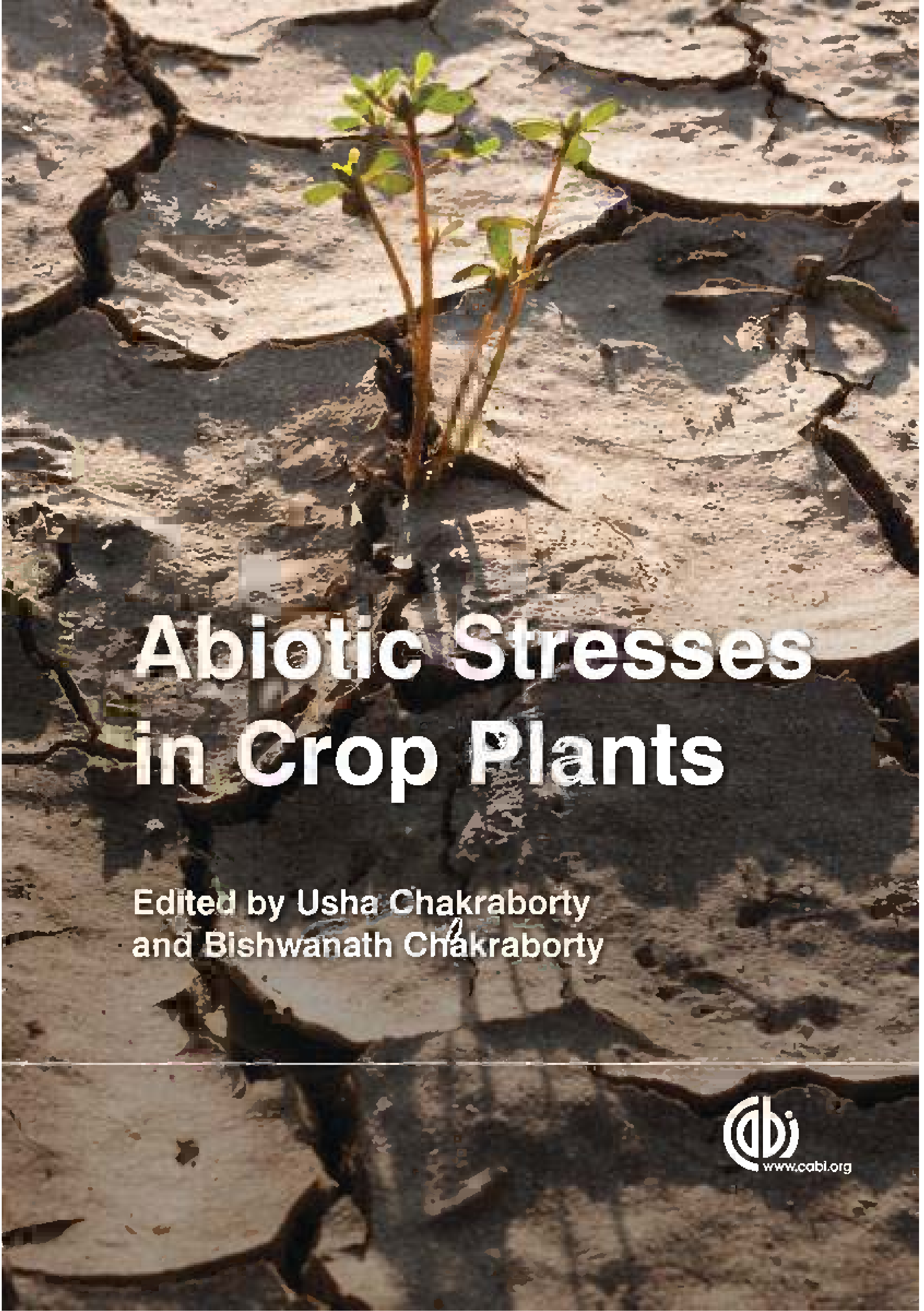

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Edited by Usha Chakraborty
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A catalogue record for this book is available from the British Library, London, UK.

