patterns of the studied proteins that explain certain characteristics of water stress responses in barley. The results contribute to a better understanding of the molecular mechanisms and behaviors of such proteins under different types of abiotic stresses, which can allow studying and analyzing genes that are involved in abiotic stress tolerance mechanisms in this agriculturally important food and feed crop.

Acknowledgement: This project was supported by the Scientific Research Projects Commission of Bahçeşehir University BAP.2023-01.28 project.

Keywords: *Barley, salinity, drought, aquaporins, dehydrins*



Valorization of Steel Slag as a Safe Soil Amendment for Sustainable Crop Production

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The studies related to the evaluation of steel slag have accelerated in recent years due to the promising physicochemical characteristics of the material for agricultural use, particularly for fertilizer and soil amendment purposes. In this study, the effects of electric-arc-furnace slag on different parameters including growth, yield, and nutritional value of bread wheat (*Triticum aestivum* cv. Nusrat) and lettuce (*Lactuca sativa* cv. Papyon) have been investigated. Model crops have been grown on acidic or alkaline soil with standard vs. half-strength fertilization under greenhouse conditions. The impacts on soil fertility and safety parameters as a response to steel slag amendment were also documented in an independent soil incubation trial. In acidic soil, steel slag amendment significantly enhanced wheat and lettuce growth by up to 400% and 500%, respectively. Steel slag provided effective zinc fertilization in alkaline soil. The effective boron fertilization was also achieved by steel slag treatment in both soil types. Grain yield and grain number of wheat grown on acidic soil drastically increased, particularly under half-strength fertilization, which suggests the beneficial role of steel slag in terms of improvement in fertilizer use efficiency. The Zn concentration of wheat grain grown on alkaline soil was significantly enhanced whereas there was no increase in Cr, Cd, Pb or V concentration in either crop. Soil incubation trial showed that steel slag incorporation with soil significantly elevated pH, and bioavailable Fe, B, and Zn, regardless of soil types. The results demonstrated that soil applications of steel slag can be an effective strategy in increasing grain yield and nutritional quality of wheat and harvestable biomass of lettuce, which can be explained by improvements in soil properties. Steel slag treatment also showed no accumulation of toxic metals in soil, indicating the potential of steel slag use in agriculture without causing any environmental concerns or food safety issues. Further investigation is required to collect more comprehensive data on long-term risk assessment of steel slag utilization under field conditions.

Acknowledgement: This study was supported by Materials Testing and Innovation Laboratories Co. (MATIL).

Keywords: Fertilizer use efficiency, lettuce, plant growth, steel slag, soil amendment, sustainability, wheat



**Lettuce Biofortification with Selenium and Its Interactions with Sulfur Nutrition in an
Indoor Vertical Nft System**

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Selenium (Se) biofortification of vegetables is a viable technique for improving the nutritional content of vegetables and increasing the daily Se intake in human populations. Vegetable production in indoor vertical farms is increasingly preferred due to enabling local, year-round, and pesticide-free production with low water footprint and high fertilizer use efficiency. However, biofortification studies have been mostly conducted under field or greenhouse conditions. Agronomic biofortification of vertically grown crops is a niche research area. The objective of this research was to investigate the effects of selenium addition on lettuce (*Lactuca sativa* cv. Levistro) yield and nutritional quality parameters at two different sulfur (S) levels in an indoor NFT system. In a factorial design, plants were grown at 2 Se (0-2 µM) and 2 S levels (0.65 and 1.3 mM). Plants were harvested 28 days after sowing as it is also the common practice in the commercial farms of Plant Factory. The fresh weights of plants grown under high S conditions were approximately 14% higher than the fresh weights of plants growing under low S conditions. Selenium fertilization under low S conditions caused a statistically significant increase in fresh weight, but under high S conditions, Se supplementation did not significantly affect the fresh weight. In Se-fertilized plants, leaf Se contents per serving were able to provide 111% and 44% of the recommended daily intake (RDI) of Se for adults under low and high S conditions, respectively. Although tissue nitrate concentrations increased by Se application under high S conditions, they were at a nutritionally safe level in all treatment groups. The antioxidant capacity of lettuce was evaluated by FRAP and DPPH assays. The highest total antioxidant capacities were observed in plants without Se supplementation under low S conditions whereas Se fertilization high S application had a negative effect on the antioxidant capacity of lettuce. According to our findings, the biofortification of lettuce with Se in an indoor vertical NFT system requires taking S into account to optimize the treatments and reach safe target concentrations that can have a positive impact on public health.

This study was supported by Plant Factory (<https://plantfactory.company>). I also would like to thank YOK 100/2000 scholarship program.

Keywords: Antioxidant, biofortification, lettuce, nutritional quality, RDI, selenium, sulfur



Cloning the SARS-CoV-2 Structural Proteins for Transient Expression of a VLP-based Vaccine Candidate in *Nicotiana benthamiana*

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Serious effects of Coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been mitigated through employment of a wide range of vaccines worldwide. However, the equal distribution of the COVID-19 vaccines is still challenging especially in developing or underdeveloped countries. Thus, development of economic and scalable vaccine platforms has a great importance in global manner. Currently, plant-based vaccines are among the most promising platforms in terms of low production cost, scalability, and fast manufacturing process. In this study, our aim was to express all the structural proteins of SARS-CoV-2 transiently in plant as a first step of developing a virus like particle (VLP)-based vaccine candidate that may exhibit a broad range of antigenicity with the highest similarity to the structure of SARS-CoV-2 and thereby, that may protect against new variants as well. For this purpose, the expression products were directed to two different compartments (endoplasmic reticulum; ER and chloroplast) at the cellular level in *N. benthamiana* leaves and examined at the transcriptional level. The genes encoding structural proteins [Spike (S), Membrane (M), Nucleocapsid (N) and Envelop (E)] of SARS-CoV-2 were synthesized commercially after codon optimization. To direct compartmentalization, different signal sequences were used during the design process. The gene cassettes were cloned into plant expression vector, pEAQ-HT, separately. Each plasmid carrying gene of interest (GOI) was transformed into *Agrobacterium tumefaciens* by electroporation. The leaves of approximately four-week-old *N. benthamiana* plants were co-infiltrated with *A. tumefaciens* cells harboring these plasmids. As a control, leaves were infiltrated with pEAQ-HT-GFP containing *Agrobacterium* cells in parallel and visualized under UV 3-, 5- and 7-days post-infiltration (DPI). The expressions of GOIs were determined by conducting RT-PCR following mRNA extraction from the leaves which were harvested 3- and 5-DPI. Cloning of GOIs were validated by sequencing and alignment analysis. Necrosis around the infiltration sites in the leaves were observed at the end of 7 days regardless of which construct infiltration medium contains. According to the GFP fluorescence, 5- and 7-DPI showed similar expression levels. mRNA expressions of GOIs were confirmed by RT-PCR. In this study, transient expression of structural genes of SARS-CoV-2 was shown in *N. benthamiana* as a first step of vaccine candidate development through plant expression system. To the best of our knowledge, this is the first effort for production of plant-based VLPs covering all the structural proteins of SARS-CoV-2. Supported by TÜBİTAK under the Grant Number 122Z123.



Evaluation of Plant Growth Promoting Rhizobacteria Isolated from Soils of Türkiye for Microbial Biofertilizer Production

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Sustainable agriculture sits in the very heart of any sustainability discussion because food is one of the most fundamental human needs and practices that contribute to the sustainability of crop production can take us closer to multiple sustainable development goals (SDGs) at the same time, including but not limited to no poverty, zero hunger, good health and even peace. While microorganisms were once considered mostly in terms of their pathogenicity risks in agriculture, the field of plant-microbe interactions has quickly evolved in recent years to focus on beneficial soil microbiota. Plant growth promoting rhizobacteria (PGPRs) are symbiotic or non-symbiotic bacteria that can contribute to plant nutrition, growth, abiotic stress tolerance and disease resistance when found in elevated numbers in the rhizosphere. They can be used to formulate bio-based and eco-friendly products that can reduce the dependence of conventional crop production on chemical inputs such as chemical pesticides and fertilizers. GUBRETAS R&D Center has focused on PGPR research since its establishment, with the aim of discovering PGPRs and formulating them into novel products. Within the scope of these studies, the first step involved the collection of rhizosphere samples from different locations in Türkiye. Then, soil bacteria were isolated from these samples. These bacteria were later screened for plant growth promotion (N fixation, P and K solubilization, siderophore production and IAA production) and stress tolerance-related (salinity tolerance, ACCD production) traits through various biochemical characterization tests. Identification of all promising isolates was conducted by using MALDI-TOF MS. Promising rhizobacteria were selected for greenhouse experiments and field trials that were conducted at GTU. Effects of *Bacillus pumilus* (RC22, RC49, RC67, RC356) and *Bacillus subtilis* (RC631) on growth and yield parameters as well as the nutritional quality of wheat (*Triticum durum*) and rapeseed (*Brassica napus*) were evaluated in greenhouse experiments and significant positive results were observed in both species. In a field trial, *Bacillus megaterium* (RC67) and *Bacillus subtilis* (RC631, GBF79) were tested on tomato (*Solanum lycopersicum*). Formulation and scale-up operations continue one hand while the entire process is iterated for new local PGPR isolates on the other hand.

This study was supported by TÜBİTAK 2244 - Industrial PhD Fellowship Program (Project 119C030) and GÜBRETAS.

Keywords: Microbial biofertilizers, plant growth promoter, rhizobacteria, bacteria characterization, greenhouse experiment, field trial



Exosomes: The Rising Star in Medicine

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Exosomes are a population of extracellular vesicles (EVs) secreted by most cell types through the endocytic pathway. They are produced in the endosomal compartment of most eukaryotic cells, including dendritic cells, macrophages, various kinds of stem cells, and even cancer cells. Once produced, exosomes are subsequently released into the extracellular space by fusion with the plasma membrane. Studies have shown that exosomes are involved in several paracrine and intracellular signaling pathways, including transcription, proliferation, and differentiation. Their contents can be different based on their cellular origin. However, they generally contain proteins, nucleic acids (especially miRNAs), and lipids.

Also, the application of these particles as a cell-free therapeutic option in regenerative medicine has been investigated by previous studies, reporting their remarkable therapeutic advantages.

During regenerative medicine, cell-free tissue engineering eliminates the need for externally seeded stem cells and is performed using scaffolds containing certain particles as the main requirement for tissue regeneration. Wound healing, cancer treatment, neurological, cardiovascular, skeletal disorders, it can be used.

The unique properties of exosomes make them highly effective drug carriers and diagnosis. The exosome lipid bilayer is highly asymmetrical, which could be particularly advantageous for their interaction with the plasma membrane and especially with their target cells. Several studies have explored the potential of stem cell-derived exosomes [1] for alleviating skin aging. This, along with advantages over traditional pharmaceutical nanocarriers, distinguishes exosomes as a rising star in both therapeutics and diagnostics.

Keywords: *Exosome, cell-free, diagnosis, drug delivery, medicine*



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New Advances in Exosome-Based Targeted Drug Delivery Systems

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Exosomes are the smallest of the extracellular vesicles and are formed by fusing with the plasma membrane. Exosomes are delivered from the cell by exocytosis and are readily detected in the circulation by CD63, CD81 and CD9. Once exosomes have been isolated, they can either be used for biomarker studies or they can be preferred in treatment studies due to the transport of biomolecules in their structure. In recent years, exosomes have been the focus of attention in drug delivery and drug release studies, where they have the advantage of high throughput. They have advantages due to their high biocompatibility. However, they have disadvantages in terms of isolation and quantity. Exosome-related issues, from industrial production and drug stabilization to sustainable product development, are among the main topics of research in this area. In order to ensure standardization in exosomal drug delivery systems and to make drug loading efficient, our studies compare different methods. Generally, incubation, electroporation, sonication, transfection and freeze-thawing are commonly used to load drugs into exosomes. Microfluidic systems are a great advantage in our drug loading methods for isolated exosomes, as they allow us to keep both the size and the amount of drug loading constant, but microfluidic systems are not yet sufficient for large-scale laboratory production. For optimal exosome loading, what is loaded really depends on the cargo molecule and the method must be carefully chosen. At present, the lack of standardization in exosome isolation, characterization and storage techniques should be considered in the potential impact on many drug properties, such as loading capacity, drug retention and changes in the structural properties of exosomes. Although the potential of exosome research is enormous, it is the innovative approaches that provide the opportunities to make this field a reality at the point of biotechnological drug development.

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Keywords: *Exosomes, Drug delivery, Drug loading, Microfluidic Systems, biotechnological drug*



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Exosomes as Smart Delivery Systems in Nanotheranostics: Opportunities and Challenges

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Multifariousness among cells provides the ability of rapid adaptation to internal and/or external stress conditions. However, this diversity ensures also multifaceted variability of enzymes, organs, systems, etc., instigating mayhem to cure diseases in medicine. The heterogeneity that emerges from phenotypic or genotypic diversity boosts or mitigates the therapeutic or diagnostic efforts in disease treatments. Ever-progressing nature of nanotechnology offers novel solutions to the challenges associated with chaotic environments in living organisms. Stimuli-responsive targeted delivery of therapeutic agents could aid in minimizing off-target toxicities and increase efficacy via enhanced permeability. Exosomes are nanosized subpopulations of extracellular vesicles originating from cell membranes and are promising biocompatible sources to serve as scaffolds for engineering these smart delivery systems. Low toxicity, low immunogenicity, and versatility in carrier properties make them excellent candidates for next-generation delivery systems in nanotheranostics. The scope of this presentation is to provide a comprehensive literature review on the utilization of bioresponsive exosome-based smart delivery platforms. The review enlists state-of-the-art strategies for targeted delivery of distinct cargo such as drugs, nucleic acids, and proteins. The advantages and challenges are critically discussed to evaluate the opportunities it grants for next generation theranostic applications. The potential solutions to overcome these limitations are debated to investigate the feasibility of exosome carriers in patient-centered diagnosis and treatment in terms of stability, specificity, compatibility, toxicity, ease of production, and cost-efficiency parameters.

Keywords: *Exosome, smart delivery systems, nanotheranostics, personalized treatment*



Wheat Breeding for High Amylose Content for Better Human Health

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Wheat, serving as a staple for billions worldwide, is becoming more important with increasing rate of diabetes. High amylose wheat (HAW) is considered as a potential remedy against type 2 diabetes and obesity. Trait introgression (TI) through traditional methods is laborious, sensitive, resource intensive and time consuming. Here, we report a rapid introgression of high amylose trait to Pakistani wheat varieties through Marker-Assisted Speed Breeding (MASB). The integration of KASP genotyping with speed breeding method facilitated precise trait selection and accelerated breeding cycles. The resulting *SBEII* mutant line exhibited substantial increase (73%) in amylose content in comparison to the wild-type control. The MASB with KASP expedited trait introgression, reducing the number of back-crossing from 5 to 3 and shortening the time frame from 6-7 years to 2.5 years. This study exemplifies the acceleration of trait introgression into our elite cultivars through MASB.



Genome Editing in Plants: Challenges and Opportunities

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After ensuring food security for several decades, the green revolution has gained its potential and is reaching its biological boundaries which is reflected by the stagnation in yield. Meeting the growing food demands of the masses, due to increasing population, is the biggest challenge for plant scientists. CRISPR-edited crops hold tremendous potential for food security and global agriculture. CRISPR technology has become a new face of genome editing with rapidly increasing applications in academic research (functional genomics and reverse genetics) as well as applied research for developing crop varieties with new or improved traits. After successful deployment of gene editing technology to modify simple traits, more ambitious ventures to combat food security challenges in the wake of growing world population with changing climate are in progress. CRISPR may be used in several ways to develop resistance in plants; targeting host susceptibility genes/factors, inserting resistance genes, activating host immunity, breaking host-pathogen cross-talk/interaction etc. We have used CRISPR system against bacterial and viral disease and found promising results. In case of bacterial disease, bacterial leaf spot, we targeted susceptibility gene with CRISPR to enhance plant immunity against the disease in tomato, while in case of viral diseases (caused by begomoviruses), we targeted several viral genes to develop resistance. It was found that targeting multiple genes simultaneously may provide resistance up to 70-80%. Moreover, delay in symptoms, low virus titer and attenuated symptoms were observed on the CRISPR plants. Initially, we screened all gRNAs in the model plants; *Arabidopsis* and *Nicotiana benthamiana*. Later, we expressed selected gRNAs-CRISPR, transient and stable expression, in tomato and cotton. CRISPR technology has a potential to be used for genetic improvement of plants to ensure food security and safety. Moreover, producing DNA-free, non-GMO plants with CRISPR is an attractive approach for breeders of all regions of the world.

Keywords: *Plants diseases, CRISPR/CAS, GMO, new breeding techniques, climate-smart crops, diseases resistance, food security*



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Hydrogen Peroxide-Induced Stress Acclimation in Plants

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Among all reactive oxygen species (ROS), hydrogen peroxide (H₂O₂) takes a central role in regulating plant development and responses to the environment. The diverse role of H₂O₂ is achieved through its compartmentalized synthesis, temporal control exerted by the antioxidant machinery, and ability to oxidize specific residues of target proteins. Here, we examine the role of H₂O₂ in stress acclimation beyond the well-studied transcriptional reprogramming, modulation of plant hormonal networks and long-distance signaling waves by highlighting its global impact on the transcriptional regulation and translational machinery.

