CURRENT STUDIES IN PLANT AND ANIMAL PHYSIOLOGY

Editors

Assoc. Prof. Dr. Hülya DOĞAN - Prof. Dr. Hatice BAŞ

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PREFACE

Dear readers,

Current studies in plant and animal physiology, the chapters in this book have been prepared by valuable academicians who are experts in their fields. With the developments in genetics and molecular fields, our knowledge of physiological, biochemical, and genetic processes in organisms is increasing daily.

In nature, several ecosystems that are formed by the coming together of different plant and animal species where live in inanimate environments caused factors. Plant diversity is formed by these ecosystem systems and biological diversity is one of the most important elements due to adding positive value to ecology and environmental health. The development of tissues in plants is closely related to the environment in which the plant is located and the physiological events that occur accordingly. Physiological characteristics in animals are related to vital events that occur in the organism and can be affected more or less by environmental factors. Functions such as body temperature are included in this group and cannot be seen from the outside. Therefore, plant and animal physiology studies are closely related to anatomy, cell biology, and structural and functional chemistry.

This book has been presented to readers and researchers by blending current research and findings in many fields and sectors such as agriculture, molecular biology, botany, genetics, veterinary, zootechnics.

We would like to thank all the authors and the publishing team who contributed to the creation of the book.

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CHAPTER 1

THE MODEL ORGANISM: *Arabidopsis thaliana* AND ITS PHOTOSYNTHESIS-RELATED GENES

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1. A MODEL ORGANISM: *Arabidopsis thaliana*

Arabidopsis thaliana (L.) Heynh. is plant model organism in the genus *Arabidopsis* in the family Brassicaceae. The taxonomic classification of *Arabidopsis thaliana* is given below.

Kingdom:	Plantae
Phylum:	Magnoliophyta
Class:	Magnoliidae
Ordo:	Brassicales
Family:	Brassicaceae
Genus:	*Arabidopsis*
Species:	*Arabidopsis thaliana*

The main reasons for it being a model organism are low chromosome number ($2n = 10$), easy crossbreeding between ecotypes, and easy obtaining of mutant genotypes. In addition, its other advantageous features are that it is self-fertilization, completes its entire life cycle in a short time (5 to 8 weeks) and can produce a large number of seeds in a short time (Johnson and Bouchez, 2007; Szymanski, 2013).

Arabidopsis thaliana naturally ranges from all of Europe except Iceland to central Asia. However, the fact that the species is widespread in almost every country in the world makes the work of systematic botanists and ecologists difficult. In addition to its wide distribution, it can live in many habitats up to about 4300 meters, including meadows, sandy soils, rocky slopes, cultivated areas, under shrubs, slightly alkaline flats, roadsides, and wastelands (Al-Shehbaz and O'Kane, 2002). Due to its widespread distribution throughout the world, its local names are also quite diverse (Table 1) (EPPO Global Database, 2024).

Arabidopsis thaliana is preferred as a model organism due to the reasons such as having a small number of chromosomes ($2n = 10$) and a small genome (125 mb), producing high amounts of seeds, completing its life cycle in a short time, and being able to be grown easily even in a petri dish in the laboratory. It is the first plant whose genome map has been produced by six sequencing groups and one genome analysis group (Leutwiler et al., 1984; Davis, 1992; The Arabidopsis Genome Initiative, 2000).

Table 1: Local common names of *Arabidopsis thaliana* according to EPPO Global Database (2024).

Common names	Language
Common wallcress Couse-ear cress Thale cress	English
Ackerschmalwand Ackerschotenkresse Gänsekressling Gänserauke Schmalwand Thals Schotenkresse	German
Arabette des dames Arabette de Thalius Arabette rameuse Arabidopsis de Thalius Fausse arabette	French
Arabide Arabidopsis Oruga	Spanish
Arabetta comune Arabide comune Arabidopside Pelosella	Italian
Zandraket	Dutch
Arabeta Erva-estrelada	Portuguese
Backtrav	Swedish
Арабидопсис Таля Резуховидка Таля Резушка Таля	Russian
Almindelig gåsemad Svensk gåsemad	Danish

Vårskrinneblom	Norwegian
Lituruoho	Finnish
Fare kulağı teresi	Turkish
Tudranit levanah	Hebrew
Rzodkiewnik pospolity	Polish
Lúdfű	Hungarian
arabidopsisi i Talies	Albanian
Арабидопсис арабидопсис Талев	Bulgarian
Arabidopsis	Catalan
Shǔ ěr gài	Chinese
Talijin uročnjak	Croatian
Huseníček rolní	Czech
Harilik müürlook	Estonian
αραβίδοφη	Greek
Tailís	Irish
Ae gi jang dae	Korean
Tāla sīkplikstiņš	Latvian
Baltažiedis vairenis	Lithuanian
Gîscariță	Romanian
Урочњак	Serbian
Arábkovka Thalova Hydozeliček roľný	Slovak
Repnjakovec	Slovene
Різушка таля	Ukrainian
Berwr y fagwyr	Welsh

2. PHOTOSYNTHESIS-RELATED GENES

The complete sequence of the chloroplast genome of *Arabidopsis thaliana* had been deduced and the genome was 154.5 kb long (Sato et al., 1999). Due to the conserved nature of the chloroplast genome, the chloroplast genomes of *Arabidopsis thaliana* and many higher plant species contain common regions (Ahmad et al., 2015). One of the most important similarities is the presence of two 26 kb inverted repeat regions (IR-A and IR-B) separating the coding regions into an 84 kb long repeat region. The chloroplast genome of *Arabidopsis thaliana* consists of a total of 117 genes, 87 of which encode proteins and 30 of which encode RNA (Sato et al., 1999; Ahmad et al., 2015).

Photosynthesis in chloroplasts involves an electron transport chain called Photosystem I and Photosystem II, which have a photochemical reaction center. The products of chloroplast DNA genes are proteins of the reaction center (Hill and Bendall, 1960; Ohyama et al., 1986; Shinozaki et al., 1986; Blankenship, 2002). In chloroplasts, the grana is rich in Photosystem II components, while the stroma is rich in Photosystem I components (Leister and Schneider, 2003).

Photosynthesis-related genes of *Arabidopsis thaliana* are listed are listed in the Tables 2-7 below. The genes of each group are listed alphabetically. References are summarized here to avoid repetition at the end of each gene (Ahmad et al., 2015; Asaf et al., 2017; NIH, 2024; TAIR, 2024; Uniprot, 2024).

Table 2: Group I: Photosystem I genes

Gene	psaA
Protein name	Photosystem I P700 chlorophyll a apoprotein A1
Length	Number of nucleotides = 2253 Number of amino acids = 750
Lokus	ArthCp022 (*Arabidopsis thaliana*-chloroplast-022)
Function	Protein-coding gene encoding psaA protein (with psaB) forming the reaction center of Photosystem I.

Gene	psaB

Protein name	Photosystem I P700 chlorophyll a apoprotein A2
Length	Number of nucleotides = 2205 Number of amino acids = 734
Lokus	ArthCp021 (*Arabidopsis thaliana*-chloroplast-021)
Function	Protein-coding gene encoding subunit-D1 of the reaction center of Photosystem I.

Gene	psaC
Protein name	Photosystem I reaction center subunit VII
Length	Number of nucleotides = 249 Number of amino acids = 82
Lokus	ArthCp075 (*Arabidopsis thaliana*-chloroplast-075)
Function	Protein-coding gene encoding subunit-pscA of Photosystem I.

Gene	psaI
Protein name	Photosystem I reaction center subunit VIII
Length	Number of nucleotides = 114 Number of amino acids = 37
Lokus	ArthCp032 (*Arabidopsis thaliana*-chloroplast-032)
Function	Protein-coding gene encoding subunit-I of Photosystem I.

Gene	psaJ
Protein name	Photosystem I reaction center subunit IX
Length	Number of nucleotides = 135 Number of amino acids = 44
Lokus	ArthCp042
Function	Protein-coding gene encoding subunit-J of Photosystem I.

Table 3: Group II: Photosystem II genes

Gene	psbA
Protein name	Photosystem II protein D1
Length	Number of nucleotides = 1062 Number of amino acids = 353
Lokus	ArthCp002 (*Arabidopsis thaliana*-chloroplast-002)
Function	Protein-coding gene encoding D1 protein (chlorophyll binding protein) of the reaction center core of Photosystem II.

Gene	psbB
Protein name	Photosystem II CP47 reaction center protein
Length	Number of nucleotides = 1527 Number of amino acids = 508
Lokus	ArthCp049 (*Arabidopsis thaliana*-chloroplast-049)
Function	Protein-coding gene encoding subunit CP47 of the reaction center of Photosystem II.

Gene	psbC
Protein name	Photosystem II CP43 reaction center protein
Length	Number of nucleotides = 1422 Number of amino acids = 473
Lokus	ArthCp018 (*Arabidopsis thaliana*-chloroplast-018)
Function	Protein-coding chloroplast gene encoding subunit CP43 of the reaction center of Photosystem II.

Gene	psbD
Protein name	Photosystem II protein D2

Length	Number of nucleotides = 1062 Number of amino acids = 353
Lokus	ArthCp017 (*Arabidopsis thaliana*-chloroplast-017)
Function	Protein-coding gene encoding PSII D2 protein.

Gene	psbE
Protein name	Photosystem II protein V
Length	Number of nucleotides = 252 Number of amino acids = 83
Lokus	ArthCp039 (*Arabidopsis thaliana*-chloroplast-039)
Function	Protein-coding gene playing a protective role as the electron donor or electron acceptor when PSII does not optimize electron flow.

Gene	psbF
Protein name	Photosystem II protein VI
Length	Number of nucleotides = 120 Number of amino acids = 39
Lokus	ArthCp038 (*Arabidopsis thaliana*-chloroplast-038)
Function	Protein-coding gene encoding PSII cytochrome b559.

Gene	psbH
Protein name	Photosystem II protein H
Length	Number of nucleotides = 222 Number of amino acids = 73
Lokus	ArthCp052 (*Arabidopsis thaliana*-chloroplast-052)
Function	Protein-coding gene encoding a phosphoprotein of Photosystem II.

Gene	psbI
Protein name	Photosystem II protein I
Length	Number of nucleotides = 111 Number of amino acids = 36
Lokus	ArthCp006 (*Arabidopsis thaliana*-chloroplast-006)
Function	Protein-coding gene encoding PSII I protein.

Gene	psbJ
Protein name	Photosystem II protein J
Length	Number of nucleotides = 123 Number of amino acids = 40
Lokus	ArthCp036 (*Arabidopsis thaliana*-chloroplast-036)
Function	Protein-coding gene encoding PSII component.

Gene	psbK
Protein name	Photosystem II protein K
Length	Number of nucleotides = 186 Number of amino acids = 61
Lokus	ArthCp005 (*Arabidopsis thaliana*-chloroplast-005)
Function	Protein-coding gene encoding PSII K protein.

Gene	psbL
Protein name	Photosystem II protein L
Length	Number of nucleotides = 117 Number of amino acids = 38
Lokus	ArthCp037 (*Arabidopsis thaliana*-chloroplast-037)

Function	Protein-coding gene encoding PSII L protein.

Gene	psbM
Protein name	Photosystem II protein M
Length	Number of nucleotides = 105 Number of amino acids = 34
Lokus	ArthCp016 (*Arabidopsis thaliana*-chloroplast-016)
Function	Protein-coding gene encoding PSII low MW protein.

Gene	psbN
Protein name	Photosystem II protein N
Length	Number of nucleotides = 132 Number of amino acids = 43
Lokus	ArthCp051 (*Arabidopsis thaliana*-chloroplast-051)
Function	Protein-coding gene encoding PSII low MW protein.

Gene	psbT
Protein name	Photosystem II protein T
Length	Number of nucleotides = 102 Number of amino acids = 33
Lokus	ArthCp050 (*Arabidopsis thaliana*-chloroplast-050)
Function	Protein-coding gene encoding subunit-PSII-T of Photosystem II.

Gene	psbZ
Protein name	Photosystem II protein Z
Length	Number of nucleotides = 189

	Number of amino acids = 62
Lokus	ArthCp019 (*Arabidopsis thaliana*-chloroplast-019)
Function	Protein-coding gene encoding subunit-psbZ of Photosystem II.

Table 4: Group III: ATP Synthase Complex

Gene	atpA
Protein name	ATP synthase alpha subunit
Length	Number of nucleotides = 1524 Number of amino acids = 507
Lokus	ArthCp007 (*Arabidopsis thaliana*-chloroplast-007)
Function	Protein-coding gene encoding regulatory subunit-alpha of ATP synthase.

Gene	atpB
Protein name	ATP synthase beta subunit
Length	Number of nucleotides = 1497 Number of amino acids = 498
Lokus	ArthCp029 (*Arabidopsis thaliana*-chloroplast-029)
Function	Protein-coding gene encoding subunit-beta hosting the catalytic sites of ATP synthase.

Gene	atpE
Protein name	ATP synthase epsilon subunit
Length	Number of nucleotides = 399 Number of amino acids = 132
Lokus	ArthCp028 (*Arabidopsis thaliana*-chloroplast-028)
Function	Protein-coding gene encoding subunit-epsilon of

	ATP synthase.

Gene	atpF
Protein name	ATP synthase B subunit
Length	Number of nucleotides = 555 Number of amino acids = 184
Lokus	ArthCp008 (*Arabidopsis thaliana*-chloroplast-008)
Function	Protein-coding gene encoding subunit-F of ATP synthase.

Gene	atpH
Protein name	ATP synthase C subunit
Length	Number of nucleotides = 246 Number of amino acids = 81
Lokus	ArthCp009 (*Arabidopsis thaliana*-chloroplast-009)
Function	Protein-coding gene encoding subunit-C of ATP synthase.

Gene	atpI
Protein name	ATP synthase A subunit
Length	Number of nucleotides = 750 Number of amino acids = 249
Lokus	ArthCp010 (*Arabidopsis thaliana*-chloroplast-010)
Function	Protein-coding gene encoding subunit-A playing in transporting protons across the membrane of ATP synthase.

Table 5: Group IV: Cytochrome b6-f Complex

Gene	pet A
Protein name	Cytochrome f
Length	Number of nucleotides = 963 Number of amino acids = 320
Lokus	ArthCp035 (*Arabidopsis thaliana*-chloroplast-035)
Function	Protein-coding gene encoding subunit-cytochrome b(6) of Cytochrome b6-f Complex. The subunit provides electron flow between Photosystem I and Photosystem II.

Gene	petB
Protein name	Cytochrome b6
Length	Number of nucleotides = 648 Number of amino acids = 215
Lokus	ArthCp053 (*Arabidopsis thaliana*-chloroplast-053)
Function	Protein-coding gene encoding subunit-cytochrome b(6) of Cytochrome b6-f Complex. The subunit provides electron flow between Photosystem I and Photosystem II.

Gene	petD
Protein name	Cytochrome b6-f complex subunit IV
Length	Number of nucleotides = 483 Number of amino acids = 160
Lokus	ArthCp054 (*Arabidopsis thaliana*-chloroplast-054)
Function	Protein-coding gene encoding subunit-IV of Cytochrome b6-f Complex. The subunit provides electron flow between Photosystem I and

	Photosystem II.

Gene	petG
Protein name	Cytochrome b6-f complex subunit V
Length	Number of nucleotides = 114 Number of amino acids = 37
Lokus	ArthCp041 (*Arabidopsis thaliana*-chloroplast-041)
Function	Protein-coding gene encoding subunit-V of Cytochrome b6-f Complex. The subunit provides electron flow between Photosystem I and Photosystem II.

Gene	petL
Protein name	Cytochrome b6-f complex subunit VI
Length	Number of nucleotides = 96 Number of amino acids = 31
Lokus	ArthCp040 (*Arabidopsis thaliana*-chloroplast-040)
Function	Protein-coding gene encoding subunit-VI of Cytochrome b6-f Complex. The subunit provides electron flow between Photosystem I and Photosystem II.

Gene	petN
Protein name	Cytochrome b6-f complex subunit VIII
Length	Number of nucleotides = 90 Number of amino acids = 29
Lokus	ArthCp015 (*Arabidopsis thaliana*-chloroplast-015)
Function	Protein-coding gene encoding subunit-VIII of Cytochrome b6-f Complex. The subunit provides

	electron flow between Photosystem I and Photosystem II.

Table 6: Group V: NADH Dehydrogenase Complex

Gene	ndhA
Protein name	NADH dehydrogenase subunit I (or subunit 1) NAD(P)H-quinone oxidoreductase subunit I (or 1)
Length	Number of nucleotides = 1083 Number of amino acids = 360
Lokus	ArthCp079 (*Arabidopsis thaliana*-chloroplast-079)
Function	Protein-coding gene encoding subunit-I of NADH dehydrogenase or NAD(P)H-quinone oxidoreductase. NADH dehydrogenase is a flavoprotein enzyme converted nicotinamide adenine dinucleotide from reduced form to oxidized form and is used in the electron transport chain of ATP production.

Gene	ndhB
Protein name	NADH dehydrogenase subunit II NAD(P)H-quinone oxidoreductase subunit II
Length	Number of nucleotides = 1539 Number of amino acids = 512
Lokus	ArthCp068 (*Arabidopsis thaliana*-chloroplast-068)
Function	Protein-coding gene encoding subunit-IIA or -IIB of NADH dehydrogenase or NAD(P)H-quinone oxidoreductase.

Gene	ndhC
Protein name	NADH dehydrogenase subunit III

	NAD(P)H-quinone oxidoreductase subunit III
Length	Number of nucleotides = 363 Number of amino acids = 120
Lokus	ArthCp027 (*Arabidopsis thaliana*-chloroplast-027)
Function	Protein-coding gene encoding subunit-III of NADH dehydrogenase or NAD(P)H-quinone oxidoreductase.

Gene	ndhD
Protein name	NADH dehydrogenase subunit IV NAD(P)H-quinone oxidoreductase subunit IV
Length	Number of nucleotides = 1503 Number of amino acids = 500
Lokus	ArthCp074 (*Arabidopsis thaliana*-chloroplast-074)
Function	Protein-coding gene encoding subunit-IV of NADH dehydrogenase or NAD(P)H-quinone oxidoreductase.

Gene	ndhE
Protein name	NADH dehydrogenase subunit 4L NAD(P)H-quinone oxidoreductase subunit 4L
Length	Number of nucleotides = 306 Number of amino acids = 101
Lokus	ArthCp076 (*Arabidopsis thaliana*-chloroplast-076)
Function	Protein-coding gene encoding subunit-4L of NADH dehydrogenase or NAD(P)H-quinone oxidoreductase.

Gene	ndhF
Protein name	NADH dehydrogenase subunit V NAD(P)H-quinone oxidoreductase subunit V

Length	Number of nucleotides = 2241 Number of amino acids = 746
Lokus	ArthCp071 (*Arabidopsis thaliana*-chloroplast-071)
Function	Protein-coding gene encoding subunit-V of NADH dehydrogenase or NAD(P)H-quinone oxidoreductase.

Gene	ndhG
Protein name	NADH dehydrogenase subunit VI NAD(P)H-quinone oxidoreductase subunit VI
Length	Number of nucleotides = 531 Number of amino acids = 176
Lokus	ArthCp077 (*Arabidopsis thaliana*-chloroplast-077)
Function	Protein-coding gene encoding subunit-VI of NADH dehydrogenase or NAD(P)H-quinone oxidoreductase.

Gene	ndhH
Protein name	NADH dehydrogenase subunit VII NAD(P)H-quinone oxidoreductase subunit H
Length	Number of nucleotides = 1182 Number of amino acids = 393
Lokus	ArthCp080 (*Arabidopsis thaliana*-chloroplast-080)
Function	Protein-coding gene encoding subunit-VII of NADH dehydrogenase or NAD(P)H-quinone oxidoreductase.

Gene	ndhI
Protein name	NADH dehydrogenase subunit I NAD(P)H-quinone oxidoreductase subunit I
Length	Number of nucleotides = 519

	Number of amino acids = 172
Lokus	ArthCp078 (*Arabidopsis thaliana*-chloroplast-078)
Function	Protein-coding gene encoding subunit-I of NADH dehydrogenase or NAD(P)H-quinone oxidoreductase.

Gene	ndhJ
Protein name	NADH dehydrogenase subunit J NAD(P)H-quinone oxidoreductase subunit J
Length	Number of nucleotides = 477 Number of amino acids = 158
Lokus	ArthCp025 (*Arabidopsis thaliana*-chloroplast-025)
Function	Protein-coding gene encoding subunit-J of NADH dehydrogenase or NAD(P)H-quinone oxidoreductase. Transcription increases when sulphur is depleted.

Gene	ndhK
Protein name	NADH dehydrogenase subunit K NAD(P)H-quinone oxidoreductase subunit K
Length	Number of nucleotides = 678 Number of amino acids = 225
Lokus	ArthCp026 (*Arabidopsis thaliana*-chloroplast-026)
Function	Protein-coding gene encoding subunit-K of NADH dehydrogenase or NAD(P)H-quinone oxidoreductase.

Table 7: Group VI: Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO)

Gene	rbcL
Protein name	Ribulose-1,5-bisphosphate carboxylase large subunit
Length	Number of nucleotides = 1440 Number of amino acids = 479
Lokus	ArthCp030 (*Arabidopsis thaliana*-chloroplast-030)
Function	Protein-coding gene encoding large subunit of enzyme RuBisCo which catalyses two reactions. The first is the carboxylation of D-ribulose 1,5-bisphosphate, which is the first step in CO_2 fixation. The second is the oxidative cleavage of the pentose substrate.

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CHAPTER 2

RECENT DEVELOPMENTS IN THE RELATIONSHIP BETWEEN PHYTOHORMONES AND NITRIC OXIDE IN WHEAT UNDER DROUGHT STRESS

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1. INTRODUCTION

Drought significantly impacts plant yield and growth. However, it is an important abiotic stressor that causes an imbalance in the levels of reactive oxygen-containing molecules, and the antioxidant defence system, resulting in oxidative stress. Drought stress significantly impacts wheat (*Triticum aestivum* L.) growth, development, and yield by inducing various morphological, physiological, and biochemical changes. Studies have shown how nitric oxide (NO) functions in different plant signaling pathways and helps preserve cellular homeostasis in drought stress. In this section, the role of NO in wheat, its connection with phytohormones, and its effects on plant development processes are discussed up to date.

2. DROUGHT STRESS AND WHEAT

The intensifying effects of climate change, extreme heat, rainfall, and drought conditions increase the need for implementing climate change adaptation policies in agriculture. The use of smart technology in agricultural food systems against stresses such as climate change and water scarcity and their transformation into climate tolerance with green practices are of critical importance for the future of agriculture. On the other hand, sustainable practices that protect soil ecosystems and effectively evaluate resources are needed in resilient and sustainable food systems. Furthermore, the Food and Agriculture Organization (FAO) predicts that the demand for agricultural products is expected to continue to rise in line with the growth of the global population. This situation indicates a need for food of 60% more than the current situation by 2050 (FAO, 2022). Accordingly, there is a need for resilient and sustainable agricultural production to ensure sufficient and affordable food supply (Figure 1).

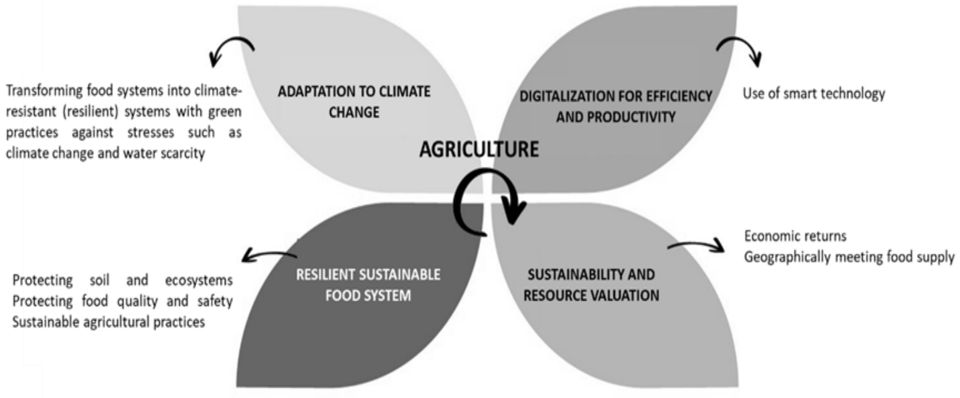

Figure 1: Agriculture of the future (edited from Cost Action AgriFood4Future, 2024).

However, global climate change, which has become visible in the last 40 years, has increased the severity of drought and its effect on plants. Negative impacts of stress on plants vary with the development stage of the plant, duration, and sternness of the stress. Water scarcity significantly affects crop yields by disrupting basic physiological processes in plants such as light harvesting, ATP synthesis, and carbon dioxide fixation (Lawlor et al., 2002). It also affects plant development by reducing nutrient uptake and transport from the soil to stems (Dietz et al., 2021). Adaptation of plants to stressful environments is achieved by complex biological reactions with physiological (transpiration, respiration, and photosynthesis), morphological (leaves, shoots, roots, and cellular structures), and metabolic (primary and secondary metabolites) changes (Jiang and Zhang, 2002; Ncama et al., 2022) (Figure 2).

When the plant's transpiration rate is excessively high or its water uptake into the roots is restricted, drought stress is triggered at the physiological level. Drought has a major impact on photosynthesis, a biological process essential to plant survival. Photosynthetic activity can be restricted by low water availability and an imbalance between light absorption and utilization, which can cause oxidative stress in the plant. A reduction in plant water content, stomatal closure, turgor, and growth are characteristics of drought stress (Balkan 2019, Demirbaş and Acar 2023) (Figure 2). One of the indicators used to define tolerance to water stress in plants is leaf relative water content (RWC) (Sanchez-Rodriguez et al. 2010). RWC are indicators of a plant’s ability to withstand drought stress. For example, RWC and chlorophyll content in wheat leaves under drought stress are significantly reduced compared to control plants, but this reduction differs between susceptible and tolerant wheat cultivars (Günay and Acar 2022).

At the biochemical level, the first response of plants to drought is the reduction in transpiration by the regulation of stomatal aperture, thereby preserving water within the plant. Photosynthesis reduces $NADP^+$ by decreasing carbon dioxide (CO_2) concentration in chloroplasts, which causes more electron leakage to oxygen (O_2), forming superoxide anions ($O_2^{\cdot -}$) and consequently resulting in ROS accumulation. In the event of severe drought, chloroplasts produce high levels of ROS that, lead to oxidative damage to cellular components. (Figure 2). As the severity and duration of drought increase, the accumulation of radicals such as $O_2^{\cdot -}$ increases more in drought-sensitive wheat cultivars than in drought-tolerant cultivars (Günay and Acar 2022).

At the molecular level, a number of genes and signaling pathways play a crucial role in the response to drought stress. For this purpose, studies have been conducted on the role of ROS, NO, abscisic acid (ABA), and calcium (Ca^{2+}) signaling in plant responses to drought stress (Uzilday et al., 2024). There are two groups of drought-responsive gene families: The first of ABA-dependent genes, LEA, enzymes (involved in osmolyte production, detoxification, and metabolism), transporters (channel proteins and ion transporters), protein kinases and transcription factors. However, some genes are not responsive to ABA. Secondly, DREB2 proteins play a significant role in the ABA-independent pathway in response to drought stress conditions. NADPH oxidases (NOXs), also known as respiratory burst oxidase homologs (RBOHs), are essential sources of ROS in plants. NOXs/RBOHs are involved in complex signaling networks, interacting with Ca^{+2}, kinases, and hormones to regulate plant stress responses (Hu et al., 2020) (Figure 3). Plants under drought stress have been reported to exhibit decreased expression of genes related to antioxidant enzymes and those encoding RBOH (Khator et al., 2023) (Figure 3). In addition to altering gene expression, drought stress also causes genotoxic changes such as DNA damage and mutation (Lau et al., 2021). Plants use different defense mechanisms to deal with these negative effects of drought stress. Stress-induced ROS accumulation is detoxified by enzymatic (catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidases (GP_X) and glutathione S-transferase (GST)) and non-enzymatic (ascorbate, α-tocopherol, flavonoids, and carotenoids) antioxidants (Mittler et al., 2004).

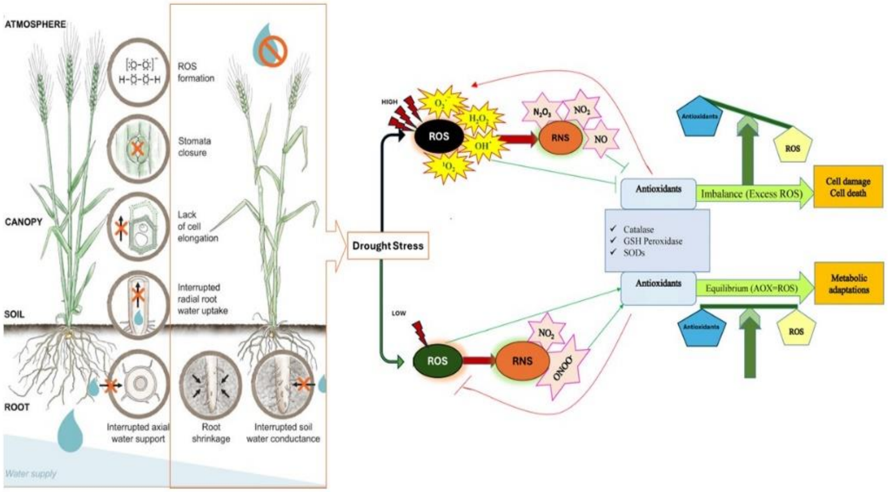

Figure 2: An illustration of the physiological responses of wheat to drought stress is represented in the schematic diagram. RNS signaling in wheat under drought stress. The interplay between NO and ROS influences the antioxidant defence system. In conditions of low drought stress, moderately produced ROS acts as a signal for NO synthesis, which strengthens the antioxidant defense system to provide protection to stressed plants. In conditions of severe drought stress, the overproduction of ROS promotes the production of RNS, which can cause an imbalance in the antioxidant system and ROS. This imbalance causes cell damage or cell death (Chang-Brahim et al., 2024).

Table 1: Overview of some of the biologically important ROS/RNS

ROS		RNS	
Hydroxyl radical	$OH^{\cdot}$	Nitric oxide	NO
Singlet oxygen	$^1O_2$	Peroxynitrite	NOOO-
Superoxide	$O_2^{-\cdot}$	Nitrosonium cation	NO^+
Hydrogen peroxide	H_2O_2	Nitroxylanion	NO^-
		Nitrogendioxide	NO_2
		Nitroso glutathione	GSNO
		Nitrous acid	HNO_2
		Dinitrogentetroxide	N_2O_4
		Dinitrogentrioxide	N_2O_3

Enzymatic antioxidants work in coordination to scavenge superoxide ($O_2^{\cdot}$), hydrogen peroxide (H_2O_2), and other free radicals. Hydroxyl radicals ($OH^{\cdot}$) and singlet oxygen ($^1O_2$) depend on non-enzymatic antioxidants for detoxification. Tocopherols effectively scavenge $OH^{\cdot}$ in biological membranes, while carotenoids scavenge $^1O_2$ (Foyer and Noctor, 2009; Mittler et al., 2011). In addition, reactive nitrogen species (RNS); Peroxynitrite (ONOO–) formed because of the interaction of ROS and NO is also used to describe molecules such as nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3), S-nitroso glutathione (GSNO), S-nitrosothiols (RSNOs), derived from NO (Gutteridge and Halliwell, 2018, Gupta et al., 2020) (Table 1, Figure 2). Nitrosative stress, which occurs in the same way as oxidative stress, means the uncontrolled and excessive production of RNS compounds with physiological reactions. RNS production or oxidative stress in plants causes damage or cell death. It is also known to play a role in defense against stress by stimulating antioxidant defense at low concentrations. Depending on the concentration, these reactive species can have both positive and negative effects on plants. Depending on their concentration in plant cells, ROS and RNS can cause oxidative bursts or act as signaling molecules that reduce stress (Figure 2 and Table 1).

A series of signaling molecules, ROS, NO, and Ca^{2+}, control plant growth, development, and defense responses to stress. These signals, along with kinases, phosphatases, chaperones, hormones, lipid molecules, and transcription factors, work synergistically to promote plant survival under stress. Phytohormone signaling integrates with ROS and Ca^{2+} induced mechanisms to control stress response. Signaling molecules such as ROS and NO play a central role in maintaining the redox balance of mitochondria and regulating DNA repair pathways. Similarly, NO participates in epigenetic controls by interacting with various metabolic pathways in DNA damage repair (Benitez Alfonso et al., 2023).

Wheat is one of the world’s top three agricultural crops. According to FAO data, 734 million tons of wheat are produced in 214 million hectares of land in the world (FAO, 2022).

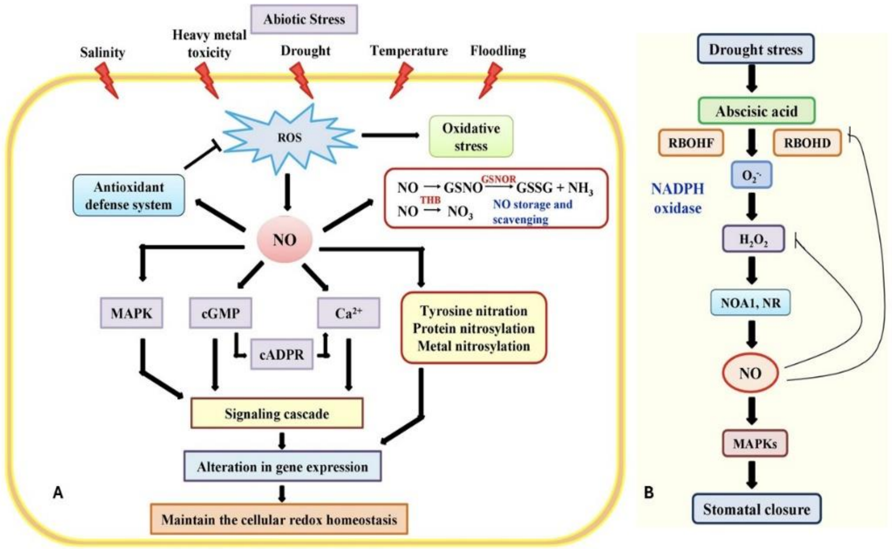

Figure 3: An overview of NO's regulating role in plants tolerance to drought stress. The NO interacts with numerous target molecules, including cyclic adenosine diphosphate ribose (cADPR), cyclic guanosine monophosphate (cGMP) and calcium (Ca^{2+}), and mitogen-activated protein kinases (MAPKs), to prevent the plant's oxidative burst caused by ROS (A). Respiratory burst oxidase homologs (RBOHs) or NADPH oxidases trigger ROS accumulation during drought stress and play a role in ABA signaling and stomatal closure, which are essential for plant drought tolerance. This activation could potentially trigger an increase in hydrogen peroxide (H_2O_2) levels, which in turn could lead to the production of NO. In addition, NO has been shown to activate the MAPK signaling cascade via the process of stomatal closure (B) (Khator et al., 2023).

The demand for wheat is increasing steadily with global population growth. Accordingly, 30% of the world's total grain production is met by wheat. The heat and drought triggered by global warming negatively affect wheat production worldwide (Asseng et al., 2015). Therefore, due to the increasing food demand, new approaches are needed for sustainable wheat production in changing climate conditions (Hossain et al., 2021; Dietz et al., 2021).

2.1. What is Nitric Oxide? and NO Relation Between Drought Stress

NO is a lipophilic molecule that plays a role in signaling in cells and its biosynthesis is endogenously produced in plants. Being a gas molecule composed of nitrogen (N) and oxygen (O), NO easily passes through cell membranes and functions as an excellent intracellular and intercellular signaling molecule in both plants and animals under normal and stressful conditions. NO plays an important role in intracellular redox signaling, ion homeostasis and activation of antioxidant defense mechanisms as a protective or stress-inducing agent (Desikan et al., 2004; Wang et al., 2011; Asgher et al., 2017; Krasuska et al., 2017). Moreover, NO regulates various developmental events in plants, such as seed dormancy and germination, plant growth and development, chlorophyll biosynthesis, stomatal movements, symbiosis, nodule formation, pollen tube elongation, flowering, fruit ripening, programmed cell death, and senescence plays an important role in responses to environmental stresses (Simontacchi et al., 2015, Nabi et al., 2019, Kumar and Ohri, 2023; Khan et al., 2023, Khator et al., 2023).

2.2. Source and Biosynthesis NO in Plant

RNS is produced as a consequence of several chemical reactions taking place within the cellular compartments of plants, including the chloroplast, mitochondria, cytosol, and peroxisomes. These reactions encompass respiration, photosynthesis, oxidation-reduction processes, and cellular photorespiration. Two different pathways are defined in the production of RNS. The oxidative pathway consists of NO synthase-like activity (NOS), polyamines (PA), and hydroxylamine-based production. The reductive pathway is based on the reduction of nitrite to NO. In the plant life cycle, NO is a molecule in signaling and can be produced by various pathways.

The main sources of NO production in plant cells are the plasma membrane, chloroplast, mitochondria, peroxisome, apoplast and cytoplasm. Changes in endogenous NO concentration led to the activation/deactivation of NO signaling and NO-related processes (Graska et al., 2023).

It has been reported that NO production in mitochondria is produced in all pathways such as the electron transport chain, complex I, II, III, and IV, alternative NAD(P)H dehydrogenases, alternative oxidase, and cytochrome c (Gupta et al., 2020). NO production from different enzymatic and non-enzymatic pathways involves nitric oxide synthase (NOS)-like activity, nitrate reductase (NR), nitrite reductase (NiR), and xanthine oxidoreductase (XOR). NR catalyzes the reduction of nitrate to nitrite, which in turn is reduced to NO, depending on NADPH. XOR catalyzes the conversion of nitrite to NO using NADH or xanthine as a reducing substrate (Khator et al., 2024). ROS are synthesized in four pools, mainly apoplastic, chloroplastic, mitochondrial, and peroxisomal pools, during drought. ROS synthesis also regulates the level of RNS (mainly NO), which regulates the signaling mechanism in the cell. It is known that H_2O_2 increases the production of NO by NADPH oxidase, which prevents excess ROS accumulation in the cell. Under drought stress, ROS synthesis also increases the production of RSS (GSH or H_2S). NO reacts with GSH to form GSNO, which is further reduced to oxidized glutathione (GSSG) by GSNO reductase. RNS interacts with H_2O_2 in the plant cell and balances the level of RNS and ROS in the cell (Mangal et al., 2023).

Nitric oxide reduction mechanism occurs mostly in the chloroplasts, peroxisomes, mitochondria, cytoplasm, apoplast, and plasma membrane via the reduction of nitrate (NO_3^-) or nitrite (NO_2^-) by nitrate reductase (NR). PA initiated NO production via reductive and oxidative mechanisms. NO production via oxidative mechanism; oxidation of L-Arginine occurs in peroxisomes and chloroplasts. In chloroplasts, oxidation of L-Arg requires NADPH and Ca^{2+}, while in peroxisomes, oxidation of L-Arg requires flavin mononucleotide, flavin adenine dinucleotide and Ca^{2+} (Lau et al., 2021). NO is known to play many roles in the regulation of plant physiological processes (Graska et al., 2023). It is known to reduce the level of ROS produced by environmental stress. It has been reported that increased levels of ROS ($O_2^{\bullet-}$, H_2O_2, OH, $^1O_2$) in stress support the production of RNS (NO, $ONOO^-$, N_2O_3, NO_2).

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2.3. Effects of NO on the Development of the Plant

Nitric oxide plays a crucial role in numerous physiological processes in plants, including seed dormancy and germination, stomatal opening and closure, plant growth and development, pollen tube elongation, senescence, and responses to environmental stresses. NO, a free radical, is also a vital signalling molecule that activates the increase in antioxidant enzyme synthesis, reduces lipid peroxidation (LP) and electrolyte leakage. NO plays a regulatory role in various physiological processes by interacting with various enzymes and hormones (Saddhe et al., 2019). Furthermore, it functions as a central molecule in plant defense from abiotic and biotic stresses and can modulate the expression of several genes. As a molecular messenger, NO has been demonstrated to interact with cGMP, Ca^{2+}, and particularly ROS-related signaling pathways (Astier et al., 2018). Additionally, it can function as a potent antioxidant by participating in the nitrogen assimilation pathway and the regulation of post-translational modification in the plant cell (Khator et al., 2024). During stress, the antioxidant system is activated when NO, ROS, and their interaction causes a moderate rise in ROS and NO levels. According to Kapoor et al. (2019), if ROS production is slightly more than NO generation, cells can experience oxidative stress due to RNS synthesis. Increased stress increases NO, promoting the formation of NO_2 and N_2O_3 from NO and thus nitrosative stress. Under these conditions, ROS and RNS cause plant cells to become damaged and die by inhibiting the antioxidant system (Yavaş et al., 2020) (Figure 3).

3. NO SIGNALING AND ITS CROSSTALK WITH OTHER PLANT GROWTH REGULATORS IN PLANT RESPONSES TO DROUGHT STRESS

Stress conditions disrupt the redox state in plants, thereby precipitating oxidative stress. In response, plants possess several physiological, morphological, biochemical, and molecular adaptations that make it possible for them to cope with and adapt to stress. The use of plant growth regulators represents a key strategy for regulating plant homeostasis when it is disrupted by abiotic stress factors and for decreasing the negative effects of environmental stress. In the process of stress adaptation, NO functions as a signaling molecule (Lau et al., 2021). Crosstalk between NO and hormones is essential for redox homeostasis in cells, altering the expression of several transcription factors and thereby escalating the plant stress response (Bhatt et al., 2023). Interaction of NO with various phytohormones positively affects the growth and productivity of plants (Kaur et al. 2022). Accordingly, NO, a signaling molecule, has been shown to functionally actively interact with phytohormones such as ABA, brassinosteroid (BR) and cytokinin (CK). ABA is one of the hormones involved in the termination of seed dormancy and the emergence of seedlings. This process is regulated by the ratios of ethylene and gibberellin (GA) concentrations during the germination phase. In studies examining the interaction between NO, ABA, and GA, it was observed that NO accumulation in the seed typically resulted in a reduction in ABA content. NO initiates ABA catabolism and promotes GA biosynthesis, which is a key step in the germination process (Sun et al., 2021). Conversely, auxin (AUX) is involved in the activation of growth and serves as a signalling molecule in ABA-controlled stomatal movements. Exogenous applications provided through growth regulators help plants grow more tolerant to stress conditions, high yields, and health.

NO and Auxin (AUX)

Auxins regulate a variety of processes associated with the development of normal and stressed plants. It has been shown that auxin and NO interact to regulate several plant responses, including root growth, gravitropic responses, root nodule formation, cell division, formation of embryonic, and NR activity stimulation (Allagulova et al., 2023).

NO and Cytokinin (CK)

On the metabolism of cytokinin (CK), NO has been shown to have both antagonistic and synergistic effects. Under hypoxic conditions, NO has been shown to regulate the activities of cytochrome-c-oxidase (CytOX) and alternative oxidase (AOX) (Gupta et al., 2012). In response to cytokinins during callus and meristem development, they found that NO is a prominent mediator in signaling the programming of mitotic cycles (Shen et al., 2013).

NO and Abscisic Acid (ABA)

While ABA stimulates stomatal closure, NO may contribute to stomatal opening. This is an important mechanism that regulates the water balance of the plant. ABA usually accumulates in plants during drought stress (Neill et al., 2008). The interplay between ABA, ROS, and NO in regulating stomatal closure highlights the complexity of plant responses to water stress. ABA is involved in the mechanisms controlling stomatal movement as well as in seed dormancy, germination, and plant development. ABA or NO is known to initiate the actions of many signal transduction components in plant cells. Both have been reported to influence the phosphorylation of proteins in cells mediated by effects on protein kinases and phosphatases (Hancock et al., 2011). Many studies have been conducted, the relationship between the ABA and NO, which are known to function antagonistically (Leon et al., 2014). As a form of defense in adverse conditions, ABA is known to suppress seed germination and promote seed dormancy. NO has an antagonistic effect with ABA as it promotes seed germination by breaking seed dormancy. NO-treated seeds were found to have lower ABA content. Both NO and ABA act as regulators of ROS metabolism.

NO and Gibberellins (GA)

Gibberellins are phytohormone that promotes plant development, including cell elongation. It also regulates gas exchange and water loss by affecting the opening of stomata. Furthermore, gibberellic acid (GA_3) has been shown to have to alleviate the negative effects of environmental stresses on plant growth (Hasanuzzaman et al., 2018; Zhang et al., 2023). The combination of GA_3 at 5 µg/ml and 100 µM SNP has been demonstrated to exert a synergistic effect, thereby mitigating the detrimental impact of heat stress on wheat. It has been demonstrated that the combined application of SNP and GA3 effectively regulates antioxidants, which play a crucial role in managing stomatal conductance and enhancing chlorophyll content (Zhang et al., 2023).

NO and Ethylene (ET)

It is known that NO can increase ethylene production. ET is involved in many physiological processes. This interaction can occur especially in processes like flowering, fruit formation, senescence and response to abiotic stresses. A notable antagonistic relationship is observed between NO and ET. For instance, the regulation of senescence is mediated by NO, which functions as an antagonist of ethylene. Furthermore, ET and NO have been demonstrated to neutralize the effect of ABA in seeds, thereby enhancing dormancy release and germination (Arc et al., 2013).

NO and Salicylic Acid (SA)

Nitric oxide and salicylic acid (SA) induce defense mechanisms of plants against pathogens and stress conditions. The applications of NO and SA in plants significantly increase crop yield by regulating photosynthesis and plant water content. A combined application of NO and SA has been shown to result in a notable reduction in the negative effects of Cd on wheat (Han et al., 2023). It has been reported that in wheat seedlings applied with NO and SA and then exposed to stress, the amount of stress-induced ROS ($O_2^{\cdot -}$ and H_2O_2) and malondialdehyde (MDA) increased stress tolerance together with increased antioxidant enzyme activities (Esim and Atıcı 2015).

NO and Brassinosteroid (BR)

Brassinosteroids (BRs) and NO regulate plant life cycle and stress responses, but function differently in these processes. BR and other phytohormones increase antioxidant capacity and act as secondary messengers such as ROS and NO for defense against stress. It seems reasonable to suggest that the combined use of BR and NO at specific concentrations may result in enhanced physiological effects. Indeed, the combined application of 24 Epi-Brassinolid (24-EBL) and SNP has been shown to positively influence the drought resistance of several plant species (Karpets and Kolupaev, 2018; Gupta et al., 2023).

NO and Strigolactones (SLs)

Strigolactones are a class of plant hormones derived from carotenoids. They are involved in the plant’s response to a range of biotic and abiotic stresses (Arıkan and Karaman, 2021). SLs that leak from plant roots into the soil play an important role in shoot and root development and the germination of parasitic plants such as *Orobanche* (Arıkan and Karaman, 2021). A few articles on SL-NO interactions focus on the root system.

NO and Jasmonic acid (JA)

Jasmonic acid is a phytohormone that initiates defense responses in plants. NO is also known to activate plant defense mechanisms against pathogens, pests etc. stress conditions synergistic interaction between JA and NO was observed under conditions of drought (Wang et al., 2021). NO plays a role in the regulation of exogenous JA in the leaves of wheat seedlings subjected to drought stress. The effect of exogenous JA in wheat has been demonstrated to enhance drought tolerance by stimulating NO production and regulating the Foyer-Halliwell-Asada cycle (Shan et al., 2015).

4. POTENTIAL EFFECT OF NO ON DROUGHT TOLERANCE OF WHEAT

Sodium nitroprusside is a NO donor applied exogenously to plants due to its small size, short lifespan and high cellular distribution. Many studies have shown that plants benefit from the protective role of SNP during various environmental stresses (Saddhe et al., 2019). Applications of nitric oxide have been shown to potentially enhance plants' resistance to abiotic stressors including drought (Ekinci et al., 2018). For example, exogenous NO application in wheat has been reported to increase drought tolerance by increasing the activities of antioxidant enzymes that scavenge ROS (Tian and Lei, 2006). Accordingly, NO application improves cellular damage in wheat plants in drought stressed. But it was found to be more effective at low concentrations (100-150 µM) and ineffective at high concentrations (0.5-1 mM) (Table 2). The increase in tolerance to PEG-induced water deficit by NO applications has been associated with PSI activity. The increase in chlorophyll content in response to NO and drought application has shown that it is regulated by NO under drought stress. In addition, the biomass and relative water content (RWC) decreased with drought application and increased with NO application (Shao et al., 2018).

Khan et al., (2017) showed that exogenous SNP application increased RWC in wheat plants under PEG (15%)-induced drought stress. In another study, it was shown that −0.4 MPa pressure reduced LP with 100 µM SNP priming application and provided antioxidant protection by increasing SOD and GR activities (Naser Alavi et al., 2014). Many studies reported that PEG (15%)-induced drought stress causes excessive ROS production and oxidative damage in wheat, and NO application increases drought tolerance by enhancing antioxidant systems (Table 2). It was found that low concentrations of NO (0.2 mM SNP) can maintain water status and enhance the growth of wheat, but high concentrations (2 mM SNP) have the opposite effect (Tian and Lei 2006). It has been shown that PEG (15%)-induced drought stress causes a decrease in SOD and CAT activity in wheat seedlings and excessive ROS production in leaves, thus increasing LP. However, SNP (0.3 mmol L) application has been shown to LP by preserving SOD and CAT activity. It has also been shown that NO scavenger (0.5 mmol cPTIO) application reverses these effects (Tan et al., 2008). Collectively, these studies show that exogenous application of NO donors such as SNP can effectively increase the tolerance of plants to abiotic stresses by regulating various physiological and biochemical processes. When the studies are examined, SNP applications in wheat plants affect plant development positively or negatively depending on the application concentration. These findings show that the effectiveness of NO donors in improving abiotic stress tolerance in wheat is dependent on concentration, with lower concentrations generally providing beneficial effects. According to the applications, it was determined that low concentrations stimulate the antioxidant system more effectively and develop tolerance to drought stress, but high concentrations trigger radical formation in wheat, causing toxic effects.