Library of Congress Cataloging-in-Publication Data

Abiotic stresses in crop plants / edited by Usha Chakraborty and Bishwanath Chakraborty.
pages cm
ISBN 978-1-78064-373-1 (hbk : alk. paper) 1. Crops--Effect of stress on. 2. Crops--Physiology. I. Chakraborty, Usha. II. Chakraborty, Bishwanath. III. C.A.B. International.

SB112.5.A24 2014
631.5'82--dc23

2014011777

ISBN-13: 978 1 78064 373 1

Commissioning editor: Sreepat Jain
Editorial assistant: Emma McCann
Production editor: Shankari Wilford

Typeset by SPi, Pondicherry, India.
Printed and bound in the UK by CPI Group (UK) Ltd, Croydon, CR0 4YY.

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Introduction

The existence of life on earth depends on the interaction of environment and living organisms and unless this is maintained at a steady balance this whole existence is at risk. Fast changing environment, increasing population, urbanization and a multitude of related factors affect food productivity by plants – the main food factory of the earth. What is of major concern is the ever-increasing population, projected to be around 9.2 billion by 2050, making demands on food production on the one hand, coupled with decreasing crop productivity on the other. In this scenario, looking for ways and means of maintaining sustainable food production seems a daunting task. Abiotic stresses, mainly due to changing climatic conditions, provide the main challenge to sustainable agriculture. Plant abiotic stressors include: fluctuating temperatures, from very low to extremely high; water level shifts, ranging from water scarcity to flooding; excessive soil salinity, caused in part by prolonged use of irrigation water and low quantities of rainfall, combined with rising saline groundwater levels; heavy metals and other pollutants in soil and air etc. Fortunately for life on earth, many plants are resilient and have developed degrees of tolerance to such stresses. The major thrust for increasing food productivity would be to accelerate such tolerance or resistance mechanisms in the plant at physiological, cellular or molecular levels, leading to improved crop health. However, sufficient caution has to be exercised while dealing with the intricate molecular mechanisms in the plant, as interference in nature's mechanisms may sometimes be counter-productive. To this end, scientists across the globe are working on developing tools for engineering enhanced resistance of plants against abiotic stresses with subsequent increase in productivity.

This book is a compilation of articles that focus on the above problem and will give an overall perspective on the current progress being made in the area of abiotic stresses and their management for sustainable agriculture. The 15 chapters in the book are divided into three sections: Temperature, water and salinity stress; Heavy metals and ozone; and General abiotic stresses and their alleviation by microbes.

The section on temperature, water and salinity stress covers seven chapters and occupies the bulk of the book. All the three major abiotic stresses have been clubbed together as there is a definite interrelationship among all three. Elevated temperatures can lead to rapid water loss, which in turn leads to drought conditions. Similarly, excess salinity also reduces available water to the plant, leading to symptoms related to water deficiency. The first chapter in this section deals with heat-shock proteins (Hsps), which show accelerated synthesis and accumulation in eukaryotes immediately following hyperthermia and confers thermotolerance as well as the capacity to withstand subsequent exposure to lethal temperature and other metabolic insults.

Many of the Hsps on the other hand, are molecular chaperones with vital functions in metabolic pathways, signal transduction, cell proliferation, differentiation and apoptosis under permissive growth conditions. Understanding the role of Hsps in thermotolerance can lead to development of strategies for induction of heat tolerance in plants. The reproductive phase in crops is particularly vulnerable to heat and drought stress and their combination, and in the second chapter the authors discuss how the interplay between leaf senescence, oxidative stress and sugar signalling in reproductive tissues contributes towards reduction in growth and yield in heat-stressed plants.

