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ICABB 2024 CONGRESS

BOOK of PROCEEDINGS

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Editors

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Dear Scientist,

The 8th International Congress on Advances in Bioscience & Biotechnology (icabb) was organized in Aksaray, TÜRKİYE. We are very happy for organizing this congress in such a beautiful city and country that we have strong historical ties.

We wanted to make this conference little bit special by bringing scientist together from different disciplines of biology and biotechnology area and also to open new research and cooperation fields for them. In this sense, we desired to bring the distinguished scientist together to get know each other and to develop and implement new joint projects.

The scientist joined the congress was from different country. Total over the hundered scientist were registered in the congress. The total number of submissions were 80 and after a careful evaluation 39 submissions were accepted by our scientific committee and 9 of them were accepted as poster presentation and, 30 of them were accepted as oral presentation and all those presentations was taken place in the conference booklet.

I would like to thank Prof. Dr. Hesham El Enshasy (Plenary/Orientation Lecture), Dr. Malik Altaf Hussain (Plenary Lecture), Prof. Dr. Mysoon Al-Ansari (Keynote Lecture for their valuable presentations.

We would like to send our special thanks to Mr. Musa Köse and Mr. İsmet Uzun, ZENITH Group workers for their special efforts. And finally, the most importantly I would like to thank to all the participants individually who came from far away to join this conference.

President

Prof. Dr. İlker Camkerten

Dear colleagues,

We are honor to welcome you this year in the 8th. International Congress on Advances in Bioscience and Biotechnology (ICABB). We are happy this year to have conference in Aksaray "The historic and wonderful city in the heart of Antolia, Türkiye. The conference this year is attended by many colleagues from all over the world to share their novel research with the scientific society. ICABB is also now considered as international hub for networking and building new partnership in research and to establish new cooperation agreements among researchers and between researchers and industries as well. I am happy to see that our ICABB family is growing from year to year in terms of number and diversity representing many countries around the world. The conference this year covers most of the biotechnology colors (Green: Agriculture and Environment; White: Industry; Red: Medical). In addition, some topics this year present very interesting integrated research between these three main fields of biotechnology.

I wish you all colleagues who are attending the conference physically in Aksaray or on-line a very nice conference and successful networking with other colleagues toward providing solutions to challenges we are facing nowadays to improve the quality of life on the earth.

With you all nice conference, successful network, and enjoying the beautiful culture of Aksaray

Prof. Dr. rer. Nat. Hesham Ali El Enshasy

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Dr. Avnish Pareek, Universiti Technology and applied Sciences-Sur Campus, Sultanate of Oman

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INVITED SPEAKERS

ANTIBIOTIC RESISTANT MICROBES (THE NEW GENERATION OF SMART KILLERS): ARE WE PREPARED FOR?

Hesham Ali El Enshasy[1,2,3]

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Abstract:

Antibiotics have been used for decades as the most potent therapy for the treatment of many infectious diseases based on their high antimicrobial properties. By definition antibiotics are chemical substances produced by living organisms and inhibit the growth of other microorganisms when used at low concentration. Since the discovery of antibiotics in the mid of 1930s, these group of compounds saved live of hundred of millions. It was also assumed that the victims of the second world war could be tripled if there were no antibiotics discovered and used in the treatment. Nowadays, antibiotics are widely used not only for human health sector but also in agriculture, aquaculture, and animal feed industries. This widespread of application of antibiotics for non-medical uses in addition of misuse and mis-dose of antibiotics create big problem related to antibiotics resistance. This will lead to that antibiotics resistant pathogens will be the main human killers by 2050. Therefore, new approaches need to be carried out to minimize this risk. At first, the control of antibiotic applications in non-medical fields to minimize the creation of antibiotic resistance by continued exposure to sub-therapeutic doses of antibiotics. Second, the integration of new strategies on controlling human and animal pathogens such as natural (plant based and animal based) anti-infective, probiotics, immunomodulators can be applied as first line in the treatment before using antibiotics. This presentation, highlights the potential new trends for utilization of new anti-infective classes in human and animal health care industries.

Keywords: Antibiotic resistance, human health, food security

MICROBIOLOGICAL FOOD SAFETY CHALLENGES ASSOCIATED WITH NOVEL PLANT-BASED FOOD PRODUCTS

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Abstract:

Contamination of foods with pathogenic microorganisms, chemicals, allergens, or physical hazards can pose health risks for consumers and economic loss to the industry. Several known food safety hazards are associated with animal-based food products. Plant-based food products have emerged as a major group of novel foods in the global food supply. Similar to animal-based or other foods, plant-based foods could naturally contain certain biological or chemical contaminants. Food safety hazards including bacteria, viruses, parasites, mycotoxin, heavy metals, and pesticide residues could be introduced in new plant-based food products through external factors or cross-contamination. Furthermore, the processing of plant-based ingredients can also create their unique type of food safety risks. For example, applying extreme temperatures and mechanical energy to manufacture extruded products may generate unwanted chemical residues. Undoubtedly, plant-based food products offer promising substitutions for animal-based food choices to meet the future protein demand. The major challenge is the availability of limited information on the microbiological risk assessment of new plant-based food products entering the market. This presentation will discuss microbiological food safety issues, challenges, and uncertainties in manufacturing plant-based food products.

Keywords: Alternative proteins, Plant-based foods, Food safety, Microbiological hazards, Food processing, Health risks

ORAL PRESENTATIONS

ESCHERICHIA COLI AND PSEUDOMONAS AERUGINOSA INTERACTIONS WITH BREAST CANCER CELLS: IMPLICATIONS FOR THERAPEUTIC TARGETS

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Abstract:

This study explores the influence of the breast microbiome on breast cancer (BC) cell metabolism, focusing on *Escherichia coli* (*E. coli*) and Pseudomonas aeruginosa interactions with breast cancer cell lines. We hypothesized that *E. coli*, prevalent in BC tissues, secretes metabolic molecules that modify the metabolism of BC cells, thereby supporting their survival. Through untargeted metabolomics, we identified significant alterations in metabolites following *E. coli* secretome treatment, revealing dysregulation in pathways critical for BC pathogenesis. Additionally, we investigated how breast cancer cells influence the metabolism of *Pseudomonas aeruginosa*, elucidating the crosstalk between these bacteria and BC cells. Our findings highlight the potential roles of bacterial metabolites in BC progression and suggest therapeutic avenues targeting the microbiome to enhance treatment strategies for breast cancer.

Keywords: *Escherichia coli,* breast cancer, microbiome

STADY OF POTENTIAL PROBIOTIC OF LACTOBACILLUS STRAINS ISOLATED FROM ALGERIAN TRADITIONAL FERMENTED MILK AND THEIR ANTAGONISTIC ACTIVITY AGAINST RESISTAN STAPHYLOCOCCUS AUREUS

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Abstract:

Background: Probiotics have gained significant attention due to their potential health benefits, including their ability to modulate the gut microbiota and positively influence various aspects of human and animal health. Lactobacillus plantarum is a well-known probiotic strain known for its beneficial effects on gastrointestinal health. In Algeria a country traditionally a consumer of fermented milk products made from raw milk the failure to comply with hygienic conditions, these products become a frequent cause of S. aureus food poisoning.

Objective: This study was conducted in order to evaluate the probiotic properties of thirty lactic acid bacteria (LAB) isolated from the traditionnal fermented milk. Also the strains of Lactobacillus were tested against S. aureus resistant.

Methods: The major properties, including gastric juice (pH2) and bile salts tolerance (0,3%), antibiotic susceptibility (10 antibiotics are tested by antibiogram test), auto-aggregation, their adhesion to polystyrene microplates and in vitro antioxidant capabilities (DPPH and ABTS test) were investigated. The strains of Lactobacillus were tested for their antagonistic activity against S. aureus resistant via spot and wells test.

Results: 50% of the strain presented a viability rate of 100% at acid pH, adhere strongly to microplates, tolerance to bile stools and sensitivity to the antibiotics tested. The results obtained show that 50% of the l'ben samples are contaminated with S. aureus, in fact, 60 S. aureus strains are identified. The study of the sensitivity of these strains with respect to cefotaxime and ceftazidime made it possible to select 20 strains of S. aureus with a 32.62% strength of resistance. The study of the antibacterial activity of the 15 strains of Lactobacillus revealed zones of inhibitions which vary between 15 and 35 mm. However, the well test showed that 5 Lactobacillus strains showed inhibitory zones that exceeded 15 mm after pH neutralization of the supernatant.

Conclusion: isolated Lactobacillus strains exhibited several characteristics to prove it's excellent as a potential probiotic candidate for developing quality fermented milks.

Keywords: Probiotics, Lactobacillus, antibacteriel activity, Staphylococcus aureus, antibiotic's resistance

WATER IN PLANT-MICROBE INTERACTIONS AND THEIR SUSTAINABILITY

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Abstract:

Water is a critical determinant of plant and microbial life, influencing the dynamics of their interactions and the sustainability of ecosystems. It acts as a medium for nutrient transport, a solvent for biochemical reactions, and a habitat for various microorganisms, in the rhizosphere. Water as water is also a habitat for microorganisms which facilitate plant-microbe interactions.

The paper explores the multifaceted role of water in plant-microbe interactions and its implications for the sustainability of these interactions. By examining the mechanisms through which water influences symbiotic and pathogenic relationships between plants and microbes, as well as the impact of water availability and quality on these interactions.

The role of water in plant-microbe interactions and their sustainability was investigated, a comprehensive review of the existing literature was conducted. Relevance to the role of water in plant-microbe interactions, focusing on studies that addressed the mechanisms through which water influences these interactions, the impact of water availability and quality, and strategies for sustainable water management. Drought stress can negatively impact both plants and microbes, leading to reduced plant growth, altered microbial communities, and impaired nutrient cycling. Intricate role of water in these interactions is essential for enhancing plant productivity, maintaining soil health, and ensuring the sustainability of ecosystems in the face of climate change and increasing water scarcity. This research highlights the importance of water management in sustainable agriculture and ecosystem health.

The study shows that the sustainability of these interactions is highly dependent on water availability and quality. Understanding the mechanisms through which water affects plant-microbe interactions can inform water management practices in agriculture and ecosystem conservation, ultimately promoting plant health, soil fertility, and ecosystem stability. Sustainable water management is essential to support micro for healthy and productive ecosystems.

Keywords: Water, Plant, Microbe, sustainability, drought stress

*Nil

GLOBAL CARP FISH DATABASE UTILITY FOR ZOONOTIC DISEASES TRANSMISSION DND PREVENTION

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Abstract:

Many global and regional fisheries information systems and databases have been developed throughout the world to assess the economic potential and disease prevalence in various fish species. All fish carry pathogens and parasites, making them susceptible to various types of diseases. Carp are the common fish species found throughout the world. The Carp are raised for food especially in Europe and Asia because of its high per acreage potential. With its 28,668 million tons global production of 12 major cultured carp species, the Carp has high economic potential. The Carp are sensitive to zoonotic human transferable disease. Human infections caused by pathogens transmitted from fish or the aquatic environment are quite common depending on the season, patients‟ contact with fish and related environment, dietary habits and the immune system status of the exposed individual. Conventionally, agar plates and phenotypic and serological properties of pathogens or histological analysis were used for fish disease diagnosis. The main zoonotic agents of fish are Gram-negative bacteria and few Gram-positive bacteria. One of the most effective means of reducing risk is by freezing or heat-inactivation. Fish-borne Zoonotic Trematodes (FZTs) affect the health of millions of humans worldwide and causes loss of productivity and for the prevention and treatment of these diseases there is a need to establish a fish disease specific database to prevent human transferable diseases especially for the zoonotic diseases, associated with fish contact. The paper examines the effect of fish transferable zoonotic diseases on human and its impact and need for databases for prevention of zoonotic diseases in common Carp. The paper also suggested a simple database structure for zoonotic disease information.

Keywords: Carp fish, zoonotic diseases, human and its impact, database

**Study is the part of PhD thesis*

MICROALGAE/PLA BIOPOLYMER SYNTHESIS: A COMPREHENSIVE PROCESS INVOLVING MICROALGAE OPTIMIZATION AND BIOPOLYMER ANALYSIS

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Abstract:

Blending microalgae biomass with certain biopolymers to improve the biodegradation rate is an emerging technique in polymer science. However, the primary issue of utilizing microalgae is the expense of growing and harvesting biomass, as well as the possibility of affecting the biopolymer's characteristics. Therefore, this study aims to reduce the production cost of microalgae through microalgae optimization and to synthesize microalgae/PLA biopolymer to explore its physical, thermal, and biodegradation characterization. Chlorella vulgaris were cultivated in N.P.K. fertilizer and urea and optimized by the Box-Behnken design, revealing a high tolerance of urea of up to 1.2 g/L for the first time. The average cell density and dry biomass weight were 1.7 million cells/mL and 0.226 g/L, respectively, costing less than 0.018 USD/L. The biomass was harvested using centrifugation and synthesized magnetic nanoparticles in a successful attempt to lower the cost of the synthesized magnetic nanoparticles by eliminating the sonication process and reducing microwave radiation during microalgae's detachment and synthesis of the nanoparticles, respectively, notably, the harvesting efficiency was 92.9%. Finally, the biomass was recycled to produce biopolymers by combining C. vulgaris biomass with PLA using solvent-casting. For the first time, two annealing phases at 105 °C were employed with a comparative approach with the biopolymers that are not annealed. The applied method increased the tensile strength to a maximum value of 15.6 MPa, and as far as we know, it is the highest tensile strength that has been achieved so far using C. vulgaris/ PLA in the solvent casting technique and no impact on the thermal properties was observed. Ultimately, a quick analysis of the biodegradation profile of the produced biopolymer in Aegean seawater revealed a reduction in weight of up to 0.9–3.5 mg after 20 days.

Keywords: Microalgae, Chlorella vulgaris, Biopolymer, PLA, Polylactic acid, Bioplastic

EFFECTS OF EUGLENA-DERIVED CAROTENOID AND EXTRACT ON HIGH-FAT DIET FED-MICE

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Abstract:

Obesity is an abnormal condition with an inflammatory process in adipose tissue and liver. Chronic inflammation is considered to be important for the development of insulin resistance. Euglena is unicellular green algae, which contained several nutrients. Euglena extract was reported to have an ability to inhibit the early stage of adipocyte-differentiation. Thus, Euglena is a promising candidate for the development of a new therapeutic treatment for obesity. Diatoxanthin (Dtx) is one of major characteristic carotenoids contained in euglena, however, there are only a few reports on its functionality. Therefore, in this study, we aim to identify the effects of euglena-derived carotenoid and extract on high-fat diet fed-mice, especially for the purpose of finding new functionalities. Mice were preliminary raised for ten days and randomly divided into 4 groups: Normal Diet (ND) group, High Fat Diet (HD) group, HD+diatoxanthin fraction (DF) group, HD+hot water extract of Euglena (HWE) group. DF was found to suppress the increase in blood glucose level by high fat diet. Dietary DF reduced the expression of lipogenesis-related genes and enhanced the expression of β-oxidation related genes in the liver. We also successfully detect the accumulation of DF in the tissues. These results suggested that functional ingredients were present in the DF fraction.

Keywords: euglena extract, diatoxanthin, high fat diet, glucose

RELATIONSHIP BETWEEN EMBRYO QUALITY AND BLOOD VALUE IN CATTLE

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Abstract:

The purpose of this study; The aim of this study is to determine the relationship between corpus luteum, number of transferable embryos, embryo quality and hematological values in Holstein cattle undergoing superovulation. As a donor in the study, 10 head of Holstein breed cattle, 2.5-4 years old and 60-90 days postpartum, were used. Progesterone-based (9-day duration) estrus synchronization protocol was applied to selected donors. Starting from the 7th day of progesterone administration, FSH was injected in decreasing doses at 12-hour intervals for 4 days. On the 9th day of progesterone application, PGF2α was applied in the morning and progesterone was removed from the vagina in the evening of the same day. On the 11th day, artificial insemination was performed on the donors every 12 hours. Artificial insemination day: Blood samples were taken from all donors to determine hematological values. Uterine flushing was performed on the 7th day after artificial insemination. The cattle were divided into two groups according to the number and quality of embryos obtained as a result of uterine flushing and the number of corpus luteum on the ovary. The first group was determined as the group in which 6 or more corpus luteum and quality embryos were obtained. The second group was grouped as the number of 3 or fewer quality embryos, degenerated embryos and corpus luteum. In the group where a higher number of quality embryos and corpus luteum were obtained; WBC, LEN, MON, GRAN, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, PDW, PCT, Embryo Number, CL Number respectively; 9.60, 3.80, 0.90, 4.90, 5.96, 10.28, 29.20, 48.84, 17.24, 35.48, 16.00, 351.80, 6, 20, 16.70, 0.22, 7.60, 8. In the group in which a lower number of quality embryos and corpus luteum were obtained; WBC, LEN, MON, GRAN, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, PDW, PCT, Embryo Number, CL Number respectively; 6.84, 2.20, 0.60, 4.16, 6.10, 9.84 28.16, 46.30, 16.10, 34.90, 17.68, 509.00, 5.84 , was found to be 16.08, 0.30, 1.40, 2.40. As a result, it was concluded that there may be a relationship between hematological values and the number of transferable embryos, embryo quality and the number of unfertilized embryos in Holstein donors.

Keywords: Cattle, Hematological value, Embryo quality

NEW SEROLOGIC MARKERS AS AN ALTERNATIVE TO MOLECULAR METHODS IN CHRONIC HEPATITIS B PATIENTS

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Abstract:

Objective: Studies on markers that can be an alternative to the gold standard test HBV DNA detected by Real Time PCR method are limited. In our study, we aimed to investigate the relationship between HBV DNA and hepsidin, pentraxin-3, zonulin and copeptin levels which can be quantitatively detected by ELISA method.

Matherial and Method: The study included 105 patients diagnosed with chronic hepatitis B with HBsAg positivity in serum for more than 6 months and 35 healthy controls without any acute or chronic disease. HBV DNA levels were determined by Real Time PCR method using Bosphore HBV Quantification kit. The patients were divided into three groups, 35 patients with viral load between 1-100 were divided into mild viral load group, 35 patients with viral load between 1.000-1.00000 were divided into moderate viral load group and 35 patients with viral load between 1.000000-1.00000000 were divided into severe viral load group, taking into account the viral load indicating biopsy indication and the viral load with a significant increase in mortality. The levels of pentraxin-3, zonulin, copeptin and hepcidin measured quantitatively from the remaining sera of the patients were investigated using Human Elabscience ELISA kits.

Results: There was a statistically significant difference in serum hepcidin levels between the control group and the heavily viral loaded group ($p=0.01$). There was a significant difference in pentraxin-3 levels between the control group and all patient groups ($p<0.001$). A significant difference was found between the group with heavy viral load and all groups in terms of copeptin levels ($p<0.05$). There was no significant difference in serum zonulin levels between the groups ($p>0.05$).

Conclusion: We think that studies on prognostic markers that can be an alternative to gold standard tests in the diagnosis and treatment follow-up of chronic diseases will both reduce laboratory costs and save time with easier-to-apply tests such as ELISA.

Keywords: Pentraxin-3; Hepsidin; Copeptin; Zonulin

This study is supported by NEU Scientific Research Project Coordination with project number 23TU18006.

INVESTIGATION OF VIRAL AGENTS IN PATIENTS WITH PREDIAGNOSED MENINGITIS

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Abstract:

Objective: Meningitis is an infectious disease with high complications requiring timely diagnosis and treatment. In most cases of meningitis, empirical treatment should be initiated rapidly before the causative agent is detected. Empirical antimicrobial therapy should be initiated in accordance with the age of the patient and the most common pathogens in the geographical region where the patient is located. The aim of this study was to investigate viral agents by molecular methods in cerebrospinal fluid (CSF) samples obtained from patients with a prediagnosis of meningitis during a five-year period.

Material-Method: The data in the hospital information system were retrospectively analyzed and included in the study. Viral agents were detected using the Allplex Meningitis-V1 Assay kit (Seegene, South Korea) on a BIO-RAD CFX96 (California, USA) PCR device.

Results: Of the 1588 CSF samples included in the study, 825 (51.9%) were from males and 763 (48.1%) were from females. Of all CSF samples, 64 (4.03%) were positive for any agent. The minimum age of the patients in our study was 0 years, the maximum age was 91 years and the median age was 18 years. The mean age of the patients included in our study was 27.76 years. 17 (1.07%) patients were positive for HHV-7, 13 (0.81%) for EBV, 8 (0.50%) for HSV-1, 7 (0.44%) for CMV, 6 (0.37%) for VZV and 3 (0.18%) for HSV-2. Two patients were HHV-7 and EBV positive, two patients were HHV-7 and VZV positive, one patient was HHV-7 and HSV-2 positive, one patient was CMV and EBV positive, one patient was EBV and VZV positive, one patient was HHV-7 and HSV-2 positive, and one patient was HHV-7, HSV-2 and VZV positive simultaneously.

Conclusion: Since it is very important to correctly identify the agent in order to apply appropriate and sufficient antimicrobial treatment to patients with pre-diagnosis of meningitis, molecular epidemiological studies should be conducted on the distribution of agents.

Keywords: Meningitis; Viral Agent, PCR

SYSTEMS ANALYSIS FOR SUSTAINABLE BIOPROCESS ENGINEERING AND BIOTECHNOLOGY

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Abstract:

Clean energy can support the circular economy to achieve Sustainable Development Goals while tackling climate change. Biomass is promoted as an eco-friendly feedstock for biofuel production to facilitate energy transition. However, sustainable production of this renewable energy is only possible if the appropriate biomass and engineering approaches are combined to work well for people and ecosystems worldwide. Thus, system thinking approaches are urgently needed to go beyond the silo mentality, green chemistry, green biotechnology and "safe and sustainable by design", among others. Systems thinking underpins bioresource systems analysis, and one example is the promising production of bioethanol from arid plants at an industrial scale. This study joined agriculture and a biorefinery to create an agrobiorefinery system whose energy efficiency was assessed by applying energy balances, energy returns on investment and cumulative energy demand. Prickly pear cactus (Opuntia ficus-indica) is farmed in arid regions and used as raw material in biorefineries for ethanol production. The fertilisers considered are inorganic or organic, whereas the biorefineries considered are traditional (with acid pre-treatment) or enhanced (recycled ionic liquids). The new agrobiorefinery is cleaner and showed an energy return on investment three times that of the traditional agrobiorefinery. The yield of ethanol per gramme of prickly pear cactus outcompeted that of rice straw, algae, bagasse and corn stover. This novel agrorefinery concept reduced by a third the energy used by the traditional biorefinery, specifically in the stages of ethanol purification, biomass pretreatment and conversion. Systems thinking can support sustainability efforts based on biotechnology and bioprocessing engineering to improve energy efficiency. Sustainable agrobiorefineries could deliver green energy, sustainable products and services for climate risk mitigation and sustainable development.

Keywords: biomass, bioprocess engineering, clean energy, circular economy, biorefineries, energy efficiency, sustainability

POTENTIAL REVERSIBLE INHIBITORS OF LYSINE-SPECIFIC HISTONE DEMETHYLASE 1 IDENTIFIED VIA STRUCTURE-BASED VIRTUAL SCREENING

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Abstract:

Lysine-specific histone demethylase 1 (LSD1) is a flavin-dependent monoamine oxidase that removes methyl group from mono- and di-methylated lysine residues at the lysine 4 of histone H3 (H3K4me1/2) and lysine 9 of histone H3 (H3K9me1/2), acting as a transcriptional corepressor or coactivator, respectively. LSD1 is overexpressed in about 60% of cases of acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML) and myelodysplastic syndrome (MDS), and more. This overexpression in a variety of tumor cells is associated with compromised differentiation, proliferation, migration, and invasion. Inhibitors of LSD1 reverse the malignant phenotype where LSD1 is overexpressed. A potent monoamine oxidase inhibitor drug tranylcypromine exhibits potent LSD1 inhibition ability, however, due to irreversible nature of the inhibition, the drug is associated with adverse effects. In search of reversible LSD inhibitors, structure-based virtual screening of ZINC library containing over 17 million drug-like compounds was conducted using multilayered scoring functions and filters in Glide. The top hit compounds were subjected to MD simulation coupled with molecular mechanics with generalized Born surface area (MM/GBSA) binding energy calculations. Based on structural diversity and good physicochemical properties, hit compounds that display potential inhibition of LSD1 were identified, subject to further experimental validation.

Keywords: LSD1, LSD1 inhibitors, structure-based virtual screening, MD simulation, MM/GBSA calculations

MEDICINAL PLANTS AS BIOLOGICAL AGENTS FOR CROP PROTECTION AND CONSERVATION

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Abstract:

There have been rapid advances in the field of plant biotechnology in recent years, increasing the potential for medical and agricultural application. The limitations of chemical crop protection products, along with their high development costs and the presence of toxic pesticide residues in raw materials, raise significant concerns about environmental and human health risks. As a result, over the past few decades, there has been a growing need to discover alternative methods for controlling plant pests and diseases.

Medicinal Plant Biotechnology is an essential field for researchers in plant biology and biotechnology, including plant tissue culture, secondary metabolite production, molecular farming, medical sciences and pharmacology.

Compounds derived from medicinal plant parts are generally used in traditional medicine to cure human diseases. Their potential action against plant pests and diseases is recently under researcher's scientific study.

Recent studies have shown that extracts from medicinal plants can reduce crop pest populations by up to 40%, significantly lowering the need for synthetic pesticides.

Keywords: Medicinal plants, extracts, fungicides, pesticides, plant protection.

**This study is supported by MESRS-Algeria.*

ALTERATION IN ANTIOXIDANT ENZYME ACTIVITIES OF TETRAPLOID WHEATS UNDER DROUGHT STRESS

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Abstarct:

Tetraploid wheats include Triticum durum, Triticum polonicum and Triticum turanicum, which are of significant nutritional and vegetative value. Drought tolerance of these wheat varieties should be investigated. Tolerant plants accumulate soluble sugars, proline content, amino acids, enzymatic and non-enzymatic antioxidants in order to cope with the stress. In this study, it is aimed to define the changes of enzyme activities of tetraploid wheat genotypes under drought stress. The research was conducted with five landraces of Triticum durum, five Triticum polonicum and five Triticum turanicum in 2023-2024 growing season in Eskişehir. Genotypes were analyzed to variance of electrolyte leakage, hydrogen peroxide (H2O2) and protein content as well as antioxidant enzyme activities such as superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX) and catalase (CAT) under drought stress in vegetative and generative period. The effects of drought stress on both vegetative and generative stages resulted in notable variations among genotypes in electrolyte leakage, H2O2 and protein content, as well as antioxidant enzyme activities. The degree of damage caused by electrolyte leakage differed significantly among genotypes subjected to drought stress at the vegetative and generative stages. Upon examination of the increase in H2O2 content, it was observed that genotypes exhibiting elevated H2O2 levels during the vegetative period demonstrated a reduction in accumulation during the generative period, and vice versa. While some genotypes exhibited an increase in antioxidant enzyme activity during drought stress in the vegetative period, others demonstrated a decrease. However, during drought stress in the generative period, there was a notable increase in antioxidant enzyme activity. A comparable alteration was observed in soluble protein content. While there is some variation between genotypes, it can be concluded that Triticum polonicum wheats is drought tolerant during both the vegetative and generative stages.

