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Recent Scientific Reserach in the Field of Biology

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CHAPTER 0

ALGAE IN MEDICAL LABORATORY APPLICATIONS

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Introduction

Algae, in addition to being photosynthetic organisms, have recently become an important focus of medical and biomedical research due to their high biochemical diversity and metabolic flexibility (Borowitzka, 2013, p. 743; Pulz & Gross, 2004, p. 635). Microalgae and macroalgae can be utilized in a wide range of medical laboratory applications, particularly because of their rich content of biologically active molecules such as proteins, lipids, polysaccharides, pigments, and phenolic compounds (Lordan et al., 2011, p. 1056; Wijffels & Barbosa, 2010, p. 796). The biochemical diversity offered by these organisms constitutes a significant potential that can be exploited in both diagnostic and therapeutic laboratory studies (Shannon & Abu-Ghannam, 2016, p. 81). Recent

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studies in this field have demonstrated that different algal species possess considerable biological activity potential, with particular emphasis on their antiproliferative and antimicrobial effects, which have been extensively investigated in recent years (Demiriz Yücer, 2024, p. 1026; Pabuçcu & Demiriz Yücer, 2022, p. 927).

The application areas of algae in medical laboratories encompass numerous interdisciplinary fields, including the investigation of antimicrobial and anticancer agents, the development of biosensor systems, biomarker production, and the execution of various biotechnological analyses (De Jesus Raposo et al., 2013, p. 233; Raja et al., 2008, p. 77). In particular, the ability to cultivate microalgae under controlled laboratory conditions and to standardize their production makes them highly suitable for experimental studies (Brennan & Owende, 2010, p. 557; Chisti, 2007, p. 294). Furthermore, the low toxicity profiles and biocompatibility of bioactive compounds derived from algae support their use in pharmaceutical and clinical research (Holdt & Kraan, 2011, p. 543; Plaza et al., 2009, p. 7159).

The importance of algae in medical laboratory applications is not limited to the compounds they contain; studying their effects on biological systems also contributes to a better understanding of disease mechanisms (Kim & Wijesekara, 2010, p. 1). For instance, the immunomodulatory effects of algal polysaccharides enable the modeling of inflammation and infection processes under laboratory conditions (Fitton, 2011, p. 1731). Similarly, algal pigments such as carotenoids and phycobiliproteins are widely used in the investigation of oxidative stress mechanisms (Guedes et al., 2011, p. 625; Pangestuti & Kim, 2011, p. 255).

Another important reason for the preference of algae in medical laboratory applications is their ability to provide sustainable and cost-effective biomass production (Spolaore et al., 2006, p. 87). Compared to traditional biological sources, their rapid growth rates

and the possibility of cultivation under controlled conditions make algae advantageous in both research and application processes (Wijffels & Barbosa, 2010, p. 796). In addition, the ability to enhance the production of specific metabolites through the manipulation of environmental factors presents a significant opportunity for targeted biotechnological studies (Markou & Nerantzis, 2013, p. 1532).

Studies in this area have increased significantly in recent years, progressing in parallel with advancements in analytical techniques (Lauritano et al., 2016, p. 1). The development of high-resolution chromatographic and spectrometric methods has enabled more precise and reliable analysis of algal-derived compounds (Cifuentes, 2009, p. 7109). This has made it possible for algae to be used more effectively in various fields, including pharmaceutical research, toxicity analysis, and preclinical studies (Plaza et al., 2009, p. 7159). Therefore, algae are gaining increasing importance in medical laboratory applications due to their rich biochemical composition, their producibility under laboratory conditions, and their broad spectrum of biological effects.

In this section, the role of algae in medical laboratory applications will be briefly discussed.

Biochemical, Cellular Properties and Production Techniques of Algae

Algae are highly valuable biological systems for medical laboratory studies due to their cellular organization and the diversity of biomolecules they contain (Altuner et al., 2002, p. 1; Borowitzka, 2013, p. 743; Kim & Wijesekara, 2010, p. 1). Microalgae are generally unicellular and exhibit either prokaryotic (cyanobacteria) or eukaryotic organization, whereas macroalgae are multicellular and display relatively more complex structures (Pulz & Gross, 2004, p. 635; Raja et al., 2008, p. 77). This cellular diversity enables algae

to possess broad metabolic capacities and to synthesize a wide range of biological compounds (Wijffels & Barbosa, 2010, p. 796).

Algal cells typically contain fundamental structures such as a cell wall, plasma membrane, cytoplasm, and, in eukaryotic species, organelles including chloroplasts, mitochondria, and a nucleus (Graham et al., 2009, p. 720). The composition of the cell wall varies significantly among species and may consist of polysaccharides such as cellulose, hemicellulose, alginate, agar, and carrageenan (Holdt & Kraan, 2011, p. 543). These polysaccharides not only provide structural support but also exhibit biological activity, playing important roles in medical applications (Lordan et al., 2011, p. 1056).

One of the most remarkable features of algae is their possession of a broad spectrum of pigment systems. Pigments such as chlorophylls, carotenoids, and phycobiliproteins play significant roles in biomedical research not only due to their involvement in photosynthetic processes but also because of their antioxidant properties (Guedes et al., 2011, p. 625; Pangestuti & Kim, 2011, p. 255). In particular, carotenoids (β-carotene, astaxanthin, lutein) are widely used in oxidative stress studies due to their free radical scavenging properties (Plaza et al., 2009, p. 7159). Phycobiliproteins, on the other hand, are utilized in biological labeling and imaging techniques owing to their fluorescent properties (Sekar & Chandramohan, 2007, p. 113).

The biochemical composition of algae is highly rich in proteins, lipids, carbohydrates, and various secondary metabolites (Becker, 2007, p. 207). In microalgae, protein content can reach levels of 40–70%, and these proteins provide a balanced profile of essential amino acids (Spolaore et al., 2006, p. 87). This makes algae a suitable source for both nutritional supplementation and biomedical research (Becker, 2007, p. 207).

In terms of lipid content, algae are particularly important sources of polyunsaturated fatty acids (PUFAs) (Hu et al., 2008, p. 621). Omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) play crucial roles in cellular membrane structure and inflammatory processes and are frequently investigated in medical laboratory studies (Adarme-Vega et al., 2012, p. 96). These fatty acids are also considered important biomolecules in research on cardiovascular and neurodegenerative diseases (Hu et al., 2008, p. 621). Indeed, detailed characterization of fatty acid compositions in certain microalgae and diatom species has revealed their relationship with biological activity (Demiriz Yücer & Pabuçcu, 2023, p. 73).

The carbohydrate fraction of algae mainly consists of sulfated polysaccharides, which exhibit antiviral, anticoagulant, and immunomodulatory properties (De Jesus Raposo et al., 2013, p. 233; Fitton, 2011, p. 1731). In particular, polysaccharides such as fucoidan, laminarin, and ulvan play roles in regulating various biological responses by influencing cellular signaling pathways (Holdt & Kraan, 2011, p. 543). Due to these properties, such compounds are among the key research subjects in functional analyses conducted under laboratory conditions (Lordan et al., 2011, p. 1056).

Algae also produce a variety of secondary metabolites, including alkaloids, terpenoids, phenolic compounds, and sterols (Shannon & Abu-Ghannam, 2016, p. 81). These compounds are generally synthesized as part of defense mechanisms and possess strong biological activities (Raja et al., 2008, p. 77). While phenolic compounds are particularly notable for their antioxidant activities, certain terpenoids exhibit antimicrobial and anticancer effects (Kim & Wijesekara, 2010, p. 1).

Understanding the biochemical structures of algae enables their controlled production under laboratory conditions and the

optimization of target compounds. Therefore, production techniques should be considered in direct relation to their biochemical properties (Wijffels & Barbosa, 2010, p. 796).

One of the reasons algae are preferred in medical laboratory applications is their ability to be produced under highly controlled conditions and the possibility of standardizing their biomass (Brennan & Owende, 2010, p. 557; Chisti, 2007, p. 294). The rapid growth capacity of microalgae and their metabolically flexible nature allow for the targeted production of conditions (Wijffels & Barbosa, 2010, p. 796). For this reason, algal culture and production techniques are of critical importance in obtaining reliable and reproducible results in medical research (Pulz & Gross, 2004, p. 635).

The media used in algal culture studies must be optimized according to the physiological requirements of the species. Among the most commonly used media are BG-11, Bold's Basal Medium (BBM), and f/2 medium, all of which provide balanced contents of nitrogen, phosphorus, trace elements, and vitamins (Guillard, 2005, p. 117; Stanier et al., 1971, p. 171). Since the composition of the medium directly affects algal growth rate and metabolite production, medium optimization is of great importance for medical applications (Markou & Nerantzis, 2013, p. 1532).

Key physical parameters affecting algal growth include light, temperature, pH, and carbon dioxide concentration (Brennan & Owende, 2010, p. 557). Light is the main determinant of photosynthetic activity, and adjustments in light intensity and photoperiod can significantly alter the biochemical composition of algae (Carvalho et al., 2006, p. 1490). High light intensities generally increase carotenoid production, whereas low light conditions may enhance protein synthesis (Guedes et al., 2011, p. 625).

Temperature has a determining effect on enzymatic activities and cellular metabolism, with an optimal range of 20–30°C reported for most microalgal species (Richmond, 2003, p. 1). Maintaining a stable pH is also critical for nutrient uptake and metabolic balance (Carvalho et al., 2006, p. 1490).

Sterility and contamination control in algal cultures are indispensable for medical laboratory applications. Contaminations can negatively affect biomass yield and product purity; therefore, aseptic techniques and regular monitoring processes must be implemented (Anderson, 2005, p. 2).

Laboratory-scale production is generally carried out using batch, fed-batch, and continuous systems (Chisti, 2007, p. 294). These systems offer different advantages in terms of growth dynamics and metabolite production and are selected based on experimental objectives (Richmond, 2003, p. 1).

Both open systems and closed systems (photobioreactors) are used in algal production. Photobioreactors are more commonly preferred in medical applications due to their ability to provide higher control and product purity (Carvalho et al., 2006, p. 1490; Chisti, 2007, p. 294). In these systems, parameters such as light distribution, gas transfer, and mixing are optimized to increase production efficiency (Pulz, 2001, p. 287).

Harvesting of algal biomass and scale-up processes are also critical stages in the production chain. Methods such as centrifugation, filtration, and flocculation are used for harvesting, while maintaining system parameters during scale-up is of great importance(Molina Grima et al., 2003, p. 491; Uduman et al., 2010, p. 1).

The biochemical and cellular properties of algae, together with their production techniques, constitute two complementary components that form the basis of medical laboratory applications.

Evaluating these two aspects together enables the achievement of more reliable and targeted results in algae-based biomedical research.