Both an excess and a deficit of water are abiotic stressors. Three chapters are devoted to this specifically. Chapter 3 will detail our understanding of the roles of nitric oxide, ethylene and haemoglobin in flooding stress and consider how this can be exploited in breeding programmes and sustainable agricultural practice. Nitric oxide (NO) has been shown to trigger the biosynthesis of ethylene during stress and also play key roles in programmed cell death and the hyponastic response. It is discussed as to how the expression of non-symbiotic haemoglobins which oxidize NO to NO_3 play an important role in controlling NO production and thus ethylene-mediated responses to submergence. In Chapter 4, the authors focus on the defence mechanisms against stresses at the molecular level, with special reference to oxylipin metabolism, which according to the authors, represents one of the main defence mechanisms employed by plants. One of the members of this family, jasmonic acid, is well known to be involved in resistance to both abiotic and biotic stresses. Authors have taken the specific example of chickpea hybrids to illustrate the roles. In Chapter 5, the authors discuss how, in contrast to conventional breeding techniques, genetic engineering offers a fast and efficient tool to produce drought-resistant and drought-tolerant plants and thus improved water uptake, use and retention by plants. In order to genetically manipulate plants to be drought tolerant or resistant, genes from the plants that are tolerant or even from other organisms can be selected, which can be grouped into three drought-tolerance engineering strategies: the engineering of functional proteins, manipulating the expression of transcription factors and the regulation of signalling pathways involved in drought tolerance. Chapters 6 and 7 deal with salinity. In Chapter 6, the authors provide an overview of the physiological, biochemical and molecular mechanisms underlying salt tolerance, combining knowledge from classic physiology with information from recent findings. Special emphasis has been given on salt signal perception and transduction and mechanisms related to maintenance of osmotic, ionic, biochemical and redox homoeostasis in salt-stressed plants. A fundamental biological knowledge in conjunction with the understanding of the salt-stress effects on plants is necessary to provide additional information for the dissection of the plant response to salinity and in trying to find future applications for reducing the deleterious effects of salinity on plants, improving the productivity of species important to agricultural sustainability. In Chapter 7, based on results from sugarcane, the authors discuss the results that indicate that the salt tolerance of a variety depends on the stage of development and the level considered. Consequently, salt tolerance of a given cultivar at whole-plant level does not guarantee salt tolerance of tissue or cell cultures issued from this cultivar.

The section on heavy metals, ethylene and ozone consists of four chapters, which deal with the negative effects of heavy metals and air pollutants. Chapter 8 deals with ozone phytotoxicity caused mainly because of its high oxidation potential to generate reactive oxygen species in exposed plant tissue. The balance between the production and the scavenging of activated oxygen is crucial to plant growth maintenance and overall environmental stress tolerance. While increased accumulation of plant secondary metabolites in leaves in response to ozone exposure has been reported, the changes on crop plants' composition and nutritional quality needs to be further studied and discussed to guide our efforts to select ozone-tolerant crops in an attempt to provide a secure food supply for a developing world. Chapters 9 and 10 deal with heavy metal toxicity including cadmium and arsenic among others. In Chapter 9, the authors have mainly focused on the interactive role of ethylene, sulfur, antioxidant system and tolerance of cadmium in plants. Ethylene is the gaseous plant hormone and is now considered to regulate many plant developmental processes throughout the plant's life from germination to senescence and also mediate the plant's responses to abiotic and biotic stress. The basic mechanisms and functional genomics perspective underlying heavy metal toxicity in plants, knowledge of which is essential for development of sustainable agriculture, are dealt with in Chapter 10. Several genetic studies have revealed major signalling pathways that are interconnected and lead to multiple responses in plants under heavy-metal stress. Functional genomics is now considered as an important dissecting tool to understand heavy-metal toxicity as well as tolerance in plants. In Chapter 11, the author has dealt with the negative effects of arsenic, which is a naturally occurring highly toxic metalloid to all forms of life, taking the example of the growth and metabolism of cereals and pulses. Combined application of phosphate with arsenate can ameliorate the damaging effects caused by arsenate treatment alone in cereal and legume seedlings. Hence, the use of phosphate-enriched fertilizers in arsenic-contaminated soil may help normal growth of cereals and legumes.