Keywords: antioxidant enzyme activity, drought stress, membrane damage, tetraploid wheats

A STATISTICAL PARSIMONY NETWORK RESOLUTION FOR THE POLYTOMIES IN THE COI-BASED PHYLOGRAMS OF ARHOPALUS RUSTICUS (COLEOPTERA, CERAMBYCIDAE)

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Abstract:

Arhopalus rusticus (Coleoptera, Cerambycidae) is a sombre-colored longnorned beetle. Although its natural distribution area is the Northern Hemisphere, it has gradually distributed to the Afrotropical, Neotropical and Australian regions through human-mediated transport. This beetle is a vector that spreads pine wood nematodes, thus damaging coniferous trees. Population explosions have caused it to be the subject of some recent studies. Despite these studies attempting to resolve phylogenetic relationships of the genus Arhopalus within the other spondylidines based on the mitochondrial cytochrome c oxidase I (COI) barcode region, utilizing conventional phylogenetic tree hypothesising algorithms Neighbor-Joining, Maximum likelihood and Bayesian Inference, they could not provide an intraspecific resolution for the A. rusticus and remarkable polytomies remained. The aim of the study is to understand whether these polytomies occurred as a result of a lack of sufficient synapomorphic characters of the COI barcode region, missing the ancestral gene sequences in the analysis or due to inadequate analysis techniques used. We constructed a statistical parsimony network for the COI global dataset, incorporating sequences from GenBank and our previous research to identify any potentially missing haplotypes and unveil potential geographic links and branching patterns among the haplotypes. Our statistical parsimony network suggested that 21 haplotypes were missing in the previous analysis, and the polytomies may be attributed to branching patterns. The branchings are not dichotomous but rather reticular and radial, indicative of ongoing gene flow and bottleneck effects, respectively.

Keywords: cerambycid pest, bottleneck effects, ongoing gene flow, Spondylidinae, TCS network

ENHANCING THE GASTROINTESTINAL STABILITY OF SPINACH-DERIVED MIRNAS THROUGH SODIUM ALGINATE ENCAPSULATION

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Abstract:

Dietary microRNAs (miRNAs) have emerged as potential key regulators of gene expression across kingdoms with promising implications for human health. However, their stability and bioavailability when ingested, particularly from plant sources, remain relatively unexplored. This study investigated the stability of three spinach-derived plant miRNAs (ath-miR159, ath-miR166, and ath-miR168) and explored the protective effects of sodium alginate encapsulation during in vitro digestion. miRNA levels were quantified following post-digestion by employing the simulated gastrointestinal digestion model (INFOGEST). miRNAs were isolated and then quantified through quantitative real-time PCR. Secondary structure predictions were conducted under physiological conditions to correlate miRNA stability with thermodynamic properties. Sodium alginate encapsulation significantly improved the stability of ath-miR159 and ath-miR166 compared to the non-encapsulated form. Secondary structure predictions revealed a more stable conformation for ath-miR168 compared to ath-miR159 and ath-miR166. These findings underscore the complex interplay between miRNA structure, encapsulation, and the gastrointestinal environment. While sodium alginate encapsulation has been demonstrated to be beneficial for enhancing the stability of certain miRNAs, tailored strategies are essential for accomplishing the most effective miRNA delivery.

Keywords: dietary miRNA, In vitro digestion, plant miRNA, sodium alginate encapsulation

CORDYCEPIN AND ITS BIOLOGICAL ACTIVITIES

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Abstract:

Cordyceps militaris is a medicinally important mushroom species. It is commonly employed in East Asian countries to cure different diseases and health problems. The mushroom is also used to heal the aging and senescence-associated health problems, such as weakness in the loins and knees, impotence and seminal emission, hyposexuality, fatigue, and night sweating. Cordycepin (3-deoxyadenosine), an adenosine analog, is a water-insoluble organic compound synthesized by Cordyceps militaris. It displays many bioactive properties such as: anticancer, antioxidant, anti-inflammatory, anti-aging, anti-bacterial, antifungal, anti-malarial, anti-hyperuricemic, antiviral, antidiabetic, immunostimulating, hypolipidemic, anti-osteoporosis, anti-arthritic and hypoglycemic activities. Because of its antioxidant activity, cordycepin alleviates age-related oxidative stress and neurodegenerative disorders. Cordycepin has a great therapeutic potential in many cancer types. It targets different signaling molecules including kinases and inhibits kinases in cancer cells. It also inhibits matrix metalloproteinases 2 and 9 and shows antimetastatic activity. Cordycepin increaes programmed cell death mechanisms, apoptosis and autophagy and decreases cancer cells growth in tumor microenvironment. Cordycepin also prevents collagen-induced platelet aggregation by decreasing calcium ion and thromboxane A2 levels. It inhibits cell attachment and decreases focal adhesion. It has strong activity in the inhibition of mammalian target of rapamycin (mTOR) signalling and as a result of this, it decreases tumor proliferation. Thus, it has many healthy biological activities and bioactivity and production of cordycepin should be improved for its commercial use as a medical drug in medical fields.

Keywords: Cordycepin, Biological Activity, Cancer, Apoptosis, Autophagy

INVESTIGATION OF NUCLEOLUS ORGANIZER (AGNOR) REGIONS IN THE CEREBELLUM OF CHICKENS*

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Abstract:

In this study, the numbers, localizations, sizes and areas of silver-stained nucleolus organizer regions (AgNORs) in the nuclei of Purkinje neurons of laying Nick Chick chicken breed and broiler Hubbard line were determined during the embryonic period in both breeds and at the 6th post-incubation period in broiler chickens. By being determined up to 32 weeks; changes in these parameters were determined. Comparisons were made between periods in both breeds, between sexes and between races in layers in each period. The cerebellum Purkinje cells of broilers had more nuclei in the 11th and 14th days of hatching, and no difference was observed between broiler and layer hens in terms of nücleus area in other periods. The nücleus diameter is also greater in broilers on the 14th day of hatching. AgNOR number and AgNOR diameter of broilers and layers do not change in any period. Broilers have higher AgNOR area than layers on the 11th day of hatching. Looking at the data obtained in this study, it was concluded that a direct correlation could not be established between growth and egg production and AgNOR parameters of Purkinje neurons; It was concluded that it would be beneficial to carry out studies including AgNOR parameters together, as well as the yield records of animals, especially in the period after hatching.

Keywords: AgNOR, Broiler, Nucleus, Nucleolus, Layer Chickens.

** This study is summarized from a part of his doctoral thesis." AgNOR, Broiler, Nucleus, Nucleolus, Layer Chickens. Supported by Harran University Research Fund.*

DEVELOPMENT AND COMPERATIVE ANALYSIS OF INTERGENIC SPACERS CPDNA, RPOB-TRNC, PETN-PSBM AND MATK FOR CRYPTOCORYNE SPECIES

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Abstract:

Cryptocoryne, an aquatic plant genus, belongs to Araceae family and is well-known for its remarkable diversity with intricate morphology. In addition, frequent occurrences of natural hybridisation have been indicated in this genus and make it a catalyst for the evolution, thus generating new morphological characteristic that disperse throughout the dynamic river systems. This research aims to disentangle some of the intricacies by selectively targeting specific intergenic spacers, rpOB-trnC and petN-psbM, developed from chloroplast DNA of Cryptocoryne nurii. Chloroplast genomes are suitable for evaluating plant phylogenetic analysis and species identification because of their simplistic structure, lack of genetic recombination, and uniparental inheritance traits. Six Cryptocoryne species were collected from different localities in Indonesia and were extracted using standard CTAB DNA extraction protocol. PCR optimization and amplification were performed to obtain visualised band. Furthermore, universal primer, which is matK, was also was tested with Cryptcoryne species. The PCR products were sent for Sanger sequencing to obtain the sequencing data. All the sequences were edited, analysed, and aligned using MEGA-X software. The phylogenetic trees were constructed according to the sequence data matrix. The results emphasise significant knowledge about the genetic variation and phylogenetic complexities of this genus. This contributes to a more profound comprehension of plant genetics and conservation approaches.

Keywords: Cryptocoryne, plant, intergenic spacers, chloroplast, phylogenetic,

UTILIZING NEXT GENERATION SEQUENCING TO CHARACTERIZE SIMPLE SEQUENCE REPEAT LOCI FROM DICRANOPTERIS LINEARIS VAR. LINEARIS (GLEICHENIACEAE)

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Abstract:

"Dicranopteris linearis (Burm.f.) Underworld. (Gleicheniaceae) is a common forked fern found in Malaysia. However, the abundance of this fern across recreational forests and crop plantations in Malaysia has raised concern due to its aggressive nature in competing for natural resources and its allelopathic effect that inhibits the growth of other species, which later may pose a threat to the regeneration of native species or the growth of the crops. This emphasizes the need for tools to effectively manage and control its capability to spread and successfully establish in new environments. Research into the population genetics of the species can provide fundamental insights into understanding its evolutionary history and adaptation mechanisms and is essential for planning, management, and monitoring measures. However, there were limited molecular markers available for the species. Therefore, the current study aims to develop simple sequence repeat (SSR) markers for the species using next-generation sequencing (Illumina NovaSeq 6000 sequencing platform) from genomic DNA of D. linearis var. linearis. After de novo assembly, 35 550 contigs were used to mine the potential SSR motifs using the MISA tool. Among the other SSR types, trinucleotide repeats were the most common. A total of 462 SSR primer pairs were designed using Primer3, but only 20 primers were randomly selected for polymerase chain reaction amplification. Of these, 8 SSR loci were successfully amplified with polymorphic alleles in 30 examined individuals. The set of SSR markers described in this study will help assess the genetic diversity of fern species at the population level and enable targeted management strategies to identify high-risk populations and understand their adaptability and dispersal potential."

Keywords: Forked fern, genetic diversity, next-generation sequencing, simple sequence repeat markers

**This study was supported by the Ministry of Higher Education for Fundamental Research Grant Scheme*

SCREENING AND CHARACTERISATION OF POLYMERS FOR LACCASE CO-ELECTROSPUN NANOFIBRE

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Abstract:

The high levels of non-biodegradable phenolic pollutants have caused significant environmental damage and pose a risk to public health. Laccase is a sustainable biocatalyst that can effectively degrade various phenolic compounds. However, industrial application of free laccase is hindered by issues such as enzyme instability, low recovery, and non-reusability which lead to the need for laccase immobilisation. Due to its high surface area-to-volume ratio and tunable physicochemical properties, entrapping laccase using co-electrospinning is a promising immobilisation method. This study aimed to screen and characterise the best polymer for synthesising immobilised laccase co-electrospun nanofibre. Several support materials such as polyvinyl alcohol (PVA), polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP) were considered as potential support materials. It involved mixing laccase with a polymer solution and co-electrospinning to synthesise laccase-polymer co-electrospun nanofibre mat. The results demonstrated that the choice of polymer significantly affects the immobilised laccase's enzymatic activity and structural properties. Besides, it also highlighted the importance of solvent conditions, polymer concentration and enzyme concentration. It shows that 10% PVA and 14% PVP concentrations were optimal for fibre formation and detachment through FESEM. The diameter distribution of PVA and PVP was in the range of 30 to 600 nm. Entrapping laccase in PVA showed the highest specific enzyme activity (5.94 U/mg). In conclusion, immobilised laccase co-electrospun nanofibre show considerable potential as a biocatalyst for various applications, including phenolic compound degradation. This study provided valuable insight for optimising polymer selection and co-electrospinning parameters, contributing to development of more effective and sustainable environmental degradation technologies.

Keywords: Laccase; Co-electrospinning; Co-electrospun nanofibre; Immobilisation

**This study is supported by Fundamental Research Grant of University Teknology Malaysia*

MODELLING POTENTIAL DISTRIBUTION OF DICRANOPTERIS LINEARIS OF PENINSULAR MALAYSIA

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Abstract:

Widely found in the old world's tropical and subtropical regions, Dicranopteris linearis (Burmf.) Underw. is a clonal mat-forming fern that can dominate forest understory and is frequently viewed as a direct competitor for forest regeneration? It is commonly found fern in Peninsular Malaysia such as on roadsides and hiking trails. However, little is known about the occurrence and potential distribution of D. linearis in Peninsular Malaysia. This study aims to predict the distribution of D. linearis using maximum entropy (MaxEnt). Data on elevation and bioclimatic conditions were imported into the model as predictor variables retrieved from an online database. 30% of the presence data were used as test data and 70% of the data were used as training for the model. According to the model's results, the receiver operating characteristics of training have an area under the curve (AUC) of 0.892, which suggests that the model is useful and informative for predicting the forked fern's species distribution. The Jackknife test was used to analyse the model and determine the relative contributions of each predictor variable. The distribution of D. linearis is influenced by 9 out of 20 environmental variables applied, with the mean temperature of the coldest quarter contributing the most. The information can be useful to prevent future economic and biodiversity loss.

Keywords: Dicranopteris linearis, fern, Peninsular Malaysia, species distribution

**This study was supported by the Ministry of Higher Education Malaysia for Fundamental Research Grant Scheme*

ENHANCING BIOACTIVE PROFILES OF ELDERBERRY JUICE THROUGH YEAST FERMENTATION: A PATHWAY TO FUNCTIONAL BEVERAGES

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Abstract:

Elderberry juice (Sambucus nigra L.) is widely acknowledged for its bioactive compounds, yet its high sugar content poses health risks, particularly for metabolic disorders. This study employed starters-assisted starter fermentation with ten yeasts belonging to distinct species (Saccharomyces cerevisiae, Metschnikowia spp., Hanseniaspora spp., Candida zemplinina/Starmerella bacillaris) to reduce sugar levels and enrich bioactive profile of elderberry juice, while maintaining its sensory qualities and low ethanol content. After initial screening, five promising yeast strains were used as single starters or in binary combinations (seven combinations) to ferment elderberry juice for 36 h at 30°C. Mixed cultures, particularly EB8 (Saccharomyces cerevisiae and Hanseniaspora spp) and EB11 (Hanseniaspora opuntiae and Hanseniaspora spp), effectively reduced residual sugars while maintaining low ethanol levels. Post-fermentation analyses showed notable increase in GABA, alongside a shift in polyphenolic composition which is marked by reductions in protocatechuic acid and rutin, with increases in quercetin and isoquercetin. Volatile organic compound analysis revealed that non-Saccharomyces strains increased ethyl acetate and acetate esters, improving aroma complexity. Sensory evaluations confirmed that EB8 and EB11 had superior fruitiness and acceptability, linked to their enhanced volatile profiles. This research highlights the pivotal role of yeast diversity in optimizing the nutritional and functional attributes of fermented elderberry juice, offering new perspectives for the development of functional beverages.

Keywords: elderberry juice, fermentation, yeast, bioactive compounds, functional beverage

INVESTIGATING THE POTENTIAL OF PHENOLIC COMPOUNDS IN THE STRUCTURE OF ONOSMA ROSTELLATUM ON CERVICAL CANCER CELLS

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Abstract:

The incidence and mortality rates of diseases such as cancer, Alzheimer's disease and diabetes have been increasing rapidly in recent years. High exposure to disease-causing agents, genetic disorders and dietary habits are among the reasons why these diseases are so common. In addition to low treatment success, the severe side effects of the drugs used are a major problem. Therefore, the use of natural ingredients in treatment is becoming increasingly popular. In the present study, the protective properties and therapeutic potential of extracts of different parts of Onosma rostellatum plant used in traditional medicine against cancer were investigated. For this purpose, antioxidant activity, enzyme inhibition activity, anticancer activity and phenolic components of the extracts were determined. Thus, the potential of the studied extracts against all these diseases was revealed. While methanol extract showed high activity in antioxidant activity tests, ethylacetate extract was found to be more active in enzyme inhibition tests. As a result of cell culture experiments, it was determined that the most effective extract against cancer cells was the ethyl acetate extract obtained from the leaves with an IC50 value of 167.2±0.32 µg/ml at 72 hours. According to our findings, it is thought that the extracts of the studied plant may be an important natural source for the pharmacology industry due to their strong antioxidant, enzyme inhibition and anticancer activities.

Keywords: Anticancer activity, Enzyme inhibition, Bioactivity, Biological compounds, Traditional medicine

**TUBİTAK*

DYSBIOSIS AND HEMORRHAGIC SHOCK: RESPIRATORY RESPONSE

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Abstract:

Dysbiosis refers to the imbalance or disruption in the normal composition of microbial communities, particularly in the gut microbiota. Hemorrhagic shock is a condition characterized by inadequate tissue oxygenation due to circulatory failure, triggering compensatory mechanisms, including respiratory adjustments. Recent studies highlight the gut-lung axis, emphasizing the bidirectional relationship between gut microbiota and respiratory health. While research indicates that gut dysbiosis can affect the respiratory system, its specific role in respiratory responses during the reversal of hypotension remains unclear.

Six pregnant Sprague Dawley rats were divided into two groups: the dysbiosis group, treated with oral antibiotics (amoxicillin+vancomycin;n=3), and the control group, treated with oral saline (n=3) during the last week of pregnancy. Treatments continued until the offspring were weaned at 4 weeks of age. Dysbiosis was induced in the dysbiosis group offspring (n=7) by administering antibiotics for an additional 4 weeks, while control group offspring (n=7) received saline. At 12 weeks, respiratory parameters, including tidal volume (TV), respiratory rate (RR), and minute ventilation (RMV), were measured before and after hemorrhagic shock. Statistical analyses were performed using two-way RM-ANOVA followed by post hoc Bonferroni tests ($p<0.05$).

According to the findings of the study, in the control group, TV decreased from 3 ml to 2.5 ml during hemorrhagic shock, while in the dysbiosis group, it dropped to 2.3 ml. In the control group, the RR increased from 70 breaths/min to 78 breaths/min following hemorrhagic shock, and was recorded at 75 breaths/min after one hour. In the dysbiosis group, the RR increased to a maximum of 74 breaths/min and was recorded at 73 breaths/min after one hour. As for the RMV values, in the control group, the baseline value of 210 ml/min decreased to 195 ml/min after hemorrhage and to 187.5 ml/min after one hour. In the dysbiosis group, these values were recorded as 170.2 ml/min and 167.9 ml/min, respectively

These findings suggest that gut dysbiosis impairs the respiratory system's recovery following hemorrhagic shock, potentially limiting the compensatory mechanisms that restore blood pressure.

Keywords: Dysbiosis, Hemorrhagic shock, TV, RR, RMV

**This research was funded by Bursa Uludag University Scientific Research Project Foundation, grant number TGA-2022-1216.*

INVESTIGATION OF THIOL/DISULFIDE BALANCE IN FELINE LEUKEMIA VIRUS INFECTION

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Abstract:

Feline Leukemia Virus (FeLV) is one of the most significant infectious diseases affecting cats worldwide. In FeLV-positive cases, the average life expectancy is 2-4 years, with 50% of infected cats dying within 2 years and 80% within 3 years. The development of the infection requires prolonged and repeated exposure to the virus. Cats infected with FeLV may develop anemia, cancer, immunosuppression, autoimmune diseases, and neurological disorders.

Oxidative stress can be simply defined as an imbalance between the body's antioxidant defense and the production of free radicals. Thiols, which contain sulfhydryl groups, play a crucial role in coordinating the antioxidant defense network. In FeLV infection, the thiol/disulfide balance should be measured to assess oxidative stress and further investigate the pathogenesis of the disease. This study aims to evaluate oxidative stress in FeLV-infected cats by measuring the thiol/disulfide balance using a novel technique.

The study group consisted of 7 cats diagnosed with FeLV using rapid diagnostic test kits, while the control group included 7 cats with no health issues and a negative FeLV test result from the same test kits. The 'Erel' method, a novel and specific technique, was employed to determine thiol/disulfide concentrations. Total thiol, native thiol, and disulfide levels were measured in both the control and FeLV-positive groups. Ratios of disulfide to total thiol, disulfide to native thiol, and native thiol to total thiol were calculated based on these parameters. The differences between the groups were statistically analyzed.

Compared to healthy cats, FeLV-positive cats exhibited significantly lower serum TTL and NTL levels, as well as a reduced native thiol/total thiol ratio ($p<0.05$). Additionally, the disulfide/total thiol and disulfide/native thiol ratios were significantly higher in FeLV-infected cats ($p<0.05$). Imbalances in thiol/disulfide homeostasis may play a role in the pathogenesis of Feline Leukemia Virus, and increased oxidative stress could significantly impact disease prognosis.

Keywords: Cat, Leukemia virus, Oxidative stress, Thiol/disulfide balance

**Tübitak 2209*

SCIENTIFIC EVALUATIONS ON THE MACROANATOMY OF ANATOLIAN LION AKSARAY MALAKLI DOGS

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Abstract:

Dogs, which have been mankind's closest and most loyal friends from past to present, have many breeds around the world. Our country, Turkey, which is a bridge between Asia and Europe, has various dog breeds. These dog breeds have many dog breeds distributed according to various regions of Turkey. The Aksaray Malaklı dog breed, which is bred intensively in the Aksaray region, is distinguishable from Kangal dogs, the other dog breeds in the region, with its large body structure, gray coat color, drooping lip structure, and large and thick paw parts. In this review, many scientific articles and literature on the Aksaray Malaklı dog's skull skeletal structure, upper and lower extremities, organ systems such as respiratory, excretory, digestive and nervous systems were examined and the scientific differences between this dog breed and other dog breeds in terms of macroanatomical aspects were examined.

As a result, a compilation was made by bringing together the literature findings of the similarities and differences of macroanatomical structures in Aksaray Malaklı Dogs, known as Anatolian Lions.

Keywords: Aksaray Malakli Dogs, Macroanatomy, Systems.

PRACTICES TO INCREASE FERTILITY IN SMALL RUMINANTS DURING THE BREEDING SEASON

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Abstract:

Small ruminants are very important animal species that contribute to the production of converting non-agricultural, rugged and infertile lands into products such as meat, milk and wool / mohair. Today, in sheep and goat breeding, practices aimed at increasing maximum productivity and reproductive performance per unit animal with less cost are aimed. In order to increase meat and milk production in economical farming, reproductive fertility must increase. In other words, meat and milk yield increases depending on fertility and thus the desired productivity level is reached. Increasing reproductive efficiency in small cattle breeding; Environmental impact is much more important than genetic characteristics. Efforts are being made to increase fertility by improving the environment and genetics. Since the heritability of fertility is low, studies conducted to date have attempted to increase fertility by improving environmental conditions rather than genetics. In order to increase fertility, in addition to correcting environmental factors, attempts have been made to increase fertility through hormone applications. In this review, the correction of environmental factors and hormonal applications to increase fertility during the mating season are mentioned.

Keywords: Small Ruminant, Fertility, Hormone, Environment and Genetics

SPLENIC HISTIOCYTICOMA IN A CAT: A CASE REPORT

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Abstract:

Histiocytomas are neoplasms arising from the histiocyte cells of the immune system and are usually benign, self-limiting, cutaneous epitheliotropic neoplasms. Histiocyticoma, which is more common in dogs, has histologic diversity and has become more recognized in the veterinary literature in recent years. Diagnosis and treatment processes vary due to the presence of histologic subtypes. Histiocyte proliferative diseases in cats are very rare and there are limited number of case reports in the existing literature. This situation indicates that the diagnosis and management of feline histiocytomas require special attention for veterinarians. This study aims to contribute to the existing knowledge on the diagnosis and management of splenic histiocytomas, which are rarely observed in cats.

The case of the study was a male tabby cat, 7 years old, brought to Aksaray University, Faculty of Veterinary Medicine, Animal Hospital, Surgery Clinic with complaints of anorexia and pica. Clinical examination revealed tenesmus, abdominal swelling, pain on palpation and porcelain white mucosal color. Blood samples were taken and hematologic and biochemical analyses were performed. According to hematologic results, anemia was detected and renal and liver function tests were evaluated in biochemical analysis. Radiographic examination revealed that the spleen was larger than its normal size. Ultrasongraphic examination revealed a markedly enlarged spleen and the onset of torsion. Cholangiohepatitis and splenomegaly were detected and surgical operation was decided. The tissue sample taken after splenectomy was fixed in 10% formol and sent to the pathology laboratory. Histopathologic examination revealed splenic histiocytoma. After the treatment, the clinical course of the patient was followed and recorded.

This case contributes to the existing knowledge on the diagnosis and management of histiocytomas, which are rarely observed in cats. This study demonstrates that histiocytoma should be considered as a potential pathology in cases with non-specific clinical signs of anorexia. This methodological approach has enabled a systematic follow-up of the steps necessary for the accurate diagnosis and effective management of rare splenic histiocytomas in cats. It also emphasizes the importance of a multidisciplinary approach for tissues with abnormal tissue appearance.

In conclusion, when encountering splenomegaly and related clinical signs in cats, the inclusion of rare tumors such as histiocytoma in the differential diagnosis list is critical for accurate diagnosis and effective treatment. A detailed review of the case is important to guide veterinarians who encounter similar clinical situations.

Keywords: Cat, Histiocyticoma, Spleen

THE EFFECT OF DIFFERENT DURATIONS OF PROGESTERONE-BASED ESTRUS SYNCHRONIZATION ON PREGNANCY RATES IN GURCU GOATS DURING THE BREEDING SEASON: A REVIEW OF STUDIES FROM 2016 TO 2024

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Abstract:

The Gurcu goat, a breed at risk of extinction, is an important local genetic resource raised in the North Anatolian region. This study aims to evaluate the effect of progesterone-based estrus synchronization of different durations on pregnancy rates in Gurcu goats during the breeding season. For this purpose, data on pregnancy rates were compiled from ten estrus synchronization studies conducted between 2016 and 2024. A total of 326 clinically healthy Gurcu goats, each of which had given birth at least once, were used in all studies. The first reports on estrus synchronization in Gurcu goats began in 2016, and research in this field continues to the present day. In this conference paper, studies involving the application of intravaginal progesterone for 7, 9, and 11 days (using vaginal sponges or controlled internal drug release devices) for estrus synchronization were considered. The data obtained from these studies were statistically analyzed using the SPSS software. In studies on progesterone-supported estrus synchronization in Gurcu goats, the pregnancy rates for 7, 9, and 11-day protocols were 79.4% (65.7%-92.0%), 69.4% (68.8%-70.0%), and 72.8% (66.7%-77.5%), respectively, with no statistically significant differences observed among the groups ($P > 0.05$). The overall pregnancy rate across all studies was determined to be 74.2%. In conclusion, given that the pregnancy rates achieved with 7, 9, and 11-day progesterone-supported estrus synchronization protocols were similar, shorter intravaginal applications may be preferable for Gurcu goats.

Keywords: Gurcu goat, pregnancy, progesterone, synchronization

DYNAMIC THIOL DISULPHIDE HOMEOSTASIS IN DOGS DIAGNOSED WITH INFECTIOUS TRACHEABRONCHITIS

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Abstract:

Infectious Tracheabronchitis (ITB) is a highly contagious complex disease characterized by dry and hard cough in the upper respiratory tract, in which more than one dog is present in the same environment. Oxidative stress is important in ITB. Thiol/Disulfide homeostasis (TDH), which is used to identify oxidative stress and inflammation, plays a critical role in many cellular activities such as antioxidant protection, inflammation, detoxification, cell growth, apoptosis, signal transduction and enzyme activities. To date, Thiol-Disulfide homeostasis has been investigated in many diseases. The aim of the study is to obtain the TTL, NTL and Disulfide ratios in dogs diagnosed with ITB and to compare these values with the healthy dog group. 15 healthy (n=15) and 15 patient dogs with ITD diagnosis (n=15) were included in the study. The TTL, NTL and Disulfide ratios obtained as a result of the measurement were 393.46 µmol/L, 275.61 µmol/L and 58.92 µmol/L in healthy dogs, respectively; while these values were 353.58 µmol/L, 240.41 µmol/L and 56.58 µmol/L in dogs diagnosed with ITB. As a result, TTL, NTL and Disulfide ratios were measured lower in dogs diagnosed with ITB, indicating that the deterioration in dynamic TDH may be associated with lower Thiol-Disulfide ratios. Thiol-Disulfide homeostasis has been studied for the first time in dogs diagnosed with ITB, and more studies are needed in this area.

