Mohd Sayeed Akhtar
Mallappa Kumara Swamy *Editors*

Natural Bio-active Compounds

Volume 3: Biotechnology, Bioengineering, and Molecular Approaches

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This book is dedicated to

Allama Shibli Nomani (1857–1914)

A great scholar, educationist, social reformer, and statesman of the nineteenth century, and founding father of the Shibli National College, Azamgarh, Uttar Pradesh, India.

Foreword

Natural bio-active compounds play a crucial role in pharmaceutical industries in designing and developing high-value products that help overcome human and animal health problems. These bio-active compounds are isolated from a wide variety of plants, microbes, algae, and several others. Due to their high therapeutic potentials and nutritional values, they are extensively used in the preparation of pharmaceutical drugs and functional foods. In view of this, comprehensive studies on various natural bio-active compounds for their potential pharmacological actions, including the identification, isolation and extraction, quality control, studies on the biological activities and mechanisms of action and clinical applications are becoming an exciting field of study in contemporary natural medication. Lately, biotechnological tools have been used in this connection. The application of such tools in natural product studies has helped in obtaining the desired compounds on a large scale. Deciphering the structure and functions of different classes of genes and enzymes involved in the biosynthetic pathways of bio-active compounds has also complemented the production of these compounds on a large scale. Similarly, molecular approaches, including genomics, transcriptomics, proteomics, and metabolomics for screening natural bio-active compounds too have augmented the discovery of new lead molecules and their large-scale production. The application of different strategies of metabolic engineering to modify existing pathways in plants and microbes have confirmed the impending prospect of producing high levels of natural bio-active compounds. Advances in technology are assisting us to a large extent in discovering new natural compounds, their biosynthesis and bioactivities. However, due to various reasons, the supply of natural bio-active compounds is still limited. On the other hand, the consumer demand is increasing progressively. Hence, there is great need to apply biotechnological and bioengineering strategies to meet the current growing demand for natural bio-active compounds.

This volume in the series titled “Natural Bio-active Compounds: Volume 3-Biotechnology, Bioengineering, and Molecular Approaches” includes 13 well-articulated chapters by academicians, scientists and researchers from different parts of the world. Chapter 1 discusses the role of bio-active peptides in plant growth and defense, whereas Chap. 2 focuses on the omics approaches related to the use of medicinal plants in human health applications. Chapter 3 presents the application of biotechnology in producing plant bio-active compounds. Chapter 4 discusses about the utility of transgenic plant cell cultures for the production of secondary metabolites. Chapter 5 describes the biotechnological approaches for the improved production of secondary metabolites from the medicinal aquatic plant, *Bacopa monnieri*. Chapter 6 highlights the prospect of plant cell culture as alternatives to produce secondary metabolites. Chapter 7 discusses the biotechnological exercises in the production of secondary metabolites and its significance in health care practices. Chapter 8 presents the biotechnological interventions in *Crocus sativus*, while Chap. 9 discusses recent advances in extraction, characterization and potential use of citral. Chapters 10 and 11 provides an update on hairy root cultures as an alternative source for the production of high-value secondary metabolites and their role in the production of secondary metabolites. Chapter 12 explains the strategies of metabolic engineering in the production of bio-active compounds from medicinal plants, while Chap. 13 describes the role of biotechnological approaches and metabolic engineering in the enhancement of rosmarinic acid content.

This volume is unique in nature. It covers various aspects of biotechnological production of high-value natural bio-active compounds and provides a deep knowledge of modern natural product research focused on producing vital native bioactive compounds of pharmaceutical importance. It also covers crucial information on the recent progress in using modern methodologies for biotechnological production of natural compounds.

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Preface

Secondary metabolites are a unique group of compounds produced by plants to protect against various biotic and abiotic factors (diseases, pests, pathogens, herbivores, environmental stresses, etc.). These compounds, however, do not influence the primary metabolic activities, such as growth and reproduction of plants. The major classes of secondary metabolites include phenolics, alkaloids, tannins, saponins, lignins, glycosides, and terpenoids. Some of these compounds have become an integral part of plant–microbe interactions toward adapting to environmental irregularities. They regulate symbiosis, induce seed germination, and show allelopathic effect, i.e., inhibit other competing plant species in their environment. Moreover, these compounds induce adverse physiological activities, such as reduced digestive efficiency, reproductive failure, neurological problems, gangrene, goiter, even death, and also possess high toxicity. The discovery of such unique compounds has inspired many scientific communities to explore their potential applications in various fields including agriculture and biomedicine. For instance, plant secondary metabolites are utilized to manufacture eco-friendly bio-pesticides and as drug sources in medicine. Due to numerous health-promoting properties, these compounds have been widely used as a source of medication since ancient times. The assessment of plant secondary metabolites for their wide-ranging therapeutic potential has led to the discovery of many drug leads in recent times. Therefore, this field of research has become a significant area for researchers interested to obtain understanding of the chemistry, analytical methodologies, biosynthetic mechanisms, and pharmacological activities of these plant secondary metabolites.

The use of natural bio-active compounds and their products are considered as most suitable and safe as an alternative medicine. Thus, there is an unprecedented task to meet the increasing demand for plant secondary metabolites from flavour and fragrance, food, and pharmaceutical industries. However, their supply has become a major constraint as their large-scale cultivation is very limited. Moreover, it is difficult to obtain a constant quantity of compounds from cultivated plants as their yield fluctuates due to several factors including genotypic variations, geography, edaphic conditions, and harvesting and processing methods. In addition, medicinal plants have become endangered due to ruthless harvesting in nature. Alternatively, plant tissue culture approaches can be well explored to produce secondary metabolites without practicing of conventional agriculture, which requires more land space. In vitro cell and tissue cultures require less space and are grown under the controlled lab conditions, and hence offer advantages of producing the desired compounds continuously without affecting their biosynthesis and quality. Furthermore, these cultures can be scaled up to produce metabolites in very large bioreactors and also, using genetically engineered cells/tissues, novel products can be obtained. The proper knowledge and exploration of these in vitro approaches could provide an optional source to produce plant secondary metabolites from many medicinal plants in large scale.

Natural Bio-active Compounds: Volume 3-Biotechnology, Bioengineering and Molecular Approaches is a very timely effort in this direction. This book volume with 13 contributions from Germany, India, Iran, Israel, Malaysia, New Zealand, Oman, Spain Turkey, and UK discusses on the Biotechnology, Bioengineering and Molecular Approaches in related to natural bio-active compounds. This book will undoubtedly encourage researchers, academicians and pharmaceutical industries towards the large-scale production of desired bio-active natural compounds using biotechnology and bioengineering approaches. Also, it will facilitate the discovery of new drugs or formulations with an improved efficacy and safety. Moreover, it is very useful for graduate students of medicinal chemistry, biotechnology and bioengineering streams, while also benefiting scientists who are keen to explore natural bio-active compounds for medical applications.