Nitric oxide interacts with plant hormones and various other signaling molecules and regulates osmo-protectants that protect against drought stress. Under drought stress, ABA-induced stomatal closure is a crucial mechanism. Complex intracellular signaling, including NO, has an impact on it (Garcia-Mata and Lamattina, 2001). The complex signaling network consisting of ROS, NO, ABA, H_2O_2 and Ca^{2+} and their interactions affect plant development and stress adaptation (Ghosh, 2020). The negative impact of drought stress on biomass and yield particularly affects strategically important crops such as wheat (Liu et al., 2019). In addition, SNP applications are available to ameliorate the negative effects of drought stress in wheat. The application of NO results in a reduction of the adverse effects of environmental stress on wheat, with the process of regulation occurring at multiple levels, including growth, mineral uptake, yield traits, and antioxidant profile. Many studies have shown that NO applications protect plants by eliminating oxidative stress factors such as LP and $O_2^{\cdot-}$ that occur in stressed plants by regulating antioxidant enzymes (GR, POX, SOD, APX, and CAT) (Kolupaev et al., 2014) (Table 2). This evidence suggests that NO functions as a scavenger of ROS. It was observed that exogenous application of SNP to plants exposed to drought stress increased enzyme activities and alleviated the negative effects of stress. NO and proline regulates the redox status of wheat plants during drought stress and protects the photosynthetic apparatus. The effects of exogenous NO on the ability of wheat plants under drought stress to maintain their photosynthetic functionality were studied. It was observed that cell damage (TBARS and H_2O_2) levels increased in plants exposed to stress, causing oxidative stress. This situation in the plant caused an increase of antioxidant enzymes and a decrease in photosynthetic properties. SNP application to wheat improved photosynthesis and reduced oxidative stress by enhancing the enzymatic antioxidant defense system and played a role in maintaining redox homeostasis by reducing TBARS and H_2O_2 levels (Sehar et al., 2023). In a study, it was stated that 200 µM SNP on wheat grown under drought conditions showed protective activity against oxidative damage caused by drought due to the increase in antioxidant enzymes activities (APX SOD, GR and CAT) (Maslennikova et al., 2020). On the other hand, it was determined that SNP applications at 150, 200, and 250 µM concentrations significantly increased the activity of antioxidant enzymes during drought stress in wheat plants grown under drought stress. Especially, 200 µM SNP significantly increased SOD, CAT, POD, and APX activities during drought stress (Boogar et al., 2014). It was stated that SNP significantly increased plant growth, photosynthesis, and chlorophyll content, and reduced ROS, H_2O_2, and TBARS accumulation. The data suggests a correlation between the increased amount of APX, SOD, and reduced glutathione was found to be more effective. On the other hand, exogenous NO (0.1 mM SNP) increased drought tolerance by inhibiting the enzymatic degradation of chlorophyll and especially Rubisco in wheat (Hassanuzman et al., 2018). Drought causes ion leakage and chlorophyll loss in leaves, and this could be partially prevented by NO. In another study, SNP pre-treatment was found to have a stimulatory and protective effect on the germination of wheat seeds water scarcity (Allagulova et al., 2023).

Sodium nitroprusside application reduces the transpiration rate and ion leakage by causing stomata to close, thus preventing water loss and making the plant more tolerant to drought conditions. NO seedlings in wheat have been shown to activate ABA and reduce water loss under water scarcity. Iqbal et al., (2022) have suggested that NO increases the accumulation of compatible solutes, such as proline, in plants that are challenged by heat and drought stress, thereby facilitating osmotic adjustment. Recent studies have indicated that the production of oxidative stress indicators like H_2O_2, MDA, and EL increases in wheat during drought stress, while NO application, which activates the antioxidant system, reduces this production. These findings indicated that NO protects proteins, lipids, and against photo-oxidative damage (Faroog et al., 2017, Maslennikova et al., 2020, Faraji et al., 2020 and Sahar et al., 2023). In this context, it can be said that NO, as a signaling molecule, is effective in the drought tolerance of wheat. The roles of NO in the amelioration of water stress are shown in Table 2. Karpets et al. (2020) investigated the effects of BR and NO applied to wheat on high temperature tolerance and antioxidant enzyme activity. It was revealed that 0.2 mM SNP and 20 nM EBL applications increased the survival rate of plants after 10 min. of heat stress at 46 °C, and their combined applications had a more protective effect. Heat stress increased the amount of MDA. It was shown that the combination applications of EBL and SNP (low concentration) applied to plants eliminated this effect, on the contrary, it increased at high concentrations. Oxidative stress caused by drought stress represents a significant limiting factor for wheat cultivation in dryland regions. The studies demonstrate that applying SNP to wheat plants under drought stress significantly increases the activity of antioxidant enzymes during dry periods. It was found to be more effective, particularly in low-concentration SNP applications. This indicates that NO application can be an effective method for decreasing the effects of drought stress and increasing stress tolerance and productivity. Identifying the source, production, and control of ROS and NO is critical to understanding the cellular response of plants to adverse conditions. Further research into how NO functions biologically is important to elucidate this process.

5. CONCLUSION

Drought-induced water constraint creates a serious risk to crop production. Plant cellular redox homeostasis is disrupted by drought, which results in oxidative stress and cell damage. Plants change their physiology, morphology, biochemistry, and molecular processes in response to drought stress. Several studies have demonstrated the part of NO as a signaling molecule in triggering ROS scavengers under abiotic stress factors such as drought (Table 2). According to the report NO protects the plant from the damaging effects of stress by causing transcriptional alterations in various pathways related to signal transduction, ROS metabolism, and plant defense (Lau et al., 2021). Given that wheat is the primary food source in the world and that global warming is getting worse, more research is needed to create wheat varieties that are more resilient to environmental stressors. According to studies on NO production, functions, and mechanisms control in response to drought stress NO increases drought tolerance by induced stress defense genes and the antioxidant defense system of plants. (Sidana et al., 2015). The first notable aspect of the interaction between NO, hormones, and ROS is the ABA-induced stomata closure.

Table 2: NO applications that regulate antioxidant defense and ROS detoxification in wheat plants under drought stress.

Stress	**Concentration**	**Application method**	**Effect of NO application on drought stress-induced changes in antioxidant properties in wheat**	**References**
Heat Stress 40°C 6h	100µM SNP and 50mM Proline	Exogen	High H_2O_2 and $TBAR_S$ under oxidative stress. Increase activity of GR, SOD and APX. Enhance photosynthetic capabilities	Sahar et al., 2023
Heat Stress 40°C, 6h, 15d	100µM SNP and ABA	Exogen	Modulates redox homeostasis, increase photosynthetic activity, ameliorate the negative effects of stress	Iqbal et al., 2022
Heat Stress 46°C10min	20-200nM EBL 0.1-2.0mM SNP	Exogen	Effective application concentrations of 20 nM EBL and 0.2 mM SNP	Karpets et al.,2021
Drought stress	10^{-4} and 10^{-5} M SNP	Priming	Increase RWC and chlorophyll content. Highest increase in germination speed, germination percentage (10^{-4}M SNP)	Bibi et al., 2020
Drought stress 12% PEG	200µM SNP	Exogen	Increase GSH content, decrease stress-induced MDA, and decreased GST activity	Maslennikova et al., 2020
Drought stress 15% and 30% PEG 9 d	100µM SNP	Exogen	Increase antioxidant capacity Increase the endogenous RWC, NO, and proline content	Hassanuzman et al., 2018
Drought stress 75% and 50% soil moisture 6h	100µM SNP	Exogen	Increase in seedling length, total proteins, net photosynthetic rate and intercellular CO_2 concentration, decrease in H_2O_2 and MDA content	Faraji et al., 2020
Drought stress 7.5% PEG 5d	5µM SNP	Hoagland Nutrient Solution	Increase in physiological parameters, especially root length	Wu et al., 2019
Drought stress PEG 24h	100µMSNP (12h before stress)	Exogen	Increase of AOX_1a and alternative pathway	Wang et al., 2016
72 h of PEG stress, recovery	0.2 mmol l^{-1} SNP	Exogen	Increasing antioxidant enzymes activities, as well as protecting PSII	Wang et al., 2011
Drought stress 15% PEG	0,2mM- 2mM SNP	Exogen	Stimulation of the antioxidant system and osmotic adjustment	Tian and Lei, 2006
Drought stress 15% PEG	0,2mM SNP	Exogen	Regulation of water status	Khan et al., 2017
Drought stress	0,3mmol L SNP	Exogen	Modulation of antioxidant system	Tan et al., 2008
Drought stress	SNP	Exogen	Regulation of water status Protective effects on photosynthesis Increase in biomass and BSI	Shao et al., 2018
Drought stress −0.4 MPa	100µM SNP	Priming	Decrease in lipid peroxidation, antioxidant protection by increasing SOD and GR activity	Naser Alavi et al., 2014

Drought stress 35%water holding capacity	50, 100, and 150µM SNP 50, 100, and 150µm H_2O_2 (7d ago)	Foliage applied stress	Improved the accumulation of phenolics, proline and GB, Reduction in MDA contents High chlorophyll contents Sustain the membranes Decreased lipid peroxidation increased yield, proline, and chlorophyll content	Farooq et al., 2017
Drought stress 72h PEG 48h recovery	0.2 mmol l^{-1} SNP	Exogen	RWC decreased after 72 h of PEG stress, along with an obvious decrease in chlorophyll content, increase in SOD, CAT, APX and MDA content. Enhance the stress resistance of wheat plant increasing antioxidant enzymes activities protecting important gene transcription in PSII	Whang et al., 2011
Drought stress 12% PEG	200µM SNP	Exogen	It promoted germination and seedling growth. Preserved RWC and ABA content MDA reduced.	Allagulova et al., 2023

Hormones such as ABA, AUX, GB, ET, and others have been found to interact either antagonistically or synergistically with RNS (NO) to regulate drought stress (Saddhe et al., 2019; Yavaş et al., 2020).

These findings suggest that signaling via crosstalk with NO and phytohormones contributes to stress tolerance (Iqbal et al., 2022).

They support the ability of NO to participate in plant defense against oxidative stress under drought. However, the knowledge about the role of phytohormones and NO in the functioning of the signaling network of plant cells is insufficient and requires further research. Understanding the basic mechanisms of plant adaptation and developing new biotechnological strategies for developing plants resistant to stress factors are prominent.

The need to implement climate change adaptation policies in agriculture is heightened by the intensifying effects of climate change, extreme heat, rainfall, and drought conditions. The use of smart technologies in agricultural food systems against stresses such as climate change and water scarcity and their transformation into climate tolerance with green practices are of critical importance for the future of agriculture. On the other hand, resilient and sustainable food systems require sustainable practices that preserve soil ecosystems and efficiently assess resources.

6. ACKNOWLEDGEMENT

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CHAPTER 3

MORPHOLOGICAL AND MOLECULAR CYTOGENETIC STUDIES IN HEMP (*Cannabis sativa* L.)

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1. INTRODUCTION

Hemp is an ancient plant that is agriculturally important. It is a cultivated plant grown for the fibers obtained from its stem and oil obtained from its seed. Hemp (*Cannabis sativa* L.), whose homeland is Central Asia, is widely used in many areas around the world. The leaves, flowers, seeds, roots, extracts and leaves obtained from the plant are used in many areas. emp serves a multitude of purposes across various industries. Female hemp flowers contain trichomes that are potent phytocannabinoids for instance cannabidiol (CBD) which have been extensively studied in clinical trials for their medical applications. The stalks of hemp are valuable for manufacturing textiles, paper, and construction materials. Traditionally, its roots have been utilized in herbal remedies. Besides, hemp seeds can be processed to produce biofuel or pressed to extract oil suitable for human consumption. Furthermore, the protein-rich seed residues left after oil extraction can be repurposed as animal feed, highlighting hemp's versatility (Schilling et al., 2020; Yılmaz and Yazici, 2022).

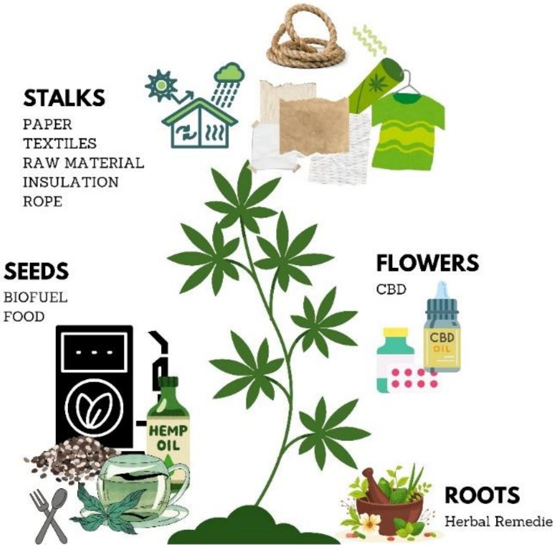


Figure 1: Hemp is versatile crops (Modified from Schilling et al., 2020).

Hemp (*C. sativa* L.) is an annual herbaceous plant with a wide range of products in the industrial area due to its versatile use. The taxonomic classification of *C. sativa* L. is given below.

Kingdom: Plantae
Division: Magnoliophyta
Class: Equisetopsida
Order: Rosales
Family: *Cannabaceae*
Genus: *Cannabis*
Species: *Cannabis sativa* L.

The flowers and leaves of *C. sativa* L. synthesize abundant terpene-derived compounds. In addition, 10% of the resin secreted from the trichomes (hair-like projections) is composed of terpenoids (Farag & Kayser, 2017). The leaves of *C. sativa* L. are covered with feathers. These feathers are divided into glandular and cytolytic. Glandular feathers produce and store resin, while cytolytic feathers store calcium bicarbonate. The terpenes with the highest volatility are myrcene, limonene and pinene (Jia et al., 2013). Research suggests that hemp contains more than 200 terpene-derived compounds. These terpenes are the part that gives the plant its characteristic odor. Although terpenes are found in the leaves, the amount is higher in the root and seed than in the leaves. For this reason, when the oil of the seed is used, the properties gained thanks to the terpenes are also provided in the oil form. Therefore, terpenes and their properties are important (Souto-Maior et al., 2017).

According to studies, 565 compounds have been isolated from hemp (*C. sativa* L.). More than 120 of these compounds are more specific to *Cannabis* species and are phytocannabinoids with cannabinoid activity (ElSohly and Gul, 2014). Phytocannabinoids, such as cannabigerol, cannabichromene, cannabidiol, and tetrahydrocannabinol, are among the most extensively studied secondary metabolites in plants. These organic compounds interact with the body's cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2), triggering various pharmacological effects. Additionally, they play a crucial role in the plant's defense mechanisms (Yıldırım and Çalışkan, 2020). Thanks to its phytocannabinoid content, it has been reported that the hemp can improve diabetic symptoms, show anti-inflammatory effects, and is also used as a drug in the treatment of various types of cancer, nervous system diseases such as Alzhemir, Parkinson's (Booth et al., 2017).

Hemp is primarily recognized for its ability to biosynthesize cannabinoids, with Δ9-tetrahydrocannabinol (Δ9-THC, commonly referred to as THC) and cannabidiol (CBD) being the most notable. Besides THC and CBD, several cannabinoids have been identified for their pharmacological effects such as 9-tetrahydrocannabivarin (THCV) (McPartland et al., 2014) and cannabigerol (CBG) (Borrelli et al., 2014).While the processes behind the biosynthesis of medicinally significant cannabinoids and terpenes are not yet fully understood, further genetic and genomic research is expected to shed light on the diverse production mechanisms exhibited by various cannabis genotypes.

THC is most concentrated in the female inflorescences (bracts) of *C. sativa* L., making female plants the primary focus for economic cultivation. Pollination significantly reduces the size of female inflorescences and their THC content (Small, 2015), emphasizing the importance of preventing male plants from growing, as they consume valuable resources, labor, and space without contributing to yield. Beyond THC production, hemp is gaining attention as a sustainable crop for producing fibers and oils. Hemp cultivars are typically low in THC, making them legal to grow in many countries where marijuana remains prohibited. The distinct characteristics of male and female hemp plants, combined with the benefits of early sexing, further highlight the importance of efficient cultivation practices (Andre et al., 2016).

C. sativa L. is a diploid plant (2n = 2x = 20), with sex determined by an XY chromosome pair in addition to nine autosomes (Sakamoto et al., 1995; Divashuk et al., 2014). Like many dioecious plants, its sexual dimorphism is minimal, and the sex of individual plants can only be reliably identified when flowering begins (Small, 2015). While several Y-linked genetic markers have been discovered and are used for sexing seedlings (Techen et al., 2010), their effectiveness across all cultivars remains uncertain. Sequencing the *C. sativa* L. sex chromosomes could provide a valuable genomic resource to enhance agricultural productivity. However, despite significant advancements in genome assembly through recent projects, sex chromosomes have yet to be successfully identified at the chromosomal level (Grassa et al., 2018; Laverty et al., 2019).

2. HEMP MORPHOLOGY and GENES

Humans have used hemp for thousands of years for its fibers, physiological and psychological effects, and its seeds in food and oil production.

Hemp was first named *Cannabis sativa* L. by Carlous Linnaeus in 1753. In 1785, the famous French biologist Jean-Baptiste Lamarck described the *Cannabis indica* species, which originated in India and was morphologically different from *C. sativa*, and in 1924, Janischevsky discovered the *Cannabis ruderalis* species in Russia, which had different characteristics from the other two species. Although some botanists think that *C. indica* and *C. ruderalis* are subspecies of *C. sativa,* it is possible to say that there are three species of hemp according to the common opinion of many botanists (Yıldırım and Çalışkan, 2020). Hemp is a woody, multipurpose industrial plant member of the Cannabaceae family of the Urticales order, whose homeland is Asia (Küçüktopçu et al., 2020). Considering the number of species in the genus *Cannabis* and the classification of these species, the classification of subspecies is as follows.

Cannabis sativa L. ssp. *sativa*

Cannabis sativa L. ssp. *indica*

Cannabis sativa L. ssp. *gigantea*

Cannabis sativa L. ssp. *ruderalis*

These four species are reported to differ from each other in terms of the way they are cultivated, flowering time, anatomical structure and the chemicals they produce. The Latins called hemp "*Cannabis sativa*", while the Ottoman Empire called it "Kendir" in Turkish. When we look at the history of hemp, it was first seen in Asia and India around 850 BC. It was found that one of the main purposes of using hemp was to obtain oil from its seed, so it was started to be cultivated (McPartland, 2018; Akpınar and Nizamoğlu, 2019; Göre and Kurt, 2020).

C. sativa L. can be dioecious (dioecious) and monoecious (monoecious). Generally, cannabis is a dioecious plant. Female and male flowers are at different plants called dioecious plants and different sex hemp's flowers bloom at different times (Aksoy and Aytaç, 2021). Hemp, which is one of the short-day plants, starts flowering at the end of two weeks regardless of day length when grown commercially (Chandra et al., 2017).

Hemp has an upright growing, tap rooted structure. The stem of the plant has a hard structure, and the length of the stem varies between 1 and 5 meters depending on the climate, soil conditions and variety. The edges of hemp leaves are toothed, veined, green in color, and the seeds are speckled in light brown-greenish color and elliptical in shape (Yılmaz et al., 2023). The plant is cultivated in two species, industrial and medical, depending on its intended use and these species have different morphological characteristics (Appendino et al., 2011 In female hemp plants, the stems tend to be thicker, resulting in a higher fiber yield. In contrast, male cannabis plants have thinner stems and produce less fiber overall. However, the fiber quality is generally superior in male plants compared to their female counterparts. In addition, the female plant is important in production for medical purposes.

Figure 2: Life cycle of *Cannabis sativa*. (a) Cotyledons and first true leaves. (b) Male cannabis flower. (c - d) Female cannabis flower before pollination. (e) Female flower after pollination. (f) Seed-bearing female flower. (g) Cannabis seeds. (Photographs taken by Doğan and Aksoy at greenhouses of Yozgat Bozok University, Cannabis Research Institute in 2024)

Hemp, with its rich phytochemical content, has been utilized since ancient times across various fields, particularly in health. Hemp, known for containing hundreds of bioactive compounds, is a source of unique secondary metabolites such as phytocannabinoids, terpenes, and flavonoids (Arnold, 2021). When plant parts or products rich in these metabolites are used for therapeutic purposes, the plant is referred to as medicinal hemp (Bridgeman and

Abazia, 2017). Δ9-THC and CBD phytocannabinoids, which have psychoactive properties, are the most researched and therapeutic effects in the plant; It shows its therapeutic effects through the endocannabinoid system in the human body, which is involved in many physiological events. Plants are classified into chemotypes I-V according to their cannabinoid profiles. Chemotype I are drug-type plants with higher THC content; chemotype II are plants containing fiber and drug-type intermediate properties; chemotypes III and IV are plants with high amounts of CBD and non-psychoactive components; chemotype V are fiber-type plants with rare cannabinoids (Pellati et al., 2018; Abu-Amna et al., 2021).

Industrial hemp is known to have low Δ9-THC and high CBD content. The legal upper limit for current industrial hemp cultivation in most European countries is 0.2% Δ9-THC. In female strains of *C. sativa* subsp. *indica,* the THC content varies between 5-35%. In general, narcotic products containing the THC necessary to produce are obtained from female hemp. However, the THC content is also affected by environmental factors such as temperature and day length. THC rate varies according to the change in environmental conditions (Shi et al., 2021).

For cannabinoid biosynthesis, there is a journey that starts with gene expression and leads to synthesis by enzymes. The biosynthesis of cannabinoids is facilitated by various oxidocyclases, with the most notable being cannabidiolic acid synthase (CBDAS), and cannabichromenic acid synthase (CBCAS) and tetrahydrocannabinolic acid synthase (THCAS). These enzymes are responsible for the production of cannabidiolic acid (CBDA), and cannabichromenic acid (CBCA) and tetrahydrocannabinolic acid (THCA), respectively, leading to their accumulation in the plant (Gülck and Moller, 2020). These enzymatic activities start with THCAS, CBDAS and CBCAS genes expression.

The Berberine Bridge Enzyme (BBE)-like gene family includes the genes responsible for synthesizing tetrahydrocannabinolic acid (THCAS), cannabidiolic acid (CBDAS), and cannabichromenic acid (CBCAS) (Taura et al., 1996; Sirikantaramas et al., 2004). These genes serve as markers for detecting cannabichromenic acid-synthase at the genomic level and enable the analysis of transcriptional profiles in *C. sativa* subsp. *sativa* and *C. sativa* subsp. *indica*. Prevalently the cannabinoid synthesis genetics are studied and discovered different genes encoding these enzymes which include single exon. THCAS and CBCAS share a high degree of amino acid sequence similarity, with 92% identity, while THCAS and CBCAS exhibit 84% and 83% identity to CBDAS, respectively. Although THCAS and CBDAS have been thoroughly researched at genetic and molecular levels, relatively little is known about the CBCAS genes (Sirikantaramas et al., 2004; van Velzen et al., 2020). According to Prentout et al. (2020), sequenced the genome of hemp and determined the chromosomal location of THC/CBD synthase genes *in silico*. While 1 genomic locus was detected on chromosomes 2, 5 and 6, 5 different genomic loci were detected on chromosome 7. The high transcription levels of tetrahydrocannabinolic acid synthase and cannabidiolic acid synthase strongly correlate with the chemical phenotype of *C. sativa* L. In contrast, the consistently low but stable transcription levels of cannabichromenic acid synthase across all genotypes indicate that these genes are active and may contribute to the overall cannabinoid content (Fulvio et al., 2021). The cannabinoid content in *C. sativa* L. is important for the added value of the plant. Therefore, cannabinoid synthase genes constitute a key point.

The diverse applications of hemp drive efforts to develop high-performing cultivars with enhanced cannabinoid production, improved fiber yield, increased disease resist, and superior food quality. The rising demand for cannabinoids presents a potential to create high yielding cultivars through innovative techniques. However, further research is required to evaluate potential trade-offs associated with these technologies. For instance, genome editing to knock out the THC acid synthase gene offers a pathway to produce THC-free, high-CBD plants, which could be of significant value in regions with stringent THC regulations (Dolgin, 2019).

A patent from Canopy Growth Corporation describes the overexpression of genes involved in trichome development. Results show significantly higher trichome density and a tenfold increase in THC production compared to unamended plants (Dolgin, 2019). Using non-transgenic genome editing techniques, the expression of a target gene can be enhanced by modifying regulatory elements, such as enhancers or promoters, located genes upstream. This approach enables increased gene expression without the need to introduce foreign genetic material. These genome editing tools also provide a precise and efficient method for developing disease-resistant cannabis varieties, offering a significant advantage over traditional breeding techniques (Mihalyov and Garfinkel, 2021).

Hemp is a common dioecious plant and sex determination is crucial for production and breeding purposes. Plant morphology, physiology and genetics differ depending on the sex of the hemp (Figure 2 and figure 3). Considering the production purpose, the sex should be considered. For example, female plants are ideal for cannabinoid synthesis, while male plants are ideal for fiber production. Sex determination in hemp is therefore an important and topical issue. The chromosome karyotyping and cytogenetic content of the hemp which contains 9 pairs of autosomes and 1 pair of sex chromosomes, is important to clarify this situation. In the next section, the cytogenetics of *C. sativa* L. will be examined from this perspective (Razumova et al., 2016).

3. CHROMOSOME BIOLOGY OF HEMP

The genome is a unit of inheritance, and its size represents one copy of chromosomal DNA, chloroplast DNA and mitochondrial DNA for plants. The genome size of the cannabis plant varies in male and female plants due to sex chromosomes. The genome sizes of the diploid male and female plants were identified to be 818 and 834 Mbp, respectively (Braich et al., 2020). The chromosomes, which ensure the correct transmission of genetic information, reach their full form during cell division, during meiosis or mitosis in the metaphase phase. *C. sativa* L., which has dioecious and monoecious forms, is a diploid plant with $2n=2x=20$ chromosomes. Of these chromosome pairs, 9 are autosomes and 1 pair is a sex chromosome. While female and monoecious plants have only X chromosomes, male plants have X and Y chromosomes.

The genetic basis of sex determination in *C. sativa* L. remains a subject of debate, particularly regarding the roles of the Y chromosome and autosomes. Some researchers suggest that *C. sativa* L. has XY sex chromosomes but follows an X:0 sex determination system, where the Y chromosome does not influence sex, and the difference between males and females is determined by the number of X chromosomes (Faux et al., 2014). Conversely, others propose that sex is determined by an active Y chromosome system, with no involvement of autosomes, resembling the mechanism found in humans (Sakamoto et al., 1998).

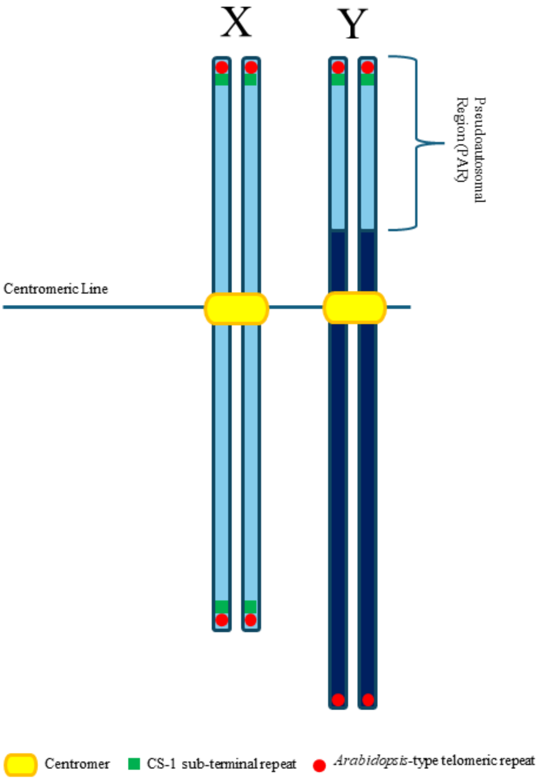


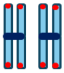
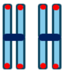
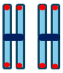
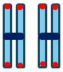
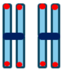
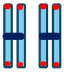
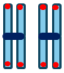
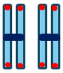
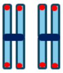
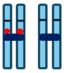
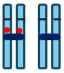
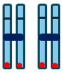
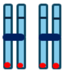
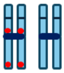
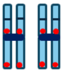
Figure 3: Ideogram of the *C. sativa* L. XY chromosomes during meiosis.hybridization sites (Modified from Divashuk et al., 2014; Karlovet et al., 2017).

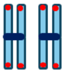
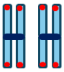
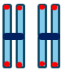
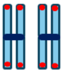
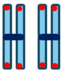
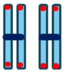
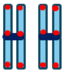
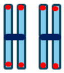
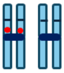
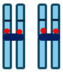
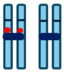
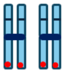
Given that *Cannabis* species belongs to the same family as *Humulus lupulus*, which follows an X:0 system, it seems plausible that *C. sativa* L. may also exhibit an X:0 effect in sex determination. However, the specific role of the Y chromosome in this process remains unclear and warrants further investigation (Skof et al., 2012; Vergara et al., 2016).

The ideogram of the sex chromosomes during meiotic division of the *C. sativa* L. plant is shown in Figure 3. In this ideogram CS-1 repeat, Arabidopsis-type telomeric repeat was used as a marker (Divashuk et al., 2013).

Sexual dimorphism in hemp (*C. sativa* L.) is relatively weak, as is common in many dioecious plants (Divashuk et al., 2013). The sex of the plant could only be definitively identified once flowering begins (Small, 2015). As a dioecious species, *C. sativa* L. determines sex through an XY chromosome pair.

Table 1: Dioecious and monoecious hemp cultivars' cytotypes. CS-1 subtelomeric repeat probe's is a marker. (Modified from Razumova et al., 2016)

Cultivar	**Igorkin**				**Zenitsa**		**Diana, Rigs, Kubanka**		
Sex	Male		Female		Male	Female	Monoecious		
Cytotype	1	2	3	4	5	6	7	8	3
Sex Chromosomes	XY	XY	XX	XX	XY	XX	XX	XX	XX
Chromosome 2									
Chromosome 9 (NOR-Bearing Chromosome)									

Cultivar	**Gentus**	**Margo**			**Ingreda**			**Maria, Tzivilsky Skorospeliy**
Sex	Monoecious	Monoecious			Monoecious			Monoecious
Cytotype	3	3	4	9	3	7	10	6
Sex Chromosomes	XX	XX	XX	XX	XX	XX	XX	XX
Chromosome 2								
Chromosome 9 (NOR-Bearing Chromosome)								

A limited number of Y-linked genetic markers have been discovered and are currently used for sexing *C. sativa* L. seedlings. But it remains unclear whether these markers are effective across all cultivars (Techen et al., 2010; Barrett and Hough, 2013; Prentout et al., 2020).

In hemp, chromosome characteristics differ depending on whether the variety is dioecious or monoecious. Therefore Razumova et al., 2016 used as a marker which is CS-1 subtelomeric repeat and compared to sex chromosomes on different *C. sativa* L. varieties. They used to dioecious (Igorkin and Zenitsa) and monoecious (Maria, Tzivilsky, Skorospeliy, Gentus, Diana, Rigs, Kubanka, Margo, Ingreda) varieties. Ideogram of sex chromosomes on different varieties and genders is shown at Table 1. This study is important for sex determination by cytogenetics and molecular tools. Cause they used male specific marker MADC2. As a PCR results only male hemp plants are positive and monoecious hemp are negative.

On the other hand, chromosome karyotyping of hemp is as important as the determination of sex chromosomes. Understanding the chromosome biology of hemp is the building block for elucidating the plant's genetic code. The study of meiosis and mitosis in the hemp has been taking place since the early 1900s. Firstly Divashuk et al. (2014), designed a karyotype marker of CS-1, 5S rDNA and 45 rDNA. Then Romanov et al. (2022), develops oligo probes which are specific to hemp. CS-1 and CS-237 repeats' chromosomal location are detected. Alexandrov et al. (2022), used to as a marker CS-237, CS-1, 5S rDNA and 45 rDNA on *C. sativa* L. Based on these studies with classical and molecular methods, we have created an ideogram of the hemp (Figure 4).

4. CONCLUSION

Although we have some information on the cytogenetics of hemp, the sex chromosomes of *C. sativa* L. and their impact on sex determination are still a matter of debate. *C. sativa* L. differ significantly morphologically in different sexes, and this affects the purpose of production. The elucidation of this situation and the discovery of the mechanism will make an important contribution to both production activities and breeding studies. In addition, increasing the synthesis of cannabinoids in female plants rich in cannabinoids will also progress by obtaining basic information such as physical mapping and characterization of the relevant genes. Therefore, molecular cytogenic studies is limited in *C. sativa* L. and we suggested to fill in the gap at literature.

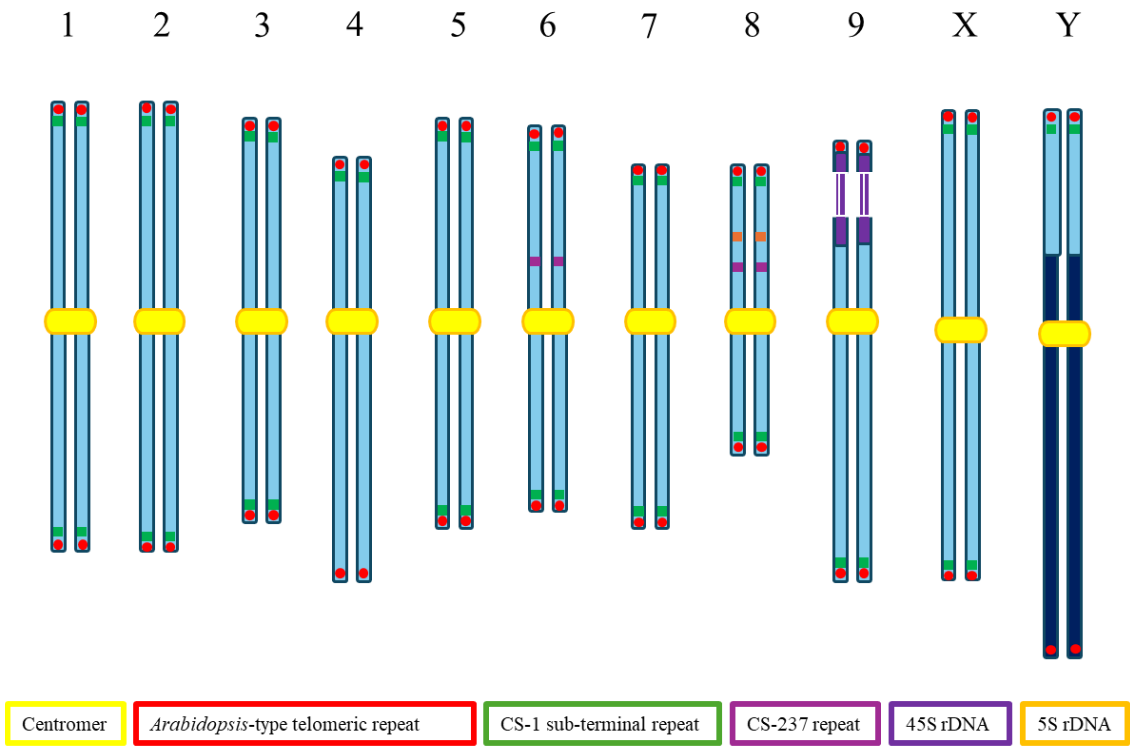


Figure 4: Ideogram of *C. sativa* L. (Modified from Divashuk et al., 2014; Alexandrov et al., 2022; Divashuk et al., 2022)

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CHAPTER 4

EFFECTS OF HYDROGEN SULFIDE ON ANTIOXIDANT DEFENCE IN DROUGHT TOLERANCE OF BARLEY

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1. INTRODUCTION

Barley (*Hordeum vulgare* L.) is a cereal crop that is widely grown worldwide and has strategic importance in agricultural production. However, it is sensitive to drought stress. Drought has a significant impact on plant growth, affecting water uptake, photosynthetic efficiency and oxidative stress, which collectively contribute to a reduction in growth and yield. Recent research has demonstrated that hydrogen sulfide (H_2S) functions as a pivotal signaling molecule within plants, linked to reactive sulfur species (RSS). This compound enhances plants' stress resilience by reinforcing their antioxidant defense systems. Plants synthesize H_2S through enzymatic and non-enzymatic means and activate cellular defense mechanisms under abiotic stress conditions such as drought. Thus, H_2S applications offer a promising biotechnological approach to ensure sustainability and increased yield in agricultural production. Research on hydrogen sulphide donors, inhibitors and scavengers reveals that this molecule plays a key role in plant stress management.

Exogenous applications of H_2S create various positive effects in plants such as increasing water use efficiency (WUE), regulating stomatal conductance and alleviating oxidative stress. Especially in barley, studies show that H_2S applications have a protective effect on water balance, photosynthetic capacity, and antioxidant defense systems of plants under drought stress. Thus, H_2S applications offer a promising biotechnological approach to achieve sustainability and yield enhancement in agricultural production.

This chapter focuses on recent advances on the role of H_2S on drought tolerance in barley and its potential impacts on agricultural production.

2. STRESS FACTORS IN PLANTS AND BARLEY

Since plants are sedentary, they encounter a variety of challenging external factors throughout their life cycle that affect their growth and productivity. “Stress” occurs when growth and development conditions are difficult for plants. These stress factors can be divided into two main groups: abiotic stresses (temperature, pH, high salt content, drought, etc.) and biotic stresses (pathogenic organisms such as viruses and parasites) (Ghosh et al., 2024).

Highly reactive oxygen metabolites that occur as a byproduct of respiration and photosynthesis in living organisms are called "Reactive oxygen species (ROS)". These species include hydroxyl radical (OH^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and superoxide anion (O_2^{*-}), free radicals, which are very harmful to the cell (Sachdev et al., 2023).

ROS is an acronym that collectively denotes a multitude of oxygen-based radical and non-radical substances that are produced in diverse organelles within cells. This includes root and shoot meristems, leaves, the apoplast, mitochondria, chloroplasts, and peroxisomes (Garcia-Caparros et al., 2021). When produced under normal conditions, detoxification is provided by the antioxidant defence system (Yavaş and İlker, 2020). Recent studies have also suggested that ROS may contribute to metabolic regulation and stress response processes, aiding in cellular adaptation (Sies and Jones, 2020). Nevertheless, research related to ROS has concentrated on regulatory systems involving ROS, including (a) cell cycle, (b) abiotic stress response, (c) systemic signalling, (d) programmed cell death, (e) pathogen defence, and (f) development (Guo et al., 2023). It is proposed that low concentrations of ROS have a cytostatic impact on cells, while elevated ROS levels are considered cytotoxic. Consequently, a basal ROS level is essential for the normal functioning of ROS and redox signalling in cells, and this level is maintained by the equilibrium between ROS production and ROS scavenging (Mittler, 2017) (Figure 1). ROS are essentially unstable and attack and damage subcellular parts to become stable. In the event of an excessive accumulation of ROS, these molecules have the potential to damage cellular components, including DNA, proteins, and lipids, and to inhibit vital plant activities (Choudhary et al., 2020). In plants subjected to severe stress factors, the antioxidant defence system is inhibited, and ROS formation can result in oxidative tissue damage/oxidative stress in the plant (Nadarajah, 2024).

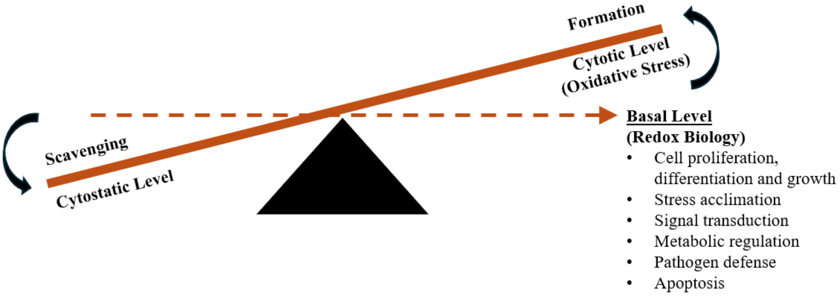

Figure 1: The impact of varying levels of ROS on the regulation of diverse cellular processes (Modified from Mittler, 2017).

Maintaining cellular homeostasis is essential for plants. Consequently, in response to changing conditions, plants produce both enzymatic and non-enzymatic scavengers to regulate ROS levels and prevent oxidative damage to cells (Garcia-Caparros et al., 2021).

Enzymatic antioxidants:

- Superoxide dismutase (SOD); The conversion of harmful O_2^{*-} radicals into H_2O_2 and oxygen (O_2) is the task of this antioxidant enzyme.
- Catalase (CAT): This enzyme facilitates the breakdown of H_2O_2 into water (H_2O) and oxygen (O_2).
- Ascorbate peroxidase (APX) catalyzes the reduction of H_2O_2 to H_2O, using ascorbate (AsA) as a reducing agent.
- Peroxidases (POX) catalyze the reduction of O_2 to either O_2^{*-} or H_2O_2. They also facilitate the oxidation of various substrates.
- Glutathione reductase (GR) catalyzes the reduction of glutathione disulfide (GSSG) to its reduced form, glutathione (GSH), as part of the ascorbate-glutathione cycle. This process is known as the AsA-GSH cycle and is essential for the clearance of hydrogen peroxide (H_2O_2; a highly reactive oxygen species).
- Monodehydroascorbate reductase (MDHAR); The enzyme in question is crucial for the synthesis and regeneration of ascorbic acid.
- Dehydroascorbate reductase (DHAR); It plays a role in the clearance of AsA by catalyzing its GSH-dependent reduction (Garcia-Caparros et al., 2021; Huchzermeyer et al., 2022; Syman et al., 2024) (Table 1).

Non-enzymatic antioxidants: GSH, α-tocopherol, ascorbic acid, carotenoids, flavonoids, and other members of the antioxidant mechanism such as plastoquinone/plastocyanin (Garcia-Caparros et al., 2021; Huchzermeyer et al., 2022).

Table 1: Intracellular localization of major antioxidant enzymes in plants (Huchzermeyer et al., 2022).

Enzyme	Localization in cell
GR	Cytosol, mitochondria and plastids
DHAR	Cytosol
MDAR	Cytosol, mitochondria, peroxisomes and plastids
POX	Apoplast, cell wall and vacuole
APX	Mitochondria, plastids, cytosol and peroxisomes
SOD	Cytosol, mitochondria and plastids
CAT	Peroxisomes and mitochondria

H. vulgare L. naturally exhibits higher abiotic stress tolerance than other crops and has significant potential as human food in drought-affected regions of the world (Rohman et al., 2020). Nevertheless, barley is among the cereal crops most affected by environmental stresses, particularly salinity and drought (Fatemi et al., 2022). According to the Turkish Grain Board (TGB) 86th Accounting Period Activity Report, the world barley (154 million tons) production in 2022/2023 ranks fourth after corn (1.165 million tons), rice (515 million tons) and wheat (804 million tons), while according to the Agricultural Economic and Policy Development Institute (AEPD) 2023 barley product report, barley ranks second after wheat in our country in terms of planting area (AEPD, 2023; TGB, 2024).

3. EFFECTS OF DROUGHT STRESS ON BARLEY

Drought is one of the most impactful abiotic stress factors influencing plant growth, metabolism, and development, often leading to substantial reductions in agricultural productivity. The adverse effects of drought on plant growth and crop yield are thoroughly documented (Saini et al., 2024). While water shortage has become the main problem of approximately 40% of the world's population, it is predicted that increasing temperatures will make dry areas even drier by 2030. The effects of drought can be mitigated by early measures (Elakhdar et al., 2022). Numerous studies have investigated the effects of excessive ROS production in barley under drought stress.

3.1. Physiological and Biochemical Effects

The physiological and biochemical damage caused by drought stress gives rise to oxidative stress because of the formation of ROS. A range of physiological and biochemical responses have been recognized as adaptations to drought stress. In C3 type photosynthetic plants, under normal conditions, low stomatal conductance, low net photosynthesis and transpiration rate, and high WUE are achieved with “water saving”. With the “water spending” strategy, stomata remain open until a serious water deficit occurs, resulting in higher net photosynthesis and transpiration rate and low WUE. In order to maintain water balance, plants are able to increase the rate of water uptake by their roots in response to stressful conditions, while simultaneously reducing the rate of water loss through cuticle and stomatal transpiration (Kosová et al., 2023). There are many important physiological and biochemical traits that alleviate the effects of drought stress on plants of the *Poaceae* family, such as barley and wheat. The main physiological changes that occur in tolerant and susceptible genotypes are shown in Figure 2.

A study using nine different Moroccan barley cultivars revealed that drought stress significantly increased water-soluble carbohydrates, H_2O_2, electrolyte leakage, malondialdehyde (MDA) and soluble protein concentrations, while simultaneously decreasing total chlorophyll content (SPAD), shoot dry weight and RWC (Ferioun et al., 2023).

In another study, in barley genotypes with different drought tolerance, significant decreases in morphological (seedling length, fresh and dry weight) and physiological (leaf turgor weight, SPAD) results were observed with increasing drought stress, especially when soil water capacity was measured at 20% and 10%, while an increase in proline amount was observed (Yetilmezler, 2023).

In the face of increasing environmental challenges, understanding plant responses to abiotic stress is crucial for sustainable agriculture. Alsamadany et al. (2024) investigated the complex interaction between salt and drought stress in barley and found that drought stress caused a significant decrease in chlorophyll levels, as well as a gradual decrease in RWC, water potential and osmotic potential under stress conditions. SOD and CAT activities, which are critical antioxidant enzyme activities in the fight against oxidative stress, were also reported to increase under drought stress.

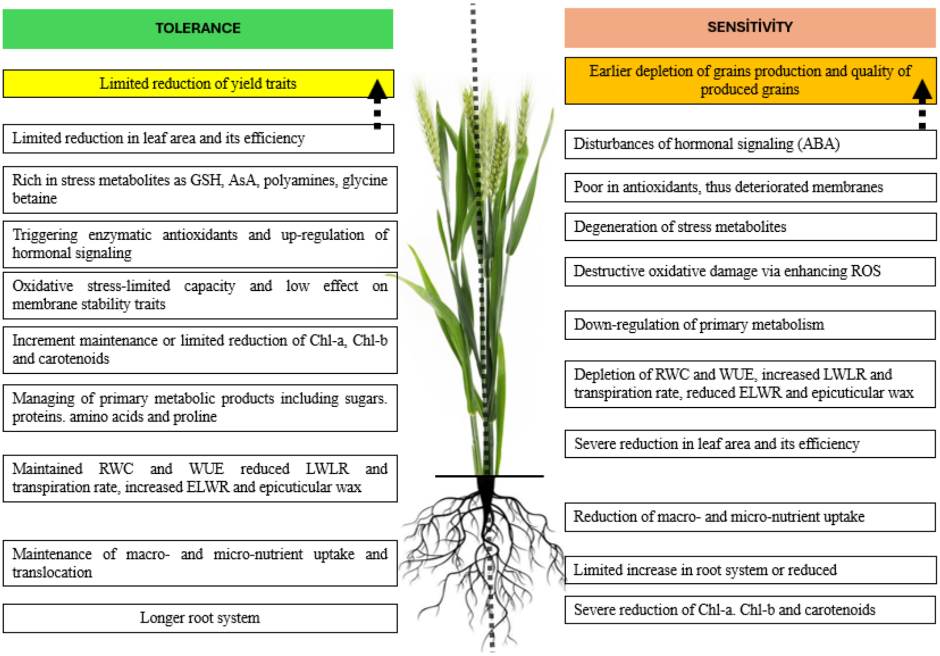

Figure 2: Physiological and biochemical responses observed in tolerant and sensitive gramineous grass genotypes under drought stress (ROS; Reactive oxygen species, GSH; reduced glutathione, LWLR; Leaf water lose rate, AsA; ascorbate, ELWR; Excised leaf water retention, RWC; Relative water content, Chl-a and Chl-b; Chlorophyll -a and -b pigments, WUE; Water use efficiency, ABA; Abscisic acid) (Modified from Sallam et al., 2019).

A field study was conducted to investigate the effects of drought stress on the physiological and morphological characteristics of Mediterranean barley cultivars, determining leaf area, free amino acids, chlorophyll and proline contents. It has been stated that SPAD, RWC and leaf area decrease with drought stress, while proline contents increase. The use of amino acids as biostimulants that significantly repair injuries caused by root growth and drought stress is well known. In the study, it was reported that the amino acid contents of especially Algerian barley varieties were high under drought stress, and the lowest percentage of decrease in terms of biochemical parameters examined under drought stress was in Egyptian and Tunisian barley varieties (Hellal et al., 2020).

In another study investigating biochemical and physiological traits in drought-resistant and susceptible wild barley genotypes grown under drought stress; the effects of drought stress were evaluated at three levels (90-95% FC (field capacity), 50-55% FC and 25-30% FC). Accordingly, drought stress (especially more at 25-30% FC) caused decreases in morphological (root length, root fresh/dry weight, seedling length/dry weight) and physiological (Chl-a, Chl-b, carotenoid, total chlorophyll) traits. Similarly, it was stated that enzyme activities (CAT, POX and SOD) increased significantly (Shirvani et al., 2024).