In the final section, which deals with abiotic stresses in general and their alleviation by microbes, four chapters have been included. In Chapter 12, the authors have focused mainly on recent information about the effects of abiotic stress on plant growth, water relations and photosynthesis, as well as mechanisms of adaptation. The higher acclimation capacity, and hence greater resistance to a given stress factor, is determined by the plant's capacity to maintain its physiological processes within the reaction norm, at a greater variation of this factor. Chapter 13 deals with small molecules such as polyamines, which may play a definitive role in protective or adaptive mechanisms that combat the potential stress-induced injuries in plants encountering abiotic stresses regularly under natural conditions apart from abrupt natural calamities for which the plant may not be prepared. Moreover, it is apprehended that PA-ROS-mediated signalling under stress may have a cross-talk with the phytohormones, figuring a further complex network of signalling for stress tolerance, analysis of which will be a challenging task in near future. The last two chapters deal with a recent, ecofriendly, cost-effective mechanism for stress alleviation through the use of beneficial soil microbes. Chapter 14 deals with the potential of *Trichoderma harzianum* to directly increase plant tolerance against abiotic stresses, such as drought, salinity and soils with low fertility, though traditionally it has been successfully used for the biological control of many plant pathogens through chemiotropic mycoparasitic interactions with the target fungal or bacterial organism. This could promote a rational and non-empirical inclusion of this important fungal species in modern agricultural sustainable practices. The possibility that soil microorganisms could play a significant role in evolving efficient low-cost technologies for abiotic stress management has been dealt with in Chapter 15. Their unique properties of tolerance to extremities, their ubiquity, genetic diversity and their interaction with crop plants can be exploited in order to develop methods for their successful deployment in agricultural production. Soil microorganisms can help crops withstand abiotic stresses, such as drought, chilling injury, salinity, metal toxicity and high temperature, through different mechanisms such as the induction of osmo-protectants and Hsps etc. in plant cells more efficiently. This ability in alleviating abiotic stress conditions in different crop systems can be used for cost-effective sustainable agriculture.

We have endeavoured to compile this book taking a holistic approach from basics to advanced technologies, with the main objective being to put together sufficient information on how to take forward sustainable agriculture in the face of mild to extreme environmental changes occurring in nature. The whole book is well focused and offers insights into the various factors reducing crop productivity and highlights different mechanisms of resistance and approaches that could be used in sustainable agriculture. The editors and authors hope that this book will be of use to agricultural scientists, the agro-industry, academicians and researchers working in the area of abiotic stress and its management.

We would like to thank all the authors who responded in time, which made it possible to bring out this book within the prescribed time. Finally, it is our pleasure to thank CABI for making this possible. Special thanks are also due to Dr Sreepat Jain, Commissioning Editor, CABI and Emma McCann, Editorial Assistant, CABI for their involvement at various stages of publication.

Usha Chakraborty
Bishwanath Chakraborty

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14 Indirect and Direct Benefits of the Use of *Trichoderma harzianum* Strain T-22 in Agronomic Plants Subjected to Abiotic and Biotic Stresses

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Abstract

Biological control of several plant diseases has been successfully achieved by the use of *Trichoderma harzianum* strain T-22, which acts through chemiotropic mycoparasitic interactions with the target fungal or bacterial organism. Since this strain can colonize the roots of most plant species across a wide range of soil types, it is particularly important for agronomic purposes. On the other hand, the study on the effect of T-22 or its derived substances against plant viruses (e.g. Cucumber mosaic virus – CMV) and the pathogenic and molecular aspects involved in this kind of three-way cross-talk between the plant, virus and antagonist are very little known. Besides the use of T-22 as a biocontrol agent, it has been reported that this fungus can also directly improve root growth and plant development in the absence of pathogens. Several mechanisms have been proposed for this, such as production of some unidentified growth-regulating compounds by the fungus, the increased availability of nutrients for plants and induction of certain root morphological changes. All these findings indicate the versatility through which T-22 can directly increase plant tolerance against abiotic stresses, such as drought, salinity and soils with low fertility. In spite of their theoretical and practical importance, the mechanisms responsible for the growth response due to the direct (growth-promoting) and indirect (antipathogenic) actions of T-22 in agronomic plants have not been investigated extensively. This chapter, based on the most significant and updated studies published in the last years by our research group, aims to contribute to a better understanding of the fundamental biochemical and physiological aspects of the antipathogenic and plant growth-promoting activities of T-22 on some economically important crops. This could promote a rational and non-empirical inclusion of this important fungal species into modern agricultural sustainable practices.