Keywords: *Chromatin remodeling, oxidative posttranslational modifications, redox signaling, stress priming.*



Potential of dsRNA Technology for Better Crop Improvement

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Potato is believed as a strategic crop from food security perspective. However, the crop is constantly exposed to the threat of insect pests and diseases. Among these pests, Colorado potato beetle (CPB) is notorious pest, larvae and adult incur significant yield losses and even result in zero economic yield in certain circumstances. Furthermore, CPB has developed resistance against chemical insecticides that are widely adopted worldwide. There is a dire need to develop an alternate approach to control CPB. RNA interference (RNAi) serves as a versatile genetic tool capable of selectively inhibiting the expression of specific genes, providing a promising avenue for preventing crops from being infested by insect pests and diseases. In our recent studies, we employed RNAi against Colorado Potato Beetle (*Leptinotarsa decemlineata* Say) and achieved promising results. Our results revealed that RNAi increased mortality rates, significant effects on larval weight gain, reduced foliage consumption, and suppression of egg-laying behaviour in *L. decemlineata*. The targeted downregulation of gene expression, as evidenced by reduced mRNA levels, confirmed the efficacy of RNAi. Overall, the studies showed that employing RNAi technology has the potential to effectively control this devastating potato pest by providing a targeted approach to suppress its population.

Keywords: *Insect pest, dsRNA technology, lethal and sub-lethal effects, CPB*



Genomics-Enabled Wheat Breeding for Improved Productivity and Nutritional Quality

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Improving the crop productivity, resilience to climate extremes, resistance to biotic stress and improving the quality are the main breeding objectives. Different tools, resources and strategies are used to precisely select the desirable cultivars in crop breeding. One of such tools is the genomics-assisted breeding (GAB), which improves selection accuracy during breeding cycles. However, practicing GAB depends on the availability of molecular markers for selecting the desired phenotypes. Once a marker is available for use in breeding, the efforts are then made to make it cost-effective and high throughput to integrate its use in applied breeding. However, different breeding scenario like gene tagging, marker-assisted recurrent selection (MARS), background selection, diversity estimates, and genomic selection require different genotyping platforms, and there is no 'one size fits all' solution. We provided an overview of the efforts around developing cost-effective, high-throughput and breeding-oriented genotyping platforms in wheat. A successful genotyping platform would have the features of high genome coverage, least ascertainment bias, high power in gene discovery studies, balance between throughput and flexibility, provide high prediction accuracy in genomic selection, and, above all, affordable for most of the crop breeding programs.



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Biologically Active Molecular Signatures of Plant Origin: Antimicrobial Proteins and Peptides

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Plants produce a variety of biologically active molecular signatures in response to various biotic and abiotic stresses. These signatures include variety of transcription factors, enzymes, peptides and proteins. Some examples include WRKY TFs, NB-LRRs, MAP Kinases, CDP Kinases, Pattern Recognition Receptors (PRRs) for different PAMPs/ MAMPs/ DAMPs; Plant antimicrobial Peptides and Proteins such as Defensins, Defensin-like peptides (Def-L), Thionins, Thionin-like peptides (Thi-L), Snakins, Cyclotides, Lipid Transfer Proteins, Albumins, Globulins and Chitinases. Here we present 5-years-progress capsule of our research group that includes isolation, identification, purification, characterization, structure prediction, transient expression and in vitro as well as in planta antimicrobial and insecticidal activity assays of some defensin-like peptides and thionin-like peptides of Arabidopsis thaliana origin recombinantly expressed in Nicotiana benthamiana leaves; a Chitinase “GgChi” isolated from Galdiolus grandifloras seeds; an 11S-Globulin/Cruciferin “CsCr” isolated from Cucumis sativus; a 7S-Globulin/Vicilin and a 2S-Albumins/Napin isolated from seeds of Cucumis melo var. momordica and couple of other proteins isolated from the seeds of Acacia nilotica and Brassica napus. This capsule will provide significant updates of various biologically active plant molecular signatures which play important role in plant innate immunity and defense.

Keywords: Antimicrobial peptides; antimicrobial proteins; albumin; cruciferin; cysteine-rich peptides; chitinase; defensin-like peptides; globulin; napin; thionin-like peptides; transient expression; vicilin



Utilization of Bioreactors for Morphine Production in the Opium Poppy

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Papaver somniferum L. (opium poppy) is a species of the *Papaver* genus and mostly produced for its seed oil and opiate content. Opiates (mostly morphine, codeine, noscapine) are mostly located to capsules and very important substance are of medicine. Opium poppy cultivation and opiate production is restricted and controlled by United Nations by law. Türkiye is one of the major producers of opium poppy.

Climate changes and its effects on agriculture are one of the global major concerns on agriculture of plants produced for food, medicine, cosmetics, industry etc. Abiotic factors which are the results of climate effects such as drought, heat and salinity are threatening conventional agriculture methods. Thus, trends of development and optimization technological alternatives for cultivation (such as hydroponic methods) and metabolite production in industry (bioreactors etc.) are increasing rapidly. These methods provide risk free, soil independent and sustainable production of plants and their metabolites. Bioreactors offer a promising alternative for producing plant metabolites in a controlled and efficient manner. By optimizing culture conditions and controlling the production of target metabolites, bioreactors can provide equal or higher yields and more consistent quality of plant metabolites than traditional methods of production.

Seeds are provided by Turkish Grain Board and used as plant explant source. Within the scope of this project, we were able to produce morphine alkaloid using cell suspension cultures derived from somatic cells in bioreactor systems. HPLC analysis confirmed that the amount of morphine produced using bioreactors was the same as the production of poppy capsule in the field with conventional methods. Moreover, bioreactor produced material is classified as natural because product is still plant based. With the method we have optimized, it will be possible to produce standardized, continuous and reproducible opiate content independent of external factors (soil, climate, pests) by using bioreactor systems in industrial scale.



Alternative Proteins as Functional Solutions: Their Sources and Applications

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The global demand for proteins which is expected to rise by 70% by 2050 (FAO, 2020) has led to exploration of alternative proteins. Alternative proteins derived from various sources such as legumes, oilseeds and cereals has garnered an attention for their versatility, applicability, and potential health benefits to be used in food and beverage applications. The mostly used application area of them are in the formulation of dairy alternatives and meat analogues where they provide consumers options that meet their dietary preferences and restrictions. In bakery products such as bread and biscuits, they contribute to improved crumb structure, volume, nutritional profile, and overall quality of the product. Due to their functional properties such as solubility, emulsifying and foam stabilizing, they are used in the beverage industry in a variety of beverages such as protein shakes, sports drinks, and meal replacements. In addition to plant proteins, emerging sources such as microalgae, fungus, and insects are considered to be comparable to the standard reference proteins regarding the amino acid quality and techno-functional properties. As edible proteins, commercial applications of these sources are still limited and need improvement due to their poor palatability, sensory properties, and consumer acceptance. This abstract provides an overview of the applications of alternative proteins, highlighting their importance in nutritional fortification and formulation flexibility. From the perspective of environmental sustainability and food security, there is a pressing need to improve the utilization of alternative proteins as human food. Despite their functional benefits, challenges such as flavor masking, texture modification and cost optimization remain as the areas for further development. Future studies should focus on optimizing extraction methods, improving flavor profiles, and exploring novel applications in food and beverage formulations.

Keywords: *Alternative proteins, sustainability, applications, flavor, consumer acceptance*



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Bio-Mimicking with Membrane Proteins for Biotechnological Applications

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Nature-friendly and nature-based technologies are regarded as one of the most attractive ways of smart and sustainable material design. Integration of cellular molecules into the structures of synthetic or man-made materials is a chance for design of composite materials mimicking natural functions. Integral membrane transport proteins (MTPs), including aquaporins, channels, and carrier proteins, play critical roles in the cell such as pathogenicity, antibiotic resistance, and survival. The discovery of novel transport proteins with new functions both extends the knowledge of membrane trafficking and increases the biotechnological application areas of channels and transport proteins. The mining and screening of these proteins in a large transporter library, the molecular dynamic simulations of particular transporters, and the wet-lab studies resolving the functions of membrane transporters result in rapid advancement in their applications in different fields including bioremediation, drug targeting, fermentation process improvements, biosensor fabrication, and biomimetic filtration membranes. This study presents a brief history on biotechnological application areas of membrane transport proteins while pointing specifically to aquaporins and nitrite/formate channels. Our recent findings showed that aquaporin of the halophilic bacterium *Halomonas elongata* has a higher capacity for salt rejection as compared to the *Escherichia coli* aquaporin, which makes it an attractive alternative for the preparation of biomimetic filtration membranes with improved water filtration capacity. Not only the rejection capacity but also the solute specificity of these channel proteins is inspiring for new biotechnological applications. For example, the nitrite channel (NirC) of *E. coli* included in liposome-based nitrite biosensors has the potential to increase the specificity of the biosensor to nitrite due to the easy entry of nitrite molecules through these channels. The fascinating properties of membrane carrier proteins will open the doors to many more biotechnological applications that can be used to solve problems encountered in the environment, health and industry.

Keywords: Membrane transport proteins, bio-mimicking, aquaporins, formate/nitrite channels



mRNA-based Cancer Vaccines

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Thanks to its favorable characteristics, mRNA is a unique tool for antigen-specific activation of the immune system. Vaccination with antigen-coding mRNA has been shown to be capable of efficiently inducing T and B cell responses paving the way for the first mRNA-based vaccine against SARS-CoV-2. mRNA-based vaccines are currently being tested in several clinical trials with promising results for various indications. This lecture will summarize the evolution of this versatile platform and its use against cancer.



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Therapies Against Mitochondrial Diseases

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Mitochondria constitute the major energy plant of the cell; genetic defects of mitochondrial bioenergetics cause an astonishingly wide spectrum of diseases. The genetic and biochemical complexity of mitochondria pose a major obstacle in devising targeted therapies for these conditions. Despite the absence of a definitive cure, supportive interventions have demonstrated efficacy in managing symptomatic manifestations. This presentation will delve into the ongoing landscape of therapeutic strategies aimed at addressing mitochondrial diseases.



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Characterization and Investigation of Biological Effects of the Microalgae-Based Microcarrier Systems Produced by Electrospraying Technique

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Spirulina, renowned for its rich nutritional content and sustainable cultivation, presents itself as a promising microcarrier for biotechnological applications. However, its inherent non-controllable degradability poses a challenge to its utility. Polylactic Acid (PLA) emerges as a viable solution to address the limitation, offering biodegradability and biocompatibility. Leveraging electrospraying technology, precise control over microparticle properties such as size, shape, and composition is achieved through the manipulation of parameters, including voltage, flow rate, and solution characteristics. In this study, Spirulina and PLA-based microparticles were fabricated via electrospraying techniques to serve as microcarriers. Various formulations were synthesized, including 3% PLA, as well as blends of Spirulina and PLA at concentrations of 0.125%, 0.25%, and 0.5%. Characterization of the microparticles confirmed the presence of Spirulina. Assessment of the biocompatibility of these microparticles was conducted using HUVEC and HaCaT cell lines through MTT assays, colony formation assays, cell survival assays, and fluorescence imaging. The results suggest that these microparticles exhibit promising properties as microcarrier systems across a broad spectrum of applications.

Keywords: *Spirulina, PLA, electrospraying, microparticle, microcarrier system.*



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The Importance and Emotional Management of Distant Learning Platforms for Life Sciences – Lessons from the Yes4elearning Erasmus+ Project

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COVID-19 pandemic has affected not only our health but also work and education capabilities throughout the world. Education at all levels were primarily conducted online throughout this period, which affected students and teachers alike. The sudden transition required not only technical capacity and infrastructure, such as software, hardware, data connection, education material suitable for online learning and teaching, but also required emotional management on both students' and teachers' side. This was particularly important for higher education that required practical training and human interactions, such as undergraduate laboratory work or graduate thesis studies in life sciences, including molecular biology and genetics, biochemistry, biotechnology, bioengineering and other similar disciplines. Erasmus+ project Yes4eLearning focuses on developing skills, knowledge and attitudes of higher education academics and workers so as to increase the quality of online courses, technical and emotional adaptation to e-learning, and to raise awareness. This is particularly important in the face of the university education concept that is currently under questioning and is expected to change and evolve in the near future. In this talk, we will introduce the assessment tool and current results of the Yes4eLearning project and discuss how the changing vies on higher education and how the integration of distant learning to all higher education curricula will affect higher education in the field of biotechnology and life sciences.

We would like to acknowledge the Erasmus+ project grant no 2023-1-LT01-KA220- HED-000166708 for funding, and our project consortium partners for the collaboration.

Keywords: *Biotechnology, life sciences, distant learning, e-learning*



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The Role of the Fgf1/Pde4d Pathway in the Regulation of Lipolysis, Glucose Levels, and Insulin Resistance

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Increases in insulin resistance, lipolysis, and hepatic glucose production (HGP) are hallmarks of type 2 diabetes. Previously, it was shown that Fibroblast growth factor-1 (FGF1) has anti-diabetic effects mediated by the adipose FGF receptor 1. In our current study, we found that FGF1 lowers HGP by suppressing adipose lipolysis.

Mechanistically, FGF1 inhibits the cAMP-protein kinase A axis by activating phosphodiesterase 4D (PDE4D), distinct from insulin's inhibitory actions via PDE3B. We identified Serine-44 as an FGF1-induced regulatory phosphorylation site in PDE4D which is also modulated by feeding/fasting.

Our findings establish the FGF1/PDE4 pathway as an alternate regulator of the adipose-HGP axis and identify FGF1 as a regulator of fatty acid homeostasis



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Role of Autophagy in Carcinoma-Associated Fibroblasts and Breast Cancer Metastasis

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Tumors do not only consist of cancerous cells, but they also harbor several normal-like cell types and non-cellular components. Carcinoma-associated fibroblasts (CAFs) are one of these cellular components that are found predominantly in the tumor stroma. Autophagy is an intracellular degradation and quality control mechanism, and recent studies provided evidence that autophagy played a critical role in tumor-stroma crosstalk. However, communication with surrounding cells and the mechanism of autophagy stimulation in the stroma are not fully documented. For this purpose, we first focused on CAFs and their role in tumor stroma. Our previous data showed that CTF1 (Cardiotrophin 1) that was secreted from cancer cells activates autophagy in fibroblasts and breast cancer-derived CAFs. We also discovered that CTF1 acted as an important tumor-derived factor regulating breast cancer cell migration and invasion (1). In line with these in vitro observations, we discovered that elevated levels of CTF1 in patient-derived tumors showed a positive correlation with lymph node metastasis (1). Our current analyses revealed that loss of autophagic activity in fibroblast causes diminished activation and trans differentiation of CAFs, and alteration in fibroblast activation signaling. Therefore, our results suggest that CTF1 is a crucial player in stromal autophagy, fibroblast activation and signaling.

This study was supported by TÜBİTAK-1001 project 122Z227.



Development of a Car-T Cell Therapy System Targeting Cd19 for the Treatment of All and Nhl

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Cancer is one of the leading causes of death worldwide. Majority of cancers are treated through surgery, chemotherapy, and radiotherapy; however, their effect is limited. Recently, a number of promising new treatment options such as targeted therapy and immunotherapy has become available. Although the use of immune cells against cancer have been subject of research for many years, it has started to be used effectively in clinical practice only in recent years. Immunotherapy have become an effective treatment option for patients resistant to conventional therapies, especially when the right candidate is selected. One of the new generation therapeutic approaches aiming at treatment efficacy with minimum side effects and maximum safety is the creation of a genetically engineered chimeric antigen receptor (CAR) on the surface of the T cells (or NK cells) using synthetic biology techniques. CAR-T receptors are formed through an antigen recognition extracellular scFv, transmembrane domain, and intracellular signalling motifs. A number of CAR-T cell therapies have been approved for the clinical use recently for the treatment of acute lymphoblastic leukemia (ALL), non-Hodgkin lymphoma (NHL), and multiple myeloma (MM). We have developed a 3rd generation CAR-T cell therapy system targeting CD19 for the treatment of ALL and NHL. The system is composed of CD8 alpha signal peptide, FMC63 scFv-light chain, whitlow peptide, FMC63 scFv-heavy chain, CD8 alpha hinge domain, CD28 transmembrane domain, and intracellular domains of CD28, 41BB, and CD3 zeta. The design synthesised and cloned into a lentivirus expression vector (pCL10.1) and packaged through a 4th generation packaging system. Lentiviral particles carrying CAR receptor were produced, concentrated to 10⁸ particle per mL and stored at -80°C until use. JURKAT T cells were transduced with lentiviral particles and CAR expression was detected at the surface of the cells. Co-culture studies with CD19 expressing NALM-6 B cells showed a great cytotoxic effect on the cancer cells. After completing characterisation studies, we aim to take the product to the Phase I clinical studies. In conclusion, we have developed a 3rd generation CAR-T cell therapy system targeting CD19 for the treatment of ALL and NHL.



**Antioxidant and Antimicrobial Activity of Grape Seed and Skin Extracts from Four
Grape Varieties**

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Research on the application of grape extracts, as alternative antioxidants and antibacterial agents in foods is constantly growing. The aim of this study was to identify and quantify polyphenolic compounds in skin and seed extracts from four Bulgarian grape varieties – Marselan, Pinot Noir, Cabernet Sauvignon and Tamyanka. The values of total phenolic contents, flavonoids, anthocyanins, procyanidins and an ascorbic acid in grape skin and seed extracts were determined. The antioxidant capacities of skin and seed extracts were evaluated by four different methods. The total phenolic content of skin extracts were 2-3 times lower than those of seed extracts. The significant difference between total parameters values of individual grape varieties were also found. The individual compounds in the grape skin and seed extracts were determined by RP-HPLC. The determined composition of skin extracts was significant different from the seed extracts composition. A correlation between phenolic contents, individual compounds and antioxidant capacity of different extracts was found. The antimicrobial activity of the obtained grape seed extracts to *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli* was evaluated by the agar diffusion test and a test to determine minimum inhibitory concentration (MIC). According to the effect on the growth of pathogens, the extracts were ranked in the following order: Pinot Noir > Marselan > Cabernet Sauvignon > Tamyanka. The tested bacteria showed high sensitivity against the extracts (MIC = 0.12 ÷ 0.50 mg mL⁻¹). According to the MIC values, the bacteria were in the order: *Staphylococcus aureus* > *Bacillus cereus* > *Escherichia coli*. The obtained results show that the studied grape seed and skin extracts have good potential as antioxidant and antimicrobial agents.