Keywords: Oxidative stress, Thiol-Disulfide Homeostasis, Infectious Tracheabronchitis, Dog

INVESTIGATION OF *TOXOPLASMA GONDII* AND *NEOSPORA CANINUM* IN ABORTED SHEEP FETUSES BY MOLECULAR METHODS IN AĞRI PROVINCE

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Abstract

Toxoplasma gondii and *Neospora caninum* are two important protozoan parasites that cause serious reproductive problems and economic losses in sheep. Both parasites are globally widespread and cause major problems in sheep farming. The final host of *T. gondii* is cats, while *N. caninum* is dogs. The aim of this study was to investigate the prevalence of *T. gondii* and *N. caninum* in the brain tissues of 100 aborted sheep fetuses from Ağrı province by PCR method. Samples were taken from the brain tissue of 100 aborted sheep fetuses and transferred to the Genetics Laboratory of the Faculty of Veterinary Medicine of Van Yüzüncü Yıl University under cold chain conditions. DNA extraction was performed using commercial kits, followed by PCR analyses using primers specific to the parasites. DNA extraction and PCR analysis were then performed. Samples were then run on a 1.5% agarose gel and images were obtained using a gel imager. After bidirectional sequencing analyses of positive PCR samples, comparisons were made with relevant reference genes in GenBank through BLAST and alignment. *T. gondii* was detected in 10% of the samples analyzed by PCR method, while *N. caninum* was not detected in any sample. The positive *T. gondii* samples showed 99.01-100% similarity with GenBank isolates PQ062231.1, MW509772.1, MW509775.1, PQ075940.1 and MH884735.1. As a result, *T. gondii* has significant effects on human and animal health and causes great economic losses. As in the example of the Ağrı province, it is important to remember that sheep meat should not be consumed undercooked or raw by humans.

Key words: *Toxoplasma gondii*, aborted sheep fetus, molecular methods

* *This study was supported by Van Yüzüncü Yıl University Scientific Research Projects Coordination Unit as a Research Project with Undergraduate Student Participation with Project Code TLO-2024-11294.*

POSTER PRESENTATIONS

REPRODUCTION OF MOLLUSKS FROM DIFFERENT LOCALITIES IN THE SOUTHERN MEDITERRANEAN COAST

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Abstract:

Mediterranean Sea biota is under great stress due to the discharges made by different activities. Mollusks are used to monitor environmental health. The aim of this study was to investigate the status of reproduction of the bivalves Donax trunculus along Annaba Mediterranean Gulf subjected to different anthropogenic ejections. Individuals have been collected in the first three months of the year from a location prone to contamination and localities subjected to organic and industrial contaminations. The sex ratio of Donax trunculus is macroscopically identifiable in February and March because gonad differentiation is possible only during the period of sexual activity. Testicular tissues showed histopathological modification in bivalves obtained from the organic and industrial polluted localities during January, February, and March. On the other hand, results demonstrated that the testicular structure of animals collected from the non-polluted site appeared unaffected. In summary, testicular histological profiles of D. trunculus have been altered by the two types of contaminations during the study period.

Keywords: Anthropogenic ejections, histopathology, bivalves, Donax trunculus, reproduction.

**This study is supported by the Laboratory of Animal ecophysiology, University of Badji Mokhtar-Annaba*

STUDY OF THE EFFECT OF WORMWOOD EXTRACTS (ARTEMISIA HERBA ALBA) ON DURUM WHEAT (TRITICUM DURUM) CULTIVATION WITH THREE COMPETING PLANT SPECIES

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Abstract:

Harmful weeds and unwanted plants are among the determinants of agricultural production due to their competition to the major agricultural crops on the basic growth components light, water, biosphere and nutrients. These unwanted weeds often controlled by using industrial chemical pesticides, which cause economic damage by reducing productivity, in addition to en-vironmental damage. Therefore, recent studies have tended to search for natural molecules of plant origin to use as alternative pesticides. Medicinal plants were chosen for their efficient alle-lopathic action.

In this paper, we selected Artemisia herba-alba, a type of allelopathic plant, in purpose of study-ing the effect of the biochemical antibody phenomenon of its extract on the interaction of hard wheat Triticum durum growth with three other plant species competing with it (cartridge, lentil and millet).

The study conducted on two laboratory experiments, the first was miniature in Petri dishes, and the second was in larger pots as a mini field experiment. The secondary metabolites also estimated to find out the contribution of allelopathic materials in explaining the phenomenon.

The growth of all plant species followed under normal watering conditions and treatment with four different concentrations of wormwood extract (0.5, 3 and 6%). The effect of these concentrations appears on the length of the root and sprouts of the treated plant species, except for wheat, the concentration 0.5 and 1% were sufficient to eliminate the sour plant only. The concentrations (3 and 6%) causes death of all studied plant species without affecting or influencing the wheat plant. The phytochemical study showed a lower content of secondary metabo-lites in wheat grown with the other three plant species compared to its solo cultivation. The most important finding of allelopathic interactions between plant species is that the phe-nomenon of biochemical contrast can be exploited in biological control of harmful weeds and unwanted plants by making use of the allelopathic effects of medicinal plants."

Keywords: chemical antagonism, chemical veto, unwanted weeds, durum wheat durum Triticum, latex sativa Avena, wormwood, Artimi

**University constantine 1*

BIODEGRADATION OF HYDROCARBURES BY INDIGENOUS HYDROCARBONOCLASTICS BACTERIA ISOLATED FROM THE EAST-ALGERIAN LITTORAL

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Abstract:

The environments contaminated by hydrocarbons contain indigenous micro-organisms which have developed resistance and potential biodegradation of this polluants. The objective of our work is to isolate and identify indigenous hydrocarbonoclast micro-organisms from the East-Algerian coastline and evaluate their potential of biodegradation.

After isolation, biochemical, and molecular identification with RNA 16S, 53 strains of microorganisms have been isolated. We selected four strains; Vibrio alginolyticus PB-WC 11099, Exiguobacterium aurantiacum strain NB11_3A, Halomonas venusta strain NY-8 and Dietzia sp CNJ898 PLO4 for the growth test in the presence of different classes of hydrocarbons: alkanes-mono-aromatics and refined hydrocarbons as unique source of carbon and energy. A total hydrocarbon assay was carried out before and after each growth by a visible UV spectrophotometer at a wavelength 436 nm after extraction with pentane, removal of the polar substances, and evaporation of the extraction solvent and an oxidative decomposition of sample.

The results of total hydrocarbon rates achieved after growth of the four selected autochthonous bacteria with the hydrocarbons tested show that all the strains appear to degrade the hydrocarbons with different rates. Thus, the maximum degradation is obtained with Exiguobacterium aurantiacum strain NB11_3A in the presence of benzene 02.21 mg/l, followed by the degradation of heptane of Halomonas venusta strain NY-8 with a rate of 3.12 mg/l and Vibrio alginolyticus PB- WC 11099 with a value of 3.76 mg/l. With Exiguobacterium aurantiacum strain NB11_3A we have the lowest degradation rate in the presence of cyclohexane, namely 21.23 mg/l.

Vibrio alginolyticus PB-WC 11099 gave the best results with all the hydrocarbons tested (alkanes and mono-aromatics). The degradation of refined petroleum gasoline was also observed with all bacterial species selected with different rates. Dietzia sp CNJ898 PLO4 and Vibrio alginolyticus PB-WC 11099 use a wide range of hydrocarbons as the sole source of carbon and energy.

Keywords: Hydrocarbons, Hydrocarbonoclastics Bacteria, Bioremediation, Algeria.

BATS AS RESERVOIRS OF ANTIBIOTIC-RESISTANT BACTERIA: A CASE STUDY IN BEJAIA, ALGERIA

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Abstract:

Introduction: Bats are unique mammals with the ability to fly, travel long distances, and have diverse diets, which influence their gut flora diversity. This study aimed to investigate the antibiotic-resistant flora in bats from the Bejaia region of Algeria.

Methods: A total of 130 samples (guano and cloacal swabs) were collected from bats in the Aokas cave. After isolation and identification, antibiotic resistance phenotypes were determined using phenotypic tests (DD-test, cloxacillin test, MIC test, Hodge test). To further confirm resistance mechanisms, genotypic identification of resistance genes was performed using PCR and sequencing techniques. Vancomycin MICs for Enterococcus spp. were determined.

Results:Six Enterobacteriaceae isolates displayed resistance to extended-spectrum β-lactamases (ESBLs). Four of these isolates were identified as Klebsiella pneumoniae and two as Escherichia coli. Genotypic analysis revealed the specific ESBLs to be CTX-M-15. Furthermore, two of the Klebsiella pneumoniae isolates exhibited carbapenem resistance, with the carbapenemase genes identified as OXA-48 and KPC-3. No vancomycin-resistant Enterococcus spp. were found.

Conclusion:Bats can be reservoirs and vectors of antibiotic-resistant bacteria, which poses a threat to public health.

Keywords: Bats, antibiotic resistance, ESBLs, carbapenems, Enterococci.

ANTIMICROBIAL POWER OF OLEASTER OIL ENRICHED WITH A MEDICINAL PLANT (ROSEMARY)

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Abstract:

Introduction: Oleaster oil, extracted from wild olives, is renowned for its numerous health benefits, including its antioxidant and antimicrobial effects. Objective: The aims of this study is to characterize a sample of oleaster oil from the Khelil region Bejaia in Algeria and enrich it with a medicinal plant, rosemary, in order to evaluate its antimicrobial activity.

Material and Methods: The study examined two samples: oleaster oil and oleaster oil enriched with Rosmarinus officinalis. It focused on determining the quality indices of the oil, including free acidity, peroxide value, UV absorbance (K232, K270), and pigment content. Additionally, antibacterial activity was evaluated against seven microbial strains.

Results and Discussion: The results, according to the C O I 2019 standards, allowed us to classify the oil in the "extra-virgin" category. The pigment content was low, with a chlorophyll/carotenoid ratio of less than one. Regarding the antimicrobial activity, we conclude that our sample of oleaster oil exhibits antimicrobial effects on the tested strains. However, we observed an increase in antimicrobial activity, achieving a bactericidal effect on some strains such us Escherichia coli, Methilicilin-resistant staphylococcus aureus (MRSA), Bacillus subtilis, and Candida albicans, with the oleaster oil enriched with the medicinal plant Rosmarinus officinalis, indicating the presence of a synergy between their components.

Conclusion: The enrichment of oleaster oil with rosemary enhances its antimicrobial activity. These results suggest that enriched oleaster oil could be an effective antimicrobial agent against certain pathogenic strains.

Keywords: oleaster oil, rosemary, quality indices, enrichment, antimicrobial activity.

BIOLOGICAL ACTIVITIES OF EDIBLE SEA URCHIN EGGS: PARACENTROTUS LIVIDUS COLLECTED IN THE ALGERIA

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Abstract:

Introduction: The sea urchin Paracentrotus lividus, is an edible echinoid commonly found in abundance on the Mediterranean coasts. This study indicates the potential richness of bioactive molecules in the gonads of the sea urchin. Objective: The aim of our study was to evaluate the antioxidant and antibacterial activity of sea urchin Paracentrotus lividus gonad extract against 10 reference strains.

Material and Methods: Gonad samples of Paracentrotus lividus were collected in the Aiguades region (Bejaia in Algeria) and subjected to extraction by maceration followed by evaporation. Phytochemical screening was performed to determine the levels of polyphenols, flavonoids, and carotenoids in the gonad extract. Antioxidant activity was evaluated by measuring the ability of the extract to scavenge DPPH and ABTS free radicals. Antibacterial activity was assessed against Gram-positive and Gram-negative bacterial strains.

Results and Discussion: The yield of the gonad extract was obtained by maceration, with a rate of approximately 13.32%. Phytochemical screening of the gonad extract revealed levels of polyphenols (5.77 mg EAG/g MS), flavonoids (1.896 mg ER/g MS), and carotenoids (1.46 mg EβC/g MS).

The evaluation of antioxidant activity confirmed the potent properties of gonad extracts in scavenging DPPH and ABTS free radicals. The results obtained revealed antioxidant activity with 50% inhibition concentrations (IC50) of 1.95 mg/ml for the DPPH test and 8.98 mg/ml for the ABTS test. The extract showed antibacterial activity against all strains except E. coli was found to be resistant to different concentrations of the extract.

Conclusion: This study highlights the potential of sea urchin gonads as a source of bioactive compounds with antioxidant and antibacterial properties. Further exploration of these properties could lead to the development of novel strategies for combating human pathogens.

Keywords: Paracentrotus lividus, bioactive substances, antibacterial activity, antioxidant activity.

SECTION OF LACTIC ACID BACTERIAL STRAINS WITH ANTIBACTERIAL ACTIVITY AGAINST STAPHYLOCOCCUS AUREUS STRAINS ANTIBIO-RESISTANT ISOLATED FROM ALGERIAN ARTISANAL DAIRY PRODUCTS

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Abstract:

Background: In Algeria, as in many other countries around the world, there are indigenous dairy products whose production methods derive from the population's cultural heritage. These products could be a valuable source of indigenous lactic bacteria of technological and probiotic interest. However, failure to follow good hygiene practices during milking makes these products unsafe in many cases, and the use of antibiotics to treat cows has contributed to the emergence of antibiotic-resistant bacterial strains.

Objective: In this context, S. aureus strains were isolated from 60 samples of different dairy ecosystems (milk, ben, raib, cheese, etc.) collected from 20 farms in Bejaia. Phenotypic and biochemical identification was carried out for these samples. An antibiotic sensitivity test (cefotaxim and ceftazidim) and an analysis of resistance phenotypes were carried out.

Methods: In order to select strains of lactic acid bacteria with antimicrobial activity against resistant S.aureus strains, 25 strains of lactic acid bacteria were tested. A spot test was carried out and in order to determine the origin of this antibacterial activity, a well test was carried out with the native supernatant, neutralised and after treatment of the supernatants with proteases.

Results: Fifty per cent of samples were contaminated with S. aureus. In fact, 30 strains of S. aureus were identified. A study of the sensitivity of these strains to cefotaxime and ceftazidime led to the selection of 20 S. aureus strains with a resistance of 32.62%. Antibacterial activity revealed zones of inhibition of between 15 and 35 mm. Only the supernatants of 17 strains showed zones of inhibition, including 10 strains after neutralisation of the supernatants. Treatment of the supernatants with proteases (trypsin, papain and chymotrypsin) and neutralisation of the pH showed that this inhibitory activity of lactic bacteria strains is not only due to the pH but probably to antimicrobial substances of a proteinic nature.

Conclusion: These results suggest that the use of lactic acid bacteria as bioconservatives could improve the microbiological quality of artisanal dairy products.

Keywords: Bioconservatives, Lactic Acid Bacteria, S.Aureus, Antibiotic Resistance, Artisanal Dairy Products

DETERMINING THE ANTIMICROBIAL ACTIVITIES OF DIFFERENT-ORIGIN GINGER EXTRACTS

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Abstract:

Ginger (Zingiber officinale), originally from Asia, is now cultivated worldwide. For over 2500 years, it has been used in ethnobotany for its therapeutic benefits, including pain relief, diarrhea prevention, and aiding digestion. Antimicrobial tests have demonstrated that ginger exhibits both antibacterial and antifungal effects, leading to the development of numerous patented ginger extracts. However, the impact of ginger's geographical origin on its biological properties has not been sufficiently studied, and comparisons of ginger from different regions have generally been overlooked. This study aims to address this gap by comparing the antimicrobial properties of ethanol extracts from ginger obtained from India, China, and Turkey using the disk diffusion method. Significant differences were observed among the ginger samples from the three countries in terms of the variety of microorganisms they affected and the level of antimicrobial activity they demonstrated. The ginger extract from India produced inhibition zones of up to 19 mm against Staphylococcus haemolyticus, while the ginger extract from Turkey showed inhibition zones of up to 17 mm against multidrug-resistant Achromobacter sp., surpassing the control antibiotics. Additionally, the ginger extract from Turkey exhibited notable antimicrobial activity against 13 different microorganisms, demonstrating a broad spectrum of effect. This suggests that ginger grown in Turkey warrants further investigation as a potential natural antimicrobial agent in the fields of health and medicine.

Keywords: Zingiber officinale, Antimicrobial Activity, Disk Diffusion Method

**This study is supported by TUBITAK 2209*

ANTIMICROBIAL ACTIVITY SCREENING OF TRACHYCARPUS FORTUNEI (HOOK.) H. WENDL

Kutay Orkun Durukan, Cenker Yaman, Gizem Gul, Ulku Tahran, Mustafa Eray Bozyel, Kerem Canli

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Abstract:

Rapidly increasing resistance seen in bacteria compared to the amount of antimicrobial agents discovered is one of the biggest predicaments surrounding human health nowadays. This crisis is mainly fueled by the misuse of antibiotics which in turn promotes antibiotic resistant organisms. In addition, our inability to match the speed of these organisms is an important factor. Our inability lies in the fact that it takes approximately 10-15 years and $1 billion to develop a new drug. In the search of finding new antimicrobial agents, this study investigates a plant known as Trachycarpus fortunei (Hook.) H. Wendl. There are various factors to study plants but two of them stand out the most. The first factor is that plants can be found abundantly in nature thus making them the perfect specimens for research. Secondly, unlike other matter which inflicts damage on both human and pathogen cells, plants rarely cause severe damage to humans. In addition, plants exhibit crucial therapeutic features as can be seen from their involvement in both the old and the new medicinal practices. This study focuses on T. fortunei because it shows immense potential with its abundance and rich nutrient results from its buds. To determine T. fortunei's antimicrobial properties disk diffusion method was used and T. fortunei's ethanol extract was tested against 48 strains which include 3 Candida species. Disks that were impregnated with T. fortunei's ethanol extract inhibited the growth of 14 bacteria strains including Staphylococcus aureus MRSA+MDR and 2 Candida species. The widest zones were seen in a clinically isolated Candida glabrata. Antimicrobial activity against this wide range of strains and its abundant nature shows that T. fortunei has great potential as an antimicrobial agent source.

Keywords: Antimicrobial activity, Disc Diffusion, Trachycarpus fortunei (Hook.) H. Wendl., Ethanol extract

FULL TEXTS

Poster Presentation

STUDY OF THE EFFECT OF WORMWOOD EXTRACTS (*ARTEMISIA HERBA ALBA*) ON DURUM WHEAT (*TRITICUM DURUM*) CULTIVATION WITH THREE COMPETING PLANT SPECIES

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Abstract

The harmful weeds and unwanted plants are among the determinants of agricultural production due to their competition to the major agricultural crops. These unwanted weeds are often controlled by using pesticides, which cause economic damage. In this study, we selected *Artemisia herba-alba*, a type of allelopathic plant, in purpose of studying the effect of the biochemical antibody phenomenon of its extract on the interaction of hard wheat Triticum durum growth with three other plant species competing with it (cartridge, lentil and millet). The study conducted on two laboratory experiments, the first was in Petri dishes, and the second was in larger pots. The secondary metabolites also estimated to find out the contribution of allelopathic materials in explaining the phenomenon. Plant species in growth followed under normal watering conditions and treatment with four different concentrations of wormwood extract (0.5, 3 and 6%). The effect of these concentrations appears on the length of the root and sprouts of the treated plant species, except for wheat, the concentration 0.5 and 1% were sufficient to eliminate the sour plant only. The concentrations (3 and 6%) causes death of all studied plant species without affecting or influencing the wheat plant. The phytochemical study showed a lower content of secondary metabolites in wheat grown with the other three plant species compared to its solo cultivation. The important finding of allelopathic interactions between plant species is that the phenomenon of biochemical contrast can be exploited in biological control of harmful weeds and unwanted plants by making use of the allelopathic effects of medicinal plants.

Key words: *Artimisia herba-alba*. chemical antagonism, chemical veto, latex *sativa Avena, Triticum durum*, unwanted weeds, wormwood.

Introduction

Undesirable plants are considered as determinants of agricultural production to compete with major agricultural crops on the elements of growth. Water, light and food (Cheng, 2015; Rice,1999), and they are often combated using industrial pesticides that cause economic and environmental damage (Chbib et al., 2020) , which necessitated the search for natural molecules with Plant origin for use as alternative pesticides (Głąb et al., 2017) . The choice fell on medicinal plants with allelopathic efficiency (Abu-Romman, 2015; Khanh, 2005). The research has counted 374 types of undesirable weeds in wheat fields. Among them, we mention wild oats, green foxtail, wild mustard and bedstraw (Nikki, 2016) which can cause yield losses in cereal crops, because they reduce the germination rate of wheat seeds (Carrara, 2004). The Allelopathic compounds are secondary metabolites produced from different plant parts (Inderjit, 2003; Li, 2010), that released into the environment in several ways (Abrahim,2000).Many *Artemisia* species have high economic value in several areas, as food plants and as medicinal plants (Abou El-Hamd, 2010). *Artemisia herba-alba* was known for its therapeutic and medicinal properties (Gehad et al., 2016; Lai et al., 2013; Lubbe, 2012). It is used in traditional and modern medicine (Abou El-Hamd, 2010), and the reported biological activities of this species are included as well as pharmacology and toxicology (Abu-Romman, 2016). Allelopathy related to *Artimisia* plants has been described (Escudero, 2000). The present study aimed to further investigate the impacts of aqueous extract of *Artemisia herba-alba* on the growth of durum wheat seedlings and its interaction with two grasses (Oats and Millet) and a legume (Lentils).

Material and methods

The study conducted on four plant species: *Triticum durum*, Avena *sativa*, *Pennisetum glucum* and *Lens culinaris*, which treated with *Artimisia herba -Alba* extract. The five plant species are acquired from different regions of the homeland (Table 1). We chose pure untreated seeds free from blemishes, holes and dark spots. We disinfected them with 5% diluted Javel water (15 mn) and soaked them in water for two hours. The collected leaves were dried at 65°C for 48 h until constant weight and ground to a fine powder. A 6% (w/v) extract was prepared by soaking 6 g of the powder in100 mL distilled water for 24 h on a horizontal shaker at 110 rpm. The extract was filtered through a double layer of cheesecloth and then filtered. The extract was stored at 4°C until use. Different concentrations (0.5, 1.0, 3.0 and 6%) of the extract were used in laboratory experiments.

The experiments in Petri dishes

The seeds of the studied species sown in Petri dishes with three replicates for each plant type of the three treatments. The number of seeds in each dish distributed

according to the number of plant species present in it is 30 grains for each plant species cultivated separately and 15:15 grains for each two types together and 10:10:10 each for three types and 8 kernels for each of four types together.

Table 1. Source of the studied plants samples.

Plant Type	The scientific name	Source
Wheat	*Triticum durum*	Institute of Field Technical (I.T.G.C) Constantine.Algeria
Oat	*Avena sativa*	Institute of Field Technical (.I.T.G.C) Constantine.Algeria
Millet	*Pennisetum glucum*	Center. Adrara Algerian Sahara Research Province
Lents	*Lens culinaris*	daily Commercial lentils for consumption from the state of .Constantine
Wormwood	Artimisia herba -alba	February from the It was picked in mountains of Constantine

The experiments in pots

The seeds of the studied species are sown in pots of 256 cm2, in two batches: a control and a treated with 0% and 6% of the wormwood extract respectively at the rate of 200 grains for each plant species cultivated separately and 100: 100 grains for each of the two species cultivated together. The experiment is repeated twice.

Monitoring of measures

For the petri dish experiment, we calculated the percentage of plant germination for the three treatments, the height of the plants for five planters of each type in the three treatments. After ten days we applied the watering with Artemisia plant extract at concentrations of 0.5 and 1% for the 3 treatments respectively. After 22 days from the start of the experiment, we measured the lengths of the legs and roots of five plants of each species for all replicates of the three treatments. We calculated the number of dead plants after re-watering with normal water for 10 days. Then we repeated the treatment with wormwood extract a second time at a concentration of 3% for the same plants previously treated with the same extract at a concentration of 0.5 and 1%, while the experiment Normal watering worked with wormwood extract at a concentration of 6%. For the pot experiment, watering was continued daily with plain water for the control and with wormwood extract at a concentration of 6% for the treated for a period of 15 days. until the seedlings reach the level of the second row of leaves in plantains and significant height in legumes. Next, we measured the stem length, length and number of roots for 10 seedlings of each plant species grown individually and 5 seedlings for every two types of plants grown together using grid paper. On the 16th day, we took the plants out of the pots and dried them in order to carry out a phytochemical study estimating the secondary metabolites (polyphenols, flavonoids

and tannins) of the species studied for the two treatments.
The Statistical analysis was done using XlStat (Adinosoft). Significant differences were computed using ANOVA after Neweman keuils test at $P< 0.05$.

Results

1st experiment: The Petri dishes

Germination Kinetic

When the cultivars are grown individually, wheat germination starts as early as thirth day and coincides with that of oats, and one day later than lentils with the rates of 22.66, 1.11 and 14.44 % respectively. While millet germination is delayed to fifth day with a rate of 1.11 %. On the twelfth day germination stabilizes with a percentage of 90.0, 86.67, 72.22 and 57.75 % respectively for wheat, lentils, oats and millet (Figure 1a). On the contrary, when the four species were planted together, germination started on the second day for the three species with a variable percentage, followed by the germination of millet on the third day. From the sixth day, the percentage of germination of lentils and oats are equal and present 87.50 %. Similarly, the percentage of wheat and millet are convergent to 50.0 and 45.83 % respectively. The germination graph stabilizes on tenth day 10 at 100 % for lentils and oats and 90.0 % for wheat and millet (Figure 1b). While planting wheat with each type separately, we notice that the germination of oats and lentils is more advanced compared to wheat from the second day and throughout the germination period with a rate of 93.33 and 95.86 % respectively, compared to wheat whose germination reaches 88.89 and 86.67 % in interference with the two species respectively (Figure 1c,d). While the germination of wheat outperformed millet from the third day until its germination was stable on the tenth day, reaching 88.89 % compared to 73.33% for millet (Figure 1e). As for the interaction of wheat germination with each of the two species, wheat and oats begin to germinate as early as fiventh day and millet germination is delayed until seventh day with an estimated rate of 53.33 %. Germination stabilizes in this case on the eleventh day, with a percentage of 86.67 % for wheat and 93.33 % for oats and millet (Figure 1f). As for the interaction of the germination of wheat with millet and lentils, both species have superiority over wheat from the eighth day until the stability of germination on the eleventh day with 90.0 % for wheat and 100 % for lentils and millet (Figure 1g). On the other hand, wheat germination starts on the sixth day interfering with lentils and oats, which are delayed to the seventh day. The germination percentages on the eighth day are equal for all three species, estimated to 60.0 %. At the end of germination, the two species, oats and lentils present 90.0 % against 83.33 % for wheat (Figure 1h).