Extraction and Analysis of Bioactive Compounds from Algae

Algae, due to their rich biochemical composition, are considered important biological resources for the production of bioactive molecules, and the extraction and analysis of these compounds constitute one of the fundamental stages of medical laboratory studies (Cifuentes, 2009, p. 7109; Plaza et al., 2009, p. 7159). Among the compounds obtained from algae are polysaccharides, lipids, pigments, proteins, and various secondary metabolites, and the isolation and characterization of these compounds involve a multi-step and carefully controlled process (Holdt & Kraan, 2011, p. 543; Shannon & Abu-Ghannam, 2016, p. 81). This process encompasses stages ranging from biomass preparation to extraction, purification, and advanced analytical characterization(Herrero et al., 2006, p. 136).

The preparation of algal biomass prior to extraction is a critical step in terms of both yield and the preservation of biological activity (Molina Grima et al., 2003, p. 491). Harvested biomass is typically subjected to lyophilization or drying under controlled temperature conditions, followed by homogenization or grinding (Plaza et al., 2009, p. 7159). Disruption of the cell wall significantly increases extraction efficiency, particularly in species with thick cell walls (Kim & Wijesekara, 2010, p. 1). For this purpose, methods such as ultrasonication, bead milling, and enzymatic disruption are widely employed (Herrero et al., 2006, p. 136).

Extraction methods vary depending on the polarity and chemical structure of the target compound (Cifuentes, 2009, p. 7109). It has been demonstrated in various studies that the biological activity profiles of extracts differ depending on the solvent used,

with methanol- and ethanol-based extracts often showing prominent antimicrobial activity (Pabuçcu & Demiriz Yücer, 2022, p. 927). Aqueous extraction is generally preferred for the isolation of polysaccharides, whereas organic solvents (such as methanol, ethanol, acetate, and hexane) are used for extracting lipids and phenolic compounds (Herrero et al., 2006, p. 136). Among these, ethanol stands out as an advantageous solvent for medical and pharmaceutical applications due to its low toxicity profile (Plaza et al., 2009, p. 7159). Parameters such as temperature, extraction time, and solvent ratio directly influence compound stability and yield (Cifuentes, 2009, p. 7109). As noted, different solvents can yield extracts with distinct biological activity profiles, with methanol and ethanol extracts frequently demonstrating higher antimicrobial efficacy (Pabuçcu & Demiriz Yücer, 2022, p. 927).

In addition to conventional extraction methods, advanced extraction techniques have become increasingly common in recent years. Supercritical CO_2 extraction enables the production of high-purity compounds and is considered suitable for biomedical applications due to the absence of solvent residues (Herrero et al., 2006, p. 136). Furthermore, ultrasound-assisted and microwave-assisted extraction techniques reduce extraction time while increasing yield (Plaza et al., 2009, p. 7159). These methods offer significant advantages, particularly in preserving thermally sensitive compounds (Cifuentes, 2009, p. 7109).

Following extraction, purification of crude extracts is necessary to isolate target compounds specifically (Holdt & Kraan, 2011, p. 543). Techniques such as liquid–liquid extraction, solid-phase extraction, and column chromatography are commonly used at this stage (Plaza et al., 2009, p. 7159). High-performance liquid chromatography (HPLC) is one of the most important methods for separating and quantitatively analyzing compounds in complex mixtures (Cifuentes, 2009, p. 7109). HPLC systems provide high

sensitivity and reproducibility, making them a standard tool in medical laboratory analyses (Herrero et al., 2006, p. 136).

Gas chromatography (GC) and GC-MS systems are widely used for the analysis of volatile compounds and fatty acid derivatives (Hu et al., 2008, p. 621). These techniques provide high accuracy, particularly in determining fatty acid profiles (Herrero et al., 2006, p. 136). In contrast, LC-MS systems are preferred for analyzing larger and more polar molecules and offer powerful capabilities for structural characterization (Cifuentes, 2009, p. 7109).

Spectroscopic analysis methods also play a significant role in the characterization of algal-derived compounds. UV-Vis spectrophotometry is commonly used for the quantitative analysis of pigments, while FTIR and NMR techniques enable detailed investigation of molecular structures (Guedes et al., 2011, p. 625; Kim & Wijesekara, 2010, p. 1). These techniques provide complementary information for identifying functional groups and confirming molecular structures (Cifuentes, 2009, p. 7109).

The analysis of bioactive compounds is not limited to chemical characterization but also includes biological activity assays (Shannon & Abu-Ghannam, 2016, p. 81). Antioxidant capacity is typically evaluated using methods such as DPPH, ABTS, and FRAP, which determine the free radical scavenging potential of the compounds (De Jesus Raposo et al., 2013, p. 233). The evaluation of antimicrobial and cytotoxic effects requires more advanced biological analyses, generally supported by cell culture and microbiological testing (Kim & Wijesekara, 2010, p. 1).

Quality control and standardization processes are of great importance in the analysis of algal-derived compounds. The composition of extracts obtained from the same species may vary depending on environmental conditions and production parameters (Markou & Nerantzis, 2013, p. 1532). Therefore, standardized

protocols and validation processes must be applied to ensure reproducibility of analyses (Cifuentes, 2009, p. 7109). The accuracy, sensitivity, and selectivity of analytical methods are critical for the scientific validity of the obtained results (Plaza et al., 2009, p. 7159). Accordingly, the extraction and analysis of bioactive compounds from algae represent a complex, multidisciplinary process. The proper selection of extraction techniques, effective application of purification methods, and use of advanced analytical tools directly influence the quality of the resulting data. Optimization of these processes is therefore essential for the effective and reliable utilization of algae.

Antimicrobial, Anticancer and Diagnostic Applications of Algae

Algae are regarded as important biological resources in terms of antimicrobial and anticancer effects due to their rich content of secondary metabolites, and the analysis of these effects under laboratory conditions constitutes one of the fundamental components of medical research (Kim & Wijesekara, 2010, p. 1; Plaza et al., 2009, p. 7159). Investigating the effects of compounds derived from algae on bacteria, fungi, and cancer cells contributes to the development of novel therapeutic agents (Raja et al., 2008, p. 77; Shannon & Abu-Ghannam, 2016, p. 81). In addition, these biological activities are known to offer significant potential not only for therapeutic purposes but also for the development of diagnostic systems (D'Orazio, 2011, p. 1749).

One of the most commonly used methods for evaluating the antimicrobial activity of algae is the disk diffusion test. In this method, disks impregnated with algal extracts are placed on agar surfaces inoculated with microorganisms, and antimicrobial activity is assessed by measuring the resulting inhibition zones (Bauer et al., 1966, p. 493; Cox et al., 2010, p. 205). The disk diffusion method is frequently preferred in preliminary screening studies due to its

simplicity and rapid application; however, it is often supported by advanced analyses because of its quantitative limitations (Cox et al., 2010, p. 205). Notably, extracts of Spirogyra prepared using different solvents have been reported to exhibit significant inhibitory effects against bacterial and fungal strains (Pabuçcu & Demiriz Yücer, 2022, p. 927).

For more precise evaluations, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests are employed(Wiegand et al., 2008, p. 163). These tests, based on microdilution methods, allow for the quantitative determination of the effects of algal extracts on microorganisms(Balouiri et al., 2016, p. 71). Differences in the effects of algal compounds on Gram-positive and Gram-negative bacteria are associated with structural differences in their cell walls (Kim & Wijesekara, 2010, p. 1).

When the mechanisms underlying antimicrobial activity are examined, it has been shown that algal-derived compounds disrupt cell membrane integrity, inhibit protein synthesis, and increase oxidative stress levels (Raja et al., 2008, p. 77). In particular, phenolic compounds and fatty acids alter microbial membrane permeability, leading to cell death (Shannon & Abu-Ghannam, 2016, p. 81). Moreover, algal extracts have been reported to inhibit biofilm formation, which may provide a significant advantage in controlling resistant infections (Cox et al., 2010, p. 205).

In evaluating the anticancer potential of algae, in vitro analyses based on cell culture constitute the primary approach (Plaza et al., 2009, p. 7159). These analyses are typically conducted on different cancer cell lines, examining parameters such as cell proliferation, apoptosis, and cell cycle progression (Kim & Wijesekara, 2010, p. 1). The MTT assay, commonly used to determine cell viability, provides quantitative information on cytotoxic effects by measuring cellular metabolic activity

(Mosmann, 1983, p. 55). Similarly, in vitro studies have demonstrated that extracts of Pseudopediastrum boryanum exhibit antiproliferative effects and suppress cell growth in various cancer cell lines (Demiriz Yücer, 2024, p. 1026).

Lactate dehydrogenase (LDH) release assays are used to evaluate cell membrane damage, indirectly indicating the loss of cellular integrity (Chan et al., 2013, p. 979). In apoptosis analyses, techniques such as Annexin V/PI staining and flow cytometry are employed to distinguish between early and late apoptotic cells (Vermes et al., 1995, p. 39). It has been reported that algal-derived carotenoids and polysaccharides can induce programmed cell death in cancer cells by activating apoptotic signaling pathways (Pangestuti & Kim, 2011, p. 255).

At the molecular level, analyses reveal the effects of algal compounds on cellular signaling pathways. Techniques such as RT-PCR, Western blot, and ELISA are used to evaluate changes at the gene and protein levels (Lauritano et al., 2016, p. 1). In particular, signaling pathways such as PI3K/Akt and MAPK have been shown to be modulated by algal-derived compounds (Kim & Wijesekara, 2010, p. 1). These analyses are of great importance for identifying potential therapeutic targets (Pangestuti & Kim, 2011, p. 255).

Dose–response relationships also represent an important parameter in evaluating the efficacy of algal extracts. Determination of IC_{50} values allows for comparative assessment of extract potency and provides quantitative insight into biological activity (Plaza et al., 2009, p. 7159). In addition, the selectivity index can be calculated to compare the effects on cancer cells versus normal cells (De Jesus Raposo et al., 2013, p. 233).

Oxidative stress parameters also play a significant role in evaluating the biological effects of algae. The production of reactive oxygen species (ROS) is measured using fluorescent probes,

allowing the determination of antioxidant or pro-oxidant effects of algal compounds (Chan et al., 2013, p. 979). This provides important insights, particularly for cancer and inflammation research (Shannon & Abu-Ghannam, 2016, p. 81). In this context, studies on Vaucheria species have demonstrated that strong antioxidant and antimicrobial activities are associated with their phenolic compound content (Uç & Pabuçcu, 2024, p. 12, 2026, p. 1)

Algae are not only important for therapeutic purposes but also show considerable potential in diagnostic systems. Algae-based biosensors are systems in which algal cells or algal-derived molecules are used as biological recognition elements (D'Souza, 2001, p. 337). These systems operate through the integration of a biological sensing component with a physical transducer, enabling the detection of target analytes (A. P. F. Turner et al., 1987, p. 1).