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Artificial Intelligence in Food Production

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The food production industry is experiencing innovative transformations with the rapid advancement of technology. Among these transformations, the integration of artificial intelligence (AI) technologies stands out as the most impactful. AI is redefining industrial food production by optimizing traditional production methods and increasing efficiency. But how is AI utilized in food production? Artificial intelligence in food production, data collection from sensors, data analysis, automatic process optimization, product design and innovation, food safety and quality control, and supply chain management.

Challenges and Future Expectations of Artificial Intelligence Technologies; The integration of AI technologies into the food production industry also brings along some challenges. To cope with challenges such as big data processing capacity, infrastructure requirements, and reliability issues, what does the industry need to do? Here are the challenges and proposed solutions; big data processing capacity, infrastructure requirements, and reliability

The integration of AI technologies into the food production industry is not just a turning point but also the beginning of an exciting journey towards the future. These technologies bring revolutionary changes in areas such as optimizing industrial processes, supporting product design and innovation processes, and enhancing food safety. However, the challenges encountered during this transformation should not be overlooked. Issues such as big data processing capacity, infrastructure requirements, and reliability are important aspects to be addressed to support the successful integration of AI technologies. With further advancements in AI technologies expected in the future, even greater and more transformative changes are anticipated in the food production industry. The future of the food production industry will be shaped in a brighter and more innovative way with AI.

Keywords: Artificial intelligence, food production, industrial processes, data analysis, food safety



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Biotechnology, Artificial Intelligence, Sustainability: Managing Innovation ,

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The world faces grand challenges in many areas including water, food, energy, natural disasters, air pollution, environmental problems, health care and medicine. Biotechnology provides solutions to these grand challenges by considering broader impacts in sustainability. The biotechnology industry grows rapidly. The pharma industry, healthcare, animal husbandry, agricultural products and environmental products have substantial fiscal contributions to the economy. Digitalization including artificial intelligence (AI) are enabling sciences and technologies for biotechnology solutions. The role of AI with respect to data processing, optimization, decision making and automation are inevitable. The efficient, innovative, cost reducing and scalable solutions are the benefits that AI can achieve for biotechnology products and services. Ethical concerns, privacy, policies and emerging laws and regulations need to be considered when AI technologies are utilized. Three examples of biotech startups, PredxBio, med-essence and Exin Health, that use AI technologies, will be presented.

A country's sustained economic growth can be created by the workforce who develop, manage, and maintain the processes and products of innovation. Such workforce mainly include engineers, scientists, and advanced-degree technologists. Science Technology Engineering and Mathematics (STEM) talent is of great importance to a country's economy and competitiveness. Biotechnology industry requires Science Technology Engineering Arts and Mathematics (STEAM) talent that must be trained as interdisciplinary thinkers and solvers through international collaborations. We have to develop, nourish and sustain both the STEAM education pipeline and the STEAM workforce pipeline for the rapidly growing biotechnology industry. A diversified STEAM talent and workforce can refuel economic growth, the base of prosperity and security.

Keywords: STEAM, workforce, economic growth, digitalization



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Deep Learning Based Sound Recognition System for Beekeeping

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Beekeeping is an important sector that contributes significantly to global food security through pollination. Traditionally, beekeepers must regularly physically inspect hives to monitor the condition of the hive, including the presence or absence of the queen-bee, activity levels, and potential disease. The sound produced in a beehive can provide critical information regarding the state of the hive and can potentially indicate the presence of a queen, activity levels. In this study, it is aimed to save time and effort by detecting abnormal situations by processing the sounds made by bees and the data received from sensors placed inside the hive, as well as increasing efficiency by reducing the need for routine inspections and allowing the management of a larger number of hives. In addition, this study was carried out in collaboration with GTU, SARAC and BRDARC.

Our research consists of two phases: data collection and processing. During the data collection, the audio data from the hive is sent to the server in the cloud using Jetson-Nano. For beekeepers to easily mark the status of the hive, a mobile application has been developed.

In data-processing, 3-second sound data of MFCC vectors were converted to 26x65 size and fed to the CNN. The network was trained separately for two different situations: 4-class and 2-class which were "Active-Day, Missing-Queen, Queen-Bee, No-Queen-Bee" and "Night, Day". For the 4-class model, training was carried out using the "bee-or-not-to-bee" dataset and test results were obtained with 97% accuracy. Since there is only the "No-queen-bee" class in our hive, only this class was tested. In night and day classification, an overall accuracy value of 80% was reached. It was understood that the wrong predictions occurred during the transition hours. As a result, it was observed that the sounds made by bees changed according to different environments and conditions.

Keywords: Bee, hive, beekeepers, deep learning, bee sound processing



Abstracts For Poster Presentations

PP1

**Characterization of Hypothetical PETase Enzyme from *Kibdelosporangium aridum*
in *E. coli***

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Polyethylene terephthalate (PET) is a common plastic utilized in various industries leading to environmental pollution due to its resistance to degradation. Conventional methods including mechanical, thermal, and chemical treatments for PET waste management can be costly, ineffective, and environmentally hazardous. Depending on these disadvantages, there is a growing interest in biological approaches providing sustainable, economical, and environmentally friendly processes by benefitting from microorganisms capable of breaking down PET polymers. Among these, enzymatic degradation having the ability of PET degradation, particularly cutinase, has emerged as a promising avenue.

This study focuses on the expression and biochemical characterization of a novel PETase-like enzyme, cutinase. In the scope study, *cutinase* gene from *Kibdelosporangium aridum* was transformed into *Escherichia Coli* BL21 (DE3) cells under the pET-22b+ plasmid after codon optimization. Cutinase was expressed in an auto-induction medium, and the enzyme was purified with the help of Ni-NTA HisTrap column chromatography. Optimum cultivation temperature (20, 25, 30 °C) for the enzyme expression, pH (range of 3.0-10) and temperature (range of 20-80 °C) for enzyme activity were determined. The optimum cultivation temperature for protein expression was determined as 30°C and the presence of the enzyme was proved with single band observation in SDS-PAGE analysis after Ni-NTA HisTrap column chromatography. Using spectrophotometric monitoring, hydrolysis of p-nitrophenyl acetate (p NPA) as a model substrate to acetate and p-nitrophenol at 405 nm was used for detection of enzyme activity and biochemical characterization results show that sodium phosphate buffer pH of 7.5 and 80°C of reaction temperature provided the most efficient reaction. Kinetic parameters were detected as 0.88 µmol/min and 1.99 mM for V_{Max} and K_M, respectively. According to obtained results, *cutinase* from *Kibdelosporangium aridum* has a huge potential to be utilized in PET waste management processes.

This study is supported by TÜBİTAK 2209-B (Project No 1139B412300335) Industry Oriented Research Project Support Programme for Undergraduate Students with the Cooperation of BAUZYME. Special thanks to BAUZYME for providing the necessary laboratory equipment.

Keywords: Polyethylene terephthalate (PET) recycling, biodegradation, cutinase, *Kibdelosporangium aridum*



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PP2

**The Effect Of Lon Protease On Secondary Metabolism and Cellular Differentiation in
*Streptomyces coelicolor***

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Streptomyces is a unique model organism due to its ability to synthesize numerous secondary metabolites of high medical and industrial value and its complex life cycle. The biosynthesis of secondary metabolites, which is closely related to the growth phase of the cell, is triggered by environmental stress conditions and regulated by complex mechanisms involving many different proteins. One of these proteins is the Lon protease, which has many important functions in all cells. In bacteria, it is an interesting candidate that affects secondary metabolism since it is involved in cellular differentiation and is part of an important stress regulon. Previously, we found that antibiotic production was dramatically increased in the Sco-pRA_{lon} strain generated by integrating the *lon* gene into the genome of *Streptomyces coelicolor* A3(2). In this study, first, the chromosomal region(s) where the recombinant plasmid was inserted into the *S. coelicolor* genome was determined by WGS, and a new integration site for the integrative vector not reported in the literature was discovered. Then, the transcriptomes of the wild-type and recombinant strains were compared, and differentially expressed genes (DEGs) in Sco-pRA_{lon} were identified. Finally, the morphological differentiation of the recombinant cell at different stages of development was examined by SEM and CSLM analyzes. The results of this study will shed light on experiments to increase the production of biotechnologically valuable bioactive metabolites in *Streptomyces* strains and other microorganisms.

Keywords: *Streptomyces coelicolor*, Lon protease, antibiotic, transcriptome, morphological differentiation



PP3

Detection Of Skin Pigmentation Associated Snps and Skin Cancer Risk By Using Loop-Mediated Isothermal Amplification (Lamp)

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Skin aging is a multifaceted process influenced by endogenous and exogenous factors. Exogenous aging, notably photoaging from UV radiation, accelerates skin aging, causes alterations on skin, such as pigmentation alterations and elevates the risk of skin cancer, particularly melanoma. Certain single nucleotide polymorphisms (SNPs) in genes like TYR, IRF4, PIGU, HERC2 and MCR1 have been shown to have affects in skin pigmentation and melanoma susceptibility through Genome-Wide Association Studies (GWAS). Loop-mediated isothermal amplification (LAMP) offers a rapid and cost-effective method for detecting SNPs associated with skin cancer risk. This study aims to employ LAMP to analyze saliva samples from females, whose age around 25-45, targeting specific SNPs linked to pigmentation and melanoma risk. Samples will be collected from consenting individuals, and specific LAMP primers which can bind and amplify the pigmentation-related SNPs. Results will also show us the individuals which have heightened susceptibility to skin cancer risk. Thus, early preventive measures can be initiated, potentially reducing the incidence and severity of skin cancer. Early detection and intervention are critical in mitigating the impact of skin cancer, emphasizing the importance of innovative techniques like LAMP in personalized healthcare approaches.

Keywords: Skin cancer, single nucleotide polymorphisms (SNP), pigmentation, loop-mediated isothermal amplification (LAMP)



PP4

**Synthesis of Antimicrobial Nanocomposite and Dental Composite
from Ciprofloxacin-Loaded Mesoporous Silica Nanoparticles and Antimicrobial
Studies**

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Nanocomposites represent a convergence of nanotechnology and materials science, wherein two or more materials are combined at nanoscale dimensions. The escalating demand for antimicrobial nanocomposites, particularly in healthcare facilities and public spaces during the pandemic, has spurred intensified research in this area. Antibiotics are commonly employed to enhance antimicrobial properties. This study focuses on utilizing Ciprofloxacin, a potent antibiotic effective against bacterial infections, to augment the antimicrobial attributes of nanocomposites.

Silica nanoparticles (SNs) are widely utilized in the biomedical domain due to their structured and adjustable porous nature, large surface area, and biocompatibility. They find applications in biomedicine, diagnostics, drug delivery, and tissue repair. Mesoporous silica nanoparticles (MSN) and amine functionalized mesoporous silica nanoparticles (MSN-NH₂) are particularly prominent in drug delivery systems and dental composites. Dental composites are crucial materials in dentistry for caries treatment, aesthetic corrections, and durable restorations. Enhancing the structural and property aspects of these composites aims at thwarting microorganism accumulation and caries development. The development of antibacterial nanocomposites assumes significance in elevating the antibacterial efficacy of dental composites. This study involves the development of antibacterial nanocomposites and dental composites using Ciprofloxacin-loaded mesoporous silica nanoparticles (MSN) synthesized through a sol-gel method. The chemical and morphological attributes of these nanocomposites and dental composites were assessed using analytical techniques such as Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). Furthermore, the antibacterial effectiveness of the nanomaterial was evaluated through MIC testing.

The results demonstrate the successful synthesis of antibacterial nanocomposites and dental composites utilizing Ciprofloxacin-loaded MSN. According to the experiments conducted, the most efficient loading was observed to be 35.3% for MSN and 36.8% for MSN-NH₂ at 0.1M. Characterization analyses revealed pertinent chemical and morphological properties, indicating the feasibility of these materials for their intended applications. When examining the SEM and FTIR results, it can be understood that the loadings have occurred. In the MIC experiment, no growth was observed at concentrations of 0.25 mg/ml, 0.50 mg/ml, 0.75 mg/ml, 1 mg/ml, 5 mg/ml, and 10 mg/ml, indicating antibacterial properties. The observed antibacterial properties signify the potential utility of these nanocomposites and dental composites in combatting bacterial infections and improving oral health. Overall, this study offers valuable insights into advancing antimicrobial nanocomposites and dental composites within the realm of nanotechnology and materials science.

This project is supported by TÜBİTAK under the 2209-A Program.

Keywords: *Ciprofloxacin, mesoporous silica nanoparticles, nanocomposites, dental composites, antimicrobial activity*



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PP5

Algae-Based Carbon Black Pigment Production and Its Use in Carbon-Neutral Printing Technologies

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The biomass-based carbon black production is an emerging issue to transform the printing sector, which uses dramatic levels of the petroleum-based carbon black pigment. Replacing the source of these carbon black pigments with microalgae or plant-based biomass will provide a promising sustainable feature in the sector's transition. In this study, we applied an improved pyrolysis method to obtain biochar from *Spirulina platensis*, known for its edible properties. We applied crystallization and acid wash to the algae to produce nanoparticles from biochar, which led to smaller particles, increasing the ink's permanence and fluidity. Following color quality and shelf-life experiments, we evaluated the cytotoxic effects of obtaining carbon-black pigment on human dermal fibroblasts. Scanning electron microscopy results showed improved quality due to the pre-pyrolysis crystallization process. Additionally, we found that the black color with approved L*a*b results was stable in 3D cured resins for at least six months without causing significant cytotoxicity to human cells. This study is promising for transforming printing and related sectors that require carbon black pigment to reduce their carbon footprints sustainably and reduce the amount of cancerogenic pigments used. This transformation can potentially affect many parts of responsible production and consumption of the industry.

Keywords: *Spirulina, carbon-black, prolysis, pigment*



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PP6

Covalent Immobilization of Bacterial α -Amylase Onto Natural Organic Polymer and Optimization by Using Box- Behnken Design

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Enzyme immobilization is an efficient application in enzyme technology for the realm of industrial biotechnology due to its capacity to offer key advantages such as improving functional efficiency, reusability, enhancing reproducibility, avoiding enzyme-product contamination, increasing enzyme stability and facile separation. α -amylase is a widely used enzyme in several industries such as food, medicine, textile, and detergent. Owing to α -amylases have several applications, they must be produced more stable in industrial scale. In the present study, α -amylase from *Priestia megaterium* was immobilized on the solid support of glutaraldehyde activated chitosan beads by covalent bonding. The experiments were designed and analyzed using Box-Behnken Design (BBD) for the optimization of factors that affect the immobilization. The process variables were included as chitosan concentration (2.0, 2.5, 3.0%) glutaraldehyde concentration (0.5, 1.0, 1.5%), activation time of chitosan beads with glutaraldehyde (1, 2, 3 h), and immobilization time of α -amylase onto activated chitosan beads (6, 16, 24 h). A total of 27 experiments were conducted and responses were obtained by measuring immobilized α -amylase activity.

As a result, 2.4% chitosan concentration, 0.86% glutaraldehyde concentration, 2h activation time and 24 h binding time was the optimum immobilization protocol. ANOVA demonstrated a critically low p-value of less than 0.0001, indicating that the quadratic polynomial model was enough to show the real relationship between the outcome and the important factors. Additionally, a satisfactory value of $R^2(0.92)$ is revealing a good correlation between the independent variables. Therefore, the beads, which will be further characterized by FTIR, SEM and kinetic studies, are promising for bringing the enzyme to industry.

This study supported by TÜBİTAK 2209B (Project No: 1139B412302428) Industry Oriented Research Project Support Programme for Undergraduate Students with the Cooperation of BAUZYME. Special thanks to BAUZYME for providing necessary laboratory equipments.

Keywords: α -amylases, enzyme immobilization, *Priestia megaterium*, chitosan beads, design of experiment (DOE)



PP7

DeepA I Acts on the Energy Metabolism of Epithelial and Endothelial Cells

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Cellular metabolism and homeostasis, and cell migration for tissue repair are very important process in all living organisms. Oxidative stress may occur as the levels of reactive oxygen species (ROS) released as a result of metabolic processes increase. This can lead to increased inflammation and impaired wound healing. Here, we aimed to analyze the roles of DeepA I, a supplement enhanced with boron, on ROS levels, cell viability, migration, and mitochondrial activity in HUVEC and MEF cells. Boron element levels in DeepA I content were determined by ICP-OES. By MTT viability test, we observed that DeepA I did not exhibit toxic effects. By fluorescent staining analysis using DAPI, MitoSPY and DCFDA, it was observed that ROS levels decreased without any change in mitochondrial activity. Also, it was observed by scratch assay that DeepA I triggered wound healing. Moreover, our analyzes at the protein level show that DeepA I did not alter the levels of cell death regulators, reduces the marker responsible for proliferation and increases the regulator that develops resistance to oxygen. According to our results, DeepA I enhanced with the boron may be a promising strategy to prevent excessive ROS levels, limit oxidative stress, and improve stalled wound healing.