We are highly grateful to all our contributors for readily accepting our invitation and sharing their knowledge and research outcomes to compose the chapters and enduring editorial suggestions to finally produce this venture. We greatly appreciate their commitment. We are also thankful to Professor Munir Ozturk Hakeem for his suggestion and for writing the foreword for this volume. We also thank the team of Springer International, especially Dr. Kapila Mamta and Raagaipriya Chandrasekaran for their generous cooperation at every stage of the publication.

Shahjahanpur, Uttar Pradesh, India — Mohd Sayeed Akhtar
Bengaluru, Karnataka, India — Mallappa Kumara Swamy

About this Book

This book provides an updated and scientifically refined information about the production of several natural bio-active compounds obtained from microbes, plants, and algae, etc., through biotechnological, bioprocess and bioengineering approaches. The latest evidences on plant cell, tissue, organ, root culture and their utilization in the production of bio-active compounds are highlighted. Scale-up procedures using different types of bioreactors and their designs, optimization of culture conditions, the genetic and biochemical stability of biocompounds, the feasibility of using transgenic microbes and plants to enhance the production of targeted bio-active compounds are discussed in detail. Moreover, this book discusses on the possible explorations of metabolic pathway manipulations to produce bio-active compounds. Some of the modern high-throughput technologies, such as genomics, transcriptomics, proteomics, epigenomics, etc., to identify the genes and proteins involved in the biosynthesis of important bio-active compounds are discussed. Overall, the information provided in this book will undoubtedly encourage researchers, academicians and pharmaceutical industries towards the large-scale production of desired bio-active natural compounds using biotechnology and bioengineering approaches. Also, it will facilitate the discovery of new drugs or formulations with improved efficacy and safety to be effectively used in the future to counter the ever-growing challenges presented by diseases and infectious agents. This text could be useful for graduate students of medicinal chemistry, biotechnology and engineering streams. It also benefits scientists, who are keen to explore natural bio-active compounds for medical applications.

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About the Editors

Dr. Mohd Sayeed Akhtar (PhD) is Assistant Professor in Gandhi Faiz-e-Aam College, Shahjahanpur, U.P., India. He received his PhD degree from Aligarh Muslim University (AMU), India, in 2008, prior to conducting postdoctoral research at the Botanical Institute, University of Basel (BIB), Switzerland (2008–2010), and the Chonbuk National University (CBNU), Republic of Korea in 2011. He was Assistant Professor, Jimma University, Ethiopia (2011–2014), and a fellow researcher at the Institute of Tropical Agriculture, Universiti Putra Malaysia (UPM) (2014–2015). Dr. Akhtar has more than 15 years of research and 10 years of teaching experience in soil microbiology, applied microbiology, environmental microbiology, molecular biology, plant pathology and plant nanobiotechnology. He is author and co-author of more than a hundred articles in peer-reviewed journals, conference proceedings, and book chapters, and has edited ten books with international publishers. He serves the scientific community as editorial board member and reviewer of several high-impact international journals. His current research is focused on the rhizospheric plant–microbe interactions and their molecular biotechnology, bioremediation, biomineralization, nano-fertilizers and nanobiotechnology.

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Pvt. Ltd., India. He also serves as the editorial board member and reviewer for several high-impact international journals. His current research is on cell and tissue culture technology for bio-active compound production, phytochemistry, phytocompounds isolation, production and their biological evaluation, and on nanotechnology for medical applications.

Bio-active Peptides: Role in Plant Growth and Defense

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Sharadwata Pan, Dominic Agyei, Jaison Jeevanandam, and Michael K. Danquah

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Abstract

The emerging beneficial characteristics of bio-active peptides have made them suitable candidates for a wide range of applications. While their usage as potent nutraceutical and pharmaceutical agents has been well-documented, applications of bio-active peptides in addressing sustainable agricultural challenges relating to biotic and abiotic stresses, plant disease control, and nutrient use efficiency have not received much attention. Bio-active peptides are specific fragments of proteins with amino acid structures capable of enhancing molecular signaling in the rhizosphere to promote nodulation, nutrient uptake, and stress management. Bio-active peptides can be formulated with agrochemicals and assimilated through the leaf system in foliar treatments to achieve a wide range of plant benefits including coloring, nutrient delivery under drought conditions, plant health, and crop protection. Harnessing the maximum potential of bio-active peptides in sustainable agriculture is a rational contemplation, since the current years have witnessed a radical upsurge in the manufacturing scale of bio-active peptides under optimum economy. The present chapter discusses the unique potential of bio-active peptides in promoting sustainable agriculture. Moreover, the molecular mechanisms of bio-active peptides in influencing plant stress relief, disease control, and nutrient assimilation efficiency and signaling routes are also elaborated. Additionally, a few advanced standpoints pertaining to optimal utilization of bio-active peptides in advancing agricultural productivity are also discussed.

Keywords

Bio-active compounds · Cyclic peptides · Disease control · Stress relief · Sustainable agriculture

1.1 Introduction

Biologically active short fragments (typically 2–20 amino acids) of parent proteins are currently at the forefront of active research and development, both under the purviews of academic and commercial domains. This is predominantly due to the profound impacts of bio-active peptides to two major domains of active lifestyle: food and health sectors. Till date, from the repertoire of literature available on the subject, the major focus of the food sector is an attempt to extract and optimize maximum benefits of bio-active peptides as nutraceuticals. On the other hand, the health sector has been busy trying to systematically elucidate and characterize the pharmaceutical characteristics associated with bio-active peptides, with an objective to fully trap the extraordinary range of health benefits associated, such as cytoregulatory, antimicrobial, antidiabetic, and antihypertensive actions, among others. On the latter perspective, the widespread critical acclaim levied on these small molecules of tremendous capabilities is a direct consequence of their outstanding capabilities to scavenge the detrimental actions of the free radicals like reactive oxygen species, which are perceived as the principal perpetrator in manifestations of a range of derogatory health complications. All these and many more insightful assertions, deliberations, comments, discussions, and recommendations are available in a wide volume of recent and past studies, both research and reviews (Agyei et al. 2015, 2016, 2017a, b, 2018; Sarethy and Pan 2017; Gnasegaran et al. 2017), to which interested readers may refer to. Although the active solicitations of bio-active peptides in medicine and food sectors are imminently noticeable and have been quite a long-standing initiative, the same cannot be ascertained regarding its applications in sustainable agriculture. This is aptly reflected in Fig. 1.1, which clearly reveals the insufficient investigations of bio-active peptide benefits in sustainable agriculture. In fact, if the current articles and news feeds are to be believed, the trial has just begun, and a "vast empty land lies ahead to be grazed." Probably the appeal of the bio-active peptides lies in their easier absorption by the host plants as compared to the free amino acids, which consequently hints at their high-class biological effectiveness and dietetic usefulness, as compared to the free amino acids. With the extension of the mechanized level of biologically active peptides and comparatively restricted manufacturing expenses, the opportunities for a widespread application in agro-based production fields are immense (Malaguti et al. 2014; Prasad et al. 2017). This is aided by the substantial benefits associated with the bio-active peptides. One of the most significant advantages of the bio-active peptide administration strategy in a sustainable agriculture is its "green and natural perspective" and the nonmandatory feature to introduce and incorporate tiresome and lengthy crossbreeding procedures to produce transgenic plants (Scheible 2018). Additionally, other benefits