All these studies point to different mechanisms in reducing the physiological and biochemical effects of drought in plants:

- Escape of drought
- Avoidance: Plants increase their water intake capacity through strong root systems.
- Osmotic adjustment: In order to maintain turgor, plants increase the elasticity of their cell walls.
- Metabolic resistance: Increased antioxidant metabolism in plants to adapt to severe stress.
- Abandonment: Defoliation under drought stress.
- Genetic mutations and gene modification: Plants are capable of evolving biochemical and physiological traits in response to prolonged periods of drought. These processes likely involve multiple or simultaneous responses to water deficit in barley (Elakhdar et al., 2022).

3.2. Agricultural Impacts

Grains during their growth period are frequently faced with various biotic and abiotic stresses that negatively affect their morphological and physiological properties. Climate change-induced global warming is responsible for the occurrence of increasingly severe and frequent droughts, which in turn intensify the abiotic impacts on agricultural productivity in many regions across the globe (Poudel et al., 2021; Ferioun et al., 2023). It is anticipated that these environmental conditions may precipitate significant challenges to plant growth in more than 50% of arable land by 2050 (Ferioun et al., 2023). The availability and suitability of water are the primary factors that determine the manner and method of utilization of agricultural lands. Consequently, the degree of agricultural efficiency is contingent upon the accessibility of water and its suitability for agricultural purposes (Singh et al., 2023; Suna et al., 2023; Alotaibi et al., 2024). Water scarcity has been identified by He and Rosa (2023) as the most significant environmental challenge facing the 21st century. The agricultural industry is responsible for consuming 80-90% of the global freshwater resources, with many of these resources being utilized for crop production (Tiwari et al., 2024). Addressing agricultural drought is of vital importance as it is considered as the most critical concern in terms of food security, social stability, and economic aspects in many countries (Benito-Verdugo et al., 2023). Water scarcity combined with climate change is expected to bring challenges to crop production and food security worldwide (Hebbache et al., 2024).

Barley is considered a cereal crop that is the main food source of many countries and is resistant to moderate environmental stress. It especially plays a critical role in terms of farmers' livelihood (Alotaibi et al., 2024). It is the fourth most productive cereal crop in the world, after wheat, rice and maize. It is susceptible to drought stress at different stages of its growth cycle (Alotaibi et al., 2024; TMO, 2024). In most regions where barley is produced, the level of production depends largely on drought conditions; this situation is reflected in both the yield amount and the general quality of the barley. Drought stress affects almost all growth stages of barley, causing a decrease in grain yield and loss of quality. It can also lead to functional deterioration and tissue loss in the plant (Hebbache et al., 2024).

Drought also causes significant changes in the hormonal balance in plants, especially leading to an increase in ABA levels, which play a critical role in regulating stress responses (Sallam et al., 2019; Poudel et al., 2021). Although increased ABA levels try to reduce water loss in the plant by triggering stomatal closure and expression of stress-sensitive genes, this process can have negative effects on yield (Margay et al., 2024). The development of barley varieties resistant to drought stress necessitates the use of breeding and biotechnological approaches to increase productivity in limited water conditions.

4. HYDROGEN SULFIDE, REACTIVE SULFIDE SPECIES AND ANTIOXIDANT DEFENSE SYSTEM

Plants have evolved a range of molecular and physiological defence mechanisms to enable them to withstand and adapt to environmental stress factors (Wen et al., 2024). One of these defense mechanisms involves the production of reactive molecules such as reactive carbonyl species, ROS, RSS and reactive nitrogen species (Zhou et al., 2021). ROS are defined as reactive chemical compounds formed because of incomplete reduction of molecular oxygen, while RSS are low molecular weight sulfur-containing compounds. These reactive species play a regulatory role as signalling molecules in plant metabolism within a certain concentration range but can damage cells if this concentration threshold is exceeded (Arif et al., 2021). These RSSs, which are highly reactive at the physiological level, are regarded as a crucial element of the intrinsic defense mechanisms of plants against oxidative stress (Ijaz et al., 2019).

While the accumulation of ROS leads to the production of O_2^{*-} and H_2O_2, which are extremely harmful to plants (Olson, 2021), various radicals such as thiyl radical ($RS^{\cdot}$), hydrogen persulfide (H_2S_2) and supersulfur radical (S_2^{-}) are formed with the accumulation of RSS (Olson, 2020). Both ROS and RSS participate in many interrelated signaling processes at the molecular level in plants and play a special role (Hasanuzzaman et al., 2020). Reactive species can also be produced under normal conditions in plants; however, cellular levels of these species increase when plants are exposed to stress conditions. Excessive production of reactive species is known to lead to cellular damage and subsequent cell death. However, in plants, these radicals are effectively scavenged by antioxidant defense systems and maintained at intracellular non-toxic levels, thus maintaining cellular redox balance. This is a critical process for optimal plant yield and growth (Kaur et al., 2019).

Hydrogen sulfide is a small and lipophilic molecule that mediates signalling networks in plants. This colorless and gaseous molecule is found in both eukaryotes and prokaryotes. It is synthesized in different intracellular regions in plants by both non-enzymatic and enzymatic pathways and can cross cell membranes without a receptor (Guo et al., 2016; Goyal et al., 2021). H_2S is produced in plants under both normal and stressful conditions (Hasanuzzaman et al., 2020). It is known to be effective in many plant processes such as growth, seed germination and development, photosynthesis, plant-water relationship, stomatal conductance, senescence, and abiotic stress resistance. In addition, it regulates various physiological and developmental processes under stress conditions through interactions between phytohormones such as ABA, jasmonic acid, auxin, salicylic acid (SA), ethylene and gibberellin. However, more research is needed on the molecular and biochemical mechanisms of H_2S in plants and its role, especially in antioxidant systems (Arif et al., 2021). H_2S plays an important role in plant development, growth and stress tolerance through crosstalk in the complex signalling network between NO, ROS, H_2O_2, ABA, secondary messengers and heat shock proteins (Bhuyan et al., 2020). These versatile properties of H_2S offer new opportunities for biotechnological applications in agriculture.

4.1. Biosynthesis of H_2S in Plants

Hydrogen sulfide is a gaseous compound that acts as an important signalling molecule in plants and is a part of plant resistance mechanisms against various environmental stress conditions. The biosynthesis of H_2S occurs via cysteine metabolism. It helps regulate plant sulfur levels in response to environmental sulfur inputs. H_2S production is activated when plants encounter exogenous sulfur compounds such as sulfate, sulfur dioxide, and cysteine (Bekturova and Sagi, 2024). However, H_2S biosynthesis in plant cells and the intracellular interactions of this process are still not fully explored. The synthesis of this molecule can occur in different cellular compartments, and various enzymes play critical roles in the process. The primary enzymes engaged in the biosynthesis of hydrogen sulfide (H_2S) in plants are sulfite reductase (SiR), cysteine synthase, L-cysteine desulfhydrase (L-DES), D-cysteine desulfhydrase and cyanoalanine synthase (CAS). These enzymes play a pivotal role in H_2S production against abiotic stresses, as well as in the growth and development processes of plants (Arif et al., 2021).

Chloroplasts are one of the main points of H_2S production due to the presence of the SiR enzyme, which plays a role in the sulfur assimilation pathway (Sharma et al., 2024). In chloroplasts, the process begins with the adenylation of sulfate via ATP sulfurylase and is reduced to sulfite (SO_3^{2-}) in the form of adenosine 5'-phosphosulfate (APS) with the help of the APS reductase enzyme. This sulfite is converted to sulfide with the help of the SiR enzyme or ferredoxin found in chloroplasts, and H_2S synthesis occurs (Vignane and Filipovic, 2023). H_2S production in the cytoplasm occurs during cysteine biogenesis. In this process, enzymes such as O-acetylserine thiol lyase (OAS-TL) and L-DES convert cysteine to sulfide and produce H_2S. At the same time, OAS-TL enzyme reduces H_2S toxicity in cytoplasm, plastid, and mitochondria by converting sulfide to cysteine (Liu and Xue, 2021; Saud et al., 2022; Huang and Xie, 2023; Sharma et al., 2024). Other important enzymes in the cytoplasm include D/L-cysteine desulfhydrase. This enzyme initiates H_2S signaling in plants by converting cysteine to H_2S, ammonia (NH_3) and pyruvate (Li, 2013). In mitochondria, H_2S production is catalyzed by β-cyanoalanine synthase enzyme. This enzyme produces H_2S by reacting with cysteine and cyanide. This process is important for cyanide detoxification in plants, especially when cyanide inhibits the mitochondrial respiratory chain (Huang and Xie, 2023). H_2S is assimilated to cysteine by the mitochondrial OAS-TL isoform and used again in the CAS pathway to detoxify cyanide (Saini et al., 2024) (Figure 3).

In response to environmental stress, plants can enhance the activity of enzymes involved in the biosynthesis of this molecule by elevating endogenous H_2S levels. Of particular significance is the role of the L-DES enzyme in maintaining H_2S homeostasis in plants subjected to abiotic stress (Santisree et al., 2019). All these processes have critical effects on H_2S biosynthesis and homeostasis by effectively regulating the sulfur metabolism of plants. In plants, H_2S biosynthesis plays an important role in both normal growth and development processes and abiotic stress conditions such as salinity, drought and heavy metal toxicity. However, the intracellular concentration of this compound increases under stress conditions. It activates antioxidant systems by interacting with other signaling compounds to protect the plant from oxidative stress. Of particular interest is the crosstalk between H_2S production and nitric oxide (NO), which is considered an important signaling mechanism in plant resistance to stress. NO and H_2S regulate each other's biosynthesis and effects, thereby enhancing the ability of plants to cope with stress (Saini et al., 2024; Sharma et al., 2024).

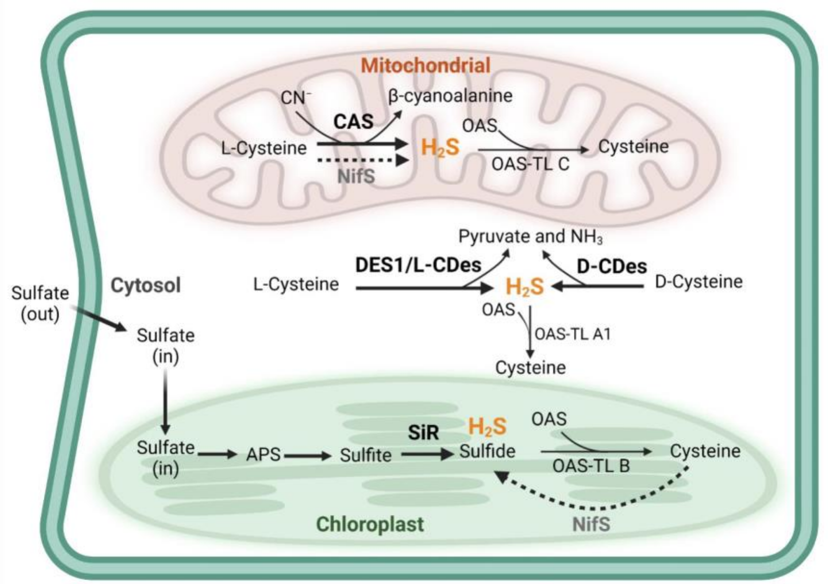

Figure 3: Schematic representation of the pathway of H_2S synthesis and metabolism in plants (CAS; cyanoalanine synthase, D-CDes; D-cysteine desulfhydrase, L-CDe; L-cysteine desulfhydrase, APS; adenosine 5’-phosphosulfate, NH_3, ammonia, OAS-TL; O-acetylserine thiol lyase, NifS; nitrogenase Fe–S cluster, H_2S; hydrogen sulfide, OAS; O-acetylserine, SiR; sulfite reductase, DES1; L-cysteine desulfhydrase) (Huang and Xie, 2023).

4.2. Hydrogen Sulfide Donors and Scavengers

Hydrogen sulfide plays an important role in regulating abiotic stress responses in plants. H_2S-releasing compounds are known as H_2S donors and these compounds are important in the regulation of various biochemical and physiological processes. However, the use of H_2S directly in water is limited by the difficulties such as low boiling point and high toxicity. Therefore, the use of H_2S donors with fewer limitations must become more widespread. Regarding the selection of H_2S donors, features such as the preference for both a fast donor and a slow-oscillating H_2S source should be considered, depending on the requirements of the situation. Ideal H_2S donors should be water-soluble, their by-products should not be harmful to the environment, and their release mechanisms should be well understood (Huang et al., 2020; Hilal et al., 2023). Nowadays, many H_2S donors have been identified and synthesized; these include compounds such as sodium hydrosulfide (NaHS), calcium sulfide, GYY4137, NOSH-aspirin, and AP39 (Antoniou et al., 2020; Huang et al., 2020; Bahmanbiglo et al., 2021).

In plant research, NaHS is applied externally by incorporating it into hydroponic solutions and in vitro culture media or by spraying it directly onto plant surfaces. NaHS is used in a variety of concentrations despite being a short-lived donor and not being able to replicate the in vivo H_2S production process (Ma et al., 2016; Zhu et al., 2018). However, further evaluation is needed on the application method of H_2S and determining appropriate dosages (Corpas and Palma, 2020). In addition, NaHS is widely preferred in plant research because it establishes a rapid equilibrium between H_2S, bisulfide ions (HS^-) and sulfide ions (S^{2-}) when dissolved in water (Corvino et al., 2021).

In addition to H_2S donors, inhibitors that prevent excessive accumulation of H_2S in the cell also play an important role. Recent studies have resulted in the discovery of several suitable inhibitors and scavengers that contribute to a better understanding of the biological functions of H_2S. H_2S inhibitors limit the production of this gas by inhibiting its biosynthesis. Due to the wide distribution of enzymes involved in H_2S biosynthesis and their complex roles in sulfur metabolism, inhibition of these enzymes may lead to a variety of broad effects. Alternatively, instead of interfering with the biosynthesis of H_2S, developing H_2S scavengers that directly target H_2S and thus eliminate its functions within the cell is also a strategy (Yang et al., 2019). The development of H_2S scavengers have received more attention in recent years. However, the most widely used H_2S scavenger in plants is hypotaurine, which has been frequently used in studies, especially in recent years (Baudouin et al., 2016; Mostofa et al., 2015; Siddiqui et al., 2021; Wang et al., 2022).

5. MECHANISM OF ACTION OF HYDROGEN SULFIDE APPLICATIONS

Drought tolerance can be defined as the capacity of a plant to capture and retain water from the soil under conditions of water scarcity, subsequently utilizing this resource in a range of biochemical and physiological processes. These processes cover basic functions such as the conversion of light energy (such as photosynthesis) into chemical energy, the subsequent use of this energy in the synthesis of plant tissues and the development of reproductive organs (Elakhdar et al., 2022). Improving drought resistance in crop plants is critical to ensuring efficiency and sustainability in agricultural production. Improving drought resistance increases the stability of crop yields, enabling high yields to be achieved even under stress conditions.

5.1. H_2S Applications in Plants

Exogenous application of H_2S is recognized as an essential signaling molecule that enhances plant resistance to various abiotic stresses. Recently studies have shown that H_2S significantly contributes to increasing plant tolerance to a range of stressors, such as osmotic stress, drought, salinity, and high temperatures. These effects are provided by mechanisms such as regulating antioxidant enzyme activities in plants, modulating hormone signalling pathways and improving osmoregulation.

According to several studies, H_2S enhances plant performance when drought stress is present. Akın and Kaya (2024), for instance, found that applying SA and NaHS separately or in combination improved grain yield and drought tolerance in wheat. Li et al. (2021) emphasized that sulfur dioxide improves drought tolerance in wheat seedlings through H_2S signalling. Kolupaev et al. (2019) also reported that NaHS application strengthens the antioxidant status of wheat plants and increases their resistance to drought stress. In addition, Ding et al. (2018) showed that H_2S causes physiological and proteomic changes in alleviating drought stress in young wheat seedlings. The effects of H_2S on drought stress occur through plant hormones and signalling pathways. In a study by Ma et al. (2016), it was stated that H_2S alleviates drought stress in wheat partially through the ABA signaling pathway. These studies reveal that H_2S supports plant defenses against drought stress at both physiological and molecular levels.

Another significant stress factor is high temperature, which has been demonstrated to impair photosynthetic efficiency and metabolic activities in plants. The application of foliar NaHS in wheat seedlings has been shown to enhance plant resilience by protecting against high temperature stress (Yang et al., 2016). H_2S acts by activating the antioxidant defense system and ensuring the protection of photosynthetic pigments in this process. Similarly, Gautam et al. (2022) showed that H_2S protects the photosynthetic metabolism of rice plants under high temperature by regulating redox homeostasis with ethylene and NO.

The protective role of H_2S in alleviating salinity stress has been extensively studied, with numerous findings indicating that H_2S supports plant growth and development under salt stress conditions. Research shows that H_2S synthesis, mediated by NO, helps counteract the harmful effects of osmotic stress in wheat seedlings by enhancing antioxidant enzyme activity and promoting osmolyte accumulation, thereby protecting the plant (Khan et al., 2017). In addition, Li et al. (2017) showed that H_2S in wheat leaves improved drought response at the transcriptome level by regulating salt stress tolerance genes. The effect of H_2S against salt stress is not only limited to the activation of antioxidant defense systems, but also has regulatory effects on ion homeostasis and water balance. Siddiqui et al. (2021) stated that the synergistic effect of H_2S and K^+ provides resistance to salt stress by regulating antioxidant defense, sugar metabolism and H^+-ATPase activity in tomato seedlings. Kaya et al. (2024) stated that H_2S is effective in coping with salinity and phosphate deficiency stress in *Capsicum annuum* plant, and H_2S supports plant growth by reducing salt toxicity. Similarly, Shoukat et al. (2023) showed that exogenous NaHS application in maize regulates ion balance, improves biochemical properties, and alleviates salt stress by increasing antioxidant enzyme activities. In a study on sunflower, Younis and Mansour (2024) emphasized that exogenous H_2S application improves photosynthetic performance against salt stress and increases osmoregulation. These results indicate that H_2S increases salinity tolerance by regulating ion homeostasis and supporting photosynthetic activity in plants. Deng et al. (2016) reported that H_2S alleviates salt stress and reduces Na^+ accumulation in wheat seedlings. The contribution of H_2S to salt tolerance in plants has also been associated with the activation of the antioxidant defense system. Ding et al. (2019) reported that H_2S improves antioxidant defenses in wheat seedlings and reduces the negative effects of salt stress by activating the "salt overly sensitive" (SOS) pathways. On the other hand, Yang et al. (2024) stated that H_2S increases salt tolerance together with strigolactones in tomato seedlings, thus increasing the plant's WUE and survival rate under stress. Kumari et al. (2023) reported that H_2S modulates the ascorbate-glutathione cycle in wheat plants and increases osmolyte production. Thus, the productivity and nutrient content of the plant increased against salt stress. In addition, Kaya et al. (2023) reported that the combination of H_2S and NO improved plant tolerance by increasing the AsA-GSH cycle against salt stress in wheat.

H_2S is not only limited to drought, salinity, and high temperature stress, but also plays a protective role against other abiotic stress factors such as heavy metals. For example, Mishra et al. (2024) studied the interaction of H_2S and NO against arsenate stress in *Oryza sativa*, showing that H_2S plays a critical role in sulfur assimilation, glutathione biosynthesis and ascorbate-glutathione cycle. These interactions are important in reducing arsenate toxicity and increase the overall stress tolerance of the plant. Similarly, Khan et al. (2023) found that H_2S regulates H^+-ATPase activity, AsA-GSH system and nitrogen metabolism in plants under chromium toxicity via melatonin and reported that these mechanisms offer significant protective effects against heavy metal stress. In addition, studies in *Triticum aestivum* plants revealed that H_2S regulates the oxidative defense system under cadmium (Cd) stress. Cd is one of the heavy metals that negatively affects plant growth and development, but the combined application of H_2S and NO alleviated Cd toxicity by strengthening antioxidant defense systems (Kaya et al., 2020). Similarly, studies on *H. vulgare* have shown that H_2S applications provide significant protection against Cd toxicity by reducing Cd uptake (Fu et al., 2019). These findings indicate that H_2S is an important agent in reducing heavy metal toxicity. Ozfidan-Konakci et al. (2020) studied the interaction of H_2S and NO against cobalt toxicity in wheat and showed that these molecules increase the resistance of plants to heavy metal stress by regulating photosynthesis, chloroplastic redox status and antioxidant capacity. This study reveals that H_2S provides a multifaceted defense against metal toxicity in plants by both protecting photosynthetic activity and strengthening antioxidant mechanisms against oxidative stress.

In addition to all these studies, in a study conducted by Yang et al. (2024), in which different concentrations of NaHS (0, 200, 400, 600 and 800 mg/L) were applied to tobacco seedlings, it was stated that as the concentration increased, there was a positive increase in root length, root wet weight, root activity and parameters such as CAT, SOD, POX. Exogenous H_2S applications protect plants from damage caused by oxidative stress and have the potential to contribute to sustainable agricultural practices by supporting plant growth under stress-free conditions.

Exogenous H_2S applications play an important role by supporting the defense mechanisms that plants develop against abiotic stress conditions. H_2S, which increases plant performance against stress factors such as salinity, high temperature and drought improves plant stress tolerance by regulating antioxidant defense systems, providing ion homeostasis and modulating hormone signaling pathways. These findings show that H_2S can be used as a biotechnological tool in agriculture and offers significant potential to increase plant productivity under stress conditions. In addition, H_2S provides an effective defense mechanism against heavy metal toxicity in plants, supporting sulfur metabolism, glutathione biosynthesis and antioxidant defense systems. These protective effects of H_2S have great potential to protect plant health and increase productivity in regions exposed to heavy metal contamination in agriculture.

5.2. H_2S-Mediated Alleviation of Drought Stress in Barley

Drought conditions lead to a decreased capacity for water uptake, which in turn reduces photosynthetic efficiency and elevates oxidative stress at the cellular level. This imbalance results in the excessive accumulation of ROS within the plant, potentially causing significant cellular damage. In response, plants have evolved a range of physiological and biochemical adaptations to cope with these stress conditions, one of which is the role of RSSs, such as H_2S, as signaling molecules.

The literature on the effect of H_2S on the alleviation of oxidative damage in barley under drought stress is limited in recent years. However, a comprehensive examination of the role of H_2S in increasing the tolerance of barley to drought and heat stress was conducted by Naz et al. (2022). The study shows that H_2S and silicon (Si) applications, when used together, increase stress tolerance by creating modulatory effects on plant hormones, antioxidant defense enzymes and cellular redox status. H_2S has been found to reduce MDA levels and improve EL by reducing ROS production, which increases under drought stress. It was reported that these applications increased overall stress tolerance by contributing to the preservation of water content, protein structure and photosynthetic pigment levels of plants (Naz et al., 2022). Furthermore, it is emphasized that the combination of H_2S and Si represents the most effective application in enhancing the performance of barley under conditions of drought stress. These findings indicate that H_2S can be considered as a crucial signaling molecule in the context of drought stress, thereby supporting the utilization of H_2S as a potential biotechnological tool to enhance drought tolerance in agricultural production.

6. CONCLUSION

The limited number of investigations into the applications of H_2S in barley under conditions of drought stress increasingly highlight the importance of further studies in this field. According to the literature, there is little (1%) research on the function of H_2S under abiotic stress conditions in comparison to general studies. However, the effects of H_2S on plant stress tolerance are attracting more and more attention. A better understanding of the interactions of H_2S with other signalling molecules, especially ROS and phytohormones, shows the potential of this molecule to be used as an agent to increase stress resistance in agricultural applications.

It has been established that H_2S plays a role in intricate signalling networks that regulate plant growth and development in the context of adverse environmental conditions, such as drought. In particular, H_2S has been observed to reinforce the antioxidant defence mechanism and interact with ROS, thereby enhancing the resilience of plants in the face of stress. Therefore, H_2S is thought to be an important signalling molecule that can provide sustainable solutions in agricultural production.

To conclude, the role of H_2S in plant development and stress tolerance is critical. Further research is needed to elucidate the role of H_2S in plant growth and development more comprehensively, thus demonstrating its potential applications in agriculture. Therefore, it is vital to gain a comprehensive understanding of the functions of H_2S in plants in order to advance the development of sustainable agricultural practices.

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CHAPTER 5

GLOBAL CLIMATE CHANGE IN THE PHYSIOLOGICAL POINT OF PLANT DEVELOPMENT ON THE POSSIBLE EFFECTS

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1. INTRODUCTION

Climate change occurs over many years and results from natural and human influences (Türkeş, 2008). The influence of humanity, via alterations in atmosphere or land usage result in the degradation of climate averages and the occurrence of extraordinary weather events. Son of the Ice Age B.C. After its end around 8000 BC, the world entered an interglacial period. However, with the acceleration of industrialization since the 19th century, the increase in greenhouse gases and the expansion of the global climate have been significantly affected.

According to measurements made at the Mauna Loa observatory in Hawaii, while the amount of CO_2 in the atmosphere was 315 ppmv in 1958, this value reached 400 ppmv in 2013. Considering the records of approximately 700 thousand years in the past, natural CO_2 accumulation varies between 180 and 300 ppmv. In this context, the effect of human activities on this increase is evident. Increasing deforestation and widespread fossil fuel consumption have elevated atmospheric CO_2 concentrations from 280 µmol/l to 400 µmol/l, with projections indicating a potential increase to 800 µmol/l by the century's conclusion. It is emphasized that CO_2 emissions are the main factor of global warming (Turkes, 2012; Vaughan et al., 2018).

Surface temperatures in the world have begun to warm since the late 19th century, and this increase has become especially evident since the 1980s. Analysis conducted between 1906 and 2005 shows that surface temperatures rise of 0.74 °C. While 1998 was the year in which the highest temperature increase was recorded with +0.548 °C, this was followed by 2005 with +0.482 °C and 2010 with +0.478 °C, respectively (Solomon et al., 2007).

This temperature increase caused the glaciers to melt excessively, causing the sea level to rise by 0.17 meters. This situation is characterized by increasing amounts and intensity of heat waves (IPCC, 2001).

2. AFFECTED PLANTS FROM GLOBAL CLIMATE CHANGE

2.1. Situations in which the Biotic Environment is Affected by Global Climate Change

2.1.1. Affected Crop Production from Global Climate Change

Climate change affects crop production directly, indirectly, or through socioeconomic means. Plant physiology tries to reveal the ways in which plants are affected by climate variability. The FAO indicated that climate change events (such as drought, flooding, increased temperatures, and hurricanes) increased significantly by 2016, and that climatic change has a direct as well as indirect effect on the farming industry. The direct effects include alterations in certain agricultural production systems due to variations in physical characteristics, like temperature and precipitation distribution. Indirect effects are elements that influence production by altering the dynamics of other species, including pollinating organisms, pests, vectors of disease, and invaders. Assessment is significantly more challenging due to a variety of interacting parameters as well as associations (Change, 2016; FAO,2017).

Boyer (1982) reported that climate changes may affect the yield of crops by up to 70%. Globally, cultivated regions are impacted by climate change, with about 3.5% of the area protected from constraints of the environment (Van Velthuizen, 2007). While correctly measuring the effects of abiotic stresses on the yield of crops is challenging, these stresses are thought to significantly influence crop productivity, dependent on the level of damage to the whole crop area.

Predictions of the future indicate a decrease in the production of staple crops across numerous countries due to warming global temperatures, water scarcity, and various environmental factors (Tebaldi and Lobell, 2018). Significant differences in vulnerabilities to contemporary climatic changes have been observed across Europe, according to national yields of crops and survey research. In Northern Europe, short crop development is the main problem, but in Southern Europe, high temperatures as well as inadequate rainfall constrain yields of crops. Nevertheless, it is projected that the most significant adverse effects will be seen in the climate of the continental part of the Pannonian region. Predictions indicate that enhanced greenhouse gas emissions and sudden climate changes could increase crop yields in northwestern Europe while decreasing them in the Mediterranean region (Bonan et al. 1994; Olesen et al. 2011). This circumstance has a significant impact on wheat productivity as well. In many countries, extreme temperature fluctuations due to climate change can reduce yields of crops by around 6% for every 1°C rise in temperature. High temperatures and droughts are the primary stressful situations significantly affecting the yield of grains (Asseng et al. 2015; Barnabás et al. 2008).

Researchers have demonstrated that the combined effect of heat and drought stress produces adverse results than either stressful event individually.

Water scarcity and extreme temperatures resulting from climate change progressively impact the reproductive stage of plant development. Flower commencement and flowering, particularly in cereal crops, are adversely impacted by water stress. Climate change, which causes temperatures to increase worldwide, poses serious risks to plant growth and agricultural yields. The Intergovernmental Panel on Climate Change (2018) reports that if present global warming rates persist, global temperatures would increase by an additional 1.5 °C between 2030 and 2052 (Winkel et al. 1997; Wang and Huang 2004; IPCC, 2018; Priya et al. 2019).

Analysis of the metabolite profiles of wheat genotypes demonstrated an important increase in sugars, and phosphates in genotypes exhibiting increased tolerance to cold. Higher temperatures at night leading to carbon loss have significantly reduced winter yields of wheat (Impa et al. 2019). A study suggests that plant growth regulators (PGRs) can offer protection against high-temperature stress (HTS). Sharma et al. (2020) suggest that plants treated with PGR indicate superior resistance to heat stress compared to plants that were not treated. This is due to their better photosynthesis, leaf hydration, and carbon distribution (Sharma et al. 2020).

Some researchers predict that maize will be the crop most adversely impacted by climate change in North America. A study relying on climatic data analysed both separate periods (1981–2010 and 2041–2070) for the North German Plains. The findings indicated that guaranteeing water availability is essential for maintaining the yield of winter wheat. Drought stress impacts wheat across all stages of development, with the periods of formation of grain and reproduction becoming the most important (Pradhan et al.2012; Hellin et al. 2014). Wheat production decreases by 1% to 30% under mild stress from drought post-flowering; however, it can decrease by up to 92% during continuous drought stress throughout grain and flowering development (De Oliveira et al. 2013).

Some researchers indicated that corn yield improves at the optimal temperature of 29 °C; however, increasing temperatures subsequently hinder yield (Araus et al. 2002; Lobell et al. 2011). Researchers have determined that each 1 °C rise in temperature adversely impacts corn production (Schlenker and Roberts, 2009). According to Lobell and colleagues, corn yield decreased by 8.3% for each 1 °C increase in temperature above the optimal growth temperature, while wheat yield declines by approximately 10% for every 1 °C increase in temperature (Brown et al. 2013; Lobell and Field, 2007). In the same way, rice yield reduces by 2.6% for each 1°C higher temperature (Easterling et al. 2007). The yield of sorghum dropped by 7.8% as a result of a 1 °C rise in temperature (Kjellstrom et al. 2018). According to Schlenker and Roberts (2009), the critical temperature for soybeans is 30°C. Lowering the temperature to the optimal level increased soybean yield; however, increasing this temperature resulted in a significant decline in productivity. Eastburn et al. (2010) indicated that increased amounts of ozone and CO_2 in the atmosphere influence disease forms and that the vulnerability of soybeans to disease increases with an ongoing increase in temperature.

Climatic conditions induce anxiety in all individuals. Extreme conditions in the environment affect plants, animals, and fish, as well as humans. Reduced efficiency of primary producers profoundly impacts all living organisms. Approximately 815 million individuals have been adversely impacted by this crisis to date. Malnutrition impairs the attainment of the sustainable development programs' fundamental goals (Richardson et al. 2018). Adverse weather conditions greatly impact food security and productivity in agriculture. The population is expected to attain around 9 billion by 2050, resulting in an increased demand for food. Climatic effects are getting worse, leading to unpredictable productivity due to increased nutrients and environmental alterations in crops (Reckling et al., 2018).

Research indicates that industrialized nations exhibit approximately 8–11% more sensitivity to climate change than countries that are developing (Dhankher and Foyer, 2018).

New developments in genetic engineering methods for reducing climate change and its adverse impacts on crops can also enhance food security. Consequently, it is essential to develop climate change-resistant crops through technological and biological approaches to deal with it (Altieri and Nicholls, 2017).

As a result, climate change negatively affects the productivity of many cultivated areas, creating the potential to produce more crops in some tropical regions while making agricultural activities difficult in other regions. Temperature instability increases the survival capacity of crop pests in cold weather, providing favorable conditions for them to emerge in outbreaks in the spring. In terms of food security, the effects of climate change on crop productivity are calculated without taking into account extreme climatic conditions, which neglects the effects of sudden climate changes as well as socio-economic factors. All these elements create negative effects that threaten global food security (Schmidhuber and Tubiello, 2007; Lesk et al., 2016). Food security is one of the basic needs of people, and climate change still poses significant challenges to food quality, supply, and safety.

2.2. Abiotic Environment Affected by Climate Change

Abiotic factors significantly influence the growth and production of plants. In natural climatic situations, plants frequently face several challenges, including waterlogging, drought, heat, salinity and cold. The (IPCC, 2014) defines the period from the 19th to the 21st century as the most experienced period. Floods resulting from warming and heavy rainfall inflict damage, but prolonged low or absent rainfall leads to drought stress (Pachauri et al. 2014; Khan et al. 2016; Benevenuto et al., 2017; Ashraf et al. 2018).

2.2.1. Effects of Climate Change on Soils

The Earth's climatic history includes distinctive cold and warm cycles. Nevertheless, these alterations have been observed quite rapidly within the globe in the past 150–200 years. Soil is increasingly important for contemporary human cultures to meet global food, and fiber demands of growing populations with constrained land resources (Fauchereau et al. 2003). Climate change undermines global food security. Nonetheless, farmers who are marginalized and small exhibit greater vulnerability due to their limited ability to cope.

Soil formation is influenced by various causes, including environment-related factors like precipitation and temperature. These climatic conditions directly influence soil formation via biomass and weathering. Regular, progressive climatic warming can result in irreversible alterations to the mineral composition of soils.

As a result, soil function declines and the demand for mineral fertilizers to sustain fertility increases. The impact of climate change on soil growth will probably primarily result from changes in soil moisture conditions as well as rises in soil temperature and carbon dioxide levels. Climate significantly influences the weathering of rocks and minerals, among the several elements that govern soil development. Climate change variables, particularly temperature and precipitation, influence the different stages of rock and mineral weathering (parent material), leading to chemical and mineralological alterations in soil-forming rocks. A single basic mineral can produce many secondary minerals under differing environmental circumstances. Consequently, similar rock types subjected to weathering under different climates can result in the development of diverse soil profiles.

a. Availability, transformation and acquisition of plant nutrients

The availability of nutrients to plants in soil depends on the soil's chemical composition, the position of the ions in relation to the root surface, and the distance the nutrients have to travel through the soil to reach the roots. The elevations in air temperature and alterations in precipitation substantially impact root zone moisture and temperature conditions.

Plants absorb nutrients from the soil's solution pool, and these nutrients must be dissolved in order to stay mobile within the soil. The biotransformation between both inorganic and organic pools is greatly influenced by moisture and temperature; therefore, climate change can significantly impact solution concentrations of elements such as nitrogen. A number of studies suggest that elevated CO_2 may not directly influence N mineralization, but the consequent warmth may increase N mineralization, potentially leading to higher solution phase N (Pendall et al. 2004; Abou Seeda et al. 2020).

b. Adaptation and mitigation strategies

Agriculture can adapt by adopting various management practices to reduce the negative effects of climate change. These practices include zero tillage, protecting crop residues, expanding fallow areas, increasing production diversity, and adjusting the amount and timing of external inputs. For example, reducing biomass burning and making energy use more efficient can reduce carbon dioxide emissions. Better management of animal waste and water in rice fields also reduces methane emissions. Proper fertilizer management can reduce nitrous oxide emissions. The global soil carbon pool is four or five times larger than biomass pools. Soil degradation in recent decades has led to the loss of 30% to 75% of previous soil organic carbon. This shows how critical soil management is in the fight against climate change.

The worldwide increase in soil carbon presents considerable mitigation potential in fighting climate change. The quantity of carbon stored in a location indicates the enduring balance between carbon absorption and emission. Effective management approaches in forestry, agriculture, as well as conservation may significantly improve soil carbon sequestration. Sustainable forestry and agricultural management are essential for enhancing soil carbon levels.

It is widely acknowledged that increased levels of CO_2 affect the release of root-derived chemicals in both quantity and quality. These changes caused an increase in microbial activity and therefore CO_2 production in the soil increased. This could lead to potential adverse effects on soil organic carbon accumulation and therefore reduce the soil's carbon sequestration capacity (Tarnawski and Aragno 2006).

Global change processes are believed to be significantly impacted negatively by C concentration, which also lowers the ability of soils to sequester carbon.

Agricultural ecosystems can significantly mitigate CO_2 emissions by biotic carbon storage in soil and vegetation. Historical carbon losses in soils are estimated to range from 41 to 55 g/t. This indicates that soils provide the capacity to sequester carbon. The carbon sequestration capacity of soil is influenced by several elements, including the kind of vegetation required by the climate, the characteristics of the initial substance, profile depth, soil drainage, edaphic conditions, organic matter content (SOM), and its rate of decomposition. Moreover, enhanced management of agricultural ecosystems can substantially increase soil carbon sequestration.

Increasing carbon (C) input in agricultural ecosystems is possible by adopting sustainable agricultural practices. Conservation farming practices help control erosion by minimizing soil disturbance. Using low-quality organic inputs can reduce soil carbon loss. In places like India, effective technological options for sequestering soil C include integrated nutrient management, incorporation of crop residues, mulch farming, and agroforestry systems. These methods create a more productive agricultural environment by improving soil agglomeration (Benbi and Kaur, 2009).

c. Climate changes and their importance in mineral accumulation

Atmospheric CO_2 levels have increased from a pre-industrial reference level of 280 ppm to over 400 ppm. Although this increase is the major cause of climate change, it could also lead to positive effects, especially on C3 plants (Ainsworth and Long, 2005; Myers et al. 2017; IPCC, 2018). This effect is known for improving crop production by increasing photosynthesis rates and water use efficiency and is called the “CO_2 fertilization effect.” This effect has been observed in many agricultural products such as wheat, corn, and rice (Ainsworth and Long, 2005; Dong et al. 2018; Fernando et al. 2012; Guo et al. 2015; Ainsworth and Long, 2005; Ziska and Bunce, 2007; Högy and Fangmeier, 2008; Bunce, 2008; Dietterich et al. 2015).

This effect has already been observed in crop plants (corn, rice, barley), including wheat, fruits (tomatoes, beans, cowpeas, potatoes, and soybeans), and vegetables (lettuce, carrots, and parsley). (Pang et al. 2006; Yang et al. 2007; Haase et al. 2007; Högy and Fangmeier, 2008; Jin et al. 2009; Fernando et al. 2012; Zong and Shangguan, 2014; Guo et al. 2015; Han et al. 2015; Bunce et al. 2008; Dong et al. 2018). Prolonged exposure to elevated CO_2 levels can result in photosynthetic acclimation, characterized by enhanced soluble sugars, which may cause an inadequate carbon-to-nitrogen ratio, leaf senescence or reduced rate of growth (Ainsworth and Long, 2005; Kaplan et al. 2012).

Climate change models for the period 2000–2100 forecast improvements in water consumption efficiency, results of biomass production, and yield, with an overall decrease in growing season duration and transpiration. Nonetheless, it is also indicated that significant differences would exist among crop models.

A rise in C concentration is anticipated to lead to a notable reduction in overall concentrations of minerals (~8%) in C3 plants, covering leaves and edible tissues (Bassu et al. 2014).

Carbon dioxide concentration can also indirectly affect the bioavailability of nutrients in soil (Ahmed et al. 2017; Kimball et al. 2002). Although the increase in carbon dioxide concentration promotes plant growth, this may affect the competition between microorganisms and nutrients in the soil. It has been shown that CO_2 enrichment conditions positively affect the bioavailability of nutrients in soil (Jablonski et al. 2002).

It has been stated that especially increasing CO_2 promotes the increase of nutrients such as P, K, Fe, Mn, and Zn in the soil (Kumeleh et al. 2009). This increase may be related to changes in soil pH improving leaching processes that increase nutrient availability. Guo et al. (2015), reported that increasing CO_2 significantly increased Fe, Mn, Mg, Ca, and Zn concentrations on the ground surface with an average of two nitrogen applications (Guo et al. 2015).

The likely cause of these changes is changes in soil pH caused by CO_2. These changes improve leaching processes that affect nutrient.

In a study, it was found that the average increased CO_2 reactions during two different N applications increased Ca, Mg, Fe, Zn, and Mn concentrations on the soil surface by 15.6% and 9%, respectively. revealed that it increased by.5, 23.4%, 138.2%, and 16.9% (Abou Seeda et al. 2020). These findings highlight the positive effects of increasing CO_2 levels on soil nutrients.

However, some studies have found the effects of increased CO_2 to be complex. When he examined the effects of long-term CO_2 conditions on different soil types, he stated that the N, P, and Zn concentrations of plants such as wheat, field peas, and canola decreased by 6%, 5%, and 10%. In this study, it was determined that Fe, K, Cu and Mn concentrations were not affected by CO_2 abundance (Jin et al. 2009).

Additionally, the potential of increased CO_2 to increase mycorrhizal colonization may indirectly support soil nutrient uptake and plant growth. Mycorrhizal fungi play an important role in agricultural productivity by helping plants uptake nutrients more effectively.

This has led to improved phosphorus (P) nutrition, especially in legumes, and increased soil organic carbon decomposition. They have investigated ways to increase the availability of some nutrients (Abou Seeda et al. 2020; Cheng et al. 2012). This can help farmers determine the agricultural management strategies needed to adapt crops to higher CO_2 levels.

However, while higher efficiency is achieved with the effect of increasing CO_2, negative side effects such as decreased mineral concentrations are also observed in some cases. For example, it has been suggested that although grain nutrient concentrations decrease under increasing CO_2, the overall availability of some nutrients on a land basis may increase (Asif et al. 2017; Fernando et al. 2012). However, this may lead to an overall decrease in elevated atmospheric N, Mg, Fe, and Zn concentrations.

Zinc deficiency has become a major global health problem (17%), and there is strong evidence that increasing CO_2 levels reduce Zn concentrations in food products (Myers et al. 2017; 2015; 2014). In meta-analyses of wheat, rice, barley, field peas, and soybeans. It was observed that Zn concentrations decreased significantly (Myers et al. 2014). Similarly, Zn concentrations in vegetables were reported to decrease by 9.4% (Dong et al. 2018).

Decreasing micronutrient concentrations in food products are predicted to put developing world populations at risk. In a study, the iron concentration in soybean seeds at the fresh edible stage decreased significantly, while zinc (Zn) and manganese (Mn) levels differed among varieties (Li et al. 2018).

It is estimated that 138 million people will be at risk of new Zn deficiency by 2050 due to the increase in atmospheric CO_2 concentrations. The populations most affected by this situation are concentrated in Africa and South Asia, especially India. Although "carbohydrate dilution" has been cited as a possible cause, this is thought to be insufficient to explain varying mineral responses for particular crops or species (Loladze, 2002; Poorter et al. 1997; Myers et al. 2015). It is also suggested that decreases in transpiration rates may reduce the mass flux of nutrients and biochemical processes may affect nutrient uptake through changes in nutrient allocation (McGrath and Lobell, 2013).

Increased CO_2 due to changes in soil pH can improve leaching processes that increase nutrient availability. It has been observed that increased CO_2 facilitates the availability of various nutrients and minerals. Because higher overall grain yield carries risks with CO_2 conditioning effects and faster depletion of mineral nutrients from the soil. Therefore, considering the long-term effects of these processes is critical for agricultural management strategies.

d. Effect of increased CO_2 on protein accumulation

High CO_2 levels have been shown to reduce the protein concentration of grains in many types of crops (Dong et al. 2018; Högy and Fangmeier, 2008; Medek et al. 2017; Myers et al. 2017). This situation directly affects human nutrition (Toreti et al. 2019). Since a large part of the world's population depends on plant proteins, millions of people may face protein deficiency (Medek et al. 2017).

Medek and colleagues observed a decrease of 7.8%, 7.6%, and 14.1% in the protein concentration of C3 grains (wheat, rice, and barley) grown under increasing CO_2 conditions (500–700 ppm) expected. A decrease of 6.4% in potatoes, 17.3% in vegetables, and 23.0% in fruits was detected (Medek et al. 2017).

The same studies in 18 countries in the Middle East and India predicted a reduction in protein intake of more than 5% under conditions of increased CO_2. At constant atmospheric CO_2 levels, globally 15% of the world's population (1.4 billion people) is projected to be at risk of protein deficiency by 2050, mainly due to demographic changes. It is estimated that CO2 levels will exceed 500 ppm worldwide by 2050 (Medek et al. 2017).

e. Effects on plant strategies to obtain phosphorus

In phosphorus-deficient soils, efficient crop production is usually achieved by the use of phosphorus fertilizers. These fertilizers make the phosphorus in the soil more easily absorbed by plants, which directly affects crop yield. However, since phosphate ore reserves are limited, it is possible that more intensive fertilization with phosphorus may pave the way for long-term maintenance in order to compensate for the increased phosphorus (P) contributions of crops under high CO_2 conditions. (Lynch, 2011). The negative effects of intensive P fertilization on the environment should not be ignored.

f. Root network mechanisms

Phosphorus (P) is an immobile nutrient in the soil, and it appears that increases in root length and root branching under high CO_2 levels can increase the plant's capacity to absorb P from the soil. Researchers found a link between the amount of P plants take in when there isn't enough of it and the growth rate, length, number, and surface area of their lateral roots in *Oryza sativa* and *Brassica oleracea* genotypes (Hammond et al. 2009).

Phosphate starvation responses (PSRs) are effective at various levels in plants, including the field, rhizosphere, and organs (Ajmera et al. 2019). Plants develop responses by combining intrinsic and extrinsic factors (Svistoonoff et al. 2007). A local system around the root tip senses the extrinsic P status, promoting root hair development and reducing primary root growth (Bonnot et al. 2016; Chiou et al. 2011).

g. Effect of plant nutrient uptake on phosphorus

Phosphorus (P) is an important nutrient affecting plant growth, and root systems develop various strategies in response to low P levels (Lambers et al. 2006, 2013). This limitation suppresses root growth and root architecture changes so that roots gain the ability to explore a more extensive soil volume (Lambers et al. 2011; Richardson et al. 2011).

Root community with arbuscular mycorrhizal fungi (AMF) is another important root trait for enhancing P uptake (Munyanziza et al. 1997). Plants form a symbiotic relationship with AMF by providing carbon in exchange for nutrients. Mycelia of AMF can enter fine soil pores, rise nutrient intake (Khalvati et al. 2005). However, approximately 18% of angiosperm species (such as Cyperaceae, Brassicaceae, Chenopodiaceae,) lack a symbiotic relationship with AMF (Brundrett, 2002; Brundrett and Tedersoo, 2019).

Knowledge gaps in this area arise particularly because most studies on plant-mycorrhiza interactions focus on a single plant growth stage. The interaction between roots and microorganisms begins in the early growth phase of the plant, but changes in root morphology and soil properties in later periods increase the importance of these interactions (Philippot et al. 2013; Wen et al. 2017; Chaparro et al. 2014; Schofield et al. 2018). Intense competition for nutrients should also be taken into account (Kuzyakov and Xu, 2013). For instance, root exudation has been shown to vary with the growth stage of the plant and enzyme activities (Chaparro et al. 2014; Kumar et al. 2018).

Plants adjust their strategies to increase nutrient uptake throughout growth periods; however, the relationship between specific root characteristics and nutrient uptake is unclear (Chen et al. 2016).

This study examines root trait plasticity in P-limited soils and their response to P availability through P fertilization. Root hairy (wild type, WT) and root hairless (rth3 mutant) maize genotypes were grown under controlled conditions for 64 days. Both genotypes were fertilized with KH2PO4 (Zhang et al. 2018). A study indicates that many plants have developed complex strategies in P uptake by changing root morphology and root hair formation (Kumar et al. 2019).

2.2.2. Water Use Efficiency Under High CO_2

Water scarcity is one of the world's biggest problems, and according to climate change estimate, it will become even more critical in the future. Since water availability and accessibility are the most important factors limiting crop production, addressing this issue is important for areas affected by water scarcity. Since there is a remarkable relationship between a country's water availability and food production capacity, assessing irrigation needs and water resource planning is of great importance so that meet food needs and prevent excessive water consumption (Balyan et al. 2017; Hussain et al. 2019).

Agricultural production increasingly necessary significant amounts of water. For this reason, it is important to improve rice genotypes by increasing water use efficiency without compromising productivity (Ambavaram et al. 2014; Mar et al. 2018; Yao et al. 2017; Shahane et al. 2019).

High yield under both optimum and environmentally induced stress conditions is a substantial crop stability trait that is planned to be improved using genetic engineering as well as conventional breeding (Century et al. 2008). Processes of integrated photosynthetic carbon metabolism (PCM), such as increasing the growth or improving photosynthetic rates and capabilities Photosynthesis is a complex process and needs a system-wide address to increase plant fertility and yields in a coordinated way (Gibson et al. 2011; Zhu et al.2010). Transcription factors (TFs), which are stable under environmental stresses, show promise in the coordinated improvement of select properties (Karaba et al. 2007). However, productivity enhancement via direct improved photosynthetic efficiencies has not yet been achieved. However, evidence that increased CO_2 can raise foliar photosynthesis in plants by up to 22.6% throughout the growth session indicates that enhanced photosynthesis can improve fertility and yields (Abdelrahman et al. 2018; Taranto et al. 2018).

Irrigation water in agricultural fields under climate changes.

Water scarcity and climate change, together with the increase in food request, reveal the necessity of sustainable approaches in agricultural water resources management. It is stated that virtual water import and export can play an important role in alleviating this demand (Qadir et al. 2003). However, the intensity of irrigation practices, especially in the summer months in countries in the Mediterranean region, creates competition between different sectors such as agriculture and tourism.