Keywords: Boron, energy metabolism, reactive oxygen species, wound healing



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PP8

**Arbuscular Mycorrhizae Protect Wheat Against Boron Toxicity and Salinity Under
Both Low and High P Conditions**

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Salinity and boron (B) toxicity are adverse soil conditions that often co-occur in semi-arid regions, especially where low quality water is used for irrigation. Their combined effects on crops may differ from their individual effects, which may also require specific management. Arbuscular mycorrhizae (AM) are fungal root-colonizing endophytes that can help plants with nutrient and water uptake, enhance plant growth and increase tolerance to abiotic stress factors. High level of P availability is known to suppress the colonization of roots by AM fungi but maximizing the activity and benefits of AM fungi requires a certain P availability in the background. While AM fungi have been extensively studied in the context of salinity, knowledge on their role in B toxicity tolerance is scarce. In the present study, the effects of AM inoculation on tolerance of wheat to co-occurring salinity and B toxicity were investigated for the first time in literature by considering P supply as an additional parameter. In a factorial pot experiment conducted under greenhouse conditions, bread wheat (*T. aestivum* cv. Nusrat) was grown in autoclaved soil at different salinity, B and P levels, with or without AM inoculation. The interactive effects of all these factors on vegetative growth, tissue accumulation of mineral nutrients, stress-induced oxidative damage and yield parameters were determined. In addition, AM colonization of roots was studied by microscopy after staining. Both stress factors significantly decreased the vegetative shoot dry weight, but their interactions did not have any significant effect on shoot growth in this experiment. AM inoculation and higher P supply increased the shoot biomass by 35% and 32%, respectively. Both salinity and AM inoculation reduced tissue B accumulation. Membrane damage caused by B toxicity was partly alleviated by salinity. Yield was limited under low P conditions. The benefits of AM inoculation on plant growth, yield and stress tolerance were observed under both low and high P conditions, suggesting that AM can contribute to the sustainable management of multiple abiotic stress factors even under high P conditions which are relevant in conventional agriculture.

Keywords: Abiotic stress, arbuscular mycorrhiza, bread wheat (*Triticum aestivum*), B toxicity, phosphorus, salinity



PP9

**Investigation of the Effects of *Cistus creticus* Exosomes on Cell Survival and Death
in Pancreatic Cancer Cell Lines**

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Cistus creticus is widely used as an alternative medicine in Türkiye, included as a tea or nutritional supplement due to its high amount and variety of phenolic content, antioxidant properties, and positive effects on health. Moreover, there are products obtained from *C. creticus* on the market, such as herbal tea and throat lozenges. Plant exosomes are thought to have anti-cancer effects in cancer cells according to the cargo materials they contain of extracellular vesicles (EVs), which play an important role in cell-cell communication. This study aims to obtain information about the therapeutic properties and effects of *C. creticus* exosome isolates grown in a greenhouse environment under controlled conditions and at a concentration of 30 mM NaCl on pancreatic cancer cell lines. Cell viability, colony formation, cell proliferation, wound healing, fluorescent staining assays were performed on AsPC-1 and BxPC-3 pancreatic cancer cell lines. Analysis results showed no significant difference in cell viability, wound healing, and cell proliferation assays.

Keywords: *Pancreatic cancer, exosomes, Cistus creticus, mammalian cell culture*



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PP10

Microalgae: Nature's Sustainable Solution for Carbon Capture

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The 13th Sustainable Development Goal aims to achieve net-zero emissions by 2050, highlighting a milestone in addressing climate change and fostering sustainable development on a global scale. However, despite international efforts and commitments, the current carbon emissions status remains concerning, with levels continuing to rise globally. This trend underscores the pressing need for innovative and sustainable solutions, such as harnessing microalgae for carbon capture, to effectively curb CO₂ emissions. The growth rate and solar energy yield of microalgae exceed that of many terrestrial plants, and their superior CO₂ sequestration potential makes them a scientifically valid and environmentally favorable option. The CO₂ sequestration capacity of microalgae unlocks a range of biotechnological applications. Their biomass can be converted into biofuels, reducing reliance on fossil fuels and mitigating greenhouse gas emissions. Additionally, microalgae play a pivotal role in wastewater treatment, absorbing nutrients and pollutants to improve water quality. Their cultivation also yields valuable bioproducts like high-protein biomass for animal feed, functional foods, omega-3 fatty acids for nutritional supplements, and bioactive compounds for pharmaceuticals and cosmetics. These applications showcase the versatility of microalgae in addressing environmental challenges while contributing to sustainable development and circular bioeconomies. As we look to the future, ongoing advancements in microalgae cultivation, genetic modification, and bioprocessing hold immense promise. Scaling up these technologies will be key to their widespread adoption, fostering a transition to a carbon-neutral economy and sustainable development. Integrating microalgae-based solutions into various sectors, supported by robust policies and investments, will not only curb CO₂ emissions but also drive innovation in circular bioeconomies. This collaborative effort paves the way for a greener, more resilient future, where microalgae play a central role in mitigating climate change and promoting global sustainability.

Keywords: *Microalgae, carbon capture, sustainability, biotechnology, climate change*



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PP11

Identification of Competitive Endogenous RNA (ceRNA) Networks during Maize (*Zea Mays*) and Fungal Pathogen *Fusarium verticillioides* Interaction

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Plant-pathogen interaction is controlled by a complex molecular mechanism via the expression regulation of many genes. Long non-coding RNA (lncRNA) and microRNA (miRNA), which are non-protein-coding RNAs, are the main regulators of gene expression in the cell. There is a concentration-dependent competition between these three RNA molecules to bind to each other via base pairing resulting in degradation of target mRNA or translational inhibition. This phenomenon has been called the competitive endogenous RNA (ceRNA) network. The role of ceRNA networks in many biological processes is being investigated. However, the roles of ceRNA networks in the interaction between maize (*Zea mays*) and *Fusarium verticillioides* have not yet been revealed. This study aimed to identify ceRNA networks that are likely to play a role in the interaction between maize and *F. verticillioides*. In this context, maize seedlings were infected by *F. verticillioides*, and the crown tissue of the plants was harvested for RNAseq analysis. The differentially expressed mRNA, miRNA, and lncRNAs were determined and their sequence was used to predict ceRNA networks by the RNAhybrid bioinformatic tool. ceRNA networks were validated by qRT-PCR. The results pointed out the role of miR159, miR166, miR169, miR172, miR396, miR408 ve miR528-ceRNA networks in the regulation of immune responsive genes during *Z. mays* and *F. verticillioides* interaction. These findings contribute to the elucidation of the regulation of the immune response mechanism and to the selection of candidate genes that will lead to the inhibition of the fungal pathogen for RNAi-based functional analyses.

This work was supported by TÜBİTAK grant number 221O321. This study is based upon work from COST Action 'RNA communication across kingdoms: new mechanisms and strategies in pathogen control (exRNA-PATH), CA20110, supported by COST (European Cooperation in Science and Technology).

Keywords: *Zea mays*, *Fusarium verticillioides*, ceRNA, miRNA, lncRNA



PP12

Gate to Gate Environmental Impact Assessment of Biotechnological Malic Acid Production from Methanol

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Demands from a growing world population and pressure from climate crisis have put sustainable development at the top of global agenda. Converting the production of certain commodity chemicals from petrochemical-based methods to more environmentally friendly processes based on industrial biotechnology is a key issue in this agenda. Biotechnological production of Malic Acid from methanol is a good example for such a conversion. One key aspect of such early-stage projects is the quantification of environmental impacts.

Here, we aim to present the environmental impact assessment of a novel process design for the manufacture of malic acid from methanol using *Bacillus methanolicus*. Also, we will be able to comment of the insights gained through the approach for an early-stage process design.

Within the framework of Environmental Impact Assessment, the evaluation method published by Heinzle et al.² has been utilized.

When examined across the entire process, classified as an input component; Malic Acid and concomitant butylamine, in chronic toxicity category, Butylamine in the Accute Toxicity category poses a greater environmental threat than other compounds. Classified as an output component; Methanol in Thermal Risk category creates a larger environmental threat compared to other components.

It is observed that the production of Malic Acid using the Novel Process exhibits lesser adverse environmental impacts compared to Conventional production. There is potential for improvement plans to mitigate the predominantly negative environmental impacts caused.

Keywords: *Malic Acid, Bacillus methanolicus, early-stage process design, life cycle assesment*



PP13

**Promising Phytosomal Delivery Systems for Aristatoside C and Davisianoside B
with Reduced Hemolysis and Sustained Release**

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Plant-derived saponins like Aristoside C and Davisianoside B hold promise for cancer therapy due to their cytotoxicity against cancer cells. However, their ability to lyse healthy especially red blood cells (hemolytic activity) limits their clinical application. Researchers are looking for ways to reduce the negative hemolytic effects while maintaining the anti-cancer benefits. This study aimed to develop phytosomal formulations (Aristoside C - ALPs and Davisianoside B - DLPs) to enhance the therapeutic profile of these saponins. We encapsulated Aristoside C and Davisianoside B within plant derived phytosomal vesicles and evaluated their size, stability, release kinetics, hemolytic activity, and cytotoxicity against A-549 cancer cells. Both ALP and DLP formulations exhibited sizes suitable for drug delivery (around 110-120 nm) and high stability over 35 days. In vitro release studies revealed sustained release profiles for both saponins, with DLPs releasing Davisianoside B for 14 days and ALPs releasing Aristoside C for 21 days. Importantly, encapsulation significantly reduced the hemolytic activity of both saponins. Notably, DLPs maintained the cytotoxic activity of Davisianoside B against A-549 cells, while Aristoside C did not exhibit cytotoxicity in its phytosomal form (ALPs). We successfully developed phytosomal formulations for Aristoside C and Davisianoside B. These formulations displayed good size distribution, stability, and sustained release properties. Encapsulation significantly reduced hemolytic activity while maintaining the cytotoxic effect of Davisianoside B. These findings highlight DLPs as a promising nanocarrier for delivering Davisianoside B with reduced hemolytic activity and sustained release, opening avenues for further investigation of its anti-cancer potential.

Keywords: *Phytosomes, saponins, hemolytic activity, sustained release, cancer therapy*



PP14

Investigation of Silencing Efficacy of Elk-1 Specific Antisense Oligonucleotides

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The COVID-19 pandemic has brought RNA-based therapies to the forefront of recognition and research, although their first use dates back much further. Many RNA therapeutics have been approved by the FDA in recent years, including siRNA and antisense oligonucleotides. Their ability to be designed in a target specific manner and to be modifiable their structures with modifications that provide protection against exonucleases/endonucleases in plasma have made these molecules good drug candidates. Depending on their structure, these molecules can target different molecular mechanisms in mRNA, and have different effects, such as increasing or decreasing the amount of protein involved in the disease without altering the gene sequence. The stability, target specificity and mechanism of action of these synthetic molecules can be altered by the addition of various modifications to their chemical structure. Elk-1 belongs to the E Twenty-Six (ETS) domain transcription family and is involved in cell survival and proliferation of the brain tumours. It has been hypothesized that the silencing of Elk-1 in cancer cells may prove to be an effective treatment for brain cancers. In this study, we aimed to demonstrate the silencing effects of the Elk-1- specific antisense oligonucleotides, which have unique sequences and modifications. The Elk-1 silencing efficiency of Elk-1-specific antisense oligonucleotides (ASOs) was demonstrated at the protein level in glioblastoma cell line, and it was determined which ASOs were more effective in silencing Elk-1. ASOs designed to target different regions of Elk-1 mRNA have been shown to result in reduced Elk-1 protein expression compared to a nonsense oligonucleotide control. Following examination of ASOs on cancer cell viability and proliferation, it is anticipated that these molecules will serve as an important drug candidate in the advancement of an efficacious cancer treatment.

Acknowledgement: This project was supported by TÜBİTAK within the scope of TÜBİTAK COST 2519, Project No:221S657.

Keywords: Antisense oligonucleotides, brain tumours, Elk-1



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PP15

**Effect of Different Activated Charcoal and 6-Benzylaminopurine Doses of the
Micropropagation of Tea Plant (*Camellia sinensis* L.)**

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Tea (*Camellia sinensis* L.), from the *Theacea* family, is the oldest non-alcoholic, caffeine-containing beverage product worldwide, and the economic importance of *Camellia* genus is primarily attributed to it. To enhance the profitability and sustainability of tea cultivation on a commercial scale, micropropagation of tea plants is economically crucial for rapid production of genetically uniform, disease-free plantlets, ensuring consistent yields of high-quality plants.

This study is an attempt to develop *in vitro* protocols for the micropropagation of *C. sinensis* L. via direct organogenesis using nodal segments. Shoot initiation was established in half-strength Murashige Skoog (MS) medium containing sucrose and 6-Benzylaminopurine (BAP). Three different concentrations of BAP were tested for shoot multiplication. The effect of different concentrations of activated charcoal on shoot development was evaluated. The results revealed that the addition of activated charcoal inhibits shoot multiplication regardless of the BAP concentration, even though it facilitates efficient and vigorous shoots. On the other hand, plants that grew in the absence of activated charcoal had higher multiplication rates as the BAP concentration was increased.

The *in vitro* shoot propagation protocol of the tea plant was established through this study. Rooting and acclimatization procedures are yet to be completed as part of the study.

Keywords: *Tea, Camellia spp., direct organogenesis, activated charcoal, plant tissue culture*



PP16

Cloning and Synthesis of Extracellular Domain of Pd-1 Towards Elisa Bioassay Development

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In the pharmaceutical sector, biotechnological drugs, especially monoclonal antibodies (mAbs) are among the largest import items in our country, and domestic production of these drugs has been encouraged in our country for the last 10 years. However, as much as the production of mAbs, the validation of the biological activities of these mAbs is of great importance, and these quality control processes are critical in terms of production costs. In our country, imported bioassay kits are used to validate the antigen-binding property of anti-PD-1 antibodies, which are on the agenda of various biotechnological pharmaceutical companies. Programmed cell death 1(PD-1) antigen was previously described in HEK293T cells in our laboratory and used in the qualitative testing of mAbs produced by a company. However, commercialization of HEK293T cells expressing PD-1 antigen, which is a membrane protein, requires special devices to take readout from the cells due to logistic costs. Therefore, in order to develop a low-cost bioassay, the part encoding the extracellular region of the protein is cloned into the bacterial expression plasmid pGEX-4T-2 by restriction enzymes from the existing PD-1 expression plasmid. Confirmation of successful cloning of PD-1-ED into the pGEX-4T-2 plasmid is achieved by sequencing of the cloning site. Expression of the extracellular domain of PD-1 (PD-1-ED) protein is verified by SDS-PAGE and expressed PD-1-ED is found in the pellet of cells. Results of osmotic shock and detergent screening methods for the isolation and location of protein in the pellet have shown that PD-1-ED could form an inclusion body which are aggregates of proteins. Results of detergent screening studies show that % 1 SDS detergent solution can solubilize most of the PD-1 protein. In conclusion, PD-1-ED was expressed in the bacteria to develop ELISA kit prototype obtained by sticking PD-1-ED.

This project was supported by G.T.U. within the scope of G.T.U. BAP Project No. 2022-A-105-31.

Keywords: mAbs, Programmed cell death 1(PD-1), bacterial expression, ELISA, antigen



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PP17

Preparation and Detailed Characterization of *Thymus kotschyanus* and *Thymus kotschyanus* var. *Glabrescens* Loaded Phytosomes as a Drug Delivery Nanosystem

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Thymus species are significant traditional herbs that are not only utilized in culinary practices as condiments but also commonly in medicinal preparations. Due to their potent biological and pharmacological activities, they find widespread applications in medicine for treating various conditions like chronic bronchitis, respiratory infections, and whooping cough. Our latest research highlighted that methanolic extracts of two thyme varieties, *Thymus kotschyanus* var. *kotschyanus* (TKK) and *Thymus kotschyanus* var. *glabrescens* (TKG) cultivated in Türkiye, exhibit antioxidant, antibacterial and anticholinesterase activity along with their rich phenolic content. Phytosomes, lipid-based vesicular carriers, are frequently employed to enhance the bioavailability and therapeutic potential of herbal extracts and phytopharmaceuticals. This study aims to prepare and characterize physicochemical and biophysical properties of phytosomal formulations of TKK and TKG as a novel drug delivery nanosystem. Phytosomal formulations of *Thymus* species were prepared by thin film hydration technique. Biophysical characterization of phytosomal preparations was achieved through dynamic light scattering (DLS) which involved the analysis of size, zeta potential, time and temperature dependent stability. Encapsulation efficiency and kinetic release profiles of phytosomes were determined via UV/Vis spectrophotometry. Phytosomes containing TKK and TKG were successfully synthesized. Dynamic light scattering analysis proved that phytosomes loaded with plant extracts exhibited small particle sizes, excellent dispersion, and high stability during different storage conditions along with a sustained release profile. These phytosomes also showed lower hemolytic activity compared to free plant extracts.

Our research suggests that phytosomes can effectively encapsulate thyme extracts like TKK and TKG, exhibiting favorable biophysical properties, high stability, and sustained release profiles that enhance their biological activity. These findings position phytosomes as promising candidates for delivering phytopharmaceuticals. Further evaluation of the antibacterial and wound-healing properties of these phytosomes is in the pipeline.