Biosensors based on photosynthetic activity represent one of the most common applications of algae. Changes in Photosystem II activity provide rapid responses to toxic substances, making these systems particularly useful in environmental and clinical analyses (Campbell et al., 1998, p. 667). Chlorophyll fluorescence measurements allow these changes to be detected with high sensitivity (Maxwell & Johnson, 2000, p. 659).

In electrochemical biosensors, algal cells or algal-derived enzymes are immobilized on electrode surfaces (D'Souza, 2001, p. 337). The presence of target compounds is determined by measuring electron transfer processes occurring in these systems (S. Singh et al., 2005, p. 73). These sensors have been reported to exhibit high sensitivity, particularly in the analysis of heavy metals and toxins (D'Orazio, 2011, p. 1749).

Phycobiliproteins derived from algae are widely used in immunoassays and imaging techniques due to their high fluorescence properties (Sekar & Chandramohan, 2007, p. 113).

These molecules provide significant advantages in biomarker analysis by offering high sensitivity and specificity in diagnostic tests (Glazer, 1994, p. 105).

Genetically modified algal systems also play an important role in biosensor technologies. In these systems, algal cells are engineered to respond specifically to certain analytes, thereby increasing sensor specificity (D'Souza, 2001, p. 337). Furthermore, algae-based sensors integrated with nanotechnology provide enhanced sensitivity and stability (S. Singh et al., 2005, p. 73). Thus, algae stand out not only as a biological resource in antimicrobial and anticancer research but also in the development of diagnostic systems, with applications ranging from microbiological tests to molecular analyses and biosensor technologies.

Safety, Toxicity and Integration of Algae into Clinical Applications

The expanding use of algae in medical research necessitates not only the evaluation of their biological activities but also a comprehensive assessment of the safety and toxicity profiles of compounds derived from these organisms (Gupta et al., 2016, p. 345; Plaza et al., 2009, p. 7159). In order for algal-derived compounds to be translated into clinical applications, it is essential to determine safe dosage ranges, identify potential toxic effects, and ensure compliance with quality standards (Holdt & Kraan, 2011, p. 543). Therefore, toxicity analyses and standardization processes constitute integral components of algae-based biomedical research (De Jesus Raposo et al., 2013, p. 233). Various studies have demonstrated that the safety profiles of algal species may vary depending on both the species itself and the extraction method used (Uç & Pabuçcu, 2024, p. 12).

In vitro cell culture-based approaches are widely used in the toxicity evaluation of algal compounds. These methods offer

significant advantages in preliminary screening studies due to their ability to produce rapid results and their controllable experimental conditions (Eisenbrand et al., 2002, p. 193). Assays based on metabolic activity, such as MTT and resazurin, are frequently used to assess cell viability and proliferation, providing quantitative data on changes in cellular metabolism(Mosmann, 1983, p. 55; Riss et al., 2016, p. 1). Additionally, lactate dehydrogenase (LDH) release assays serve as important indicators for evaluating cell membrane integrity (Chan et al., 2013, p. 65).

The assessment of oxidative stress parameters also plays a crucial role in toxicity analysis. The production of reactive oxygen species (ROS) and their effects on antioxidant defense systems contribute to understanding the cellular-level impacts of algal compounds ((Halliwell & Gutteridge, 2015, p. 1) & Gutteridge, 2015). It has been reported that certain algal compounds may exhibit antioxidant effects at low doses, while demonstrating pro-oxidant effects at higher concentrations, potentially leading to cellular damage (Kim & Wijesekara, 2010, p. 1).

Genotoxicity analyses are critical for evaluating the potential effects of algal-derived compounds on DNA. Methods such as the Comet assay and the micronucleus test enable quantitative assessment of DNA damage (Fenech, 2000, p. 81; N. P. Singh et al., 1988, p. 184). These analyses are particularly important for investigating genetic effects that may arise during long-term exposure (Eisenbrand et al., 2002, p. 193).

In vivo toxicity studies are conducted in more complex biological systems and allow for the evaluation of the systemic effects of algal compounds (OECD, 2008, p. 1). These studies include acute, subchronic, and chronic toxicity tests and contribute to the determination of pharmacokinetic and pharmacodynamic parameters (Parasuraman, 2011, p. 74). In vivo analyses provide

critical data for the transition to clinical applications (Gupta et al., 2016, p. 345).

Another important aspect to consider in the safety evaluation of algae is the potential of certain species to produce toxins. In particular, compounds such as microcystins and anatoxins produced by cyanobacteria can pose serious health risks (Carmichael, 1992, p. 445). Therefore, careful selection of algal species intended for medical use and the implementation of toxin analyses are essential (Gupta et al., 2016, p. 345). These analyses are typically performed using advanced techniques such as HPLC, LC-MS, and ELISA (Plaza et al., 2009, p. 7159).

Standardization processes play a critical role in ensuring the reliability of algal-derived products. The composition of products obtained from the same algal species may vary significantly depending on culture conditions, harvesting time, and extraction methods (Markou & Nerantzis, 2013, p. 1532). Therefore, standardization of production and analysis processes enhances both the comparability and scientific validity of the obtained data (Cifuentes, 2009, p. 7109).

Within quality control frameworks, the chemical composition of algal biomass, microbial contamination status, and heavy metal content must be thoroughly analyzed (Holdt & Kraan, 2011, p. 543). The presence of environmental contaminants, in particular, can directly affect the safe use of algal products (Gupta et al., 2016, p. 345). For this reason, quality control analyses are indispensable for medical applications (De Jesus Raposo et al., 2013, p. 233).

The integration of algae into clinical applications requires a multidisciplinary approach. The collaboration of fields such as biotechnology, pharmacology, and molecular biology contributes to enhancing the clinical effectiveness of algal-derived compounds

(Lauritano et al., 2016, p. 1). However, parameters such as bioavailability and stability must be optimized during the transition to clinical use (Shannon & Abu-Ghannam, 2016, p. 81).

Nanotechnology represents one of the key approaches used to improve clinical performance of algal compounds. Nanoparticle-based delivery systems enhance therapeutic efficacy by enabling targeted drug delivery and controlled release (Patra et al., 2018, p. 1). Algal-derived biopolymers are used as biocompatible if these systems (Kim & Wijesekara, 2010, p. 1).

Another critical stage in the integration of algae into clinical applications is the regulatory process. For algal-based products to be recognized as pharmaceutical products, quality, safety, and efficacy criteria must be met in accordance with international standards (EMA, 2013, p. 1; FDA, 2016, p. 1). This process involves the establishment of production standards and the validation of clinical data (Gupta et al., 2016, p. 345).

From a future perspective, the role of algae in biomedical applications is expected to increase significantly. Advances in genetic engineering and synthetic biology enable the high-yield production of specific bioactive compounds (Wijffels & Barbosa, 2010, p. 796). These developments will facilitate the more effective use of algae in biopharmaceutical production (Chisti, 2007, p. 294).

Furthermore, within the scope of personalized medicine, algal-derived compounds are anticipated to be used in individualized treatment strategies (Lauritano et al., 2016, p. 1). In addition, algal products used as functional foods and nutraceuticals play an important role in preventive healthcare applications (Spolaore et al., 2006, p. 87). The scientific validation of these products is made possible through medical laboratory analyses (Becker, 2007, p. 207).

In this context, the rigorous application of toxicity analyses, quality control mechanisms, and regulatory requirements ensures the

safe and effective use of algae-based products. Therefore, the integration of algae into clinical applications should be considered not only as a scientific process but also as a technological and regulatory one.

Conclusion

Algae stand out as important biological resources due to their extensive biochemical diversity, advantages in production, and versatile biological activities (Borowitzka, 2013, p. 743; Plaza et al., 2009, p. 7159). The proteins, lipids, polysaccharides, pigments, and secondary metabolites contained in these organisms provide a rich molecular repertoire that can be utilized in both analytical and experimental biomedical studies (Holdt & Kraan, 2011, p. 543; Kim & Wijesekara, 2010, p. 1). These characteristics demonstrate that algae occupy a valuable position not only in basic research but also in translational and applied sciences (Lauritano et al., 2016, p. 1). Recent studies have shown that different algal species exhibit promising results in terms of both antimicrobial and antiproliferative potential (Demiriz Yücer, 2024, p. 1026; Uç & Pabuçcu, 2026, p. 1).

The use of algae in medical laboratory applications spans a broad spectrum, ranging from culture techniques and the extraction of bioactive compounds to the analysis of antimicrobial and anticancer activities and the development of biosensor systems (De Jesus Raposo et al., 2013, p. 233; Shannon & Abu-Ghannam, 2016, p. 81). The diversity of methodologies employed in these processes, along with the integration of advanced analytical techniques, further enhances the importance of algae in biomedical research (Cifuentes, 2009, p. 7109). In particular, the high sensitivity provided by chromatographic and spectrometric methods enables detailed characterization of algal-derived compounds (Plaza et al., 2009, p. 7159).

The evaluation of the biological effects of algae under laboratory conditions provides significant opportunities for the development of new therapeutic agents. The identification of algal compounds with antimicrobial and anticancer potential contributes to the development of alternative or complementary (Kim & Wijesekara, 2010, p. 1; Pangestuti & Kim, 2011, p. 255). However, in addition to their efficacy, the safety and toxicity profiles of these compounds must also be thoroughly investigated (Gupta et al., 2016, p. 345).

The effectiveness of algal applications largely depends on the control of production and standardization processes. Optimization of culture conditions, enhancement of biomass production, and standardization of the obtained compounds are critical for ensuring the reproducibility of research outcomes (Markou & Nerantzis, 2013, p. 1532; Wijffels & Barbosa, 2010, p. 796). Furthermore, rigorous implementation of quality control processes increases the reliability of algae-based products (Holdt & Kraan, 2011, p. 543).

The integration of algae into clinical applications requires a multidisciplinary approach, involving fields such as biotechnology, pharmacology, nanotechnology, and molecular biology (Patra et al., 2018, p. 1). In particular, the development of nanotechnological delivery systems and the application of genetic engineering approaches hold significant potential for enhancing the clinical effectiveness of algal-derived compounds (Lauritano et al., 2016, p. 1). Nevertheless, critical stages such as regulation, standardization, and large-scale clinical studies must be completed for successful clinical translation (EMA, 2013, p. 1).

From a future perspective, the role of algae in medical laboratory applications is expected to expand further. Algae are anticipated to be used more effectively, especially in personalized medicine applications, biosensor technologies, and biomarker development (De Jesus Raposo et al., 2013, p. 233). These

advancements will enable algae to be considered not only as research tools but also as integral components of clinical applications (Plaza et al., 2009, p. 7159).

In conclusion, algae possess strategic importance in medical laboratory applications due to their sustainable biomass production, rich biochemical composition, and broad spectrum of biological effects. To fully realize their potential, it is necessary to employ advanced analytical methods, optimize production processes, and expand research focused on clinical applications. Scientific studies conducted in this direction will contribute to the broader utilization of algae in the biomedical field in the future.