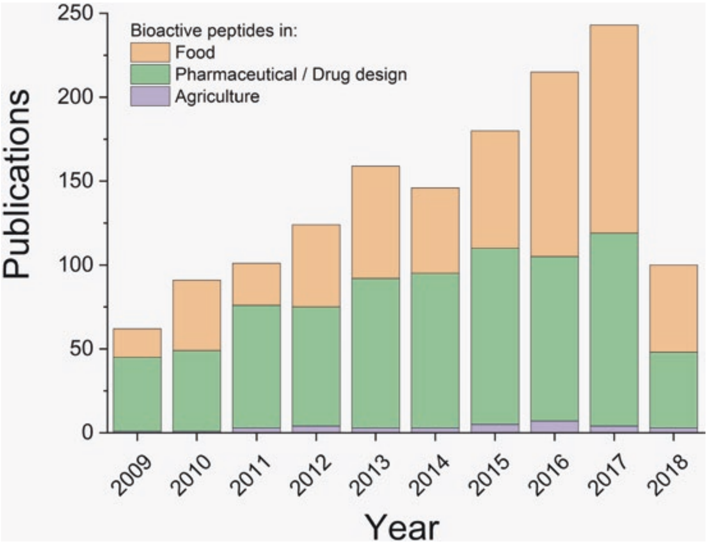

Fig. 1.1 Recent publications in the SCOPUS database (http://www.scopus.com) from 2009 to 2018, using the terms "bio-active peptides" and "food/pharmaceutical or drug design/agriculture" in the scientific literature

such as ability to synthesize peptides that mimic the original host peptide sequences, large-scale manufacturing capabilities, ability to show bioactivity even at a minuscule concentration, and an overall positive effect on plant development have only augmented the desire to achieve a mammoth-scale application of bio-active peptides in viable agriculture (Malaguti et al. 2014; Scheible 2018). On this front, Malaguti et al. (2014) have analyzed the agronomical, clinical, and biochemical viewpoints with reference to bio-active peptides and concluded that extensive trials and stringent validation of links between benefits of bio-active peptides and agronomical deliberations are necessary. Furthermore, robust quality control and assurance protocols need to be reinforced for bio-active peptides on the production front, as has been demonstrated in case of herbal medicines (Pan et al. 2013). This necessitates a careful attempt to review the current situation. Thus, the present chapter discusses the unique potential of bio-active peptides in promoting sustainable agriculture. Moreover, the molecular mechanisms of bio-active peptides in influencing plant stress relief, disease control, and nutrient assimilation efficiency and signaling routes are also elaborated. Additionally, a few advanced standpoints pertaining to optimal utilization of bio-active peptides in advancing agricultural productivity are also discussed.

1.2 Stress Sustenance Mediated by Bio-active Peptides

1.2.1 Biotic Stress Relief

One of the most significant advantages of bio-active peptides in sustainable agriculture could be their abilities to counter stress generated from invasion of foreign (mainly microbial) pathogens: bacteria, viruses, or fungi. It is no secret that failures to sustain and counter stresses from these biotic agents denounce crop productivity, mainly in terms of yield. Consequently, understanding the molecular mechanisms of biotic stress tolerance in crop plants via active involvement of biologically active proteins and peptides could be a continuous effort (Besseau et al. 2012; Scarpeci et al. 2013; Ramegowda and Senthil-Kumar 2015). In this context, functional-, biochemical-, and molecular-level investigations on plant-microbe communications have been studied which reveal significant influence of microbial associations toward biotic stress sustenance (Farrar et al. 2014). Furthermore, the interactions at the level of individual molecules and even genes have been facilitated through the synthesis of biological data founded on multi-omics strategies (Kissoudis et al. 2014). Interestingly, past studies focused on combined effects of biotic and abiotic stresses have indicated elicitation of unique responses (either positive or negative, or being liable or lenient) from the affected plant species (Ramegowda et al. 2013a, b; Ramegowda and Senthil-Kumar 2015). This is even more practical, considering the ever-changing ecological conditions and perpetual threats of global warming. The bottom line is that both biotic and abiotic stress sustenance mechanisms are interconnected to a considerable degree. Purely from the perspective of biotic stress tolerance, the implications of abscisic acid (ABA) stand above the rest, via laying out either a substantial barricade or downregulating specific signaling pathways to counter the pathogen-induced responses (Ramegowda and Senthil-Kumar 2015). The bio-active agents derived from foreign pathogens may trigger immune responses in the host plants which bear resemblance to contagions, which may ultimately assist in enabling stress sustenance (Rasmussen et al. 2013). Past studies have laid claims to evidences of host plants, with prior reactions to drought stress, to have significantly altered pathogen reactions (Ramegowda et al. 2013a). Prasch and Sonnewald (2013) demonstrated that plants of *Arabidopsis* species, subjected to viral infections, trigger an ensemble of gene-encoded small bio-active peptides with stress-protective features. Recombinant DNA technology studies have confirmed the involvement of bio-active agents to counter biotic stress through mediation via either salicylic or jasmonic acid and/or ethylene signaling pathways (Besseau et al. 2012; Scarpeci et al. 2013; Chen et al. 2013). The involvement of several bio-active agents, including biologically active peptides, over-manifestation and optimum manifestation of transcription factors, and upstream monitoring of genes and even enzymes, in countering biotic stress, have been aptly highlighted (Ramegowda and Senthil-Kumar 2015 and references therein).