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Keywords: *Thymus kotschyanus*, *Thymus kotschyanus* var. *glabrescens*, phytosomes, drug delivery, sustained release



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PP18

**Effect of Pyruvate Kinases in Enhancing Metabolic Flux to the Shikimate Pathway in
*Pseudomonas putida***

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The shikimic acid (SA) pathway synthesizes essential vitamins and aromatic amino acids like phenylalanine, tyrosine, and tryptophan. SA acts as an intermediate compound in the biosynthesis of aromatic amino acids (AAA) and serves as the starting material for the chemical synthesis of oseltamivir phosphate (Tamiflu), a drug used to treat influenza (Bo et al., 2023). Another metabolite is salicylic acid, an important drug precursor (aspirin) widely used in skincare and haircare products (Reshi et al., 2023). Because these metabolites become widely useful in pharmaceutical and cosmetic industries, various organisms are used to reduce the cost of production and to achieve eco-friendly manufacturing. *P. putida* is preferred as a chassis in synthetic biology and metabolic engineering research due to its capacity to harbor pathways designed to produce valuable chemicals or degrade/valorize environmental pollutants (Martínez-García and de Lorenzo, 2024). In this study, we investigated the effect of pyruvate kinase (*pyk*) genes on metabolic flux to the aromatic amino acid pathway. For this purpose, *P. putida* genome was edited and the effect of the alterations on both growth and the AAA pathway was examined. The results obtained will contribute to the elucidation of the role of *pyk* genes in the pathway.

This study was funded by TÜBİTAK Grant No 120Z407.

Keywords: *Pseudomonas putida*, pyruvate kinase, shikimic acid pathway, synthetic biology, metabolic engineering



PP19

An “Assured” Approach for Multiplex Detection of Bio Analytes

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The impact of the COVID-19 pandemic on the supply chain, as in many areas, has highlighted the urgent need for rapid and reliable diagnostics for sustainability. The World Health Organization (WHO) summarizes the desired device characteristics as follows: “ASSURED”: Affordable, Sensitive, Specific, User-friendly, Rapid&Robust, Equipment-free. This approach has been transformed into a requirements format that can be summarized as “REASSURED” by adding the principles of “Real Time connectivity” and “Ease of specimen collection”. As potential “REASSURED” test candidates, “Biosensors” are practical devices consisting of a bio recognition element, a signal transducer, and a reader that can be used at point-of-care locations. Aptamers are single-stranded synthetic nucleotides that can be used as bio recognition elements of biosensors, which selectively recognize and bind to a wide range of specific molecules such as small molecules, nucleic acids, pathogens, etc. In addition to the advantages of customizability, repeatability, and reusability, aptamers have significant advantages in being produced using combinatorial production methods (e.g., Systematic Evolution of Ligands by Exponential Enrichment (SELEX)), as they do not require animal hosts. This allows aptamers to be obtained under simpler infrastructure conditions, in shorter time frames, and at lower costs compared to more commonly used bio-recognition elements like antibodies, while also offering the option to develop tests that are sensitive to animal rights, vegan, cost effective and sustainable. This study aims to develop a paper-based multiplex biosensor as a sustainable, rapid screening method for analytes with minimal equipment and/or operational skills requirement. With a single diagnostic kit, targeting clinically most confused Influenza subtypes and SARS-CoV-2 can be detected from a single sample, potentially influencing treatment decisions. The applicability of this test at *point-of-care* locations will enable early diagnosis of diseases that can easily turn into pandemics in resource-limited areas, critical supply chain points, homes, fields, disaster areas, etc.

Keywords: Biosensor, “ASSURED”, “REASSURED”, aptamer, supply chain



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PP20

***In vitro* Propagation of Avocado Nodal Budswithzero Valent Iron Nanoparticles
(Nzvi)**

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Avocado, scientifically known as *Persea americana Mill*, is a fruit whose popularity has been increasing worldwide in recent years and has become a significant agricultural product with its valuable carbohydrates, protein, and fat content together with presence of wide spectrum of vitamins (A, B, C, D, E and K) and minerals. Thus, the development and widespread adoption of the avocado industry creates economic, environmental, and social impacts both locally and globally. However, avocado propagation through seeds exhibits high genetic variation, hence less appealing for orchard plantings. As potential of micropropagation has been well demonstrated for wide variety of economically important plants, *in vitro* propagation of avocado via plant tissue culture is becoming an important technique with enabling its large-scale production in a short time. Hence, in this study, shoot tip and nodal explants were used for the initiation of *in vitro* culture in MS medium containing different concentrations of BA (Benzyl aminopurine). Following the optimization of the best BA concentration, experiments will also be conducted to reveal the effect of zero valent iron nanoparticles (nZVI) on the *in vitro* propagation of avocados since nZVI can promote plant growth by increasing its photosynthesis and nutrient accumulation. The results will be evaluated after 4 weeks of incubation. It will be proven that the nanoparticle concentration with the highest effect on propagation will fasten the avocado propagation.

Keywords: Avocado micropropagation, iron nanoparticles, plant tissue culture, plant growth



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PP21

Heterologous Expression of Streptomycin Resistance Gene in *Streptomyces Coelicolor* A3(2) Caused Metabolic Change

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Streptomyces spp. are natural producers of valuable secondary metabolites, such as antibiotics, anticancer drugs, immunosuppressants, etc. In these organisms, the production of secondary metabolites is dependent on their precursors, the products of primary metabolism. To produce them on an industrial scale, it is often necessary to use metabolic or genetic engineering approaches. Antibiotic selective markers are used to select the recombinants in genetic engineering studies. Streptomycin adenyltransferase (AAD) is one such selective marker which modifies the streptomycin antibiotics by adenylating the 3'-OH position of the streptomycin glucosamine ring. There are a considerable number of studies in which the omega (Ω) fragment containing the *aadA* has been used as a selective marker. In this study, however, we show that heterologous expression of the *aadA* fragment itself, without the Ω fragment, completely altered the metabolism of *Streptomyces coelicolor* A3(2). Its presence and expression completely closed the pathways of secondary metabolism and, on the other hand, activates the primary metabolic pathways of the recombinant strain. While this study shows that the streptomycin resistance gene should not be used in *Streptomyces* genetic studies, it gives hope that the new recombinant strain can be used for the production of primary metabolites of industrial importance.

Keywords: *Streptomyces coelicolor* A3(2); streptomycin resistance; primary metabolite



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PP22

Elucidation of the Network between Stringent Response-Related Proteins and Antibiotic Production in *Streptomyces coelicolor* A3(2) through *In silico* and *In vitro* Studies

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Secondary metabolites produced by bacteria are valuable in medicine and biotechnology. *Streptomyces spp.* are important natural sources for producing secondary metabolites. The stringent response has been identified as playing a key role in the production of secondary metabolites. However, in *Streptomyces*, the stringent response is not yet fully understood. In an amino-acid-deprived *E. coli* cell, alarmones (ppGpp and pppGpp) are produced by RelA/SpoT activity. Alarmones lead to polyphosphate (polyP) accumulation in the cell by regulating the inorganic phosphate cycling proteins polyphosphate kinase (PPK) and exopolyphosphatase (PPX). polyP molecules complex with LON protease and this complex cleaves the ribosomes to provide amino acids to the cell. In our previous studies, we reported that stringent response related PPK and LON proteins are affecting antibiotic production in *Streptomyces coelicolor* A3(2): PPK has a negative effect whereas LON protease has a positive effect on antibiotic production. In this study, we aimed to elucidate the network between the stringent response proteins and antibiotic production in *S. coelicolor*. For this purpose, ensemble modelling, a *de novo* approach, was used to predict and interpret the metabolic outputs in ppk deficient cells that overexpress the *lon* gene. Our results identified pathways associated with high antibiotic production in the model. Our results identified pathways associated with high antibiotic production in the model. The *in silico* results were validated by in vitro experiments by generating recombinant *ppk*-deficient strain carrying an extra *lon* gene (*Sco-ΔppkpRAlon*). This study largely elucidates the molecular and metabolic mechanisms underlying the increase in actinorhodin production in the recombinant cell and shows that *in silico* approaches can be a starting point for *in vitro* studies in *Streptomyces*.

Keywords: *Streptomyces coelicolor* A3(2); ATP-dependent Lon protease; actinorhodin production; ensemble modelling



PP23

Effects of Watercress (*Nasturtium officinale*) Extract Grown at Different Selenium Levels on Pancreatic Cancer Cell Lines

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Functional foods have the potential to enhance wellness and reduce susceptibility to disease by providing targeted physiological advantages in addition to essential nutrients and are crucial for human health. Plants used in traditional medicine have been shown to have potential anticancer effects. Watercress (*Nasturtium officinale*) is rich in vitamins, minerals, antioxidants, and anti-cancer substances such as isothiocyanates, glucosinolates, and polyphenols. Moreover, watercress is a crucial source of vitamin C, which has been linked to a reduced risk of developing several malignancies. Selenium is an essential trace element for human health and plays a crucial role in the antioxidant defense system. Selenium enrichment is crucial for plants as it improves their antioxidant capacity, nutritional value, stress tolerance, disease resistance, and environmental remediation capabilities, thereby contributing to overall plant health and ecosystem sustainability. The association between pancreatic cancer and nutrition suggests that dietary choices and nutrient intake play an important role in both cancer prevention strategies and in supporting the overall well-being of individuals affected by this disease. In this study, we aimed to investigate the effect of watercress extract, cultivated under varying selenium concentrations, on pancreatic cancer cell lines PANC-1 and MIA-PaCa-2. Selenium-enriched watercress extracts were applied to the cells in a dose-dependent manner during cell growth and evaluated for cell viability, colony formation, and cell survival variations compared to non-enriched watercress cultivar extract. In addition, cells were stained with DCFDA, DAPI, DIOC6, PI, and MitoTracker fluorescent dyes, and cell imaging was performed. In this study, the potential of Selenium-enriched watercress grown with the hydroponic systems to be used as a functional food and their anticancer potentials were revealed. Our study continues on preliminary findings that will draw attention to the importance of healthy food produced in sustainable and easily accessible ways in people's daily lives.

Keywords: Watercress, pancreatic cancer, functional food, preventive medicine



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PP24

Molecular Interactions of Fraser's Photinia and Pgb_invit Bacterium

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Plant endophytic bacteria can improve plant growth by modulating plant growth regulators and nutrient uptake and also can target pathogens and promote plant defenses. In our former studies, an endophytic beneficial bacterium was isolated and characterized from *in vitro* grown microshoots of Fraser's Photinia (*Photinia × fraseri* Dress.) and named as "PGB_invit" (Gül-Şeker et al. 2017; Şah et al. 2019). In this study, physiological, biochemical and molecular influences of PGB_invit bacterium on Fraser's Photinia growth and development was determined. For this purpose, Fraser's Photinia shoot tips inoculated with active, inactive (incubated at 95°C for 20 minutes) PGB_invit bacterial culture at the concentration 10⁶ cfu ml⁻¹. After optimum incubation periods, amounts of photosynthetic pigments, antioxidant enzyme activities, malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) contents were evaluated. Also, the effect of PGB_invit on rooting and *in vivo* acclimatization of Fraser's Photinia was determined. In addition, RNA isolated from all these plants and the high-throughput sequencing was carried out and 14 differentially expressed genes were selected to be confirmed by qRT-PCR due to their relatively high expression levels. Our results demonstrated that proline content was significantly enhanced in response to bacterial treatment whereas cellular H₂O₂ content was decreased. In consistent with the decrease in H₂O₂ content an increase in catalase activity was also detected. Also, this study provided clear microscopic evidence of the endophytic bacterial colonization since the presence of bacterium was detected inside the cells by confocal microscopy in Fraser's Photinia. Moreover, gene expression patterns evaluated by RNA sequencing (RNA-Seq) and differentially expressed five genes between active and inactive form of the bacterium were confirmed by qRT-PCR. Among them, ubiquitin-conjugating enzyme E2 and predicted "E3 ubiquitin-protein ligase RHA1B-like genes which play important roles in plant immunity and stress were down-regulated in plant samples inoculated with bacteria which may demonstrate that PGB_invit does not evoke plant immune system and has a positive effect on reducing stress on Fraser's Photinia. Due to these beneficial traits and findings, this specific bacterium promotes *in vitro* proliferation of Fraser's Photinia microshoots and enables microshoots to be conserved at 25±2°C for up to 16 months without requiring periodic subculture. As a result of this positive effect on plant growth and development, PGB_invit can be used as a potential biofertilizer.

This study was supported by TÜBİTAK with project number 117R002.



Keywords: *Fraser's Photinia, PGB_invit, RNA-seq, gene expression, plant-bacteria interaction*



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PP25

**Epibrassinolide Inhibited Tamoxifen Resistance in MCF 7/TAMR Cell Lines through
Endoplasmic Reticulum Stress**

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Patients with estrogen-receptor-positive breast cancer are treated with tamoxifen (TAM), which is one of the commonly used chemotherapeutic drugs for inhibiting the activation of estrogen receptors. The prolonged usage of TAM leads to drug resistance and may result in elevated estrogen receptor expression and signaling. Endoplasmic reticulum stress (ER) is a suggested strategy to combat drug resistance. Our group has identified epibrassinolide (EBR), a member of brassinosteroids, as an anti-cancer agent by modulating ER stress. This study evaluated EBR as an effective agent to overcome TAM resistance through ER stress in the MCF-7/TAMR cell line.

MTT assay was performed to evaluate the effect of drugs on cell viability. The colony formation potential was analyzed in a two- and three-dimensional cell culture. Fluorescence staining by DAPI, PI, and DiOC6 was performed. Western blotting was conducted to evaluate protein expression profiles of ER stress and autophagy markers.

We found that the combined treatment of EBR and TAM increased cell viability loss in the MCF 7/TAMR cell line. The combination of EBR with TAM decreased colony formation potential in 2D and 3D cultures. The number of fragmented DNA and cell death rates were elevated. Lastly, exposure to the combined drugs enhanced expression profiles of ER stress and autophagy markers.

Our results indicated that EBR might have a critical role in increasing TAM sensitivity by reducing drug resistance through the ER stress-mediated autophagy in MCF-7/TAMR cell lines.

Keywords: Autophagy, epibrassinolide, endoplasmic reticulum stress, tamoxifen, drug resistance



PP26

Epibrassinolide Increased the Apoptotic Potential of Temozolomide by Inducing Endoplasmic Reticulum Stress in Glioblastoma Cells

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Glioblastoma (GBM) is a highly invasive and aggressive form of malignancy characterized by a median survival time of 12 months. Temozolomide (TMZ), as a DNA alkylating agent, is one of the approved chemotherapeutic agents used to treat GBM. It functions by inducing mismatches in DNA bases, ultimately leading to cell death. However, TMZ effectiveness against GBM is limited due to drug resistance. Epibrassinolide (EBR) is recognized for its anticancer properties, primarily attributed to its ability to induce endoplasmic reticulum (ER) stress in various cancer cell lines and SCID mouse colon cancer model. This study seeks to enhance the efficacy of TMZ by EBR co-treatment, via inducing ER stress.

The MTT cell viability assay was conducted to assess the effects of TMZ in combination with EBR in GBM cells. Clonogenic and soft agar colony assays were performed to elucidate the impact of drug combination on GBM cell lines. DiOC6 fluorescence staining was conducted to visualize the effects of combining TMZ with EBR. Finally, immunoblotting was performed to analyze the expression levels of proteins involved in cell cycle, ER stress and apoptosis. The combination of TMZ and EBR induced cell cycle arrest in U87 and LN18 cell lines. Furthermore, the combination enhanced ER stress. Upregulation of ER stress related proteins consequently lead to the activation of apoptotic proteins such as caspase 3 and PARP.

The findings of our study demonstrate that TMZ and EBR combination led to enhanced ER stress induction, cell cycle arrest and subsequent cell death. These results provide valuable insights into the potential of utilizing a combination of TMZ and EBR in GBM cells.

Keywords: Glioblastoma, epibrassinolide, temozolomide, cell cycle, ER stress and apoptosis



PP27

**Energy Efficiency Considerations in Biotechnological Computational Approaches: A
Case Study**

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Computers play an indispensable role in all aspects of our lives. Since their invention, they have been extensively utilized in our work and professional endeavors. Computers have progressively advanced in tandem with the requirements of advanced research as well as consumer needs, including sophisticated predictive modeling, simulations, and the increasingly prevalent use of artificial intelligence approaches. This advancement has necessitated the utilization of specialized hardware, enabling the attainment of computing speeds in the range of petaFLOPS. Through the customization of hardware and the construction of grid-based architectures comprising multiple computer nodes, these systems have reached supercomputer classification, laying the foundation for computational analyses and experiments deeply intertwined with biotechnology, such as computational biochemistry, medicine, epidemiology, geographic information systems, and others.

Beyond discussions about the role of personal computers in scientific approaches and their energy consumption, the energy consumption and efficiency of supercomputers and workstations used for advanced biotechnological analyses are undoubtedly important. They are directly or indirectly related to the pillars of sustainability, encompassing environmental and economic concerns. Additionally, these concerns are similar to those related to the efficiency and management of the laboratories where experimental studies are conducted. Given the evident importance of these aspects in the present and near future, our study compares the hardware specifications and energy consumption of computers used in our recent computational study. In this previous study, cyclin-dependent kinase inhibitor molecule targets of whole human and *C. elegans* proteomes were screened via molecular docking and molecular dynamics simulation methods on the Turkish National High-Performance Computing (HPC) facility (TRUBA) and our custom workstations. Based on this study, we accumulated approximate energy usage of the systems per given task, and we compared relative efficiencies based on energy consumption.

Our findings predominantly emphasize that computational raw power is not always dominantly important; rather, the use of software versions and algorithms tailored to the hardware is crucial. We suggest that although personal computers commonly used in our daily lives may suffice for basic analyses and studies, efficient results can be obtained only at an introductory level. Furthermore, we demonstrate that GPUs significantly reduce computation times and potentially enhance efficiency in molecular dynamics simulations.

We would like to acknowledge and thank to TÜBİTAK ULAKBİM, High Performance and Grid Computing Center (TRUBA resources), and Computational Biochemistry Laboratory (The Institute of Biotechnology, Gebze Technical University) for providing computational resources for the study.