This highlights the importance of water resource planning for a balanced allocation of water resources among economic sectors. In agriculture, determining the water needs of plants according to climatic conditions is the first step in effective irrigation practices. Techniques for monitoring irrigation needs include soil observation and various modeling methods. The models provide an effective solution for calculating water needs at on-farm and regional levels (Sinclair et al. 1984). He defined this efficiency as a measurement of biomass accumulation compared to the plant's transpiration. Nowadays, strategies such as selection of optimal irrigation systems, irrigation planning and crop management are implemented to increase water efficiency.

Irrigation systems are generally divided into gravity systems and pressurized systems. Some researchers have developed different methods to measure irrigation efficiency (Burt et al. 1997).

In general, pressurized systems have been found to be more efficient than traditional gravity systems in transporting water to facilities. (Chimonides, 1995). Therefore, increasing irrigation efficiency is of critical importance for water saving and agricultural productivity.

Recently, many irrigation systems have substantially increased implementation efficiency at the farm level by improving management of irrigation water. Although traditional gravity irrigation methods are still widely used, these systems are beginning to change, especially in the southern part of Europe (Abou Seeda et al. 2020).

Irrigation is necessary to meet the water needs of the soil in order to achieve maximum crop yields. This applies to most field crops and many orchard crops. Some researchers have stated that incomplete irrigation of some tree and vine plants can yield more profit with higher quality and less water use, even if there is a small decrease in yield (Holzapfel et al. 2009).

Improving the technical efficiency of the crop could be another strategy to solve the water problem for food purposes. Developing more drought-resistant varieties and crop management taking into account soil and climate conditions offer effective methods to increase water efficiency. Such approaches can help both ensure more sustainable use of water resources and increase agricultural productivity.

Climate change affects soil salinity in agricultural lands

Climate is characterized by several factors, such as high atmospheric CO_2 levels, rising temperatures and abrupt changes in seasonal and inter-annual temperatures.

These changes in climate patterns can dramatically terrestrial the land systems, soil characteristics, surface waters and fluvial flows. Although climate models provide a consensus on some key global trends, with uncertain, especially at regional and local scales. These models expect climate change to affect potential evapotranspiration (ET), and temperature. Additionally, changes in radiation, and ozone levels will occur.

Climate changes predict a rise in average temperature, changing weather conditions with changes and an rise in extreme climate conditions in local regions (Yeo, 1999). Rising global temperatures will cause polar ice caps to melt, raising ocean levels and bringing extreme weather conditions.

As a result, climate change is expected to increase the frequency of extreme weather events around the world, with unusually high precipitation triggering floods and low precipitation triggering higher temperatures and longer, harsher droughts.

Drought maps prepared by the National Center for Atmospheric Research using the Palmer Drought Severity Index for the period 2000-2099 reveal the expectation of extreme drought in a large part of the world. However, regions at higher latitudes, from Alaska, are likely to experience greater flooding and flooding. There is a variety of evidence from around the world about the effects of climate change on climate patterns.

Particularly in the southwestern United States, there have been periods of moderate to severe drought; but other regions, such as the Midwest and Southeast, are not exempt. One of the examples that affected the public the most is the drought experienced in California between 2011 and 2015.

Other recent drought events around the world include the once-in-a-1000-year drought in Australia from 1995 to 2009, drought in Spain, drought in northern India in the first decade of the new millennium droughts in northern China, Syria and southeastern Brazil (Dai, 2011). Ironically, the world's top crop-producing regions are also placing that experience water scarcity.

Many of the agricultural areas successfully produce crops thanks to the year-round mild climate and available surface or groundwater resources. However, climate change will change the amount and distribution of precipitation by affecting global precipitation patterns. Arid areas are more prone to negative effects such as desertification and salinization (Szabolcs, 1990; Collins et al. 2013).

Temperature and frequency of extreme weather events will negatively affect agricultural production, and the impact of climate change on agriculture is likely to be negative overall.

Increased CO_2 in the atmosphere can stimulate plant growth by increasing soil organic matter and increase water use efficiency in some crops. However, extreme weather events such as heat waves, droughts and floods can limit these potential yield increases. Recent studies have revealed that the impact of increased atmospheric CO_2 on plant and crop productivity may not be as great as previously thought. Increased ozone levels can reduce the positive effect of CO_2. Furthermore, the adverse effects of rising temperatures on plant growth can also reduce the benefit of CO_2 (Zavaleta et al. 2003; Jarvis et al. 2010; Zaehle et al. 2010).

Higher atmospheric CO_2 levels, rising temperatures, heavy rainfall, prolonged droughts and heat waves will accelerate the chemical and physical weathering of rocks and minerals in the soil. A 44-year field study by Gislason and colleagues showed that global warming increases weather conditions (Poorter and Navas, 2003; Long et al. 2005; Gislason et al. 2009; Körner, 2006).

Accelerated decomposition caused by climate change has both positive and negative effects. Accelerated weathering can expand the pool of inorganic carbon in the soil by increasing carbonate mineral formation, which can help reduce atmospheric CO_2 levels. Furthermore, the dissolution of elements that function as nutrients for microbes and plants can enhance biotic carbon sequestration by promoting microbial and plant growth (Qafoku, 2014).

Control of salinity is generally achieved through washing processes. Soil salinity is defined by the concentration and composition of soluble salts and is measured by electrical conductivity (Corwin and Yemoto, 2017).

Salinity reduces the osmotic potential, restricting plants' water uptake and making water outflow difficult. Additionally, depending on soil pH, certain ion toxicities, especially Na+ ion, can disrupt the nutrient balance of plants. The salt content of soil water affects the combination of cations on the exchange complex of soil particles, which in turn affects the perviousness of the soil.

Soil salinization is a trigger of desertification and can lead to the abandonment of arable land and soil erosion (Qin et al. 2013). The detrimental effects of increased salinity and its link to irrigated agriculture is recognized as a chemical quality of vital importance for soil health.

Salinity has a coefficient of variation of over 60%, is a spatially complex and temporally dynamic soil property, making it difficult to collect data on (Corwin et al. 2003). Salt-affected soils account for approximately 23% (approximately 350 million hectares) of cultivated land worldwide.

However, directly measured global inventories of soil salinity are not available; Most known global and regional scale inventories consist of estimates based on qualitative data, with only one exception (Massoud, 1981; Lobell, et al. 2010; Corwin and Scudiero, 2016). Past trends in climate change and crop production are clearly observed in different regions of the world. Evidence is presented that climate change affects wheat and corn yields on both a regional and global scale (Lobell et al. 2011). Expectations are that climate change will dramatically change global food consumption patterns and have adverse impacts on the yields of wheat, rice and maize at low latitudes, particularly in the tropics (FAO, 2017). Accelerating phenology and more frequent and intensive heat events with increasing water shortage will also adversely impact temperate regions (FAO, 2017).

While the impacts of climate change on crop productivity are generally negative, rainfall is projected to improve in some regions, which could lead to positive impacts. For example, studies in grain-producing regions of Central Eurasia show that higher atmospheric CO_2 levels, rising temperatures, and longer growing seasons can lead to increased yields. In the Mediterranean Basin, positive changes in crop yields are expected with changing precipitation patterns and increasing winter temperatures according to climate models (Qafoku, 2014).

However, the Gaza Strip, experiences serious water deficit problems (Massoud, 1981; Zheng et al. 2009). More than 70% of the groundwater extracted in the Gaza Strip is used in agriculture, which brings to the agenda the overuse of water resources (Corwin and Yemoto, 2017). Simulations by Loáiciga and colleagues show that groundwater extraction increases seawater intrusion (Loáiciga et al. 2012).

The spatial variation of Ca2+ and other ions together with Na+ and Cl− in the coastal areas of the Gaza Strip reveals the seawater intrusion into the aquifer, which is the source of irrigation water (Benbi and Kaur, 2009; Corwin and Yemoto, 2017). Additionally, climate change projections indicate that seawater intrusion will increase throughout the Gaza Strip and the Mediterranean Basin (Loáiciga et al. 2012).

These changes will enhance salinity deposition due to the impact of seawater intrusion, which will negatively impact yields (Corwin and Yemoto, 2017). This situation is a significant factor that threatens agricultural productivity in the region.

2.2.3. Impact of Climate Change on Soil Health

Climate change impacts on soil health are manifested through chemical and physical parameters. In this context, while many articles focus on soil physical properties, it is essential to think carefully chemical parameters as well. The main consequences of global climate change on soil health are summarized below:

1. Soil Texture and Structure: Soil texture and structure have significant effects on water leakage and the amount of water available to plants. Climate change can affect these structures, altering the movement and storage of water.

2. Soil Porosity: Porosity determines the ability of water to penetrate the soil. Climate change can affect this porosity through extreme precipitation and temperature changes.

3. Bulk Density: High temperatures and excessive rainfall can cause soil compaction and increase bulk density, which negatively affects root development.

4. Rooting Depth: Climate change can reduce the depth of roots by affecting soil water balance, which restricts plants' access to water.

5. Vegetation Types: Different plant species can respond differently to changing conditions with climate change. This situation indirectly affects soil health.

6. Soil Temperature: High temperatures can accelerate the decomposition of organic matter in the soil, leading to nutrient imbalance.

7. Salinity: Increasing temperature and changing rainfall patterns can lead to increased soil salinity, which negatively affects plant growth.

8. Water Availability: Drought and excessive rainfall can change the available water in the soil. This makes it more difficult for water to be uptake by plant roots.

9. Biodiversity: Climate change can impact the diversity of organisms living in soil. This can have negative effects on soil health.

As a result, climate change has a multifaceted impact that affects soil health. Understanding these impacts is critical to maintaining agricultural productivity and developing sustainable agricultural practices.

Soil temperature

The soil temperature regime is governed by processes such as gains and losses of solar radiation, evaporation, heat conduction, and motion of gas and water (Karmakar et al. 2016).

Effects of Soil Temperature

• Decomposition of Organic Matter: High soil temperatures can increase soil fertility by causing rapid decomposition of organic matter.

• Microbial Activity: Increased temperature also accelerates microbial activities, resulting in faster release of nutrients and increased nitrification rates.

• Mineral Weathering: The rate of chemical weathering of minerals is directly proportional to soil temperature; This helps make nutrients more accessible to plants.

Dense vegetation can reduce temperature fluctuations at the soil surface, causing soil temperatures to remain more stable. This interaction becomes even more important under conditions of climate change, as changes in vegetation can lead to significant effects on local climate and soil temperature.

As a result, soil temperature has a significant impact on agricultural productivity and ecosystem health and should be carefully monitored within the framework of climate change and adaptation management. Understanding these dynamics is critical to developing sustainable agricultural practices.

Climate change has become one of the biggest research challenges for biologists, and agronomists. Direct impacts of climate change result from changes in temperature levels and precipitation distribution. These changes can affect crop growth, water requirements and plant health. For example, rising temperatures can shorten or lengthen the growing periods of plants, which directly affects crop productivity.

Indirect effects of climate change occur through changes on other species (invasive species, disease vectors, pollinators, pests). Interactions between these species can significantly impact agricultural production. Many unknown interactions and parameters make it difficult to evaluate these indirect effects.

Climate change may increase the accumulation of soluble sugars in crops while reducing levels of protein, nitrates and some minerals (Mg, Fe, Zn). Extreme weather conditions and the effects of global warming are among the factors that threaten food security.

In C3 plants, increased CO_2 levels can increase photosynthesis rates and water use efficiency; However, during this period, unbalanced C rates and water stress can accelerate leaf senescence. Water stress, especially in the flowering stage of grains, can negatively affect yield.

Genetic mechanisms define physiological and molecular processes as well as mineral nutrient absorption. Therefore, important to develop genetic research and strategies to enhance the nutritional health of cereals affected by climate change.

In conclusion, climate change is a complex issue that threatens both food security and agricultural production. Measures need to be taken for the adaptation of agricultural systems to these changes and for sustainability.

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CHAPTER 6

NATURAL ANTIOXIDANTS DERIVED FROM PLANTS: THEIR SIGNIFICANCE IN REDUCING OXIDATIVE STRESS

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1. INTRODUCTION

Free radicals are continuously produced at low concentrations in human body. The beneficial acts of free radicals at lower or moderate concentrations are crucial for the maturation process of cellular structures in human health and in normal physiological functions. They mainly function as second messengers to regulate important cellular responses and can function as weapons for the host defence system (Giovanni et al., 2022). But excess production and accumulation of free radicals to be a natural byproduct in metabolic processes, or a decrease in antioxidant level, lead to oxidative stress in the human body (Fig.1). Because free radicals are unstable atoms that can lead to cellular harm, leading to inflammation and various chronic diseases, including cancer, neurodegenerative disorders like Alzheimer’s and Parkinson's, and heart disease. Despite endogenous stress factors such as respiratory bursts, exogenous factors such as ultraviolet (UV) irradiation, environmental pollution, cigarette smoke, radiation, and pathogens can lead to enhanced free radical levels in the body and cellular damage (Birben et al., 2012 and Isaguliants et al., 2020). Thereby, an imbalance between the creation of reactive oxygen species (ROS), the synthesis of free radicals, and the detoxification of these reactive products results in oxidative stress (Pisoschi and Pop 2015).

Reactive oxygen species are a class of bioactive compounds created when the human body normally metabolism during living things' respiratory activities. A variety environmental stresses can cause excessive generation of ROS. Overproduction of ROS and the harmful consequences of free radicals can damage proteins, enzymes, DNA, and cell membranes, leading to aging and disorders associated with aging. These conditions can cause numerous pathological conditions, including diabetes, cancer, cardiovascular disease, and neurodegenerative illnesses, all of which have sharply raised in frequency and are closely associated with oxidative stress and redox imbalance. As a consequence, there is a decrease in life span with subsequent premature death due to the activation of free radical oxidation reactions (Avery 2011). Nevertheless, this process can be modulated by antioxidants generated in origin through a natural mechanism or externally supplied by foods and/or supplementation with herbals (Pham et al., 2008). Antioxidants are compounds that assist in scavenging reactive oxygen species, neutralizing harmful free radicals in the body, and repairing cell damage, so consuming more fruits and vegetables with high antioxidant content is advised to reduce oxidative stress in the human organism (Sefren et al., 2024). Furthermore, a wide range of antioxidant, anti-inflammatory, anti-aging, anti-atherosclerosis, and anti-cancer properties were exhibited by thousands of biologically active components in each whole derived from plants, offering promising bioactive effects for promoting human longevity and healthy aging. This chapter focuses on the benefits of plant-based antioxidants that provide promising results in prevention and oxidative stress therapy.

Plants, because of their high physiology of oxygen exposure, have amazing antioxidant benefits. In actuality, there could be more ROS production sites in plants. Numerous antioxidant defense systems scavenge ROS molecules under steady-state circumstances (Honglin et al., 2019). The therapeutic importance of phytochemicals as antioxidants and pro-oxidant agents is discussed, and their role in enhanced longevity, aging-related illnesses, and healthy aging (Carlsen et al., 2010). Given that oxidative stress is a key component of inflammatory processes, plant species with antioxidant activity offer significant prospects for treating a variety of chronic illnesses. The goal of this chapter is to examine the natural antioxidant components found in different plants and fruits, since they have a unique role in medical research and in illness prevention and treatment.

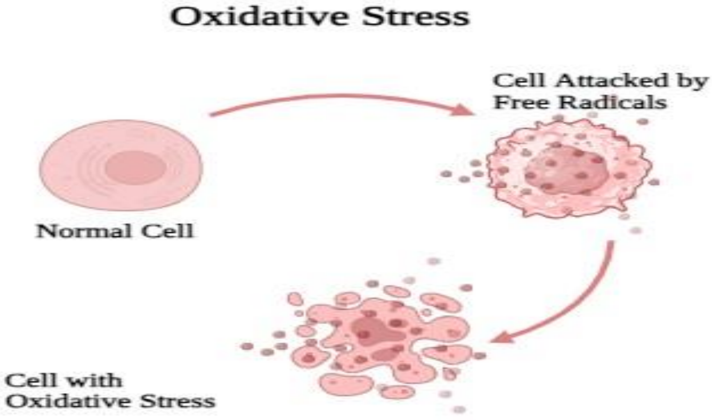

Figure 1: Schematic of oxidative stress at a cellular level

2. OXIDATIVE STRESS

An imbalance between antioxidants and reactive oxygen species (ROS) in cells is known as oxidative stress. In humans, oxidative stress is a significant pathogeneti link and contributes significantly to the pathophysiology for potentially serious illnesses. These types of stresses can result when cells are unable to sufficiently eliminate the surplus free radicals that have produced an imbalance between the production and neutralization of ROS, or free radicals, and oxidants (Bae et al., 2011). Therefore, a defect in redox homeostasis caused by high concentrations of oxidant species and concurrently low levels of antioxidant species can cause toxicity and hasten the development of harm to tissues (Cano 2018). Cancer-associated oxidative stress and decreased antioxidant levels induce mitochondrial dysfunction, decrease protein synthesis, and dysregulate autophagy (Fig. 2).

Typically, ROS byproducts of the process of oxidation, via mitochondrial respiration, but in pathological situations, ROS generation is raised by phagocytes, chronic wounds, and UV radiation exposure all through different mechanisms (Zuo et al., 2015). In particular, a moderate ROS amount has roles in a variety of biological processes, such as immunity and cell proliferation, and can stimulate platelet activation, skin cell migration and proliferation, and the activation of keratinocyte and epidermal growth factor receptors (Wang 2023). Notably, in physiological conditions free radicals and ROS act a dual function as both harmful and beneficial elements. The beneficial acts of ROS have an extensive impact on cellular constituents and have physiological roles in the operation of several cellular responses that can be modified via crosstalk between ROS and cellular signalling pathways. The generation of ROS by nonphagocytic NADPH oxidase isoforms acts crucially in the modulation of intracellular signaling pathways, including in fibroblasts, endothelial cells, cardiac myocytes, vascular smooth muscle cells, and thyroid tissue. The significance of reactive oxygen species generation by the immune system is clearly demonstrated by illnesses of granulomatous disease. Furthermore, ROS released by macrophages and neutrophils can regulate the immune response by destroying pathogens directly or by activating immunological-related receptors (Rodriguez 2008). Oxidative stress is defined by a nonphysiological rise in ROS production, which rise causes cell malfunction that can lead to permanent cell lesions by oxidatively altering proteins, lipids, RNA, and DNA. This disorder process can mediate destruction of cell structures, which leads to a number of diseases. In the long term, a rise in prooxidant factor levels can lead to structural flaws in mitochondrial DNA, changes in the functioning of enzymes or cellular structures, and the emergence of aberrant gene expression or functional or structural abnormalities (Young and Woodside 2001). In a research, D. Harman suggested that by including free radical reaction inhibitors in the diet and limiting the consumption of goods that cause these reactions, one may extend life and slow down the aging process in cell physiology. Therefore, it seems that the compounds of antioxidants can scavenge free radicals in the human system and have a significantly pivotal role in significantly reducing the occurrence of inflammation, infection, apoptosis, and dysfunction (Harman 1969). Then, Denham Harman proposed the notion that aging and age-related disorders are the deleterious consequences of the body's buildup of free radicals and the damaging effects of a phenomenon known as oxidative stress in the body (Harman 1956). Due to ROS's role in aging and aging-related disorders, including atherosclerosis, neurological and cardiovascular diseases, and others, investigation into ROS is expanding these days. Consequently, the significance of eliminating excessive ROS is being noticed, and antioxidants are frequently utilized to accomplish this. Plants, to protect themselves against oxidative stress brought on by excessive ROS formation, have evolved antioxidant defense mechanisms (Tripathy and Olmuller 2012).

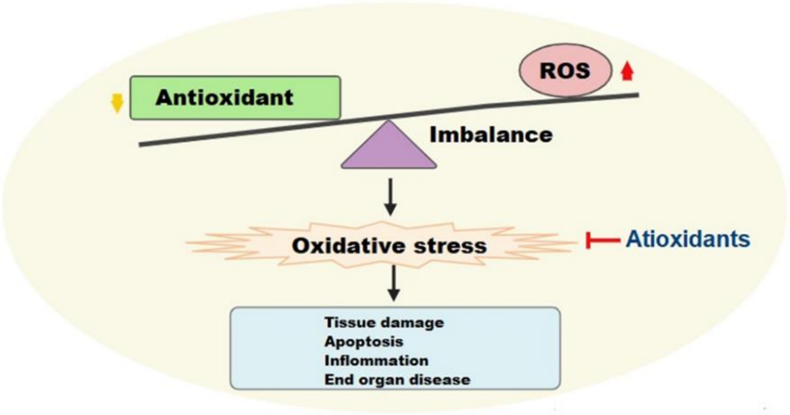

Figure 2: The schematic of oxidative stress: the result of an imbalance between the production of ROS on one side and cellular defenses on the other.

2.1. Signal Transduction Oxidative Stress and Defense Pathway in The Cell

Oxidative stress, defined as an imbalance between the body's natural defense system of antioxidants and the formation of ROS, is linked to the appearance of several disorders, such as chronic fatigue syndrome, cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure, and schizophrenia. Oxidative stress has the ability to directly oxidize macromolecules, such as proteins, lipids, and DNA, which can cause cytotoxicity, cell death, and injury to tissues or regions (Juan et al., 2021). Furthermore, inflammation brought on by a variety of outside stressors can induce mutations in components of cells via the buildup of reactive oxygen species, which can then result in cancer.

ROS, produced by a variety of extracellular and intracellular activities, play a function in cell signaling, development, differentiation, progression of cells, and cell death. ROS preserve the body's homeostasis under the physiological condition by modulating a variaty of pathways of cell signaling that are important in cellular functions (Sosa et al., 2013). Overproduction of ROS alters the structure of cellular macromolecules and results in oxidative stress. Consequently, the cellular and biological activities are disabled with the signaling within cells being modified, ultimately changing the typical physiological signal and chaining to the apoptosis or cell death signals. ROS also has a crucial role in maintaining the equilibrium between intracellular and external cellular Ca2+ levels, so is thought of as a secondary messenger for the activation of cellular signaling (Jelic et al., 2021).

Further, the antioxidant systems in the body are a dependable defensive mechanism that had to react to the collected ROS. Antioxidants are able to generate bidirectional hormetic reactions at the level of cells as well as full organisms and can function specificaly on cells and tissues in various biological circumstances biphasic impacts, stimulate, and prevent of any type of biologic activity. So that NADPH and GSH, as two important antioxidants, can preserve the oxygen and oxidation reduction in cells (Forman et al., 2014). In the human organism, the control of enzymatic antioxidants (glutathione peroxidase, catalase, SOD) and non-enzymatic antioxidants (bilirubin and albumin) natures because of the mechanism of antioxidants in a biological system permits ROS to be deactivated. If the body is submitid to a high level of ROS, exogenous antioxidants such as food, dietary supplements, or pharmaceuticals are administered in order to shield the organisms and reduce oxidative stress. However, a variety of investigations have demonstrated that many plants and the derivatives of them contain significant natural antioxidants that due to their great bioactivity, low toxicity, and widespread utilized to prevent oxidative stress. The phytochemicals, or naturally occurring antioxidant substances, gradually modulate the ROS-sensitive signaling pathway and restock the antioxidant inside the cell via preserving the redox equilibrium (Yingying et al., 2024). The triggers of such signaling pathways surged the expression of genes encoding cytoprotective proteins, such as antioxidant enzymes, growth factors, and proteins inrelated to the control of cellular energy metabolism. This section explains how antioxidant properties of plants can impact oxidative stress.

3. ANTIOXIDANTS

The term "antioxidants" originally was defined as compounds that can prevent oxidative processes in the manufacturing processes of the food and chemical industries and shield cells from the damage of free radicals produced. In a broad sense by Halliwell an antioxidant is “any substance that delays, prevents, or removes oxidative damage to a target molecule”. These substances are vital to life and are needed in the diet for wellness health. ca (Forman et al. 2014). Originally, antioxidants safeguard the cells from oxidative stress via one of two ways: (a) the chain-breaking mechanism, in which the primary antioxidant gives the free radical an electron; (b) the second mechanism, which involves quenching a chain-initiating catalyst to remove ROS/RNSinitiators. Thus, antioxidants regulate the human physiological processes by inducing and expressing antioxidant enzymes that help to prevent illness or its progression and to maintain one's health (Kumar et al., 2017). The body has a variety of processes to combat oxidative stress via producing antioxidants: a: endogenous antioxidants that can be produced naturally spontaneously in the body; b: exogenous antioxidants that are externally acquired from meals, fruits, and vegetables. Some of the antioxidants, as biochemical compounds, involve vitamins, polyunsaturated fatty acids, and minerals utilized either singly or as a combined treatment that are found in nature and reduce oxidative stress in organisms, and some of them are obtained in synthetic form commonly used as supplements in plants.

Recent pharmacological investigations demonstrated that the numerous organic antioxidants found in some plants, fruits, seeds, and natural products have a variety of health-promoting properties. Adopting useful plants with strong antioxidant capacity would enhance the treatment of illnesses attributed to free radicals more effectively while avoiding the toxicities and unfavorable side effects of traditional medicine. If these normal processes are impaired, radicals stack up abnormally and are induced to the development of several illnesses (Fromage 2012). Based on these investigations, dietary suggestions have been emphasizing the utilization of plant foods that are antioxidant-rich, numerous novel pharmacological plant antioxidants for the cure of various pathologies have been constructed, and antioxidant supplements have become a crucial component of vitamin compounds.

3.1.The Benefits of Antioxidants for Mind and Body

Incorporating plant-based antioxidants can offer a plethora of benefits for body (Fig.3).

Reduced Inflammation: Chronic inflammation is a root cause of many diseases. Antioxidants help combat inflammation by neutralizing free radicals and reducing oxidative stress in the body.

Improved Cognitive Function: Research suggests that antioxidants may play a protective role against cognitive decline and neurodegenerative diseases. Consuming foods rich in antioxidants can improve cognitive performance and promote brain health (Knockaert et al., 2015).

Enhanced Immunity: Antioxidants help strengthen the immune system by protecting cells from harmful caused by free radicals. Protecting overall health and avoiding off infections require a strong immune system.

Healthy Aging: By neutralizing free radicals, antioxidants can slow down the aging process and promote youthful vitality. They help protect against age-related conditions, including cardiovascular disease, arthritis, and vision loss (Hajam et al., 2022).

Cancer Prevention: Some antioxidants have been demostreated to have anti-cancer attributes by inhibiting the development of cancer cells and reducing the risk of tumor formation. Frequent consumption of antioxidant-high meals can reduce the risk of developing some cancers. Therefore, plant-derived antioxidant compounds have attracted significant interest in the last years because of their therapeutic value in both the avoidance and cure of illness, whether deriving from entire plants, extracts of plants, or even isolated constituents with complete phytochemical profiles (Cui et al., 2020). Therefore, daily consumption of a wide range of phytochemicals could hold promise for an adjunctive treatment for various diseases, such as cancer, cardiovascular disease, diabetes, and neurodegenerative disorders.

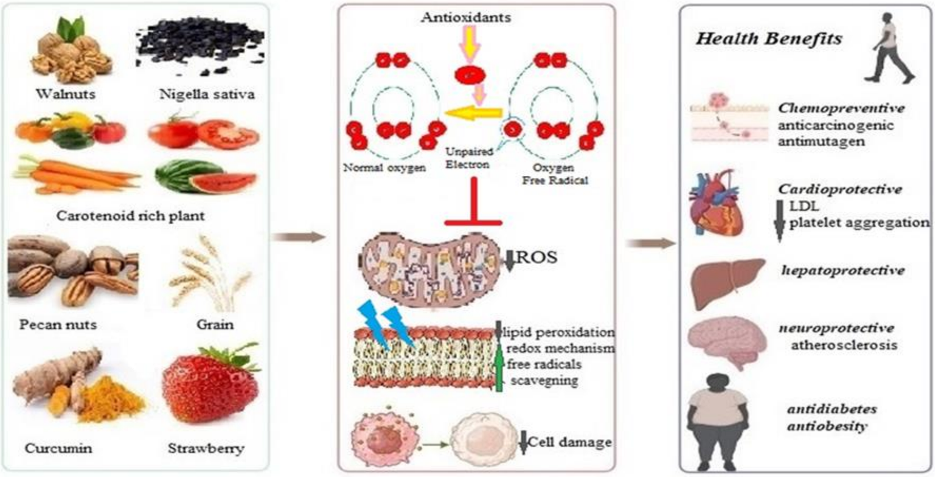

Figure 3: Schematic of antioxidant mechanisms of bioactive compounds from plant-driven sources.

3.2. Classification of Plant-Derived Antioxidants

Plants, as one excellent source of exogenous antioxidants, contain antioxidants to prevent the toxic effects of free radicals. Unquestionably, these naturally occurring antioxidants serve as reducing agents, scavengers of free radicals, quenchers of metals that produce singlet oxygen and pro-oxidants, lowerrs of localized O2 concentration, enhancers of endogenous antioxidant defenses, and prevent damage in repair systems. Additionally, they guard against oxidative stress, which aids in preserving the balance ratio of antioxidants to oxidants (Liguori et al., 2017). Thus, plants could create non-enzymatic antioxidant systems that are more advanced than humans. Incorporating plant-based rich antioxidants into diet plans can be a powerful strategy for promoting optimal health and preventing premature aging. These powerful compounds not only contribute to preventing premature aging but also function an important role in shielding bodies from the detrimental effects of oxidative stres.

Antioxidants are classified into two types: endogenous and exogenous compounds. Endogenous antioxidants are compounds produced naturally by cells and present in our body, and exogenous antioxidants are received from exogenous sources that can be acquired from a variety of natural sources as well as chemically synthesized antioxidants (Fig.4). Plants are a rich source of bioactive molecules and secondary metabolites with antioxidant activity, among several sources of antioxidants. The plant antioxidant defense mechanisms be discreted into enzymatic and non-enzymatic antioxidants Enzymatic antioxidants, like glutathione reductase, catalase (CAT), glutathione peroxidase (GPX), and superoxide dismutase (SOD), as well as minerals including Se, Zn, Cu, Mni. (Ahmad et al., 2010). Nonenzymatic or nutrient-derived antioxidants involve vitamins A, C, E, and phytonutrients that are found in a variety of foods. Non-enzymatic antioxidants, including phytochemicals, depend on the kind and source of the plant. Curcumin, the primary ingredient of Curcuma longa, is one of the healthiest phytochemicals obtained from plants and has pharmacological advantages, including anti-inflammatory and antioxidant capabilities. Resveratrol, as a phenolic compound, offers protection against metabolic, cardiovascular, and cancer disorders (Yang et al., 2018).

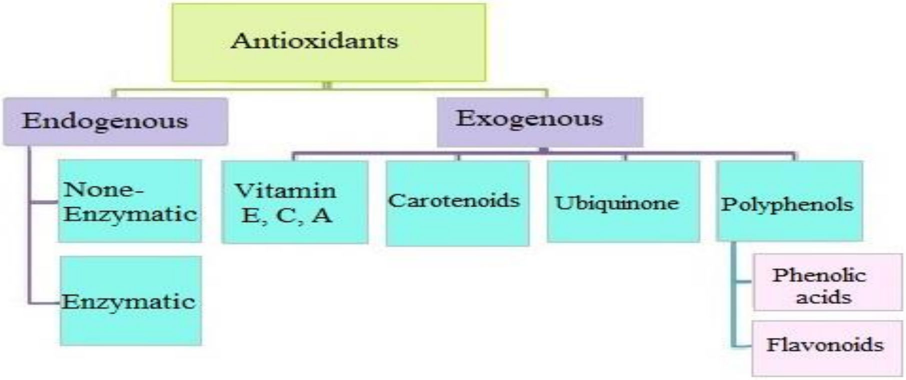

Figure 4: Schematic of classification of antioxidants

3.2.1. Endogenous Compounds

Endogenous compounds in cells can be divided into two types: enzymatic and non-enzymatic antioxidants. (Irato and Santovito 2021). The first-line antioxidant defense system enzymes, which are the main antioxidant defense system enzymes in the neutralization of ROS, include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GRx), that are found in cellular cytoplasm and mitochondria (Ighodaro and Akinloye 2018). The second-line protective antioxidant system involves non-enzymatic antioxidants, subdivided into metabolic and nutritional antioxidants. Nonenzymatic exogenous antioxidants are mostly btained from fruits, vegetables, plants, and photosynthetic organisms and constituent to several families like melatonin, carotenoids, natural flavonoids, thiol antioxidants (lipoic acid, glutathione, and thioredoxin), and vitamins E and C. Dietary antioxidants like flavonoids, α-tocopherol, ascorbic acid, carotenoids, amino acids, peptides, proteins, and other phenolic compounds can be beneficial in the enhancement of the antioxidant system. The non-enzymatic system's function is trapping and neutralizing free radicals in order to prevent the radical initiation reaction (Ullah et al., 2020).

Metabolic antioxidants belonging to endogenous antioxidants are created by metabolism in the body, which quickly neutralizes oxidants like ROS, lipoid acid, glutathione, L-ariginine, coenzyme Q10, melatonin, uric acid, bilirubin, metal-chelating proteins, and transferrin (Miro´nczuk et al., 2018).

Nutrient antioxidants, which belong to exogenous antioxidants, have a crucial function in helping endogenous antioxidants neutralize oxidative stress. The deficiency of nutrient antioxidants is one of the causes of chronic and degenerative pathologies and illnesses (Wang et al., 2018).

3.2.2. Exogenous Compounds

Exogenous compounds, known as nutrient antioxidants, are substances that the body is unable to produce on its own and must obtain from food or supplements. Examples of these substances include ubiquinone, carotenoids (such as beta-carotene), trace metals (such as zinc, manganese, and selenium), phenolic acid (flavonoids and phenolic acids), and omega-3 and omega-6 fatty acids. The function of exogenous compound antioxidants is to avoid or reduce the harm to cells caused by the free radicals. It is noteable that some naturally occurring exogenous compounds that contain the flavonoids interact directly with the ROS to produce less reactive or stable complex molecules, also can act as a system of repair for the enzymes that are damaged by ROS (Middleton 2000). So, this section goes into detail about the natural antioxidants, the evaluation of antioxidant activity at the cellular levels, and their primary sources, which are food and medicinal plants.

3.2.2.1. Phenolic compounds (flavonoids and phenolic acids)

Phenolic compounds are the products of a varied classof secondary metabolites that comprises approximately 8,000 naturally occurring compounds (Tabart et al., 2009). Phenolic compounds are potent chemical antioxidants, active reductants, and free radical scavengers due to their bearing one or more aromatic chains with a number of hydroxyl groups. The studies that have shown that polyphenols and their bioactive compounds offer protection by scavenging a variety of ROS, such as peroxyl and hydroxyl radicals, hypochlorous acids, superoxide anions, and peroxynitrite signaling pathways. Additionally, they inhibit the activities of free radical enzymes, which ultimately protects oxidative stress and apoptosis from causing damage to cells (Zargoosh et al., 2019). In addition to the antioxidant role of phenolic compounds in plants, the importance and health benefits of them provide in diets as well. For instance, epigallocatechin and epigallocatechin gallate (EGCG) that were found to be the most abundant in tea and curcumin that is obtained from the rhizome of Curcuma longa are the phenolic compounds (Tabart et al., 2009).

Phenolic compounds can be grouped into two major categories: phenolic acids (non-flavonoids, such as resveratrol, sesamin, and ellagic acid) and flavonoids (such as curcumin, epigallocatechin gallate, apigenin, and quercetin) (Mukhtar et al., 2000). Some of the best food sources of phenolic compounds, including cranberry, apple, red grape, strawberry, pineapple, banana, peach, lemon, orange, pea grapefruit, broccoli, spinach, yellow onion, red pepper, carrot, cabbage, potato, lettuce, celery, cucumber, and others are shown in Figure 5.

Figure 5: Schematic of most valuable dietary sources of phenolic compounds

3.2.2.1.1. Flavonoids

Flavonoids are substances of polyphenolic compounds that are found in most plants. Each plant contains specific flavonoid compounds, which is why various plants, rich of these compounds, have rather diverse physiological effects (Hanneken et al., 2006). Flavonoids have been acknowledged for their unique antioxidant beneficial properties on human health. Flavonoids by inhibiting xanthine oxidase and chelating transition metals can stop the creation of oxidants. They can also stop oxidants from attacking cellular targets by electron donation and scavenging activities, stop oxidative processes from spreading by breaking the chain of antioxidant activity, and increase the antioxidant capacity of cells by protecting other antioxidants and promoting the expression of endogenous antioxidants (Ullah et al., 2020). Flavonoids also, by blocking pertinent enzymes and signaling pathways, have anti-inflammatory and anti-platelet aggregation capabilities that eventually lead to a decrease in the generation of oxidants. Finally, flavonoids used as vasodilator and have alot medicinal values. The therapeutic properties of flavonoid-rich sources as antioxidants increase free radical trapping/scavenging, nitric axide and xanthine oxidase activity, and decrease DNA damage. The antioxidant-rich plants of flavonoid that help to battle against free radicals and enhance longevity are illustrated in figure 6.

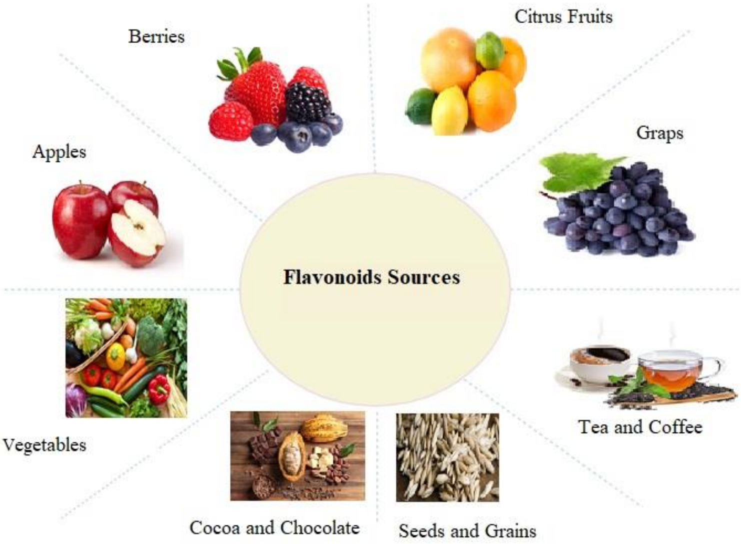

Figure 6: Schematic of best dietary sources of flavonoid compounds.

The major plant-baised sources of flavonoids involve apples, cocoa (chocolate), ginkgo biloba, soybeans, curcuma, berries, onions, and broccoli, grapes, and green tea. For example, catechins and quercetin are flavonoids found in green tea. Green tea has several health advantages, including anti-inflammatory, anti-carcinogenic, anti-hypercholesterolemic, and antibacterial properties. (Pham-Huy et al., 2008). Some studies have reported that flavonoids are linked to the prevention or postponement of the effects of cancer, heart disease, arthritis, aging, cataracts, memory loss, stroke, Alzheimer's disease, inflammation, and infection.

3.2.2.1.2. Carotenoids

Carotenoids (Crt), as very potent natural antioxidants, are a class of pigmented substances that are naturally synthesized by microorganisms and plants but not by animals. Carotenoids, being a lipid-soluble substance, are usually situated in the apolar center of lipid membranes, the mitochondria, and the nucleus and are associated with the inner and outer cell membranes. Carotenoids are classified as β-carotene (green leaf plants, yellow and orange fruits; carrots, pumpkin, mango), lycopenes (ex; tomatoes), and xanthophyls (fruits and vegetables; apples, apricots, mandarins) (Camara et al., 2013 and Gammone et al., 2015). Different types of carotenoids have roles in antioxidant activities. The antioxidant functions of carotenoids in biological systems are based on their singlet oxygen quenching capabilities and the activity to scavenge free radicals in lipid phases. Thereby, they actively trap lipid and water-based phase radicals and funtion a crucial role in protecting free radical harm to cell membranes and lipoproteins, which can lead to harm to lipids and DNA and of being mutagenic. Furthermore, the main protective mechanism in carotenoids is their lipophilicity, which enables them to interpenetrate via the cellular lipid bilayer membrane and pass the bloodbrain barrier to perform their biological roles throughout the human body, such as the brain. These effects are mostly solely associated with the triggering of apoptosis and the generation of ROS by carotenoids, which could avert programmed cell death by reducing the creation of ROS and lipid peroxidation byproducts (Jiri et al., 2024).

3.2.2.1.3. Trace elements

Trace elements are be in the natural world in a variety of forms. These elements are crucial for biological processes and are directly related to the metabolic and physiologic processes of the body. As cofactors of antioxidant enzymes, the trace elements zinc, copper, manganese, selenium, and manganese shield the organism against oxygen-free radicals created in oxidative stress. Also, a number of trace elements have roles in redox processes. Some other trace elements stabilize protein and enzyme structures and function as co-factors for certain enzymes, enabling them to cooperate in important biological processes. The deficiency of trace elements can lead to severe bodily malfunction, heightened sensitivities to systemic infections of the mouth, postponed mental and physical development, and reduced efficiency (Marcin et al., 2023).

3.2.2.1.4. Selenium

Selenium (Se) is an important trace mineral that is ingrediet in the active regions of various antioxidant enzymes, such as thioredoxin synthase and glutathione peroxidase. Also, selenium is the precursor of the enzyme superoxide dismutase, which acts with ascorbic acid, enzyme, and oxidative dismutase to create one of the main and most significant oxidative defense strategies (Rayman 2012). The health benefits of selenium can be immunomodulatory, anti-carcinogenic, and antioxidant properties. Selenium can affect autoimmune reactions by altering mediator and transmitter release or making immune cells more resistant to peroxidation (Pham-Huy et al., 2001). Selenium is present in soil, water, vegetables (garlic, onion, cereals, nuts, soybeans), seafood, meat, liver, and yeast.

3.2.2.1.5. Ubiquinone (COQ10)

Ubiquinone (COQ10) is a required element for every cell in the body that functions as an antioxidant to shield cells from the aging process, combat oxidative stress, and avoid harm to tissues. CoQ10 in its active form is called ubiquinone or ubiquinol. Ubiquinone, or ubiquinol, is an active state of CoQ10. Coenzyme Q10, as a potent antioxidant, can boost absorption of other critical nutrients and helps recycle vitamin C and vitamin E, thereby enhancing their effects, and as a “coenzyme,” CoQ10 also helps other enzymes operate efficiently (Saulo et al., 2022). Also, coenzyme Q10 is not only essential for generating cellular energy but also for protecting cells from damaging free radicals and counteracting their effects. The deficits of CoQ10 are impacted by the damaging effects of oxidative stress and cause heart disease, diabetes, cancer, fibromyalgia, and cognitive decline (Li et al., 2015). The richest natural sources of vegetarian options dietary that supply CoQ10 include broccoli, cauliflower, oranges, strawberries, beans, and some vegetables.

3.2.2.1.6. Vitamins

Vitamins, as a large category of organic components, are necessary nutrients that play important functions in human health and can be found in certain foods. The human body is unable to produce vitamins, so they are utilized as dietary supplements. They perform a variety of crucial activities in the body to keep a healthy metabolism in the body. Thereby, the advantages of vitamin-rich foods and plants for enhancing metabolism and health-promoting activities are important (Combs 2016). The A, C, and E vitamins have roles as antioxidants. These nutrient antioxidant vitamins (A, C, and E) significantly function in the lowering and management of oxidative stress and infectious illnesses by neutralizing free radicals. Thus, it is suggested to take vitamins as supplements in addition to a regular diet because their deficiencies can impair the immune system (Keservani 2016).

Vitamin A

One of the fat-soluble vitamins, vitamin A, is considered a crucial nutrient in the food of mammals, and it enters tissues in the form of retinol. It is having a significant impact on the development, flexibility, growth, and differentiation of brain tissue as well as the visual cycle. The antioxidant properties of vitamin A are important functions in trapping the species of free radicals. Because vitamin A is autooxidized when under elevated oxygen concentrations, and it functions best as an antioxidant in tissues with biological oxygen tensions (Dao et al., 2017). Vitamin A has three sorts: retinal, retinol, and retinoic acid. Among them, retinoic acid (RA) is the active metabolite of vitamin A and, by scavenging free radicals, acts as a strong antioxidant. (Barbara et al., 2009). Thereby, vitamin A demonstrates direct antioxidant activity by functioning as a chain-breaking antioxidant by reacting with peroxyl radicals, which stops lipid peroxidation from spreading within cells and the production of hydroperoxides, so lipids within cell membranes are better able to withstand endogenously generated peroxidation (Chen et al. Citation2020). Furthermore, a lack of vitamin A as as a marker of oxidative stress is believed to control or mitigate oxidative stress by decreasing the ROS (Maxim et al., 2011)

Vitamin C

Vitamin C (ascorbic acid) is a soluble in water vitamin that plays a critical role in immune function. This vitamin is crucial to human health, but as the body is unable to generate it endogenously, thus the body obtains it via the intakes of foods high in vegetables and fruit. Acid fruits and green vegetables (such as broccoli, red and green peppers, tomatoes, cabbage, sprouts, grapefruit, lemons, limes, blackcurrants, oranges, kiwi, and sweet potatoes) are organic sources of vitamin C (Michels 2013). Vitamin C, as as a chain-breaking antioxidant, is an immunomodulator and anti-carcinogenic that traps superoxide and hydroxyl radicals. Vitamin C's protective effects include preventing the progression of peroxidative processes, reacting synergistically with attached to the membrane oxidized vitamin E to squelch free radicals, and regenerating the decreased form of the vitamin (Li 2007). Consequently, vitamin C acts as an agent against oxidative stress and has an important role for scavenging free radicals at the whole of the human body (Padayatty et al., 2003). Also, in biological systems, the antioxidant properties of ascorbic acid, which acts as a reducing agent by donating electrons to a variety of enzymatic and non-enzymatic activities, are extremely efficient. Vitamin C Induces the Reduction of Oxidative Stress. So, vitamin C antioxidant capacity induces the reduction of oxidative stress (ROS), paradoxically preventing apoptosis gene expression under increasing oxygen concentrations (Ye et al., 2013). A number of enzymatic processes, including those that involve the formation of collagen, require vitamin C as a cofactor. Numerous investigations conducted indicate that vitamin C may lower the incidence of cataracts, heart disease, and some types of cancer. Consequently, because the human body's systems are unable to generate or retain vitamin C, a sufficient daily intake of this nutrient is necessary for good health (Colunga 2020).

Vitamin E

Vitamin E is the most important micronutrient fat-soluble vitamin, and tocopherols are the active type of vitamin E. The RBC membrane, mitochondrial membrane, and endoplasmic reticulum have rather high quantities of this vitamin. Vitamin E possesses pro-oxidant effects and interacts with free radicals to create a reactive radical in the absence of co-antioxidants (AGNİESZKA et al., 2022). Vitamin E shields polyunsaturated fatty acids in the cellular membrane against free radicals and singlet oxygen and inhibits the peroxidation of lipids. So that it can function as the body's most significant antioxidant present in lipid membranes and play a role in preventing low-density lipoprotein (LDL) from oxidizing. Thus, vitamin E is capable of being administered as a dietary supplement to trap the free radicals produced in tissues to minimize oxidative stress. Since plants are the primary source of vitamin E (tocopherols and tocotrienols), the significant amounts of this vitamin are resourced from leafy vegetables (asparagus and spinach, safflower, corn, soybeans, mango, jujube, and broccoli), nuts (almonds, sunflower seeds, vegetable oils, whole grains, olives, and beans), and vegetable oils (Brahim et al., 2019).

4. CONCLUSION

Oxidative stress is a complex process that can have long-term effects on the health and welfare of both humans and animals. Oxidative stress can lead to immune system malfunction, harm to tissues, and an elevated chance of contracting other illnesses. Thus, research in this area can play a significant role in the future in helping to comprehend and treat a variety of illnesses, and reducing and eliminating oxidative stress led to an aging decrease. In addition, to counteract ROS and stop oxidative stress, a strong antioxidant system must also be maintained. Thus, the antioxidant issue could offer an avenue strategy for improving the antioxidant capacity of cell therapy to support the treatment of degenerative and chronic illnesses over the long run. Rich sources of antioxidants found in plants and fruits have the potential to lower oxidative stress in living things and offer a substitute for treating disorders linked to oxidative stress, which are caused by free radicals attacking essential biological components like lipids or nucleic acids. Furthermore, adopting nutritious meals with high antioxidant potential can help treat free radical illnesses more effectively and affordably while preventing the toxicities and unfavourable adverse reactions of traditional medicines. Hance, it is time for us to investigate, determine, and evaluate plant-based sources and therapeutic information in light of current developments in the battle toward oxidative stress so as to afford it a due position.

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CHAPTER 7

EFFECTS OF ENVIRONMENTAL POLLUTANTS ON PHYSIOLOGICAL PROCESSES IN ANIMALS

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1. INTRODUCTION

Nowadays, environmental pollutants harm the environment and indirectly harm humans and animals. Especially in the last 10 years, indirect/direct adverse effects of many environmental pollutants have been tried to be revealed in many in vivo/in vitro experimental studies.

Environmental pollution is an extensive problem, and it is of great importance that it results in problems related to the health of human populations. The adverse effects of environmental contamination from the perspective of soil, deterioration of air quality, water pollution, and the effects of soil waste pollution on animals, humans, and plant species especially trees attract attention (Khan and Ghouri, 2011). Many environmental products cause cell damage. Toxic contamination makes people acutely or chronically ill (Wimalawansa and Wimalawansa, 2014).

Many environmental chemicals are known to cause diseases by interfering with cellular functions in living organisms. Factors like diet and lifestyle have been associated with conditions such as diabetes mellitus, cancer, neurological diseases, atherosclerosis, as shown in epidemiological and clinical research. Oxidative stress increasing, either from reactive oxygen species (ROS) elevation or insufficient ROS detoxification, has been linked to all these diseases (Limon-Pacheco and Gonsebatt, 2009).