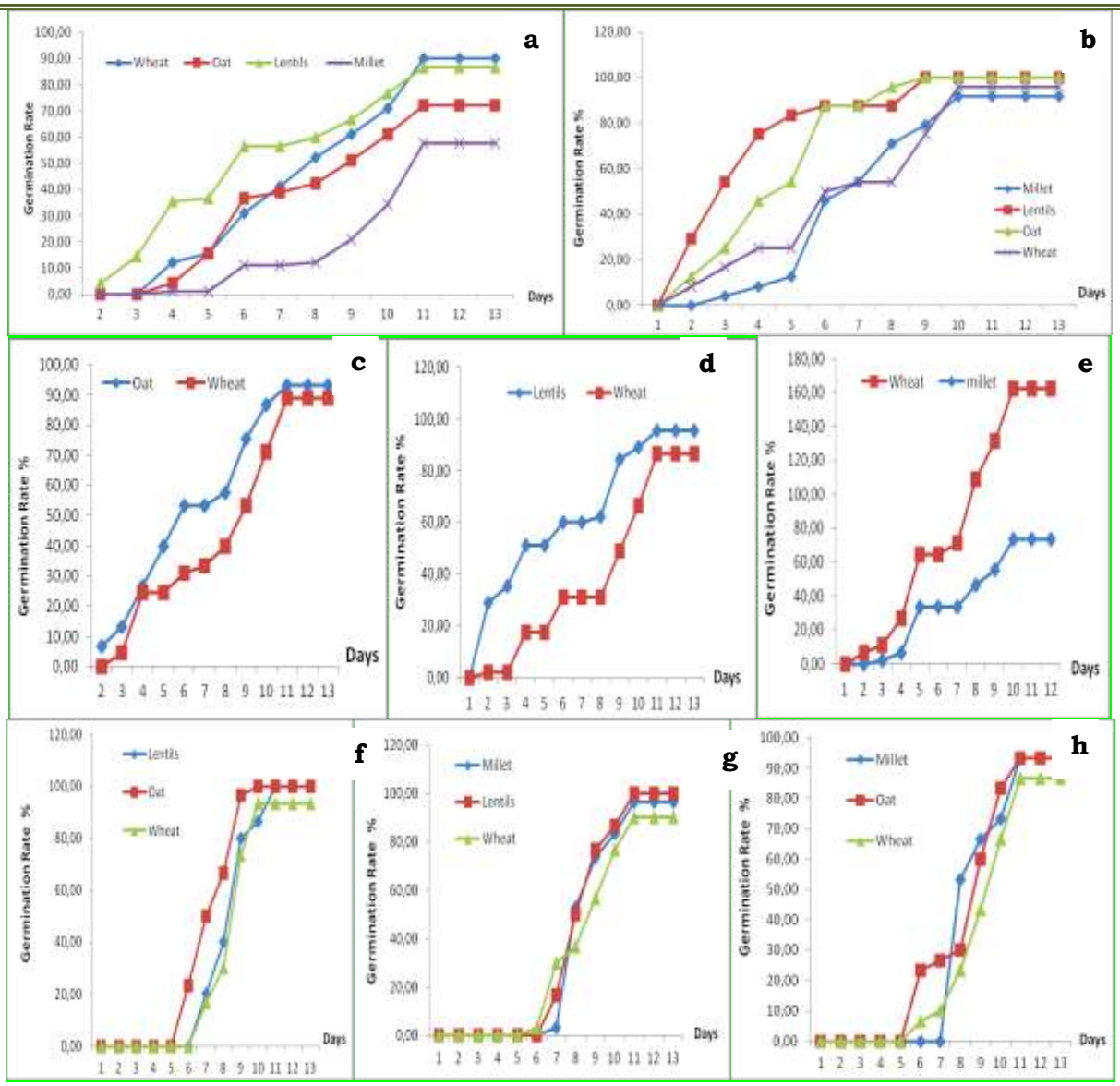

Figure 1. Wheat germination kinetics. (a) Separately of the species. (b) Common of the tree species. (c) Wheat with oat. (d) Wheat with lentils. (e) Wheat with millet. (f) Wheat germination interference with oat and millet. g: Wheat germination interference with millet and lentils h: Wheat germination interference with oat and lentils.

Length of the plant species after watering with the extract of wormwood

All plant species continued their longitudinal growth naturally in the control treatment (wormwood extract 0%). where the stems of the plants cultivated with wheat was longer than it except for millet plant. The length of oat, lentil and millet plants reached 15.74, 13.34 and 11. 87 cm respectively, compared to the wheat plant height, which reached 12.32 cm. When watering with wormwood extract at a concentration of 0.5 and 1%. The growth of the rest of the plant species stopped, while the growth of wheat was not affected and continued normally and ordinary and three treatments had recorded the lengths of 16.75, 19.56 and 17.15 cm (Figure 3a).

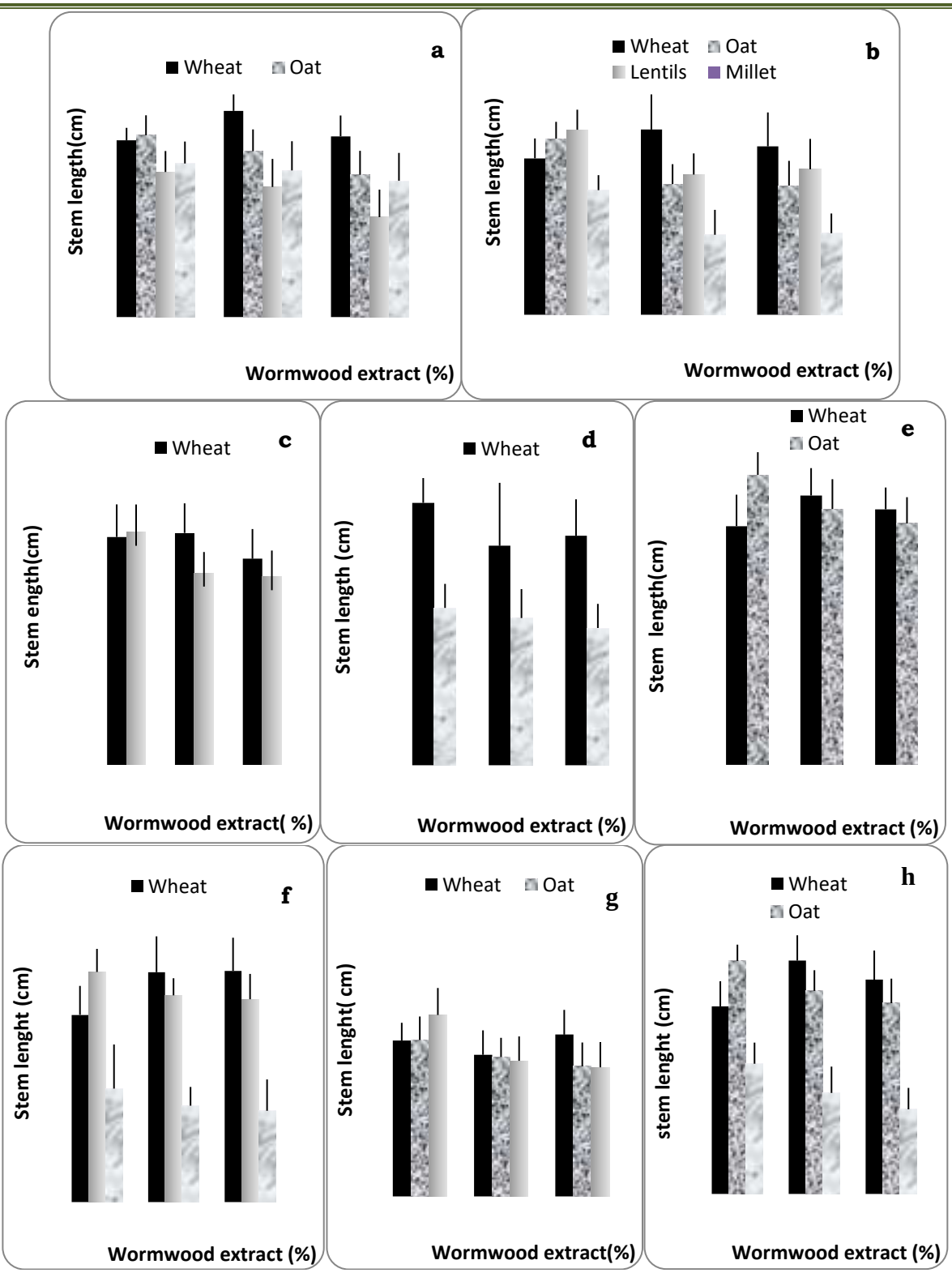

Figure 2. Plant height irrigated with *Artemisia* extract at concentrations of 0.5 and 1%. (a) for each individual plant species; (b) the four plant species cultivated together, (c) wheat with oats, (d) wheat with lentils, (e) wheat with millet, (f) wheat and lentils + millet, (g) wheat and oat + lentils, (h) wheat and oat + Millet.

The vegetative growth of the three plants cultivated with wheat was greater than during irrigation with regular water, while their growth was halted or stunted when

treated with wormwood extract at a concentration of 0.5 and 1%, which encouraged the wheat plant to continue to grow normally, as its length ranged between 13.18 and 16. 18 cm (Figure 3). We observed that the growth of the three plant species cultivated individually with wheat (oat, millet and lentils) stopped or inhibited when watered with wormwood extract unlike wheat which continued to grow naturally without a competitor. Wheat plants reached 16.99, 12.29 and 12.60 cm. respectively with lentils. Millet and oat plants of 14.06, 6.43 and 15.29 cm (Figure 2 c, d, e).

Whereas, the longitudinal growth of the stems of the two varieties cultivated with wheat was greater than during irrigation with regular water, and their growth was stopped or stunted when treated with wormwood extract at a concentration of 0.5 and 1% which encourages the wheat plant to continue to grow normally as its length ranged from 13.73 to 15.33 cm (Figure 2 f, g, h).

After two days of re-watering with regular water, we recorded the regrowth of the lentil plant after it wilted when watered with wormwood extract, with the restoration of the stem's vertical position after it was completely fallen. On the fifth day of watering, we recorded a return to growth with a small percentage of millet plants and a large percentage of lentils. As for the oat, we did not notice any change in its wilting, as the plants remained dead. As for wheat, its growth was normal and continuous in all dishes.

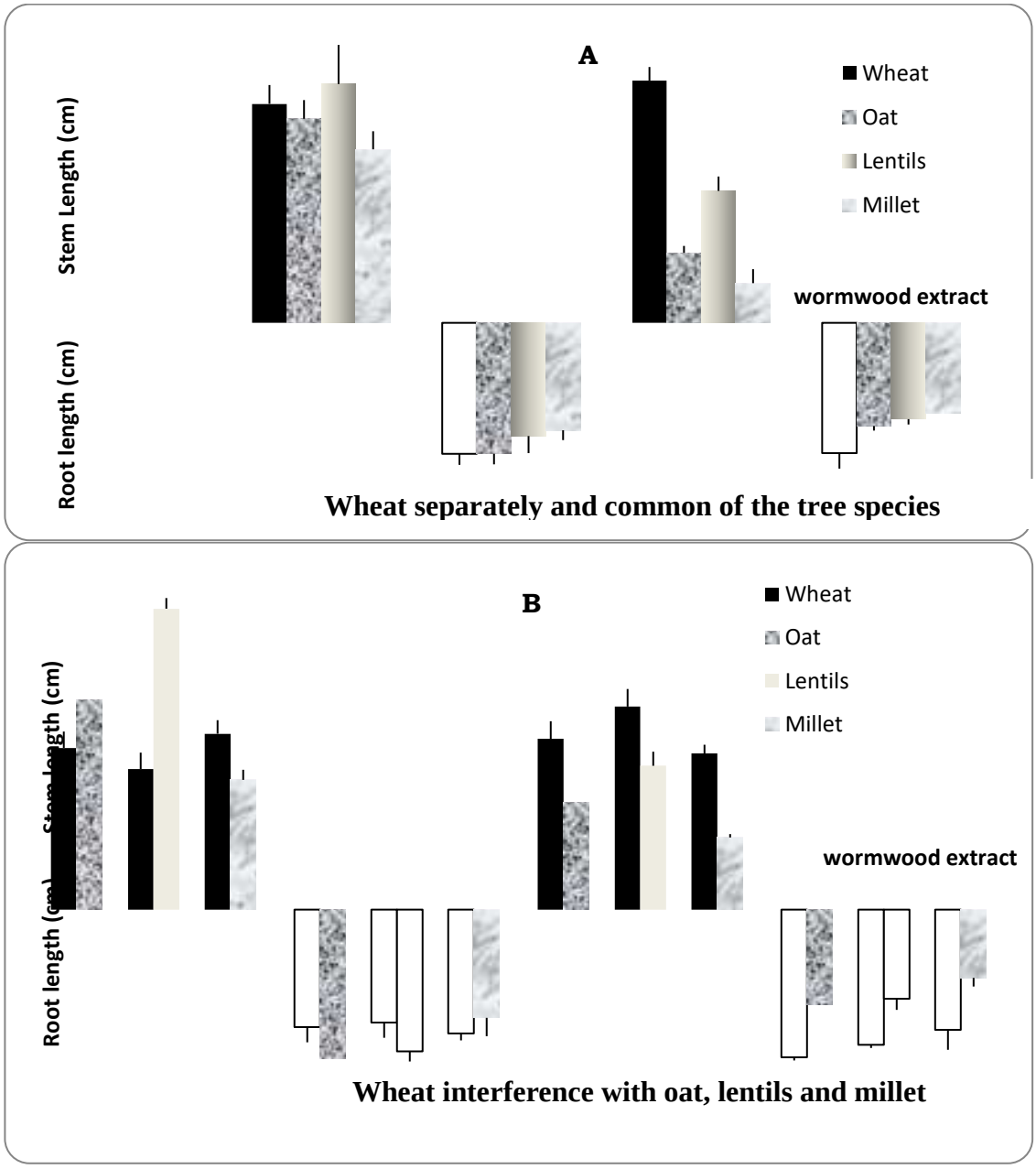

Figure 3. The length (stem and root) of the plant of each species after irrigation with wormwood extract at a concentration of 0 and 6%.

Calculating the number of dead plants for all plant species treated with different concentrations of wormwood extract with concentrations 0.5% and 1% after watering with plain water

The 1% wormwood extract has a more effective effect than 0.5% extract to eradicate the oat plant by 89% in all cases while it was enough to eradicate millet and lentils only 40 and 52 % respectively. Experimenting with the effect of the extract at a concentration of 3% increased the complete death of all plants of both species about 100%. The percentage of plant death increased significantly in all plant species except wheat

when the wormwood extract concentration increased to 6%, which ranged from 73.33 for millet to 100% for oats (Table 2) according to the cultivation method.

Table 2. The percentage of dead plants at different concentrations of the wormwood extract.

Plant species distribution		0.5 % wormwood Extract	1% wormwood Extract	% 6 wormwood extract
Each plant species is unique	Wheat	0	0	0
	Oat	71.11	89	98.89
	Lentils	30	45	98.89
	Millet	29	41	98.89
All two species are plants together	Wheat : oat	: 0 :82.22	0: 98	0 :100
	Wheat : lentils	0 :22	0 : 45	0 :97.78
	wheat: Millet	10 : 0	0: 39	0 :97.78
	wheat: lentils :oat	0: 30 : 63.33	0 : 49 : 98	0: 86.67 : 96.67
	wheat: Millet : lentils	28 : 20: 0	0 : 45 :53	0: 96.67 : 96.67
All three types together	wheat : Millet : oat:	86.67 : 0 5 : 0	0 : 39 :93.33	0:73.33 : 93.33
All three types together	Wheat : Millet :lentils: oat	0 : 23 :33 :70.83	0 : 40 :52 :89	0 : 87.50 : 95.83 :100

Second: The potted experiment

The vegetative growth length

The results of both experiments showed the phenomenon of biochemical contrast between the studied plants on wheat. This is noticeable in the germination rate of wheat to 90%grown alone and the decrease of its germination rate to 86.67 and 88.89% planted with oats and lentils respectively (Figure 1a). The height of the wheat plant decreased from 12.32 to 9.94 cm in front of the superiority of the studied plants species vegetative growth with lentils and then oat on the advance with rates 14.99 and 13.40 cm respectively. As for the root system's length each plant species cultivated with wheat has roots longer than the roots of wheat except for the roots of millet where we find the length of the root assembly of oat is 13.14 cm. lentils 12.60 cm and millet 9.54 cm respectively with the length of the root group of wheat, 10.58, 9.96 and 10.92 cm by comparing the lengths of the root system of all root system plants when grown separately. The result was radically changed when treating the four types with wormwood extract in different concentrations (0.5, 1.0, 3.0 and 6.0%). where the vegetative part of the three plant species oat. Lentils and millet was halted. With a tihith days time difference between concentrations 0.5 and 1% and the natural continued growth of wheat with the two treatments when continuous watering we recorded the return of lentil and millet to normal growth. While, we recorded a complete death of the oat plant. There is a direct correlation between the death of oat, lentils and millet, and the increase in the concentration of the wormwood extract. The

effectiveness of wormwood extract in eliminating unwanted plants growing around wheat increases with the increase in its concentration. Where the effectiveness was partial at the lowest concentration of 0.5 and 1% and it was fatal at concentration 3 and 6% except for the oat where the concentration 1% was sufficient to eliminate it (Table 3).

Table 3. Number of root in the each species and with the wheat.

	Each plant species is unique			
Wormwood extract	Wheat	Oat	Lentils	Millet
0%	6.00 ± 0.90	4.00 ± 0.47	1.00 ± 0	1.00±0
6%	6.00±1.0	4.00 ± 0.45	1.00±0	1.00±0

	Wheat with another species					
Wormwood extract	Wheat	Oat	Wheat	Lentils	Wheat	Millet
0%	5.00 ± 0.84	4 ± 0.55	4.00 ± 0.45	1.00 ± 0	6.00 ±0.89	1.00 ± 0
6%	5.00 ± 0.44	4 ± 0.55	4.00 ± 0.84	1.00 ± 0	6.00 ±0.55	1.00 ± 0

The phytochemical study

The analysis of variance of our phytochemical results shows a significant effect for the five species studied. Table 4 presents the different homogeneous groups of the three metabolites. The studied five-plant species secondary metabolites quantitative estimation study showed the superiority of wheat, oat and wormwood for compounds of phenols, flavonoids and tannins in their extracts for a plant. The polyphenol content of wheat is high compared to its content in the plants grown with it, but its quantity is always lower than that of individual grown wheat, while we find that the content of this substance is relatively high in the wormwood plant. The value of the compounds of tannins in the plant species grown separately from the plants growing together, as there was an increase in the percentage of these compounds for the wheat plant grown with the oat than its value in the latter.

Table 4. The quantification of the secondary metabolites of the plant species under study.

Secondary metabolites / Specie	Polyphenols (µg equivalent to Gallic acid from dry plant matter)	Flavonoids (µg equivalent to catechin acid from dry plant matter)	Tannins (µg equivalent to catechin acid from dry plant matter
Oat	744.44 (A)	327.33(C)	417.79(A)
Wheat	720.54 (B)	290.0(D)	217.79(F)
Wheat : oat	600.80 (C)	229.0(G)	141.11(H)
Millet : Wheat	605.39(C)	248.67(F)	242.22(E)
Oat : Wheat	598.99(C)	93.33(K)	47.79(J)
Millet	541.75(D)	374.33(A)	266.67(D)
Artemisia	502.02(E)	343.0(B)	321.11(B)
Wheat : lentils	350.51(F)	109.33(I)	76.67(I)
Lentils : Wheat	285.19(G)	149.33(H)	181.12(G)
Lentils	263.97(G)	268.33(E)	285.56(C)
wheat: Millet	143.77(H)	66.33(J)	54.44(J)

Discussion

Each species exercises its free germination power according to its genetic potential and seed reliability (René et al., 2004) hence durum wheat germination is in first position followed by lentil, oats and millet. In contrast, his results differ once we combine wheat germination with the other three species, implying the phenomenon of allopathy associated with the release of chemical compounds by plants that "suppress the growth and establishment of other plants in their vicinity" (Eichorn,2014; Inderjit et *al*.,2011; Shalinder et al., 2014). The results of germination are similar to those of Bojović and Jakovljević (Biljana et al., 2015). Different concentrations of *Artimisia* extract have a significant effect on species. Each species exerts an allalophytic effect through the secretion of secondary metabolites produced from different parts of the plant. The release of antagonistic compounds is deposited in the soil and may be taken up directly by neighboring or companion plants or undergo chemical or biological transformations that alter the properties and nature of the soil, reflecting positively (Macias et al.,1998) or negatively (Duke, 1998) on plants grown in soil as a drug (Kadawi, 2011). Some of the classes of wheat allelopathic substances were also defined, such as phenolic acids, hydroxamic acids, and short-chain fatty acids (Hanwen et al., 2001), and the content of each phenolic acid was strongly associated with the others in wheat seedling shoots. Wheat accessions with high levels of total phenolic acids identified in shoots are generally strongly allelopathic to annual ryegrass growth (Hanwen et al., 2001; Jabran et al., 2013). The amount of polyphenol content of wheat decreases when planted with other plant species, but by comparing this content of wheat with its content in the cultivated oats, with him, we find that their quantity is almost equal, and this is due to the allelopathic competition between the two plants, as the wheat plant changes effort to resist the oat plant and that is by releasing the largest amount of these substances, which are considered as a defense system against competing plants, also the same observation when comparing the polyphenol content of cultivated wheat and lentils With it, we find that wheat has resistance, which is manifested in the secretion of these compounds in abundance that exceeded their quantity in the lentil plant planted with it, alone, while we find that the content of this substance is relatively high in the wormwood plant, and this explains the effect of the extract of this plant on the species. The phenolic compounds can inhibit root elongation and cell division in plants, and cause changes in cell ultrastructure, interfering with the normal growth and development of the whole plant. This explains the different vegetative growth lengths of the plants studied. The flavonoid compound is responsible for the physiological activity that inhibits the vegetative growth of plants and prevents the elongation of stems. Given the content of flavonoids in wormwood, we find that their amount is high and their effectiveness is manifested in the inhibition of vegetative growth of competing plants of wheat (Iyad,2012).This explains, by the inhibition of the growth of oats and lentil due to flavonoid compounds

rich in phenolic groups, and this makes them able to stabilize some enzymes specific to the intermediate reactions leading to the formation of auxin, which leads to block its formation (Mizraq, 2010 ; Iyad,2012). Our results are consistent with those of Shalinder kaur and Daizy (2010) and Xu et al (2019) regarding the effect of *Artimisia scoparia* extract at different concentrations from 0 to 10% on the growth of seedlings of some plants and Salman et *al*.(2017)regarding the effect of *Artemisia annua* extract from 0 to 3.5% concentration on the growth of barley seedlings of a concentration, in general, the inhibitory effect was greater on weeds than on crops. Of the plants tested, oats were the most sensitive.

Conclusion

The results of this study showed that the extract of *Artemisia herba -alba* has strong phytotoxicity against weeds and therefore could be used as a bio-herbicide. The use of plant extracts in the control of unwanted weeds brings great benefit and success in agriculture and crop protection in addition to positive allelopathic effects (stimulation) which can be well used in the production of agricultural crops.

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Oral Presentation

A STATISTICAL PARSIMONY NETWORK RESOLUTION FOR THE POLYTOMIES IN THE *COI*-BASED PHYLOGRAMS OF *ARHOPALUS RUSTICUS* (COLEOPTERA, CERAMBYCIDAE)

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Abstract

Arhopalus rusticus (Coleoptera, Cerambycidae) is a sombre-colored longnorned beetle. Although its natural distribution area is the Northern Hemisphere, it has gradually distributed to the Afrotropical, Neotropical and Australian regions through human-mediated transport. This beetle is a vector that spreads pine wood nematodes, thus damaging coniferous trees. Population explosions have caused it to be the subject of some recent studies. Despite these studies attempting to resolve phylogenetic relationships of the genus *Arhopalus* within the other spondylidines based on the mitochondrial cytochrome *c* oxidase I (*COI*) barcode region, utilising conventional phylogenetic tree hypothesising algorithms Neighbor-Joining, Maximum likelihood and Bayesian Inference, they could not provide an intraspecific resolution for the *A. rusticus* and remarkable polytomies remained. The study aims to understand whether these polytomies occurred as a result of a lack of sufficient synapomorphic characters of the *COI* barcode region, missing the ancestral gene sequences in the analysis or due to inadequate analysis techniques used. We constructed a statistical parsimony network for the *COI* global dataset, incorporating sequences from GenBank and our previous research to identify any potentially missing haplotypes and unveil potential geographic links and branching patterns among the haplotypes. Our statistical parsimony network suggested that 21 haplotypes were missing in the previous analysis, and the polytomies may be attributed to branching patterns. The branchings are not dichotomous but rather reticular and radial, indicative of ongoing gene flow and bottleneck effects, respectively.

Keywords: Bottleneck effect, cerambycid pest, ongoing gene flow, Spondylidinae, TCS network

INTRODUCTION

Spondylidinae Audinet-Serville, 1832, is a subfamily of longhorned beetles (Coleoptera, Cerambycidae) known for their plain, somber-colored bodies and crepuscular or nocturnal activity (Švácha & Lawrence, 2014). A tribe of the subfamily Asemini Thomson, 1860, has gained attention from phytosanitary authorities as vectors and invaders. They prefer laying their eggs under the bark of dead or stressed coniferous trees, allowing them to evade phytosanitary measures in the international marine wood trade (Soydabaş-Ayoub et al., 2023). Studies have shown that the most intercepted cerambycid during cross-continental transit is the members of the genus *Arhopalus* Serville, 1834 (Haack et al., 2014; Wu et al., 2017).

The natural distribution area of *Arhopalus* is the Northern Hemisphere; however, it gradually spreads to all biogeographic regions (Linsley, 1962; Bense, 1995). Adlbauer (2001) reported the introduction of *A. ferus* (Mulsant, 1839) to Namibia; Wang and Leschen (2003) of *A. ferus*, *A. rusticus* (Linnaeus, 1758) and *A. syriacus* (Reitter, 1895) to Australasia; López et al. (2008), of *A. ferus* and *A. rusticus* to Argentina. Despite having been reported years ago that they pose a threat to *Pinus* (pine) trees all over the world (Linsley, 1962; Duffy, 1968; Webb & Eldridge, 1997), *Arhopalus spp.* had been considered a severe forest pest after the population explosions in recent years (Ciesla, 2011). Among them, *A. rusticus* has notable harm to the forests by carrying the nematodes *Bursaphelenchus* spp., which are responsible for pine-wilt disease (Ryss et al., 2005; Smith et al., 2008; Wang et al., 2021; Žunič-Kosi et al., 2019). It is important to understand the phylogeographic relationships of the species to prevent unexpected invasions. Soydabaş-Ayoub et al. (2023) attempted to resolve phylogenetic relationships of the genus *Arhopalus* utilising conventional phylogenetic tree hypothesising algorithms Maximum likelihood (ML) and Bayesian Inference (BI), both are probabilistic approaches to suggest dichotomous trees, they could not provide an intraspecific resolution for *A. rusticus* and remarkable intraspecific polytomies remained. In such situations, phylogenetic networks are a saviour to hypothesising reticulate events such as hybridisation, horizontal gene transfer, recombination, or gene duplication. The study aims to understand potential geographic links and branching patterns among the haplotypes of *A. rusticus,* utilising a network analysis.