1.2.2 Abiotic Stress Relief

A wide array of abiotic stress factors is responsible for affecting agricultural crop yield: salinity, heat, drought, temperature, and precipitation, among others (Meena et al. 2017). The authors emphasize on some crucial aspects in order to achieve a desirable abiotic stress counter effect, including recognizing the profusion of metabolic routes, detection of characteristics linked to stress reactions and subsequent associated genetic markers, and novel gene pullout approaches that could optimize the stress alleviation approaches. Abiotic stress relief involving microorganisms has also received considerable attention (Nadeem et al. 2014; Souza et al. 2015; Meena et al. 2017). The volume of data and past studies, targeted toward involvement of biologically active agents, including small proteins and peptides, toward alleviation or mitigation of abiotic stress in agricultural crops, is much broader as compared to the biotic stress sustenance. Till date, diverse strategies have been employed to decipher and extract bio-active peptides with beneficial features, including high-throughput chromatographic techniques and innovative computational biology tools (Sagar et al. 2012; Torrent et al. 2012). This is logical, since downregulation or alleviation of abiotic stress responses, which is the leading restrictive aspect for agricultural throughput, has been focused on a wide range of agricultural research initiatives. Much of these studies have been directed using genetic-level investigations in the plant *Arabidopsis thaliana*. For instance, Vie et al. (2017) have recently showed that IDL6 and IDL7, the inflorescence deficient in abscission or IDA-LIKE bio-active peptides, adversely regulate the abiotic stress reactions in these host plants. Contextually, minor signaling peptides with resemblances to IDA-LIKE peptides have also been implicated earlier to aid in the process (Vie et al. 2015). The implications of novel bio-active peptides such as OSIP108, obtained from *A.*

thaliana, have been highlighted in countering the menaces of oxidative stress by scavenging various types of reactive oxygen species or free radicals and by conferring protections against agents like hydrogen peroxide (De Coninck et al. 2013). Their work is laudable, from the perspective of identification of biologically active peptides with potent free radical scavenging properties; those may only be programmed by minor open reading frames (ORF) in the host plant genome. This actually advances the work by Brand et al. (2012), who have reported similar efforts, except that the bio-active peptides could only be encoded in the plant proteome. Several other bio-active peptides, such as plant elicitor peptide At Pep1, phytosulphokine (PSK) peptide, C-terminally encoded peptide (CEP-3), and cysteine-abundant peptide AtCAPE1, among others, have shown promise toward alleviation or mitigation of abiotic stress in plants (Yamaguchi et al. 2010; Delay et al. 2013; Chien et al. 2015). Very recently, Meena et al. (2017) have comprehensively reviewed the Omics approaches involving microbial associations and subsequent countering of abiotic stresses in plants. The review is noteworthy, since the reader is directed toward detailed, insightful discussions regarding several unique strategies, like proteomics, genomics, metabolomics, and transcriptomics, from the perspective of an initial introduction to the concepts, and later the justification and rationale surrounding these techniques to assimilate, scrutinize, and infer real-time cellular information that could be effectively transferred from the lab to the field.

1.3 Bio-active Peptides in Plant Disease Control

Plants are the important source of energy for herbivorous animals, birds, and humans. Diseases in plants will disturb the continuity in food chain and also affect the economy that depends on agriculture. Generally, plant diseases are caused by microbes such as bacteria, fungi, and viruses. Thus, efficient antimicrobials are highly in demand to control the spread of microbial infection with antibiotic resistance in plants. Bio-active peptides are proved to possess enhanced potential as antimicrobials to eradicate microbe-mediated plant diseases (Gomes et al. 2018).

1.3.1 Antibacterial Response of Bio-active Peptides

Defensive peptides are produced in organisms as antimicrobial peptides (AMPs), which are the new class of antibiotics that are formed, when microorganisms or extraneous materials encounter with the surface of host organism. Ribosomes are the significant precursors which consist of 10–60 amino acid residues that help in the production of AMPs via C-terminal amidation, cysteine pairing, and amino acid isomerization. These AMPs possess antibiotic and antiendotoxic activities against fungi, bacteria, viruses, and some parasites as well as boost innate immune systems (Ovando et al. 2018). New strategies to produce transgenic plants that are expressing AMP genes via recombinant DNA techniques facilitate the bioactivity of AMPs against bacterial and fungal plant pathogens (Wang et al. 2018). Among antibacterial peptides, cecropin B is an important peptide obtained from *Hyalophora cecropia* and *Bombyx mori* that shows antibacterial response against several Gram-positive and Gram-negative bacteria (Zou et al. 2017). Recently, cationic lytic peptide cecropin B was proved to possess antibacterial efficacy against two major pathogens of tomatoes such as *Ralstonia solanacearum* and *Xanthomonas campestris*, and also in vivo studies in transgenic tomato plants with these peptides demonstrate significant resistance to bacterial spot and wilt diseases (Jan et al. 2010). Likewise, bacteriocins, defensins, peptaibols, cyclopeptides, and pseudopeptides (Breen et al. 2015; Borriss 2016; Camó et al. 2017; Gwinn 2018) were also used to control bacterial-mediated plant diseases.

Among these wide variety of bio-active antibacterial peptides, bacteriocins, cyclopeptides, and pseudopeptides can be subclassified into further types. Bacteriocins that are produced by actinobacteria are classified into type 1 lantibiotics, which include microbisporicin and planosporicin; type 2 lantibiotics, namely, variacin, michiganin A, cinnamycin group, and actagardine; and labryinthopeptins and NAI-112 which are categorized under type 3 lantibiotics (Gomes et al. 2017). These bacteriocins help in controlling plant diseases such as tomato bacterial wilt (Konappa et al. 2015), vegetable diseases by plant growth-promoting rhizobacteria (Rizvi et al. 2017), citrus canker (Canteros et al. 2017), and Stewart's wilt of corn (Javandira et al. 2013) and also help as microbiota regulators and promote plant growth (Drider et al. 2016). Similarly, cyclic peptides or cyclotides are classified into homodetic, heterodetic, and complex based on their type of bonds within the rings (Claro et al. 2018). Cyclotides such as iturin, gramicidins, and lipid peptides help to control fire blight diseases (Habbadi et al. 2017); tailed lipid cyclotides, namely, polymyxins, putisolvins, and corpeptins inhibit the growth of bacteria that causes wilt, spot, speck, and canker disease in tomato (Panneerselvam et al. 2015) and fire blight diseases (Sonawane et al. 2015). Meanwhile, pseudopeptides are used to control bacterial growth in plants to avoid spreading of diseases such as fire blight disease (Patel et al. 2017), blackleg disease in potato (Dutkiewicz et al. 2016), citrus canker (Dutkiewicz et al. 2016), and nosocomial infections (Montesinos et al. 2012). Also, bio-active peptides are beneficial in controlling other bacterial infections in plants such as bacterial crown gall (Frikha-Gargouri et al. 2017), foliar diseases (Ali et al. 2016), leaf blight (Shi et al. 2016), soft rot disease (Charkowski 2015), and root and postharvest diseases (Rahman 2016).