Kaynakça

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CHAPTER 0

INTERACTIONS OF BRASSINOSTEROIDS WITH GROWTH REGULATORS IN THE REGULATION OF PLANT SALT TOLERANCE

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Introduction

Owing to the increasing world population, food problems are likely to arise by 2050. Abiotic stress significantly affects agricultural production. Abiotic stress poses a serious threat to agricultural productivity. Reduced precipitation due to climate change leads to increased soil water loss through evaporation, which inhibits salt leaching and accelerates soil salinization. In summary, soil salinity is progressively increasing due to rising temperatures, reduced rainfall, irrigation with saline water, and improper agricultural practices (Tarolli et al., 2024: 1). Salinity has become a critical global issue for agricultural land. Therefore, enhancing agricultural productivity and plant tolerance to abiotic stress through various interventions is essential to address the impending food

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crisis. Plant adaptation, or stress tolerance, involves complex, tightly regulated metabolic networks. Although ABA is the most studied hormone in plants under stress, the roles of cytokinins, brassinosteroids, and auxins in the environmental stress process are also very important.

Brassinosteroids (BRs) are steroidal hormones that play a crucial role in initiating stress responses and regulating plant resistance to support growth under adverse conditions. BR-mediated stress responses enhance plant tolerance by modulating various biochemical pathways. This includes the activation or suppression of enzyme-catalyzed reactions within signaling pathways, stimulation of protein synthesis, and production of bioactive compounds involved in the plant defense system (Miao et al., 2024: 4).

BRs also interact synergistically with other phytohormones to regulate plant growth and development. The signaling pathways of BRs and other phytohormones involve multiple components, and their coordinated action enhances plant tolerance and promotes development under salt stress (Özdemir et al., 2004: 204; Ali et al., 2007: 217). Therefore, investigating the molecular mechanisms under BR-induced abiotic stress tolerance and identifying the key components involved are of significant importance. As these components are characterized, the mechanisms that reveal the existing link between brassinosteroids and other hormones will become clearer.

Salt Stress

Sodium chloride, the primary contributor to soil salinization, is the most common and highly soluble natural salt found in nature. Salinity typically occurs in arid and semi-arid regions and shares many characteristics with soils found in these climates. In addition to drought, salinity reduces soil water potential, prompting plants to develop adaptive mechanisms to tolerate these conditions. Both

glycophytes and halophytes exhibit tolerance to osmotic stress. Salt stress adversely affects plant growth and development by inducing osmotic and ionic stresses (Parida & Das, 2005: 334). Although roots are the first plant organs to encounter salinity, leaf growth is generally more affected than root growth (Munns & Tester, 2008: 655). In response to moderate salinity, many plants synthesize compatible solutes-organic compounds that help maintain a cellular water potential lower than that of the surrounding soil solution. This mechanism prevents the accumulation of toxic salt levels in plant tissues. Salt may also enter and exit the root zone with seasonal precipitation, leading to a phenomenon known as "temporary salinity" (Rengasamy, 2002: 353). This form of salinity has not been extensively studied in agricultural and engineering applications. Genetic improvement of crops is a preferred strategy to mitigate the effects of salinity and enhance productivity.

Brassinosteroids

Importance in Metabolism

Although phytohormones are synthesized in minute quantities, they play critical roles in mediating plant responses to abiotic stressors. These molecules act as key messengers, integrating complex transduction pathways that govern the perception of stress signals and initiate adaptive responses (Vob et al., 2014: 313; Wani et al., 2016: 164). Exogenous application (foliar spraying or seed treatment) of plant growth regulators (PGRs), has been reported to enhance plant tolerance to a range of environmental stresses, including drought, salinity, and extreme temperatures (Awan et al., 2017: 17; Banerjee & Roychoudhury, 2018: 1029; Verma et al., 2016: 2). Plant-based extracts containing natural growth regulators provide a sustainable and eco-compatible alternative to synthetic chemicals, consistent with the objective of minimizing environmental harm (Ashraf et al., 2016: 2). Among these,

brassinosteroids (BRs) are particularly noteworthy for their dual function: they act as growth-promoting hormones and confer protection against a broad range of abiotic stresses (Fig. 1) (Ashraf et al., 2016: 4; Ahammed et al., 2020a, b: 1452, 129; Hussain et al., 2020: 142).

Research on brassinosteroids has spanned several decades, culminating in the identification of brassinolide, the most active form, and related compounds. This discovery led to the formal recognition of brassinosteroids as the sixth major class of plant hormones, highlighting their importance in plant physiology and in stress resilience (Fig. 1).

Figure 1. *Importance of Brassinosteroids in Plants.*
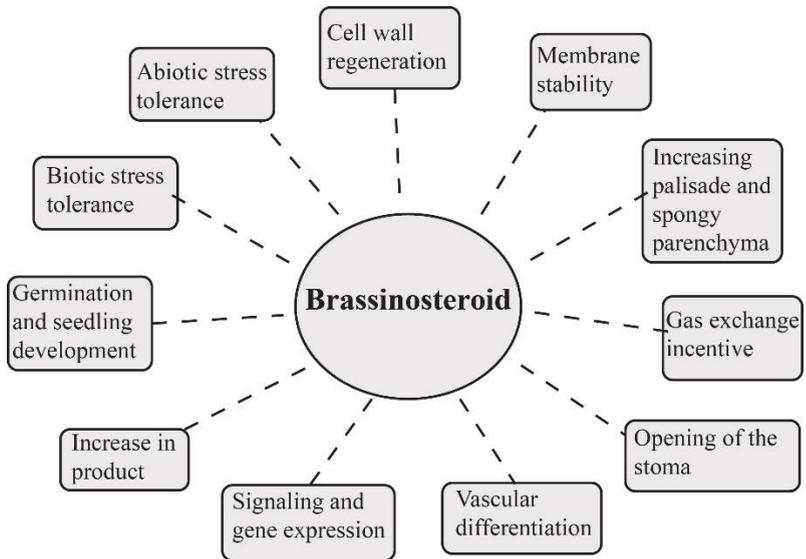
28-homobrassinolide (hBL) and 24-epibrassinolide (eBL) are two BR isomers that regulate plant metabolism (Hayat et al., 2011: 397; Siddiqui et al., 2018: 208). 24-epibrassinolide has been identified as the active form of BR (Ikekawa et al., 1988: 407). BR isomers play important role in enhancing tolerance in plants, helping

them cope with abiotic stress by acting as nutrients. They are crucial for providing plants with tolerance to abiotic stress and improving their productivity. Given that plants are often exposed to stress throughout their life cycle, it is essential to fully defined the stress-related mechanisms to develop species with better tolerance. In this review, I discuss the effects of BR applications on molecular and biochemical events under stress conditions. BR binds to the membrane-localized receptor Brassinosteroid Insensitive1 (BRI1) and forms dimers with its co-receptor BRI1-Associated Receptor Kinase1 (BAK1), leading to mutual phosphorylation and the initiation of signal transduction. When BR does not bind to the receptor (BRI1), BRI1 binds to an inhibitor (BKI1) instead. This inhibition activates the BIN2 kinase. This activation leads to the phosphorylation and inactivation of transcription factors. In the absence of a BR, the response formation is suppressed. The binding of BR to the receptor ensures that BAK1 and BRI1 receptors phosphorylate each other and that BSK (brassinosteroid signaling kinase) is phosphorylated. BSKs activate BSU1 (BRI1-Suppressor1) by phosphory. This chain of interactions ensures the inactivation of Brassinosteroid Insensitive2 (BIN2). In the absence of BIN2, the target transcription factors become dephosphorylated and active. Thus, response formation occurs through gene expression (Zhu et al., 2013: 1616; Belkhadir & Jaillais, 2015: 529). Most of the genes known to be regulated by brassinosteroids are related to growth events, such as cytoskeleton formation, cell wall modification, and hormone synthesis (Vert et al., 2005: 192). However, little is known about how brassinosteroids regulate gene expression. In stressed plants, eBL plays a crucial role in coordinating the expression network of ion transport-related genes, making it a promising option for developing salinity-tolerant genotypes. The application of 0.052 or 0.104 μM BR to seedlings grown in 120 mM NaCl medium resulted in increased wheat yield (Ali et al., 2008: 107). However, applying epibrassinolide to the growing medium is challenging

because it requires optimization of both the eBL concentration and application timing. Using epibrassinolide at incorrect concentrations may harm plants because of the responses it triggers (Ashraf et al., 2010: 109). Determining the optimal concentrations of brassinosteroids for soil or growing media can also be costly. Additionally, applying epibrassinolide to the environment may involve some uncertainty, particularly when spraying bean plants grown in a 150 mM NaCl medium with 5 μM eBL 15 d after planting. This treatment helps detoxify stress effects and degradation caused by microorganisms while increasing antioxidant enzyme activity and proline levels (Rady, 2011: 235). Furthermore, it reduces electrolyte leakage and lipid peroxidation, thereby enhancing membrane stability and relative water content (RWC). Shahid et al. (2011: 505) also grew bean plants in NaCl and applied eBL to seeds and seedlings both before and after NaCl exposure, which reduced the harmful effects of salinity. Liaqat et al. (2020: 1548) determined that the combination of salinity stress and potassium deficiency is particularly detrimental to plants. The application of 24-eBL to seedlings under stress conditions alleviated the negative effects of combined stress and promoted seedling growth. The beneficial effect of 24-eBL is attributed to increased antioxidant enzyme activity and enhanced osmolyte activity, such as proline and sugar levels, which support the oxidative system. Under saline conditions, the application of brassinosteroids before planting many seeds has a healing effect by increasing germination and seedling growth, shoots, and biomass (Ashraf et al., 2010: 172). Before germination, the protective effect of brassinosteroid (BR) application to the seed plays a significant role in preventing a decrease in cytokinin levels and reducing stress-induced abscisic acid (ABA) levels (Avalbaev et al., 2010: 101). The application of 24-epibrassinolide (24-eBL) to *Eucalyptus camaldulensis* seeds positively influenced germination and seedling growth under salt stress conditions. In a 150 mM NaCl medium, 24-eBL reduced seed

germination inhibition, resulting in an increased germination rate. However, the uptake of 24-eBL from the roots of seedlings grown hydroponically under salt conditions was more impaired (Sasse et al., 1995). In rice (*Oryza sativa*), brassinosteroids counteract salinity-induced inhibition of both seed germination and seedling growth. BRs also promote chlorophyll levels and nitrate reductase activity under saline conditions. This enzyme plays a crucial role in plant growth and productivity, particularly in the nitrogen supply in cereals. For the effective use of brassinosteroids in local agricultural and environmental settings, an appropriate BR solution concentration and optimal application timing must be determined in advance. The roles of hormones in cellular responses to adverse environmental conditions have been investigated, revealing that they contribute to salinity stress tolerance. In this review, we will examine the important role of brassinosteroids, along with auxin, cytokinin, gibberellin, abscisic acid, ethylene, strigolactones, and polyamines, in salt tolerance.