MATERIAL AND METHODS

The dataset included *COI* barcode sequences of six *Arhopalus* species: *A.asperatus* LeConte, 1859, *A. ferus, A. foveicollis* (Haldeman, 1847), *A. productus* LeConte, 1850, *A. rusticus* and *A. syriacus. Distenia phaeocera* Bates 1880 (Coleoptera Disteniidae) (BOLD ID: ASSCR5853-12) was used as an outgroup. The NCBI (National Center for Biotechnology Information) or BOLD (Barcode of Life Database) accession numbers of sequences analysed are listed in Table 1.

Table 1. Haplotype codes, accession numbers and localities of the sequences analysed

#	Species	Haplotype	Accession number(s)	Locality	References
1	*A. asperatus*	AAS1	CERPA067-08*	Canada	Anonymous
2	*A. asperatus*	AAS2	CERPA066-08*	Canada	Anonymous
3	*A. ferus*	AFE1	MK689187	India	Behere et al. (Unpublished)
4	*A. ferus*	AFE2	KM286046	France	Rougerie et al. (2015)
5	*A. ferus*	AFE3	OM681091,92	Türkiye	Soydabaş-Ayoub et. al. (2023)
6	*A. ferus*	AFE4	KY357757	Italy	Wu et al. 2017
7	*A. foveicollis*	AFO1	KJ203018	Canada	Woodcock et al. 2013
8	*A. foveicollis*	AFO2	KJ203029	Canada	Woodcock et al. 2013
9	*A. productus*	APR1	CERPA071-08*	Canada	Anonymous
10	*A. productus*	APR2	CERPA069-08*	Canada	Anonymous
11	*A. productus*	APR3	CERPA070-08*	Canada	Anonymous
12	*A. rusticus*	ARU1	KM439641	Germany	Hendrich et al. (2015)
13	*A. rusticus*	ARU2	HQ559250	Finland	Pentinsaari et al. (2014)
14	*A. rusticus*	ARU3	KJ965325	Finland	Pentinsaari et al. (2014)
15	*A. rusticus*	ARU4	KY357658	n/a	Wu et al. 2017
16	*A. rusticus*	ARU5	KJ964579	Estonia	Pentinsaari et al. (2014)
17	*A. rusticus*	ARU6	OM681072	Türkiye	Soydabaş-Ayoub et. al. (2023)
18	*A. rusticus*	ARU7	KY357666	n/a	Wu et al. 2017
19	*A. rusticus*	ARU8	OM681071	Türkiye	Soydabaş-Ayoub et. al. (2023)
20	*A. rusticus*	ARU9	OM681076	Türkiye	Soydabaş -Ayoub et. al. (2023)
21	*A. rusticus*	ARU10	OM681086	Türkiye	Soydabaş-Ayoub et. al. (2023)
22	*A. rusticus*	ARU11	GU003934	n/a	Wu et al. 2017
23	*A. rusticus*	ARU12	OM681077	Türkiye	Soydabaş-Ayoub et. al. (2023)
24	*A. rusticus*	ARU13	OM681075	Türkiye	Soydabaş-Ayoub et. al. (2023)
25	*A. rusticus*	ARU14	OM681069	Türkiye	Soydabaş-Ayoub et. al. (2023)
26	*A. rusticus*	ARU15	KU907357	Germany	Rulik et al. (2017)
27	*A. rusticus*	ARU16	OM681073	Türkiye	Soydabaş-Ayoub et. al. (2023)
28	*A. rusticus*	ARU17	KM440404	Germany	Hendrich et al. (2015)
29	*A. rusticus*	ARU18	OM681078,79	Türkiye	Soydabaş-Ayoub et. al. (2023)
30	*A. rusticus*	ARU19	KU918848	Germany	Rulik et al. (2017)
31	*A. rusticus*	ARU20	KJ964380	Finland	Pentinsaari et al. (2014)
32	*A. rusticus*	ARU21	OM681080	Türkiye	Soydabaş-Ayoub et. al. (2023)
33	*A. rusticus*	ARU22	OM681083	Türkiye	Soydabaş-Ayoub et. al. (2023)
34	*A. rusticus*	ARU23	OM681085	Türkiye	Soydabaş-Ayoub et. al. (2023)
35	*A. rusticus*	ARU24	OM681074	Türkiye	Soydabaş-Ayoub et. al. (2023)
36	*A. rusticus*	ARU25	KY357682	n/a	Wu et al. 2017
37	*A. rusticus*	ARU26	KU915404	Germany	Rulik et al. (2017)
38	*A. rusticus*	ARU27	OM681084	Türkiye	Soydabaş-Ayoub et. al. (2023)
39	*A. rusticus*	ARU28	KU914249	Germany	Rulik et al. (2017)
40	*A. rusticus*	ARU29	KU908164	Germany	Rulik et al. (2017)
41	*A. rusticus*	ARU30	KM439145	Germany	Hendrich et al. (2015)
42	*A. rusticus*	ARU31	KM451619	Germany	Hendrich et al. (2015)
43	*A. rusticus*	ARU32	KM286239	France	Rougerie et al. (2015).
44	*A. rusticus*	ARU33	JF889719	Germany	Anonymous*
45	*A. rusticus*	ARU34	KY357660	n/a	Wu et al. 2017
46	*A. rusticus*	ARU35	KU914469	Germany	Rulik et al. (2017)
47	*A. rusticus*	ARU36	KU917780	Germany	Rulik et al. (2017)
48	*A. rusticus*	ARU37	KM286380	France	Rougerie et al. (2015)
49	*A. rusticus*	ARU38	KY357662	n/a	Wu et al. 2017
50	*A. rusticus*	ARU39	KU915398	Germany	Rulik et. al (2017)
51	*A. rusticus*	ARU40	KM440454	Germany	Hendrich et al. (2015)
52	*A. rusticus*	ARU41	OM681081,82,87	Türkiye	Soydabaş-Ayoub et. al. (2023)
53	*A. rusticus*	ARU42	KM447391	Germany	Hendrich et al. (2015)
54	*A. rusticus*	ARU43	KU914752	Germany	Rulik et al. (2017)
55	*A. syriacus*	ASY1	OM681090	Türkiye	Soydabaş-Ayoub et. al. (2023)
56	*A. syriacus*	ASY2	KY357827	Türkiye	Wu et al. 2017
57	*A. syriacus*	ASY3	OM681088	Türkiye	Soydabaş-Ayoub et. al. (2023)
58	*A. syriacus*	ASY4	OM681070	Türkiye	Soydabaş-Ayoub et. al. (2023)

* searched on http://www.boldsystems.org/ while others could be searched on https://www.ncbi.nlm.nih.gov/taxonomy

Haplotype codes used the same as Soydabaş-Ayoub (2021). MUSCLE v3.8.425 (Edgar, 2004) aligned the *COI* barcode region 658 bp in length. The best-fitting substitution model was selected according to AICc by greedy search using PartitionFinder v2.1.1 (Lanfear et al., 2016; Stamatakis, 2014). The GTR+G substitution model was used for ML and BI analyses. ML analysis was conducted in PhyML (Guindon et al., 2010) with 10,000 bootstrap iterations and BI analysis was conducted in MrBayes (Ronquist & Huelsenbeck, 2003), with 10 million generations of two runs, one cold three hot Monte Carlo Markov Chain (MCMC), sampled every 5000 generations, of which 25%were discarded as burn-in. Statistical parsimony network (TCS, Templeton, Crandall, Sing; Templeton et al., 1992) was drawn in PopART v1.7 (Leigh & Bryant, 2015).

RESULTS

ML and BI phylogenetic trees were compatible topologically and statistically. All haplotypes of *A. rusticus* species are grouped on the branch, supported by BI and ML (PP 1, BS 96%). However, the internal nodes of the branch mainly remained unsolved, including two samples with *A. syriacus* haplotype codes (ASY3-ASY4) which are reported to be carriers of the mitochondrial COI gene of *A. rusticus* by Soydabaş-Ayoub et al. (2023) Another group consisted of the remaining five species, *A. asperatus, A. ferus, A. foveicollis, A. productus*, and *A. syriacus* were clustered together at the BI tree (PP 0.97, Figure 1). At the same time, they separated into sub-branches at the ML tree (Figure 2).

TCS network suggested reticular and radial branchings (star-shaped twigs) in addition to the dichotomies (Figure 3). Two star-shaped clusters were remarkable. One of them was consisted of mostly haplotypes from Türkiye (ARU23, ARU 28, ASY3, ASY4 ARU9, ARU10, ARU21, ARU24, ARU14, ARU27, ARU8, ARU22, ARU30), and two from Germany (ARU15, ARU26) and two from an unknown origin (ARU11, ARU25) intercepted in a port of United States of America (USA) reported by Wu et al. (2017), and seven hypothetical haplotypes providing reticular connections. The second star-shaped cluster consisted of mostly haplotypes from Germany (ARU1, ARU17, ARU29, ARU31, ARU36, ARU35, ARU39, ARU40 ARU42, ARU43), two from France (ARU32, ARU37) and two from unknown origin (ARU34, ARU38). A third group notable with dichotomous branchings included haplotypes from Türkiye (ARU16), from Germany (ARU19, ARU33), Finland from (ARU2, ARU3, ARU5) from Estonia (ARU5) from an unknown origin, intercepted in a port of USA (ARU4). Four hypothetical haplotypes provided connections between countries. Reticular twigs provided the connections between the groups.

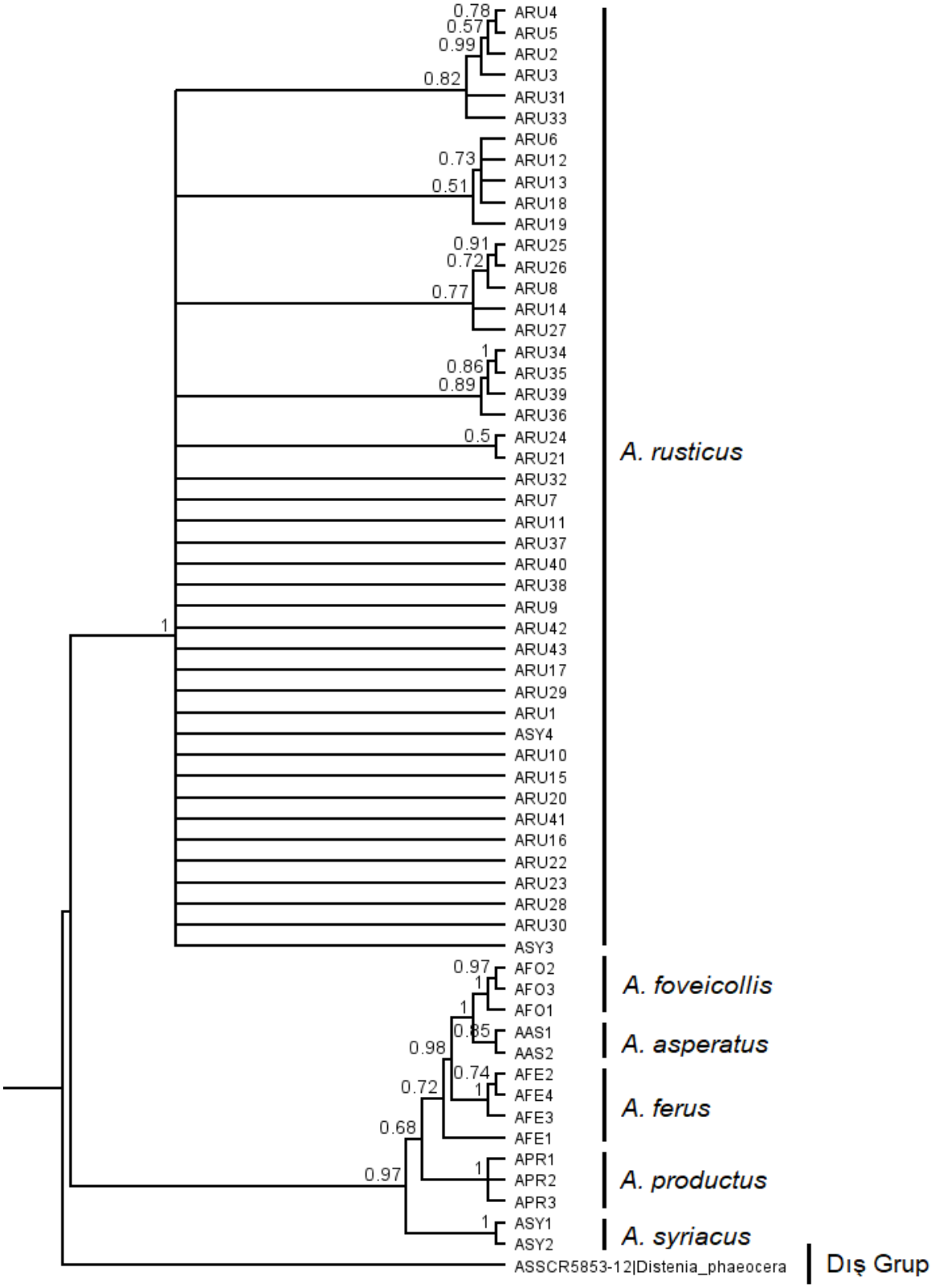

Figure 1. Bayesian tree of the *COI* haplotypes (658 bp) of *Arhopalus* spp. The numbers on nodes indicate posterior probabilities.

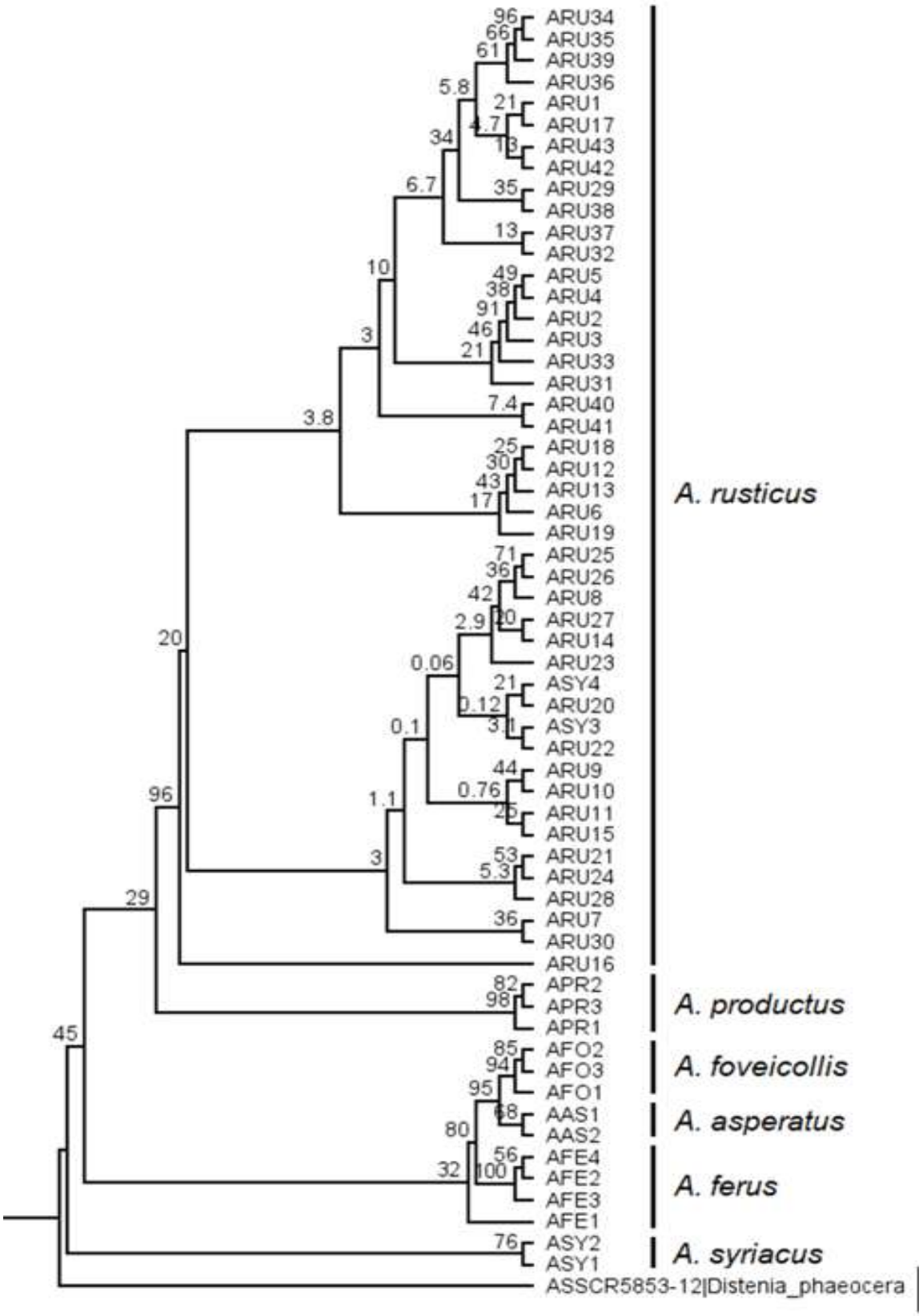

Figure 2. Maximum Likelihood tree of the *COI* haplotypes (658 bp) of *Arhopalus* spp. The numbers on nodes indicate bootstrap support.

Three *A. productus* haplotypes (APR1-APR 3), all from Canada, were closely related in the network, as in all other analyses (Figure 1, Figure 2, Figure 3). Three haplotypes of *A. ferus*, AFE2 from France, AFE3 from Türkiye and AFE4 from Italy, showed dichotomous branching (Figure 3). Two *A. syriacus* haplotypes, ASY1 and ASY2, intercepted in a port of USA in a trade wood from Türkiye (as stated by Wu et al., 2017), were linked to a hypothetical haplotype, which is a bridge between *A. syriacus* and *A. productus* (Figure 3). The haplotypes of *A. asperatus* (AAS1 and AAS2) and the three haplotypes of *A. foveicollis* (AFO1-AFO3) were associated with multiple connections, all of them from Canada (Figure 3).

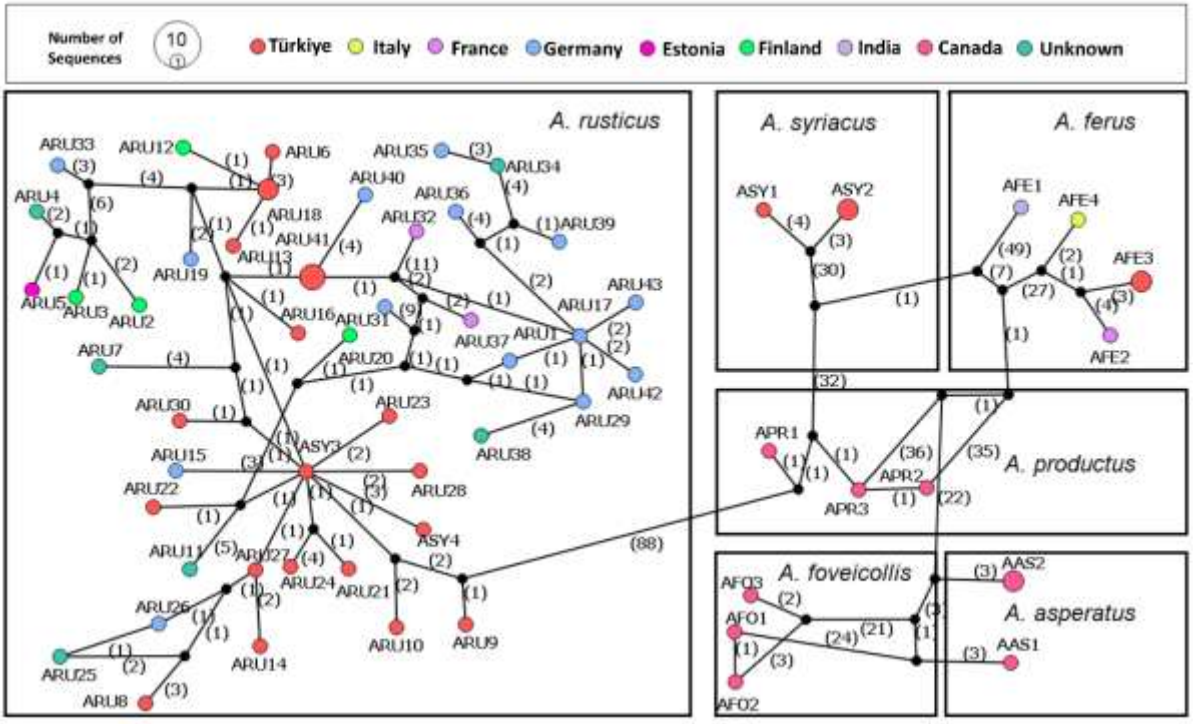

Figure 3. Statistical parsimony network of the *COI* haplotypes (658 bp) of *Arhopalus* spp. The numbers in the parathesis indicate mutational steps. Each colour represents a country. Black points are the hypothetical haplotypes.

DISCUSSION

The phylogenetic relationships among taxa are typically depicted using phylogenetic trees. These trees serve as hypotheses for evolutionary relationships. However, they are limited in their ability to represent complex evolutionary scenarios (Blair & Ané, 2020). Intraspecific polytomies resulting from ML and BI trees of *Arhopalus rusticus* might be a good example of this limitation, which the dichotomous branching could not solve (Figure 1, Figure 2). These polytomies might be due to a lack of sufficient synapomorphic characters of the *COI* barcode region having a limited number of variations (Simon et al., 1994), missing the ancestral gene sequences in the analysis, or both. (Townsend & Lopez-Giraldez, 2010). From another point of view, polytomies

might be a sign of a complex evolutionary process, which cannot be explained by dichotomous branching (Lewis et al., 2005; Sayyari & Mirarab, 2018).

In cases where phylogenetic trees fall short, phylogenetic networks serve as a valuable tool for capturing reticulate events such as hybridisation, horizontal gene transfer, recombination, and gene duplication or loss (Templeton et al., 1992). Moreover, to evaluate the various connectivity probabilities that can be possible with networks and figure out phylogeographic relationships (Cassens et al., 2003).

Our statistical parsimony network suggested that 21 haplotypes were missing analysis within the *A. rusticus* species group (Figure 3). The dichotomies branching appeared in the network similar to the phylogenetic trees (Figure 1, Figure 2, Figure 3). Moreover, the TCS network suggested reticular and radial branchings in addition to the dichotomies. (Figure 3). The star-shaped formations appeared in two clusters; one consisted mostly of haplotypes from Türkiye, and the other consisted of haplotypes from Germany (Figure 3). This radial pattern is mainly attributed to the bottleneck effect (Richards et al., 2019). The reticular formation between the clusters is a sign of ongoing gene flow (Wollenberg et al., 2019), which is also supported by the scantiness of the mutational steps between haplotypes (Pogson et al., 1995; Karthika et al., 2019).

CONCLUSIONS

Based on the branching patterns suggested by the TCS network, it can be inferred that *A. rusticus* likely has a complex evolutionary background. This is evidenced by several events, including continuous migrations and the bottleneck effect.

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Conflicts of Interest Statement

As authors Havva Kübra SOYDABAŞ-AYOUB and Fevzi UÇKAN, we declare that **NO known** competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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Oral Presentation

ALTERATION IN ANTIOXIDANT ENZYME ACTIVITIES OF TETRAPLOID WHEATS UNDER DROUGHT STRESS

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Abstract

Tetraploid wheats include *Triticum durum*, *Triticum polonicum* and *Triticum turanicum*. Drought tolerance of these wheat varieties should be investigated. Tolerant plants accumulate enzymatic and non-enzymatic antioxidants in order to cope with the stress. In this study, it is aimed to define the changes of enzyme activities of tetraploid wheat genotypes under drought stress. The research was conducted with five landraces of *T. durum*, five *T. polonicum* and five *T. turanicum* in 2023-2024 growing season in Eskişehir. Genotypes were analyzed to variance of electrolyte leakage, hydrogen peroxide (H_2O_2) and protein content as well as antioxidant enzyme activities such as superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX) and catalase (CAT) under drought stress in vegetative and generative period. The effects of drought stress on both stages resulted in notable variations among genotypes. The degree of damage caused by electrolyte leakage differed significantly among genotypes under drought stress at the vegetative and generative stages. Genotypes exhibiting elevated H_2O_2 levels during the vegetative period demonstrated a reduction in accumulation during the generative period, and vice versa. While some genotypes exhibited an increase in antioxidant enzyme activity during drought stress in the vegetative period, others demonstrated a decrease. However, during drought stress in the generative period, there was a notable increase in antioxidant enzyme activity. A comparable alteration was observed in soluble protein content. While there is some variation between genotypes, it can be concluded that *T. polonicum* wheats is drought tolerant during both the vegetative and generative stages.

Keywords: antioxidant enzymes, drought stress, growth stage, hydrogen peroxide, membrane damage, tetraploid wheats

Introduction

Drought stress can be defined as a condition of water scarcity that results in morphological, biochemical, physiological, and molecular alterations in plants. These changes have an impact on plant growth and yield. Drought stress can manifest at any stage of the growth cycle and its effects may vary depending on the genotype, the environment and their interaction (Adel and Carels, 2023). Consequently, some genotypes may demonstrate tolerance to drought conditions at the germination or seedling stage, while others may exhibit sensitivity to drought at the flowering stage, and vice versa. The determination of drought tolerance can be achieved through the identification of traits that can be employed to quantify the impact of drought stress on plants. Such traits should be capable of differentiating between genotypes that are tolerant and those that are susceptible (Khadka et al., 2020). The determination of the content of damage indices such as hydrogen peroxide (H_2O_2) and electrolyte leakage (EL) is a valuable tool for the investigation of the extent of induced tolerance of plants to drought conditions. Plants capable of withstanding drought exhibit reduced levels of oxidative stress and increased levels of antioxidant enzymes, including glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX). These enzymes are capable of scavenging reactive oxygen species (ROS) (Nawaz et al., 2020).

Tetraploid wheats (2n = 4× = 28; AABB genome) are the progenitors of modern durum and bread wheat varieties and they have been pivotal in the advancement of agriculture and human civilization. The presence of numerous favorable agronomic features in both wild and domesticated ancestors may prove effective in durum wheat breeding efforts aimed at enhancing the quality and productivity of this crop, as well as stress tolerance (El Haddad et al.,2021). Old landraces and wild ancestors represent a rich source of genetic diversity that has not been fully exploited. Using the potential of these genetic resources in the modern breeding methods could facilitate genetic progress, thereby enabling the advancement of sustainable agriculture and the resilience of crops in the face of climate change (Pour-Aboughadareh et al., 2021). Notwithstanding the impact of genetic drift and other environmental challenges, wild and domestic tetraploid wheat subspecies demonstrate a markedly superior capacity to retain genetic diversity compared to durum wheat (Yadaw et al., 2023). Furthermore, *dicoccoides*, *dicoccum*, *paleocolchicum*, *polonicum*, *turgidum*, and *turanicum* are among the subspecies that possess advantageous genes that enhance their resistance to drought (Fatiukha et al., 2021). Wheat landraces are capable of withstanding harsh and stressful conditions due to their genetic composition, capacity to act as a buffer, and agro-physiological characteristics. Furthermore, landraces exhibit genetic variability for a multitude of genes that are absent or undetectable in commercial cultivars (Zencirci et al., 2024). The discovery of wheat landraces with the

requisite genetic composition to withstand drought stress could facilitate the development of a novel cultivar. Consequently, they may prove useful in the development of innovative breeding strategies that yield materials with modified characteristics, such as enhanced stress tolerance (Chaouachi et al., 2024).

The major objective of this study was to elucidate the enzymatic antioxidative responses of some tetraploid wheat landraces (*Triticum durum, Triticum polonicum*, and *Triticum turanicum*) at the tillering and flowering stage which are two critical affecting grain yield.