1.3.2 Antifungal Response of Bio-active Peptides

Similar to antibacterial effect, bio-active peptides also possess antifungal properties toward various fungal infections. Peptides such as alfAFP, Pn-AMP2, CEMA, MSI-99, and polyoxins from various sources possess antifungal activity against several phytofungal diseases (Keymanesh et al. 2009). Plant sources, namely, *Medicago sativa* and *Pharbitis nil*, help to fabricate antifungal peptides such as alfAFP and Pn-AMP2 that help to control fungal species such as *Verticillium dahliae* (Maróti et al. 2011) that causes wilt disease (Ilyas et al. 2017). Pn-AMPs are hevein-like peptides that are also used to control the growth of phytopathogenic fungi that causes disease in *Lycopersicum esculentum* (tomato) (Slavokhotova et al. 2017). CEMA and MSI-99 peptides are originated from synthetic sources such as hybrid chimeric form of cecropin-melittin (Li et al. 2015) and magainin analog (Białkowska et al. 2017), respectively. CEMA peptides possess enhanced antifungal property that helps to control fungal-mediated plant diseases such as huanglongbing (HLB, citrus greening), canker (Dutt et al. 2015), Pierce's disease (Li et al. 2015), and *Verticillium* and *Fusarium* wilt in cotton (Zhang et al. 2016). Likewise, MSI-99 peptide helps to control phytofungal diseases such as blue mold and sour rot diseases in citrus fruits (Wang et al. 2018), rice blast fungus (Wang et al. 2015), fungi that attack *Brassica juncea* (Rustagi et al. 2014), and aflatoxigenic fungi in maize (Schubert et al. 2015). Other novel peptides such as cathelicidin (Scarsini et al. 2015), 14-helical β-peptides (Raman et al. 2015), histatin 5-halocidin hybrid (Han et al. 2016), human β-defensin 3-C15 (Lim et al. 2016), and ABP-dHC-cecropin A (Zhang et al. 2015) also possess potential antifungal ability. These peptides help to control plant diseases such as dollar spot, brown patch disease in tall fescue, powdery mildew, root rot of kidney beans, and gray mold diseases (Zhou et al. 2016; Kusch and Panstruga 2017; Tian et al. 2017; Tong et al. 2017).

1.3.3 Antiviral Response of Bio-active Peptides

Viruses, especially bacteriophages, also cause wide variety of diseases in plants (Tepfer et al. 2015). Peptides such as entry blocker (Datta et al. 2015), RRKKLAVLLALLA, P1 (NDFRSKT), FluPep (Mendoza-Figueroa et al. 2014), N-modified peptide with palmitic acid (Aronin et al. 2015), and retrocyclins (Chen et al. 2014b) are proved to possess antiviral properties (Skalickova et al. 2015). Lactoferricin is an important peptide that possesses enhanced antiviral activity against viruses such as tomato yellow leaf curl virus (Mendoza-Figueroa et al. 2018) and potato virus X (Taha et al. 2015). Also, polysaccharide peptide (PSP) (Zhao et al. 2015), anthrax peptides (McComb et al. 2015), and RhoA peptide (Ortega-Berlanga et al. 2016) help to control the growth of famous tobacco mosaic virus. Also, plant elicitor peptides, aracins, and other novel peptides help to control plant diseases that are caused by insects and other pathogens (Huffaker 2015; Toopaang et al. 2017). Table 1.1 is a summary of different bio-active peptides that are used to control plant diseases. Thus, the peptide-based transgenic plants are highly in demand as they reduce the risk of pathogenic diseases which affect agriculture, economically (Lucht 2015). However, disruption of biodiversity and blockage in the food chain are the major drawbacks of using these transgenic plants (Abiri et al. 2015). These drawbacks, which can be unveiled by using formulation of bio-active peptides and nanomaterial encapsulated peptides to treat plants, instead of transgenic plant development, will reduce their environmental impact (Subbarao et al. 2015).

Table 1.1 Bio-active peptides as control agents for pathogen-mediated plant diseases

Peptides	Source	Benefits in controlling plant disease and pathogens	Reference
Antibacterial peptides			
Cecropin B	Giant and domesticated silk moth	*Ralstonia solanacearum* *Xanthomonas campestris* Controls bacterial wilt and spot disease	Jan et al. (2010)
Bacteriocins	Actinobacteria	Tomato bacterial wilt Vegetable diseases by plant growth-promoting rhizobacteria Citrus canker Stewart's wilt of corn	Javandira et al. (2013), Konappa et al. (2015), Canteros et al. (2017), and Rizvi et al. (2017)
Defensins	Vertebrates and invertebrates	Alfalfa crown rot Snow mold Wilt disease	Sasaki et al. (2016), Hsiao et al. (2017)
Peptaibols	Fungi and bacteria	*Botrytis cinerea* Basidiomycetes Trichoderma species	Vos et al. (2015), Bisen et al. (2016), and Hamid and Wong (2017)
Cyclotides	Plants	Fire blight diseases Wilt, spot, speck, canker disease in tomato	Panneerselvam et al. (2015) and Habbadi et al. (2017)
Pseudopeptides	Bacteria, fungi, and plants	Fire blight disease Blackleg disease in potato Citrus canker Nosocomial infections	Montesinos et al. (2012), Dutkiewicz et al. (2016), and Patel et al. (2017)
Antifungal peptides			
alfAFP	Alfalfa, *Medicago sativa*	Wilt disease	Ilyas et al. (2017)
Pn-AMP2	*Pharbitis nil*	*Verticillium dahliae* Diseases in tomato	Maróti et al. (2011) and Slavokhotova et al. (2017)
CEMA	Hybrid chimeric form of cecropin-melittin	Citrus greening Canker Pierce's disease *Verticillium* and *Fusarium* wilt in cotton	Dutt et al. (2015), Li et al. (2015), and Zhang et al. (2016)

(continued)

Table 1.1 (continued)

