PREFACE

The word auxin has a Greek origin: auxein means to enlarge or to grow. Auxins are an important category of plant hormones, regulating many aspects of plant growth and development throughout the plant cell cycle, from cell division, cell elongation and vascular differentiation to root initiation, somatic embryogenesis, apical dominance, tropistic responses, flowering, fruit ripening, senescence, parthenocarpy, dormacy and pollen maturation. Common purification procedures for the determination of auxins include column chromatography, solid phase extraction, liquid-liquid and mass spectrometric detection. This book discusses the production, biosynthesis and role of auxin in plant development. The topics include the effects of inoculation of auxin producing bacteria on root development in model plants and semihardwood olive cuttings; auxin's role in plant defense; auxin's influence on plant developmental responses during salinity; the history, synthesis, transport, determination, metabolism, properties and physiological effects of auxins in plant growth; and the inclusion compounds of auxins in cyclodextrins.

Chapter 1 – Organic cultivation has nowadays spread out around the world owing to its environment preservation, contrary to the overuse of pesticides and fertilisers. Plant growth-promoting bacteria (PGPB) produce indole-3 acetic acid (IAA), a plant hormone implied in root development. In this study, several bacterial isolates were selected by their *in vitro* plant growth promoting features and evaluated for their effects on rooting of model plants. Both supernatants and cells were tested independently on *Vigna unguiculata* cuttings. While low concentrations of IAA produced stimulating effects on the root elongation, a higher number of short adventitious roots were displayed by cuttings inoculated with highly IAA producers. The selected strains inoculated in semi-hardwood cuttings of four olive cultivars (arbequina, hojiblanca, picual and koroneiki) showed different rooting abilities, and innocuous effects on plant after one year. The successful effectiveness of olive tree propagation under real nursery conditions shown by the selected PGPB, together with efficiency, ecology and plant harmlessness in a long-term confirmed its suitability in organic farming system and broaden the field of application of this method into low rooting ability cultivars and even in direct competition with the conventional propagation method.

Chapter 2 – Auxin is a well-known phytohormone that plays important roles in several physiological processes in plants. Currently, many studies have indicated that multiple hormonal signals, including auxins, are implicated in plant defense signaling pathways, but the roles of these signals in plant defense remain unclear. These phytohormones regulate the gene expression of plant defense and eventually trigger the production of defense molecules, such as phenylpropanoids, phytoalexins and pathogenicity-like proteins (PR). Studies of defense signaling against root-knot nematode invasion in resistant soybean genotype suggest that auxin and gibberellin interactions modulate ROS levels and include the participation of DELLA-like proteins, thereby possibly controlling plant immune and stress responses. Auxins promote the degradation of a family of transcriptional repressors known as Auxin/Indol-3-acetic acid (Auxin/IAA). These repressors bind to auxin repressor factors (ARFs) and inhibit the transcriptional-specific auxin response genes. Most of the total auxin in plants is found in the conjugated form and the formation of auxin conjugates is an important regulatory mechanism in the activation and inactivation of IAA. To regulate plant growth and development, auxin induces the expression of three groups of genes: Aux/IAA, *GH3* and small auxin-up RNA (SAUR) families. Auxin responsive *GH3* genes have been shown to play important roles in plant defense response in *Arabidopsis thaliana* and rice. Recently, it was shown that GH3.5 acts as a bifunctional modulator in both SA (salicylic acid) and auxin signaling during pathogen infection. *A. thaliana* auxin mutants evaluated against *Pseudomonas syringae* had compromised systemic immunity, demonstrating that indole-derived compounds, but not auxin itself, are involved in the maintenance of the systemic immunity. Furthermore, it is suggested that the repression of auxin signaling through either mutations in the auxin pathway or pharmacological interference with the auxin response impairs resistance to the necrotrophic fungi *Plectosphaerella cucumerina* and *Botrytis cinerea*. This chapter describes the influence of auxin on plant responses to stress and defense reactions, including the establishment of effective immunity in the plant-pathogen interaction and the interplay of auxin and other plant hormones.

Chapter 3 – Plants integrate intrinsic developmental programs and quickly adjust growth to environmental stimuli. Plant growth and development largely depend on the phytohormone auxin which exerts its function throughout partially redundant F-box receptors, TIR1-AFBs. Several studies using pharmacological and genetic approaches demonstrated that auxin response is down-regulated under salinity. However, how auxin regulation impacts on growth and developmental programs is still under study. Developmental plasticity of roots is modulated by auxin and also is critical for tolerance to stress. Reasonably, plant acclimation to salinity could involve root growth reprogramming depending on the intensity of the stress whereby plant growth is redirected to diminish stress exposure. It is hypothesized that stress-induced morphological responses (SIMR) are mediated by auxin-mediated redox metabolism. However, one emerging question is how salinity transduces stress signals to regulate root plasticity during plant acclimation. The entwined mechanisms involving miRNAs and Salt Overly Sensitive (SOS) pathway in the regulation of auxin homeostasis will be also discussed.