Materials and Methods

The plant material comprised 15 tetraploid wheat genotypes, obtained from the USDA gene bank. These included five T. *durum* landraces, five T. *polonicum*, and five T. *turanicum* genotypes. Further details on the wheat genotypes used are provided in Table 1.

Table 1. Information on tetraploid genotypes used in the study

Plant ID	Plant Name	Collected from
Triticum durum		
PI 73376	2529/Kara Kilchik	Azerbaijan
PI 172562	Akbasak	Türkiye
PI 166706	Kunduru	Ankara
PI166243	Sahman-6	Konya
PI 341353	Sari Kilcik	Ankara
Triticum polonicum		
CItr 14139	CI 14139	Unknown
CItr 14140	CI 14140	Unknown
PI 306548	2939	Romania
PI 306549	2941	Romania
PI 167622	Mika	Balıkesir
Triticum turanicum		
PI 67343	Australian Pouland	Victoria, Australia
PI127106	Dandan-i-Shutur	Fāryāb, Afghanistan
PI 210386	Heath	Iranian
PI 192658	Meknes	Morocco
PI 166959	Turnadili	Çankırı

The experiment was conducted in both irrigated and rainfed conditions in accordance with the split-plots experimental design. The planting was conducted using a plot planting drill in 4-row plots with a planting density of 500 plants per square meter, measuring 4 meters in length with a 20 cm distance between rows. A total of 70 kg ha^{-1} of P_2O_5 and 40 kg ha^{-1} of N were applied at the time of sowing. A nitrogen fertilizer at a rate of 40 kg ha^{-1} of N was applied as a top dressing prior to the stem elongation

period. Climatic data for the growing season are shown in Table 2.

The field capacity of the research area soils was determined in accordance with the methodology proposed by Danish et al. (2020). In order to optimize yield and quality in wheat planting, an average of 500–600 mm (or at least 400 mm) of water is required. In order to maintain the soil with a determined field capacity at 80% field capacity for normal growing conditions (irrigated conditions), the moisture value was gauged with a Delta-T Devices HH2 Soil Moisture Meter, and the supplementary irrigation time and volume were determined in addition to the water received from natural rainfall. In addition to the 240 mm of rainfall, 260 mm of supplementary irrigation was applied to fulfil the 500 mm water requirement throughout the growing season. The plants were cultivated in an environment characterized by rainfed conditions, with the provision of natural rainfall serving as the primary source of irrigation. The evaluation of drought tolerance was conducted through the analysis of enzymes in both the vegetative (end of tillering period, Zadoks 30) and generative (flowering period, Zadoks 65) phases.

Table 2. Climatic conditions in growing season

	Growing season (2023-2024)		Long Years (1991-2022)	
Month	Mean Temperature (°C)	Total Precipitation (mm)	Mean Temperature (°C)	Total Precipitation (mm)
November	9.1	47.8	5.9	33.4
December	4.6	32.4	1.9	44.4
January	2.0	46.6	-0.1	33.0
February	4.4	17.0	1.6	28.2
March	6.5	33.2	5.2	29.9
April	14.1	11.8	9.9	44.1
May	13.8	50.4	14.9	42.3
June	22.9	5.6	18.9	24.2
July	23.7	4.4	21.9	15.0
Mean/Total	*11.2*	*249.2*	*8.9*	*294.5*

The leaves belong to wheats at the irrigated and rainfed conditions, three 2 cm diameter disks were excised, cleaned with distilled water, dried, and placed in test tubes. Each test tube was filled with 20 ml of distilled water, and the samples were permitted to rest at room temperature for four hours while being shaken at 250 rpm. An initial reading (RV1) was obtained using an EC meter (Mettler Toledo, S230-K) in order to assess electrolyte leakage. Then, the tubes were subjected to autoclaving for a period of 15 minutes at a temperature of 121°C, with the objective of achieving complete tissue destruction. Subsequently, the electrolyte leakage measurement (RV2) was conducted once the samples had reached room temperature. The formula employed to calculate the electrolyte leakage was as follows:

(% Electrolyte Leakage = (RV1/RV2) × 100).

The levels of H_2O_2 were determined using the methodology described by Velikova et al. (2000). A homogenate was prepared by macerating 500 mg of leaf tissue in 5 mL of 0.1% TCA. The homogenate was subjected to centrifugation for a period of 15 minutes at a speed of 12,000 g. Thereafter, one ml of 1 M KI, five ml of a 10 mM potassium phosphate buffer solution (pH 7.0), and five ml of the supernatant were combined. The absorbance of the supernatant was determined using a spectrophotometer with a wavelength of 390 nm. A standard curve was employed to determine the quantity of H_2O_2 present.

A bovine serum albumin (BSA) standard was employed to ascertain the soluble protein concentrations in leaf samples from all treatments, utilizing Bradford's methodology (1976). The absorbance values of the solution were measured at a wavelength of 595 nm using a Thermo-Aquamate spectrophotometer. The quantity of protein content was then determined by means of a standard curve created with the use of BSA standards.

The Cakmak and Marschner (1992) method was used to evaluate antioxidant enzyme activity. 0.2 g of plant leaves were homogenized in K-P buffer (pH 7.6) and centrifuged at 15,000 g for 20 minutes at 4 °C to extract enzymes. Reactions were configured for each enzyme activity following the extraction of the supernatant. All enzyme activity measurements were conducted using the supernatant.

The solution for calculating SOD activity is: 0.5 mL sodium bicarbonate, 0.5 mL methionine, 0.5 mL NBT, 0.5 mL riboflavin, 0.1 mL sample, and 2.9 mL K-P buffer (pH 7.6). Glass test tubes were filled with the solution and mixed. They were left for ten to fifteen minutes until the color changed to blue. The spectrophotometer was used to ascertain the absorbance at 560 nm. The quantity of enzyme present per milligram of protein was determined in micromoles.

To ascertain GR activity, the following protocol was followed. Initial assessment at 340 nm: 0.7 ml K-P buffer (pH 7.6), 0.1 ml oxidized glutathione (GSSG), 0.1 ml supernatant, 0.1 ml NADPH. The total GR enzyme activity and absorbance readings were obtained using a spectrophotometer for one minute. After deducting non-enzymatic oxidation, the reaction's starting rate was determined to be nmol mg^{-1} protein min^{-1}.

After adding K-P buffer, H_2O_2, the sample supernatant and ascorbic acid, the absorbance was read for one minute at 290 nm to determine APX activity. The enzyme quantity was determined in micromoles per minute per milligram of protein.

Following the addition of 0.8 mL of K-P buffer (pH 7.6), 0.1 mL of sample supernatant, and 0.1 mL of H_2O_2, absorbance measurements were taken at 240 nm for a duration of one minute. This was done in order to ascertain the CAT activity. The quantity of

enzyme, expressed as nmol/mg protein/min, that reduced the absorbance by µmol in one minute at 25 °C was determined.

The IBM SPSS 26 software was employed for the analysis of the study's data, in accordance with the split-plot experimental design, which was used to examine the variance. The data are presented as error bars and bar graphs, which illustrate the mean and standard error of the data set. The figures were created using IBM SPSS 26.

Results

The values for electrolyte leakage and H_2O_2 content, as determined during the vegetative and generative periods, are presented in Figure 1. The measured membrane damage parameters of plants grown under irrigated and rainfed conditions demonstrate considerable variability. The electrolyte leakage values of wheat plants in the vegetative period varied between 4.40% (Australian Pouland) and 12.64% (Akbasak) under irrigated conditions. Under rain-fed conditions, electrolyte leakage values increased as expected. The lowest value (6.58%) was measured in the Dandan-i-Shutur genotype and the highest value (26.32%) in the Akbasak genotype. The electrolyte leakage values determined during the generative period are comparatively higher than the vegetative period. The electrolyte leakage was ranging from 10.25% (Australian Pouland) to 25.76% (2529/Kara Kilchik) under stress conditions. The Akbasak, Australian Pouland and Meknes genotypes exhibited reduced electrolyte leakage in comparison to the vegetative period (Figure 1).

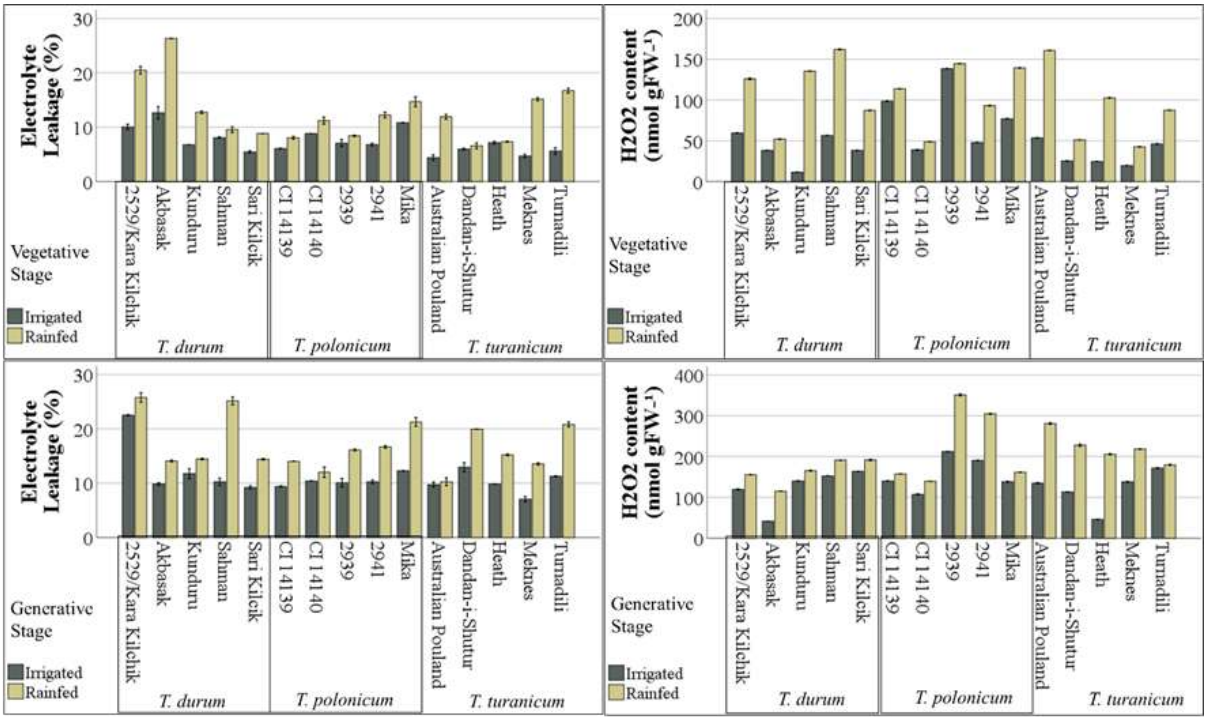

Figure 1. Changes of electrolyte leakage and H_2O_2 content of tetraploid wheat genotypes under drought stress.

The H_2O_2 content of wheat genotypes revealed a higher accumulation during the generative period. The H_2O_2 content of some genotypes was observed to be higher under irrigated conditions than under stress conditions in both periods. The H_2O_2 content of certain genotypes was observed to accumulate at a lower level in stressful conditions than in irrigated conditions, when compared to the accumulation of H_2O_2 in other genotypes (Figure 1). Genotypes with high H_2O_2 levels under irrigation also had high levels under stress. However, the increase differed between genotypes when they were switched from normal to stress conditions. The Kunduru genotype exhibited the greatest increase in H_2O_2 during the vegetative period, with a 91.44% rise. During the generative period, the H_2O_2 content of the Heath genotype increased from 46.23 nmol gFW^{-1} to 205.84 nmol gFW^{-1}, representing a 77.54% increase (Table 3).

Table 3. Percentage values of membrane damage and H_2O_2 accumulation in tetraploid wheat genotypes exposed to drought stress

	Membrane Damage (%)		H_2O_2 % increase	
Plant Name	Vegetative	Generative	Vegetative	Generative
2529/Kara Kilchik	10.41	3.24	52.73	23.08
Akbasak	13.67	4.24	26.63	63.73
Kunduru	5.96	2.70	91.44	15.19
Sahman	1.47	14.89	65.06	20.16
Sari Kilcik	3.38	5.18	56.10	14.66
T. durum Mean	**6.98**	**6.05**	**58.39**	**27.37**
CI 14139	1.98	4.63	13.39	10.82
CI 14140	2.39	1.60	19.94	22.96
2939	1.34	6.04	4.20	39.57
2941	5.44	6.43	48.62	37.41
Mika	3.85	8.98	44.76	14.34
T. polonicum Mean	**3.00**	**5.53**	**26.18**	**25.02**
Australian Pouland	7.52	0.52	66.56	52.01
Dandan-i-Shutur	0.61	7.00	50.25	50.21
Heath	0.16	5.38	75.91	77.54
Meknes	10.44	6.48	53.99	36.78
Turnadili	11.10	9.52	47.14	4.45
T. turanicum Mean	**5.97**	**5.78**	**58.77**	**44.20**

The calculated damage percentages-based electrolyte leakage exhibited considerable variation between genotypes and growth stages. In the vegetative period, damage percentages ranged from 0.16% in the Heath genotype to 13.67% in the Akbaşak genotype, while in the generative period, damage percentages ranged from 0.52% in the Australian Pouland genotype to 14.89% in the Sahman genotype. Some genotypes had higher damage percentages during the vegetative period than during the generative period, and vice versa. A comparison of the mean values of wheat

subspecies revealed that the damage percentage of *T. durum* was higher. *T. turanicum* accumulated a greater quantity of H_2O_2 when subjected to stress (Table 3).

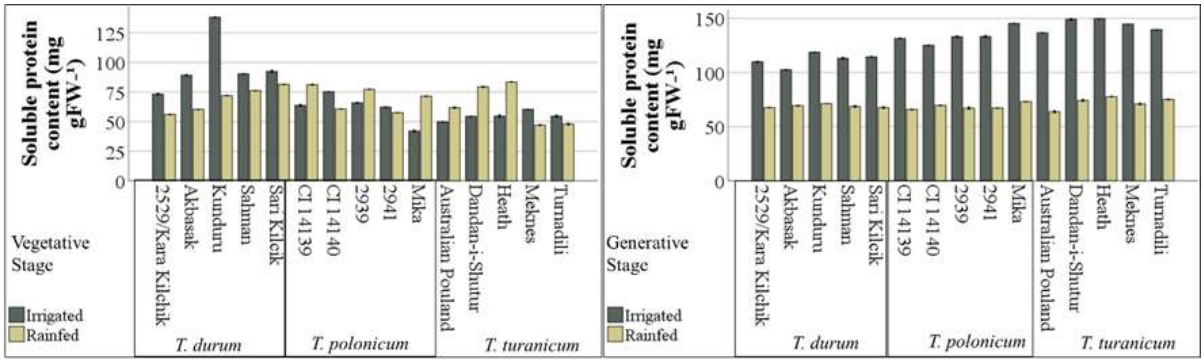

Figure 2. Changes of soluble protein content of tetraploid wheat genotypes under drought stress.

Soluble protein content in fresh leaves of wheat plants varied between 41.95 mg gFW^{-1} (Mika) and 138.05 mg gFW^{-1} (Kunduru) during the vegetative period under irrigated conditions (Figure 2). The protein ratios of certain genotypes demonstrated an increase in response to drought stress. The Mika genotype exhibited an approximate 90% increase under the *T. polonicum* subspecies. The lower and upper limits of the protein ratio in the vegetative period of rainfed wheat plants were found to be similar to those observed under irrigated conditions. During the generative period, the protein ratio was higher under irrigated conditions. The protein ratio of the Kunduru genotype was similar to that observed under irrigated conditions in both periods. However, the protein ratio decreased significantly under drought conditions. In drought conditions during the generative period, the protein ratio was close to that observed during the vegetative period, but the protein loss compared to irrigated conditions was approximately 45% on average (Table 4).

The activity of SOD varied between 1.10 and 5.56 µmol $mgprotein^{-1}$ during the vegetative period and between 0.93 µmol $mgprotein^{-1}$ and 3.90 µmol $mgprotein^{-1}$ during the generative period (Figure 3). The Mika genotype showed the highest level of SOD activity during the vegetative period under irrigated conditions. In conditions of drought, the Meknes genotype had the highest value, with 3.91 µmol $mgprotein^{-1}$. The Kunduru genotype exhibited the lowest SOD activity in both experimental conditions. The profile of GR activity was similar to that of SOD activity. The Mika genotype had the highest GR activity under irrigated conditions; however, its activity decreased considerably under drought stress. The CI 14139 genotype had the lowest GR activity (40.80) and the highest activity (64.23) under irrigated conditions.

Table 4. Percentage values of protein content decrease in tetraploid wheat genotypes exposed to drought stress

	Soluble Protein Content % decrease	
Plant Name	Vegetative	Generative
2529/Kara Kilchik	20.87	38.51
Akbasak	26.19	38.44
Kunduru	40.32	38.88
Sahman	22.03	39.34
Sari Kilcik	8.92	44.53
T. durum Mean	**23.67**	**39.94**
CI 14139	-24.71	52.61
CI 14140	11.06	41.22
2939	-8.45	51.56
2941	6.43	51.37
Mika	-90.67	48.71
T. polonicum Mean	**-21.27**	**49.09**
Australian Pouland	-20.81	52.47
Dandan-i-Shutur	-45.87	45.34
Heath	-54.63	44.53
Meknes	16.23	51.93
Turnadili	5.81	43.46
T. turanicum Mean	**-19.85**	**47.54**

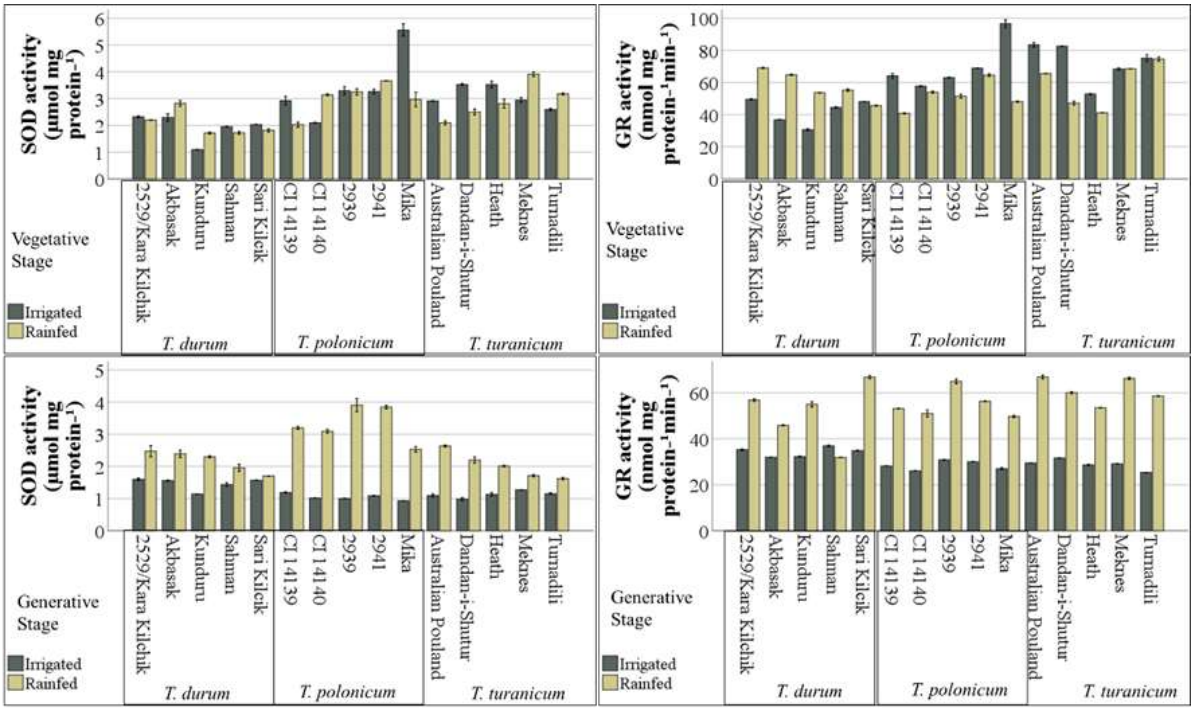

Figure 3. Changes of SOD and GR activities of tetraploid wheat genotypes under drought stress.

During the generative period, both SOD and GR activities were lower than the vegetative period in both conditions. However, the drought response of the genotypes in the generative period resulted in an increase in SOD and GR activities. The mean increase in SOD activity for *T. durum* varieties during the generative period was 30.97%, while the mean increase for *T. polonicum* varieties was the highest at 67.86%. The greatest increase in GR activity was observed in *T. turanicum* varieties, with an average increase of 52.54% (Table 5).

Table 5. Percentage values of increase of SOD and GR activities in tetraploid wheat genotypes exposed to drought stress

	SOD activity %increase		GR activity % increase	
Plant Name	Vegetative	Generative	Vegetative	Generative
2529/Kara Kilchik	-5,55	35,28	28,26	38,01
Akbasak	18,79	34,84	43,11	30,30
Kunduru	36,00	50,35	42,85	41,36
Sahman	-13,54	26,52	19,54	-15,90
Sari Kilcik	-11,84	7,88	-5,57	47,81
T. durum Mean	4,77	30,97	25,64	28,32
CI 14139	-44,57	62,86	-57,44	46,99
CI 14140	33,29	67,08	-6,78	48,85
2939	-1,43	74,31	-22,46	52,43
2941	10,93	71,72	-6,75	46,66
Mika	-87,35	63,31	-100,90	45,66
T. polonicum Mean	-17,83	67,86	-38,86	48,12
Australian Pouland	-38,87	58,39	-27,25	55,92
Dandan-i-Shutur	-40,93	55,20	-74,69	47,40
Heath	-25,45	43,73	-28,26	46,51
Meknes	24,68	25,81	0,16	56,06
Turnadili	18,26	28,83	-0,82	56,80
T. turanicum Mean	-12,46	42,39	-26,17	52,54

APX activities varied between 14.66 µmol mgprotein^{-1} (Kunduru) and 45.31 µmol mgprotein^{-1} (Mika) under irrigated conditions and between 9.12 µmol mgprotein^{-1} (Heath) and 35.54 µmol mgprotein^{-1} (Turnadili) under drought conditions (Figure 4). While there was an increase in *T. durum* ranging from 4.87% to 43.36%, a decrease was observed in three varieties of *T. polonicum* and *T. turanicum*. Especially Dandan-i-Shutur and Heath genotypes exhibited a notable decline in APX activity. During the generative period, APX activities increased exceeding 30% in all genotypes (Table 6). The highest APX activity was observed in the 2529/Kara Kilchik genotype, with a value of 18.23 µmol mgprotein^{-1} under irrigated conditions and 29.41 µmol mgprotein^{-1} under drought conditions. The highest increase was recorded in the Meknes genotype, with 52.55%.

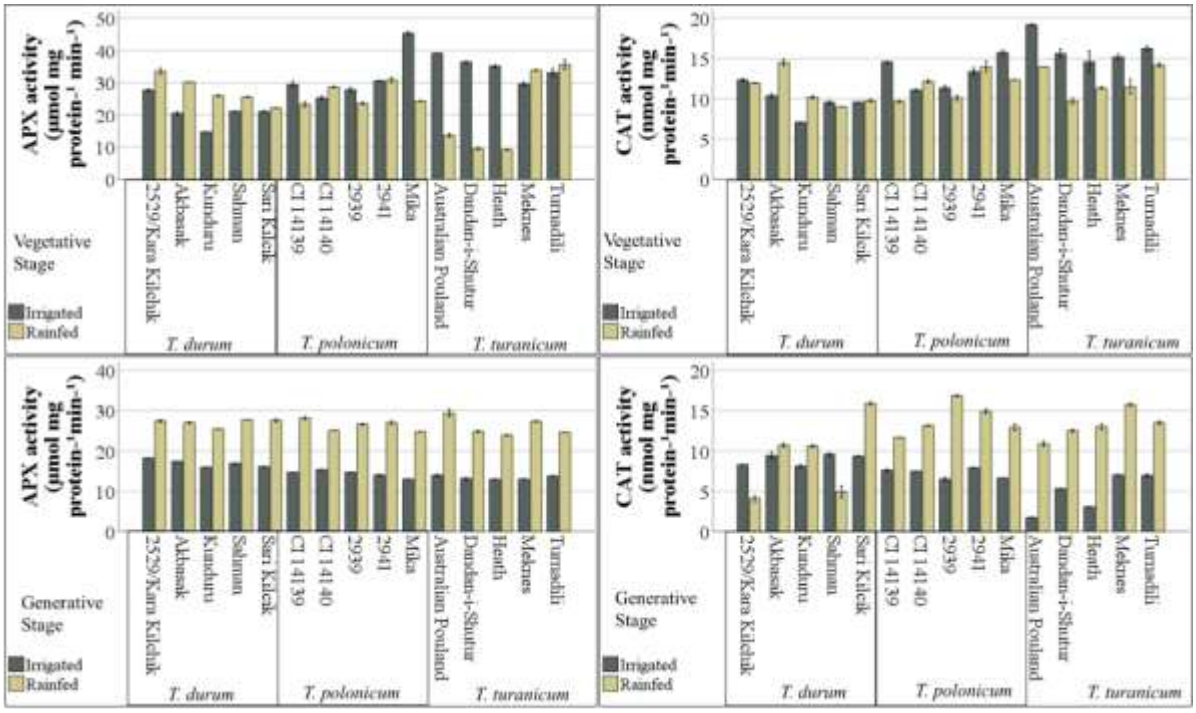

Figure 4. Changes of APX and CAT activities of tetraploid wheat genotypes under drought stress.

The level of CAT activity is greater during the vegetative period than the generative period when plants are irrigated. Indeed, the lowest value observed during the vegetative period is nearly equivalent to the highest value recorded during the generative period. Australian Poluand genotype had the highest (19.18 nmol mgprotein^{-1}) CAT activity in the vegetative period under irrigated conditions and the lowest (1.73 nmol mgprotein^{-1}) CAT activity in the generative period (Figure 4). Under drought conditions, CAT activities ranged from 8.99 nmol mgprotein^{-1} (Sahman) to 14.48 nmol mgprotein^{-1} (Akbasak) in the vegetative period and from 4.07 nmol mgprotein^{-1} (2529/Kara Kilchik) to 16.86 nmol mgprotein^{-1} (2939) in the generative period. While CAT activity decreased in most genotypes in the vegetative period, it decreased in 2529/Kara Kilchik and Sahman genotypes in the generative period (Table 6).

Discussion

The threat to the world's food supply posed by droughts brought on by climate change is set to persist in the years to come. In light of these circumstances, it is of paramount importance to develop new high-yielding cultivars that are well-suited to different geographical locations. Plant breeders should initially select promising germplasm with high genotypic variability to enhance drought tolerance. It is essential to incorporate ancient tetraploid species and old landraces as gene sources, given the declining genetic diversity observed in modern durum wheats. One of the most

effective indicators of drought resistance is the presence of variations in membrane injury parameters and antioxidant enzyme activity. The objective was to identify genotypes with enhanced tolerance by examining the impact of drought stress on electrolyte leakage, H_2O_2 content, and crucial antioxidant enzymes in 15 tetraploid wheat genotypes (comprising five *T. durum*, five *T. polonicum*, and five *T. turanicum*). In addition, the genotypes were evaluated in terms of their resistance to drought during both the vegetative and generative periods.