## Significance of Brassinosteroids' Interaction with Other Hormones in Stress Conditions

To cope with various environmental stresses, plants have developed adaptive strategies, including redox changes, hormonal changes, and epigenetic control of stress-related genes. The role of brassinosteroids (BR) in plant responses to abiotic stress has been demonstrated in recent years. However, little is known about the specific contribution of BR to stress tolerance, its interactions with other stress hormones, and their signaling pathways. Although there is considerable evidence that brassinosteroids increase ethylene and jasmonic acid levels under favorable growth conditions (Yi et al., 1999: 444; Kitanaga et al., 2006: 2411), few studies have linked the increase in abscisic acid (ABA) levels under stress conditions to brassinosteroids (Bajguz, 2009: 885). Recent studies have shown that ABA inhibits the brassinosteroid signal transduction pathway by

phosphorylating BES1 (Zhang et al., 2009: 4544). Further research is needed at the genetic level to understand how brassinosteroids interact with other hormones under stress. Some, but not all, genes responsive to jasmonic acid (JA)/ethylene, salicylic acid (SA), and abscisic acid (ABA) are regulated by brassinosteroids in both wild-type and mutant plants. These results indicate hormonal interactions. Under salt stress conditions, the signaling pathways of plant hormones and calcium ions (Ca^{2+}) are activated in coordination to regulate osmotic regulation or homeostasis, which regulates plant growth and development. 24-epibrassinolide abolished salinity stress-induced inhibition of seed germination in *B. napus* (Kagale et al., 2007: 359). In mutants deficient in BR and defective in BR signaling, germination was inhibited under salt stress. In summary:

1. The increase in BR-mediated tolerance to stress occurs due to the interaction with other hormones.

2. ABA inhibits the effects of brassinosteroids on abiotic stress responses.

3. BR overcomes salt stress-induced germination inhibition.

4. A few genes that respond to certain hormones also respond to brassinosteroids. Generally, BR mediates stress responses via the salicylic acid, ethylene, and ABA signaling pathways.

The MAP (mitogen-activated protein) kinase cascade plays a crucial role in various abiotic stress and hormone responses, including ROS signaling in plants (Fujita et al., 2006: 438). Molecular studies have suggested connections between brassinosteroids and other hormones (Fig. 2). Brassinosteroids influence changes in the expression of genes involved in hormone biosynthesis and in signal transduction pathway molecules.

Figure 2. *Plant stress signaling networks.*
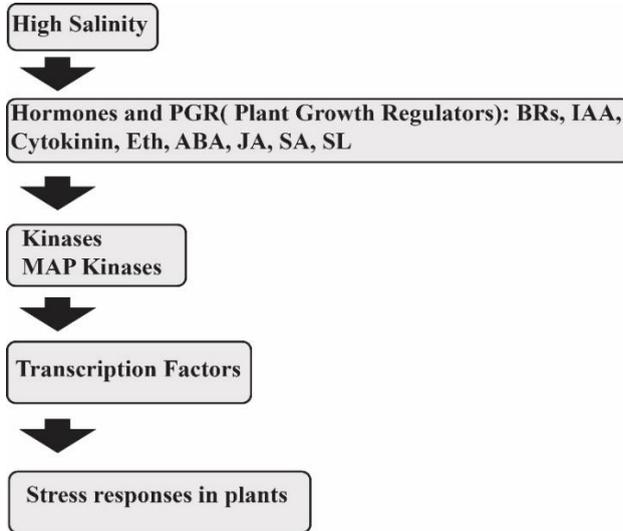
Brassinosteroids and Auxin

Auxin reacts to salinity stress in plants. The auxin signaling pathway is closely related to the early seedling development processes in plants. A decrease of approximately 75% in IAA levels was observed in tomato seedlings grown under salinity stress (Dunlap & Binzel, 1996: 383). Under these conditions, auxin accumulation is altered, and plant growth is reduced, which can be the result of altered auxin accumulation and redistribution. Exogenous auxin application may be a highly preferred approach to counteract stress conditions in agriculture. The MAP (mitogen-activated protein) kinase cascade plays a crucial role in various abiotic stress and hormone responses, including reactive oxygen species (ROS) signaling in plants (Fujita et al., 2006: 438). Molecular studies have suggested a connection between brassinosteroids and other hormones. Brassinosteroids influence changes in the expression of genes involved in hormone biosynthesis

and signal transduction pathway molecules. Although endogenous and exogenous BRs have been shown to regulate ROS in recent years, detailed molecular research continues on the mechanisms by which they induce or control the stress response.

The brassinosteroid signaling pathway is known to be linked to other signaling pathways, such as those of auxin, abscisic acid, and phytochrome, in response to environmental stimuli (Wang et al., 2014: 148). Furthermore, the synergistic relationship between BL and IAA in cell metabolism is now well understood. Recent studies have shown that several auxin signal transduction pathways and response genes are regulated by brassinosteroids (Nakamura et al., 2003: 1845; Vert et al., 2008: 9832). To reveal the physiological interaction between BRs and auxin, the connections between hypocotyl elongation and root development have been examined (Hardtke, 2007: 1121; Teale et al., 2008: 230). Previous studies have shown that brassinosteroids (BRs) act synergistically with auxin to promote cell elongation in plants. In contrast, the data obtained show that basipetal auxin transport is inhibited by brassinosteroids [as in 1-N-Naphthylphthalamic acid (NPA)], whereas lateral auxin transport is increased. It has been found that brassinosteroids interact with auxin and enhance ethylene production in *Arabidopsis thaliana* (Richard and Jeannette 2008: 3024). Yi et al. (1999: 450) reported that BR and auxin differentially regulate the expression of three members of the 1-aminocyclopropane-1-carboxylate synthase (ACS) family in *Vigna radiata* L.

Brassinosteroids and Cytokinins

One of the most important questions when studying plant mechanisms is how phytohormone pathways interact. Studies have been conducted on the mechanisms by which brassinosteroids and cytokinins affect ethylene biosynthesis. Ethylene biosynthesis is regulated in response to the levels of various phytohormones and a

wide range of endogenous and exogenous signals. Cytokinins stabilize ACC synthase, which catalyzes ethylene biosynthesis. Similar to cytokinin, brassinosteroids also increase the stability of ACS5, a component of ethylene biosynthesis that exerts a post-transcriptional effect. Auxin administration increases ACS5 protein stability of cytokinin while causing an increase in ACS transcript levels (Liang et al., 1992: 11048; Yamagami et al., 2003: 49107; Wang et al., 2004: 947). Bera et al. (2013: 182) applied 4 µM brassinosteroid (BR) to a nutrient solution containing 150 or 300 mM NaCl and found that BR alleviated the negative effects of salt stress on soluble protein, nucleic acid, proline levels, and peroxidase activity in plants. The most obvious protective effect of BR application to plants under stress is a decrease in cytokinin levels. This protective effect is antagonistic to that of ABA (Avalbaev et al., 2010: 101). When brassinosteroid was added to the growth medium of barley seedlings grown in a 0.35 to 0.4 M NaCl solution, a significant decrease in the mitotic index and the number of chromosomal abnormalities was observed compared to untreated seedlings (Tabur & Demir, 2009: 120).

Brassinosteroids interact with ethylene under environmental stress conditions (Wang et al., 2014: 150; Li et al., 2016: 1024). Studies have shown that brassinosteroids can increase the activity of 1-aminocyclopropane-1-carboxylic acid synthase (ACS), which stimulates the accumulation of ethylene (Hansen et al., 2009: 610). However, it remains unclear whether brassinosteroids and ethylene collectively contribute to plant resistance under stress conditions and what their relationship is. Tomatoes, one of the most widely cultivated crops globally, were studied, and it was found that BR application increased ACS activity, thereby promoting ethylene accumulation in tomato plants (Zhu et al., 2016: 2). As a result of this increased activity, tomato seedlings resistant to salt stress were developed in this study. These researchers conducted virus-induced

gene silencing (VIGS) experiments and demonstrated that brassinosteroid signaling molecules and ethylene signaling molecules can interact under salt stress conditions. In recent years, the relationship between brassinosteroids and ethylene signaling has been extensively studied to mitigate stress-induced damage.

Brassinosteroids and Abscisic Acid

ABA is one of the key hormones that has a healing effect on the growth of seedlings that are unable to grow due to salinity stress. The increase in ABA levels under salinity contributes to membrane integrity and regulates ion uptake and transport in plants (Parida & Das, 2005: 332). When salt stress and ABA-promoted proline synthesis were examined, a strong correlation was found with a significant activation of the P5CS1 expression. In contrast, the induction of P5CS2 was reduced and inhibited to varying degrees by ABA and salinity in *Arabidopsis thaliana*.

In a study conducted on wheat (*Triticum aestivum* L.), it was determined that under salt stress, brassinosteroids did not affect ABA content but only increased lectin accumulation in the roots of wheat seedlings. This indicates that the lectin content is regulated by brassinosteroids independently of ABA. A classic cereal seed lectin (agglutinin: WGA) was elevated in plants under salt stress. The rapidly increasing accumulation of ABA and WGA in the salt medium caused a 5-fold increase in lectin levels. Brassinosteroids have been shown to have a protective effect by reducing salinity-related BR and WGA accumulation in the roots by 50% (Shakirova & Bezrukova, 1998: 1021; Shakirova & Bezrukova, 2002: 227). Together with ABA, the level of WGA is thought to be under the control of brassinosteroids. In all physiological and genetic studies, BRs and ABA have been shown to work together in plant development. One of the protective effects of the external application of brassinosteroids is that it reduces the level of ABA,

which increases rapidly under stress (Avalbaev et al., 2010: 101). BRs antagonize the effect of ABA, which causes seed dormancy and inhibits seed germination, possibly by accelerating embryo growth and seed germination. Studies have also shown that mutants of potential components of the ABA signaling pathway, such as gpa1, agb1, and gcr1, are more sensitive to ABA during seed germination. These mutants also exhibit varying susceptibilities to brassinosteroids (BRs). BRs and ABA together regulate the expression of hundreds of genes (Nemhauser et al., 2006: 473). Exogenously applied ABA has been found to have a similar effect as the BR receptor mutant bri1 (Zhang et al. 2009: 4547). However, the ABA-insensitive mutants abi2 and abi1 eliminated the inhibitory effect of ABA on BR signaling outputs. These researchers identified the role of the negative regulator BIN2 after BR bind to the receptor. This study suggested that BR-responsive genes are also regulated by ABA (Zhang et al., 2009: 4547). Although significant progress has been made in studies on hormonal interactions, most of these studies remain at the physiological level of analysis. In a study on the guard cells of *Vicia faba*, it was found that brassinosteroids, in combination with ABA, regulate stomatal movements by inducing stomatal closure and inhibiting stomatal opening (Haubrick et al., 2006: 135; Banerjee & Roychoudhury, 2018: 1027). New studies on plants exposed to salt stress will provide further insights into the molecular and biochemical relationships between brassinosteroids (BRs) and other hormones. In recent years, studies aimed at understanding the mechanisms of abiotic stress tolerance in cultivated plants have made progress. However, the gene activation sequence responsible for tolerance to abiotic stress has yet to be determined. Plants are sometimes subjected to multiple biotic and abiotic stresses simultaneously, which makes coping challenging for them to cope. Understanding the response mechanisms of tolerant plants to abiotic stresses will help improve agricultural yields in the near future.