We carry some of the many chemical pollutants commonly found in nature in our bodies. How these chemicals affect our health is a very important question. We can collect these pollutants under the following headings pesticides, heavy metals, nanoparticles, microplastics, and plastics (Limon-Pacheco and Gonsebatt, 2009). Once ingested and bioaccumulated, environmental particles cause a range of toxicological alterations (like gut dysbiosis, oxidative damage, intestinal effects, etc.) and can also threaten human and animal health via the food chain (Chang et al., 2022).

Figure 1: Major environmental pollutants that harm living things (Limon-Pacheco and Gonsebatt, 2009)

2. EXPOSURE TO PESTICIDES

Pesticides rank among the most significant environmental pollutants. When pesticides leach into water, soil, and surrounding ecosystems, they contribute to environmental pollution, harming various soil microorganisms and leading to several plant diseases (Laghari et al., 2020). Exposuring to pesticides induces ROS formation and oxidative stress, which can impair the immune system, disrupt cell membrane structure, degrade cellular integrity, trigger inflammatory responses, damage DNA (Zeng et al., 2021). Organophosphate pesticides (OPs), used in agriculture, industry, and medicine widely, have left considerable residues in vegetables, soil, and fruits (Uzunbayir and Apaydin, 2021). They are also known to inhibit acetylcholinesterase (AChE) activity (Galal et al., 2019).

With the increasing world population, food control has become an important issue pesticide are often used for this purpose. The inappropriate use of pesticides leads to problems in the production of agricultural products, some of which, such as the resistance of insect pests to chemical poisons, severe soil erosion, and increased incidence of skin diseases and other chronic diseases, including cancers (Sayadi et al., 2024).

Alterations which are induced by pesticides in target enzymes, many of which are involved in metabolism of neurotransmitters, occur in some cases in parallel with these prooxidant effects (Limon-Pacheco and Gonsebatt, 2009). Another example of an oxidative stress-inducing insecticide is dimethoate, which leads to free radical formation and changes in antioxidant systems in reproductive organs in the rat (Uzunbayır and Apaydın, 2021).

Most of the mechanisms of action of organophosphates involve the nervous system and are based on inhibiting their target enzyme acetylcholinesterase (AChE) (Demir et al., 2011). Acetylcholinesterase inactivation leads to the aggregation of acetylcholine at synapses in the central and peripheral nervous systems, which results in excessive cholinergic receptor excitation (Thiermann et al., 1997). One of the most widely used commercial organophosphates is fenamifos (FNP). Accumulation in aquatic organisms has been observed in long-term FNP exposures. While FNP exposure can occur in many ways, it is usually dermal, inhalation, and drinking contaminated food and water (Qader et al., 2019). Pesticides are known to induce oxidative stress in various tissues by elevating of levels of reactive oxygen species (ROS). Lipid peroxidation (LPO) is one of the key mechanism of pesticide caused toxicities (Demir et al., 2011). And malondialdehyde (MDA), the primary marker of LPO in cells, is a major oxidation peroxidized polyunsaturated fatty acid product. Increasing in MDA value indicates cellular damage caused by pesticides. In general, elevated ROS levels and resulting lipid peroxidation weaken mitochondrial antioxidant defenses (Karaboduk et al., 2024).

Epidemiological studies show that pesticides not only harm the environment but also have numerous adverse effects on reproductive organs (Mega et al., 2022). Previous research has linked pesticide-induced cytotoxicity driven by increased ROS and alterations in activities of antioxidant enzymes (Apaydın et al., 2023).

CAT and SOD are the two main subcellular defences of the antioxidant defence that works to neutralize the free radicals generated when exposed to experimental pollutions. Superoxide dismutase (SOD) enzyme is responsible for the dismutation of superoxide, so it converts superoxide radicals into H_20_2, while catalase (CAT) then transforms H_20_2 into H_20. Glutathione peroxidase (GPx) can also reduce hydrogen peroxide to H_2O. Additionally, glutathione S-transferase (GST) facilitates the conjugation of various electrophilic compounds to the thiol group of glutathione, creating less toxic substances. These enzymes play critical roles in cells by breaking down numerous environmental pollutants, including pesticides (Uzun and Kalender, 2013).

Studies have also shown that pesticides disrupt blood biochemical parameters in experimental animals (Uzun and Kalender, 2013). For example, the organophosphate insecticide chlorpyrifos (CPF) has been reported to cause hepatotoxicity by altering liver marker enzymes such as alanine transaminase, aspartate transaminase and lactate dehydrogenase. For this reason, biochemical parameters have been widely used as bioindicators in toxicological studies.

Increased levels of these enzymes may be caused by liver dysfunction, which disrupts the metabolism of these enzymes and alters the sensitivity of the hepatic barrier. The increased serum lactate dehydrogenase level may also be due to hepatic cell necrosis. This leads to leakage of the enzyme into the bloodstream. The animals treated with CPF also had significantly lower albumin and total protein levels than the control rats. The liver synthesizes albumin, and is protein. The level of albumin may be reduced in animals with liver dysfunction following exposure to CPF (Uzun and Kalender, 2013).

When we look at the mechanism of action of some pesticides, we can see differences in their mode of action. For example, Fipronil is known to act on the central nervous system of insects through non-competitive antagonism of gamma-aminobutyric acid ionotropic receptors, blocking the typical passage of chloride ions and the transmission of normal neural impulses (AlBasher et al. 2020).

Pesticides also have adverse effects on hematological parameters. In some researchers’ study, chlorpyrifos-treated rats significantly increased white blood cells and platelet counts. These data are supported via histological analysis showing mononuclear cell infiltration in their study (Uzun and Kalender, 2013). After administration of toxic doses of chemicals, differences in blood parameters indicating haematological defects may occur. In addition, researchers reported that leukocytes, which play a key role in preserving immune cells and are highly reactive to toxic chemicals, are also reduced. This may be due to migration into the area of damage or a decline in the number of blood cells (Karaboduk et al., 2024a,b).

Pesticides have also been shown to cause changes in body weight in experimental animals. This can be related to pesticide that may disrupt general metabolism and hemostasis in rats (Karaboduk et al., 2024b).

Various pathological changes in tissues can be caused by organophosphate and other pesticides (Uzun and Kalender, 2013). If we give examples of such studies: Chlorpyrifos has been reported to cause many toxicological changes in laboratory animals. It is a lipophilic molecule, so it can easily cross the cell membranes and enter the cytoplasm. When administered at 1/25 of the oral LD50 dose over the experimental period, changes such as sinusoidal enlargement, mononuclear cell infiltration, central vein congestion, nuclear pyknosis, increased Kupffer cell count, and eosinophilic cytoplasm were observed in rat liver tissues (Uzun and Kalender, 2013). Fenamiphos (FNP) is another insecticide widely used in agriculture, applied to control pests on various crops, including apples, bananas, and cotton. When fenamiphos was applied to experimental animals at low doses, many cellular damages were detected like sinusoidal dilatation, hemorrhage, infiltration, and congestion in the liver. Also, glomerular atrophy, infiltration, and congestion were shown in FNP-treated rats kidney tissues. It is generally accepted that the primary pathophysiological due to an unbalance between oxidants and antioxidants is the major mechanism of OP toxicity (Karaboduk et al., 2024b).

Pesticides are also known to damage lung tissue. In their studies, some researchers have shown the subacute toxicity of the pesticide chlorpyrifos on the lungs. In their study, chlorpyrifos treatment resulted in mononuclear cell infiltration, emphysema, epithelial degenerations, hemorrhage, and increased interalveolar septa of connective tissue. These may be due to increased ROS in the lung tissue. They also observed that the less histological differences in the ameliorative substances-treated groups (Uzun et al., 2010).

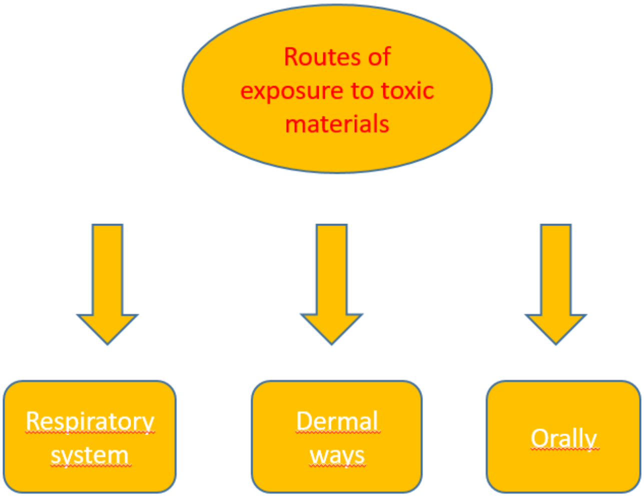

Figure 2: Potential exposure and toxicological routes (Prata et al., 2020)

3. EXPOSURE TO MICROPLASTICS

Microplastics (MPs) have recently been recognized as a significant environmental pollutant. The amount of plastic in the environment has sharply increased due to rising synthetic plastic production and inadequate plastic waste management (Chang et al., 2022). Further research on MP effects should take into account factors such as animal feeding systems, diet, exposure duration, as well as MP size and dosage. MPs not only impact the physical and chemical properties of soil but may also affect food safety for both animals and humans (Chang et al., 2022; Akhtar, 2015; Machado et al., 2019).

Microplastics are a widespread environmental hazard that inevitably leads to human exposure to them (Prata et al., 2020).

Exposure to microplastics can lead to toxicity across biological systems, including oxidative stress, inflammation, and increased particle uptake or translocation. When synthetic particles cannot be cleared by the immune system, chronic inflammation may result, raising the risk of neoplasia. Microplastics contribute to oxidative stress due to their high surface area, the release of oxidant species adsorbed on their surfaces (such as metals), or reactive oxygen species (ROS) released during inflammation. This particle-induced toxicity can lead to oxidative damage and inflammation. Circulating microplastics may also reach organs like the liver and kidneys, which play key roles in metabolizing and excreting foreign substances. Besides particle toxicity, microplastics pose chemical risks, as their matrix can leach harmful chemicals and monomers, such as phthalates and bisphenol A, into the body. These substances, known endocrine disruptors, interfere with hormones even at low concentrations (Prata et al., 2020). Exposure to agents that increase ROS formation can accelerate pathological changes (Limon-Pacheco and Gonsebatt, 2009).

Exposure to pollutants linked to neurodegenerative diseases can lead to neurotoxicity. This may occur due to oxidative stress and the activation of microglia in the brain, triggered either by direct contact with airborne particles or by circulating inflammatory cytokines, which can potentially cause neuronal damage (MohanKummar et al., 2008).

Microplastics, particularly polystyrene microplastics (PS-MPs), have emerged as a significant environmental threat to both animals and humans. Long-term exposure to PS-MPs has been associated with neurotoxicity, carcinogenesis, and reproductive toxicity. A recent study investigated the impact of chronic PS-MP exposure on metabolic and reproductive functions in female rats, finding that subchronic exposure resulted in oxidative stress, endocrine disruption, and chronic inflammation, ultimately leading to metabolic and hormonal imbalances (Saeed et al., 2023).

4. EXPOSURE TO PLASTICS

Plastics are produced through the sequential polymerization of various monomers and other materials, leading to a variety of materials with different properties, including those with glass-like characteristics (Chang et al., 2022).

Bisphenol A (BPA) [2,2-(4,4-dihydroxydiphenol) propane] is an environmental compound commonly used in numerous environmental products. BPA is known to act as a xenoestrogen, which is known to affect the reproductive system. As food and drink make up most of daily human intake, BPA enters the body through the oral route. In addition, changes in testicular functions and heart parameters are induced by BPA exposure (Apaydın et al., 2019; Kalender et al., 2019).

Researchers have reported that exposure to BPA causes various histopathological changes in the testes. These changes include necrosis, edema, and undulation in testes tissues. The pathologic changes seen in the BPA-treated groups in this study are likely to result from increased ROS production, causing oxidative stress (Apaydın et al., 2019). Many environmental chemicals have also been shown to cause cellular damage in the testicular tissue, linked to changing the antioxidant mechanism (Uzunbayır and Apaydın, 2021).

Previous studies have shown that bisphenol A (BPA) increases malondialdehyde (MDA) levels and alters antioxidant enzyme activities in various rat tissues. These adverse effects are likely due to its estrogenic properties (Apaydın et al., 2019; Kalender et al., 2019). In these studies, rats were exposed to BPA via gavage for 4 weeks, and the researchers examined MDA levels, antioxidant enzyme activities (GPx, GST, SOD, CAT), as well as histopathological and cytopathological changes in the heart. Significant differences in MDA levels and antioxidant enzyme activities were observed in BPA-exposed rats, along with various histo/cytopathological changes. Electron microscopy revealed sarcoplasmic reticulum dilatation, cytoplasmic edema, mitochondrial vacuolization, and swelling in myocardial cells from BPA-treated rats. The increased MDA levels and reactive oxygen species (ROS) generation are likely responsible for these pathological changes in the heart (Apaydın et al., 2019).

5. EXPOSURE TO NANOPARTICLES

Nanoparticles (NPs) are attracting increasing attention from scientists due to their unique optical, mechanical, and electrical properties and their wide range of applications in fields as diverse as organ and cell imaging, drug delivery, biomedical sciences, manufacturing, cosmetics, and ceramics. As nanotechnology advances, there has been growing interest in understanding the biological effects of nanoparticles (NPs) on living organisms and the environment (Fidan et al., 2024).

Research has demonstrated that nanoparticles can lead to a variety of pathological conditions, including cellular damage, as they pass through cell membranes and interact with macromolecules, causing nuclear defects and disrupting the structural integrity of proteins (Ahamed et al., 2008; Fidan et al., 2024). Key indicators of oxidative stress, apoptotic markers, and histopathological changes are crucial for assessing the clinical signs of toxicity and play an essential role in toxicological evaluations (Apaydın et al., 2023).

Nickel oxide nanoparticles (NiONPs) are used in various industrial applications, including photoelectric materials, recording devices, catalysts, and sensors. Their use is rapidly expanding across multiple industries (Fidan et al., 2024). Nickel, a naturally occurring silvery metal, is found in many products, including animals, plants, air, oceans, soil, lakes, drinking water, and living organisms (Fidan et al., 2024). Recent studies have focused on the toxicity of nickel oxide nanoparticles, particularly their adverse effects on the respiratory system. Research has shown that both nickel oxide (NiO) and NiONPs increase the likelihood of damage to the bronchi and alveoli, induce oxidative stress, and upregulate p53 gene expression in male rats. Intravenous exposure to NiONPs resulted in significantly more oxidative damage and histopathological changes compared to NiO exposure and caused greater up-regulation of the pro-apoptotic p53 gene (Fidan et al., 2024).

Interleukin-1β (IL-1β) is a key initiator of inflammation and plays a role in the pathogenesis of various diseases (Cano-Cano et al., 2022). Several studies have reported significant increases in IL-1β levels in rats exposed to nickel oxide (NiO) and nickel oxide nanoparticles (NiONPs) compared to control groups. Additionally, upregulation of apoptotic markers such as Bax, caspase-3, and p53, along with downregulation of Bcl-2, was observed in these studies (Karaboduk et al., 2024a). Research has also shown that nanoparticles can cause kidney tissue damage through cellular histopathological changes and biochemical disruptions (Karaboduk et al., 2024a). Nickel compounds, including nickel oxide and nickel nanoparticles, have been investigated in vivo for their potential to induce inflammation, oxidative stress, apoptosis, and pathological changes in lung and kidney cells (Fidan et al., 2024; Karaboduk et al., 2024a). In conclusion, Nickel oxide microparticles and nickel oxide nanoparticles have been observed to induce inflammation by inducing oxidative stress in renal tissues (Karaboduk et al., 2024a).

6. EXPOSURE TO HEAVY METALS

Heavy metals are widely used in industries around the world (Bas et al., 2021). Even at low doses, metals such as lead and mercury can be harmful and accumulate in the food chain (Garcia-Nino and Pedraza-Chaverri, 2014). These metals are significant environmental pollutants, with their concentrations in air, soil, and water steadily increasing due to human activities. Regardless of their density or atomic mass, all heavy metals are toxic. When present in excessive amounts, they can disrupt the metabolic functions of vital organs and glands, leading to damage in both the body and plants (Timothy and Williams, 2019).

Lead and cadmium, in particular, have been linked to decreased antioxidant and acetylcholinesterase activity, increased malondialdehyde levels, changes in serum biochemical parameters, and various pathological conditions (Bas et al., 2021). The kidneys, which are critical for detoxification, are particularly vulnerable to heavy metal poisoning, especially from lead and cadmium. Both acute and chronic exposure to toxic metals can alter the structure and function of the liver, another organ susceptible to the toxic effects of heavy metals due to its role in detoxifying harmful substances. In the toxicity of cells, tissues, and organs induced by heavy metals, oxidative stress plays a crucial role (Bas et al., 2021).

Lead has long been recognized as a highly toxic metal for humans and animals (Rainio et al., 2015). Lead is among the most widely used metals in industrial areas. Primary lead sources, such as lead batteries, mining, ceramics, and crystal industries, have harmed the environment and humans. Airborne industrial deposition can accumulate in sediments and soils (Bas et al., 2021). Like lead, cadmium is a metal used in industry as a fertilizer, in nickel-cadmium batteries, electroplating, and smelting. For this reason, living things are exposed to cadmium in various ways. Previous research has shown that lead and cadmium can alter the antioxidant capacity of enzymes by inhibiting functional sulfhydryl (SH) groups, as they have a high-affinity for -SH groups in these enzymes (Bas et al., 2021).

The common toxicological effect of heavy metals is believed to stem from the inactivation of enzymes and functional proteins through direct binding to them. However, recent studies suggest that the toxicity may also be due to oxidative damage caused by the formation of reactive oxygen species (ROS) in the presence of heavy metals (Tsuji et al., 2002).

Exposure to lead has been shown to increase the production of intracellular reactive oxygen species and lipid peroxidation, which can result in tissue damage, particularly in the reproductive systems of animals. Additionally, lead has been reported to affect the activities of cellular antioxidant enzymes, such as catalase, glutathione peroxidase, and superoxide dismutase, in various laboratory animals. Lead, a heavy metal to which humans are often exposed, induces lipid peroxidation, which may be caused by the formation of free radicals. The observed increase in antioxidant activity following exposure to lead nitrate could be a protective response aimed at removing the harmful lead nitrate (Apaydın et al., 2015).

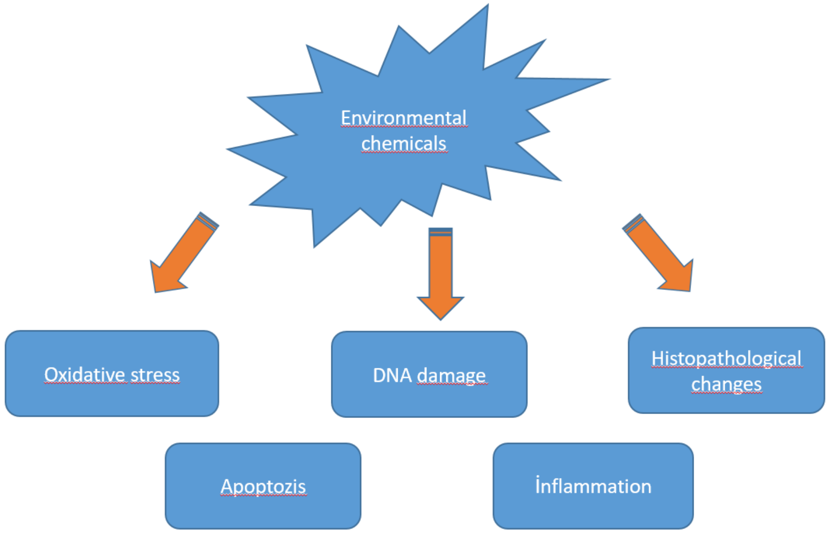

Figure 3: Toxic pathways for many environmental products (Karaboduk et al., 2024a,b)

7. PROTECTIVE MECHANISMS AGAINST THE TOXICITY OF ENVIRONMENTAL POLLUTANTS TO LIVING ORGANISMS

Many in vivo precautions must be taken against damage caused by environmental pollutants (Boya and Apaydın, 2023). Naturally derived antioxidant compounds are essential in modulating diseases related to environmental pollutants that cause oxidative stress by scavenging free radicals. Today, to investigate their pharmacological role, they are used in many in vivo and in vitro studies, particularly toxicology (Bas et al., 2021).

Many diseases caused by environmental pollutants in animals are now linked to free radical damage. Antioxidants are compounds that prevent or counteract this damage. Numerous natural antioxidants, including flavonoids, vitamins, and certain therapeutic supplements, have been used to mitigate the toxicity of environmental pollutants on cells (Apaydın et al., 2015; Bas and Kalender, 2016). Substances rich in antioxidants, such as gallic acid, quercetin, vitamins, and other natural agents, are commonly used to protect against the toxicity of xenobiotics (Uzun et al., 2010; Donmez et al., 2019; Tekeli et al., 2023).

Plant-derived compounds play a crucial role in decreasing the xenobiotics' adverse effects due to their antioxidant properties. Earlier studies have shown the sesamol's therapeutic effects, highlighting its benefits in metabolic regulation, anti-mutagenic, anti-inflammatory, antioxidative, chemopreventive, antihepatotoxic, and antiaging effects (Nayak et al., 2013).

Flavonoids play crucial roles in the plants where they are found, including protection from UV rays, resistance to drought and frost, defense against environmental stress, and protection against harmful environmental substances (Apaydın et al., 2023). For example, Fenamiphos (FNP) is an organophosphate pesticide with a broad range of toxic effects on non-target organisms. Naringenin (NAR) is known for its therapeutic effects in experimental animals and has effective protective roles against oxygen radicals. NAR treatment modulated and blocked the increase in tissue caspase-3 and TNF-alpha expression, IL-17 levels, 8-OHdG, and reduced biochemical markers, as well as renal/hepatic AChE levels. It also alleviated histopathological, ultrastructural, and immunohistochemical damages. Electron microscopy of FNP-treated rats showed swelling in the mitochondria of cells in both tissues, while the FNP plus NAR-treated rats showed fewer ultrastructural changes (Karaboduk et al., 2024b). These protective effects of Naringenin may stem from its anti-inflammatory, antioxidant, and DNA-protective properties, which help reduce inflammation and cell death (Khaled et al., 2023).

Cells have various mechanisms to cope with oxidative stress, supported by both enzymatic/nonenzymatic antioxidants. These chemicals are known to scavenge ROS. Studies have demonstrated that exposure to chlorpyrifos, a commonly used pesticide, induces ROS production and alters the activity values of SOD, CAT, GPx, GST, and MDA levels in various tissues of animals. In one such study, researchers examined the protective effects of quercetin and catechin (Uzun and Kalender, 2013).

Quercetin, in particular, is more effective than other flavonoids at scavenging ROS. This antioxidant potential is attributed to the presence of two pharmacophores: the catechol group in the B-ring and the hydroxyl group, which enhance its ability to neutralize free radicals (Bagheri et al., 2021). These beneficial effects may also be due to their ability to diffuse more readily into cell membranes, facilitating the scavenging of free radicals (Uzun and Kalender, 2013). Catechins, which are found in various plant-based foods like green tea, black tea, and other food sources, contribute to similar antioxidant effects (Baba et al., 2001).

Many studies highlight the healing and protective effects of flavonoids in the medical field, emphasizing their potential to shield against cellular damage caused by various foreign substances (Apaydın et al., 2023). Similarly, the therapeutic effects of vitamins in protecting experimental animals from numerous environmentally harmful substances have been well-documented. Several studies have shown that alpha-tocopherol, a form of vitamin E, inhibits the formation of free radicals and effectively minimizes lipid peroxidation in biological models. Vitamin C, a well-known antioxidant, helps to prevent lipid peroxidation in vitro by directly scavenging radicals and working in synergy with alpha-tocopherol. In some studies, both vitamins C and E were found to reduce pesticide toxicity (Kalender et al., 2010).

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CHAPTER 8

THE “CLEAN GREEN ETHICAL” CONCEPT IN SMALL RUMINANT REPRODUCTION

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1. INTRODUCTION

The earth's population is increasing daily, and in parallel with the increased population, the demand for animal-sourced food (ASF) is also rising. Livestock animals are the main source of this food chain. Animal-sourced foods serve the best available high quality, nutrient-rich food, especially in developing countries. The content of higher quality protein, vitamins, minerals, and essential fatty acids of ASF is more than of plant-sourced foods. Thus, in order to feed the world adequately and be healthy, the sustainability of ASF should be guaranteed. (Adesogan et al., 2020). The human population is predicted to be 9.6 billion by 2050, and the ASF demand will increase by 70% in the same period. The agricultural sector production has predicted to increase by more than 60% (Rojas-Downing, 2017; OECD/FAO, 2020). Although the increase in the agricultural system is essential for human nutrition, the agricultural production process has some deleterious effects on natural sources. During livestock production, greenhouse gases (GHG) release by directly, which can be as enteric fermentation, respiration, and excretion, or indirectly by feed crops, manure applications, farm operations, livestock product processing, transportation, and land use allocation (Baki Acar and Tunç, 2022). The environmental impact of the livestock production systems is defined as ecological footprints (EF) and separated by the carbon footprint (CF), blue water footprint (BWP), economic footprint (E$F), and social footprint (SF) according to the affected sections. Current studies aim to decrease all those EF with sustainable and improved agricultural production systems (Villarreal-Ornelas et al., 2022).

Cattle have a major effect on GHG emissions (62.2% of total GHG emissions), while pigs (10.1%), buffalo (9.5%), and small ruminants (sheep and goats) (7.4%) have less than the cattle (Cheng et al., 2022) in livestock production. Small ruminants tend to grow and reproduce better than cattle even under challenging and complex conditions, and they show more adaptive potential to climatic changes. Therefore, regarding climatic changes and ecological footprints, small ruminant breeding gains importance for ASF in the world, especially in developing and hard-conditioned countries. According to the FAO reports, sheep have a crucial economic value in meat, milk, and wool production of 68,117 M€ (89,000 M$). The main producers in globally are China (34%), Algeria (6%), Türkiye (6%), Australia (5%), Iran (4%), and New Zealand (4%) (FAO, 2021; Villarreal-Ornelas et al., 2022).

In the last few decades, the livestock production industries have needed to give a point of view to avoid being less harmful to nature and climate change. For this reason, the "clean, green, ethical" (CGE) concept was developed by Martin and colleagues (Martin et al., 2004; Martin and Kadokawa, 2006). The productivity and sustainability of meat and the production of milk directly depend on reproductive performance in ruminants (Baki Acar, 2023). Therefore, the CGE studies focused on improving reproductive management and techniques by natural methods. CGE management purposes minimize or finish the usage of chemicals and exogenous hormonal treatments of animals (clean), to minimize the impact of animal industries on the environment (green), and to increase animal welfare (ethical). Nonetheless, to ensure the CGE concept successfully, animal reproduction physiology should first be well understood. Enhancing reproductive parameters and prolificacy of small ruminants by natural methods without using exogenous hormones, the physiological status of females and males, and environmental factors such as season, nutrition, and daylight should ideally be controlled and managed. Otherwise, the fertility and prolificacy results could not be up to the mark (Martin et al., 2004; Martin, 2022).

2. REPRODUCTIVE PHYSIOLOGY OF SHEEP AND GOATS

Sheep and goats are seasonally polyestrous animals, and the onset of sexual activity is stimulated by declining the length of daylight. The average length of estrous cycle in sheep is 17 days, and 21 days in average in goats. The exhibition of spontaneous estrus and ovulation during periods of short daylight is described as breeding season, however period of long daylight is not defined as a non-breeding season. The decrease in daylight and less ultraviolet light transmission through the retina increases melatonin secretion from the pineal gland. Melatonin inhibits the hypothalamic inhibitory A15 dopamine neurons, and hypothalamic sensitivity to estradiol-17β decreases. This situation stimulates the release of kisspeptin from the hypothalamic arcuate and preoptic nuclei. Then gonadotrophin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) secretion gets stimulated. FSH initiates ovarian follicular development, then estradiol-17β secretion increases from granulosa cells of follicles, GnRH and LH pulse frequency increase, and preovulatory LH surge induces ovulation of Graaf follicle in the breeding season, respectively. The increasing daylight length affects the endocrine mechanism of the reproductive system quite the opposite during non-breeding season. Melatonin secretion declines, dopamine release increases, and then secretion of kisspeptin inhibits during this period. The gonadotropic hormone (GnRH, FSH, and LH) secretion decreases, the ovarian activity becomes static, and estradiol-17β levels remain low in the blood circulation. The animal shows no sexual behaviors or reproductive activity, and it is called as the anestrous stage (Noakes et al., 2002; Canooğlu and Sarıbay, 2019; Habeeb and Anne Kutzler, 2020).

The latitude directly affects the season, and at the equator, sheep and goats do not show seasonality, but they continue to show the cyclic activity at any time of the year. The season is restricted and distinct in northern and southern hemispheres, with an anestrus phase after parturition. The breed of animal also influences the duration of the breeding season. The Dorset Horn ewes have a distinctly longer season than other breeds, and Welsh Mountain and Scottish Blackface ewes have shorter season. Some local breeds of Europe and Merino in Australia may not show an annual anestrus. The age of the ewe differs in the length of the season; the ewe-lambs and yearlings have shorter seasons (Noakes et al., 2002).

The estrus cycle is divided into four stages in small ruminants. Proestrus and estrus stages are called the follicular phases. Follicular development emerges by the stimuli of FSH, and estradiol-17β secretion increases in the proestrus stage. The behavioral signs of estrus are observed due to the increasing estradiol-17β concentration. At the end of the estrus stage, preovulatory LH surge induces oocyte maturation. The ovulation of the Graaf follicle occurs 17-24 hours after the preovulatory LH peak, and estradiol-17β concentration decreases rapidly. Metestrus and diestrus stages are called the luteal phase of the cycle, and corpus luteum (CL) forms in few days following ovulation. Progesterone concentration rises during the diestrus stage and decreases to the basal level after luteolysis, which is controlled by

PGF2α from uterine endometrium (Noakes et al., 2002; Canooğlu and Sarıbay, 2019).

3. THE ARTIFICIAL CONTROL OF REPRODUCTIVE ACTIVITY IN SMALL RUMINANTS

Due to the restrictive nature of small ruminant seasonality, alternative control applications are needed to improve reproductive productivity and prolificacy. For this reason, estrus and ovulation synchronization treatments are performed in animals. Artificial control of cycles helps to increase fertility and genetic improvement, collect parturitions in a planned time, increase prolificacy, and plan flock vaccinations and antiparasitic treatments in a scheduled time. It also helps to provide uniform product to markets (Martin and Kadokawa, 2006; Uçar and Özyurtlu, 2019).

The most used and proven estrus induction and synchronization method is exogenous hormone administration protocols worldwide. The usage of these protocols differs according to the season. In the non-breeding season, reproductive control protocols are performed to have more lambs/kids in a year. In anestrous, the ovarian activity needs to be controlled by progesterone first. The melatonin hormone implants also support the progesterone effect. Equine chorionic gonadotropin (eCG/PMSG) and PGF2α administration control the follicular stimulation and luteolysis, respectively, in addition to progesterone usage during the non-breeding season. In the transition period, between the end of the non-breeding season and the beginning of the breeding season, the protocols, including melatonin, progesterone, GnRH, eCG, and PGF2α, are used to mate animals earlier in the season. In the breeding season, synchronization protocols are administered to sync the estrous and ovulation in a short, determined period and to schedule mating and parturitions in a planned period. Progesterone, GnRH, eCG, and PGF2α hormones are in use for this purpose (Baki Acar et al., 2013; Skliarov et al., 2021; Arya et al., 2023).

Nowadays, consumers in the world demand for healthier products, especially in ASF markets. The farm animal production industry is forced to use hormone-free production methods to support public health and animal welfare (Laclef et al., 2023). Therefore, non-medical, hormone-free, natural methods for controlling and improving reproduction in ruminants are researching widely. Controlling lighting and ambient temperature, supporting nutrition, and using the male effect and pheromones are the non-medical synchronization methods in small ruminants (Uçar and Özyurtlu, 2019).

4. “CLEAN, GREEN, ETHICAL” CONCEPT IN ANIMAL REPRODUCTION

Due to the changes in society's expectations of ASF contents and production techniques, the farm animal industries began to change their view to a “clean, green, ethical” production concern. CGE concept defines as Clean: to minimize or eliminate the use of drugs, exogenous hormones, and chemicals; to avoid hormonal and drug residues. Green: to minimize the harmful effect on the environment, make the farm animal industry more sustainable, control the animal-sourced GHG and animal waste, decrease the processing of products from farms (transport, abattoirs, milk factories, etc.), and guarantee the long-term future of the industry. Ethical: to avoid practices that compromise animal welfare (Martin, 2009).

The CGE concept focuses on improving reproductive productivity by controlling environmental challenges using natural methods. Photoperiod, nutrition, and socio-sexual stimuli factors should be enhanced to cope with environmental challenges on reproduction. This is because these factors directly affect the gonadotropic and endocrine control of female reproduction, and play a crucial role in folliculogenesis, ovulation, and prolificacy (Martin and Kadokawa, 2006). External environmental inputs affecting sheep and goat reproduction provide management opportunities and enhance fertility. Control of the timing of the reproductive process, nutrition management, maximizing offspring survival, and genetic selection could be done through CGE practices (Martin, 2022).

4.1. Control of the Timing of Reproductive Process

In the small ruminant reproduction, some physiological handicaps prevent the productive efficiency. Puberty of females, seasonal breeding, and postpartum anestrus limit the producers. In conventional farm practices, exogenous hormone administrations hasten puberty and animals may breed if they have reached sufficient body weight, induce estrus and breed in the out of season or postpartum anestrus. However, exogenous hormone and drug usage are not acceptable in the CGE concept, and alternative practices have been developed to manage physiological handicaps (Martin and Kadokawa, 2006).

4.1.1. Changing the lighting durations

In small ruminants, decreasing photoperiod induces ovarian activity, and the breeding season can be changed. The daylight control is performed with exposure to artificial lighting for 16-18 hr/day for eight weeks during the winter solstice. In the mid-winter, following the eight-week period, the length of the light exposure decreases to 8 hr/day for 6-8 weeks until the start of estrus cycles (Rautela and Katiyar, 2016). In addition, daylight control mostly affects ovarian induction insufficiently; thus, supportive practice, such as the male effect or short-term nutrition support (flushing), should be needed (Martin, 2022).

The stimulation by light treatment is not only used for females but also for males in small ruminants. The exposure of 2 months of extra light treatment for the bucks and rams in the autumn and winter; the sheep and goats do not show seasonal anestrous following the introduce of light treated rams and bucks. Light-treated males positively affect the prevention of seasonal anestrus in females, and ovarian and estrus activity can be prolonged (Delgadillo et al., 2022; Abecia et al., 2022). The mechanism of prolonged ovarian and estrus activity by light-treated sexually activated males involved the induced LH secretion and preovulatory LH surges in the seasonal anestrus (Abecia et al., 2019; Abecia et al., 2020). The studies on the light exposure effect of rams reveal that socio-sexual interactions could be used to restrict out-of-season reproduction in small ruminants (Palacios et al. 2023).

4.1.2. The Male Effect

In the non-breeding season or lactation anestrus, the ovarian activity of sheep and goats can be induced by the sudden introduction of rams or bucks into the flock. This male effect is used in small ruminant breeding for a long time as a natural estrus synchronization method. The pheromones of the rams affect female anestrus by olfactory signals and the hypothalamic-pituitary-gonadal (HPG) axis being activated. The ovulations occur in anovulatory ewes and goats in a few days. The male effect also helps to induce puberty earlier in the nulliparous ewes and goats (Martin et al., 1986; Martin et al., 2004).

The chemical information of pheromone is delivered to neuronal networks in the preoptic hypothalamic cells. This system includes kisspeptin, dynorphin, and neurokinin (KDNy system) in the arcuate nucleus, and KDNy controls GnRH/LH pulse frequency. When females are exposed to males, arcuate nucleus cells, the ventromedial nucleus of the hypothalamus, and the organum vasculosum of the lamina terminalis are activated in the first two hours. The “olfactory memory” also plays a crucial role in the ram effect; the novel and unfamiliar males elicit more effective ovulatory responses in the females. The familiar males cannot evoke an increase in cell proliferation in the hippocampus. Females can recognize the odor of males in the same way that they remember their offspring through olfactory memory (Martin, 2022; Robertson and Martin, 2022).

The response of sheep and goats to the male effect differs in relation to the breed. The Dorset rams are more responsive than the Suffolk, Romney, Romney x Finn, or Coopworth rams. However, the Merino rams are less effective than the Dorset but more than the Romney rams. This difference may be a result of the photoperiod sensitivity, ability of reproductive hormone production, or nutrition factors of rams. Anestrus ewes need intense olfactory, behavioral, visual, and auditory stimulation for 48-72 hours by suddenly introduced males for successful stimulation (Martin et al., 2004; Martin and Greeff, 2011). Age and body condition scores (BCS) of females and males, milk production level, time between drying-off and mating, flock performance, and management factors also vary the male effect response (Laclef et al., 2023).

4.1.3. Nutrition and Focused Feeding

Nutrition is a core environmental factor in animal reproduction and fertility success. Nevertheless, feeding constitutes more than 70% of the total cost of farming management systems. Therefore, farm industries often target maximum feed conversion with minimal forage cost. Nutritional factors directly affect reproductive efficiency in ruminants through HPG axis and endocrine alterations. Inadequate nutrition and negative energy balance inhibit GnRH and LH pulsatility; in females, this situation results deleterious effect on folliculogenesis and oocyte quality, and ovulation failures. In males, malnutrition affects negatively HPG axis, testosterone secretion, and sperm production (Scaramuzzy et al., 2011; Martin and Ferasyi, 2016; Rietema et al., 2019).

In the CGE concept, the "focused feeding" strategy is performed to affect the HPG axis positively, to increase ovulation rates, to maximize prolificacy, to avoid early embryonic and fetal losses. It also helps toprogram the future productivity of the developing fetus, maximize postnatal survival and development in females, and boost sperm production before mating in males. In the rams and bucks, the nutritional supplements are used for short periods, for eight weeks before mating, to increase testicular size, sperm production, sperm motility, and quality. The nutritional stimulus affects the KNDy system and controls the frequency of GnRH pulse. The kisspeptin neurons are activated by supplemental nutrients, then LH pulse frequency increases. Thereby, the fertility of males is maximized, and the pregnancy rate of the flock improves. However, the nutrition and supplementation of the males should be controlled to avoid overnutrition. Because of the expectation of focus feeding is "fit but not fat" males, otherwise, the overweight males' fertility decreases, and reproductive production of farm fails (Martin, 2009).

The focused feeding aims to increase ovulation rates, prolificacy, and productivity with nutrient supplements for about four days in the final stages of the estrous cycle of females without any exogenous hormones or chemical administration. Enhanced nutrition supports glucose supply and metabolic factors such as insulin, growth factors, leptin, and glucosamine. The increased level of these factors in the blood and follicular fluid promotes folliculogenesis, estradiol secretion from granulosa cells, oocyte development and maturation, and the number of gonadotrophin-responsive follicles. Nutritional influence on reproductive functions also includes negative and positive feedback mechanisms of HPG axis (Scaramuzzy et al., 2011; Martin, 2022).

The focused feeding strategy also helps to decrease embryonic loss and increase the future productivity of fetus. Nutritional mineral and vitamin supplements reduce the early embryonic mortality rate when minerals and vitamins are deficient. The strategic nutrition of the mother initiates the future quality and quantity of wool, milk, and meat production of the fetus.

Malnutrition of the mother during placental development and the last trimester of pregnancy negatively affects fetal development, also causes permanent damage (Martin et al., 2004). Nutrient restriction during pregnancy affects growth restriction, glucose intolerance, insulin and hepatic enzyme mechanism deficiencies, peripheral glucose utilization reduction, and alteration in glucose transporter expression in rats (Desai and Hales, 1997). In sheep, undernutrition during the last weeks of pregnancy results in a decrease in secondary follicle initiation and development in the fetal skin and wool production with increased fiber diameter. The development of fetal muscle fibre declines, which negatively affects offspring growth and carcass quality. The puberty age is directly affected by weight gain and BCS; therefore, the puberty age delays. The Sertoli cell number and sperm production capacity are significantly reduced in malnourished male fetuses. Malnutrition causes an insufficient reproductive axis development in male offspring, and future fertility is restricted in these individuals (Martin et al., 2004; Martin, 2022).

One of the crucial issues of reproductive efficiency is newborn survival in the livestock industry. The feed supplement of the mother during pregnancy supports fetal development and enhances the quality and quantity of colostrum. Colostrum is a vital element for newborn survival for all mammalian species. Therefore, another essential step of focus feeding is increasing the colostrum production and quality during pregnancy nutrition. Supporting of the mother especially in multiple pregnancies, avoids stillbirths, fetal losses due to the metabolic problems such as pregnancy toxaemia (Martin and Ferasyi, 2016; Ji et al., 2023). However, overfeeding the mother during pregnancy may cause unintended consequences. The over-adipose tissue storage in the birth canal leads to dystocia. Uncontrolled over-feeding induces fetal weight gain, and dystocia occurs due to the incoordination between fetal and birth canal size. Controlled and sufficient nutrition is very important to avoid malnutrition or overnutrition in the mother and fetus (Martin and Ferasyi, 2016).

4.1.4. Genetic Selection for Future Reproductive Performance

Managing and improving reproductive performance and livestock production using CGE practices are long-term processes. Ovulation rate, embryo survival, udder size, and lamb survival rate are affected by environmental factors, but mostly, they are heritable traits. Therefore, genetic selection is an essential stage for sustainable and effective livestock industry. The genetic selection studies on CGE concept focuses on enhancing the prolificacy, fecundity, fertility rates, and colostrum quality (Martin and Greef, 2011).

Mother-young bonding varies between sheep and goat breeds, and with this, neonatal survival differs genotypically. In Merino sheep, the mother and her newborn lamb recognition takes a longer time. Therefore, the newborn loss rate is higher in Merino ewes than in British breeds (Bickell et al., 2010). Mother temperament is another factor in reproductive effectiveness. Calm ewes have higher ovulation rates (1.83 v 1.57), and twin pregnancies (1.39 v 1.29) than the nervous ewes (Hart et al., 2008). Genetic selection according to the mother temperament has some classifications, such as fearfulness of animals, reactivity to humans, and strange, novel, or threatening environments. Different test techniques, such as the arena test and the box test, are used for selection. For sheep, the Allandale Flock method was created specifically against the "calm" and "nervous" lines of sheep (Martin et al., 2004).

5. CONCLUSION

The earth has been threatened by climate change, overpopulation, limited food sources, and malnutrition for the last few decades. The livestock industry might be a solution for limited food sources and malnutrition. However, this industry is also partly responsible for climate change and global warming due to GHG production. Clean, green, ethical production practices try to reduce the negative effects of livestock production using natural methods to improve reproductive efficiency. The applications of the CGE concept enhance livestock production without any harmful effects on the environment and climate change. Male effect, nutritional supplements, focused feeding practices, and genetic selections are effective practices for this purpose. However, the CGE concept may have yet to have successful fertility rates as exogenous hormone and drug-using synchronization protocols. Therefore, CGE practices need more research to improve reproductive and fertility gains.

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CHAPTER 9

A GLANCE AT THE GUT MICROBIOTA AND ITS EFFECT ON ANIMAL PHYSIOLOGY

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1. INTRODUCTION

A microbiota is a collection of all the microorganisms that are present in an organism. The entire population of microorganisms identified in the gastrointestinal system is called the gut microbiota. The species that constitute the gut microbiota include bacteria, archaea, yeast, and fungi. Bacteria make up more than 98 percent of the microbiota that lives in the digestive tract (Turner, 2018). When bacteria ferment nutrients, they produce metabolites that are essential to the organism's health. Due to this, the intestinal microbiota is considered one of the most important structures for the digestive system overall.

Both the number of microorganisms that constitute the microbiota and the genetic material that these bacteria possess are regarded as components of the microbiome. Several important metabolic activities are regulated by the microbiome. Examples of these tasks include the breakdown of nutrients, metabolizing of these nutrients for energy, supervising the immune system, and forming a barrier to short-lived pathogens. The microbiome, in addition to performing all of its functions, is responsible for preventing inflammation in the event of an infection by virtue of its anti-inflammatory and pro-inflammatory effects, and it also supports the preservation of homeostasis (Küllük et al., 2021). Up to date microbiome research aims to elucidate the complex connection between animal well-being and microbial organisms.

2. INTESTINAL MICROBIOTA DYNAMICS

2.1. Microbiota Composition

Research on microbiota indicates that the density of bacteria escalates from the stomach to the colon. It is recognized that both the concentration of microorganisms and their diversity and populations are increasing. The research of the Human Genome Project, launched in 2007, uncovered 2,172 species inside the intestinal microbiome, grouped into 12 phyla. Among the dominant species are Actinobacteria, Bacteroidetes Firmicutes, as well as Proteobacteria. These species constitute 93.5% of the microbiota (Figure 1) (Hugon et al., 2015).

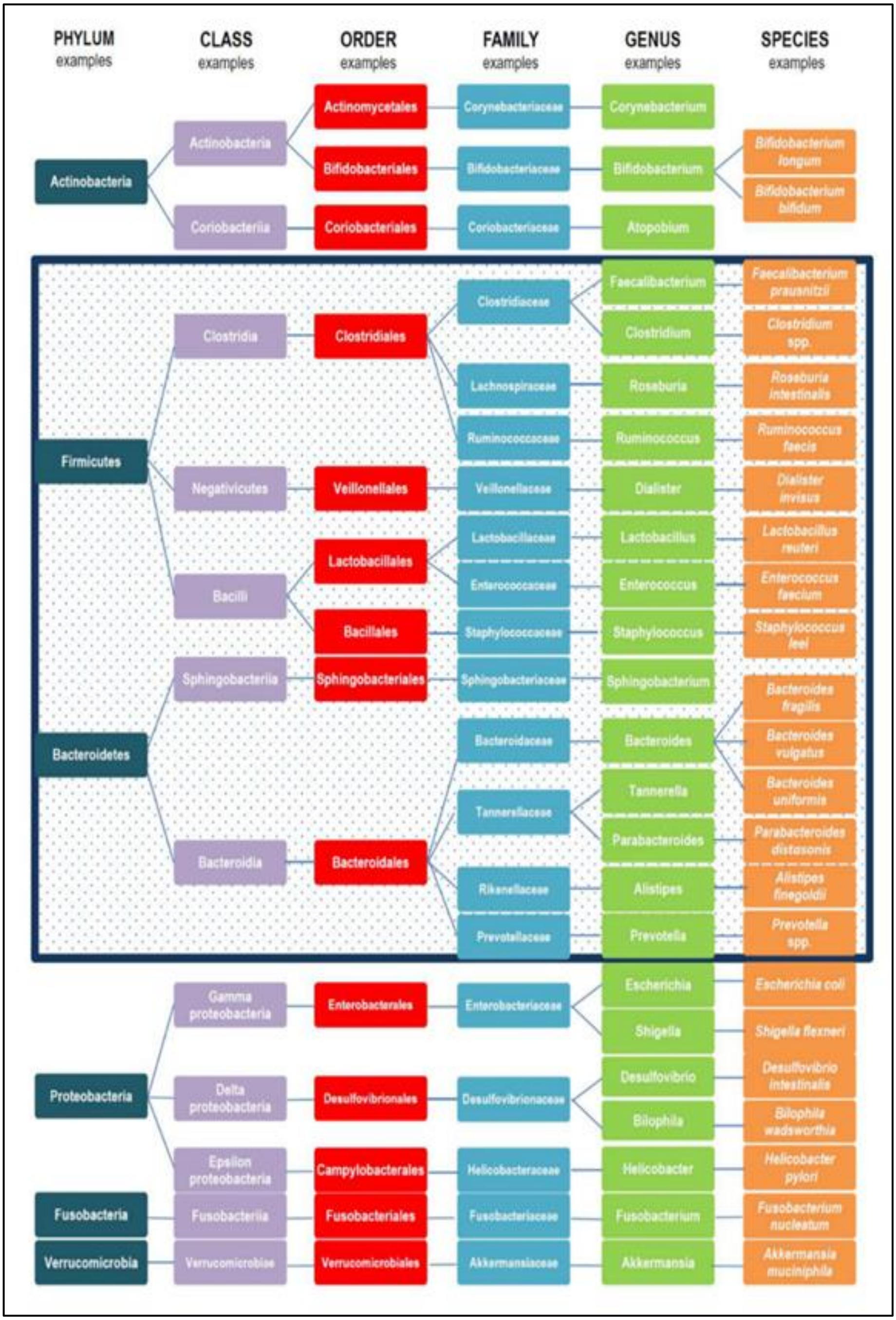

Figure 1: Classification of the bacteria (Rinninella et al., 2019).

A variety of functions are performed by the intestinal microbiota. These include improving food absorption by metabolizing indigestible dietary substances, inhibiting pathogen colonization by creating antimicrobial agents, strengthening the intestinal epithelial barrier, and preventing germs from penetrating tissue. Other functions of gut-based bacteria include vitamin synthesis, bile acid and steroid conversion, xenobiotic metabolism, and intestinal motility control. Through synthesis of vitamin K, biotin, riboflavin, pantetoic acid, and folate, the bacteria also significantly influence host nutrition.

Extensive studies have identified the members of the microbiota involved in some metabolic pathways. Accordingly, the phylum Firmicutes, bacteria found in the colon, participates in glucose metabolism. The phylum Bacteroidetes participates in energy supply, transport, amino acid and carbohydrate metabolism (Ottman et al., 2012).