Peptides	Source	Benefits in controlling plant disease and pathogens	Reference
MSI-99	Magainin analog	Blue mold and sour rot diseases in citrus fruits	Rustagi et al. (2014), Schubert et al. (2015), Wang et al. (2015, 2018)
		Rice blast fungus	
		Fungi that attack *Brassica juncea*	
		Aflatoxigenic fungi in maize	
Antiviral peptides			
Lactoferricin	Milk	Tomato yellow leaf curl virus	Mendoza-Figueroa et al. (2014) and Taha et al. (2015)
		Potato virus X	
Polysaccharide peptide (PSP)	Plants, animals, bacteria, and fungi	Tobacco mosaic virus	McComb et al. (2015), Zhao et al. (2015), and Ortega-Berlanga et al. (2016)
Anthrax peptides			
RhoA peptide			

1.4 Effects of Bio-active Peptides on Nodulation and Nutrient Utilization

1.4.1 Promoting Biofertilizer Actions

As a general notion, biofertilizers are envisaged as vigorous agents assisting in maintaining the ecological area surrounding soil abundant in both macro- and micronutrients by promoting inherent cycles of nitrogen fixation, secretion of plant growth regulators (PGR) or hormones, mineral assimilation and volatilization, and synthesis of active compounds with antimicrobial activities (Sinha et al. 2014). For a detailed discussion on the range of microbes, both fungi and bacteria, that have been used as potent biofertilizers, including *Azotobacter*, *Rhizobium*, *Phosphobacter*, *Azospirillum*, and *Rhizobacter*, among others, readers are encouraged to see the review by Bhardwaj et al. (2014). The authors have outlined probable solicitations of microbial biofertilizers in sustainable agriculture, their proposed high degree of optimization to improve the crop profile and productivity, and commented on possible mechanisms of biofertilizers actions. Past studies have highlighted the involvement of bio-active compounds and ligands in promoting nodulation and nutrient utilization in crop plants, including proposed machinery that aids in such processes. For instance, bio-active ligands known as Nod or Myc factors have been reported to activate the secretion of calcium ions in cytosol as a consequence of activation of signaling routes from the rhizosphere, mediated by intermediate receptors in *Rhizobium* and mycorrhiza (Bonfante and Genre 2010; Roberts et al. 2013). A hypothetical schematic depicting this action is reproduced from Bhardwaj et al. (2014) in Fig. 1.2. The onset of calcium release is also known to be facilitated by the

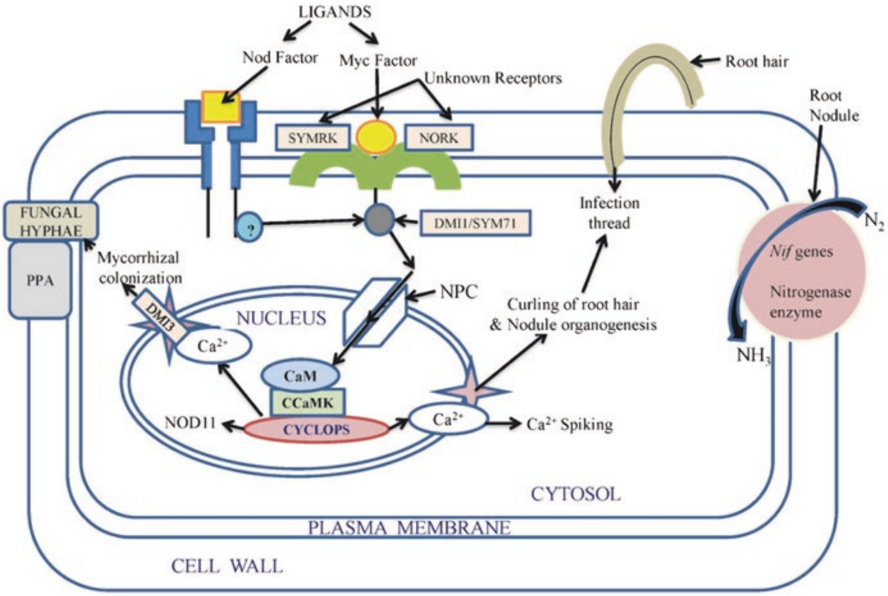

Fig. 1.2 Action mechanisms of bio-active ligands in a plant root cell: a conjectural representation. (Reproduced from Bhardwaj et al. (2014) with the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0))

participation of nuclear pore complex and related proteins and kinases and kinase-associated proteins like SYM71 and DMI (Sieberer et al. 2009; Maillet et al. 2011). The nodulation process has also been linked to be benefited from the involvement of the enzyme calmodulin-dependent protein kinase or CCaMK (Maillet et al. 2011). A stimulating discussion related to the various factors and active molecules involved in nitrogen fixation and nodulation has been collated in the review by Zhuang et al. (2013). For instance, the authors report that nodulation and nitrogen fixation are promoted by bio-active molecules like exopolysaccharides and lipochitooligosaccharides such as Nod factors, mainly sourced from *Rhizobium* sp. Additionally, bio-active compounds such as lysophosphatidylcholine have been known to assist in phosphate acquisition (Drissner et al. 2007).

1.4.2 Protein Hydrolysates as Biostimulants

Protein hydrolysates represent a cohort of mixed sequences of amino acids and oligo- and polypeptides generated via fractional hydrolysis of source proteins (Schaafsma 2009). Bio-active peptides in the form of protein lysates, especially of plant origins, have been reported to offer service as “biostimulants,” mainly due to their role in facilitating sprouting, improved yield and quality of agronomic crops, and laying a positive impression for countering abiotic stresses such as drought, heavy metal contaminations, and/or salinity (Colla et al. 2017). Contextually, the biostimulant actions of protein hydrolysates, in the form of overall growth promotion and nitrogen acceptance, derived from corn and tomato plants, have been recently demonstrated by Colla et al. (2014). The authors report that the increased rate of nitrogen uptake could be due to a strong auxin- or gibberellin-like response, the widespread root machinery development, and enhanced nitrogen acclimatization procedures, as shown by the protein hydrolysates. The improved nitrogen incorporation may be a consequence of enhanced secretion of distinct enzymes such as glutamine synthetase and nitrate reductase, as observed previously (Ertani et al. 2009). The beneficial effects of bio-active peptide solicitations have been reflected in the form of laudable nitrogen contents in the leaves of vegetable crops (Liu and Lee 2012; Tsouvaltzis et al. 2014). Since this may optimize the efficacy of nitrogen consumption, the bio-active peptides in the form of protein hydrolysates could also be considered as active plant growth promoters. Incidentally, the roles of biologically active intrinsic peptides such as systemin, CLE, phytosulfokine, SCR/SP11, etc., in advancing cellular split and differentiation, including abilities to counter proteases, have been well-documented (Colla et al. 2014). Very recently, Colla et al. (2017) have systematically reviewed the biostimulant activities of protein hydrolysates and their effects on general plant functioning. The authors have collated recent references which highlight the positive and direct influence of plant-isolated protein hydrolysates, including their strong implications in facilitation of carbon and nitrogen absorption; in regulation of the activities of key enzymes like malate and isocitrate dehydrogenase, and citrate synthase, which are central to nitrogen uptake process; and in general advancement of root and foliar growth (Matsumiya and Kubo 2011; Colla et al. 2014, 2015; du Jardin 2015; Lucini et al. 2015; Nardi et al. 2016. It may be noted that although the phyto-protein hydrolysates have been associated with a wide variety of advantages, their animal counterparts may not be granted similar distinction and have been linked with growth clampdown and phytotoxic outcomes (Cerdán et al. 2009; Lisiecka et al. 2011).