Brassinosteroids and Strigolactones (SL)

There is an interaction between BRs and SLs (Wang et al., 2013: 681). A transcription factor (BES1), which plays an important role in the BR response pathway, is a proteolytic target of SL/SCFMAX2 signaling pathway. Studies have shown that SL biosynthesis gene expression increases under salt stress, and SL can act as a positive regulator (Ha et al., 2014: 854; Min et al., 2019: 100). Aroca et al. (2013: 52) found that an increase in SL production in a salt-stressed lettuce plant accelerated fungal growth and helped the plant cope with stress. Responses to drought and salinity in *Arabidopsis* were observed to be mediated by SL. The links between SL and brassinosteroids (BR) are not yet clear. Foo et al. (2014: 1041) found that SLs and BRs together had a positive effect on the nodule in a study they conducted on peas. Little data are available on strigolactone and brassinosteroid signaling pathways. In genetic and molecular mechanism studies, it has been determined that the strigolactone and brassinosteroid signaling pathways antagonistically regulate rice branching (Wang et al., 2013: 683; Faizan et al., 2020: 224). The findings from research on this new class of phytohormones will be further hormonally influenced by SL and BR. There are very few studies on the effects of SLs on plant growth, and the information remains insufficient.

Brassinosteroids and Jasmonic acid

Jasmonates are important components of defence response signals (mediators of defence response signals) during the senescence process. They also play a crucial role in plant salt tolerance. The jasmonate levels in the tissues of seedlings treated with salt were examined, and a higher jasmonate level was found in salt-tolerant tomato cultivars than in salt-sensitive tomato cultivars (Hilda et al., 2003: 153). Similarly, Kang et al. (2005: 273) cultivated rice plants in a 20–40 mM NaCl medium and applied jasmonic acid

(JA). JA resulted in a decrease in Na^+ uptake, an increase in the photosynthetic rate, and a more negative leaf water potential.

Brassinosteroids and Polyamines

The importance of using natural polyamines to protect plants from salinity stress has been highlighted by several researchers (Ashraf & Harris, 2004: 7; Kuznetsov et al., 2006: 595; Ndayiragije & Lutts, 2007: 228-231). Brassinosteroids not only regulate the growth of plants exposed to stress but also interact with auxin, gibberellin, cytokinin, ethylene, ABA, salicylic acid (SA), and jasmonic acid to regulate growth and development. The relationship between polyamines and brassinosteroids has been studied, and it was found that epibrassinolide treatment alleviated copper toxicity in *Raphanus sativus* (Choudhary et al., 2012: 5661), playing an important role in regulating polyamine (PA) functions. When brassinosteroid analogs were applied to lettuce plants grown under salt stress, PA levels were maintained, helping the plants recover from stressful conditions (Serna et al., 2015: 110).

Brassinosteroids and Salicylic Acid

The interaction between BR and SA is likely mediated by shared signaling components involved in cellular signal transduction pathways associated with gene regulation. Previous studies have suggested that reactive oxygen species (ROS) may act as common mediators in the signaling processes of both BR and SA (Xia et al., 2009: 803). The effects of SA and BR conjugates on lipid peroxidation (LPO) have been investigated, demonstrating that various types of stress can induce LPO in plants (Apel & Hirt, 2004: 389). BR treatment reduces oxidative damage in seedlings and enhances protective antioxidant activity. Notably, the conjugates of 24-eBL and SA were highly effective in mitigating oxidative damage in plants exposed to salt stress.

Physiological and Protective Effects of Brassinosteroids on Plants Under Stress Conditions

Brassinosteroids enhance tolerance to stress conditions, promote seedling growth, and accelerate seed germination. However, determining the optimal concentration of eBL, the appropriate timing of application, and whether to add eBL to the growth medium is challenging because of the high cost. Incorrect dosing can lead to plant damage owing to adverse responses (Ashraf et al., 2010: 175). It remains unclear whether brassinosteroids are degraded by microorganisms when applied to soil, which is why the spraying method is generally recommended for their application. When seedlings exposed to a saline environment were treated with brassinosteroids, seeds of barley, wheat, rice, pepper, and beans germinated, and the treatment had a beneficial effect on seedling growth. Additionally, it was found that seed yield increased as both the shoot and root biomass of the crops grew (Ashraf et al., 2010: 173-174). When brassinosteroids (BR) are applied before sowing, they may be responsible for their enhanced protective effect, as they prevent a decrease in cytokinin levels. It also contributes to the healing effect by reducing the level of the stress hormone ABA (Avalbaev et al., 2010: 101). The BR solution, which is sprayed according to the appropriate concentration and correct timing rule, makes a great contribution to the growth of the plant, due to this feature, it is a very suitable hormone for agricultural applications. Stress damages cell membranes. BR application increases CO^2 uptake, promotes stomatal opening and hydrolytic enzyme activity and reduces lipid peroxidation levels (Farooq et al., 2009: 266). Thus, under stress conditions, seedlings continue to grow (Shu et al., 2016: 347). By enhancing the expression of antioxidant genes, brassinosteroids (BR) increase the activities of antioxidant enzymes (APX, SOD, POD, CAT, and GR). BR strengthens plant defense systems and improves tolerance to stress. Brassinosteroids offer a

potential solution to the damage caused by Na^+ ions in plants when applied exogenously (Khripach et al., 2000: 444). The application of BR to plants grown in stressful environments induces positive changes at the cellular, biochemical, morphological, and physiological levels. These changes are linked to alterations in antioxidant metabolism, gas exchange, cell division, and growth rate (Yuan et al., 2012: 208-210). Ekinci et al. (2012: 634) demonstrated that salt stress triggers K^+/Na^+ imbalances, and the application of 24-eBL to these plants helps increase K^+ content in both roots and leaves. Other studies have also highlighted the positive interaction between eBL and salt stress. For example, oxidative damage was reduced in *Triticum aestivum* treated with BR (Talaat & Shawky, 2013: 737), and photosynthetic activity improved as a result of enhanced gas exchange in *Cicer arietinum* (Wani et al., 2017: 50). Kolomeichuk et al. (2020: 156-157) investigated the eBL-mediated reduction of photosynthetic damage caused by NaCl toxicity in their study on potato plants. In this study, the salt medium inhibited photosynthetic pigment content, photosynthetic electron transfer, the efficiency of photosystem II (PSII), and the oxidation of plastoquinone (PQ). The application of 24-eBL under salt stress helped preserve the decrease in PSII photochemistry efficiency, PQ pool oxidation, and leaf osmotic potential. Similarly, in another study, 24-eBL provided tolerance to NaCl (100 mM) stress in soybean seedlings (Alam et al., 2019: 1). In these seedlings exposed to salt stress, growth, biomass yield, chlorophyll fluorescence, and photosynthetic pigment content decreased, while MDA, H2O2, CAT, SOD, proline accumulation, glycine betaine, total antioxidant activities, total flavonoids, and phenols increased. Oliveira et al. (2019: 563) revealed in a study that eBL reduced the harmful effects of salt stress in young *Eucalyptus urophylla* plants. The study emphasized that the improvement occurred through the homeostasis of the K^+/Na^+ ratio and nutrient content in the tissues. The benefits of this steroid on gas exchange and growth were also related to leaf

anatomy. Examination of the leaf anatomy showed increased stomatal density, palisade parenchyma, and spongy parenchyma. Molecular changes induced by BR under stress conditions indicated that it contributed to increased stress tolerance in treated plants compared to untreated plants, as stress-sensitive genes were expressed at higher levels (Kagale et al., 2007: 358). The molecular changes that occurred can be listed as follows: protein synthesis continued (Dhaubhadel et al., 2002: 683), photosynthetic efficiency increased (Krishna, 2003: 292; Koh et al., 2007: 462), and other hormone responses and antioxidant enzyme activities were enhanced (Vert et al., 2005: 186), while osmoprotectant accumulation occurred (Özdemir et al., 2004: 208; Divi & Krishna, 2009: 123). In conclusion, it was determined that 24-eBL plays an important regulatory role in response to salinity toxicity through epigenetic modification and induction of methylation.

Practices Influencing BR-Mediated Abiotic Stress Tolerance

Arabidopsis gene expression studies, both with and without BR, have shown that BR affects tolerance both directly and indirectly, through interactions with other hormones, enhancing tolerance under both stress and non-stress conditions. While exogenous BR application has not yet been incorporated into greenhouse or field agricultural practices, it presents a promising alternative for application. After obtaining more detailed information about brassinosteroids, the optimal application time should be determined, the correct concentration should be selected, and sufficient care should be taken during application. It should be applied with consideration of environmental and health risks, and by individuals with adequate technical expertise. Research on this topic also offers an alternative approach for enhancing crop growth and increasing stress tolerance by modulating endogenous BR activity through transgenic technology.

In adverse environmental conditions, plants organize their cells and tissues to grow in a coordinated manner. The expression of genes, supported by molecules in signal transduction pathways once conditions are detected, explains this pattern. Gene and cellular events are interrelated. This pattern also applies to the salinity tolerance mechanism.

Conclusion

The aim of this review is to provide a basic biological understanding and information flow to support the development of alternative solutions to future climate challenges. Supporting research on salinity tolerance, mitigating the negative effects of salinity stress on plants, and enhancing plant responses to salinity are crucial for ensuring future nutrient supply. To ensure agricultural and environmental sustainability for human health, it is necessary to improve the performance of plant species. Although significant information is presented in this study, many questions remain on the topic. The role of BR signaling in cellular processes such as cytoskeletal organization and the maintenance of cell wall integrity is not yet fully understood. There is still considerable uncertainty regarding endogenous brassinosteroid levels in plant tissues and organs under abiotic stress conditions, the interaction of brassinosteroid with other signaling pathways during growth and development, and whether brassinosteroids play a significant role in cell differentiation and morphogenesis. To address these uncertainties, future research will need more information on the plant's defense pathways, BR-mediated signal transduction pathways, and the growth processes under various biotic and abiotic stress conditions.