Chapter 4 – The word auxin has a Greek origin: *auxein* means to enlarge or to grow. Auxins are an important category of plant hormones, regulating many aspects of plant growth and development throughout the plant cell cycle, from cell division, cell elongation and vascular differentiation to root initiation, somatic embryogenesis, apical dominance, tropistic responses, flowering, fruit ripening, senescence, parthenocarpy, dormacy and pollen maturation. Common purification procedures for the determination of auxins include column chromatography, solid phase extraction, liquid-liquid and mass spectrometric detection. The four naturally occurring (endogenous) auxins are indole-3-acetic acid (IAA), 4-chloroindole-3-acetic acid, phenylacetic acid and indole-3-butyric acid (IBA); only these four were found to be synthesized by plants. Synthetic auxin analogs include 1-naphthaleneacetic acid (α-NAA), 2,4-dichlorophenoxyacetic acid (2,4-D) and many others. The most important member of the auxin family is IAA. Most of the IAA produced within plants is conjugated to other compounds to form esters, amides or glycosyl esters. The most commonly occurring IAA-conjugates are indole-3-acetylaspartic acid (IAAsp) and a range of IAA glucose esters (IAAGlu). Conjugation seems to be a mechanism for storing IAA in cells, stabilising the level of free auxin in the plant, and metabolising its excess. Auxin in conjugated molecules is protected from oxidative breakdown and may be released again through the action of enzymes. Natural and synthetic auxins are low-molecular weight, organic substances, containing either an indole or an aromatic ring. They are crystalline and only slightly soluble in water, but readily soluble in organic solvents (ethanol, methanol, acetone, diethyl ether and dimethyl sulphoxide, DMSO) or, as weak acids, in alkaline watery solutions. In intact plants, the highest level of IAA occurs predominantly in or near the apex and IAA is transported basipetally; thus, IAA concentration decreases from the top to the bottom. The above is especially true for monocotyledons, whereas in dicotyledons the highest concentration of IAA occurs in the subapical zone, which also grows most rapidly. Auxin is also abundant in young leaves, floral organs and developing fruits and seeds. IAA is photo-oxidized rapidly (50 % in 24 h) while IBA is oxidized slowly (10 %) and NAA is very stable. IAA concentrations are high in young, fast growing organs and decline with age. The indole amino acid, tryptophan (Trp) is the precursor of IAA. There are several possibilities as to how plants may convert Trp into IAA: via indole-3-pyruvic acid, via indole-3-acetaldoxime and *via* tryptamine. Auxins exhibit active transport in a polar manner in addition to long-distance movement via vascular tissues.

Chapter 5 – The low aqueous solubility of the auxins causes major problems in their administration. In order to overcome these problems water soluble salts of auxins are used or auxins are dissolved in ethyl alcohol (or another organic solvent). It has been shown that ethyl alcohol may have modifying effects in auxin action even at low concentration (Allerup, 1962). In addition, the alcohol evaporates during use, making the solution more and more concentrated and thus stunted or abnormal growth is observed.

An alternative way to increase the water solubility of the auxins is via their encapsulation inside suitable amphiphilic molecular capsules. Cyclodextrins (CDs) are oligosaccharides consisting of six, seven or eight α-(1→4)-linked D-glucopyranose units called α-, β- or γ-CD respectively, having a toroidal shape with a lipophilic inner cavity and a hydrophilic outer surface. As a result of their molecular structure and shape, they possess a unique ability to act as molecular containers by entrapping guest molecules in their hydrophophic cavity and thus exerting a profound effect on the physicochemical properties of guest molecules (Del Valle, 2004). Moreover, due to the weak non-covalent interactions between the host and the guest molecules, these supramolecular complexes are not fixed or permanent but rather in a dynamic equilibrium with their free components in solution, thus offering a slow release of the guests and sustaining their action.

CDs are used in agriculture, food, pharmaceuticals, cosmetics, environment protection and it is generally acceptable that the inclusion complex formation of auxins with CDs could improve their bioavailability, enhance their stability and reduce considerably the side effects following administration. The low cost, biocompatible and effective degradation makes β-CDs the best candidates for such molecular encapsulants. Commercial available derivatives of β-CDs are also very interesting because of their increased aqueous solubility and inclusion capacity.

In this chapter, studies on the molecular encapsulation of eight auxins (1-NAA, 2-NAA, 4-CPA, MCPA, 2-NOA, IBA, 2,4-D and 2,4,5-T) have been extensively reviewed. The molecules that have been used as hosts are mainly native β-CDs, methylated or hydroxypropyl β-CD derivatives and in a few cases α- or γ-CDs. The combined use of different analytical techniques has provided a complete characterization of the inclusion complexes. The structures of some of these complexes have been accurately determined in crystalline state by X-ray crystallography. NMR, spectrofluorometric and phase solubility studies have given solid evidences about the formation of the complexes in solution and estimations of the binding constants. DSC and FT-IR studies have checked the formation of a true solid inclusion compound and they have determined the stoichiometry of the formed complexes in the solid state.

This inclusion complexation could be regarded as an important step in the design of novel formulations of auxins with enhanced chemical properties and the gathered results can be regarded as an essential step to the development of controlled release agricultural formulations containing auxins. In addition, the inclusion of the auxins inside the CDs' hydrophobic cavity can be regarded as a simple prototype for ligand-enzyme systems, involving mainly van der Waals and hydrophobic interactions and therefore their structural study can provide some insight about the auxins binding into the pockets of the protein receptors.