1.5 Role of Bio-active Peptides in Phyto-signaling Pathways

Aside their roles in stress mitigation and disease control, bio-active peptides also play a crucial role in the metabolic signaling network of plants. In all life forms, signal transduction is important in a cellular communication, and without it metabolic processes which give rise to growth, defense, and survival will not occur (Banerjee and Sengupta 2011). Signal transduction involves the initiation and transmission of molecular events in the form of chemical or physical signals leading to a cellular response. In the field of botany, the role of signal transduction mediators, such as plant hormones and integrin-like receptors, are well described in the literature, and are always treated as chemicals, i.e., organic acids (e.g. salicyclic acid, jasmonic acid, indole-3- acetic acid), polyhydroxysteroids (e.g., brassinolide), hydrocarbons (e.g., ethylene), and lactones (e.g., 5-deoxystrigol). Interestingly, an increasing number of studies have shown that certain biologically active peptides also act as signaling molecules, hence the name “plant peptide

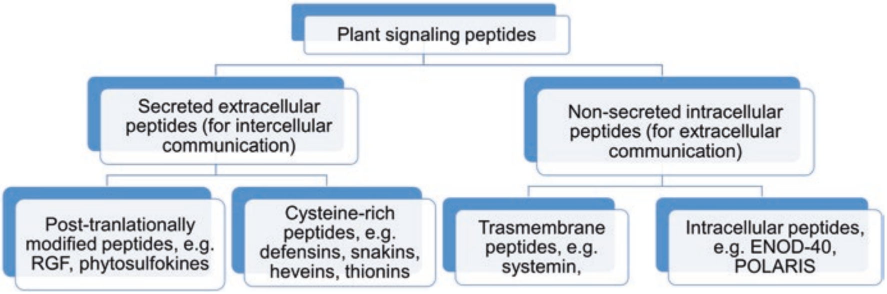

Fig. 1.3 Classification of signaling peptides or plant-based peptide hormones

hormones" (Pearce et al. 2001a; Marshall et al. 2011; Matsubayashi 2014; Oh et al. 2018). The sequencing of the *Arabidopsis* genome has shown that there are over 1000 potential signaling peptides (Oh et al. 2018), but the bulk of these has not been characterized biochemically (Czyzewicz et al. 2013). These peptides are involved in a number of plant processes and mechanisms, the most important being cell division, defense, and reproduction (Lindsey 2001). A structural classification of peptide phytohormones is given in Fig.1.3. The non-secreted peptides are located inside the cells, but their target functions could be either extracellularly or intracellularly (Guo et al. 2015; Xu et al. 2018). The secreted peptides differ based on the time of processing strategy that precedes the proteolytic cleavage used to release the matured peptide. It is either the peptides undergo posttranslational modifications (PTM) (such as sulfation) giving PTM peptides or intramolecular disulfide bond formation giving cysteine-rich peptides (Matsubayashi 2014; Oh et al. 2018). This section focusses on some of the characteristics and functions of signaling peptides responsible for processes such as root and foliar development. The distinct courses of bio-active peptide actions in effective crop management and a holistic development are captured in Fig. 1.4.

1.5.1 Root Signaling Machinery

Root development and growth were described in detail for the first time using *Arabidopsis* as a model. The process is precise and consists of a set of rapidly dividing stem (or "initial") cells that surround another set of infrequently dividing cells. The rapidly dividing initial cells include the ground tissue cells (cortex/endodermis), the central portion of root cap (columella), and the outer portion of root cap (epidermal or lateral root cells). In contact with the abovementioned initial cells are the quiescent centers which consist of nondividing cells (Scheres et al. 2002; Scheres 2013). The development, growth, and differentiation of root cell are mediated by several signaling molecules, some of which are peptides. The root meristem growth factor (RGF) is one example of such peptide. It is encoded by a family of 11 genes to polypeptides with conserved C terminal that are processed into