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UPDATED BRYOPHYTE CHECK-LIST FOR B9 SQUARE OF TÜRKİYE
CHAPTER 0

MEVLÜT ALATAŞ[1]

SERHAT URSAVAŞ[2]

Introduction

Studies on bryophytes (mosses, liverworts, and hornworts) in Turkey were initiated by foreign researchers (Tchihatcheff, 1860: 1; Juratzka & Milde, 1870: 589; Wettstein, 1889: 389; Barbey, 1890: 82; Schiffner, 1896: 274, 1897: 125; Fritsch, 1900: 219; Schiffner, 1903: 1, 1908; Penther & Zederbauer, 1905: 385; Bornmüller, 1908: 1, 1909: 440; Czeczott, 1939: 1; Henderson & Muirhead, 1955: 29; Henderson, 1957: 189, 1958: 611, 1961a: 263, 1961b: 279, 1964: 279; Jovet-Ast, 1957: 667; Walther, 1967: 129, 1970:167; Henderson & Prentice, 1969: 235). Since the 1980s, however, research on bryophytes has been predominantly conducted by local researchers and continues to this day.

In these studies, the Henderson grid system, which is accepted by Turkish bryologists, is utilized spatially. The Henderson grid system, which is the basis for mosses, liverworts, and hornworts on the map of Turkey, consists of 15 squares (Henderson, 1961a: 263). According to this system, the B9 square is located in the eastern part of Turkey (38°-40° N; 38°-42° E) (Figure 1). This square encompasses the entirety of the Tunceli, Bingöl, Elazığ, and Erzincan provinces; the eastern parts of the Sivas and Malatya provinces; the southwest of the Erzurum province; the west of Muş

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and Bitlis; the north of Diyarbakır and Adıyaman; and the northwest of the Siirt province.
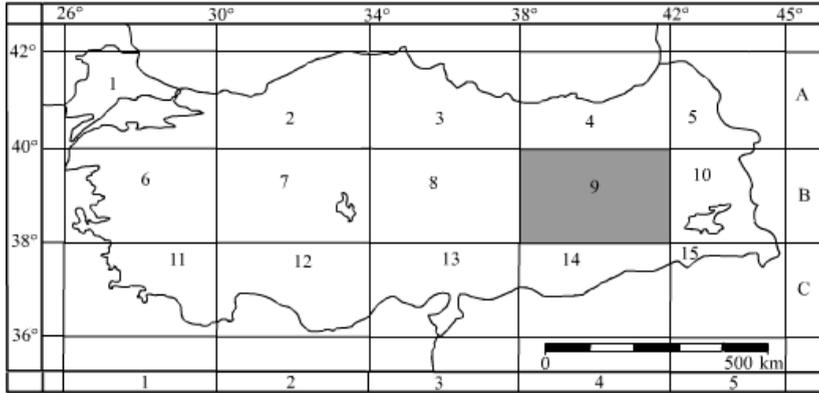
Figure 1. Henderson's Türkiye Grid System (Henderson, 1961a: 263)

Alongside general bryophyte checklists prepared for the entire country [(Çetin, 1988a: 12-1988b: 15), (Uyar & Çetin, 2004: 203), (Kürschner & Erdağ, 2005: 95, 2023: 1), (Özenoğlu Kiremit & Keçeli, 2009: 343), (Erdağ & Kürschner, 2017: 1, 2024: 20), (Yücel et al., (2025: 131)], bryophyte checklists containing only specific squares have also been prepared in recent years. In this context, Özdemir first prepared a list containing bryophyte taxa distributed in the A4 square (2000: 60, 2009: 1). Subsequently, Ursavaş and Abay (2009: 33) for the A2 square, Ursavaş et al. (2009: 604) for the A1 square, Abay et al. (2009: 613) for the A3 square, and Şahin et al. (2009: 620) for the A5 square published checklists regarding the class Bryopsida. With these publications, the moss lists for the squares covering the northern parts of Turkey (A1-A2-A3-A4-A5) have been completed.

The first study containing the bryophytes of the B9 square was conducted by Alataş and Ursavaş in 2021 (Alataş & Ursavaş, 2021: 53). In this study, conducted five years after the initial list, an expanded and updated bryophyte list containing the mosses and liverworts of Turkey's B9 square is presented.

Material and Method

This checklist was prepared by examining bryofloristic and bryosociological research conducted within the B9 square according to the Henderson (1961a: 263) Turkey grid system. The studies conducted on bryophytes in the B9 square to date are, in chronological order: Henderson, 1958: 611; Robinson & Godfrey, 1960: 244; Henderson, 1961a: 263, 1963. 279; Henderson & Prentice, 1969: 235; Erdağ et al., 2003: 349; Papp, 2007: 71; Kürschner & Erdağ, 2009: 441; Erdağ & Kürschner, 2009a: 183, 2009b: 54; Alataş et al., 2014: 148; Alataş & Batan, 2015: 59, 2016a: 102, 2016b: 47, 2017: 31; Alataş & Ursavaş, 2019: 81; Alataş et al., 2019: 23; Alataş et al., 2020: 55; Ellis et al., 2022: 87; Alataş et al., 2023: 24; and Alataş et al., 2024: 1. In addition to those identified in these studies, Özenoğlu Kiremit and Keçeli (2009: 343) and Yücel et al. (2025: 131) were utilized for the identification of other liverwort taxa falling within the B9 square.

While preparing this checklist, control lists covering Turkish mosses and liverworts such as Çetin (1988a: 12-1988b: 15), Uyar & Çetin (2004: 203), Kürschner and Erdağ (2005: 95, 2023: 1), Özenoğlu Kiremit & Keçeli (2009: 343), Erdağ & Kürschner (2017: 1, 2024: 20) and Yücel et al. (2025: 131) were examined to investigate whether the listed taxa were present in our country's bryophyte list. During the preparation of the plant list, Hodgetts et al. (2020: 1) was taken into account regarding valid names, synonymy status, and the arrangement of taxa according to systematic categories.

Results

In the bryofloristic list formed based on research conducted to date regarding bryophytes within the B9 square according to the Henderson (1961a: 263) grid system, there are 154 taxa belonging to 18 families and 61 genera for mosses, and 6 taxa belonging to 4 families and 5 genera for liverworts, totaling 160 species and infraspecific taxa belonging to 22 families and 66 genera.

These 160, taxa represent more than one-tenth of the approximately 1280 bryophyte taxa (5 hornworts, 224 liverworts and 1051 mosses) identified in studies conducted throughout Turkey (Çatak et al., 2025: 1). In the B9 square, where fewer studies have been conducted compared to other squares, it is probable that this number will increase further with new studies to be carried out in unstudied areas.

In the B9 square bryofloristic list, the richest families in terms of the number of genera they contain are: Pottiaceae (15), Brachytheciaceae (10), and Amblystegiaceae (7), whereas the richest families in terms of the number of taxa they contain are Pottiaceae (48), Brachytheciaceae (21), Bryaceae (18), Grimmiaceae (17), Orthotrichaceae (15), and Amblystegiaceae (10). The richest genera in terms of taxa diversity are *Ptychostomum* (13), *Grimmia* and *Syntrichia* (12), *Orthotrichum* and *Didymodon* (10), and *Tortula* (9).

This updated checklist, resulting from the examination of bryofloristic and bryosociological research conducted within the B9 square according to the Henderson (1961a: 263) Turkey grid system, will determine the current status and reveal the opportunity for comparison with similar studies conducted or to be conducted in other squares of Türkiye. If such studies are realized for all squares, they will constitute an important resource for studies regarding the chorology of mosses, liverworts, and hornworts in Türkiye.

Bryophyte Check-List For B9 Square

Marchantiophyta Stotler & Crand.-Stotl.
Jungermanniopsida Stotler & Crand. Stotl.
Metzgeriales Chalaud.
Aneuraceae H.Klinggr.
1 *Riccardia* Gray.
    1 *multifida* (L.) Gray.
Pelliales He-Nygrén.
Pelliaceae H.Klinggr.
2 *Apopellia* (Grolle) Nebel & D.Quandt
    1 *endiviifolia* (Dicks.) Nebel & D.Quandt

3 *Pellia* Raddi.
    1 *epiphylla* (L.) Corda.
Marchantiopsida Cronquist, Takht. & W.Zimm.
Lunulariales H.Klinggr.
Lunulariaceae H.Klinggr.
4 *Lunularia* Adans.
    1 *cruciata* (L.) Dumort. ex Lindb.
Marchantiales Limpr.
Marchantiaceae Lindl.
5 *Marchantia* L.
    1 *polymorpha* subsp. *polymorpha* L.
    2 *polymorpha* subsp. *montivagans* Bischl. &   Boissel.- Dub.

Bryophyta Schimp.
Bryopsida Pax.
Encalyptales Dixon.
Encalyptaceae Schimp.
6 *Encalypta* Hedw.
    1 *alpina* Sm.
    2 *ciliata* Hedw.
    3 *streptocarpa* Hedw.
    4 *vulgaris* Hedw.
Funariales M.Fleisch.
Funariaceae Schwägr.
7 *Entosthodon* Schwägr.
    1 *muhlenbergii* (Turner) Fife
    2 *pulchellus* (H.Philib.) Brugués
8 *Funaria* Schwägr.
    1 *hygrometrica* Hedw.
9 *Physcomitrium* (Brid.) Brid.
    1 *pyriforme* (Hedw.) Bruch & Schimp.
Dicranales H.Philib. ex M.Fleisch.
Dicranellaceae M.Stech
10 *Dicranella* (Müll. Hal.) Schimp.
    1 *subulata* (Hedw.) Schimp.
    2 *varia* (Hedw.) Schimp.
Fissidentaceae Schimp.