Table 6. Percentage values of increase of APX and CAT activities in tetraploid wheat genotypes exposed to drought stress

	APX activity %increase		CAT activity % increase	
Plant Name	Vegetative	Generative	Vegetative	Generative
2529/Kara Kilchik	17,37	33,64	-3,25	-103,81
Akbasak	32,22	35,52	28,52	12,00
Kunduru	43,36	37,27	30,41	23,47
Sahman	17,40	38,95	-6,03	-94,83
Sari Kilcik	4,87	41,60	1,96	41,13
T. durum Mean	**23,05**	**37,40**	**10,32**	**-24,41**
CI 14139	-26,55	47,92	-50,76	34,84
CI 14140	11,99	38,78	8,68	43,06
2939	-17,82	44,95	-12,18	61,38
2941	0,93	48,05	4,26	46,95
Mika	-86,43	47,64	-27,66	48,78
T. polonicum Mean	**-23,58**	**45,47**	**-15,53**	**47,00**
Australian Pouland	-187,38	52,47	-37,41	84,08
Dandan-i-Shutur	-284,35	47,21	-60,53	57,55
Heath	-285,60	45,81	-28,82	76,60
Meknes	12,58	52,55	-31,96	55,36
Turnadili	6,85	44,08	-14,54	48,44
T. turanicum Mean	**-147,58**	**48,42**	**-34,65**	**64,41**

One of the principal indicators of the stress response in intact plant cells is electrolyte leakage. This phenomenon is frequently employed as a measure of plant stress tolerance and as a test for stress-induced tissue damage in plants (Maurya, 2020). The occurrence of electrolyte leakage is elevated in plants subjected to stress due to drought. This is caused a considerable increase in the percentage of membrane damage. The present study revealed that the percentage of electrolyte leakage and membrane damage was notably elevated in the vegetative period for the 2529/Kara Kilchik, Akbasak, Meknes, and Turnadili genotypes, as well as in the generative period for the Sahman genotype. Several researches has demonstrated that significant damage and, in some cases, even plant cell death occurs when electrolyte leakage increases by more than 50% (Rolny et al., 2011; Guadagno et al., 2017; Naderi et al.,

2020; Savage et al., 2024). The degree of increase in electrolyte leakage may be indicative of the function of ROS signaling in wheat plant cells, rather than being a direct consequence of actual cell damage. Furthermore, it may demonstrate the direct and indirect impacts of drought stress on proteins and the composition of fatty acids in membranes (Naderi et al., 2020).

The excessive accumulation of H_2O_2 serves as a marker for assessing the extent of oxidative damage that occurs under drought-stress conditions (Huseynova et al., 2015). The genotypes examined in the study also have varying levels of H_2O_2 content under irrigated conditions. Some genotypes have higher H_2O_2 contents under irrigation than under drought. At low concentrations, H_2O_2 functions as a signaling molecule, regulating a range of biological and physiological processes, including photosynthetic functions, the cell cycle, growth and development, and plant responses to biotic and abiotic stresses (Černý et al., 2018; Jahan et al., 2023). It is important to note, however, that as with many other traits, the accumulation of H_2O_2 will also vary according to the genotypes involved. Accordingly, it would be a more logical criterion to consider the extent to which H_2O_2 content increases when the plant is exposed to drought stress. It can be observed that the H_2O_2 accumulations of the 2529/Kara Kilchik, Kunduru, Sahman, Sarı Kilcik, Australian Pouland, Dandan-i-Shutur, Heath, and Meknes genotypes exceed 50% when exposed to drought stress during the vegetative period. During the generative period, a notable increase in H_2O_2 content was observed in the Akbasak, Australian Pouland, Dandan-i-Shutur, and Heath genotypes. In drought-stressed plants, the sustained increase of H_2O_2 did not result in cell damage considering membrane damage by electrolyte leakage. This suggests that H_2O_2 acts as a signal molecule to convey the effect of drought and induce the necessary physiological responses.

Changes are also observed in the amount of soluble proteins in plants under drought stress. Plant proteins are associated with a number of processes, including modifications in plant metabolism and the initiation of defensive responses to stress. A higher protein concentration typically indicates an enhanced capacity to withstand drought stress (Sattar et al., 2020; Hussain et al., 2023). Some genotypes showed increased soluble protein content during the vegetative period. During the generative period, drought stress reduced soluble protein content of all genotypes. The soluble protein content of T. *polonicum* and *T. turanicum* was observed to be lower during the vegetative period than the generative period in irrigated conditions. However, *T. durum* varieties demonstrated a decrease in protein content in response to drought stress during both periods. While some researchers have indicated that drought stress increases the total soluble protein content, others have reported that protein structure is degraded and protein levels are reduced in response to stress (Ahmad et al., 2018; Sattar et al., 2020; Alsamadany et al., 2023; Khalvandi et al., 2024). Fluctuations in

soluble protein content in wheat leaves during drought stress may be influenced by the genotype, the level of stress severity, and the duration of the drought. Furthermore, the simultaneous occurrence of heat and drought stress results in a deterioration of the structural integrity of the protein content (Zulkiffal et al., 2021). The damage caused by stress does not only affect the protein synthesizing machinery, but also accelerates protein hydrolysis due to increased protease activity (Alsamadany et al., 2023). The reduction in soluble protein levels observed in all genotypes during the generative period may be attributed to the impact of elevated temperatures during this phase. A high soluble protein content serves as a protective mechanism for the plant against stress. It can be posited that genotypes exhibiting augmented soluble protein content during the vegetative period and those demonstrating reduced soluble protein loss during the generative period may exhibit superior resilience to drought conditions. Nevertheless, this hypothesis should be corroborated by evidence of increased antioxidant enzyme activity.

Drought-related changes in antioxidant enzyme activity are necessary to preserve the delicate balance between intracellular ROS generation and detoxification, particularly H_2O_2. Genotypes with high antioxidant capacity mitigate damage from oxidative stress and preserve cellular integrity. Plants use SOD, APX, CAT, and GR to eliminate ROS-mediated damage (Ahmadi et al., 2018; Chaouachi et al., 2023). SOD and GR constitute the initial line of defense against oxidative stress. During the vegetative period, there was a decrease in SOD activity in some genotypes, while in others there was an increase. During the generative period, all genotypes exhibited an increase in SOD activity. The GR activity of *T. turanicum* and *T. polonicum* exhibited a decline during the vegetative period, whereas it demonstrated an increase in all genotypes except Sahman during the generative period. The alterations in antioxidant enzyme activities in response to drought stress depend on plant species, cultivar, level of stress, and duration of exposure. In the initial stages of drought, plants exhibit a rapid surge in CAT and APX activities as they accumulate H_2O_2, which is produced following the dismutation of O_2^− by SOD. The presence of elevated CAT and APX activities accompanied by diminished H_2O_2 accumulation in plants suggests the existence of an enhanced redox defense potential against drought stress (Rajput et al., 2021). The APX activity increased in response to drought stress during the vegetative period in all *T. durum* varieties, including CI 14140 and 2941 derived from *T. polonicum*, as well as Meknes and Turnadili derived from *T. turanicum*. The activity of CAT increased in Akbasak, Kunduru, Sari Kilcik, CI 14140, and 2941. The activities of both APX and CAT were observed to increase in all genotypes during the generative period. A number of researchers have reported that low electrolyte leakage and H_2O_2 accumulation, along with increased and high levels of antioxidant enzymes, are characteristics of tolerant genotypes. These characteristics have been proposed as an

effective selection criterion (Naderi et al., 2020; Chaouachi et al., 2023; 2024).

Conclusion

The findings of this study indicate that drought stress has a detrimental impact on certain tetraploid wheat varieties, with the severity of these effects varying according to the genotype and the plant growth period. During the vegetative period, the Akbasak, Kunduru, CI 14139, and 2939 genotypes were identified as tolerant based on their low electrolyte leakage and H_2O_2 accumulation, coupled with high soluble protein content and antioxidant enzyme activity. In the generative period, Kunduru, Mika, Australian Pouland, and Turnadili can be considered tolerant genotypes. The fluctuating results of genotypes in the vegetative period may indicate that drought sensitivity can be better distinguished. In contrast, the results obtained in the generative period were closer in the genotypes due to the prolongation of drought stress and adaptation to drought. Among the compared subspecies, it was concluded that tolerance was higher in *T. polonicum*. In contrast, *T. durum* was more stable within the subspecies.

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Oral Presentation

RELATIONSHIP BETWEEN EMBRYO QUALITY AND BLOOD VALUE IN CATTLE

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Abstract

The purpose of this study; The aim of this study is to determine the relationship between corpus luteum, number of transferable embryos, embryo quality and hematological values in Holstein cattle undergoing superovulation. As a donor in the study, 10 head of Holstein breed cattle, 2.5-4 years old and 60-90 days postpartum, were used. Progesterone-based (9-day duration) estrus synchronization protocol was applied to selected donors. Starting from the 7th day of progesterone administration, FSH was injected in decreasing doses at 12-hour intervals for 4 days. On the 9th day of progesterone application, PGF2α was applied in the morning and progesterone was removed from the vagina in the evening of the same day. On the 11th day, artificial insemination was performed on the donors every 12 hours. Artificial insemination day: Blood samples were taken from all donors to determine hematological values. Uterine flushing was performed on the 7th day after artificial insemination. The cattle were divided into two groups according to the number and quality of embryos obtained as a result of uterine washings and the number of corpus luteum on the ovary. The first group was determined as the group in which 6 or more corpus luteum and quality embryos were obtained. The second group was grouped as the number of 3 or fewer quality embryos, degenerated embryos and corpus luteum. In the group where a higher number of quality embryos and corpus luteum were obtained; WBC, LEN, MON, GRAN, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, PDW, PCT, Embryo Number, CL Number respectively; 9.60, 3.80, 0.90, 4.90, 5.96, 10.28, 29.20, 48.84, 17.24, 35.48, 16.00, 351.80, 6, 20, 16.70, 0.22, 7.60, 8. In the group in which a lower number of quality embryos and corpus luteum were obtained; WBC, LEN, MON, GRAN, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, PDW, PCT, Embryo Number, CL Number respectively; 6.84, 2.20, 0.60, 4.16, 6.10, 9.84 28.16, 46.30, 16.10, 34.90, 17.68, 509.00, 5.84 , was found to be 16.08, 0.30, 1.40, 2.40. As a result, it was concluded that there may be a relationship between hematological values and the number of transferable embryos, embryo quality and the number of unfertilized embryos in Holstein donors.

Keywords: Cattle, Hematological value, Embryo quality

INTRODUCTION

Improving the reproductive performance of farm animals is a high priority for breeders worldwide.

Embryo transfer is the process of transferring embryos obtained from the uterus of a donor animal in vivo or from ovaries collected from a slaughterhouse in vitro (Karaşahin, 2017). Embryo transfer (ET) is the process of transferring embryos obtained from donor animals (in vivo) or from laboratory conditions (in vitro) to carrier animals (Sağırkaya, 2009). Embryo production and transfer is an advanced reproductive technique that has been widely used in developed countries, especially in cattle and buffalo, for many years (Karaşahin, 2021). Today, in vivo and in vitro embryo production techniques have become routine, and research and applications are now being conducted on advanced reproductive techniques and methods in embryos.

Embryo transfer; It is a method that will meet the need for dairy or beef cattle with the desired characteristics and high quality in a short time. Another strength is that it is an alternative option for solving the infertility problem in cattle (Karasahin, 2017). Bovine embryo transfer technology involves the selection and management of donor and recipient animals both physically and pharmacologically and the collection and transfer of embryos in a short period after estrus (Mapletoft, 2002). While 1 oocyte is normally expelled from the ovaries as a result of a single follicle ovulation, in the in vivo embryo production technique, hormones such as FSH, PMSG and HcG are used to trigger the development of a greater number of follicles and therefore oocytes. Embryo production and transfer technology is one of the advanced reproductive techniques and although it has a very complex application process, the benefits it provides in animal breeding increase the use of this technology. The most commonly used method for in vivo embryo production is to perform superovulation using FSH hormone. Genetic selection and reproductive efficiency play a key role in the success of dairy and beef farms (Baruselli et al. 2015). Embryo transfer, one of the reproductive biotechnology methods, is widely used in beef and dairy cattle to rapidly increase the number of animals with high productivity and superior genetic capacity (Mapletoft 2018). The most commonly used method for in vivo embryo production is superovulation using FSH hormone. In cattle, the main purpose of superovulation application is to obtain the maximum number of transferable embryos (Bo and Mapletoft 2014). Over the past 40 years, many studies have been conducted to improve the superovulation regime in embryo transfer programs in cattle (Bo and Mapletoft 2014, Moore and Hasler 2017). However, studies have shown that superovulation response and embryo yield in cattle vary depending on many reasons. The difference in the number of

superovulation responses and quality embryos between donors is the most important problem in the spread of cattle embryo transfer programs (Abdel Aziz et al. 2017).

Factors affecting the superovulation response can be classified as factors affecting the donor's ovulatory response, factors affecting fertilization and embryo viability, and factors related to the programming and management of animals. Another classification that affects the number and quality of embryos to be obtained from donor cattle is that age, breed, presence of dominant follicles, subclinical infections, lactation, repeated superovulation applications, abnormal sperm transport, ovulation and hormone levels are effective in the formation of differences in the superovulation response, which is the most important step of embryo transfer technology. In addition to these factors; There may be differences in the superovulation response depending on the type and dose of gonadotropin hormone used (FSH or PMSG), duration of administration of gonadotropin hormones, route and time of administration, nutrition and season (Baruselli et al., 2006; Taşdemir et al., 2016).

MATERIALS AND METHODS

In the study, 10 Holstein cattle aged 2.5-4 years, 60-90 days postpartum were used as donors. A progesterone-based (9-day) estrus synchronization protocol was applied to the selected donors. A progesterone-based synchronization protocol was applied to the selected donors. For this purpose, first, a progesterone source (1.38 g, Eazi-Breed CIDR, Zoetis, USA) was placed intravaginally and at the same time, a GnRH analog (10 µg, Buserelin Acetate, Receptal, MSD, USA) was injected intramuscularly (day 0). While the synchronization protocol was continuing, FSH (Stimufol, Reprobiol SPRL, Belgium) injection was started for superovulation 7 days after the placement of the progesterone source. FSH was administered intramuscularly for 4 days and at decreasing doses (100-100, 75-75, 50-50, 25-25 µg) at 12-hour intervals, totaling 500 µg. PGF2α was administered intramuscularly 9 days after progesterone administration in the morning and progesterone was removed from the vagina in the evening of the same day. Artificial insemination was performed on donors 2 days after PGF2α administration, in the morning and evening of the 11th day of the synchronization and superovulation protocol. Uterus flushing was performed on the 7th day after artificial insemination. For this purpose, first, donors underwent rectal and ultrasonographic examinations to determine the number of corpus luteum in both ovaries. Donors with a total corpus luteum count of ≥3 in both ovaries were evaluated as superovulation positive. Donors responding to superovulation were given upper epidural anesthesia (5-8 ml, Lidocaine HCl, Adokain, Sanovel, Turkey). Then, a balloon catheter (2-Way Foley Catheter, Silicone, 16-20 inch) was placed in the uterine horn. The uterus washed several times with lactated Ringer's (%2 calf serum + 200 mg kanamycin) and possible embryos were collected in a filter (EmCon Filter, 75 micron). After the washing process, the filter was taken to the laboratory and examined in petri

dishes. The developmental stages and quality of the embryos obtained were evaluated according to IETS criteria (Bo and Mapletoft, 2013). Hematological values of donor animals were performed in the blood counting device at the Aksaray University Embryo Transfer Center.

RESULTS AND DISCUSSION

In blood samples taken from donor cows on the day of embryo collection after synchronization and superovulation application, in the group in which a higher number of good quality embryos and corpus luteum were obtained; WBC, LEN, MON, GRAN, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, PDW, PCT, Embryo Number, CL Number were; 9.60, 3.80, 0.90, 4.90, 5.96, 10.28, 29.20, 48.84, 17.24, 35.48, 16.00, 351.80, 6.20, 16.70, 0.22, 7.60, 8. In the group in which a lower number of good quality embryos and corpus luteum were obtained; WBC, LEN, MON, GRAN, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, PDW, PCT, Embryo Number, CL Number were found as 6.84, 2.20, 0.60, 4.16, 6.10, 9.84 28.16, 46.30, 16.10, 34.90, 17.68, 509.00, 5.84, 16.08, 0.30, 1.40, 2.40, respectively. It was observed that leukocyte counts were higher in the group in which a higher number of quality embryos and corpus luteum were obtained than the other group. HGB, HCT, MCV, MCH, MCHC, PLT, MPV, PDW values were found to be higher in group 1 than in group 2. RBC, RDW and PCT values were found to be higher in Group 2 than in Group 1. Both the number and quality of embryos obtained in Group 1 were higher than those obtained in Group 2. Corpus luteum numbers were also higher in Group 1.

CONCLUSION

As a result, it was concluded that the hematological values of donors who underwent superovulation are closely related to the embryo yield and quality obtained. It is also thought that measuring the hematological values during the determination of donors may be useful in terms of the answers to be obtained later.

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Oral Presentation

THE EFFECT OF DIFFERENT DURATIONS OF PROGESTERONE-BASED ESTRUS SYNCHRONIZATION ON PREGNANCY RATES IN GURCU GOATS DURING THE BREEDING SEASON: A REVIEW OF STUDIES FROM 2016 TO 2024

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Abstract

The Gurcu goat, a breed at risk of extinction, is an important local genetic resource raised in the North Anatolian region. This study aims to evaluate the effect of progesterone-based estrus synchronization of different durations on pregnancy rates in Gurcu goats during the breeding season. For this purpose, data on pregnancy rates were compiled from ten estrus synchronization studies conducted between 2016 and 2024. A total of 326 clinically healthy Gurcu goats, each of which had given birth at least once, were used in all studies. The first reports on estrus synchronization in Gurcu goats began in 2016, and research in this field continues to the present day. In this conference paper, studies involving the application of intravaginal progesterone for 7, 9, and 11 days (using vaginal sponges or controlled internal drug release devices) for estrus synchronization were considered. The data obtained from these studies were statistically analyzed using the SPSS software. In studies on progesterone-supported estrus synchronization in Gurcu goats, the pregnancy rates for 7, 9, and 11-day protocols were 79.4% (65.7%-92.0%), 69.4% (68.8%-70.0%), and 72.8% (66.7%-77.5%), respectively, with no statistically significant differences observed among the groups ($P > 0.05$). The overall pregnancy rate across all studies was determined to be 74.2%. In conclusion, given that the pregnancy rates achieved with 7, 9, and 11-day progesterone-supported estrus synchronization protocols were similar, shorter intravaginal applications may be preferable for Gurcu goats.

Keywords: Gurcu goat, pregnancy, progesterone, synchronization

1. Introduction

Goats are valuable livestock that provide a variety of economically beneficial animal products, such as meat, leather, milk, and fiber, while also serving as an important source of nutrition. Additionally, goats contribute to pasture productivity by efficiently utilizing parts of the rangeland that cattle and sheep cannot graze (Akmaz & Çağlayan, 2021; Ergün et al., 2001). One of the first domesticated livestock species, modern goat breeds have evolved with the influence of wild goats such as *Capra aegagrus*, *Capra falconeri*, and *Capra prisca*. In classifying goats, two main criteria are generally considered. The first is based on their production characteristics, and the second is according to the countries where they were developed (Akmaz & Çağlayan, 2021). Compared to other regions in Türkiye, goat farming is observed to be more prevalent in the Aegean, Mediterranean, and Southeastern regions. The primary factors contributing to this concentration include the economic and social structures of farmers engaged in livestock production in these areas, traditional livestock farming preferences, and the geographical characteristics of the regions (Kuru & Boğa Kuru, 2020; Özdemir & Keskin, 2018).

The Gurcu goat is one of the local breeds raised in the Northern Anatolia region. This indigenous breed, originally from the Caucasus, is also referred to by local communities as the Tbilisi goat or Caucasian goat (Kuru et al., 2017b; Kuru & Boğa Kuru, 2020). Today, this local breed, whose population has significantly declined, is being protected through government-supported community-based breeding projects in an attempt to prevent further reduction in numbers. However, despite these efforts, the breed remains at risk of extinction as it is only maintained by a limited number of breeders (Boğa Kuru et al., 2024a; Kuru & Boğa Kuru, 2020). During the breeding season, a significant proportion of Gurcu goats exhibit estrus and achieve successful pregnancies. The gestation period in Gurcu goats is approximately 150 days, and reproductive issues are generally uncommon. However, dystocia can occasionally occur, leading to losses of kids. Young breeding Gurcu does usually give birth to single kids, and parturition predominantly takes place during daylight hours. The multiple birth rate in Gurcu goats is around 45.5%, although this can vary depending on factors such as nutrition and breeding age. Furthermore, the proportion of male kids born tends to be higher in this breed (Kuru et al., 2017b).

Various hormonal and management techniques have been developed to enhance reproductive efficiency in livestock. These protocols involve the careful application of hormones to regulate the reproductive cycle and overcome seasonal constraints (Abecia et al., 2012; Kuru et al., 2022a; 2020b; 2018a; 2017c). Sheep and goats are seasonal polyestrous animals, and ovulation activity is significantly suppressed outside the breeding season (Kuru et al., 2020a; 2018a). In these species, hormonal manipulations, such as progestagens and equine chorionic gonadotropin (eCG), are

commonly used to stimulate ovulation and estrous activity outside the breeding season (Abecia et al., 2012). These methods are crucial for ensuring year-round reproductive efficiency and optimizing herd management. The literature indicates that hormonal protocols offer significant benefits, including synchronization of estrus, increased pregnancy rates, and reduced intervals between births (Gordon, 1983, 1997).

In small ruminants, progesterone and its analogs are widely employed to effectively induce and synchronize estrus (Boğa Kuru et al., 2024b; Kuru et al., 2017a; 2018a; 2018b; Taçyıldız et al., 2023). Progesterone-impregnated sponges are typically inserted intravaginally for periods ranging from 5 to 14 days, depending on the specific protocol used. Estrus is generally detected approximately 30 to 37 hours following the removal of the progesterone-impregnated sponge, which facilitates a predictable and controlled onset of estrus (Kuru et al., 2020a, 2022a; 2017d; Silva et al., 2021). For optimal synchronization outcomes, it is essential to combine progesterone treatment with eCG, a hormone that exhibits both follicle-stimulating and luteinizing hormone-like activities. The administration of eCG enhances the effectiveness of progesterone therapy by stimulating follicular development and promoting ovulation. This combination ensures that adequate concentrations of gonadotropins are present to initiate pre-ovulatory events, thereby maximizing the efficiency of the synchronization process (Abecia et al., 2012; Kuru, 2022b, 2022a; Kuru et al., 2022a; Kuru & Boğa Kuru, 2023). The integration of these hormonal treatments has been shown to improve estrus synchronization and overall reproductive performance in small ruminants, contributing to more predictable and manageable breeding outcomes. Such advancements are crucial for optimizing reproductive efficiency and managing breeding programs effectively (Abecia et al., 2012; Gonzalez-Bulnes et al., 2020; Kuru, 2022b, 2022a; Kuru & Boğa Kuru, 2023; Martinez-Ros et al., 2019).

The initial investigations into estrus synchronization in Gurcu goats commenced in 2016, and research efforts in this domain continue to progress. This report will compile data derived from ten distinct estrus synchronization studies conducted between 2016 and 2024. The primary objective of this study is to assess the impact of various progesterone-based estrus synchronization protocols, implemented for different durations during the breeding season, on pregnancy rates in Gurcu goats. By analyzing these protocols, the study aims to elucidate their effectiveness and optimize reproductive management practices in Gurcu goat populations. The findings are anticipated to contribute significantly to improving reproductive efficiency and informing future research directions in this field.

2. Estrus Synchronization Studies in Gurcu Goats from 2016 to 2024

2.1. Location

The studies were conducted at the Kafkas University Faculty of Veterinary Medicine Education, Research, and Practice Farm. Situated at an elevation of 1751 meters above

sea level, the farm is located at coordinates 40°34'23"N latitude and 43°02'27"E longitude, within the Kars province near the Armenian border. Kars is situated in the northeastern region of Anatolia and experiences a continental climate.

2.2. Animals

In the studies, 326 healthy Gurcu goats were selected. These goats were between 3 and 5 years of age, weighed between 40 and 50 kg, and had body condition scores ranging from 2.5 to 3.5 (where 1 = emaciated and 5 = obese). All selected goats had previously given birth at least once and had successfully completed the postpartum period. Additionally, Gurcu bucks were used for mating during estrus detection. The selected goats and bucks received routine internal and external parasite treatments, including 250 mg of oxyfenbendazole, 750 mg of oxyclozanide, 10 mg of ivermectin, and 100 mg of closantel. They were also vaccinated at least 20 days prior to synchronization.

2.3. Estrus Synchronization

Estrus synchronization procedures have been conducted throughout the breeding season using sponges containing medroxyprogesterone acetate or Controlled Internal Drug Release (CIDR) devices, along with eCG and a combination of d-cloprostenol or dinoprost. On Day 0, a progesterone-impregnated sponge or CIDR was inserted into the vagina. Two days prior to the completion of progesterone treatment, eCG and d-cloprostenol or dinoprost were administered via intramuscular injection. Progesterone treatment was terminated after 7, 9, or 11 days, and the progesterone-releasing device was removed from the vagina. Subsequently, the goats were exposed to a buck 12-24 hours after removal of the device.

2.4. Pregnancy Diagnosis

Pregnancy diagnosis in goats was conducted using transrectal ultrasonography (5–7.5 MHz) approximately 30-35 days post-mating. The presence of pregnancy was confirmed through the visualization of the embryo (Kuru et al., 2018c). To ensure the accuracy of the pregnancy diagnosis, a follow-up examination was performed 45-60 days after mating. This secondary assessment provided additional confirmation of pregnancy status, thereby enhancing the reliability of the initial diagnosis.

2.5. Statistical analysis

Statistical differences in pregnancy rates obtained on different days were determined using the chi-square test. Statistical analyses were conducted using GraphPad Prism (Version 9.5.1, GraphPad Software Inc., USA). A significance level of $P < 0.05$ was established for group comparisons.

3. Results from the Studies Conducted Between 2016 and 2024

Table 1 presents the details of the protocols employed, the number of animals used, and the pregnancy rates achieved in the studies. For the progesterone-supported

estrus synchronization protocols in Gurcu goats, pregnancy rates varied based on the duration of treatment: 65.7% to 92.0% for the 7-day protocol, 68.8% to 70.0% for the 9-day protocol, and 66.7% to 77.5% for the 11-day protocol (Table 1). During the breeding season, the total pregnancy rates obtained with the 7-day, 9-day, and 11-day progesterone-based estrus synchronization protocols were 79.4%, 69.4%, and 72.8%, respectively. Statistical analysis revealed that these differences were not significant (Figure 1, $P > 0.05$). Overall, the cumulative pregnancy rate across all estrus synchronization studies was determined to be 74.2% (Table 1 and Figure 1). These results suggest that while individual protocol durations yielded varying success rates, the overall effectiveness of the progesterone-supported estrus synchronization protocols in improving pregnancy rates was consistent across different durations.