Keywords: *Supercomputers, sustainable computing, efficiency, computational biology*



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PP28

**Exploring the Regulatory Role of Mmu-Mir-3535: Its Impact on Lpin1/LepR
Expression in Adipogenesis**

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Obesity is becoming an epidemic and it is affecting almost 40% of the world population. In obesity, the most visible distinction in adipocytes is the fat deposition. This causes endoplasmic reticulum stress and affects the viability of the cells. Adipocytes are derived from a specialized cell lineage in the mesoderm. Adipocyte differentiation, adipogenesis, occurs in 4 stages which are commitment, growth arrest, mitotic clonal expansion, and terminal differentiation. In the adipogenesis process, lipin-1 gene is highly expressed, and it is proved that Lpin1 deficiency in mice severely reduces the fat deposition. Even though leptin hormone is considered to have anti-adipogenesis effects on the cells, leptin receptor has an opposite effect. This gene, LepR, has proven to be an adiposity sensor that promotes adipogenesis. Together, they are essential to decide the fate of the cell in adipogenesis. In the adipogenesis, in addition to outside signals, miRNAs also take part. It was observed that miRNAs regulate adipocyte differentiation by acting on transcription factors which are PPARs and C/EBPs, and by regulating signaling pathways, including MAPK, PI3K/Akt, cAMP/PKA/CREB, and Wnt/b-catenin pathways. miR-3535 is a miRNA that is mainly studied on chicken fat deposition. mmu-miR-3535 is not studied in terms of the adipogenesis process. In our study we tried to make a connection of mmu-miR-3535 in the adipogenesis process. It was observed that in the differentiated 3T3-L1 cells mmu-miR-3535 expression decreases as the Lpin1 and LepR expression increases. To conclude, mmu-miR-3535 expression decreases significantly in the adipogenesis process.

Keywords: *Obesity, adipogenesis, miRNA, lipin, leptin*



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PP29

**Examination of the Individual or Combined Effects of *A. officinarum* and *G. glabra*
Herbal Extracts on the Tauopathy Model**

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Alzheimer's disease (AD) presents a significant challenge in modern healthcare due to its prevalence and debilitating effects on cognitive function. The neuropathological hallmarks of AD include the accumulation of hyperphosphorylated tau proteins leading to neurofibrillary tangles and the formation of amyloid plaques. Cholinergic neuron dysfunction is also implicated in AD pathology. This study aimed to explore the potential therapeutic effects of herbal extracts *A.officinarum* and *G. Glabra* in an AD cellular model using SH-SY5Y neuroblastoma cells. The cellular model of AD was established through transient transfection with tauopathy-inducing plasmids, followed by treatment with herbal extracts. Employing techniques such as MTT viability assays, plasmid transfection, and fluorescence microscopy, the study evaluates the effects of herbal extracts on cell viability and tauopathy progression. Furthermore, this study investigated the mechanism underlying the protective effects of these herbal extracts by assessing cellular survival, propidium iodide staining for cell death, and reactive oxygen species (ROS) determination. The effects of decreased cell viability with tau formation and the individual or combined effects of herbal extracts were modelled. Overall, this study provides insights into the potential therapeutic benefits of herbal extracts in mitigating the cytotoxic effects of tau pathology in AD. Further research into the molecular mechanisms underlying these effects may uncover novel therapeutic targets for AD treatment.

Keywords: *Alzheimer's Disease, neuroblastoma cells, tauopathy model, herbal extracts*



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PP30

**Exploring the Impact of Plasma-Activated Water on Seedling Emergence of Tea,
Leek, Eggplant, and Dill Seeds**

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Cold Atmospheric pressure Plasma Jet (CAPPJ) device (10 kV, 20 kHz) was used to prepare plasma-activated water (PAW). The distance between the CAPPJ nozzle and the water surface was 2,5 cm. Approximately 400 mL of distilled water was exposed to plasma for 90 minutes to prepare PAW (PAW-90). The nitrate (NO₃⁻) concentration was measured with ion chromatography and a spectrophotometer. The maximum content of NO₃⁻ was found to be 71 ppm. Then, the sown seeds of tea, eggplant, leek and dill were fertigated by distilled water (controlled group) and PAW treated distilled water. Positive results were obtained in all seeds. The tea, eggplant and dill seeds irrigated with PAW started to emerge 5 days earlier than the control group, while no difference was observed in leek seeds. The average height of leek seedlings was approximately 1 cm higher than the control group. The seedling emergence percentage of eggplant seeds of the controlled group and PAW-fertigated group are 67% and 74%, respectively. Furthermore, PAW-90 reduces the time to 50% emergence (t/50) to 21 days compared to 30 in the control. It is possible that exposure to and the possible generation by CAPPJ of RONS within the seed accelerated dry after-ripening, a known effect of reactive oxygen species (ROS) resulting in reduced dormancy and enhanced seed vigour. Germination experiments were also carried out with eggplant, dill and leek seeds and the control group gave better results than the PAW group. This is thought to be due to the presence of ROS in PAW.

This work is financially supported by TÜBİTAK 1512 Grant (No:2220369) and TÜBİTAK 1001 Grant (No:223Z020).

Keywords: *Plasma agriculture, plasma-activated water fertilizer, seed vigour, seedling growth*



PP31

**Investigation of the Physiological Effects of Selenium-Enriched Watercress
(*Nasturtium officinale*) Extract on *Caenorhabditis elegans***

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Watercress (*Nasturtium officinale*), from the Brassicaceae family known to have high selenium accumulation, is a semi-aquatic plant that is rich in nutrients and has high anticarcinogenic and antioxidative effects. Antioxidant activity and total phenolic content are highest in watercress compared to other plants in the same family. Studies have proven that the anticarcinogenic and antioxidative activities of plants enriched with selenium are significantly increased. In this study, the physiological changes of *Caenorhabditis elegans* treated with extracts obtained from unenriched and 50% selenium-enriched watercress grown by hydroponic farming were examined. The experiments carried out in this context are chemotaxis, swimming speed and egg retention. As a result of chemotaxis experiments, strain CL2120 showed positive affinity towards unenriched watercress. In assessing swimming speed, positive effects were seen in strain CL2120 treated with selenium-enriched and unenriched watercress. In more stressful environments, worms exhibit egg-retention behavior in their ovaries. Less egg-retention behavior was observed in unenriched watercress compared to selenium-enriched watercress extract of *C. elegans* strain CB1370, which contains the DAF-2 gene encoding insulin-like growth factor (IGF-1) which is known to regulate the rate of aging. In conclusion, selenium-enriched watercress extracts gave significant results in strain CL2120. With the significant results obtained in the CL2120 strain, which is an Alzheimer's disease model, it can be predicted that selenium-enriched watercress can be used in the research of Alzheimer's and other neurological diseases.

Keywords: *Caenorhabditis elegans*, *Nasturtium officinale*, Se-enriched watercress, hydroponic farming



PP32

Investigation of the Effect of *H. italicum* Extracts and *H. italicum* Loaded Chitosan Nanoparticles on Wound Healing

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Helichrysum italicum (*H. italicum*), an aromatic shrub that can grow between 30-70 centimeter, contains chemical components such as essential oils, flavonoids, diterpenes, and various phenolic compounds. *H. italicum* promotes healing by reducing inflammation around the wound, removes free radicals in the wound area due to its antioxidant components, and accelerates wound healing by increasing the proliferation of fibroblast cells. Extracts derived from *H. italicum* have a low solubility and bioavailability in living systems due to their chemical structure. Therefore, integration into nanoparticulate systems using natural polymers is targeted to enhance their efficacy. Chitosan, the main derivative of chitin, a long-chain polymer, is a copolymer of glucosamine and N acetylglucosamine units linked by 1-4 glycosidic bonds. Chitosan (CS) polymer, a natural polymer that can be obtained from organisms such as shrimp, crab, squid, and crayfish, is preferred due to its hydrophilic nature, biocompatibility, biodegradability, and non-toxicity. In the current study, chitosan derivative nanoparticles were selected as the natural polymer-based nanoparticle primarily for their antimicrobial activity and accelerated wound healing properties. The present study serves as research demonstrating the potential of designing biotechnological systems containing natural components to enhance the efficacy of molecules with low bioavailability. The current study involves the following steps:

1. Extraction of *H. italicum* extracts using Soxhlet and SFE methods, 2. Synthesis of *H. italicum*-loaded and blank chitosan nanoparticles using the ionic gelation method, 3. Determination of antioxidant activity in vitro using the DPPH assay, 4. Determination of encapsulation efficiency and drug loading capacities of chitosan nanoparticles using UV-Vis spectrophotometry, 5. Analysis of particle size, polydispersity index, and zeta potential values of chitosan nanoparticles, 6. Examination of polymer-drug interactions in chitosan nanoparticles using FT-IR spectroscopy, 7. Cytotoxicity analysis of the extracts in cell culture using the MTT assay, 8. Conducting wound healing tests for efficacy analysis on wound healing. Effective nanoparticles suitable for topical formulations have been obtained by adapting low bioavailability and solubility yet beneficial *H. italicum* extracts for wound healing.

Keywords: Chitosan, *H. italicum*, wound healing, antioxidant activity, nanoparticle



PP33

**Agronomic Biofortification of Basil with Selenium is Affected by Sulfur Rate in an
Indoor Vertical NFT System**

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Agronomic biofortification can increase the nutritional content of vegetable products and help tackle hidden hunger in human populations. Selenium is an essential element for human health but not essential for crops. Yet, Se can provide plants with some growth benefits at non-toxic levels. In soilless systems, Se is not a standard component of fertilizer solutions but can be added to stock solutions for biofortification purposes. Indoor vertical farms are becoming increasingly popular for producing vegetables in urban environments as they allow year-round, local, pesticide-free, and climate-resilient cultivation. The number of published studies on the biofortification of vegetables in plant factories is, however, limited. This study aimed to examine how adding Se (2 μ M) affects basil yield and quality characteristics at two distinct S levels (low S: 0.65 mM; high S: 1.3 mM) in an indoor vertical NFT system that represents a commercial plant factory. Regardless of Se application, high S application increased the fresh weight of basil. Basil grown under high-S conditions had 20% more fresh weight than those grown under low-S conditions. On the other hand, Se fertilization under low-S conditions reduced the fresh weight by 14% but this negative effect of Se disappeared under high-S conditions. Tissue Se concentration results showed that high S limited excessive Se accumulation in tissue. In our experimental setting, one serving of Se-biofortified basil grown under high S conditions could provide 20% of the recommended daily intake (RDI) while this value increased to 37% under low S conditions. These results showed that Se and S rates in plant factories must be adjusted together by considering their interaction for the best results in terms of yield and nutritional quality. Plant- and system-specific optimization studies are needed for the commercial production of biofortified leafy greens in plant factories.

This study was supported by Plant Factory (<https://plantfactory.company>). I also would like to thank YOK 100/2000 scholarship program.

Keywords: *Biofortification, basil, nutritional quality, plant factory, RDI, selenium, soilless, sulfur*



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PP34

**Investigating the Soil Regulatory and Plant Growth Promotion Capabilities of
Biochar Generated from Waste Biomass Gasification**

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Biochar, which is obtained from waste biomass through pyrolysis or gasification, is increasingly recognized in sustainable agriculture for its potential as a soil amendment. Carbon in biochar is highly resistant to microbial decomposition. Therefore, biochar production helps sequester carbon in soils while contributing to sustainable crop production through the improvement of soil properties. It is used to control soil acidity, enrich soils with organic matter, enhance the beneficial soil microbiota, improve water retention, and thus increase crop yield and productivity. However, the exact properties of biochar depend very much on the type of biomass the process parameters. Based on the hypothesis that biochar generated as a by-product of gasification has improved properties when compared to biochar obtained by conventional pyrolysis, this study focused on biochar produced by gasification of oak waste biomass. Fine or coarse biochar were applied to two different soils with contrasting pH levels (5.0 vs. 8.0) at two different rates (1% or 2%). Effects of treatments on the growth and mineral nutrition of alfalfa (*Medicago sativa* cv. Sunter) and lettuce (*Lactuca sativa* var. *crispa* cv. Levistro) were investigated. Application of fine biochar strongly enhanced lettuce growth in acidic soil. In the case of alfalfa, the harvested biomass was maximized with the application of fine biochar at 2% in both soil types. Significant changes in the tissue concentrations of both macro- and micronutrients were detected in alfalfa and lettuce. Particularly, biochar acted as a K fertilizer due to its high extractable K content as demonstrated by mineral analysis of biochar. The results suggest that biochar from waste biomass gasification facilities, which primarily serve as power plants, may be a safe and effective way to improve soil fertility and sequester carbon at the same time although effects may vary according to crop species and soil type.

The research was funded by the project TTO-AR-GE-21-163, carried out in partnership with Gebze Technical University and GÜBRETAŞ.

Keywords: Biochar, plant growth promoter, lettuce, alfalfa, soil pH, plant nutrition



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PP35

**Willow (*Salix babylonica*) Extracts Can Act as Plant-Based Biostimulants for
Overcoming Salinity Stress of Maize Grown in Soilless Culture**

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Biostimulants obtained from natural sources like plant extracts can be used in crop production to improve productivity. Their affordability and safety make them appealing choices, but the processes underlying their effectiveness are still being investigated. Willow tree (*Salix* sp.) has been important for society due to its varied uses since the beginning of history. Extracts from the weeping willow tree (*Salix babylonica*) are rich in phenolics, terpenoids, and most importantly salicylates, including salicin, saligenin and salicylic acid. One of the most widely seen abiotic stresses that pose a major threat to the agricultural industry and the world's ability to sustainably feed its expanding population is salinity stress. Biostimulants can enhance the salinity tolerance of plants and contribute to sustainable agriculture. In this study, salicylic acid (SA) (25 µM) and willow bark (WB) (0.1 and 0.2%) and leaf (WL) extracts (0.1 and 0.2%) were tested as plant-based biostimulants to enhance the growth of maize (*Zea mays* cv. *Caramelo*) under both normal and salinity stress conditions (0 or 60 mM NaCl). The experiments were conducted by using soilless culture under growth chamber conditions. According to the results, willow tree extracts were reported to have a positive impact on growth parameters like plant height and dry weight of the shoots and roots, under both control and saline conditions. Within the tested biostimulant applications, 0.2% WB, 0.1% WL and 0.2% WL gave the most promising results, for enhancing stress tolerance of maize. Furthermore, these extracts decreased the Na⁺ uptake and the Na⁺/K⁺ ratio and raised K⁺ uptake as well as protein concentration, consequently mitigating the adverse effects of salinity. Willow extracts are thought to have beneficial effects because of their rich bioactive compounds. The findings suggest that willow extracts can be used as effective and eco-friendly biostimulants for sustainable agriculture applications.

Keywords: *Biostimulant; hydroponic culture; salicylic acid; salinity stress; sustainability; willow leaf extract; willow bark extract*



PP36

Pre-Sowing Treatment with Various Extracts from the Brown Seaweed (*Cystoseira barbata*) Influences Wheat Seedlings Growth

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Seaweed-derived biostimulants used as pre-sowing treatments represent a promising and important approach for sustainable agricultural production as the demand for novel and greener solutions increases worldwide. It is well recognized that extracts obtained from seaweed, one of the most significant natural sources of biostimulants, include rich bioactive chemicals that positively impact a variety of plant metabolic processes. In this study, the brown seaweed species *Cystoseira barbata* (*C. barbata*), which is common along the Mediterranean coasts, was used to obtain biostimulants by using hot water, alkali, and acid extraction. The biostimulant activity of these extracts was evaluated using seed or substrate applications on perlite media in a growth chamber with wheat (*Triticum durum* cv. Sarıcanak-98) seeds. The study investigated the effects of extracts on seedling growth, root morphology and mineral nutrient concentrations. The findings suggest that seaweed extracts may function as a biostimulant to improve wheat seedling performance and have a favorable impact on various growth parameters. High concentrations of all seaweed treatments enhanced the shoot length and fresh weight by 20% and 25%, respectively. Substrate experiments resulted in a 20% increase in root length, 25% increase in root area, and 30% increase in root volume, when treated with a medium concentration of water extract. The results suggest that, in comparison to their direct applications as seed treatment agents, seaweed extracts added to the growth medium were more successful in improving seedling performance. *C. barbata* extracts have great potential as a bioeconomy resource and their different compositions can be used as promising, effective, eco-friendly, and reliable agents. Moreover, these extracts may contribute to the reduction of economic losses in agricultural production, global food security, and sustainable agriculture practices. This study was supported by TÜBİTAK (Project number: 121Z215).

Keywords: Bioeconomy, biostimulant, *cystoseira barbata*, seaweed extract, seed treatment, sustainable agriculture, wheat



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PP37

**Comparison of Growth Performance of Green and Purple Basil in Deep Water
Culture Versus Peat Substrate**

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Basil (*Ocimum* L.) belongs to the diverse Lamiaceae family and is cultivated in both home gardens and commercial farms. Following lettuce, basil is among the most popular high-value leafy greens cultivated in soilless greenhouses and indoor farming environments. Basil serves as a crucial source of essential oils, which are used in the food, cosmetics, and pharmaceutical industries. Valued for its medicinal attributes, this plant is rich in essential oils and phenolic compounds. Basil phenolic acids are particularly valued for their potential in addressing a range of health concerns, such as cardiovascular diseases. This study involved growing green and purple basil plants for six weeks by using peat or deep-water culture, all under identical artificial lighting conditions with the Vahaa Smart Garden system. The nutritional quality was evaluated by analyzing mineral and metabolite concentrations using ICP-OES and UV-Vis spectrophotometry. Results indicated that the fresh weights of basil plants grown in deep water culture surpassed those grown in peat by approximately 100%. The chlorophyll and carotenoid concentrations in plants cultivated within the Vahaa system were notably higher compared to those grown in peat. Furthermore, the vitamin C concentrations of plants cultivated through deep water culture were significantly elevated in comparison to their counterparts grown in peat. The protein concentration, antioxidant capacity, and total phenolic content of both green and purple basil plants cultivated in deep water culture exhibited higher levels compared to those grown in peat. These findings suggest that deep water culture presents a promising technique for achieving both high yield and superior quality in basil production.