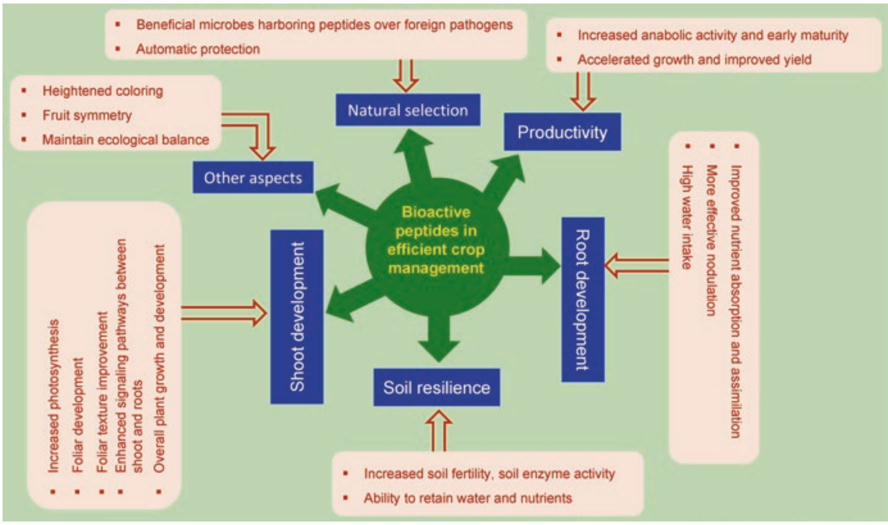

Fig. 1.4 Efficacy of biologically active peptides in overall crop management

RGF1–RGF11. RGF1 is a trideca-peptide hormone that is responsible for the post-embryonic development and maintenance of root meristem stem cells (Matsuzaki et al. 2010), but overexpression of RGF peptides results in wavy roots (i.e., a series of undulation shapes of roots) (Matsubayashi 2014). RGF is posttranslationally modified to contain a sulfonated tyrosine unit and works via the PLETHORA (PLT) stem cell transcription factor pathway (Shinohara et al. 2016). Receptors for the RGF peptides have not been identified, but the peptides share C-terminal sequence similarity with CLE18-type A, another class of secreted peptide hormones. CLE peptides are a group of secreted posttranslationally modified peptides with functions that fall into two categories, namely, the development of shoot and roots (type A) and control of xylem differentiation (type B) (Matsubayashi 2014). An example of type A CLE peptides that slows down root growth is CLE-RS (CLAVATA3/embryo surrounding region-related) peptides. CLE-RS peptides are a 13-amino acid glycopeptide that perform root-to-shoot signaling for the control of nodulation in plants. CLE-RS shows some degree of structure-function relationship as the arabinosylation of the hydroxyproline residue is vital for the hormonal functions of this peptide (Okamoto et al. 2013). Another group of plant peptide hormones with similar functions are phytosulfokines (PSK) and plant peptide containing sulfated tyrosine 1 (PSY1). PSKs are disufalted pentapeptides found in all higher plants (Sauter 2015). PSY1 on the other hand is an octadecapeptide secreted as a sulfated glycopeptide and responsible for cell growth. PSK is an autocrine growth factor, which controls root cells elongation in the elongation/differentiation zone (Oh et al. 2018). There are two receptors that recognize PSK, namely, PSKR1 and PSKR2, both of which are leucine-rich repeat receptor kinases (LRR-RK). PSY1R is also a LRR-RK and serves as the receptor for PSY1 (Mosher and Kemmerling 2013). PSK and

PSY1 perform physiological functions ranging from growth to defense (Mosher and Kemmerling 2013; Sauter 2015). In fact, PSK peptides have bio-active properties that control the formation and growth of lateral roots (Oh et al. 2018).

Early nodulin (ENOD-40) is another peptide growth factor with a high mitogenic activity and therefore responsible for the early stages of nodule development, as well as controlling sucrose metabolism by regulating the activity of the enzyme sucrose synthase (Farrokhi et al. 2008; Germain et al. 2006). ENOD-40 peptides are usually 10–13 amino acids long and is synthesized as an intact peptide and not in the form of a large precursor which needs to be processed to release the active peptide (as in the case for PSK) (Schaller 2001). Aside from root nodule formation, ENOD-40 is also responsible for other physiological processes in plants such as the formation and differentiation of vascular bundles. The existence and functions of ENOD-40 have been demonstrated in genetic studies, but their biochemical structural characteristics are yet to be deciphered (Lindsey 2001), and it is still debated whether ENOD-40 should be classified as a signaling peptide or an allosteric regulator of sucrose synthase (Germain et al. 2006). 5 kDa rapid alkalinization factor (RALF)-like peptides have also been isolated from tobacco, tomato, and alfalfa leaves and found to perform a negative regulatory role in the growth and development of lateral roots and pollen tube elongation (Pearce et al. 2001b; Murphy and De Smet 2014). RALF peptides monitor cell division in the pericycle and initiate the development of lateral roots by mediating a temporal increase in cytoplasmic calcium ion concentration with concomitant effect of rapid increase in alkalinity of extracellular space. The extracellular alkaline pH is picked up by cell surface receptors mitogen-activated protein kinase (MAPK) which signals the DNA in the nucleus to halt root development through the (Raf/MEK/ERK) pathway (Murphy and De Smet 2014). Other plant peptide growth factors not described in details in this chapter are POLARIS (for root growth, leaf vascular patterning, auxin and ethylene transport) (Casson et al. 2002) and inflorescence deficient in abscission (IDA) peptides which are responsible for floral abscission and cell separation during lateral root development (Matsubayashi 2014).

1.5.2 Foliar Development and Influence on Photosynthesis

The Devil/Rotundifolia (DVL/ROT) family of peptides have been shown to play a significant role in the proliferation of leaf cells and development of socket cells and trichomes. To date, about 24 DVL/ROT peptides which are between 41 and 145 amino acids have been discovered (Czyzewicz et al. 2013). These peptides have been shown to be nonmobile and do not use the usual plant secretary pathway (i.e., via endoplasmic reticulum-Golgi apparatus); thus, the signaling mechanism of these peptides is not fully understood (Germain et al. 2006; Valdivia et al. 2012). A few distinct peptides involved in plant signaling pathways are listed in Table 1.2.

Table 1.2 Peptides with phyto-signaling functions

Name of peptide families	Number of amino acids, sequence, and/or molecular weight	Receptor(s)	Function	Reference
Root meristem growth factor (RGF1)	Asp-Tyr(SO_3H)-Ser-Asn-Pro-Gly-His-His-Pro-Hyp-Arg-His-Asn	Unknown	Maintenance of root stem cells	Matsuzaki et al. (2010)
CLE-RS	Arg-Leu-Ser-Hyp-Gly-Gly-[Ara3] Hyp-Asp-Pro-Gln-His-Asn-Asn	Hypernodulation aberrant root formation (HAR1) rector kinase	Controlling of root nodulation	Okamoto et al. (2013)
Phytosulfokines	Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-OH)	PSKR1 and PSKR2	Proliferation of plant cells; control of immune system in response to pathogens	Matsubayashi and Sakagami (1996)
PSY1	Asp-Tyr(SO_3H)-Gly-Asp-Pro-Ser-Ala-Asn-Pro-Lys-His-Asp-Pro-Gly-Val-[Ara_3] Hyp-Hyp-Ser	PSYR	Proliferation and expansion of cells	Oh et al. (2018)
Systemin	18, Ala-Val-Gln-Ser-Lys-Pro-Pro-Ser-Lys-Arg-Asp-Pro-Pro-Lys-Met-Gln-Thr-Asp	Systemin receptor 160 (SR160)	Wound response	Pearce et al. (1991)
IDA	*E*xtended *p*roliferating cell nuclear antigen *i*nteracting *p*rotein (EPIP) domain oligopeptide	HAE and HSL2	Lateral root development and control of floral abscission	Matsubayashi (2014)
RALF-like peptides	5 kDa polypeptide; 49 amino acids	Unidentified	Cell expansion	Czyzewicz et al. (2013)

(continued)