11 *Fissidens* Hedw.
     1 *arnoldii* R.Ruthe [1]
Pottiaceae Schimp.
12 *Anoectangium* Schwägr.
     1 *handelii* Schiffn.
13 *Barbula* Hedw.
     1 *unguiculata* Hedw.
14 *Bryoerythrophyllum* P.C.Chen.
     1 *recurvirostrum* (Hedw.) P.C.Chen.
15 *Cinclidotus* P.Beauv.
     1 *aquaticus* (Hedw.) Bruch & Schimp.
     2 *riparius* (Host ex Brid.) Arn.
     3 *vardaranus* Erdağ & Kürschner [2]
16 *Crossidium* Jur.
     1 *squamiferum*
      var. *squamiferum* (Viv.) Jur.
     2 var. *pottioideum* (De Not.) Mönk.
17 *Didymodon* Hedw.
     1 *acutus* (Brid.) K.Saito.
     2 *australasiae* (Hook. & Grev.) R.H.Zander
     3 *fallax* (Hedw.) R.H. Zander.
     4 *insulanus* (De Not.) M.O.Hill.
     5 *luridus* Hornsch.
     6 *nicholsonii* Culm.
     7 *spadiceus* (Mitt.) Limpr.
     8 *tophaceus* (Brid.) Lisa
     9 *umbrosus* (Müll.Hal.) R.H.Zander.
     10 *vinealis* (Brid.) R. H. Zander.
18 *Eucladium* Bruch & Schimp.
     1 *verticillatum* (With.) Bruch & Schimp.
19 *Gymnostomum* Nees & Hornsch.
     1 *aeruginosum* Sm.
     2 *calcareum* Nees & Hornsch.
     3 *viridulum* Brid.
20 *Pseudocrossidium* R.S.Williams
     1 *obtusulum* (Lindb.) H.A.Crum &
L.E.Anderson

21 *Pterygoneurum* Jur.
    1 *ovatum* (Hedw.) Dixon
22 *Streblotrichum* P.Beauv.
    1 *convolutum* (Hedw.) P. Beauv.
23 *Syntrichia* Brid.
    1 *calcicola* J.J.Amann
    2 *caninervis* Mitt.
    3 *handelii* (Schiffn.) S. Agnew & Vondr.
    4 *laevipila* Brid.
    5 *latifolia* (Bruch ex Hartm.) Huebener.
    6 *minor* (Bizot) M.T.Gallego, J.Guerra, M.J.Cano, Ros & Sánchez-Moya
    7 *montana* Nees.
    8 *princeps* (De Not.) Mitt.
    9 *ruraliformis* (Besch.) Mans.
    10 *ruralis* (Hedw.) F.Weber & D.Mohr.
    11 *sinensis* (Müll.Hal.) Ochyra.
    12 *virescens* (De Not.) Ochyra.
24 *Tortella* (Müll.Hal.) Limpr.
    1 *tortuosa* (Hedw.) Limpr.
25 *Tortula* Hedw.
    1 *acaulon* (With.) R.H.Zander
    2 *brevissima* Schiffn.
    3 *inermis* (Brid.) Mont.
    4 *muralis* Hedw.
    5 *muralis* var. *aestiva* Hedw.
    6 *schimperi* M.J.Cano, O.Werner & J.Guerra
    7 *solmsii* (Schimp.) Limpr.
    8 *subulata* Hedw.
    9 *vahliana* (Schultz) Mont.
26 *Trichostomum* Bruch.
    1 *brachydontium* Bruch.
Grimmiales M.Fleisch.
Grimmiaceae Arn.
27 *Coscinodon* Spreng.
    1 *cribrosus* (Hedw.) Spruce.
28 *Grimmia* Hedw.

1 *anodo*n Bruch & Schimp.

2 *anomala* Hampe ex Schimp.

3 *funalis* (Schwägr.) Bruch & Schimp.

4 *laevigata* (Brid.) Brid.

5 *montana* Bruch & Schimp.

6 *orbicularis* Bruch ex Wilson.

7 *ovalis* (Hedw.) Lindb.

8 *pulvinata* (Hedw.) Sm.

9 *reflexidens* Müll.Hal.

10 *tergestina* Tomm. ex Bruch & Schimp.

11 *trichophylla* Grev.

12 *unicolor* Hook.

29 *Schistidium* Bruch & Schimp.

1 *apocarpum* (Hedw.) Bruch & Schimp.

2 *confertum* (Funck) Brunch & Schimp.

3 *helveticum* (Schkuhr) Deguchi.

4 *platyphyllum* (Mitt.) H.Perss.

Hedwigiales Ochyra.

Hedwigiaceae Schimp.

30 *Hedwigia* P.Beauv.

1 *ciliata* (Hedw.) P.Beauv.

2 *emodica* Hampe ex Müll. Hal.

Bartramiales D.Quandt, N.E.Bell & M. Stech.

Bartramiaceae Schwägr.

31 *Philonotis* Brid.

1 *calcarea* (Bruch & Schimp.) Schimp.

2 *fontana* (Hedw.) Brid.

Splachnales Ochyra.

Splachnaceae Grev. & Arn.

32 *Tayloria* Hook.

1 *lingulata* (Dicks.) Lindb.

Bryales Limpr.

Bryaceae Schwägr.

33 *Bryum* Hedw.

1 *argenteum* Hedw.

2 *canariense* Brid.

3 *gemmiparum* De Not.

34 _Imbribryum_ Pedersen.

    1 _alpinum_ (Huds. ex With.) N.Pedersen.

    2 _mildeanum_ (Jur.) J.R.Spence.

35 _Ptychostomum_ Hornsch.

    1 _capillare_ (Hedw.) Holyoak & N. Pedersen.

    2 _creberrimum_ (Taylor) J.R. Spence & H.P.
     Ramsay.

    3 _donianum_ (Grev.) Holyoak & N.Pedersen.

    4 _elegans_ (Nees) D.Bell & Holyoak

        5 _funkii_ (Schwägr.) J.R.Spence

    6 _imbricatulum_ (Müll. Hal.) Holyoak & N.
     Pedersen.

    7 _kunzei_ (Hornsch.) J.R. Spence

    8 _moravicum_ (Podp.) Ros & Mazimpaka.

    9 _pallens_ (Sw. ex anon.) J.R. Spence.

    10 _pallescens_ (Schleich. ex Schwägr.) J.R.Spence

    11 _pseudotriquetrum_ (Hedw.) J.R.Spence &
     H.P.Ramsay ex Holyoak & N.Pedersen

    12 _schleicheri_ (DC.) J.R.Spence ex D.Bell & Holyoak

    13 _torquescens_ (Bruch & Schimp.) Ros &
     Mazimpaka.

Mniaceae Schwägr.

36 _Pohlia_ Hedw.

    1 _elongata_ Hedw.

    2 _wahlenbergii_ (F.Weber & D.Mohr)
     A.L.Andrews.

    3 _wahlenbergii_ (F.Weber & D.Mohr)
     A.L.Andrews var. _calcarea_ (Warnst.)
     E.F.Warb.

Orthotrichales Dixon.

Orthotrichaceae Arn.

37 _Lewinskya_ F.Lara, Garilleti & Goffinet.

    1 _affinis_ (Schrad. ex Brid.) F.Lara, Garilleti & Goffinet.

    2 _rupestris_ (Schleich. ex Schwägr.) F.Lara, Garilleti &
Goffinet.

    3 _speciosa_ (Nees) F.Lara, Garilleti & Goffinet.

    4 _striata_ (Hedw.) F.Lara, Garilleti & Goffinet.

38 _Orthotrichum_ Hedw.

1 *anomalum* Hedw.

2 *bistratosum* (Schiffn.) Guerra

3 *cupulatum*

  var. *cupulatum* Brid.

  4 var. *riparium* Huebener.

  5 *diaphanum* Brid.

  6 *pallens* Bruch ex Brid.

  7 *pamiricum* Plášek & Sawicki.

  8 *pumilum* Sw. ex anon.

  9 *sprucei* Mont.

  10 *stramineum* Hornsch. ex Brid.

39 *Zygodon* Hook. & Taylor.

1 *rupestris* Schimp. ex Lorentz.

Hypnales W.R.Buck & Vitt.

Fontinalaceae Schimp.

40 *Fontinalis* Hedw.

1 *antipyretica* Hedw.

Amblystegiaceae G.Roth

41 *Amblystegium* Schimp.

1 *serpens* (Hedw.) Schimp.

42 *Campyliadelphus* (Kindb.) R.S.Chopra.

1 *chrysophyllus* (Brid.) R.S.Chopra.

43 *Cratoneuron* (Sull.) Spruce.

1 *filicinum* (Hedw.) Spruce.

44 *Hygroamblystegium* Loeske.

1 *humile* (P. Beauv.) Vanderp. Goffinet & Hedenäs

2 *tenax* (Hedw.) Jenn.

3 *varium* (Hedw.) Mönk.

45 *Leptodictyum* (Schimp.) Warnst.

1 *riparium* (Hedw.) Warnst.

46 *Palustriella* Ochyra.

1 *commutata* (Hedw.) Ochyra.

2 *falcata* (Brid.) Hedenäs

47 *Pseudocampylium* Vanderp. & Hedenäs

1 *radicale* (P.Beauv.) Vanderp. & Hedenäs

Pseudoleskeellaceae Ignatov & Ignatova.

48 *Pseudoleskeella* Kindb.

1 *nervosa* (Brid.) Nyholm.

2 *rupestris* (Berggr.) Hedenäs & L.Söderstr.

Brachytheciaceae Schimp.

49 *Brachytheciastrum* Ignatov & Huttunen.

    1 *collinum* (Schleich. ex Müll.Hal.) Ignatov & Huttunen.

    2 *olympicum* (Jur.) Vanderp. et al.

    3 *velutinum* (Hedw.) Ignatov & Huttunen.

50 *Brachythecium* Schimp.

    1 *albicans* (Hedw.) Schimp.

    2 *glareosum* (Bruch ex Spruce) Schimp.

    3 *mildeanum* (Schimp.) Schimp.

    4 *rivulare* Schimp.

    5 *rutabulum* (Hedw.) Schimp.

51 *Homalothecium* Schimp.

    1 *aureum* (Spruce) H.Rob.

    2 *lutescens* (Hedw.) H.Rob.

    3 *philippeanum* (Spruce) Schimp.

    4 *sericeum* (Hedw.) Schimp.

52 *Kindbergia* Ochyra.

    1 *praelonga* (Hedw.) Ochyra.

53 *Oxyrrhynchium* (Schimp.) Warnst.

    1 *hians* (Hedw.) Loeske.

    2 *speciosum* (Brid.) Warnst.

54 *Plasteurhynchium* M.Fleisch.

    1 *striatulum* (Spruce) M.Fleisch.

55 *Rhynchostegiella* (Schimp.) Limpr.

    1 *tenella* (Dicks.) Limpr.

56 *Rhynchostegium* Bruch & Schimp.

    1 *confertum* (Dicks.) Schimp.

    2 *riparioides* (Hedw.) Cardot.

57 *Sciuro-hypnum* (Hampe) Hampe

    1 *populeum* (Hedw.) Ignatov & Huttunen.

58 *Scleropodiu*m Bruch & Schimp.

    1 *cespitans* (Wilson ex Müll. Hal.) L. F. Koch.

Pylaisiaceae Schimp.

59 *Calliergonella* Loeske.

    1 *cuspidata* (Hedw.) Loeske.

60 *Pylaisia* Schimp.

    1 *polyantha* (Hedw.) Schimp.

Antitrichiaceae Ignatov & Ignatova
61 *Antitrichia* Brid.
    1 *californica* Sull.

Additional Explanations

[1] First recording from Türkiye; *Fissidens arnoldii* R. Ruthe Erdağ & Kürschner.

[2] First recording from Türkiye; *Cinclidotus vardaranus* Erdağ & Kürschner.

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