This investigation was carried out under a university-industry partnership project involving VAHAA Vertical Farming Solutions and Technology A.S and Gebze Technical University. Funding for this study was provided by TÜBİTAK project number 130614.

Keywords: *Basil, deep water culture, hydroponic, nutritional quality, peat, purple basil, smart garden, soilless agriculture*



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Investigating the Impact of Selenium Biofortification on Watercress

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Watercress (*Nasturtium officinale* R. Br.) is a member of the Brassicaceae family, esteemed for its nutritional value and safety for consumption. It is abundant in minerals and vitamins such as folic acid, carotenoids, glucosinolates, vitamins B, C, and E, and pro-vitamin A. Additionally, watercress is a rich source of essential minerals like Ca, Fe, and S, while boasting elevated dietary fiber content and being low in calories. Although it is not essential for plants, selenium (Se) is a vital mineral required by human bodies. It plays a crucial role in the formation of selenoproteins, specialized proteins that regulate thyroid hormones, enhance immune function, and provide defense against oxidative stress. Among the mineral deficiencies that contribute to hidden hunger, Se deficiency is especially prevalent and poses significant global health challenges. Biofortification, a strategy that involves increasing the uptake and accumulation of micronutrients in crops, provides an effective agricultural solution to address this issue. This study involved hydroponic cultivation of watercress plants for five weeks using a soilless farming device (Vahaa Smart Garden) under room conditions. The nutrient solution, containing essential micro and macronutrients (ready mix of Vahaa Plant Nutrients), was refreshed every two weeks. Sodium selenate (Na_2SeO_4) was added to the nutrient solution at concentrations of 0, 0.25, and 0.50 mg/L starting the second week of the growth period. After the cultivation period, watercress plants were harvested. The application of Se did not adversely affect watercress fresh weight, total phenolic content, chlorophyll and carotenoid concentrations, or antioxidant capacity. Moreover, the application of 0.25 mg/L Se and 0.50 mg/L Se resulted in selenium accumulation of 135 µg/kg and 306 µg/kg in watercress, respectively. Thus, a serving (34 g) of biofortified watercress could supply 3-6% of the Recommended Dietary Allowance (RDA) for Se, which is 55 µg/day for adults. The application of Se to plants in soilless agriculture shows potential for improving public health through the production of selenium-enriched watercress. This indicates the potential success of using functional green plants for Se biofortification in soilless agriculture.

We are grateful to Vahaa Vertical Farming Solutions for providing the smart gardens used for this study.

Keywords: Biofortification, hydroponic smart garden, nutritional quality, selenium, soilless



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PP39

**Utilization of Duckweed (*Lemna minor*) for the Bioremediation of Agricultural
Wastewater and Production of Biofertilizer**

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Lemna spp., commonly known as duckweed, are small floating freshwater plants in the Lemnaceae family. As aquatic macrophytes, they proliferate rapidly and form dense coverings on both polluted and unpolluted water surfaces. Their adaptability to various environments and wide range of growth conditions enables their easy cultivation while turning them into potentially invasive species. Duckweed has various applications, including wastewater treatment, animal feed production, and vegetarian protein production. They show significant potential for phytoremediation due to their efficient nutrient absorption. *Lemna* species are efficient at nutrient uptake, particularly nitrogen, and phosphorus. Due to their fast growth, and high demand for nutrients such as nitrogen (N) and phosphorus (P) which are considered as pollutants in aquatic habitats. Although the potential of duckweed as a bioremediation agent for wastewater of different origins has been extensively studied, its potential for removing valuable nutrients from the drainage waste of greenhouses or plant factories and turning them into biofertilizers has not been investigated. In this study, common duckweed (*Lemna minor*) was cultivated in nutrient solutions with different electrical conductivity (EC) levels (200, 400, 800, 1500 $\mu\text{S}/\text{cm}$) to determine the optimum growing condition of this species and mimic agricultural wastewater. During the cultivation period, EC and pH values were measured periodically. After harvesting, the wet and dry weights of duckweed were recorded. The mineral and nitrate concentrations in nutrient solutions and plant tissues were determined by using ICP-OES and UV-Vis spectrophotometry. The results showed that duckweed consumed N and P in nutrient solutions by up to 100% in 7 days. Additionally, it was observed that boron (B), copper (Cu), iron (Fe), and manganese (Mn) were nearly completely depleted by duckweed in solutions with initial EC values of 200, 400, and 800 $\mu\text{S}/\text{cm}$. According to the mineral analysis of plant tissues, duckweed accumulated high levels of essential minerals which makes the biomass potentially valuable as a biofertilizer. This study highlights the potential of duckweed to recycle nutrients from agricultural wastewater. Cultivation of duckweed in agricultural wastewater and its utilization as a biofertilizer may provide a perfect example for a circular bioeconomy application that can contribute to sustainable agriculture while helping in the fight against pollution and eutrophication.

Keywords: Biofertilizer, duckweed, eutrophication, *Lemna*, nutrient consumption, sustainable agriculture, wastewater treatment



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PP40

Biochar-Perlite Mixtures as Novel Substrates for Soilless Systems

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In controlled environment agriculture (CEA) facilities such as greenhouses or plant factories, soilless systems are often preferred over soil-based systems due to their management, efficiency and yield advantages. Commercial CEA facilities, including most modern greenhouses employ substrate-based systems where the soil is replaced by a solid substrate that provides plant roots with water, nutrients and oxygen as well as mechanical support. Cocopeat and cococoir, both being by-products of the coconut industry, are popular soilless substrates in commercial settings as they offer excellent water retention and aeration at the same time. However, most temperate countries, including Türkiye, which dominates the global greenhouse sector, must import this critical and expensive input from a few tropical countries. Tomato, the most important vegetable globally, is also the most widely grown vegetable in greenhouses in Türkiye, and the vegetative biomass waste of tomato greenhouses is currently not utilized in any way. In collaboration with Mavruz Tarım, a commercial soilless greenhouse tomato producer located in Çanakkale, the semi-woody vegetative biomass waste of tomato plants was converted into biochar through gasification. To deal with the salinity and alkalinity problems of the biochar, a pre-treatment method was devised and optimized. Then, by mixing pre-treated biochar (PTB) or non-treated biochar (NTB) with perlite at various ratios, substrates were obtained and tested as soilless germination media with cocopeat serving as the control substrate. Pepper (*Capsicum annuum* Toygar F1) and tomato (*Solanum lycopersicum* Ezgi F1) seeds were sown in the experimental substrates under greenhouse conditions. Substrates were regularly irrigated with deionized water and no fertilizer was applied during the 3-week experimental period. In most NTB-perlite mixtures, no germination was observed whereas seedlings grown in 15-85% and 25-75% PTB-perlite mixtures had the highest shoot and root dry weights. The shoots of both tomato and pepper seedlings were about 4 times larger in these PTB-perlite mixtures than in the control substrate. Mineral analysis of biochar and plant tissue samples indicated that PTB in the right ratio with perlite could provide seedlings with most essential nutrients and thus serve not only as a soilless substrate but also as a source of mineral nutrients. Further research is needed to evaluate the comparative effects of these promising substrates on plant performance in the long term. This study was funded by the Research Universities Support Program (Project No: GTU-2023-A-113-05), Mavruz Tarım and KSV (Kaleseramik Dr. Ibrahim Bodur Egitim, Saglik ve Sosyal Yardım Vakfi). The presenter was supported by YOK 100/2000 and TÜBİTAK 2211/A scholarship programs.

Keywords: Biochar, cocopeat, greenhouse, soilless, substrate, pepper, perlite, tomato



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**Investigation of a Suitable Protein Extraction Method from *Spirulina platensis*
Biomass and Investigation of Effects of the Selected Method on the Functional
Properties of Proteins**

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Becoming the most produced microalgae biomass around the world, *Spirulina* has been indicated as a potential protein source with several bioactive and technofunctional properties. Extraction methods such as mechanical cell disruption, enzymatic, thermal and chemical treatments can improve protein extraction by complete or partial disruption of the microalgae cell wall, increasing the accessibility of intracellular proteins. Therefore, choosing an appropriate method is necessary to maximize protein extraction from algal biomass. The current research investigated the effect of different protein extraction methods from *Spirulina platensis* biomass in order to increase the protein extraction yield. For this purpose, *Spirulina platensis* biomass was subjected to enzyme (lysozyme) assisted extraction, freeze and thaw, high pressure homogenization, ultrasonic bath extraction, and ultra-turrax extraction techniques. The, the total soluble protein contents of extracts were revealed and compared with each other. Accordingly, protein contents of five different extracts were 7.59, 17.62, 9.26, 7.33, 6.64 g/mL, respectively. The results showed a significantly higher protein content obtained by freeze and thaw technique, followed by high pressure homogenization, enzyme assisted extraction, ultrasonic bath, and ultra-turrax protein extractions. Therefore, freeze-thaw was chosen as the most effective method for protein extraction from *Spirulina* biomass. Subsequently, proteins were extracted by using freeze-thaw method and the obtained extracts were used for protein precipitation. Precipitated proteins were lyophilized, and the obtained powder has been evaluated in terms of protein solubility and total amino acid composition. Understanding how different extraction methods affect the physicochemical and functional properties of *Spirulina* proteins can guide their application in several food and nutraceutical formulations. Functional properties also make microalgal proteins promising candidates for various food industrial applications, thus further extraction-method-based functional properties need to be investigated and optimized enough for large-scale production.

Keywords: *Microalgae, alternative protein, sustainability, solubility, amino acid profile*



PP42

**Possible Roles of *Cellulose synthase A (Cesa)* Genes in K-Deficiency Pathways of
*A. thaliana***

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Potassium (K) plays a crucial role in plants, aiding in carbohydrate, water uptake, and nutrient transport within the plant. When plants experience a K deficiency, they often exhibit symptoms like yellowing or browning of leaf edges, reduced turgor pressure, and they finally become wilt. The *Cellulose synthase A 4 (CESA4)* gene has a significant role in cellulose biosynthesis in plant metabolism. In many plant species, cellulose biosynthesis has a critical function in maintaining the axial tension and geometric rigidity to upright their stems, leaves, and branches. Differences in *CESA4* gene expression levels were observed in the *A. thaliana* genotypes grown under different K deficiency conditions. Some genotypes showed higher *CESA4* expression under K deficiency. Physiological tests, including biomass measurement, shoot elongation, and chlorophyll measurement, were performed on these plants. Elemental analysis using ICP-OES was performed to assess the nutritional conditions, and all tests were confirmed. Based on these data, the research focused on functionally characterizing the gene using novel gene editing techniques. The aim was to reveal the relationship between the gene of interest and the potassium nutrition mechanism or potassium utilization pathways of the plant. The CRISPR/Cas9 system was designed to knock out the gene and was applied using floral dipping transformation. This method was mediated by polyethyleneimine-functionalized single-walled carbon nanotubes (PEI-SWNT) and *Agrobacterium tumefaciens* as gene delivery agents to compare different delivery methods and obtain a higher yield. The designed four different gRNA sequences will be applied to achieve non-synonymous knock-out to gain insight into the function of the gene. The research will continue with T₁ generation seeds that will be cultivated and grown under K deficiency and control conditions. However, to determine whether there will be a change in the function of the CESA enzyme complex formed by the expression of the gene, an enzyme activity test will be performed using the produced CESA enzyme assay. The analysis is expected to contribute to the literature on the roles of the CESA4 gene in the potassium mechanism by highlighting its unpredictable expression under potassium deficiency.

Keywords: *Potassium deficiency in plants, A. thaliana, PEI-SWNT mediated gene delivery, cellulose synthase A*



PP43

Understanding the Microbial Degradation Pathways of Urea Aldehyde Fertilizers

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The flow of nitrogen into and out of the atmosphere, biosphere, hydrosphere, and geosphere is referred to as the nitrogen cycle. A vital component of nucleic acids, which are the building blocks of genetic material (RNA and DNA), and amino acids, is nitrogen. The amount of accessible nitrogen frequently limits ecosystem production and biomass in situations where other resources, like light and water, are ample. This is the main justification for nitrogen's importance in fertilizers meant to improve the quality of the soil for farming purposes. Slow-release fertilizers are fertilizers in which all the elements in the nutrients are not released at the same time, but at certain time intervals. In this way, nitrogen and phosphorus sources are in a form that the plant can take up without getting into the air or water. This method, used to ensure the efficient release of nitrogen, plays an important role in preventing environmental problems. Cross-linking polymerization by trapping urea nitrogen with aldehyde-based chemicals is one of the ways to develop slow-release nitrogen fertilizers. However, concerns regarding their environmental impact have prompted extensive research into their breakdown processes, particularly influenced by soil microbial communities. The crucial role of microbial consortia in transforming urea aldehyde compounds into biologically usable forms is examined, elucidating key metabolic pathways such as hydrolysis, ammonification, and assimilation. Furthermore, the influence of environmental factors like pH, temperature, and moisture on microbial activity and degradation rates is discussed. The insights obtained from this investigation not only enhance our understanding of the fate of urea aldehyde fertilizers in soil ecosystems but also provide significant implications for sustainable agricultural practices and environmental preservation strategies.

Keywords: *Urea aldehyde, microbial degradation, soil ecosystems, sustainable agriculture, slow-release fertilizer*



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**Stimulating Yield and Nutritional Value of Broccoli with Different Seaweed
(*Cystoseira barbata*) Extracts**

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Given the global food crisis resulting from population expansion and climate change, prioritizing sustainable agriculture practices over conventional practices is imperative. Biostimulants obtained from seaweed species can boost crop growth and resilience thanks to their bioactive components while reducing the dependence of cultivation on other inputs. A brown seaweed species known as *Cystoseira barbata*, which is widely distributed in coastal regions of Türkiye, is an untapped bioeconomy resource for the preparation of extracts with biostimulant properties. In the present study, experimental *C. barbata* extracts were tested on broccoli, which is a high-value vegetable with a high nutritional value and health benefits due to its beneficial phytochemical content. The objective of this study was to examine if three different *C. barbata* extracts could enhance the growth and nutritional value of broccoli (*Brassica oleracea* L. cv. Maraton) through repeated soil applications. Hundred days after sowing, plants were harvested, and their growth and nutritional characteristics were assessed. The alkali extract significantly enhanced the yield of broccoli by up to 35 %, the water extract significantly enhanced the shoot dry biomass by up to 40%. The total antioxidant capacity, vitamin C concentration which contribute to nutritional quality, were found to be enhanced by seaweed extract treatments. This research demonstrated the potential biostimulant effects of different *C. barbata* extracts on broccoli grown in soil under greenhouse conditions. Further research is needed to identify which bioactive component in which experimental extract contributed to the observed positive responses and the associated mechanisms of action. These findings underscored that local seaweed species may be useful in agriculture and depending on the method of extraction, they may affect not only the growth and yield but also the nutritional quality and organoleptic properties of vegetables.

This study was supported by TÜBİTAK 2244 project number 119C030 and YOK 100/2000 Scholarship Program.

Keywords: *Biostimulant, broccoli, Cystoseira barbata, nutritional quality, seaweed extract, sustainable agriculture*



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**Foliar Application of Brown Seaweed (*Cystoseira barbata*) Extracts Stimulate Durum
Wheat Growth and Yield**

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In crop production, biostimulants are environmentally friendly substances that are mostly of biological origin and applied at low rates to enhance plant growth and vigor. They often lead to physiological changes, improve stress tolerance in plants, boost metabolism in plants, and increase the absorption of essential minerals. The brown seaweed *Cystoseira barbata*, which grows commonly in the coastal regions of the Marmara Sea and can form dense stands in winter months, can be sustainably harvested and processed into various extracts. Wheat, one of three major cereals globally, was grown on 220 million ha of land in 2022. Although it is not considered a high-value crop, it is of great strategic importance in terms of food and nutrition security. How locally sourced and foliarly applied seaweed extracts could affect the growth and yield of durum wheat (*Triticum durum* cv. Saricanak-98) was investigated in the present study under greenhouse conditions. In the first experiment, different rates of different experimental extracts were tested on wheat plants to determine the most promising treatments in terms of growth stimulation. Then, the effects of the selected applications on the yield parameters of durum wheat were studied in the second experiment. The growth stimulation observed at vegetative stages translated into higher grain yield and increased grain number in the long term. The observed improvements in yield parameters were as high as 25% with the most effective treatment, which involved the experimental water extracts. Mineral analysis results indicated that the observed benefits cannot be solely attributed to the essential mineral contents of the extracts. Field studies are needed to assess the potentials of these extracts at a large scale and their economic feasibility. However, the results obtained so far are promising and suggesting that the *C. barbata* biomass from the Marmara Sea may be processed into a valuable plant nutrition product.

This study was supported by TÜBİTAK 3501 project number 121Z215 and YOK 100/2000 Scholarship Program.