Table 1: Pregnancy rates achieved in Gurcu goats with progesterone-supported estrus synchronization protocols of different durations

Protocols	Pregnant / Total	Pregnancy rate (%)	Reference
11 days CIDR + PGF2α and eCG on the 9th day	31/40	77.5	(Kuru et al., 2016b)
11 days CIDR + PGF2α and eCG on the 9th day	30/40	75.0	(Kuru et al., 2016a)
11 days sponge + PGF2α and eCG on the 9th day	20/30	66.7	(Kuru et al., 2018c)
11 days CIDR + PGF2α and eCG on the 9th day	28/40	70.0	(Kuru et al., 2020c)
11 days sponge + PGF2α and eCG on the 9th day	9/12	75.0	(Kuru et al., 2022b)
9 days sponge + PGF2α and eCG on the 7th day	22/32	68.8	(Kuru et al., 2022c)
9 days sponge + PGF2α and eCG on the 7th day	21/30	70.0	(Boğa Kuru et al., 2024a)
7 days sponge + PGF2α and eCG on the 5th day	10/12	83.3	(Kuru et al., 2022b)
7 days sponge + PGF2α and eCG on the 5th day	23/25	92.0	(Kuru et al., 2023)
7 days sponge + PGF2α and eCG on the 5th day	25/30	83.3	(Boğa Kuru et al., 2023)
7 days sponge + PGF2α and eCG on the 5th day	23/35	65.7	(Kuru et al., 2024)
Total	242/326	74.2	-

CIDR: Controlled internal drug release devices, eCG: equine chorionic gonadotropin

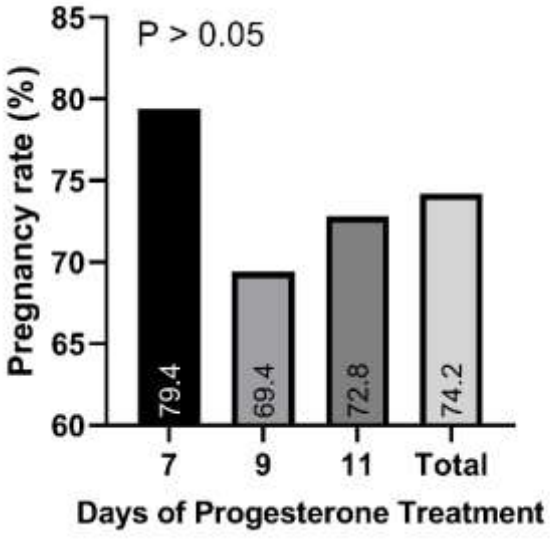

Figure 1: Pregnancy rates obtained with progesterone-supported estrus synchronization protocols of different durations in Gurcu goats

4. Conclusion

The results indicate that progesterone-based estrus synchronization protocols of varying durations can be effective in achieving high pregnancy rates in Gurcu goats. However, the duration of the protocol does not significantly impact the success rates. Consequently, the selection of protocol duration may be more influenced by practical considerations rather than differences in pregnancy outcomes. Additionally, shorter-duration protocols might be preferred to minimize stress associated with prolonged intravaginal treatments. Future complex studies could further elucidate these relationships and provide a more comprehensive understanding of the factors affecting synchronization efficacy and reproductive performance in Gurcu goats.

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Oral Presentation

INVESTIGATION OF NUCLEOLUS ORGANIZER (AGNOR) REGIONS IN THE CEREBELLUM OF CHICKENS*

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Abstract:

In this study, the numbers, localizations, sizes and areas of silver-stained nucleolus organizer regions (AgNORs) in the nuclei of Purkinje neurons of laying Nick Chick chicken breed and broiler Hubbard line were determined during the embryonic period in both breeds and at the 6th post-incubation period in broiler chickens. By being determined up to 32 weeks; changes in these parameters were determined. Comparisons were made between periods in both breeds, between sexes and between races in layers in each period. The cerebellum Purkinje cells of broilers had more nuclei in the 11th and 14th days of hatching, and no difference was observed between broiler and layer hens in terms of nücleus area in other periods. The nücleus diameter is also greater in broilers on the 14th day of hatching. AgNOR number and AgNOR diameter of broilers and layers do not change in any period. Broilers have higher AgNOR area than layers on the 11th day of hatching. Looking at the data obtained in this study, it was concluded that a direct correlation could not be established between growth and egg production and AgNOR parameters of Purkinje neurons; It was concluded that it would be beneficial to carry out studies including AgNOR parameters together, as well as the yield records of animals, especially in the period after hatching.

Keywords: AgNOR, Broiler, Nucleus, Nucleolus, Layer Chickens.

** This study is summarized from a part of his doctoral thesis." AgNOR, Broiler, Nucleus, Nucleolus, Layer Chickens. Supported by Harran University Research Fund.*

Introduction

Nucleoli are nuclear regions where the precursors of ribosomal subunits are biosynthesized. rRNA genes (rDNA) are carried on specific chromosomes in different organisms. The parts of these chromosomes carrying rRNA genes gather in specific regions within the nucleus during interphase, forming darkly stained regions called nucleoli (Goodpasture et al. 1976, Alberts et al. 1989, Fischer et al. 1991, Schwarzacher and Wachtler 1993). Therefore, the regions of DNA carrying rRNA genes and forming the nucleolus are called nucleolus organizer regions (NOR).

The structural details and shapes of nucleoli can vary greatly in different cells. In cells that synthesize ribosomes at a high rate, the nucleoli are large and complex. In cells with low activity, the nucleoli are usually small and simpler. In many types of nucleoli, three main structural components can be seen with the help of electron microscopy (Jordan 1979). These are the fibrillar center (FC), the diffuse fibrillar component (DFC), and the granular component (GC). FCs stain well with silver salts, depending on the method used (Bourgeois et al. 1979, Hernandez-Verdon et al. 1980, Ellinger and Wachtler 1980). DFCs also show affinity to silver salts under certain conditions and provide good contrast (Jordan 1979). Because of the high concentration of ribonucleoproteins (RNPs) in GCs, GCs stain strongly with basic dyes provided that the pH is carefully controlled (Pischinger 1926). Specific nucleolar proteins, many of which have enzyme properties, are found in the nucleolus. The majority of these specific nucleolar proteins, which react positively with silver, are found attached to NORs during mitosis and are responsible for the silver staining of NORs in mitotic chromosomes. These proteins are also called AgNOR proteins. AgNORs contain C23, B23, AgNOR-protein (Hubbell et al. 1979) and RP I nucleolar proteins (Hoyo et al. 1993, Lischwe et al. 1979, Williams et al. 1982, Ochs et al. 1983).

The amount of silver positive proteins in a cell, which play an important role in rDNA transcription and ribosomal biogenesis, is a good indicator of the protein synthesis activity of the cell, since it is related to ribosome synthesis in the cell. There is a common strong opinion that there are significant differences between the numbers of active NORs in the genomes of cells of different organisms or different cells of an organism, and that the numbers of NORs vary according to the protein synthesis needs of the cell and environmental conditions (Goodpasture et al. 1976, Mikelsaar et al. 1977, Alberts et al. 1989).

There is no comparative study on the changes in the nucleus diameter, nucleus area, AgNOR number, AgNOR diameter and AgNOR area of each Purkinje cell in the cerebellum tissue during the embryonic period and after hatching in broiler and layer chickens. In this study, the areas, sizes, localizations and numbers of AgNORs in the

nuclei of Purkinje neurons of Nick Chick layer chicken breed and Hubbard line broiler chickens were determined during the embryonic period in both chicken breeds and up to 6 weeks after hatching in broiler chickens and 32 weeks in layers; and the changes in these parameters were determined. Comparisons were made between male and female layer chickens during the rearing period after hatching

Materials and Methods

Incubation Procedures: All eggs were weighed with a scale (Sartorius, PT 120) before incubation. Eggs were disinfected by fumigation for twenty minutes. Incubation procedures were carried out in the incubator at the Department of Histology and Embryology, Faculty of Veterinary Medicine, Selcuk University (Gostyn, Poland) under optimal conditions, turning once every 2 hours. Embryonic development was observed in the eggs examined every other day.

Collection and Processing of Tissue Samples: For the investigation of AgNORs in the embryonic stage, 10 eggs from each group were opened on the 11th, 14th and 17th days of incubation. The developmental stages of the embryos were determined according to the Hamburger Hamilton scale (Hamburger and Hamilton 1951) and the embryos were weighed. For the investigation of AgNORs, the nervous system organ cerebellum tissues were taken from the embryos. After the incubation period, cerebellum tissue samples were taken from 10 animals from broilers on the first day of hatching and at the end of the 1st, 4th and 6th weeks following the hatching. Cerebellum tissues were taken from layers on the 11th, 14th and 17th days of incubation, on the day of hatching and at the end of the 6th, 18th and 32nd weeks following hatching.

The hatched broiler and layer chicks were placed in separate compartments and fed under optimal conditions throughout the study. Broilers were given standard feed, layers were fed chick feed between weeks 0-8, and chicken grower feed between weeks 8-18. Since egg production started from these weeks, they were fed with layer feed and continued until week 32.

The tissue samples taken for our study were firstly fixed in 10% buffered (0.1 M) formalin saline (pH: 7.4). Then, the tissues were dehydrated by passing through an alcohol series and blocked in paraffin after passing through a xylene series and polishing. Sections taken from the paraffin blocks at 4 µm thickness were stained with the silver staining method (Ploton et al., 1983, Korek et al., 1991) for the investigation of AgNORs.

Preparation of AgNOR Staining Solutions: Gelatin-Formic acid solution was prepared by dissolving 2 g of gelatin (Merck) in 100 ml of 1% formic acid (Merck). This process was carried out by heating the solution in an incubator at 58-60°C to ensure complete dissolution of the gelatin in formic acid. Then, this solution was

cooled to room temperature. Silver Nitrate solution was prepared by dissolving 50 g of crystalline silver nitrate (Botafarma Laboratory, Ankara, Turkey) in 100 ml of distilled water.

Staining of Sections with AgNOR Staining Method: Fresh staining solution was prepared by mixing Gelatin Formic acid solution and silver nitrate solution prepared just before use. Therefore; the staining solution was prepared by mixing one volume of gelatin formic acid solution and two volumes of 50% silver nitrate solution. Sections were kept in the freshly prepared staining solution just before use, at 20^{o} C room temperature and in the dark for 30 minutes. At the end of the period, sections were washed three times in distilled water. Then, sections were dehydrated, transparent and covered with Entellan (Merck).

Examining the Prepared Preparations and Analyzing the Recorded Images with the Image Analysis System: The preparations were examined with a light microscope (Nikon Eclipse, E-400) at X40 and X100 magnifications, and digital images of the required areas were recorded with the help of a digital camera (Nikon DS Camera Control Unit DS-L1 with DS Camera Head DS-5M) attached to the same microscope. The evaluation of the images obtained was carried out using an image analysis program (BS200 PRO).

The nuclear diameter, nuclear area, AgNOR number, AgNOR diameter and AgNOR area of each Purkinje cell were measured. From the obtained data, AgNOR area/nuclear area ratios (relative AgNOR area) were calculated. The results were analyzed with statistical methods and the significance levels of the differences between the examined parameters of two different chicken breeds were determined.

Statistical Analyses: The obtained data were analyzed on the computer with the help of the Standard statistics program (SPSS 10.0) using statistical methods (one-way analysis of variance -ANOVA- and Tukey test). Data obtained from the cerebellum tissues of a race at different periods were analyzed with the Tukey test. The analysis of differences between races and between different genders of a race was evaluated with the t-test. $P<0.05$ was considered statistically significant.

Results

Results Obtained from the Cerebellum of Broilers: The large nuclei of Purkinje neurons in the cerebellum were oval-round in shape and were mostly localized in the middle of the cell body. The shape and localization of AgNORs, which varied in number from 1 to 3, were very similar to those of pyramidal neurons in the deep cortical region of the cerebral hemispheres (Figure 1).

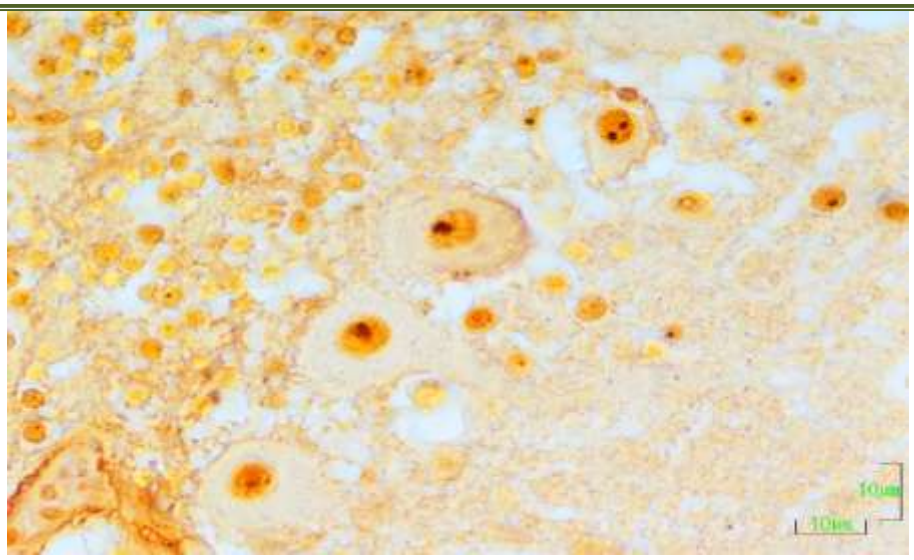

Figure 1. AgNORs as brown-black spots in the nuclei of Purkinje neurons in the cerebellum of an eighteen-week-old broiler. AgNOR silver staining, Magnification line: 10 µm.

The mean nuclear area was quite close to each other on the 11th and 14th days of incubation (P>0.05). The mean nuclear area increased on the 17th day of incubation and reached the highest value (11.7 µm²) on the day of hatching. The mean nuclear area value, which decreased significantly (P<0.05) in the 1st week of post-hatching, continued to increase until the 6th week. The nuclear diameter was significantly (P<0.05) lower than in the other periods on the 11th, 14th and 17th days of incubation. The nuclear diameter, which increased significantly (P<0.05) on the day of hatching, did not change in the following periods. The mean AgNOR numbers had similar values throughout the examined periods. The mean AgNOR area and AgNOR diameter values were significantly (P<0.05) lower than in the other periods on the 11th and 14th days of incubation. The ratio of AgNOR area within the nuclear area was at the highest level (17.38%) in the 1st week of the post-hatching period, while it gradually decreased in the following periods (Table 1).

Table 1. Results of statistical analysis of values determined in Purkinje neurons in the cerebellum of broilers in different periods.

Parameter n=100	Periods						
	Incubation period			Post-Incubation period			
	Day 11 $\bar{X}$ ±SE	Day 14 $\bar{X}$ ±SE	Day 17 $\bar{X}$ ±SE	Exit Day $\bar{X}$ ±SE	1.th week $\bar{X}$ ±SE	4.th week $\bar{X}$ ±SE	6.th week $\bar{X}$ ±SE
Nucleus area(µm²)	3.80±0.22^{d}	3.54±0.26^{d}	6.07±0.45^{c}	11.7±0.80^{a}	9.03±0.46^{b}	10.5±0.56^{ab}	11.3±0.67^{a}
Nucleus diameter (µm)	2.74±0.13^{b}	2.55±0.09^{b}	3.24±0.13^{b}	4.27±0.34^{a}	4.17±0.28^{a}	4.19±0.11^{a}	4.39±0.10^{a}
Number of AgNOR	1.40±0.16^{a}	1.62±0.18^{a}	1.53±0.14^{a}	1.66±0.23^{a}	1.41±0.19^{a}	1.33±0.11^{a}	1.62±0.32^{a}
AgNOR area (µm2)	0.55±0.07^{b}	0.43±0.05^{b}	0.77±0.09^{ab}	1.52±0.63^{a}	1.57±0.14^{a}	1.15±0.10^{ab}	1.21±0.19^{ab}
Ag NOR diameter (µm)	1.05±0.07^{bc}	0.95±0.05^{c}	1.32±0.08^{abc}	1.58±0.27^{ab}	1.61±0.12^{ab}	1.74±0.13^{a}	1.76±0.16^{a}
The ratio of AgNOR area to the nucleus area (%)	14.47	12.14	12.68	12.99	17.38	10.95	10.70

a-d: Differences between values with different letters in the same row are statistically significant (P<0.05).

Results Obtained from the Cerebellum of Layer Hens: Purkinje neurons had a round nucleus centrally located in the neuron body and the number of AgNORs varied between 1 and 4. The shape and localization of AgNORs were very similar to those of broilers.

The mean nuclear area and nuclear diameter values gradually increased from the 11th day of incubation to the 18th week of post-hatching and decreased after this period. The mean AgNOR number had the highest value (2.32 pieces) at the 32nd week of hatching. The AgNOR area and AgNOR diameter, which had the lowest values at the 11th and 14th days of hatching, had the highest values at the 6th week of post-hatching. The ratio of AgNOR area within the nuclear area was at the highest level (17.44%) at the day of hatching, and gradually decreased in the following periods and decreased to 6.57% at the 32nd week (Table 2).

Table 2. Results of statistical analysis of the values determined in Purkinje neurons in the cerebellum of laying hens at different periods.

Parameter n=100	Periods						
	Incubation period			Post-Incubation period			
	Day 11 $\bar{X}$ ±SE	Day 14 $\bar{X}$ ±SE	Day 17 $\bar{X}$ ±SE	Exit Day $\bar{X}$ ±SE	6.th week $\bar{X}$ ±SE	18.th week $\bar{X}$ ±SE	32.th week $\bar{X}$ ±SE
Nucleus area(µm²)	2.30±0.08^{b}	2.35±0.15^{b}	6.26±0.33^{ab}	8.54±0.02^{ab}	10.3±0.58^{ab}	12.9±1.66^{a}	9.74±0.61^{ab}
Nucleus diameter (µm)	1.98±0.05^{b}	2.03±0.07^{b}	3.31±0.09^{a}	3.98±0.03^{a}	4.15±0.47^{a}	4.58±0.23^{a}	4.17±0.15^{a}
Number of AgNOR	1.33±0.33^{b}	1.64±0.15^{ab}	1.29±0.14^{b}	1.33±0.21^{b}	1.17±0.06^{b}	1.38±0.06^{b}	2.32±0.17^{a}
AgNOR area (µm2)	0.39±0.05^{c}	0.36±0.02^{c}	1.04±0.08^{abc}	1.49±0.01^{ab}	1.69±0.13^{a}	1.27±0.11^{ab}	0.64±0.10^{bc}
Ag NOR diameter (µm)	0.89±0.03^{c}	0.90±0.05^{c}	1.46±0.08^{abc}	1.55±0.01^{ab}	1.94±0.07^{a}	1.59±0.07^{ab}	1.08±0.08^{bc}
The ratio of AgNOR area to the nucleus area (%)	16.95	15.31	16.61	17.44	16.40	8.94	5.57

a-c: Differences between values with different letters in the same row are statistically significant (P<0.05).

While the mean nuclear area values were close to each other on the day of hatching and at 6 weeks after hatching in both sexes; roosters had higher values than hens at 18 weeks (P<0.05). However, hens had higher nuclear area than hens at 32 weeks (P<0.05). While the nuclear diameter values were close to each other on the day of hatching and at the following 6 and 32 weeks (P>0.05) in both sexes; roosters had higher values than hens at 18 weeks (P<0.05). The mean AgNOR number was close to each other on the day of hatching and at 6, 18 and 32 weeks in both sexes and the differences between the periods were found to be insignificant (P>0.05). While the AgNOR area and AgNOR diameter values of both sexes had close values on the day of hatching and at the following 6 weeks (P>0.05), roosters had higher AgNOR area and diameter than hens at 18 weeks. However, at 32 weeks post-hatching, hens had greater (P<0.05) AgNOR area and diameter than roosters (Table 3).

Table 3. Statistical analysis results of the values determined in the Purkinje neurons in the cerebellum of male and female layer hens during the rearing period after hatching.

Parameter n=100	Gender	Periods			
		Exit Day	6 th week	18 th week	32 th week
Nucleus area (µm²) $\bar{X}$ ±SE	♀	8.57±0.04[a]	9.71±0.98[a]	8.59±0.68[b]	12.85±0.35[a]
	♂	8.52±0.04[a]	8.28±1.09[a]	17.32±3.39[a]	10.83±0.39[b]
Nuclesur diameter (µm) $\bar{X}$ ±SE	♀	4.03±0.01[a]	3.98±0.24[a]	3.84±0.13[b]	4.88±0.10[a]
	♂	3.93±0.06[a]	3.80±0.23[a]	5.37±0.45[a]	4.57±0.15[a]
Number of AgNOR $\bar{X}$ ±SE	♀	1.50±0.28[a]	1.12±0.12[a]	1.30±0.09[a]	2.10±0.34[a]
	♂	1.25±0.25[a]	1.37±0.18[a]	1.46±0.11[a]	2.70±0.26[a]
AgNOR area (µm²) $\bar{X}$ ±SE	♀	1.49±0.08[a]	1.37±0.18[a]	0.95±0.07[b]	1.27±0.24[a]
	♂	1.50±0.01[a]	1.10±0.13[a]	1.63±0.22[a]	0.35±0.05[b]
Ag NOR diameter (µm) $\bar{X}$ ±SE	♀	1.56±0.02[a]	2.03±0.19[a]	1.34±0.05[b]	1.60±0.17[a]
	♂	1.54±0.03[a]	1.56±0.05[a]	1.82±0.14[a]	0.84±0.08[b]

a-b: Differences between values bearing different letters in the same parameters of different genders are statistically significant (P<0.05).

Table 4. Statistical analysis results of the values determined in the Purkinje neurons in the cerebellum of broiler and layer hens in different periods.

Parameter n=100	Breeds	Periods				
		Day 11	Day 14	Day 17	Exit Day	6 th week
Nucleus area (µm²) $\bar{X}$ ±SE	Broilers	3,80±0,22[a]	3,54±0,26[a]	6,07±0,45[a]	11,7±0,80[a]	11,3±0,67[a]
	layers	2,30±0,08[b]	2,35±0,15[b]	6,26±0,33[a]	8,54±0,02[a]	10,3±0,58[a]
Nuclesur diameter (µm) $\bar{X}$ ±SE	Broilers	2,74±0,13[a]	2,55±0,09[a]	3,24±0,13[a]	4,27±0,34[a]	4,39±0,10[a]
	layers	1,98±0,05[a]	2,03±0,07[b]	3,31±0,09[a]	3,98±0,03[a]	4,15±0,47[a]
Number of AgNOR $\bar{X}$ ±SE	Broilers	1,40±0,16[a]	1,62±0,18[a]	1,53±0,14[a]	1,66±0,23[a]	1,62±0,32[a]
	layers	1,33±0,33[a]	1,64±0,15[a]	1,29±0,14[a]	1,33±0,21[a]	1,17±0,06[a]
AgNOR area (µm²) $\bar{X}$ ±SE	Broilers	0,55±0,07[a]	0,43±0,05[a]	0,77±0,09[a]	1,52±0,63[a]	1,21±0,19[a]
	layers	0,39±0,05[b]	0,36±0,02[a]	1,04±0,08[a]	1,49±0,01[a]	1,69±0,13[a]
Ag NOR diameter (µm) $\bar{X}$ ±SE	Broilers	1,05±007[a]	0,95±0,05[a]	1,32±0,08[a]	1,58±0,27[a]	1,76±0,16[a]
	layers	0,89±0,03[a]	0,90±0,05[a]	1,46±0,08[a]	1,55±0,01[a]	1,94±0,07[a]

a-b: Values bearing different letters in the same parameters of different breeds are statistically significant (P<0.05).

Discussion

In the first study conducted on metaphase chromosomes in poultry, two types of chromosomes were identified: macrochromosomes and microchromosomes. Almost half of poultry genes are found in microchromosomes. In previous studies (Habermann et al. 2001), it was observed that microchromosomes are located in the center of the cell nucleus and macrochromosomes are located in the periphery of the nucleus in neurons and fibroblasts. The chicken karyotype consists of a total of 78 chromosomes, consisting of 9 pairs of macrochromosomes and 30 pairs of microchromosomes. Since the rDNA genes in chicken are located on chromosome number 16, this chromosome is known as the NOR chromosome. For this reason, it

can be thought that there may be two NORs and two nucleoli in a diploid chicken cell (Miller et al. 1996, Masabanda et al. 2004).

Schmid et al (1982) suggested that the silver staining properties of NORs are an indicator of the transcriptional activity of ribosomal RNA genes. Therefore, the AgNOR numbers of cells forming different tissues also vary. There is a common view that there are significant differences between the active AgNOR numbers present in the genomes of different similar cells of an organism or cells of different organisms, and that the active AgNOR numbers may vary according to the protein synthesis needs of the cell and environmental conditions (Goodpasture et al 1976, Mikelsaar et al 1977, Alberts et al 1989).

In chickens, information on the number, localization, size of AgNORs in normal tissue and the relationship between nuclear size and NOR parameters is limited. Su and Delany (1998) studied broiler chicken breeds that were subjected to selection for growth performance improvement. The researchers (Su and Delany 1998) found that there were significant differences in the number and type of NORs both within and among pure lines, and that these NOR types contained different numbers of rRNA genes with different nucleolus formation capacity.

Manuelidis (1984) reported that AgNORs in mature central nervous system cells generally show fixed localization in the nucleus. While there is always a single centrally located AgNOR in large neurons, there are several small and peripherally located AgNORs in the stratum granulosum of the cerebellum. This fact shows that in highly differentiated cells, AgNORs are definitely located in the nucleolus, and that localization of different types of NORs on different chromosomes is not important in their localization within the nucleus. In this study, the large nuclei of Purkinje neurons in the cerebellum were oval-round shaped and mostly localized in the center of the cell body. NORs were seen as oval-round shaped brown-black spots in broilers and layers during the incubation period and post-hatching rearing periods. The shape and localization of AgNORs, whose numbers ranged from 1 to 4, were very similar to those of pyramidal neurons in the deep cortical region of the cerebral hemispheres.

In vivo and in vitro study results show that the balance between protein synthesis and degradation rates in broilers has changed towards protein synthesis (Klasing et al. 1987). Increase in growth and protein synthesis rates at the cellular level requires an increase in the number of ribosomes in the cytoplasm. The transcription of rDNA genes and the increase in the half-life of ribosomes contribute significantly to the increase in ribosome levels in the cell cytoplasm (Larson et al. 1991). However, there is no sufficient experimental data to determine whether there is an increase in the number of rDNA copies in specific organ system cells in flocks subjected to unidirectional selection. In the present study, no regular and distinctive differences

were detected in the AgNOR number and AgNOR diameter parameters of the Purkinje cells of the cerebellum tissue of 2 different chicken breeds developed for meat or egg yields. However, there are also significant differences between some of the parameters examined in both embryonic and post-hatching periods.

Conclusion

In this study, cerebellar Purkinje cells of broilers had more nuclear area on the 11th and 14th days of incubation, and no difference was observed between broilers and layers in terms of nuclear area in other periods. Nucleus diameter was also higher in broilers on the 14th day of incubation. AgNOR count and AgNOR diameter of broilers and layers did not change in any period. Broilers had higher AgNOR area than layers on the 11th day of incubation. No statistical difference was found between AgNOR counts determined in Purkinje neurons in the cerebellum of male and female layers during the rearing period after incubation. Considering these results, although it is not possible to establish a direct correlation between the productivity characteristics of layers and broiler breed chickens and the parameters examined, it was concluded that it would be useful to conduct studies including AgNOR parameters together with the productivity records of animals, especially in the period after hatching. In addition, basic data was also provided for future studies on the nuclear and AgNOR parameters of the two chicken breeds in different periods.

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