Short-chain fatty acids (SCFAs) are synthesized by beneficial bacteria to secure the intestinal lumen from harmful bacteria. SCFAs provide an acidic environment by reducing the intestinal pH; hence, they limit the survival of pathogens inside the intestinal lumen. Zhang et al. (2015) found that propionate, butyrate, and acetate are the primary SCFAs that are released when carbohydrates are degraded. The diversity of SCFAs can be affected by the fiber content of dietary intake and intestinal microbial diversity (Krishnamurthy et al., 2023).

The flora bacteria include both beneficial and pathogenic microorganisms. There is a balance between the bacteria living in the intestinal lumen. This equilibrium is one in which beneficial bacteria are predominant and pathogenic bacteria are less abundant. Pathogenic microorganisms may disrupt this balance in the intestine if they become dominant in number. This disruption of homeostasis results in microbial dysbiosis. Metabolic disorders are caused by the metabolic pathways affected by dysbiosis (Table 1). Intestinal infections, allergies, obesity, celiac disease, cancer, diabetes and Parkinson's disease can be observed due to these metabolic disorders.

Table 1: Metabolism of intestinal microbial flora and dysbiosis (Altuntaş et al., 2017).

Metabolism of microbial flora	Dysbiosis
Production of vitamins and SCFAs	Allergy
Synthesis of amino acids	Inflammatory gut disease
Biotransformation of bile acids	Cancer
Fermentation and hydrolyzation of indigestible nutrients	Diabetes, obesity, and cardiovascular diseases
Production of conjugated linoleic acids	Lupus
Modulation of immune system	Asthma
Transformation of ammoniac to urea	Multiple sclerosis
Detoxification	Coeliac

2.2. Metabolites Of Gut and Gut Bacteria

Intestinal microbiota metabolites are basically divided into three groups. These include SCFAs and indole metabolites released by direct bacterial utilization of dietary products, metabolites modified by intestinal microorganisms, and metabolites produced de novo (Postler et al. 2017). Metabolites from the gut microbiota are categorized into nine structural classes. SCFAs, gases, bile acids, indole derivatives, neurotransmitters, vitamins, choline metabolites, lipids, and various metabolites are included in these structural classes.

2.2.1. SCFAs

SCFAs are one of the most important and functional compounds synthesized by the gut microbiome. Structurally, SCFAs are known as saturated aliphatic acids, ranging from one to six carbon numbers. SCFAs include acetate, propionate, butyrate, isovalerate, 2-methylpropionate, haxonite, and valerate. The majority of SCFAs are generated by fermentation of undigested carbohydrates in the stomach as well as small intestine, while a small proportion are the fermentation products of undigested protein-derived branched-chain amino acids (BCAAs). BCAAs such as 2-methylbutyrate and isovalerate are formed by the metabolism of undigested proteins and peptides containing BCAAs (Liu et al., 2022).

The predominant role of SCFAs is to supply energy for colonocytes. The residual SCFAs are conveyed via the circulatory system to many tissues, including the brain, heart, and lungs. SCFAs generally promote the conservation of intestinal barrier integrity, the regulation of energy balance, circadian rhythms, intestinal hormone synthesis, hunger control, the inhibition of proinflammatory cytokines, as well as modulating the systemic immune response (Pan et al., 2021; Romaní et al., 2021). Colonocytes of the large intestine, mediated by hydrogen- and sodium-dependent monocarboxylate transporters, absorb SCFAs following their production.

2.2.2. Bile acid (BA)

The intestinal microbiota is an essential player in bile acid synthesis, influencing the biochemical processes involved. Bile acids are identified under two main groups: primary and secondary BAs. The synthesis of primary bile acids occurs through two distinct routes. The first of these pathways is the traditional pathway in which cholesterol is synthesized by 7α-hydroxylase. The other pathway is the alternative pathway controlled by sterol-27-hydroxylase. The traditional mechanism involves the formation of cholic acid or chenodeoxycholic acid through the action of the enzyme sterol 12α-hydroxylase. The alternate process involves the synthesis of chenodeoxycholic acid (Schwarz et al., 2001). Subsequent to consuming, the duodenum secretes bile acids. Upon reaching the colon, bile acids may be transformed into bile acids conjugated with amino acids, which are vital in intestinal function. The resultant conjugated acids function as agonists of farnesoid X receptors and pregnane X receptors, which are implicated in the immune system, hence aiding in its defense (Aleti et al., 2023). Bile acids are deconjugated by microbial bile acid hydroxylase enzymes. Deconjugated bile acids undergo biotransformation to form secondary bile acids (Krautkramer et al., 2021). Exchanges of deconjugated secondary and primary bile acids impact the mechanisms of various diseases of the immune and central nervous systems.

2.2.3. Gases

Intestinal bacteria create gases including hydrogen sulfide (H_2S), hydrogen, carbon dioxide, methane, as well as nitric oxide (Kalantar et al., 2019). These metabolites produced by microbiota have some functions. For instance, CH_4 decreases intestinal motility. H_2S modulates intestinal motility, epithelial secretion, infection susceptibility, and the suppression of intestinal inflammation. Nitric oxide realises mucosal blood flow by providing gastric mucosal protection. Consequently, gases contribute to regulating the physiological processes of the host.

2.2.4. Tryptophan

Tryptophan is utilized biochemically by intestinal bacteria in several processes. The metabolites formed at the end of the biochemical process of tryptophan act as ligands of aryl hydrocarbon receptors of the immune system's T helper cells. Metabolites that interact with aryl hydrocarbon receptors act a part in the formation of the interleukin-22 cytokine. This cytokine is involved in the inflammation response. Furthermore, it is known that tryptophan enters the kynurenine pathway and produces various intermediate metabolites. It has been reported that the regulation of mucosal homeostasis and microbiota immune tolerance is performed by kynurenin metabolites (Aleti et al., 2023). Research involving quinolinic acid and kynurenic acid has indicated a potential association between these metabolites, and schizophrenia as well as depression. Moreover, a study involving mice with Huntington's and Alzheimer's diseases revealed dysregulation of the kynurenine pathway (Krautkramer et al., 2021).

The intestinal bacteria synthesize tryptophanase, which transforms tryptophan into tryptamine, an indole derivative. Serotonin (5-HT), a metabolite of tryptophan, is synthesized by tryptophan hydroxylase. Neurons of the central and enteric nervous systems have isoforms of the enzyme tryptophan hydroxylase. They are therefore able to synthesize 5-HT. In addition, enterochromaffin cells in the intestinal lumen can synthesize 5-HT. Over 90% of 5-HTs are produced by enterochromaffin cells. Various bacteria, including *Escherichia* spp., *Enterococcus* spp., and *Streptococcus* spp., within the intestinal lumen are recognized for their ability to synthesize 5-HT. With the release of serotonin, gastrointestinal system motility can be regulated. Consequently, in individuals with irritable bowel syndrome, tryptamine may be utilized therapeutically due to its modulation of type 4 serotonin receptors (5HT4R) (Bhattarai et al., 2018). Additionally, indole can be metabolized into indoxyl sulphate by the liver and afterwards enter the portal circulation. Indoxyl sulphate is removed from the body by the kidneys. It has been reported that high amounts of indoxyl sulphate are among the causes of chronic kidney disease. In a study on chronic kidney disease with germ-free mice, it was observed that the use of *Bacteroides* species that produce the tryptophanase enzyme, which contributes to the regulation of indoxyl sulphate level, may be useful in the cure of this disease (Devlin et al., 2016). The effects of tryptophan metabolites and its relationship with the intestinal microbiome on the formation and treatment of various diseases, especially inflammation, can be observed.

2.2.5. Histidine

The amino acid histidine can undergo decarboxylation by the enzyme histidine decarboxylase, resulting in the formation of histamine. Intestinal bacteria, including *Escherichia coli, Morganella morganii*, *Citrobacter freundii*, and *Proteus vulgaris*, possess the ability to synthesize histamine through the action of the enzyme histidine decarboxylase. Histamine involves in gastric acid secretion, immune response control, and intestinal motility. Furthermore, non-oxidative deamination metabolizes histidine to imidazole propionate (ImP), via producing ammonia and urocanate. It has been reported that ImP disrupts the insulin signal, and therefore this metabolite is high in type 2 diabetes patients (Koh et al., 2018).

2.2.6. Tyrosine

Tyramine can be produced by intestinal lumen bacteria such *Enterococcus faecalis, Staphylococcus, Lactobacillus plantarum*, and *Lactobacillus bulgaricus* using the aromatic L-amino acid decarboxylase on tyrosine. Tyramine acts in the regulation of blood pressure. An increase in tyramine resulting from intestinal dysbiosis correlates with hypertension. In addition, a study including patients with ulcerative colitis is indicated an elevation in tyramine levels (Santoru et al., 2017).

2.2.7. Choline metabolites

Choline metabolites include trimethylamine (TMA), methylamine, dimethylglycine, and dimethylamine. Choline metabolites may stimulate thrombosis, limit bile acid synthesis, inflame mitochondrial dysfunction, and stimulate inflammation. The molecular mechanism of these functions has not been fully elucidated. Furthermore, NF-κB can be activated by protein kinase C and NLRP3 inflammasome. Its function is thought to be mediated by this mechanism (Liu et al., 2022).

2.2.8. Lipids

Lipid metabolites produced by intestinal bacteria include conjugated fatty acids, lipopolysaccharide (LPS), cholesterol, triglycerides, and phosphatidylcholines. Among these metabolites, the functions of conjugated fatty acids include regulation of the immune system, hyperinsulinemia and lipoprotein profiles. Cholesterol acts as a basic biomolecule for the formation of bile acids. LPS also takes place in the systemic inflammatory response (Liu et al., 2022).

2.2.9. Neurotransmitters

Catecholamines, serotonin, dopamine, and γ-aminobutyric acid (GABA) are neurotransmitters synthesized or indirectly produced by intestinal microorganisms (McCarville et al., 2020). These synthesised neurotransmitters function by binding to adrenergic receptors, 5-HT receptors, and GABA receptors as ligands. The roles of these metabolites include the regulation of immune system functions and bowel motions, modulation of stress responses, as well as regulation of memory.

3. THE IMPACT OF INTESTINAL MICROBIOTA ON METABOLISM

3.1.The Significance of the Gut-Brain Axis

In recent years, with the understanding of the importance of microbiota, physiological mechanisms of action have started to be investigated. When the mechanisms of its effects are tried to be understood due to the abundance of intestinal microbiota, first of all, studies are drawing attention to the bidirectional interaction between the intestines and the brain. This communication regulates gastrointestinal homeostasis and controls cognitive behaviours, including emotions and motivation. The 'gut-brain axis' (GBA) (Figure 2) executes these control mechanisms (Morais et al., 2021). Recent studies on the GBA interaction seek to reveal the complicated connections between the brain, gut, metabolites as well as physiology.

The GBA includes the autonomic (ANS), central (CNS), enteric (ENS) nervous system as well as hypothalamic pituitary adrenal axis (HPA) (Carabotti et al., 2015). The ANS regulates the two-way connection between the intestinal lumen and the CNS. The communication between the intestinal lumen and the

CNS is carried out through the transport of afferent signals via the enteric, spinal, as well as vagal pathways. The ANS also controls the transmission of efferent signals from the CNS to the intestinal lumen. It is widely recognized that the disruption of communication through these pathways is the cause of CNS disorders, such as anxiety, depression, as well as stroke.

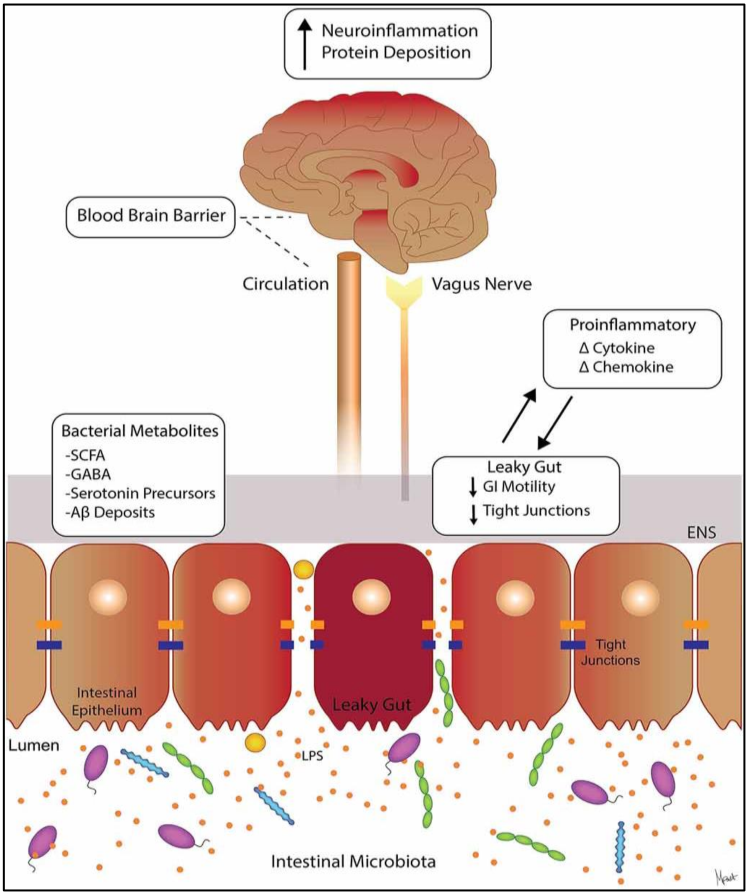

Figure 2: Gut-Brain axis and interactions (Ambrosini et al., 2019)

The HPA, which is an element of the limbic system, governs adaptive responses to stress. Cortisol is known as a stress hormone. The HPA leads to the synthesis of adrenocorticotropic hormone through the pituitary gland, resulting to release of cortisol. Consequently, both the nervous and hormonal systems can communicate. This communication has the ability to affect the activities of enteric neurons, smooth muscle cells, and epithelial cells of the gut and brain. Additionally, research has demonstrated that the gastrointestinal microbiota influences the same cells in GBA (Chakrabarti et al., 2022).

The vagus nerve (CN X) acts as a motorway, providing the physical connection between the ENS and CNS (Sandhu et al., 2017). It is not only associated with the CNS and ENS but also has sensory functions in internal organs such as the tongue, ear, and liver, and motor functions in the heart, diaphragm, and stomach. The vagus nerve enables the brain to understand gut chemistry through neurotransmitters and gut metabolites generated by the microbiota and can affect the immune and endocrine systems. A systemic inflammation can occur when microorganisms and their components are transported into the body due to a breakdown of the intestinal barrier. This condition is a sign of a leaky gut. By stimulating the vagus nerve, the integrity of the intestine can be maintained and restored.

3.2.The Significance of the Gut-Liver Axis

While the gut and liver have distinct morphological and functional roles, they also exhibit characteristics of collaboration. The phrase "intestine-liver axis" was developed to explain the relationship among the intestine and the liver. The connections of this axis are established through the portal vein, systemic circulation as well as biliary tract. The portal vein establishes a circulatory link between the gut and the liver, with 75% of the necessary blood for liver nourishment being given from the intestine. Therefore, the liver is the primary recipient of nutrients that are absorbed via the intestine. Hence, the liver has a prominent part in the transportation of nutrients to other tissues and is regarded as a vital metabolic organ (Ringseis et al., 2020).

The digestion of dietary nutrients generates multiple metabolites in the intestinal tissue, which are formed by the microbiota. SCFAs, acetylcholine, hydrogen sulfide, indole, and phenol compounds are among these metabolites. The production of microbiota-produced metabolites may lead to various effects on the organism. The triggering of the immune system is the most critical of these effects. The liver's production of lipopolysaccharide (LPS) and microbial associated molecular pattern (MAMP) is reduced by the inhibition of intestinal permeability. It also assists the immune system in fulfilling its function by producing hepatic immune cells, natural killer cells (NKC) as well as Kupffer cells (Balmer et al., 2014) (Figure 3).

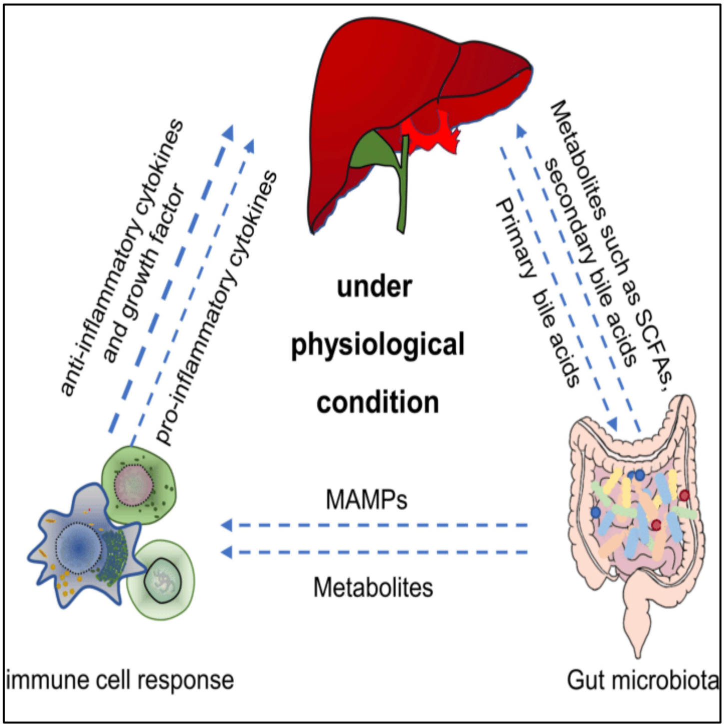

Figure 3: Gut-liver axis (Chen et al., 2021a)

Not only does the intestine communicate with the liver, but there is also a reverse communication. For example, bile acids synthesized by the liver contribute to the uptake of fat-soluble nutrients. Bile acids also take a part in regulating the gut microbiota and preventing the growth of pathogenic microorganisms (Inagaki et al., 2005).

Immunoglobulin A (IgA) antibodies are also synthesized by the liver. The clearance of intestinal microorganisms that travel to the liver is facilitated by IgA (Moro-Sibilot et al., 2016). Therefore, the host is effective in the modulation of the gut-liver axis composition and the establishment of microbial communities in the gut, as well as the secretory functions of the liver.

4. INTERACTION OF GUT MICROBIOTA WITH PHYSIOLOGICAL SYSTEMS

4.1.The Endocrine System

The functioning of the physiological system is regulated by the control of the glands and the hormones produced by these glands by the endocrine system. From this perspective, the endocrine system is essential for the proper functioning of organisms. Recent studies have proved the complex relationship between the gut microbiota and the endocrine system in animals. The gut microbiota has exhibited its impact in the regulation of the endocrine system by the production of metabolites that alter hormone levels and signaling pathways (Clarke et al., 2014). These microbial metabolites, like LPS and SCFAs, can get into the bloodstream and affect distant organs, changing metabolic homeostasis and endocrine regulation (Meyer and Duca, 2023).

The GBA is essential in mediating the impacts of gut microbes on the endocrine system. Enteroendocrine cells (EEC) are a significant component in the functions of the GBA. Some of the microorganisms in the gut, for instance, the phylum *Bacteroidetes*, are capable of the synthesis of specific molecules, including SCFAs. These molecules can have different effects, like increasing insulin release or initiating neuroendocrine responses through binding to related receptors on the surface of EECs (Dalile et al., 2019). Studies in different fish species and livestock animals have shown that EEC is regulated by microbial metabolites, resulting in alterations in lipid metabolism and the expression of the related genes. Thus, these metabolic activities end up with both weight gain and growth (Sheng et al., 2018; Butt and Volkoff, 2019; Chen et al., 2021b).

The gut microbiota can synthesize important neurotransmitter agents including acetylcholine and GABA as well as hormones like serotonin and melatonin. By this way, it can even reach the CNS and directs the attitudes of the organism (Luck et al., 2021). The shift in the heterogeneity of gut microbiota can also affect serotonin secretion in rats, mice and dogs, causing obesity (Hata et al., 2017; Sun et al., 2018; Morelli et al., 2022).

Various studies have demonstrated the regulatory role of the gut microbiome on sex hormones like estrogen and androgens (Guo et al., 2016; Qi et al., 2021). In a study conducted in rodent models of polycystic ovary syndrome (PCOS) caused by androgen-induced dysbiosis, it was observed that glucolipid metabolism and consequently, endocrine system functions were impaired, suggesting a bidirectional communication between gut microbiota and sex hormone levels (Han et al., 2021).

The diversity of gut microbiota has a structure that can change with the reproductive cycle. In a study with sows, it was shown that *Limosylactobacillus reuteri* and *Prevotella* spp. in the gut microbiota affect estrus return by regulating testosterone degradation and estrogen biosynthesis, and that the microbial community in the gut may vary at different gestational stages, thus affecting reproductive performance (Liu et al., 2023a).

Bo et al. (2022) demonstrated that gonadotropin-releasing hormone (GnRH) secretions in early-breeding female mice on a high-fat diet (HFD) ended in an escalation in certain phyla, including *Desulfovibrio* and *Anaerotruncus*, in the gut microbiota. Previous studies have detected these two phyla in obese mice, suggesting that they cause obesity by increasing intestinal inflammation (Zhang et al., 2020). Bo et al. (2022) concluded that both phyla also take part in sexual development.

The gut microbiota can also exhibits its impact on reproductive physiology through transforming the structures of relevant hormones through their enzymatic activities. Steroid hormones that are planned to be conjugated and excreted from the body can be reused by being deconjugated with some enzymes produced by the gut microbiota. For instance, β-glucuronidase prevents the attachment of estrogen to glucuronic acid, causing the escalated levels of estrogen in circulation (Hu et al., 2023). Apart from these, there are also studies which have observed that high levels of hormones in circulation contribute the way for the formation of some types of cancer (Sun et al., 2023).

As a result of the studies, it would be wrong to think that other members of the endocrine system (e.g. thyroid gland) will be unrelated to the gut microbiota. The primary focus of research in microbial endocrinology should be to clarify the development and direction of these interactions and consequences.

4.2.The Immune System

The evolution of the host's immune system can be affected by the gut microorganisms. With these effects, it has a place in important activities of the immune system, such as providing the host's defense against pathogens, regulating inflammatory responses, and optimizing immune responses (Wiertsema et al., 2021; Shao et al., 2023).

The ongoing association between the host and the gut microbiota is symbiotic. Briefly, the host provides habitat and nutrients to the microbial community, and the microbial community aids glucose and lipid metabolisms in the host. Meanwhile, it ensures the maturation of the immune system with the metabolites it produces (Belkaid and Hand, 2014).

Germ-free (GF) animal models are utilized enlighten this complex relationship. Fruit flies, mice, and zebrafish are among the animals that are used as GF models. These animals are isolated from the environment from birth, and their interaction with any microorganism is strictly prevented. Through this, any effects of the microbiota on the animal's natural and acquired immunity can be observed without other causes (Luczynski et al., 2016).

In infancy, the delivery method, breastfeeding status, and environmental factors impact the development of the human gut microbiota. According to Zhang and his colleagues' research from 2021, the microbiota of babies born by vaginal delivery is more diverse than that of babies born by cesarean delivery, which affects the early improvement of the immune system (Zhang et al., 2021).

During early childhood, the gut microbial diversity increases the ability of immune cells to recognize pathogens, which leads to healthy immune system development. According to the conducted studies, increased microbial diversity supports the immune system in later ages and increases the body's resistance to infections (Ronan et al., 2021).

SCFAs synthesized by the gut microbiota are important metabolites that have an immunoregulatory effect. SCFAs, especially butyrate, propionate, and acetate, have an anti-inflammatory effect, reducing the inflammatory response in immune cells and preventing systemic immune imbalances (Yao et al., 2022; Liu, et al., 2023b). They are used as an energy supply within the intestinal epithelium, strengthening the intestinal barrier and preventing pathogens from passing through this wall (den Besten et al., 2013). In addition, according to some studies butyrate supports mechanisms that prevent the development of autoimmune diseases by affecting T cells (Kim, 2023).

Lipopolysaccharides (LPS), one of the molecules synthesized by pathogenic bacteria in the intestine, can trigger inflammatory responses in the host (Ngkelo et al., 2012). LPS can travel from the intestine to the blood and cause systemic inflammation (Tulkens et al., 2020). This can result with the emerging chronic inflammatory diseases (Tucureanu et al., 2017).

Diet type can indirectly cause differences in the immune system by affecting the gut microbial diversity. Western-style high-fat and low-fiber diets can cause an increase in pathogenic bacteria. It has been determined that such diets reduce the ratio of beneficial microorganisms in the gut microbiota, which enhances the inflammation levels. Conversely, a diet rich in fiber increases microbial diversity and promotes the production of SCFAs (Zhang, 2022).

5. CONCLUSION

The gastrointestinal system, in addition to undertaking many important tasks in animal physiology, also serves as a host for many microorganisms in the gut and this biological interaction is an example of symbiosis. The gut microbiota can act like another organ in the body through the different types of microorganisms that make up the microbiota and the metabolites they produce. It can communicate with the brain, like the organs in the body, and it can even affect the animal's behavior with the metabolites it secretes. The fact that it has various effects on the animal's most critical physiological systems gives more importance to the intestinal microbiota. This structure develops with the animal's other physiological systems from birth, so understanding its components and functions can help solve anomalies and diseases. In such studies, which require detailed laboratory and informatics work, the greatest assistants of the researchers will be new sequencing and omics technologies which are developing day by day.

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CHAPTER 10

USE OF ECDYSONE AGONISTS IN PEST CONTROL

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1. INTRODUCTION

The knowledge of negative effects of the pesticides on human and environmental health has led to both an increase in the demand for safe food and the search for the alternative control methods. Although it is not possible to completely eliminate the chemicals used against pests in modern agricultural production, control methods have changed to narrower and target specific pesticides. One of the methods used for this purpose is insect growth inhibitors (Insect Growth Disruptors-IGDs), called third-generation insecticides, which act by disrupting the metamorphosis and molting mechanism of insects. As in all animals, development and growth in insects is under the control of hormones, one of the most important of which is ecdysone, the metamorphosis hormone in insects. Ecdysone agonist insecticides are new generation insect control agents that bind to ecdysone receptors in insects, disrupting the hormone balance chemically and mechanically, and killing insects by inhibiting their development, chitin formation and metamorphosis functions of the larvae. Ecdysone agonists mimic the hormone ecdysone in insect larvae and cause premature molting, cessation of feeding and ultimately death. Other effects include increased egg mortality and reduced reproductive rate. This group of drugs includes tebufenocide, which is used to control butterflies, and halofenocide, which is used to control butterflies, beetles and coleopterans (Tarrant et al., 2011).

Although chemical control is in many cases the most effective method of pest control, its unconscious and indiscriminate use leads to the pests being harmed as well as the beneficial insect fauna, the development of resistance in the pests and many environmental and health problems. For this reason, the importance of alternative control agents that are harmless or less harmful to environment and other organisms has increased in pest control, and over time neurotoxic insecticides have been replaced by more targeted substances (Beament et al., 2011).

As in humans and animals, the growth and development of insects is also controlled by hormones. Mainly, the most important hormones that allow insects to develop from egg to adult are the juvenile hormone and the metamorphosis hormone, which allows insects to moult and thus grow and develop their organs. This activity depends mainly on which of these hormones is most abundant in the insect's blood. These hormones must be released regularly so that the insects can continue their lives normally (Retnakana et al. 2011). This order is found in the brain and in the body. The hormone is secreted by the brain from a small organ called corpora allata. The idea of disrupting the hormone balance of insects and thus preventing the development and formation of the chitin substance that protects their bodies from external influences was developed in 1956. Insect Growth Disruptors (IGDs) or Insect Growth Regulators (IGRs) accumulate in the chitin layer in the insect's integument and stop the development of the larvae by preventing chitin synthesis and inhibiting their moulting functions. These compounds, which have been developed over the last 30 years specifically for insecticidal purposes but act differently from classical insecticides, also cause the larvae to become paralyzed and immobile by stopping feeding. Insect growth inhibitors from these compounds In addition to their larvicidal effect, they also have an ovicidal effect. These compounds can prevent biological processes that are part of the vital activities of insects, such as development in the egg, the building of various body structures from hatching to adulthood, moulting, hibernation, metamorphosis and reproduction. Insect growth regulators or insect growth inhibitors are used against many species of butterflies and warehouse moths at the egg and larval stages, and the use of these agents is becoming more widespread. Insect growth inhibitors have the advantage of having fewer undesirable side effects on the environment and beneficial insects. These compounds, which belong to two groups, protect the natural balance and are suitable for integrated control programs (Keskin et al. 2021).

2. ECDYSONE HORMONE AND METAMORPHOSIS

In insects, the process of periodically changing the exoskeleton (integument = cuticle) covering the outer surface of the body and especially the head capsule is called moulting, as it limits the growth of the pre-adult periods (larvae or nymphs). The time between two moults is called the "first larval period" and the "second larval period". The number of moults and thus the number of larval stages varies depending on the insect group. The number of larval stages is 1 in the Apterygota, 3 in the Diptera, 5 (sometimes 6-7) in the Lepidoptera and 22 in the Ephemeroptera. To prevent damage to the epidermis, the only living layer of the insect integument, during moulting and the simultaneous deposition of a new cuticle under the old cuticle, i.e. destruction, chitin is synthesised at almost all stages of the integument modification chain (Palli, 2009).

The production of hormones and their secretion into the insect body occurs thanks to the coordination of the nervous system and endocrine system. While the nervous system creates rapid responses by transmitting both physiological and environmental (peripheral) stimuli through neurons, the endocrine system is responsible for producing the necessary amount of hormones in response to these stimuli and mixing them into the blood (hemolymph). Juvenile hormone (= youth = JH) and ecdysone (= 20 hydroxyecdysone = 20 E) hormones are mainly effective in molting and metamorphosis. The levels of these two hormones in the hemolymph play a role in the formation of nymph-nymph, larva-pupa, nymph /larva-adult metamorphosis in insects. Shirt changing is a complex event in which many physical and biochemical events occur simultaneously, and depending on insect groups, there are changes in some genes, protein structures and hormone amounts (Tarrant et al., 2011).

The moulting hormone 20-hydroxyecdysone (20E) is secreted as ecdysone from endocrine glands, namely the prothoracic glands, located in the prothorax of Lepidoptera. In many insects, these are located in the ventral of the head and are often referred to as ventral glands. Ecdysone, sometimes referred to as insect excretory hormone, is converted to 20E, the primary excretory hormone that triggers excretion in various tissues. It could therefore be assumed that ecdysone can be considered a prohormone. The common name for Ecdysone, 20E and other related steroids is simply ‘Ecdysones' (Scott- Dupree et al., 2009).

3. ECDYSONE AGONISTS (EA)

Oath Haas Co. at Rohm in Spring House, Pennsylvania, developed the benzoylhydrazines that act as ecdysone agonists with tremendous developmental potential. Surprisingly, these compounds had no structural similarity to the steroid replacement hormone 20 Ecdysone (Seccacini et al., 2008). Ecdysteroids in plants, called phytoecdysteroids, and in animals, often called zooecdysteroids (Palli et al., 2009).

Ecdysoid are synthetic substances derivatives of ecdysone. They trigger the development of a damaged cuticle and lead to the death of the insect. The integument is without scales or wax layer, as the growth processes are accelerated and numerous typical events are bypassed (Song et al., 2017).

First non-steroidal EA (ecdysone agonist), came from the research laboratories of Haas Co. They reported a compound belonging to the bisacylhydrazine (BAH; also referred to in the literature as diacylhydrazine) class of chemistry, codenamed RH-5849. This compound has been found to be effective mostly by ingestion of lepidopteran, dipteran and coleopter larvae from holometabolous insects. This substance allowed a rapid inhibition of feeding in the larval stages and, more importantly, caused an early but unsuccessful moult, disrupting cuticle and leading to death of the larvae. RH-5849, *Drosophila* possibly ecdysone receptor (EcR) proteins extracted from cellular extracts of Kc cells and Dried fruit moth *Plodia* provided the first evidence that interpunctella (Lepidoptera: Pyralidae). Because this compound replaced ponasterone A, a potent phytoecdysteroid, in competitive ligand binding radiometric assays (Beament et al., 2011).

The molecular mechanism of RH-5992 and related analogues is needed to understand the processes of 20 Ecdysone release and regulation. 20 Ecdysone is the ligand that binds and activates the ecdysone receptor and the ultra-spiracle. The active component of the ligand and heterodimer binds to a specific DNA sequence, DNA binding domains that initiate the expression of a number of genes. All these cascade genes activated by 20 Ecdyson are upregulated genes for triggering the molting process. The event that initiates this molt consists of, among other things, the division of epidermal cells, the secretion of ecdysial fluid with the old cuticle, the synthesis of cuticle and epicuticle, apolysis, the displacement of the head capsule and the synthesis of the ecdysone hormone ventro-medially in the brain in neurosecretory cells, the displacement along the ventral nerve cord and in the proctodeal nerves. At the end of the decline of the 20E peak, the larva enters the pharate stage, i.e. the new larval stage within the old cuticle envelope, which is finally expelled. Ecdysis starts in the up-regulated region of 20E (Retnakana et al. 2011).

4. ECDYSONE AGONIST COMPOUNDS USING IN PEST CONTROL

It has been shown that methoxyfenozide, tebufenozide , halofenozide and chromafenozide [3H] can replace ponasterone A (Pon A). It is the natural moulting hormone of insects. Competitive ligand binding assays with [3H] Pon A use either protein extracts from insect cell lines (containing the EcR complex) or EcR complex proteins expressed in vitro from DNA sequences cloned from insect representatives of different insect species (Beament et al., 2011; Retnakana et al. 2011).

From an application point of view, all commercial EAs, with the exception of halophenoside , which is also toxic to Coleoptera larvae, can also be used against Lepidoptera larvae. Halofenozide is registered in the US turf market for lepidopteran pests. Commercial forms of chromafenozide (labelled ANS-118, CM-001) are largely specific to Lepidoptera. Yanagi et al. (2006) reported that chromaphenoside is registered in Japan, Thailand, Indonesia, Brazil and other countries for the control of lepidopteran pests in rice, tea, apples, pears, cabbage, lettuce, strawberries, eggplant, tomatoes, peppers and gall onions, sugar beet and some ornamental plants. It is effective as a foliar application (25-50 ppm) in the control of the *Spodoptera litura, S. exigua, Mamestra brassicae, Heliothis armigera, Caloptilia theivora, Ascotis selenaria, Archips fuscocupreanus* and *Adoxophyes oranana fasciata*. These authors also report good efficacy of chromaphenoside in the control of *S. litura* and *Cnaphalocrocis medinialis* the rice leaf miner (Wing, 1988; Wing et al., 1988; Scott- Dupree et al., 2009; Beament et al., 2011; Retnakana et al., 2011).

Hamamura et al. (2006) compared the effects of various insecticides on spiderlings of the wolf spider *Pardosa* in vivo. Yanagi et al. (2006) found that chromaphenazidine is safe against non-lepidopteran insects, but it is safe against mammals, ecotoxicologically and reported no data on residual metabolic profiles. In a laboratory bioassay, Zhang et al. (2003) found that fufenozide is active against the larvae of *Plutella xylostella* and *Ostrinia* sp.

5. RESISTANCE AND SUSCEPTIBILITY OF INSECTS TO ECDYSONE AGONIST INSECTICIDES

The insect excretory hormone 20E is used by all insects from the larval and nymphal stage after hatching to adulthood. This hormone exerts its moulting-inducing effect by interacting amino acid residues of the EcR protein. The fact that the insecticidal BAH compounds are true agonists of 20E and yet their predominant selective toxicity to Lepidoptera insects remained unresolved for a long time. This paradox of the very high insect-selective toxicity of BAH insecticides with the same molecular target site (EcR complex) for 20E is explained by (1) the very high binding affinity of these compounds for 20E. EcR complex proteins of susceptible insects compared to those of inactive or non-binding insects of non-susceptible insects; (2) the metabolic stability of the compounds in susceptible insects is much greater than 20E (Beament et al., 2011). The BAH class insecticides are true agonists of 20E, as each of their agonists exerts its effect by interacting with the EcR (Scott- Dupree et al., 2009).

Since Williams (1967) proposed the concept of using insect hormones or their mimics as “third generation pesticides” for insect control, analogs and agonists of the two major insect growth and development hormones, JH and 20E, respectively, have been developed. On the other hand, the physiological, biochemical and molecular basis for the action of 20E as non-steroidal BAH agonists is well understood. In addition, the insecticidal compounds 20E and BAH were found to exert their effects by binding to EcR proteins. These are the crystal structure of EcR complex proteins ponasterone A and the BAH active compound was obtained and the amino acid interacting with 20E pocket of ecdysone receptor protein residues and the mechanism of BAH formation and action were understood (Oouchi et al., 2005).

The EAs have introduced new modes of action and tools that primarily target insect pests rather than beneficial insects, predators and parasitoids; however, in some cases, beneficial insects are also sensitive to these active ingredients. There are also some insects that are resistant to these hormone analogues. Since the introduction of EA insecticides, there are a few reports mentions on the development of resistance and cross-resistance (Dhadialla et al., 1998, 2005, 2010). Gomez et al. (2011) found that *Plutella xylostella*, a Chinese laboratory strain selected for resistance to EA fufenoside, exhibited

17.2- and 28.7-fold resistance to diflubenzuron and hexaflumuron, respectively and this shows clear cross-resistance. The stability and biochemical basis of resistance to fufenoside in the laboratory selected strain (JSR) of *P. xylostella* selected by Sun et al., 2012. When the studies with the JSR strain began, its resistance to fufenoside had reached 320.3-fold. However, when this strain was kept under laboratory conditions without selection pressure, the resistance level dropped from 320.3 to 20.5-fold within six generations (Tang et al., 2011). Over the course of 28 generations, the JSR strain did not return to the original level of susceptibility that existed before the selection experiments. While increasing resistance to fufenoside was associated with increased fitness costs, this decreased after the removal of selection pressure. In experiments aimed at understanding the mechanism of resistance development, Tang et al. (2011) demonstrated that monooxygenases, glutathione S-transferases, carboxylesterase, etc. were involved in fufenoside metabolism in the resistant JSR strain of *P. xylostella* and that these substances may play a role.

6. CONCLUSION

Ecdysteroids (Ec) are signalling molecules that are widely distributed throughout the animal and plant kingdoms. However, they do not occur naturally in a vertebrate, which makes them suitable as ligands for medical gene-switching applications due to the lower likelihood of pleiotropic effects. Ecdysteroids fulfil a variety of functions and act as hormones, pheromones or insect repellents. However, their most common and important role is that they act as ‘moulting hormones’ and thus regulate the development of insects and other arthropods as well as reproductive functions and other physiological processes. (Palli et al., 2005). The hormone ecdysone has a very crucial role in regulating the growth and development of adult organs. And it also influences the overall timeframe of this process (Nogueira Alves et al., 2022). The limited direct effects of tebufenozide on non-target organisms can be attributed to its structural similarity to the insect moulting hormone 20-hydroxyecdysone. This causes lepidopteran larvae to undergo a premature and incomplete moult (Edge et al., 2022).

Tebufenozide mimics the natural ecdysone of the target insect and it binds to the ecdysone receptor in the intestine of the larvae. The moulting process is initiated by the binding of ecdysone to its receptor in the larval intestine. This initiated process is not completed and leads to the death of the larvae. These agonists differ from conventional insecticides such as organophosphates in that they have a high specificity against Lepidoptera, while they have a low toxicity for non-target arthropods. Tebuconazole shows toxicity to a wide range of lepidopteran species (Roscoe et al., 2020).

Methoxyfenozide (Runner 24 SC) has proven to be the most effective analogue against *Spodoptera littoralis* and *Spodoptera exigua*, as well as against *Culex pipiens* and *Musca domestica*. In addition, methoxyfenozide safe for mammals birds and fish (Ahmed et al., 2022).

Selective use of halofenocide (Mach2) in turf management has been shown to be effective in controlling of *Popillia japonica* and *Exomala orientalis* with no toxicity on other insect populations (Joseph & Braman, 2016). Chromafenozide has been shown to have toxic effects on the larvae of several Lepidoptera species. These effects are primarily due to the impairment of digestion by the substance. Therefore, the efficacy of chromafenozide in protecting agricultural crops from infestation by serious Lepidoptera pests is well established. The benefits of this pesticide have been observed in protecting a variety of crops, including vegetables, fruit trees, tea, rice, ornamentals and many others (Yanagi et al., 2006; Nollet and Mir 2023)).

The relatively safe mammalian and ecotoxicological profiles of ecdysone agnosides make them ideal tools for integrated pest management programmes and insect resistance control programmes, thanks to the above-mentioned characteristics of selective toxicity and mode of action. In contrast to neurotoxic pesticides, EA pesticides kill insects much more slowly. However, BAH insecticides work within hours of EA ingestion and inhibit feeding much faster than other hormones. Therefore, BAH insecticides stop feeding damage to plants by killing pests within hours of EA ingestion. Apart from this, there is a possibility of discovering more insecticidal compounds that are agonists of 20E in the future. The extensive research on the BAH insecticides to elucidate the 20E pathway coupled with the advances in the use of dsRNA inhibition (RNAi) of specific genes in insects also provide the opportunity to expand the concept of chemical IGD that can be exploited through the use of molecular and biotechnological tools. It is therefore expected that in the next decade or two, more macromolecules will be available for insect control against susceptible pests and that it will be possible to use molecular biology methods more effectively to inhibit insect growth and development by interfering with the targets of hormone signalling and chitin synthesis.

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CHAPTER 11

EFFECTS OF HEAT STRESS ON FEEDING PHYSIOLOGY IN FARM ANIMALS

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1. INTRODUCTION

The animal farming sector, a rapidly growing component of the agricultural economy, is transforming at an unprecedented rate, presenting new opportunities for agricultural growth, poverty alleviation, and enhanced food security (Chaidanya et al., 2017). Projections indicate that by 2050, global temperatures could rise by up to 1.5°C due to climate change. With the world's population on the rise, the demand for food production will inevitably increase (Lima et al., 2022; Silveira et al., 2024). This trend highlights a paradox that calls for a deeper exploration of how climate change affects the thermoregulatory functions of livestock and impacts their productivity (de Castro Júnior et al., 2023; Silveira et al., 2024). In order to mitigate the effects of heat stress on cattle productivity, effective management strategies must be developed, rooted in a solid understanding of this condition. The global temperature increase of 1.5°C predicted between 2030 and 2050 is expected to significantly challenge the profitability and efficiency of livestock production systems.

Farm animals possess an intricate and well-structured thermoregulatory system that includes various bodily fluids, tissues, and organs (Leite et al., 2021). Adaptation research in animals focuses on several critical factors such as age, nutrition, production level, body condition, sex, and physical characteristics, all of which influence the extent of heat stress experienced by mammals (de Castro Júnior & Silva, 2020). Species differ in their responses to heat stress and exhibit distinct geographical distributions (Gilbert et al., 2018). For instance, goats tend to be more resilient to heat stress (Sejian et al., 2021; Darcan and Silanikove, 2018), while dairy cattle, with their higher metabolic rate, are more vulnerable (Mauger et al., 2015). A bibliographic study on heat tolerance in livestock by McManus et al. (2023) found a larger volume of research focused on cattle compared to goats or sheep. As the future of animal agriculture hinges on adopting both innovative and existing practices, understanding these effects remains crucial for the sector's sustainability and profitability. This study explores the impact of heat stress on the feeding physiology of farm animals.

2. RUMINANTS

Extreme temperatures significantly affect animals' energy use, leading to higher production costs and raising concerns among farmers, especially in tropical and temperate regions affected by global warming (Lima et al., 2022). Research by Maia et al. (2016) and de Castro Júnior and Silva (2021) indicates that animals are more efficient at utilizing feed nutrients when they are within their thermal comfort zone. However, elevated temperatures cause a range of physiological and behavioral changes in ruminants, such as reduced dry matter intake, increased water consumption, electrolyte imbalance, and alterations in blood volume and osmolarity. These changes negatively impact rumen function and microbial composition, ultimately lowering productivity (Spiers et al., 2004; Ferreira et al., 2021). Chaidanya et al. (2017) investigated how temperature and feeding stress independently and together influenced goats, focusing on rumen fermentation, histopathology, and heat shock protein (HSP70) gene expression. Their findings revealed that temperature stress alone caused less disruption to feed intake and weight fluctuations compared to feeding stress, suggesting that goats handle temperature stress better when feeding is not restricted. However, combining both stresses led to notable declines in feed intake, body weight, and rumen fermentation. Similarly, Marai et al. (2007) highlighted that heat stress leads to decreased feed consumption in animals, as heat triggers thermal sensors that reduce appetite through signals to the hypothalamus. This reduction in feed intake was also observed in studies on Malpua sheep by Sejian et al. (2010) and Maurya et al. (2015), where both temperature and feeding stress contributed to decreased feed consumption, underlining the compounded negative effects of these stresses. Animals under temperature stress were less able to obtain energy and nutrients that their bodies could absorb, according to Beede and Collier (1986). Reduced dry matter intake, a minor increase in digestibility from extended intestinal retention time, and maybe a decrease in blood flow and nutrient absorption were the causes of this. Additionally, the negative effects of reduced nutrient intake and absorption typically offset the little improvement in digestibility, resulting in a reduced total nutritional availability for the animal under heat stress. It has been discovered that physiological alterations brought on by abrupt or extended exposure to extremely high or low temperatures have an impact on how the body uses and processes nutrition and energy sources.

Cows exposed to temperatures outside their ideal range of 5 to 25°C experience increased metabolic demands. To regulate their body temperature, they pant, sweat, and exhibit a higher respiration rate (Collier et al., 1982; McDowell, 1972). One common tool used to assess temperature stress in dairy cows is the temperature-humidity index (THI), which has been linked to changes in behavior, health, and productivity (Becker et al., 2020). Research suggests that productivity declines when the THI exceeds 72 (Armstrong, 1994; Ravagnolo et al., 2000), and behavioral changes have been observed when THI reaches 68 or higher (De Rensis et al., 2015).

Najar et al. (2011) aimed to model the impact of heat stress on cows' feeding behavior and physiological responses. Their findings indicated that the effects of heat stress varied depending on the cows' diet and physiological characteristics. Heat stress impacted animal behavior, physiology, and productivity when the THI ranged from 80 to 90, with respiratory rate and rectal temperature showing the strongest correlation to THI and ambient temperature. The study found that heat stress significantly reduced dry matter intake (DMI), with higher THI and neutral detergent fiber (NDF) associated with reductions of 4.88 grams per kilogram of DMI and 11.5 grams per kilogram of metabolic body weight (MBW). Moderate reductions in feed intake were noted in cows fed low-fiber, high-concentrate diets. Digestibility of both dry and organic materials was also found to decrease under heat stress.

Heat stress-induced reductions in feed intake are a key factor behind lower milk production and negative energy balance (Sammad et al., 2020). Dairy calves, in particular, are highly sensitive to heat stress, presenting a significant global challenge for the dairy industry (Tian et al., 2015). Heat stress can also lead to ruminal acidosis, caused by the accumulation of lactic acid, which lowers the rumen's pH (Yadav et al., 2013). The acidic environment reduces the ability to digest fiber due to a decrease in fibrolytic bacteria (Baek et al., 2020). When concentrated feed replaces forage, less rumination occurs, reducing saliva production, which serves as a buffer in the rumen, exacerbating the drop in pH. This decrease in milk production may be linked to increased lactate levels and a reduction in acetate-producing bacteria in the rumen (Zhao et al., 2019).

According to Kim et al. (2022), heat stress causes significant losses to the global dairy industry by altering the rumen microbiota, increasing metabolic diseases, and decreasing overall animal performance. Cattle exposed to heat stress showed elevated ruminal lactate concentrations and reduced ruminal pH and acetate levels. The number of lactate-producing bacteria, such as *Streptococcus* and unclassified *Enterobacteriaceae*, increased significantly, while the abundance of soluble carbohydrate-utilizing bacteria, such as *Ruminobacter* and *Treponema*, also rose. Interestingly, heat stress led to the proliferation of heat-tolerant cellulolytic bacteria, particularly those from the phylum *Fibrobacteres*. In contrast, the abundance of acetate-producing bacteria like *Actinobacteria* and *Acetobacter* decreased under high-temperature conditions. Heat stress also affected blood parameters, metabolites, and rumen fermentation processes, with notable decreases in levels of isoleucine, methionine, myo-inositol, lactate, tryptophan, tyrosine, 1,5-anhydro-D-sorbitol, 3-phenylpropionic acid, urea, and valine. These reactions affect the quantity of food consumed as well as the production efficiency in terms of growth rate, reproduction, and milk yield (Kim et al., 2022). Rhoads et al. (2013) discovered that one practical way to help animals adjust to hotter climes is through nutrition. They found that animals under heat stress often change how they use energy by using more carbs and using less lipids for oxidation. Moreover, it has been proposed that diets or nutritional supplements that improve glucose utilisation may be beneficial, and that sufficient insulin activity may be essential for adjusting to heat stress. Because heat stress enhances the production of heat shock proteins (HSP), which improve insulin action by lowering the phosphorylation of IRS-1 serine, it has been shown to promote insulin sensitivity. In fact, it has been demonstrated that applying therapeutic heat stress improves insulin sensitivity in a variety of animals. However, the exact amount of insulin activity that must be increased to maintain output under heat stress is still unknown. To create more appropriate and effective treatment strategies, additional research is needed to determine how heat affects variables linked to energy metabolism (Rhoads et al., 2013).