Keywords: *Biostimulant, cystoseira barbata, seaweed extract, sustainable agriculture, wheat, yield*



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PP46

**Identification of Phenotypic, Biochemical and Transcriptomic Responses of
Sunflower Seedlings (*Helianthus annuus* L.) to Drought Stress for the Development
of Tolerant Parental Lines**

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Sunflower (*Helianthus annuus* L.) is one of the most important annual oilseed plants in the world and has become a strategic plant due to the increasing demand for sunflower oil in recent years. The main goal of this project is to develop new high-yield parent lines of sunflower, which has a significant cultivation area in Türkiye, that can be grown under drought stress conditions. Drought inhibits plant growth due to its detrimental effects on major physiological and biochemical processes in plants. Water availability mostly affects leaf and root growth, stomatal conductance, photosynthesis and dry matter weight. One of the first responses of plants to water stress is a decrease in leaf growth rate and the closure of stomata. Accumulation of some organic compounds such as sugars, betaines and proline is also one of the first reactions of plants to water stress. Stomatal limitation in response to water deficit is associated with a reduction in both the rate of photosynthesis and the internal CO₂ concentration. The decrease in CO₂ concentration in the leaves of plants under drought stress and the dissipation of excess light energy in the PSII pathway causes the formation of reactive oxygen species such as H₂O₂ (hydrogen peroxide) and O₂⁻ (superoxide). Oxidative Stress, which causes metabolic damage by promoting excessive ROS production, increases lipid peroxidation, which is the biggest indicator of membrane damage. Enzymatic and non-enzymatic antioxidants in plant cells play an important role in avoiding the harmful effects of free radicals. Lipid peroxidation, H₂O₂ (hydrogen peroxide), O₂⁻ and proline amounts of 4 female parent (78, 105, 109, and 88) and 3 male parent (26, 19, 1) sunflower lines grown at 3 different water levels. Relative water contents and stem dry weights were compared. The biochemical analyzes (NOX, CAT, APX, and GR) were performed on the selected tolerant and susceptible lines for control and drought groups. Following the enzyme determination of tolerant and susceptible sunflower lines, RNA sequencing (RNAseq) analysis has been performed for leaf tissues after mild and severe drought stress treatments. Through comparative transcriptome analyses, it may be possible to develop new molecular markers. The molecular markers will be helpful in enabling the rapid and effective



identification of drought-tolerant sunflower lines and contribute to understanding and combating the impact of climate change on sunflower plant productivity.

This Project has been supported by the General Directorate of Agricultural Research and Policies (TAGEM) grant (#TAGEM/ 20 / AR-GE / 21) to BCKeskin

Keywords: Drought; sunflower; transcriptome; RNAseq; oxidative stress; antioxidant



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PP47

**Establishment an Indirect Competitive Elisa System for the Detection of Aflatoxin
B1 in Food**

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Aflatoxin B1 (AFB1) is one of the most harmful and carcinogenic mycotoxins, causing contamination in many foods (nuts, peppers, corn, etc.) and animal feed. The amount of aflatoxin in foods and feeds has been limited by legal regulations in many countries. Chromatographic methods such as HPLC and LC-MS/MS used for aflatoxin detection require technical staff, comprehensive clean-up procedures, and expensive equipment that are not suitable for a large number of sample analyses. For these reasons, immunoassays are often used for the detection of mycotoxins in foods and feeds because they are economical and suitable for comparison with analytical methods. This study aims to develop an Enzyme-Linked Immuno Sorbent Assay (ELISA) kit based on the competitive system for rapid and practical detection of the low amount of AFB1 in contaminated foods.

The main method used which is indirect competitive ELISA (IC-ELISA) based on prevention of the interaction between immobilized antigen and labelled antibody in the presence of the analyte. By increasing the analyte concentration in the solution, the signal detected due to the interaction between the immobilized and labelled components is reduced this study, the IC-ELISA test was performed with test reagents (Anti-AFB1 monoclonal antibody, AFB1-BSA-HRP conjugate) prepared in our laboratory.

As a result, it was determined that the developed ELISA system could detect flour and fig extract samples contaminated with AFB1 in the range of 0.5-0.05 ppb. Accelerated aging tests to determine the shelf-life of our system were performed and it was shown that the system maintained its shelf life up to 1 year at +4°C. This IC-ELISA would be a useful tool for screening the AFB1 in food.



PP48

**Altering Alkaloid Production in *Papaver somniferum* Suspension Culture via
Macerozyme Enzyme Assisted Virus-Mediated Gene Silencing (Vigs) Technique**

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Papaver somniferum L., a well-regarded medicinal plant esteemed for its abundant pharmaceutical alkaloids, has intrigued humans since ancient eras. This research investigates the potential for modifying secondary metabolite production in medicinal plants through conventional breeding or molecular biology approaches. By harnessing the Plant Defense System, particularly Post-Transcriptional Gene Silencing (PTGS), this study concentrates on utilizing the Virus-Induced Gene Silencing (VIGS) method to selectively silence genes linked to morphine biosynthesis. Notably, the study introduces a novel approach employing the macerozyme enzyme for efficient genetic manipulation on somatic embryos. The experimental process involves the cultivation of plant leaves from sterilized seeds on somatic embryo-inducing medium, leading to the generation of white dispersible somatic embryos and suspension cultures. Quantitative analysis of the expression levels of five key genes (CODM, T6ODM, COR, 4OMT, DIOX2) involved in morphine biosynthesis were conducted. The distinctive aspect of our study lies in intentionally reducing CODM gene expression, inhibiting morphine conversion, while simultaneously enhancing codeine synthesis through upregulation of the COR gene. Importantly, T6ODM gene expression remains unaffected, ensuring the pathway remains open for codeine production. The targeted design of specific regions in T6ODM, CODM, and DIOX2 genes paves the way for future modifications in codeine, morphine, and thebaine alkaloids. In this study, silencing of the Morphine gene was studied using the VIGS technique. Total RNA was isolated from plants obtained from *Papaver somniferum* Ofis1 (TMO). The cDNA was amplified using gene-specific designed primers and then the terminal ends of this cDNA were cut with restriction enzymes and inserted into the TRV2 vector in *Agrobacterium tumefaciens* as a silencing fragment. Plant leaves obtained from sterilized seeds were transferred to somatic embryo-inducing medium and subcultured once a week to obtain white dispersible somatic embryos in approximately 5 weeks. These embryos were cultured into hormone-free MS medium and suspension cultures were formed. When the suspension cultures reached a sufficient amount, they were infected by *Agrobacterium* cultures containing TRV1 and TRV2 silencing fragments in the prepared combinations. At this point, we present a new interpretation with the macerozyme enzyme for gene transfer to somatic embryos. Then infected cells were lyophilized for further analysis and preservation. And finally, for the



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control of gene expression level (CODM, T6ODM, COR, 4OMT, CNMT, 7OMT, DIOX2), real-time PCR was used with primers designed specifically for genes.

Keywords: *Papaver somniferum L., virus-induced gene silencing (VIGS), morphine biosynthesis, somatic embryo suspension cultures, macerozyme enzyme, real-time PCR, alkaloid production*



PP49

Alternative Protein Production Using *Schizochytrium limacinum* Microalgae and the effect of Fermentation on the Techno-functional Properties of the Obtained Protein Concentrates

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Nowadays, the importance of replacing unsustainable animal-derived proteins with sustainable alternative proteins to meet future global needs is strongly emphasized. Microalgae biomass is considered as a significant alternative to terrestrial biomass, offering advantages such as a high growth rate, low water consumption, carbon-neutral emissions. The proposed project aims to obtain a protein concentrate from the de-oiled biomass of microalgae *Schizochytrium limacinum* using a biorefinery approach. The supernatant obtained after homogenization in the alkaline extraction process of de-oiled *Schizochytrium limacinum* biomass was precipitated at its isoelectric point, resulting in the extraction of protein. Subsequently, obtained protein extracts were used for protein precipitation. Precipitated protein powders were lyophilized, and its protein content was found as 40% according to Dumas method. However, the protein precipitation yield was calculated as 43.2% through the Lowry protein assay. Since there were soluble proteins and other nutritional compounds remaining in the supernatant, this extract were utilized as culture media for the fermentation process. The fermentation medium was prepared by adding 0.2% of the lyophilized powdered protein to the supernatant used as the medium. Specifically, the protein obtained will undergo fermentation with *Lactobacillus helveticus* bacteria aiming to enhance its properties. The highest number of viable cells was 8.41 log CFU/mL reached after 24 h of fermentation.

To assess the techno-functional properties of the protein, antioxidant activity and solubility analyses were conducted. Solubility analysis is crucial for understanding the physicochemical characteristics and shelf stability of protein-rich foods. Antioxidant activity was determined according to DPPH (2,2-diphenyl 1-picrylhydrazyl) radical scavenging assay, yielding the EC₅₀ value for protein concentrates. The fermented product exhibited a lower EC₅₀ value of 595 µL compared to the control group's 669 µL, indicating enhanced antioxidant activity through fermentation. This study will contribute to the alternative protein market to obtain foods with high nutritional value.

Keywords: Microalgae, Sustainability, Biorefinery, Techno-functional properties

Acknowledgment

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PP50

Investigating the Effects of a Novel Lignocellulose-Based Biodegradable Superabsorbent Polymer on Growth and Nitrogen Use Efficiency of Wheat under Different Irrigation Regimes

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Maximizing both water- and nutrient-use-efficiency by using novel technologies is critical for sustainable agriculture. With their remarkable swelling capacity and ability to retain water, superabsorbent polymers (SAPs) can effectively maintain soil moisture while minimizing losses caused by leaching. This study was conducted to evaluate the effects of a novel lignocellulose-based biodegradable SAP on the growth, photosynthetic parameters, mineral uptake and N use efficiency of wheat (*Triticum aestivum* cv. Nusrat) under control, leaching and drought conditions, depending on the N fertilizer form. Soil experiments were set up under greenhouse conditions. The newly developed SAP was applied to the pots at two different levels: 0.05% and 0.10%. Half of the pots received N fertilizer in the form of calcium nitrate, while the other half received N fertilizer in the form of urea. Throughout the 8-week experiment, the pots were weighed daily to apply different irrigation regimes. The control pots were maintained at 80% field capacity while the pots exposed to drought experienced a weekly reduction in field capacity. In the leaching group, 100 ml of leachate samples were taken from the saucers once a week. Chlorophyll and photosynthetic parameters were measured. At the end of the experiment, shoot dry weights and tissue mineral concentrations of the plants were determined. Moreover, nitrate, ammonium and urea analyses were performed on leachate samples. In both control and leaching groups, increasing levels of SAP application positively affected plant growth. On the other hand, drought stress negatively affected wheat growth and shoot biomass. However, the negative effects of the drought were alleviated by increasing levels of SAP. This effect was observed more clearly in plants fertilized with urea compared to plants fertilized with calcium nitrate. It was determined that the high level of SAP significantly increased the photosynthesis rate although it reduced the chlorophyll content. Moreover, in SAP applied plants, the shoot K concentration and content increased while the Na concentration decreased significantly. These findings demonstrate that the novel SAP is potentially a promising input for sustainably enhancing plant growth through improved mineral nutrition.

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Keywords: bread wheat (*Triticum aestivum*), drought, mineral homeostasis, photosynthesis, plant growth, superabsorbent polymers.



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PP51

Mycelium Biocomposites: Utilizing Sugar Cane and Wheat Straw Substrates for Fully Bio-Based and Biodegradable Material Solutions

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The increasing pollution caused by plastic waste has prompted a search for eco-friendly alternatives, especially in packaging and construction. Styrofoam, a commonly used material in these sectors, worsens the problem due to its non-biodegradable nature. Mycelium biocomposites present promising sustainable alternatives to non-biodegradable foams, thanks to their dimensional stable structure and foam-like texture. However, enhancing their strength remains a critical challenge.

In this study, we thoroughly examined mycelium biocomposites, analyzing their various properties such as strength, morphology, flammability, thermal resistance, and biodegradability. We use different types and sizes of substrates, like sugar cane and wheat straw, along with oyster mushroom mycelium, either separately or mixed in equal parts. The UL-94 test was carried out for the flammability properties of biocomposites. Our results show biodegradability rates of 65.48% to 77.80% over 12 weeks, depending on the substrates used. The compression strength of the biocomposites ranges from 4.71 MPa to 13.24 MPa, all surpassing that of Styrofoam (1.73 MPa). Additionally, certain biocomposites achieved a 5VB class rating for inflammability. Enhancing the mechanical properties represents a crucial advancement, paving the way for these materials to become viable eco-friendly alternatives to non-biodegradable foams in packaging and construction.

Keywords: *Mycelium biocomposites, biodegradability, flammability, eco-friendly packaging and construction materials.*



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PP52

Using CRISPR /Cas9 System for Accelerated Breeding in Plants

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CRISPR/Cas9 system was first identified by Francisco Mojica in the 1990s. The use of CRISPR/Cas9 has revolutionized plant breeding by providing a very effective and precise method for enhancing the genetic traits of crops. Conventional methods of crop breeding are often hindered by long developmental cycles and inadequate genetic diversity, which may obstruct the improvement of crucial traits like yield, stress resistance, and nutritional quality. The CRISPR/Cas9 technology provides a groundbreaking way of allowing accurate manipulation of genes, which speeds up the breeding process and overcomes the genetic limitations of traditional approaches. This research investigates the efficacy of CRISPR/Cas9 in reducing breeding cycles and improving the efficiency of choosing desired features by leveraging its accuracy and speed. Furthermore, the use of CRISPR/Cas9 in accelerated breeding not only expedites crop development cycles but also streamlines the process of domesticating untamed species and enhancing plant physiological characteristics under various climatic situations. The potential of CRISPR/Cas9 to address the demands of a growing global population against shifting environmental challenges is a promising field in agricultural sciences. It indicates a future where crop improvement is rapid and strongly aligned with global food security objectives.

Keywords: CRISPR/Cas9, plant breeding, accelerated breeding, food security



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PP53

Genomics and Speed Breeding of Cotton and Wheat under Genome Center Project

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In the face of a rapidly growing global population, ensuring food security and promoting sustainable agriculture are paramount to meeting future nutritional demands. The integration of advanced genomic techniques and speed breeding strategies to enhance the breeding efficiency and crop improvement of cotton and wheat. The National Crop Genomics and Speed Breeding Center for agriculture sustainability Project at MNS University of Agriculture, Multan aims to leverage cutting-edge biotechnological tools to address key challenges in agriculture, such as climate change, disease resistance, and yield optimization. A significant achievement under the project is the whole genome sequencing of 40 varieties of cotton and 16 varieties of wheat, representing the local germplasm of Pakistan. The insights gained from the sequencing data will design the future breeding programs for cotton and wheat. Additionally, molecular markers for several genes related to drought tolerance, oil content, and other important traits have been designed. By identifying and utilizing beneficial genetic variations, genomic technologies—such as whole-genome sequencing, marker-assisted selection, and CRISPR-Cas9 gene editing are employed to accelerate the breeding process, enabling the development of superior crop varieties in a significantly reduced timeframe compared to traditional breeding methods. In conclusion, the Genome Center Project exemplifies the potential of integrating genomics and speed breeding to revolutionize crop improvement. The collaborative efforts showcased at the congress underscore the importance of interdisciplinary research and international cooperation in addressing global agricultural challenges.

Keywords: *Genomics, speed breeding, cotton, wheat, genome center project, sustainable agriculture, biotechnology, crop improvement, whole genome sequencing, molecular markers*



PP54

Silver Nanoparticle-mediated Regeneration in Potato

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Potato is the fourth major staple food crop. It is a good source of minerals, vitamins, carbohydrates and proteins but its response towards tissue culture approaches is variety depend. Variety dependency can be overcome and maximum regeneration potential can be achieved by optimizing various factors like PGRs and environmental factors. The special qualities of nanoparticles make nanotechnology attractive for use in agriculture and biology. Numerous aspects of plant biology, such as genetic modification, large-scale multiplication, conservation and the production of bioactive chemicals depend on plant tissue culture. Prior research has shown the advantageous effects of silver nanoparticles (Ag NPs) on the development of calluses, somatic embryogenesis, soma clonal variation, genetic modification and the production of secondary metabolites. This research aims to investigate the roles that AgNO₃ and Ag NPs play in the growth and development of potatoes. In potato tissue culture, we investigated the role of silver nanoparticles upon callus induction and regeneration. Several regeneration media containing different concentrations of Ag NPs and AgNO₃ (0.5, 2, and 5 mg/l) were applied to the stem and root tissues. We have identified optimum concentration of nanoparticles that resulted in maximum vegetative growth of potato.

Keywords: *Potato, callus, regeneration, silver nanoparticles, somatic embryogenesis*



PP55

GWAS for Mapping Genetic Variations associated with Grain Yield Contributing Traits in Bread Wheat

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Wheat (*Triticum aestivum*, $2n = 6x = 42$; AABBDD) belongs to Poaceae family is a self-pollinated crop. It is well-known as a vital food crop for 95 developing countries including Pakistan. The world population is increasing day by day and it is expected that it will increase up to 9.8 billion in 2050 but the wheat yield is almost same. To meet the expected increase in demand for food by 2050 an estimated 50% increase in food production is required. This requires continuing genetic gain in yield improvement by approximately 1.1% per annum. In this study important variations associated with grain yield were identified using Genome Wide Association Studies (GWAS). DNA of 168 wheat genotypes including land races and varieties was extracted and Liquid Chip (37K) was utilized for genotyping. The chip was developed on historical set of Pakistani wheat germplasm. At the same time phenotypic data was collected for the traits days to heading, NDVI, number of productive tillers, plant height and spike length under normal field conditions. Analysis of Variance (ANOVA) was performed to check the significance of the phenotypic data. The genotypic data from 37K liquid Chip was associated with the phenotypic data using GWAS model FarmCPU. Quantitative Traits Nucleotides (QTNs) related to grain yield contributing traits in historical set of Pakistani wheat germplasm were identified in this study. We will develop SNP based molecular markers on the identified QTNs in future. The identified markers will have the ability to be used in Marker Assisted Breeding by research institutes.

Keywords: *Wheat, GWAS, 37K liquid chip, FarmCPU, QTNs, SNP based marker(s)*



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