Meneses et al. (2021) set out to investigate how heat stress and dietary energy level affected ruminal characteristics, dry matter intake, water intake, digestibility, physiological parameters, and digestive behavioural parameters in beef cattle. Therefore, regardless of whether the animals were kept in feedlots or on pasture, it was demonstrated that higher critical temperature levels resulted in higher water consumption and lower dry matter and other nutrient components including organic matter (OM) and crude protein (CP). Furthermore, this study discovered that heat affects the rumen's basic physiology and metabolism. The synthesis and fermentation patterns of volatile fatty acids, which are critical for digestion, are changed by heat stress. Additionally, it influences the rumen's capacity to break down food and encourages improved nutrient absorption and utilisation in the gut. These results imply that an improvement in digestibility and nutrient utilisation was the primary adaptation response to heat stress in this investigation. According to Conte et al. (2021), professionals frequently choose to increase the energy content of the diet by adding more grain or concentrate in order to offset the decline in nutrient and energy consumption brought on by heat stress (HS) and the metabolic heat produced during feed fermentation. This technique should be used with caution, though, as it could cause the pH of the rumen to drop. The development of rumen acidosis during heat stress (HS) can be caused by a number of causes. These include greater quantities of fermentable carbohydrates and a decrease in feed rate coupled with a decrease in dry matter intake (DMI). Saliva production, which typically supplies bicarbonate for buffering, and rumination are also reduced. The increased CO2 excretion brought on by panting is the reason of this drop in buffering capability. Additionally, the effectiveness of fibre digestion is adversely affected by the drop in rumen pH. It has been noted that the fibrolytic bacteria in the rumen are most impacted when the pH falls below 6.0.

3. POULTRY

Cattle that grow rapidly and produce large amounts of food may be more vulnerable to the effects of climate change, particularly as temperatures rise, due to their increased internal heat load. This heightened vulnerability could have negative consequences for both the health and welfare of farm animals (McMichael and Lindgren, 2011; RSPCA, 2020). Several studies suggest that while rising temperatures could increase the risk of animal mortality, it may also improve the health and well-being of animals in regions that experience harsh winters (Nardone et al., 2010). Despite these findings, little is known about how animal production practices can adapt to a changing climate across different scenarios (Al-Amin and Alam, 2011; RSPCA, 2020). Therefore, it is crucial to have reliable guidelines for maintaining cattle production systems in diverse agro-ecological regions under changing climate conditions (Erensoy and Boz, 2022).

In poultry farming, feed and water consumption are generally lower (Uçar, 2022). When considering feed requirements for live weight gain, cattle need 8-10 kg of feed per kilogram of live weight gain, while sheep require 4-6 kg. In comparison, broiler chickens need 1.5-2 kg, layers need 2 kg, turkeys and quails require 2.5-3 kg, ducks and geese need 3.5-4 kg, and pheasants, partridges, and guinea fowls need 4-5 kg of feed for the same weight gain (Ertuğrul, 1997; Sarıca and Erensayın, 2018; Yamak et al., 2016; Yamak et al., 2018; Yamak et al., 2020).

Poultry are warm-blooded animals, with adult birds maintaining a body temperature of 41-42°C. The body temperature of day-old chicks is lower, and it can fluctuate by around 1.5°C depending on their daily activities. The critical lethal temperature for poultry is reported to be 47°C (Şenköylü, 2001; Smith, 2002; Arslan and Duru, 2004). Studies indicate that poultry thrive at temperatures between 5°C and 25°C, referred to as their "thermoneutral zone." Exposure to temperatures outside this range, either too hot or too cold, results in stress. Extreme temperatures, particularly below 0°C and above 30°C, are considered especially dangerous (Smith, 2002; Şenköylü, 2002; Yardibi, 2002). High temperatures can negatively impact poultry growth and productivity by reducing appetite and feed intake. This leads to lower live weights, and animals exposed to heat stress during growth may reach sexual maturity later. Chicks, however, are better able to tolerate heat than adults and typically respond to heat stress by increasing water consumption (Koçak and Yalçın, 1990; Erganiş, 2002).

With rising temperatures due to global warming, poultry are increasingly at risk of heat stress, a significant environmental stressor (Boz, 2022). Heat stress occurs when there is an imbalance between the heat energy produced by the animals and the heat energy transferred to the environment. Factors that contribute to this imbalance include mobility, sunlight, thermal radiation, air temperature, humidity, and their interactions (Şentürk et al., 2020). Heat stress in poultry can lead to physiological changes, including increased mortality rates, reduced feed efficiency, lower live weights, and a decline in reproductive performance (Wasti et al., 2020).

Along with heat stress, temperature stress is also a criterion taken into consideration. Temperature stress, which is very important for poultry, is generally the reaction of the animal to the hot environment where different or abnormal physiological responses such as rapid breathing are obtained (Lesson, 1986; Alarslan, 2000; Arslan and Duru, 2004).

Chickens attempt thermoregulation in hot weather by altering their behaviour and physiological balance because they lack sweat glands (Lara and Rostagno, 2013; Kapetanov et al., 2015). Under hot climatic conditions, poultry try to remove excess heat from their bodies by radiation, convection, evaporation, egg and faeces (Renaudeau et al., 2012; Pawar et al., 2016). Blood in their bodies is sent more to the body organs such as crest, beard and feet to cool these areas (Safdar and Maghami, 2014). One of the most obvious behaviours seen during heat stress is the spreading of the wings to the side and fluffing of the feathers. After this point, since the removal of heat stress by radiation and convection is insufficient, excessive heat is tried to be removed by evaporation. To elaborate on this issue, poultry try to cool their bodies by breathing and panting (Muğlalı, 2002; Şenköylü, 2002; Rath et al., 2015).

In poultry, sudden changes or increases in ambient temperature can negatively impact the intestinal mucosa and gut flora. The intestinal epithelial cells become damaged, and harmful microorganisms proliferate in the intestinal lumen, leading to digestive and absorption disorders. High temperatures also cause a reduction in appetite and feed intake, resulting in issues such as growth stunting, and a decline in egg production and quality (Zhang et al., 2012; Ölmez et al., 2021).

In the event that heat stress has a negative impact, feeding-related actions are implemented. Increasing poultry's nutritional intake is the main objective. Nonetheless, increasing the feed's nutrient density and guaranteeing sufficient nutrient intake are essential (Bozkurt et al. 2000; Arslan and Duru, 2004).

Improvement measures can be taken with feed additives against the effects of temperature stress. In this context, many studies have been carried out and continue to be carried out. In poultry raised or forced to be raised under heat stress, especially probiotic feeding is performed and epithelial cell development is supported against intestinal pathogens. In this way, the performance of poultry under heat stress can be improved (Tang et al., 2017; Fathi et al., 2018; Mazanko et al., 2018; Yan et al., 2019; Zulkifli et al., 2000; Ölmez et al., 2021).

Increasing the amount of protein in the diet may be the first idea that springs to mind to satisfy the daily protein needs because animals subjected to heat stress consume less feed. Nevertheless, research on broilers has demonstrated that extra amino acids are eliminated even when the feed contains a high protein content. Therefore, lowering the amount of protein in the diet and balancing the amino acids in the available protein are the most crucial nutrient arrangements to be made against heat stress. Here, methionine and lysine are the most crucial amino acids. The addition of amino acids to the ration will lower its protein content while lowering heat production and improving performance. This trait should be highlighted in hot conditions since lysine deficit will raise body temperature. (Muğlalı, 2000; Arslan and Duru, 2004).

During heat stress, the depletion of animals' vitamin stores is generally minimal. However, heat stress leads to a reduction in vitamin C synthesis and lower plasma levels of this vitamin. To cope with heat stress, poultry release corticosterone to mobilize their energy reserves. This process compromises the immune system, lowers disease resistance, and increases mortality rates, partly due to the shrinkage of the Bursa of Fabricius. When poultry under heat stress receive adequate vitamin C, their performance improves, mortality is reduced, and their immune functions are strengthened (Koçak and Yalçın, 1990; Türker, 2000; Konca and Yazgan, 2002; Yardibi, 2002).

Vitamin E, a vital antioxidant that helps protect biological membranes, is also linked to heat stress management. Located in the membrane's second layer, vitamin E plays a role in preventing oxidative damage by counteracting free radicals (Şahin and Küçük, 2001). Supplementing with vitamin E during heat stress has been shown to improve feed intake and productivity. For example, laying hens exposed to 34°C benefited from vitamin E supplementation at 125 and 500 mg/kg, which enhanced their feed intake and egg production (Bollengier-Lee et al., 1998), and at 60 IU/kg, egg production was also improved (Kirunda et al., 2001). In another study, adding 250 mg/kg of vitamin E to the diet of hens at 32°C led to better egg weight, shell thickness, and Haugh unit scores (Şahin et al., 2002). High doses of vitamin E (85 IU/kg) increased egg production and feed efficiency even under heat stress in laying hens, though a lower dose of 65 IU/kg was suggested to avoid liver damage (Ehran and Bölükbaşı, 2011).

For broilers, supplementing with 250 mg/kg of vitamin E improved their antioxidant and immune responses under prolonged heat stress (29–36°C) (Rehman et al., 2017). When quails were exposed to 34°C, dietary supplementation with vitamin E (125, 250, and 500 mg/kg) and vitamin C (100 and 200 mg/kg) improved digestibility, feed conversion, and growth performance (Şahin and Küçük, 2001). Çiftci et al. (2005) obtained similar results with 125 mg/kg of vitamin E and 200 mg/kg of vitamin C. Adding vitamins E and C to the diet of laying hens under heat stress notably enhanced their antioxidant and immune capabilities (Asli et al., 2007). Even though vitamin E and C injections prior to hatching did not show any improvement in the performance of chicks exposed to 38°C during the first 7–14 days, they did reduce the risk of stress-related peroxidation (Altan et al., 2017). Furthermore, supplementing Japanese quails with vitamin C (250 mg/kg) and folic acid (1 mg/kg) helped mitigate the adverse effects of heat stress (Olgun et al., 2021; Şahin et al., 2003).

Recent studies have indicated that adding both vitamin E and selenium to broiler diets under heat stress enhances their performance, reduces mortality, and improves meat quality by lowering MDA levels and increasing α-tocopherol in breast meat (Calik et al., 2022; Pecjak et al., 2022).

Temperature stress, on the one hand, increases the nutrient requirement due to the increase in metabolic activities, and on the other hand, it negatively affects the amount and quality of yield due to the decrease in feed consumption and efficiency, plasma protein and calcium in the blood (Gençoğlan, 2023). The decrease in feed intake in temperature stress also causes a decrease in the minerals taken from the feed. Exposure to high temperatures in laying hens causes a decrease in potassium retention in the body and losses in minerals such as sodium, sulphur, selenium and copper. As a result, electrolyte balance (Na + K - Cl) is disturbed (Smith, 2002; Arslan and Duru, 2004; Gençoğlan, 2023). Changes in electrolyte balance are of great importance in terms of nutrition. Acceleration of respiration causes alcoholosis and thus loss of bicarbonate from the kidneys. Deterioration of electrolyte balance also causes water loss in tissues (Roche- Damla, 1987; Arslan and Duru, 2004).

As the temperature in the surrounding environment rises, birds respond by increasing their respiration rate in order to enhance cooling through evaporation. As birds inhale and exhale, they have a tendency to expel a greater amount of CO_2, which can lead to fast alterations in their acid-base equilibrium. Alkalosis, ranging from mild to severe, can cause blood pH levels to vary between 7.2 and 7.5. In extreme cases, blood pH levels can reach up to 7.7. The alteration in blood pH, along with the depletion of bicarbonate ions, can impact on the quality of eggshells and the overall health and metabolism of chickens. In instances of heat stress, the availability of bicarbonate is the primary factor that affects the synthesis of eggshells. This availability is determined by the acid-base balance, renal function, and respiratory rate (Leeson and Summers, 2005).

If conventional management measures are unable to minimise heat stress, it may be advantageous to modify the ration by adjusting the electrolyte levels. Nevertheless, the approach should vary for juvenile birds in contrast to mature laying hens. It is necessary to preserve the bicarbonate buffer system in laying hens, since it has an impact on the quality of eggshells. Thus, the use of sodium bicarbonate for ration or water treatment may prove advantageous, underscoring the importance of fulfilling the minimal chloride requirements. However, using acidifiers like NH_4Cl to treat respiratory alkalosis in laying hens may relieve respiratory distress but could lead to a decrease in shell quality. Electrolyte therapy is typically advantageous for immature pullets, requiring less focus on bicarbonate buffering. Including up to 0.3% NH_4Cl in the diet can enhance the development rate of birds under heat stress. However, it remains uncertain whether this impact is influenced by electrolyte balance and blood pH or is only a result of increased water consumption. According to Leeson and Summers (2005), adding salt to the drinking water of young birds in commercial settings has been found to reduce bird discomfort and enhance growth.

A normal electrolyte balance is approximately 250 mEq/kg, which can be achieved by either increasing the levels of sodium (Na) or potassium (K) in diet, or by decreasing the level of chloride (Cl). In real-world scenarios, maintaining electrolyte balance becomes more challenging in the presence of elevated chlorine levels. Alternatively, substituting $NaHCO_3$ for NaCl, as occasionally suggested during heat stress, may result in a shortage of chlorine.

Alterations in dietary electrolyte balance typically arise from significant modifications in the composition of the ration, particularly when animal-derived protein sources are substituted for soybean meal, or vice versa (Leeson and Summers, 2005).

Ammonium chloride (NH_4Cl), sodium bicarbonate ($NaHCO_3$), sodium chloride (NaCl), potassium chloride (KCl), and potassium sulfate (K_2SO_4) have been shown to improve the performance of broiler chickens under heat stress conditions (Ahmad and Sarwar, 2005). According to Ahmad and Sarwar (2005), adding electrolytes such as sodium (Na), potassium (K), and chloride (Cl) to the feed of broilers can help reduce the negative effects of heat stress. Olgun et al. (2021) suggest that increasing the electrolyte content of broiler diets by 1.5% for each degree Celsius above 20°C can help prevent a decrease in feed intake during heat stress.

In another study, Ubosi et al. (2003) found that adding 0.1% hydrochloric acid (HCl) to the drinking water of laying hens under heat stress significantly improved both egg quantity and quality. Gamba et al. (2015) concluded that the ideal electrolyte balance for broilers under heat stress ranged from 230 to 250 mEq/kg, while Ahmad and Sarwar (2006) recommended 250 mEq/kg for laying hens. A recent study by Cruvinel et al. (2021) showed that feeding laying quails an electrolyte balance of 265 or 315 mEq/kg under 33°C improved bone mineral density, egg production, and overall egg quality.

Heat stress leads to a reduction in both feed and energy consumption, but it simultaneously increases the energy requirements for basic bodily functions. As a result, only a small portion of the energy consumed is available for production (Açıkgöz and Özkan, 2001). In addition to lowering the protein content in the diet, substituting fat for carbohydrates can help mitigate the heat-producing effects of feed. At this stage, selecting high-quality oils and ensuring proper feed processing techniques are important. Moreover, adding fat to the diet may also stimulate increased feed consumption (Arslan and Duru, 2004).

To cool down through evaporation, poultry under heat stress attempt to regulate their body temperature by increasing their respiratory rate. This can lead to respiratory alkalosis in hens, as increased breathing expels more carbon dioxide. To balance the acid-base disturbances caused by this, the animal compensates by excreting more bicarbonate and retaining more hydrogen through the kidneys (Borges et al., 2007). Consequently, less calcium carbonate is available for the formation of eggshells and bones, leading to impaired bone growth and poor eggshell quality, both of which contribute to financial losses (Cruvinel et al., 2021).

Temperature stress negatively impacts egg production in hens (Deng et al., 2012). The reduction in feed intake leads to insufficient nutrients for egg formation, particularly lower blood calcium (Ca) levels and protein, which in turn causes a decrease in egg production (Mashaly et al., 2004). While the decline in eggshell quality is linked to respiratory alkalosis during heat stress, the decrease in egg production and egg weight is more directly connected to the reduction in feed intake (Olgun et al., 2021).

It is essential to understand the balance between decreased feed intake and the heightened nutritional needs to effectively manage animal health and performance in different environmental conditions. The rationale behind this distinction is that when environmental factors cause changes in feed intake, the animal's adverse effects must be lessened by suitably modifying the ration's nutritional levels to make up for the changed daily consumption. It is feasible to claim that nutrient requirements alter if equivalent daily feed consumption under various environmental circumstances does not result in comparable efficiency. This idea can be demonstrated using data from a study by March and Biely (1972) (Figure 1), where Leghorn chicks were fed rations with varying levels of lysine at 20 and 31.1°C. The results showed that feed consumption at the two temperatures differed significantly, with chicks at 31.1°C consuming about 20% less feed. Thus, it may be said that high temperatures reduce feed intake, which could result in decreased body weights. Plotting the data according to body weight growth and lysine consumption, however, reveals a similar reaction in both settings. This highlights the importance of adjusting the nutrient composition of the ration when feed intake fluctuates due to external factors. The birds' response is primarily influenced by the extent and duration of feed intake reduction. Research by Polin and Wolford (1972, 1973), Snetsinger and Zimmerman (1974), and Davis et al. (1972) has shown that a decrease in feed consumption of up to 15 percent may not impact egg production or quality, even within the thermoneutral zone.

Wilson (1949) and Payne (1966), two of the first researchers in the subject, recognised that hens' decreased energy consumption in warmer climates was the cause of their decreased egg production. Dale and Fuller

(1980) then noted that at 31°C, grill weight loss might be somewhat mitigated by foods with a high fat content or high fat-high density. According to other studies, feeding a limited quantity of feed results in very little consumption, and changing the nutritional makeup of diets does not guarantee that regular production will return. For every degree Celsius that temperatures climb above the thermoneutral zone, there is a corresponding 4% decrease in energy maintenance and basal metabolic rate (Shannon and Brown, 1969) (Leeson et al., 1973). For example, when exposed to 33–34°C, the heat loss of Rhode Island Red and White Leghorn hens is 51 and 58 percent, respectively, as opposed to their values at 18.3°C (Ota and MeNally, 1961). Research on laying hens under feed limitation (Jackson, 1972) or breeds with lower feed intake (Morrison and Leeson, 1978) shows that a portion of this reduction can also be ascribed to decreased feed consumption.

There is a well-established understanding that chickens consume a less amount of feed when the energy level of the feed is higher. This is because fowl strive to maintain a specific energy intake on a daily basis. Nevertheless, the system is not flawless, and when the energy level rises, the precise regulation of the decrease in feed intake is sometimes imprecise, resulting in a 'overconsumption' of energy. As the ambient temperature rises, the capacity to regulate food consumption according to energy requirements is hindered, thus boosting the calorie content of the diet is commonly contemplated as a means to enhance energy intake. Leeson and Summers (2005) reported that Payne (1967) observed the classic effect in brown laying hens that were provided with a diet with 2860 to 3450 kcal ME/kg at temperatures of 18°C or 30°C. This information is presented in Table 1. At a temperature of 18°C, birds demonstrate a notable adaptation as their feed consumption declines in a sequential manner with increasing energy levels, while energy intake remains constant. At elevated temperatures, the birds' ability to regulate their feed intake efficiently is diminished, leading to an excessive use of energy. Using these extreme levels of ration energy commercially is not advisable. Instead, it is recommended to maximise energy intake by using the highest possible level of ration energy. It is important to consider the inclusion of additional fat in order to raise the energy level of the diet. The usage of ration fat has the benefit of enhancing flavour and simultaneously decreasing the heat created during its utilisation in production (Leeson and Summers, 2005).

In order to prevent water loss in poultry under heat stress, animals should consume more water. Because evaporation of water from the lungs is the leading method of reducing body temperature. Providing cool drinking water to animals increases the survival rate by helping to reduce body temperature. Feeding water at a temperature similar to the ambient temperature to animals under heat stress leads to an increase in respiratory rate, an increase in respiratory rate leads to a decrease in blood pH (alcoholosis), and alcoholosis leads to deterioration of egg shell quality (Roche- Damla, 1987; Arslan and Duru, 2004). In addition, during heat stress, fresh and cool drinking water at around 15℃is reported to increase feed consumption by 5-10% compared to warm water (29℃) (Esmail, 2002; Muğlalı 2002). Similarly, Gutierrez et al. (2009) investigated the feeding of uncooled (23.0 ± 2.5°C) and cooled water (16.0 ± 0.5°C) in laying hens. As a result, they determined that feed intake and egg production of the hens drinking chilled water were 11.64% and 11.27% higher than the non-chilled group, respectively.

Table 1: Water balance of layers at 22°C and 35°C (Leeson ve Summers, 2005)

	22°C	**35°C**
Water intake	210	350
Faecal water content	85	150
Feed water content	50	50
Number of respirations	75	150

Recent studies have demonstrated that lower feed intake is not the only factor contributing to the decreased output of hens subjected to temperature stress. The detrimental effect on the fowl's digestive tract is another explanation. Temperature stress leads to significant changes in the digestive morphology of the small intestine, reducing the absorptive surface area responsible for nutrient absorption (Liu et al., 2019). Simultaneously, heat stress adversely affects the composition of gastrointestinal peptides and the intestinal microbiota (Wang et al., 2021), further impairing the small intestine's ability to absorb nutrients (Liu et al., 2019). Additionally, heat stress triggers the production of harmful free radicals, such as hydrogen peroxide, hydroxyl, and superoxide anion, which damage healthy cells (Sivakumar et al., 2010). Moreover, heat stress decreases the number of lymphocytes and lymphoid follicles, leading to a reduction in the relative weight of immune organs, such as the spleen, thymus, and Bursa Fabricius (Aengwanich, 2010).

Calcium (Ca) plays a critical role in mineral metabolism and has a significant impact on egg production in chickens, particularly in laying hens. The amount of calcium in the diet is directly correlated with egg production (Borges et al., 2003). Calbindin, a protein that binds to calcium and is essential for calcium absorption, is less readily absorbed by the intestines during heat stress (Ebeid et al., 2012). Egg production is negatively impacted by decreased blood calcium levels caused by reduced calcium intake under heat stress circumstances (Allahverdi et al., 2013). Research has shown that when chickens are subjected to heat stress, their intake of calcium and feed decreases. However, the nutrient balance is disturbed when they consume large amounts of calcium. Grit can be added to the feed to raise the calcium content and lessen this problem. Because grit is held in the gizzard for a long time, the colon and bloodstream can receive a steady supply of calcium (Olgun et al., 2021).

Phosphorus (P) is a crucial mineral for chicken growth and development. However, during heat stress, reduced feed intake leads to a diminished need for phosphorus (Persia et al., 2003). Plasma phosphorus levels are lower in chickens exposed to heat stress at 35°C (Olgun et al., 2021). Coon (2002) suggests that laying hens under heat stress should receive around 400 mg of bioavailable phosphorus daily. Excess phosphorus intake can affect eggshell quality, but this issue rarely arises under heat stress due to decreased feed intake. Adjusting the calcium-to-phosphorus ratio can help mitigate the effects of sudden heat stress (Leeson and Summers, 2005).

Zinc (Zn) is vital for animal growth and plays an important role in the structure of enzymes like carbonic anhydrase, which is involved in eggshell formation (Balnave and Muheereza, 1997; Nys et al., 2001). Zinc can negatively impact eggshell quality by inhibiting carbonic anhydrase. Additionally, zinc helps protect birds from heat stress by increasing antioxidant enzyme activity (e.g., catalase and glutathione peroxidase), which neutralizes free radicals (Olgun et al., 2021). Without enough zinc, the production of free radicals increases, making heat stress more severe.

Selenium (Se) is another essential nutrient for various bodily functions, with over 25 selenoproteins and enzymes relying on it (Zhou et al., 2013; Surai, 2018). In heat-stressed broilers, adding selenium improves feed intake, weight gain, and feed conversion by influencing the metabolism of proteins, fats, and carbohydrates (Habibian et al., 2014). It also shields the pancreas and small intestine from oxidative damage caused by heat stress, lowering harmful MDA levels and boosting antioxidant enzyme activity (Olgun et al., 2021). Selenium supplementation has been shown to reduce the detrimental effects of heat stress on immunity (Arthur et al., 2003; Surai, 2018).

In a study on male geese, Baş et al. (2023) and Taşkesen et al. (2023) looked into how adding vitamin E, selenium, and zinc to their diets during heat stress affected testicular health and semen quality. The results showed that the control group, which was exposed to heat stress, experienced a significant decrease in testicular histology and semen quality. However, when supplemented with selenium, vitamin E, or both, these parameters significantly improved compared to the control group.

Copper is a component of many enzymes involved in poultry immunity and has antioxidant qualities. Reduced feed intake from heat stress results in a decrease in copper uptake. The body thus suffers from copper deficiency. By affecting T cell formation, antibody synthesis, and phagocytic index, copper deficiency negatively affects the immune system (Olgun et al., 2021). When exposed to high ambient temperatures, the body's antioxidant activity decreases. However, taking copper supplements, which are a part of many antioxidant systems, can lessen the negative effects (Olgun et al., 2021). The quality of laying hens' eggshells was negatively impacted by heat stress and inadequate copper (Cu) concentration (8.73 mg/kg). Furthermore, copper is important for the development of eggs, and deficiencies in copper may be observed. By adding 50% more nano Cu-oxide to grill feed than is permitted, the negative effects of heat stress on performance and immune response were successfully mitigated. In 2021, Olgun et al. reported that this was seen in broilers that were exposed to 33°C.

4. CONCLUSION

Heat stress undeniably wreaks havoc on the energy balance of animals, leading to increased production costs and diminished productivity. The impact is especially pronounced in ruminants, where heat stress disrupts their physiology, hormones, and biochemistry, primarily affecting their energy metabolism. Despite the clear evidence of these losses, a deeper, more comprehensive understanding of the underlying mechanisms is still needed. More research is critical to unravel these complex processes and offer solutions to mitigate the effects of heat stress. This knowledge will be vital to ensure the long-term sustainability and profitability of animal production.

When it comes to poultry, heat stress management is essential. A variety of strategies can be employed to minimize its impact. One effective method is to withhold feed during peak heat stress periods. This is because the digestion, absorption, and metabolism of feed generate heat, and withholding feed reduces this internal heat production. Additionally, depriving animals of feed can help calm them, reducing overall energy expenditure.

Feeding poultry at cooler times of day early morning or evening helps minimize metabolic heat production, which peaks 4 to 6 hours after feeding. Optimizing energy balance in poultry diets is also crucial. Feeds with high cellulose content should be avoided, and amino acids should be balanced to meet nutritional needs. Electrolyte, vitamin, and mineral levels must be properly adjusted, while the protein ratio in feed should be lowered, with restrictions on animal protein use. Increased water consumption is another key factor in heat stress management. Offering cool (10-15°C), clean water is essential for hydration and cooling. Furthermore, moldy feed must be strictly avoided, as it exacerbates the effects of heat stress. To ensure feed quality, moisture content should not exceed 12% during storage.

In conclusion, managing heat stress requires a multifaceted approach, from adjusting feeding times and nutrition to optimizing water intake and feed quality. By implementing these strategies, producers can help safeguard animal health and productivity, even in the face of extreme heat conditions.

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CHAPTER 12

EFFECTS OF PHOTOPERIOD ON ANIMAL PHYSIOLOGY

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1. INTRODUCTION

Photoperiodism, the physiological reaction of organisms to the length of day and night, is a critical environmental cue that regulates various biological processes in animals. These processes include reproductive cycles, growth patterns, immune responses, migration, and hibernation. Animals use photoperiod changes to synchronize these activities with the seasons, which ensures that key physiological functions occur under optimal conditions for survival and reproduction (Goldman, 2001; Schneider and Shaw, 2020).

The influence of photoperiod on animal physiology is achieved through complex neuroendocrine pathways, including the release of melatonin from the pineal gland, which communicates seasonal information to the brain (Yoshimura, 2004; Meng et al., 2022). This chapter will explore the mechanisms by which photoperiod influences animal physiology, the effects on different systems, and the adaptive significance of these responses across species.

2. MECHANISMS OF PHOTOPERIOD PERCEPTION

Mechanisms of photoperiod perception are integral to understanding how animals adapt to changing seasons and environmental conditions. Photoreceptors play a central role in detecting light cues, which serve as indicators of day length. In mammals, this process begins with light-sensitive cells in the retina that detect environmental light changes and relay this information to the suprachiasmatic nucleus (SCN) of the hypothalamus, the body’s master circadian clock. From the SCN, the information is transmitted to the pineal gland, which regulates melatonin production in response to darkness. This hormone is secreted in prolonged durations during longer nights, signaling the length of nighttime to the organism, which in turn acts as a seasonal timer. Changes in melatonin secretion trigger physiological and behavioral adaptations that prepare animals for upcoming seasons (Goldman, 2001; Paul et al., 2020). In non-mammalian species, such as birds and reptiles, photoperiod perception can differ significantly. Birds, for instance, have specialized photoreceptors located within the hypothalamus itself. These brain-based photoreceptors can directly detect changes in day length without reliance on retinal input, allowing a more immediate response to seasonal shifts. This direct perception enables birds to adjust behaviors like migration, molting, and reproduction in sync with changing photoperiods. The hypothalamus in birds influences neuroendocrine systems responsible for regulating hormones, such as gonadotropins, which drive seasonal reproductive cycles and other photoperiod-dependent activities. Rapid adjustments in response to photoperiod are critical for survival, especially for migratory species that must precisely time their departures and arrivals with favorable environmental conditions (Yoshimura, 2004; Rani and Kumar, 2021).

2.1. Melatonin as a Photoperiodic Signal

Melatonin, frequently dubbed the "night hormone," serves as a crucial signal for photoperiodic regulation by conveying day length information to multiple physiological systems. This hormone is synthesized in the pineal gland and released primarily at night, with its secretion duration directly correlating with night length. In mammals, the production of melatonin during extended night periods in autumn and winter triggers a cascade of physiological responses, including modulation of reproductive and metabolic systems. For example, in short-day breeding species like sheep and deer, the extended duration of melatonin secretion acts on the hypothalamic-pituitary-gonadal (HPG) axis, stimulating reproductive hormone release to prepare animals for breeding in alignment with seasonal conditions. This ensures that offspring will be born in the spring, when food resources are abundant and environmental conditions support juvenile survival (Bronson, 1989; Gilmour et al., 2023). In contrast, long-day breeders, such as horses and certain bird species, experience a reduction in melatonin secretion as daylight lengthens in spring. The shorter duration of melatonin release removes the inhibitory effects on the HPG axis, allowing the release of gonadotropin and other reproductive hormones that promote breeding readiness. This adjustment to melatonin levels is adaptive, aligning reproductive activity with optimal conditions for offspring survival, such as warmer temperatures and ample food availability (Lincoln and Short, 1980; Ueda et al., 2021). Additionally, melatonin impacts other systems sensitive to photoperiod, including immune function and energy metabolism, contributing to a synchronized seasonal adjustment across various physiological domains. This multifaceted role underscores melatonin's importance not only in regulating reproductive cycles but in coordinating a broad range of adaptive responses to seasonal environmental changes.

3. EFFECTS OF PHOTOPERIOD ON REPRODUCTIVE PHYSIOLOGY

The effects of photoperiod on reproductive physiology demonstrate an intricate relationship between environmental cues and the physiological processes that control breeding cycles. Many animals, especially those in temperate regions, exhibit seasonal breeding patterns that are tightly regulated by changes in daylight length. This strategy allows animals to align reproductive timing with optimal environmental conditions, such as temperature, food availability, and overall climate, enhancing the survival prospects of their offspring (Malpaux et al., 2001; Schneider and Shaw, 2020). Photoperiodic cues are detected by the animal's photoreceptive systems, which convey information about day length to the hypothalamic-pituitary-gonadal (HPG) axis, a key endocrine pathway regulating reproductive hormone production. In response to longer nights, for instance, short-day breeders such as sheep and deer experience a surge in melatonin production, a hormone released by the pineal gland during darkness. This prolonged melatonin signal stimulates the release of gonadotropins, hormones that promote gonadal development and function, thereby initiating reproductive activity suited to ensure offspring are born in spring when conditions are more favorable. Conversely, long-day breeders like horses and some rodent species respond to decreasing melatonin levels as daylight increases in spring. The reduction in melatonin duration alleviates inhibition on the HPG axis, facilitating an increase in gonadal activity, gamete production, and mating behaviors. This allows these animals to produce offspring in late spring or early summer when resources peak. The timing of these responses is also influenced by genetic factors, as some populations or species have evolved unique photoperiodic sensitivities based on their geographic location or ecological niche (Bronson, 1989; Paul et al., 2020). Additionally, photoperiod-driven reproduction in birds demonstrates this adaptability, with brain-based photoreceptors detecting day length changes that trigger breeding readiness, ensuring offspring hatch during periods of abundant resources (Dawson et al., 2001; Bentley et al., 2017). This precise alignment between photoperiod and reproduction highlights the evolutionary importance of photoperiodism as a regulatory mechanism in diverse animal species, facilitating a synchronized approach to reproductive success and offspring survival.

3.1. Short-Day Breeders

Short-day breeders, which include species like sheep, deer, and some rodent species, rely on photoperiodic cues to time their reproductive cycles to ensure that offspring are born when conditions are most favorable. In these animals, reproductive activity is triggered by the extended melatonin secretion associated with longer nights in the fall and winter. Melatonin’s increased duration inhibits gonadotropin-inhibitory hormones in the hypothalamus, thus stimulating the hypothalamic-pituitary-gonadal (HPG) axis to promote reproductive hormone release. This process prepares the reproductive system for breeding as days shorten, ensuring that births align with springtime, a season marked by warmer temperatures and increased food availability. By timing reproduction in this way, short-day breeders maximize the chances of offspring survival, allowing young to grow during the months when environmental conditions support their development (Prendergast et al., 2002; Gilmour et al., 2023).

The photoperiodic response to melatonin can be further modulated by factors such as age, health, and genetic differences among species and even within populations. In certain rodent species, like Syrian hamsters, the sensitivity to melatonin’s effects varies with these factors. Younger individuals or those in optimal health conditions may exhibit a stronger response to short days, resulting in more consistent and synchronized breeding patterns. In contrast, older or less healthy animals may show a diminished or altered response to melatonin, potentially leading to variability in reproductive timing. This plasticity in response allows species to adjust to slight shifts in environmental cues or physiological states, though it may also result in suboptimal reproductive timing if these variations affect the synchronization with favorable spring conditions (Malinowski and Roussel, 2022).

3.2. Long-Day Breeders

Long-day breeders, including horses, certain bird species, and some rodents, have reproductive cycles that are activated by increasing day lengths, typically in spring and early summer. This group of animals depends on longer daylight to initiate reproductive hormone production, aligning their breeding season with environmental conditions that support offspring growth and survival. In these species, the gradual shortening of melatonin secretion as days lengthen lifts the suppression on the hypothalamic-pituitary-gonadal (HPG) axis, facilitating the release of reproductive hormones such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These hormones trigger ovulation and spermatogenesis, signaling the start of reproductive cycles (Lincoln and Short, 1980; Ueda et al., 2021). This photoperiodic strategy ensures that offspring are born during summer when warmer temperatures, longer days, and increased food availability provide ideal conditions for growth and development. Moreover, abundant vegetation and shelter in summer increase the likelihood of offspring survival, particularly in herbivorous species like horses, whose dietary needs are directly tied to seasonal plant growth (Rani and Kumar, 2021).

In avian species, the influence of photoperiod on reproduction is complex, as it often interplays with other seasonal behaviors such as molting and migration. For many migratory bird species, longer daylight hours in spring trigger not only breeding readiness but also initiate molting cycles necessary for replacing worn feathers. The synchronization of molting and reproductive preparation allows birds to replace feathers needed for long migratory journeys while concurrently readying for mating and egg-laying, which are energetically demanding processes. Longer daylight periods stimulate neuroendocrine changes that coordinate these processes, enabling birds to build energy reserves for migration while optimizing reproductive success (Gwinner, 1996; Taylor et al., 2024). This dual response to photoperiod cues illustrates how tightly integrated reproductive and survival strategies are in long-day breeders, ensuring that physiological changes are in sync with the seasonal environment and resources.

4. EFFECTS OF PHOTOPERIOD ON GROWTH AND METABOLISM

Photoperiod exerts a significant impact on growth and metabolic processes across numerous species, allowing animals to align their physiological states with seasonal changes in resources and environmental demands. During long photoperiods, such as those in spring and summer, extended daylight stimulates growth hormone production and accelerates metabolic rates. This increase in metabolic activity supports behaviors and physiological processes that require higher energy expenditure, like foraging, mating, and physical development. For juvenile animals, this period of heightened growth is crucial, as it allows them to reach maturity more quickly, enhancing their chances of survival and successful reproduction in future seasons. In contrast, shorter photoperiods in fall and winter trigger physiological shifts that favor energy conservation. Many animals reduce their metabolic rates during these times, entering states of lower activity or even torpor or hibernation in some cases, thereby conserving energy when food is scarce. These adjustments often involve hormonal changes, such as reductions in thyroid hormones, which are key regulators of metabolism, as well as decreased production of insulin-like growth factors that support growth (Hazlerigg and Loudon, 2008; Paul et al., 2020).

In addition to seasonal influences on growth rates, photoperiod also plays a role in lipid metabolism and energy storage. Animals in temperate climates often build up fat reserves during longer days, preparing for the energy demands of winter or migration. For instance, birds accumulate significant fat stores to fuel long migratory flights, while hibernating mammals build up body fat to sustain them through months of low food availability. Photoperiod-induced changes in metabolic processes, therefore, allow animals to efficiently manage energy resources across different seasons, contributing to overall fitness and survival. Recent studies highlight how the interplay between light cycles and genetic factors can influence individual responses to photoperiod, with some animals showing variation in metabolic adaptations based on their latitude or the specific demands of their ecological niche (Prendergast et al., 2002; Zaidan et al., 2017). These adaptive responses demonstrate the critical role photoperiod plays not only in immediate energy balance but in long-term survival strategies, aligning growth and metabolic cycles with the rhythm of the natural environment.

4.1.Growth Regulation

Growth regulation in mammals is closely linked to seasonal changes, with photoperiod playing a crucial role in modulating growth hormone (GH) levels. For instance, in species like Siberian hamsters, long photoperiods typical of spring and summer stimulate the secretion of GH, which promotes rapid growth and development. This acceleration in growth is vital for building the energy reserves needed to endure the harsher conditions of the winter months when food availability is reduced and thermoregulatory demands increase. In these species, the photoperiod acts as a cue to prepare the body for survival during a period of reduced activity, ensuring that they have sufficient energy stores for maintenance, hibernation, or other metabolic processes (Prendergast et al., 2002; Malinowski and Roussel, 2022). Conversely, exposure to short-day conditions, which occur in the fall and winter, leads to a reduction in GH secretion, resulting in slower growth rates. This physiological response conserves energy by slowing down metabolism and growth, which aligns with the body's need to preserve resources during times of limited food intake and increased environmental stress.

Additionally, the photoperiod has been shown to influence other physiological parameters such as muscle mass and bone density. Under long-day conditions, which promote active behaviors like migration, reproduction, or increased foraging activity, animals often exhibit enhanced muscle development and bone mineralization. This is particularly noticeable in species that need to prepare for migration, where increased muscle mass supports prolonged flight or locomotion. Similarly, during reproductive periods, the body may enhance bone density to support the physical demands of mating behaviors and offspring rearing. These physiological changes highlight how photoperiod-induced shifts in growth and metabolic processes are not just a matter of energy storage, but also preparation for seasonally specific activities (Simpson et al., 2023; Paul et al., 2020). Thus, growth regulation via photoperiod allows animals to optimize their physiological functions based on the environmental conditions they are likely to encounter, supporting both survival and reproductive success.

4.2. Energy Metabolism and Fat Storage

As days shorten in the fall, many animals, including ground squirrels, bears, and certain rodents, undergo significant metabolic shifts to prepare for winter hibernation or periods of reduced activity. This process, regulated by photoperiodic cues, triggers a series of physiological changes that help animals accumulate energy reserves and reduce their energy expenditure during the harsh winter months when food is scarce. As daylight decreases, these animals increase their fat storage while simultaneously reducing their metabolic rates to conserve energy. The photoperiod plays a crucial role in regulating the release of hormones like leptin, which helps control appetite and fat storage, and insulin, which modulates glucose metabolism. These hormonal adjustments ensure that animals store enough fat to maintain their energy levels throughout the winter, during which they may undergo hibernation or enter a state of torpor (Nelson, 2005; Simpson et al., 2023). In many species, including bears, fat reserves can reach substantial levels before the onset of winter, enabling them to survive prolonged periods without food.

Additionally, photoperiodic changes can influence thermoregulation, which further supports energy conservation. Research has shown that the transition to shorter days triggers alterations in metabolic rates that help animals adapt to colder ambient temperatures. For example, certain species, like ground squirrels, experience a drop in their core body temperature, which slows down metabolic processes and reduces overall energy expenditure. These animals can enter a state of hibernation, where their metabolic rate decreases to a level that allows them to survive for months without food, relying solely on their fat stores (Bauer et al., 2020). Similarly, studies on species such as deer and other large mammals have revealed that reduced metabolic rates during colder months help them conserve energy for vital functions like maintaining body temperature and supporting basic physiological processes (Meng et al., 2022). Thus, photoperiod-induced metabolic shifts are integral to winter survival, allowing animals to optimize their energy use, store sufficient fat, and adjust thermoregulatory mechanisms to respond effectively to seasonal temperature fluctuations.

5. PHOTOPERIOD AND IMMUNE FUNCTION

Photoperiod significantly influences immune function, with many animals adjusting their immune responses seasonally to better cope with the challenges of changing environmental conditions. Research has shown that animals like white-footed mice exhibit enhanced immune activity during short-day conditions, typically associated with winter. This seasonal adjustment likely serves as a strategy to offset the physical stress and potential exposure to pathogens during colder months when food availability may be low, and environmental challenges increase. Under these conditions, the immune system ramps up activity to ensure greater resilience against infections and injuries that could arise from the harsher environment (Nelson and Demas, 1996; Demas and Carlson, 2022).

A critical mediator of this seasonal immune adjustment is melatonin, the hormone primarily secreted by the pineal gland in response to darkness. Known for its antioxidant and anti-inflammatory properties, melatonin plays a pivotal role in regulating immune function throughout the year, particularly during the winter months. Melatonin influences various immune cells, including T lymphocytes, macrophages, and neutrophils, enhancing their activity when environmental conditions make individuals more susceptible to infections. This response is especially vital in species that experience colder climates, where the risk of infectious diseases may be elevated due to crowding, weakened immune function from food scarcity, or colder temperatures (Rani and Kumar, 2021; Gilmour et al., 2023). Furthermore, melatonin's ability to modulate inflammatory responses helps reduce the damage caused by prolonged stress or exposure to harmful pathogens during these high-risk periods. Thus, photoperiodic regulation of melatonin not only helps animals prepare physiologically for seasonal changes but also boosts their immune defenses, ensuring they are better equipped to handle the challenges posed by changing environments and seasonal diseases.

6. MIGRATION AND PHOTOPERIODIC CUES

Migration, particularly in avian species, is strongly influenced by photoperiodic cues, as birds rely on changes in day length to initiate their migratory behavior. This reliance on photoperiod helps synchronize migration with the seasonal availability of resources and optimal environmental conditions. The onset of migration is regulated by complex physiological and behavioral changes, including "zugunruhe" (migratory restlessness), a period of heightened activity and restlessness that precedes migration. Zugunruhe is believed to be an innate behavior triggered by photoperiodic changes, signaling that birds are preparing for the long journey ahead. As days shorten, birds become more restless, displaying increased activity and feeding, signaling the start of migratory preparations (Gwinner, 1996; Taylor et al., 2024).

At the physiological level, the hypothalamus and pineal gland play key roles in regulating this behavior by responding to the length of daylight. These brain regions process the photoperiodic information and translate it into a signal that coordinates the timing of migration. This ensures that migratory birds leave their breeding grounds at the optimal time to travel to their wintering grounds and that they arrive at their destinations when conditions are most favorable. These conditions include abundant food sources, suitable temperatures, and reduced predation risk. The ability to precisely time migration maximizes the chances of survival and reproductive success by aligning migratory timing with ecological factors such as resource availability and predator behavior (Schneider and Shaw, 2020; Harrison and Alliston, 2021). Moreover, this synchronization of migration with seasonal cues enables birds to avoid the harsh winter conditions of their breeding grounds and find more suitable habitats, ensuring that they are in optimal condition for survival and reproduction. Thus, photoperiodic regulation is a crucial element in the migratory success of birds, illustrating the profound connection between environmental cues and the evolutionary strategies that animals use to thrive in varying conditions.

7. ADAPTIVE SIGNIFICANCE OF PHOTOPERIODIC RESPONSES

Photoperiodic responses offer significant adaptive value to animals, enabling them to anticipate and prepare for seasonal changes in their environment. By adjusting their behavior and physiology to changes in day length, animals can optimize their survival and reproductive success. For instance, many rodent species, such as voles and mice, use photoperiodic cues to time reproduction so that offspring are born during periods of peak food availability in the spring or early summer. This ensures that young animals have access to abundant resources during critical growth stages, thereby increasing their chances of survival (Bronson, 1989; Schneider and Shaw, 2020). Similarly, long-day and short-day breeders have evolved strategies where the timing of reproductive cycles is closely linked to the availability of food and favorable climatic conditions, which reduces the risk of offspring being born during harsh or resource-scarce seasons.

The adaptive significance of photoperiodic responses is becoming increasingly important in the face of climate change. As global temperatures rise and seasonal patterns shift, the timing of photoperiodic cues may be disrupted, potentially affecting species' fitness and altering ecosystem dynamics. For example, some species might find that their reproductive cycles no longer align with peak food availability or that migratory timing is off, leading to mismatches between animal needs and environmental conditions (Harrison and Alliston, 2021). Climate change may also cause species to face new environmental challenges that they are not evolutionarily prepared to handle, potentially leading to population declines or shifts in migration patterns. The ongoing research in this area focuses on understanding how species might adapt—or fail to adapt—to these new environmental realities. Some studies suggest that while some species may show plasticity in their responses to changing climates, others may struggle to cope with the rapid rate of environmental change, highlighting the importance of continued monitoring and study of photoperiodic adaptations in a warming world (Taylor et al., 2024; Gilmour et al., 2023). By understanding how photoperiodic responses are influenced by climate change, scientists hope to develop strategies to mitigate the effects on species that are at risk of maladaptation, ensuring that they continue to thrive in rapidly changing ecosystems.

8. CONCLUSION

Photoperiod is a fundamental environmental cue that regulates a wide range of physiological functions in animals, from reproduction to metabolism, immunity, and migration. Through hormonal signaling, primarily via melatonin, animals synchronize their physiology with seasonal light changes, optimizing survival and reproductive success. Recent studies continue to deepen our understanding of photoperiodic regulation, revealing complex mechanisms that vary across taxa.

Understanding photoperiodic responses is increasingly important in the face of climate change, as altered seasonal patterns may disrupt the finely tuned responses of photoperiod-sensitive species. Future research will play a critical role in identifying which species are most vulnerable to these changes and how adaptive mechanisms may help some species survive in shifting environments.

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CHAPTER 13

SATELLITES IN PLANT VIRUSES: STRUCTURE, FUNCTION AND IMPACT

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1. INTRODUCTION

Satellites are subviral entities that infect host cells through a helper virus (HV) and relying on them for replication within cells. Catalysis of this process requires replicase enzymes of the host cells. Additionally, these satellites rely on their HV for movement, transmission, and encapsidation.

Satellites are primarily categorized into two groups: satellite viruses (satviruses) and satellite RNAs (satRNAs). Satviruses, prevalent in plants, mammals, arthropods, and bacteria, encode distinctive structural proteins which are different from the helper virus coding sequences. In contrast, the other group of satellites does not carry genetic information for structural proteins and instead relies on the HV to encode a protein that is necessary in their encapsulation.

The first satellite was identified in *Tobacco necrosis virus* (TNV) in 1962, equipped with the ability to construct its own protective shell and displaying no serological relation to the virus. Subsequently, a second satellite was uncovered in *Tobacco ring spot nepovirus* (TRSV) after some years. Investigations suggested that the origin of satellites could potentially be traced back to the host cell, the helper virus, or any transmission vector. In recent times, numerous satviruses and satRNAs have been discovered, with many being sequenced. Nevertheless, there remains a scarcity of comprehensive information regarding their biology.

They differ from defective interfering RNAs (DI-RNA) in that they do not have significant sequence similarity with their HV (Simon et al., 2004). Occasionally, we encounter other small RNAs that resemble satellites, although they are distinct. Unlike satellites, these RNAs exhibit similarity in sequences with their HV. Examples include the DI-like satRNA chimera of *Turnip crinkle carmovirus* (TCV) and certain RNAs that are needed for the life cycle of HV, such as *Groundnut rosette virus* (GRV) (Simon and Howell, 1986).

Satellites exhibit a size range from < 200 nt to 1500 nt. The largest among them includes an open reading frame (ORF), yet the function of their gene products remains unidentified. In contrast, the smaller satellite RNAs tends to possess complex structures and can survive in linear or circular forms. Despite their small size, these RNAs have the potential to influence the symptoms caused by the HV. They have the ability to alter, lessen, or worsen the helper virus's symptoms. Since these molecules utilize a replication mechanism similar to that of the helper virus and do not encode their own replicase, they serve as valuable tools to study the replication system of the helper virus (HV) (Zhang et al., 1991). Studies indicate that they typically reduce the aggregation of the HV in host cells. When accumulated in substantial quantities in certain host plants, they can function as expression vectors for foreign genes.

Nevertheless, these distinctive characteristics render them a well-suited biological system for in-depth studying of molecular biology of different viruses. This chapter primarily focuses on the importance of studying satRNAs and satviruses, exploring aspects like their replication mechanisms, pathogenic effects, and evolutionary patterns.

2. WHAT ARE SATELLITES?

Some plant viruses carry non-infectious short RNAs in their genome, with one subgroup referred to as satellites. Satellites function as subviral agents, relying on their HV for replication, encapsulation, movement, and transmission. Predominantly non-coding RNAs, satellites often influence the interaction between the HV and host cell, impacting helper virus accumulation and modulating virus symptoms, either weakening or exacerbating them. Notably, satellites exhibit sequence differences from their helper viruses. Encapsulation in its encoded structural proteins creates a distinction between coding satellite viruses and non-coding satellite RNAs. Research indicates that while most satellite viruses and satellite RNAs do not impede the helper virus infection process in hosts, a subset of satellite RNAs may play roles in insect transmission of the virus or in facilitating movement. Satellites are broadly categorized into two groups: Satellite viruses and Satellite RNAs (Hu et al. 2009; Briddon et al. 2012; Palukaitis 2016).

2.1 Satellite Viruses

Satviruses, like satRNAs, rely on their HV for multiplication, transmission, and movement. A distinguishing feature is that satellite viruses can encode their own coat protein (CP). In-depth studies on the replication mechanisms of two different satviruses, *Satellite Tobacco Mosaic virus* (STMV) and *Satellite Tobacco Necrosis virus* (STNV), revealed interesting findings. Specifically, the untranslated regions in 5' and 3' ends of STNV-C exhibited merely 47% and 36% similarity, respectively, with the compatible regions in its helper TNV-D. Remarkably, these regions were interchangeable with equivalent sequences of TNV-D or multiplication, committing that STNV and its helper virus utilize a similar mechanism for replication (Leung et al., 1979; Bringloe et al., 1998). While there is limited information about the impact of satellite viruses on their helper viruses and host plants, it is conceivable that they may influence helper virus accumulation or contribute to the pathogenicity in the host.

However, the majority of studies have primarily focused on the impact of non-coding sequences on the translation of satellite virus RNAs. A notable example is the satellite virus of TMV, which was firstly identified as a Virus-Induced Gene suppressing (VIGS) vector (Liou et al., 2017; Gossele and Metzlaff, 2005; Gossele et al., 2002; Dodds, 1998). This satvirus has been employed for inducing gene suppression, effectively silencing various endemic plant genes (Lave et al., 2002). Its ability to silence genes was proved by suppressing the expression of fourteen endogenous genes such as chalcone synthase, phytoene desaturase, cellulose synthase involved in different biochemical pathways in *Nicotiana tobacum* (Liou et al., 2017).

In single-stranded satviruses, there are generally two subclasses. One is satvirus associated with *chronic bee paralysis virus* (CBPV). This subclass comprises only one member, which is the CBPV. The other one is the satellite of *Tobacco necrosis virus*. This subgroup encompasses 4 members including *Panicum mosaic satellite virus* (PMV), *Maize white-line mosaic satellite virus* (MWLMV), *Tobacco mosaic satellite virus* (TMV), and *Tobacco necrosis satellite virus* (TNV).

SatRNAs can be classified into 3 classes: large linear satRNAs, small linear satRNAs, and small circular satRNAs.

2.1.1. Large linear satellite RNAs

These molecules exhibit a size range between 800 to 1500 nt and all encode a protein that plays a different role in associated helper viruses. This group is classified as messenger-type satRNAs due to coding a minimum of one protein that is not structural. *Arabis mosaic virus* (ArMV) large satRNA, *Bamboo mosaic virus* (BMV) satRNA, *Chicory yellow mottle virus* (ChYMV) large satRNA, *Tomato black ring virus* (TBRV) satRNA, *Grapevine fanleaf virus* satRNA (GFLV), *Beet ring spot virus* (BRSV) satRNA are the notable members of this group. In Nepoviruses, the encoded protein is necessary for the replication of satRNAs. Conversely, in *Bamboo mosaic virus* (Potexvirus), the encoded protein has a crucial role in the systemic movement of the satellite virus. Interestingly, unlike their roles in the mentioned viruses, no encoded protein has been identified in association with *Groundnut rosette virus* (Umbravirus). It has been determined that, for most large satRNAs in nepoviruses such as AMV and GFLV, there is no impact on viral symptoms, and there is no correlation between viral symptoms and satRNAs.

2.1.2. Small linear satellite RNAs

These satRNAs, which have a high rate of nucleotide pairing in their structures, are shorter than 700 nucleotides and do not code for any protein. Studies have indicated that this extensive base-pairing serves to protect satRNAs from the RNA silencing system. The effects of the helper virus may be reduced or worsened by these satRNAs, or have no impact on them. Some members of this group include *Cucumber mosaic virus* (CMV) satRNA, *Pea enation mosaic virus* (PEMV) satRNA, *Grapevine ring spot virus* (GRSV) satRNA, *Turnip crinkle virus* (TCV) satRNA.

2.1.3. Small circular satellite RNAs

Circular satRNAs share a common feature with viroids in having internal base-pairing, yet they typically require a helper virus for replication, especially for encapsidation. These satRNAs are characterized by their small size, ranging between 220 to 388 nt, and lack significant biological messenger activity. Members of this group include *Arabis mosaic virus* small satRNA, *Cereal yellow dwarf virus-RPV* (CYDV) satRNA, ChYMV satRNA, *Solanum nodiflorum mottle virus* (SnMV) satRNA, *Subterranean clover mottle virus* (SCMV) satRNA, Lucerne *transient streak virus* (LTSV) satRNA, *Tobacco ringspot virus* (TRSV) satRNA , and *Velvet tobacco mottle virus* (VTMV) satRNA. They replicate through a rolling-circle mechanism, and through the activity of a hammerhead ribozyme, they undergo self-cleavage into monomers.

3. SMALL satRNAs ASSOCIATED WITH INTENSIFYING VIRUS PATHOGENICITY

The phenomenon of enhancing helper virus symptoms by satRNAs is not widespread, but studies on *Turnip crinkle virus* (TCV) have revealed intriguing insights. In the case of TCV, it has been demonstrated that TCV-associated satC reduces HV accumulation by approximately 25–50%. Paradoxically, despite this reduction, it intensifies the symptoms induced by the HV and enhances virus movement in both short and long-distance (Li and Simon, 1990; Zhang and Simon, 2003). By preventing TCV virion formation in host cells, satC leads to the build-up of free coat proteins, which has been identified as an RNA silencing agents in *Arabidopsis thaliana*.

Additionally, the effects of *Cucumber mosaic virus* (CMV) satRNAs vary based on the host and satRNA isolate. Generally, they either attenuate the virus symptoms or have no significant effect on them (Gal-on et al., 1995; Habili and Kaper, 1981; Waterworth et al., 1979). However, certain CMV satRNAs may have detrimental consequences on the host cells, inducing chlorosis in tobacco and pepper (*Nicotiana tabacum*, *Capsicum annuum*), yellow leaf or white leaf chlorosis and necrosis in tomato (*Solanum lycopersicum*) and its wild relatives (Kuwata et al., 1991). The variations in nucleotide sequences in particular regions of these non-coding RNAs are attributed to these differences (Sleat and Palukaitis, 1992).

4. SMALL satRNAs INDUCE CHLOROSIS IN TOBACCO

Studies conducted by Wang and his colleagues in 2004 demonstrated that yellow chlorosis was primarily attenuated in tobacco plants when inoculated with CMV+satRNA Y (Devis et al., 1990; Jaegle et al., 1990). The accumulation of the virus and satRNA complex was found to be influenced by the presence of HC-Protein. The expression of this protein, HC-Pro, proved to be effective in eliminating the negative effects of the virus and satRNA complex (Wang et al., 2004a, b; Zhang et al., 1994; Masuta et al., 1989).

5. INDUCTION OF NECROSIS IN TOMATO BY SMALL satRNAs

CMV-satRNA D-induced necrosis in tomatoes triggers a plant cell death response in phloem cells (Taliansky et al., 1998; Xu et al., 2000). This response was due to the accumulation of the minus-stranded RNA of satRNA D, along with the plus-stranded RNA of HV. The comparison of transcriptome analysis of tomato plants infected with CMV+satRNA D and plants infected with a non-necrogenic mutant of satRNA D showed 401 variations in gene expression profile. Genes related to ethylene production and ethylene-induced defensive responses were among these alterations. (Sleat and Palukaitis, 1990). The exact mechanism behind this defense response is not fully understood yet (Irian et al., 2007).

6. ATTENUATION OF PATHOGENICITY BY SMALL satRNAs

In general, decreasing the symptoms of the HV is often associated with a reduction in the accumulation level of HV RNAs. There are certain exceptions, though, as the *Cucumovirus tomato aspermy virus* (TAV) demonstrates, where satRNAs compete with the helper virus to access the replicase. This competition results in reducing HV accumulation levels and subsequently leads to the development of mild disease symptoms (Tien and Wu, 1991).

7. SMALL satRNAs AS ANTI-SUPPRESSOR OF RNA SILENCING

Various RNA silencing suppressors (RSS) play a crucial role in pathogenicity by inhibiting microRNAs responsible for the turnover of transcription factors. In the case of CMV, the 2b protein has been identified as the RSS in severe strains of CMV. This protein contributes to the development of severe disease symptoms in Arabidopsis thaliana through alteration of mRNAs encoding different transcription factors. Studies have shown that coinfection of severe CMV strains with attenuating satellite RNAs can reduce the effects of HV on the accumulation of multiple transcription factors, similar to the effects observed when the 2b gene is deleted from the severe CMV strain. Therefore, it suggests that the 2b RSS is at least partially responsible for some of the deficiencies caused by CMV. Moreover, satellite RNAs might serve as a source of small interfering RNAs (siRNAs) that compete with microRNAs and other siRNAs in binding to the 2b protein. Due to this competition, protein 2b cannot bind to microRNAs., thereby attenuating symptoms caused by 2b protein and obstructing microRNA-mediated regulation of mRNAs (Shen et al., 2015; Feng et al., 2011, 2012).

8. INTERFERENCE OF SMALL satRNAs IN ACCUMULATION OF SPECIFIC HELPER VIRUSES

In this context, research has demonstrated that TBSV-defective interfering RNAs (DI-RNAs) and CMV satellite RNAs, when present alongside extra viral RNA, cause a significant reduction in the aggregation of specific subgenomic RNAs of the HV (Xu et al., 2004). This model was proposed as a mechanism for CMV satD to diminish the aggregation of the 2b protein for mitigating symptoms (Liao et al., 2007). Additionally, it was committed that small interfering RNAs produced from satRNAs could diminish the level of CMV-RNA 4A encoded by 2b, targeting specific regions in the 3' non-structural ends (Xu et al., 2003).

9. SatRNAs REDUCING HELPER VIRUS ACCUMULATION

Depending on the particular virus and the infected host, study results can differ. Research conducted with two strains of CMV, severe and mild, along with two potyviruses, revealed that in certain hosts, infection with CMV induced an increase in virus aggregation and consequently disease severity. However, this pattern was not observed with potyviruses, which was attributed to the RNA silencing suppressor (RSS) activity of potyviruses. Therefore, factors such as the host, the specific helper virus (HV), satellite RNA, and the interactions between them collectively contribute to the modulation of symptoms or the reduction of virus accumulation. These intricate relationships underscore the complexity of the interactions in viral infections and highlight the need for a nuanced understanding of the specific host-virus-satellite RNA interactions in different systems.

10. THE ADVANCES IN satRNAs

10.1.Replication Mechanism

satRNAs are a valuable tool for studying various biological functions, including replication and translation, by leveraging the replication mechanisms of their HVs. The 3'-terminal region of TCV and its satRNA, satC, is particularly well-characterized and can be utilized for such studies (Song and Miller, 2004). However, it is important to note that while TCV and satC share similarities in their 3'-terminal regions, they are not fully interchangeable.

Known for its dynamism, this region can alternate between translation and replication structures based on how the replicase interacts with TCV. Similar phenomena have been observed in other satellite viruses. For example, it has been demonstrated that the replication of certain DI-RNAs originating from the genome of CIRV or related *Cymbidium ring spot virus* (CymRSV) is aided by the expression of the replication protein of CIRV in yeast cells, but not those initially linked to CymRSV. This suggests that satRNAs adopt a replication system similar to that of their helper viruses but distinct from it. As a result, satRNAs do not compete directly with their helper viruses, and this lack of direct competition contributes to the observed differences and limited similarity between satRNAs and their HVs.

10.2. RNA Silencing in Host Plants is Involved in the Pathogenicity of satRNA

As previously discussed, satRNAs exhibit diverse effects on disease symptoms, ranging from attenuation to intensification, or even having no noticeable impact. Recent researches have emphasized the critical role of RNA silencing in pathogenicity of viruses. To counteract the host plant's defense system, viruses often encode proteins that act as RNA silenencers, thereby inhibiting the plant RNA suppression mechanism (Fig. 1). Additionally, satRNAs, much like viroids, have been observed to be resistant to RNA-silencing-mediated disruption. This resistance could potentially serve as a helper mechanism for the evolution of satRNAs.

This interplay between RNA silencing, viral proteins acting as suppressors, and the resistance of satRNAs to silencing mechanisms highlights the complex molecular strategies employed by viruses and subviral agents in their interactions with host plants. Understanding these mechanisms is crucial for gaining insights into the pathogenicity and evolution of these agents.

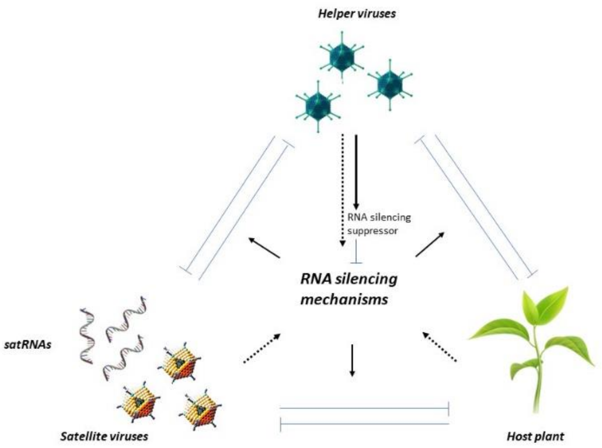

Figure 1: An illustration of the intricate relationship between the host plant, helper viruses, satRNAs /satviruses and the RNA silencing mechanism of the host

10.3. The Origin of satRNAs

While many studies suggest that satRNAs can emerge spontaneously under certain experimental conditions, satRNAs infection cycle reveals 3 potential sources that could serve as origins for these subviral RNAs.

Genome of the helper virus or co-infecting virus: The genome of the helper virus or another co-infecting virus can serve as a source for the origin of satRNAs. This is particularly relevant in cases where a host organism is infected by multiple species of viruses or satellites simultaneously.

Host organisms: Host organisms themselves can be a source of satRNAs. The regulatory small RNAs, including siRNAs and microRNAs, produced in response to viral infection in host plants could potentially be assembled into satRNAs by viral replicases.

Vectors: Insects and other organisms that can transmit viruses between plants could also play a role in the origin of satRNAs. The interaction between vectors and the viruses they transmit might lead to the generation of satRNAs and other siRNAs.

In natural settings, it is not uncommon for a single plant to be infected by multiple species of viruses or satellites simultaneously. Using a simple method, multiple infections can be elucidated by dsRNA extraction (Khabbazi et al., 2017). This complexity in viral interactions and co-infections contributes to the potential origins and diversity of satRNAs. The assembly of satRNAs from host-produced small RNAs underscores the intricate interplay between the host defense mechanisms and the strategies employed by viruses and subviral agents in their evolution.

10.4. Molecular evolution

Most satRNAs, lacking obvious coding potential, are thought to express their biological activities through direct interactions between the secondary structures or nucleotide sequences of satRNAs and host factors. The secondary structures present in the genome of satRNAs, as illustrated in figure 2, contribute to their resistance against the RNA suppressing mechanism of the host plant. These structural elements are highly conserved and play a critical role in the survival and persistence of satRNAs (Ban et al., 1995). The resistance to RNA silencing is a notable feature shared by satRNAs and viroids. This resistance allows them to evade the host plant's defense mechanisms and establishes them as unique entities capable of influencing host-virus interactions. The conservation of secondary structures in satRNAs highlights their functional significance and suggests that these structures are important in the adaptation and evolutionary success of satRNAs.

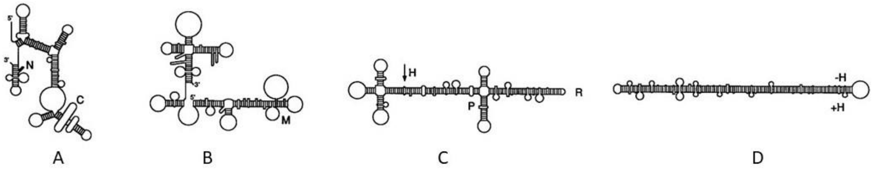

Figure 2: Secondary structure types of satRNAs: A) linear B2 satellite RNA of CMV; B) linear satRNA C of TCV; C) rolling circle replication intermediate of the linear satellite RNA of TobRV; and D) the circular satellite RNA of LTSV

11. THE APPLICATIONS OF satRNAs

SatRNAs could be used to create satRNA-based vector systems, especially for the expression of foreign genes in plants. While efforts have been made to harness their unique features, such as small size and stability due to secondary structures, to create efficient vectors, the practical application of satRNA-based vectors has faced challenges, and many studies have not succeeded in this regard. However, the limited applications have been achieved regarding expression of foreign genes in plants, functional studies and gene silencing. SatRNAs small size makes them amenable to manipulation, and their ability to aggregate to high levels in hosts offers potential for increased expression of foreign genes. SatRNAs can be utilized as vectors for functional studies, allowing researchers to investigate the impact of these subviral agents on gene expression and other cellular processes. SatRNA-based vectors can also be employed for gene silencing studies, contributing to the understanding of RNA silencing mechanisms and their effects on host genes. Despite these promising applications, the development of satRNA-based vectors has proven challenging, and further research is needed to overcome the limitations and unlock their full potential in various biotechnological applications.

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CHAPTER 14

PLANT REPETITIVE ELEMENTS

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1. INTRODUCTION

The genome is an entire set of genetic material inside a cell. The genome consisting of DNA is a gold mine of information for life and heredity. The plant genome which has a complex and continually evolving structure, is composed of a great number of elements that are synergistically affect its structural organization and functional capabilities. Plant genomes are organized in the structure of chromosomes that include conserved features like as centromeres, telomeres, and chromatin packaging. Chromosomes provide steady structure to genetic information allowing it to have accurate replication. Also, its transcription and transmission of genetic information is maintained. These features are essential for all the steps that genetic information goes through (Heslop-Harrison and Schwarzacher, 2011). The plant genome comprises repetitive DNA elements covering over 90% and their distribution in genome as shown Figure 1 (Mehrotra and Goyal, 2014). Also, repetitive sequences play a significant role in numerous processes of cell cycle.

1. Motion of chromosomes and pairing
2. Condensation process at centrometric region
3. Recombination of chromosomes
4. Pairing of sister chromatids
5. Association of chromosome with mitotic spindles
6. Chromosomal arrangement
7. Interaction of proteins in chromatin thread
8. Histone binding
9. Stability of chromosomal structure
10. Karyotype evolution
11. Regulation of stimuli-responsive mechanism to environmental and physiological changes

All of these are essential components of evolutionary mechanisms and karyotype differentiation. Accordingly, repetitive DNA elements have an important role in the evolution of plant species. They are all predicted to form genes having regulatory patterns that result in phenotypic variations.

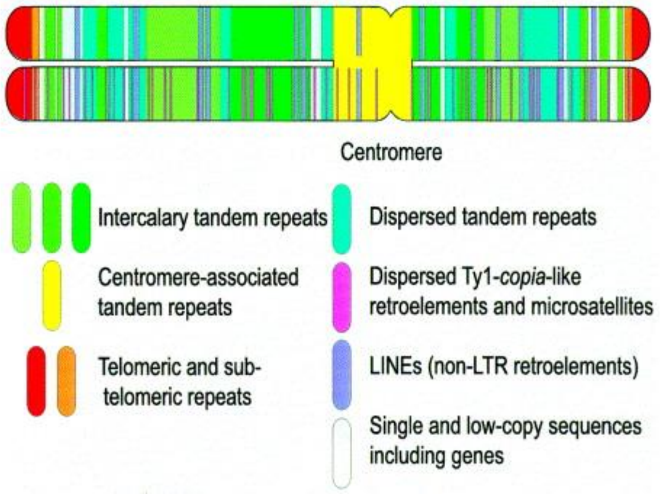

Figure 1: Genomic Distribution of Repetitive Elements (Schmit and Heslop-Harrison, 1995)

Repetitive DNA segments are representing significant portion in plant genomes, like other elements including transposable elements, tandem repeats, and Ribosomal DNA (rDNA) sequences. These mentioned elements have contributions to variations of genome size and play roles in genome evolution and function (Ubis et al., 1998).

Basically, repetitive DNA elements may be divided into encoding and non-coding repetitive elements shown at Figure 2. Ribosomal DNA (rDNA) genes are repetitive DNA elements that are transcribed and tandemly repeated. 5S rDNA and 45S rDNA exhibits a high conservative structure as being members of this gene family. Non-coding repetitive elements are tandem and interspersed repeats. Microsatellites, minisatellites, and satellite DNA repeat tandemly in the genome. Unlike satellites, transposons are interspersed in the genome as DNA and RNA transposons. The mechanism of DNA transposons is copy-paste while RNA transposons have a cut-paste mechanism. This review focused on their genomic structure and features.

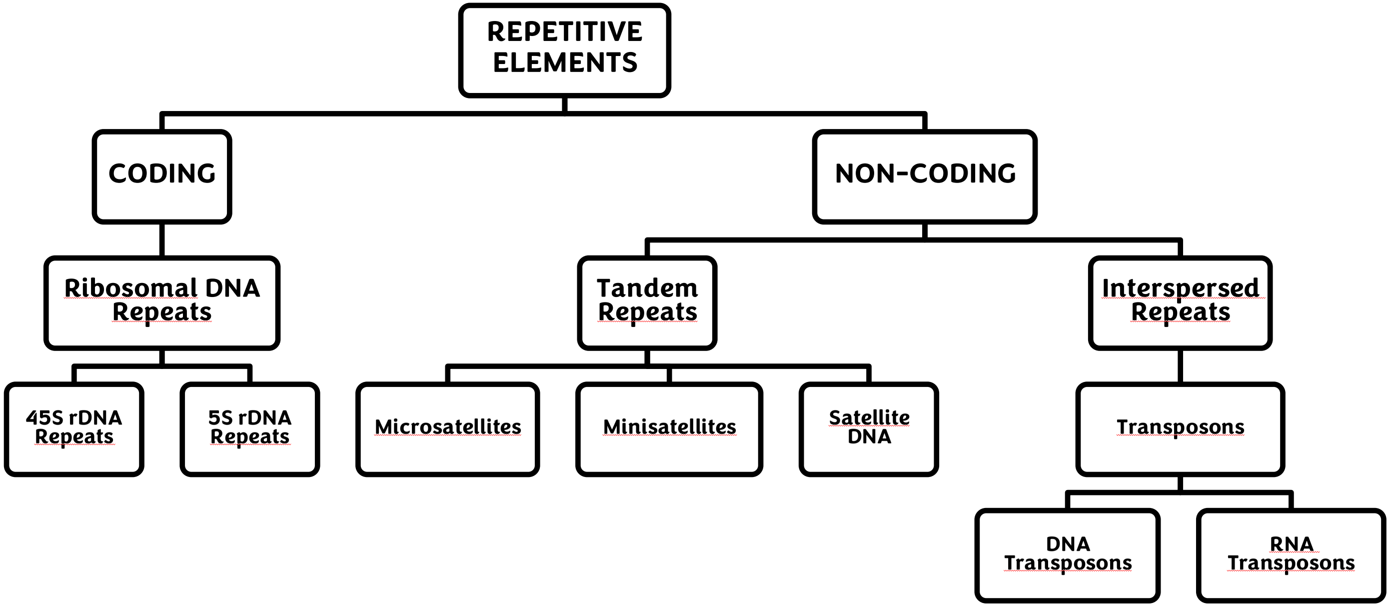

Figure 2: Representing Repetitive Elements as a Concept Map

2. CODING REPETETIVE DNA ELEMENTS RIBOZOMAL GENES

There are unique chromosomal regions named as nucleolar organizer regions (NORs), where ribosomal DNA (rDNA) is placed and plays a very important role in forming of the nucleolus. rDNA sites are generally located at the endings of chromosomes, specifically in the NORs. 45S rDNA in Arabidopsis thaliana is located as tandem arrays at the termination region of 2 chromosomes. In the chromosome 2 it is located in the NOR2 where in the chromosome 4 it takes its places in NOR4. The place of these rDNA sites on the chromosomes has an influence on the transcriptional activity in the genome. In the acrocentric chromosomes the sites that are placed in the short arms are being more actively transcribed (Kutashev et al., 2020).

The rDNA regions consist of subunits of 45S, 5.8S and 26S rDNA with ITS1, ITS2 and IGS intervals. In addition to this, 5S rDNA has non-transcribed sequence (NTS) intervals. In general, there is a uniformity in rDNA units, but with the timeline of evolutionary period, it showed some changes in DNA sequence. The main reason after this evolution is the inequality during the process of crossing over. There are mainly two rDNA families in plants which are 45S rDNA and 5S rDNA (Figure 2). 45S rDNAs are separated with some elements called intergenic spacer (IGS). Additionally, 45s rDNA includes 5.8S, 18S and 25S rDNA subunits. Internal Transcribed Spacer 1 (ITS) is located right between 5.8S and 18S while ITS2 is located between 26S and 5.8S rDNA subunits. Monomers of 5S rDNA are splitted off by Non-Transcribed Spacer (NTS) (Eickbush and Eickbush, 2007; Britton-Davidian et al., 2012).

45S rDNA tandem repeats exhibits a quite conserved repeat element structure. They generally appear on the short arm of the chromosomes as they are condensed clusters of one or two in angiosperms. Nevertheless, they can be placed in distinct locations and in varying numbers. Number and placement of the rDNA units are thought to be the consequence of a mechanism that is beneficial in selection or can have a relation to the construction of chromosomes (Roa and Guerra, 2012). 5S rDNA sites are found in most of the plants which provides a great system to study and understand the evolutionary dynamics at molecular level like as 45S rDNA. With the help of rDNAs the taxonomic relation between the plants can be understood better (Sastri et al., 1992).

2.1. Importance of rDNA

Ribosomal DNA (rDNA) repeats are crucial genomic components that encode ribosomal RNA (rRNA) that are one previous necessary step for protein synthesis. rDNAs are shows high repetitiveness and take a significantly big portion of the genome. In plants, these repetitive elements exhibit considerable variations in their organization, number of copies, and evolutionary dynamics. Understanding the role of rDNAs in stability of genome is crucial, as their instability can result into various changes in genome and impact on cellular functions. Instability of rDNA is linked to maintain whole genome integrity and can cause aging processes (Kobayashi, 2008).

Copy number of rDNA also has an impact on genome stability. A reduced number of rDNA copies can be provoking genomic instability, directing to chromosomal duplications and alterations in gene expressions. This can also affect response mechanism of plant to pathogens. In case of rDNA over expression reduction in the interference of transcription together with DNA repair occurs (Picart-Picolo et al., 2020; Ide et al., 2010). Overall, rDNA repeats show behaviours such as a central region for protect genome integrity and guarantee cellular functions operate properly.

5S and 45S rDNA are generally taken as a reference in the studies regarding plant taxonomy and evolution because of their conserved structure and changing intergenic spacers (IGS) (Volkov et al., 2017). Most plant species show very high levels of intragenomic uniformity in 35S rDNA sequences because of the collective evolution in homogenizing the rDNA units throughout the plant genome. Cycas revoluta species exhibits considerable 35S rDNA repeat diversity, with an average of 12% nucleotide sequence divergence among different copies. (Wang et al., 2015).

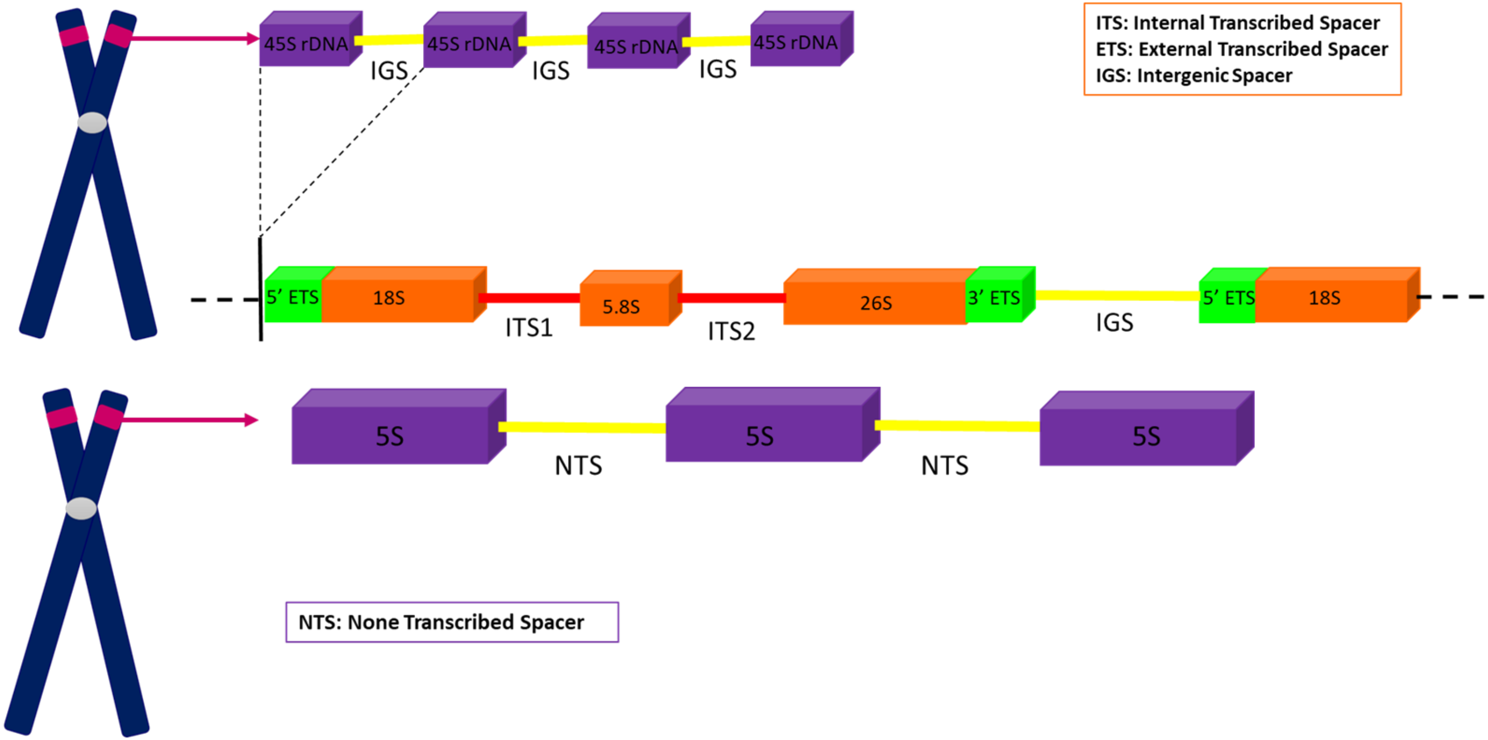

Figure 3: Schematic Representation of Ribosomal Genes

homogenization can create paralogous sequence relationships, misleading phylogenetic inference. Intragenomic variation in ITS regions is common, and multiple copies complicate phylogenetic and DNA barcoding efforts. However, major variants are usually sufficient for species identification. (Alvarez and Wendel, 2003; Song et al., 2012). There may be some limitations in the use of these rDNAs in constructing phylogenetic trees. 18S rDNA sequences are widely used for phylogeny reconstruction at higher taxonomic levels but may provide insufficient resolution due to conservative evolutionary rates. 26S rDNA sequences evolve more rapidly than 18S rDNA and provide more phylogenetically informative characters, making them more useful for resolving relationships at various taxonomic levels. 35S rDNA cistrons are used as cytogenetic markers, but interpreting these markers can be difficult due to the variability of genetic architecture and various molecular changes (Figure 3) (Kuzoff et al., 1998; Soltis et al., 1999; Rossello et al., 2022).

3. NON- CODING REPETETIVE DNA ELEMENTS: TANDEM REPEATS

Tandem repeats are used different techniques and technologies such as karyotype evolution, cytogenetic mapping, comparative genetics and genomic analyzes are used to detect changes (Metrotra and Goyal, 2014; Yu et al., 2017) Therefore, understanding the tandem repeats will guide studies in genome, taxonomy, phylogeny and similar fields. Understand to distribution, genomic organization, chromosomal location and evolutionary origin of Repetitive DNA Sequences to provide elucating of repetitive sequencing's organization, evolution, behavior and functional potential (Jurka et al., 2007; Yu et al., 2013; Metrotra and Goyal, 2014).

Tandem repeats are tandemly repeated elements in genome. They divided into 3 groups and main difference is reapeted count and lenght in genome as represented in Figure 4. Microsatellites are the shortest tandem repeat which is between 2 and 5 bp. Its lenght might be 10 - 100 unit. Minisatellites' units are longer than microsatellites. Each unit is between 6-100 bp and total lenght might be 0.5 - 300 kb. Satellite DNA is the most lenght tandem repeat. One unit is between 150-400 bp and its lenght arrays up to 100 Mb (Metrotra and Goyal, 2014).

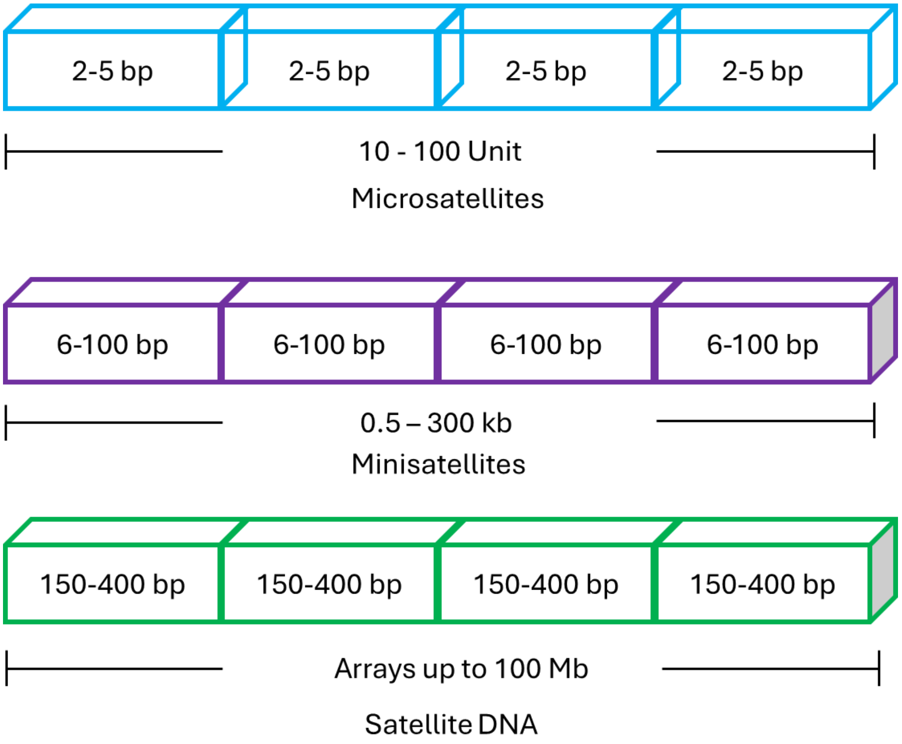

Figure 4: Schematic Representation of Tandem Repeats

4. TRANSPOSONS

Transposable Elements (TEs) are immanent and shows a high divergence in plant genomes. They also have influence on genome structure and its function by causing genomic variation to happen and by organizing gene expression (Flavell et al., 1994).

4.1. Types of Retrotranposons

Retrotransposons are one of the most plethora types of transposons in the genome and reproduce by "copy-paste" mechanism by converting RNA back into DNA. This process results in an increase in genome size. Retrotransposons of plant could be divided into three main groups. These are long terminal repeat (LTR) retrotransposons, non-LTR retrotransposons, Penelope-like elements (PLEs), and Dictyostelium intermediate repeat sequence (DIRS) elements

LTRs are found at either end of retrotransposons or are repetitive DNA sequences.

LTR retrotransposons are divided into three superfamilies based on sequence similarity: Ty1-copia, Ty3-gypsy, and endogenous retroviruses (ERVs). Retrotransposons without LTRs are divided into two main groups: LINE and SINE. Retrotransposons in this group do not have LTR sequences at their ends. In addition, thanks to today’s technology, changes in the genome caused by LTR retrotransposons can now be easily detected. As a result of selecting appropriate regions in the genome, retroviruses and retrotransposons replicate and integrate their DNA into target regions.

As a result, it is evaluated that these targeted integration mechanisms may have very important applications in the field of genetic engineering (Ramakrishnan et al., 2023).

4.2. LTR Retrotransposons

Activated via RNA-mediated transcription, LTR retrotransposons are highly plethora in the plant genome, however less abundant in the animal genome. Their sizes can vary from several hundred base pairs to an unusual 25 kb (Wicker et al., 2007). They generally include open reading frames (ORFs) encoding GAG and POL proteins. They encode the Pol protein, reverse transcriptase (RT), aspartic proteinase (AP), RNase H (RH), and DDE integrase (INT) domains. In some cases, an additional ORF of obscure function may be present.

LTR retrotransposons rapidly increase their copy numbers during the replication process by using the RNA-mediated “copy-paste” mechanism and are therefore considered one of the most common repetitive genetic elements (Orozco-Arias et al., 2019). For instance, approximately 80% of the maize genome occurs of repetitive elements, and a large portion of these elements are known to be LTR retrotransposons. (Sipahi and Yumurtacı, 2020).

4.3. Ty1-copia Group

These retrotransposons are abundant in plant genomes and are named after Ty1 and copia retrotransposons. LTR retrotransposons have terminal repeat sequences that are approximately 100 to 6000 base pairs long. These long terminal repeats act as promoters for the transcription of LTR retrotransposons but do not have any protein coding properties. Ty1-copia retrotransposons consist of protein coding regions located between these LTR regions (Figure 5).

LTR retrotransposons contain the genes gag (Group-specific antigen), pol (Polymerase), and env (Envelope). The gag gene is a protein that arranges the transcription and production process of the retrotransposon and is responsible for the production of proteins specific to the interior of the retrotransposon. The pol gene has an important role in enzyme coding, and these encoded enzymes are very important. Because these enzymes are responsible for the transcription of the retrotransposon from RNA to DNA, DNA synthesis, and the integration of the resulting DNA into the target genome. These enzymes are reverse transcriptase, RNase H and integrase enzymes. The env gene is responsible for synthesizing envelope proteins that facilitate the passage of retrotransposons through the cell membrane.

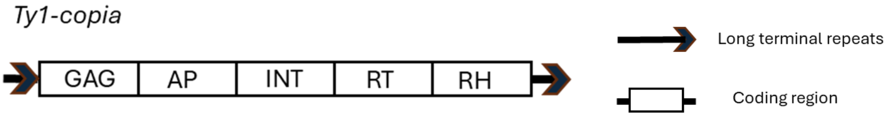

Figure 5: Structure of the Ty1-copia element (Modified from the Wicker et al., 2007).

4.4. Ty3-gypsy Group

These retrotransposons are plenty in genome of plant and are named after Ty1 and Copia retrotransposons. LTR retrotransposons have terminal repeat sequences. These terminal repeat sequences are approximately 100 to 6000 base pairs long. Although these long terminal repeats serve as promoters for transcription of LTR retrotransposons, they do not have protein coding properties. Ty1-Copia retrotransposons contain protein coding regions located between these LTR regions.

These retrotransposons contain the genes gag (Group-specific antigen), pol (Polymerase), and env (Envelope). The gag gene plays an momentous act in the production of proteins specific to the internal structures of the retrotransposon; these proteins regulate the transcription and replication processes of the retrotransposon. The pol gene plays a key role in coding enzymes; these enzymes are responsible for the transcription of the retrotransposon from RNA to DNA, DNA synthesis, and integration of the copied DNA into the target genome. In this process, reverse transcriptase, RNase H and integrase enzymes are involved, respectively. The env gene synthesizes envelope proteins that facilitate the passage of the retrotransposon through the cell membrane.

In general, Ty1-Copia retrotransposons have similar properties to the Ty3-Gypsy group; however, they show structural and functional differences. Gypsy and Copia elements be different in the reverse transcriptase (RT) and integrase (INT) sequences in the pol region. Ty1-Copia retrotransposons are more common in genome of plant. In addition, the LTR lengths of the Ty1-Copia group are shorter and more similar to each other than the LTR lengths of the Ty3-Gypsy group (Figure 6).

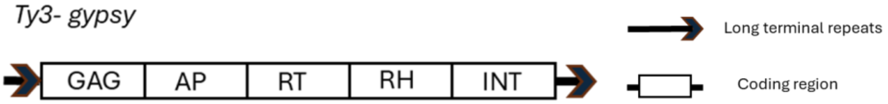

Figure 6: Structure of the Ty3-gypsy element (Modified from the Wicker et al., 2007).

4.5. Retroviruses

There is a close relationship between retroviruses and LTR retrotransposons in evolutionary terms. Although long included in the virus family, retroviruses are also included in the retrotransposon classification. However, they differ from retroelements in some fundamental ways. Like LTR retrotransposons, retroviruses also perform reverse transcription of RNA into cDNA and translation of encoded products in their replication cycle (Ramakrishnan et al., 2023). Retroviruses can also transform into an LTR retrotransposon as a result of events such as deletion or gene loss (Wicker et al., 2007). LTR retrotransposons generally do not have the env gene (except for the Ty3/Gypsy superfamily). The env gene, which acts a serious role in the infection process in retroviruses, encodes the envelope protein, which facilitates the retroviruses to be transported from cell to cell. Since most plant

LTR retrotransposons lack this envelope protein, they do not cause infection like retroviruses. The cycle of retroviruses continues with them infecting a cell and from there spreading to other cells (Wicker et al., 2007).

Figure 7: Structure of the retrovirus element (Modified from the Wicker et al., 2007).

4.6. Non-LTR Retrotransposons

Retrotransposons lacking LTRs function by using a promoter for their transcription. These retrotransposons initiate DNA synthesis from a poly-A tail at the 3' end of the transcript via the RT domain instead of the INT domain and then ligate to the end of newly synthesized DNA (Orozco-Arias et al., 2019). While LTR retrotransposons are widely distributed in plant genomes, LTR-free retrotransposons are more limited. Non-LTR retrotransposons have a poly-A tail at the 3' end of the transcription cycle (Orozco-Arias et al., 2019). This group is divided into two subclasses: LINEs and SINEs.

4.7. Long Interspersed Nuclear Elements (LINE)

LINE retrotransposons can longue several kilobases in length and are found throughout the eukaryotic kingdom (Wicker et al., 2007). Similar to LTR retrotransposons, LINEs, which include the gag and pol genes, play critical roles in both enzymatic and structural functions through these genes. Sequence diversity analyses suggest that LINE elements represent the oldest retrotransposon class of eukaryotes and that LTR retrotransposons are likely derived from these elements (Casacuberta and Santiago, 2003).

LINE elements, which vary in prevalence and diversity among eukaryotes, are more prevalent than LTR retrotransposons in many animals. LINEs, which usually integrate into genomes through retrotransposition, ensure that their RNA is transcribed into DNA and that this DNA is then incorporated into genomic sequences. LINEs are divided into five main superfamilies: Jockey, RTE, R2, I, and L1. These superfamilies are further divided into many families, showing genetic diversity. For example, approximately 20% of the human genome includes of LINE elements belonging to the L1 family, of which there are approximately 105 copies in mammals (Figure 8) (Wicker et al., 2007).

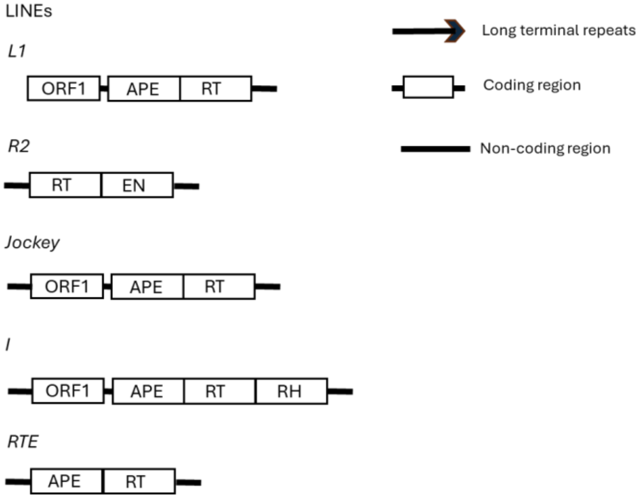

Figure 8: Structure of the LINE elements (Modified from the Wicker et al., 2007).

4.8. Short Interspersed Nuclear Elements (SINE)

SINE elements have lengths ranging from 75 to 662 base pairs (bp). These elements are formed of different rRNA, tRNA and other polymerase III transcripts such as 7SL RNA (Orozco-Arias et al., 2019). The most important property that distinguishes SINEs from other types of retrotransposons is that they do not have coding or self-replicating ability; therefore, they are dependent on the mechanism of LINE retrotransposons (Casacuberta and Santiago, 2003).

SINE elements have a Pol III promoter (Wicker et al., 2007) and are usually terminated with a poly(A) tail or A to T nucleotide-rich sequences (Schmidt, 1999). In addition, SINEs integrate into genomes via retrotransposition, generating target site duplications (TSDs) of 5 to 15 bp in length. This integration process is similar to LINEs and produces short target site duplications as they integrate (Figure 9) (Schmidt, 1999).

The best example of a SINE element is Alu. There are at least 500,000 copies of the Alu element in the genome of human (Wicker et al., 2007).

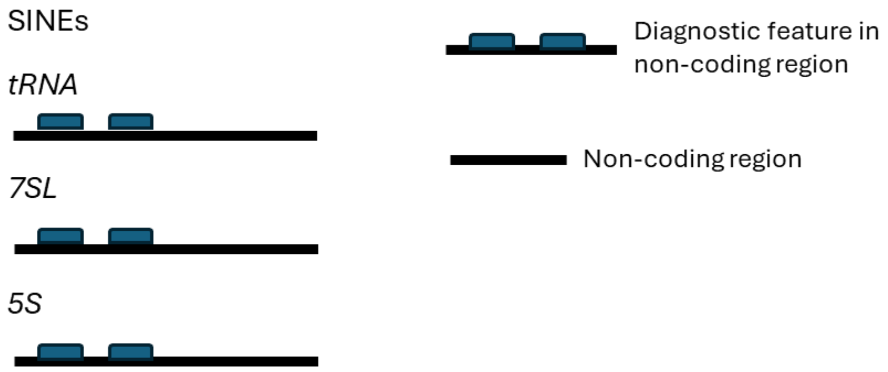

Figure 9: Structure of the SINE elements (Modified from the Wicker et al., 2007).

4.9. Penelope-like Elements (PLE)

PLE elements were first discovered in the species *Drosophila virilis* (Wicker et al., 2007). Although these elements are not found in mammals, they have been defined in more than 50 species containing protozoa, plants and fungi. One of the most striking features of PLEs is that the reverse transcriptase (RT) domain is similar to telomerase (Orozco-Arias et al., 2019).

PLE elements consist of an open reading frame (ORF) region. This region encodes several domains such as endonuclease (EN) and reverse transcriptase (RT). In addition, PLEs encode an endonuclease dependent on the GIY-YIG intron-encoded endonuclease and the bacterial DNA repair protein UvrC protein (Figure 10) (Makałowski et al., 2012).

Some PLE members have LTR-like sequences that may have a functional intron and may be located in the right or reverse orientation (Wicker et al., 2007). Similar to other retrotransposons, PLEs form target site duplications (TSDs); however, the length of these TSDs varies (Orozco-Arias et al., 2019).

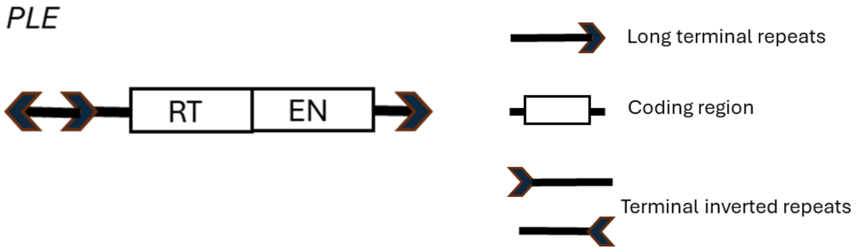

Figure 10: Structure of the PLE element. (Modified from the Wicker et al., 2007)

4.10. Dictyostelium Intermediate Repeat Sequence (DIRS)

DIRS (Dictyostelium internal repeat sequences) were discovered in the genome of the species *Dictyostelium discoideum* in the early 1980s (Makałowski et al., 2012). DIRS elements are found in many organisms, including plants and vertebrates. These elements have a tyrosine recombination (YR) gene in place of an integrase (INT) gene, so they do not produce target site duplication (TSD) (Figure 11) (Orozco-Arias et al., 2019; Wicker et al., 2007).

The terminal structures of DIRS elements are similar to inverted repeats or split direct repeats (SDRs). This feature suggests that DIRS elements have a different integration mechanism than other retroelements.

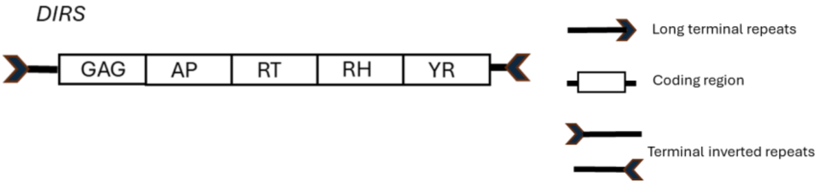

Figure 11: Structure of the DIRS element. (Modified from the Wicker et al., 2007)

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