14.1 Introduction

Plant life on emerged land has made been possible by the symbiosis between plants and related microorganisms. Mycorrhization is the demonstration of the importance in establishing symbiosis between root system and some microorganisms, which makes possible an enduring protection of cultivated plants and a better use of nutrients, so improving plant tolerance to diseases. It is a symbiotic relationship between the mycelium of a fungus

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and the roots of a plant (Lynch, 1990). In soils, numerous microorganisms co-exist in association with plant roots, inducing morphological and physiological changes in the roots in order to promote the adaptability and survival of both symbionts (Rigamonte *et al*., 2010). Some microorganisms live specifically in the rhizosphere or on plant root surfaces, and these can have many effects on plant performance and may also affect plant community structure. The plant root surface is surrounded by a specific microflora, and the microorganisms distributed there have specific roles in the decomposition of organic matter. Diverse substances are secreted and deleterious microorganisms, which could inhibit plant growth, may be suppressed (Hyakumachi and Kubota, 2004). Mycorrhizated plants are generally able to tolerate pathogens and compensate for root damage and photosynthate drain by pathogens because mycorrhiza are able to enhance host nutrition and the overall plant growth. Arbuscular fungi (e.g. *Glomus* spp.) are known to enhance plant tolerance to pathogens but also to abiotic stresses (Hrynkiewicz and Baum, 2012), enhancing photosynthetic capacity and delaying senescence.

The microorganisms that populate soils, as mycorrhizae, endophytes, saprophytes, but also phytopathogens and entomopathogens, represent a good resource in transformation of organic matter, offering products of enormous potential, such as secondary metabolites, antibiotics and catabolic enzymes (Arora, 2003). Among them, some species of bacteria and fungi are effective also as biocontrol agents (BCAs). These fungal antagonists reduce the growth of plant pathogens by antibiosis, competition and parasitism (Mathivanan *et al*., 2008). They also induce various defence responses in host plants, such as systemic acquired resistance (SAR) and/or induced systemic resistance (ISR). For this purpose, many scientists proposed the use of mycorrhizae associated to biocontrol microorganisms as a solution for increasing plant tolerance/resistance against both biotic and abiotic stresses, for increasing plant productivity in degraded soils and for reducing agricultural environmental impact. The use of microorganisms for biocontrolling plant pathogens has been shown to be very efficacious for some fungi of the genus *Trichoderma*, *Glomus*, *Streptomyces* and some species of bacteria (e.g. *Bacillus subtilis* and *Agrobacterium radiobacter*). In fact, some of these fungi interact with other fungi in a mechanism called mycoparasitism, wherein one fungus directly kills and obtains nutrients from other fungi. Mycoparasitism is one of the most important biocontrol mechanisms (Mukherjee, 2011).

Besides the use of *Trichoderma* as a biocontrol agent, this fungus can directly stimulate root and shoot growth without the presence of pathogens (Sofo *et al*., 2012). This direct effect could be due to some growth-regulating compounds produced by the fungus, the increased availability of nutrients for plants, and some induced change in root morphology. All these findings indicate the versatility through which *Trichoderma* can directly increase plant tolerance to different kinds of abiotic stresses. In the context of plant defence by biotic stresses, understanding biochemical and molecular mechanisms deriving from the host–pathogen–*Trichoderma* interaction is without doubt essential for investigating the dynamics of infectious processes. This knowledge could be also useful for the development of new strategies for controlling phytopathogens, particularly viruses, against which chemical treatments have no effect.

Thanks to recent studies, it is now possible to develop new strategies based on the use of peptaibols, a class of linear peptides biosynthesized by many species of *Trichoderma* (Daniel and Filho, 2007). Trichokonins, which are antimicrobial peptaibols isolated from *Trichoderma pseudokoningii* SMF2, have been reported to induce tobacco systemic resistance against tobacco mosaic virus (TMV) through activation of multiple plant defence pathways. This is based on an elicitor-like cellular response, i.e. enhancement of production of superoxide anion radical and peroxide in tobacco plants and also enhancement of enzymes such as peroxidase (POD), which are involved in resistance, up-regulation of antioxidative enzyme genes known to be associated with the reactive oxygen species (ROS) intermediate-mediated signalling pathway, and of salicylic acid (SA)-, ethylene (ET)- and jasmonic acid (JA)-mediated defence pathway marker genes (Luo *et al*., 2010). This finding implies the antiviral potential of peptaibols, supporting the hypothesis of using them as biocontrol antiviral agents. Therefore, *Trichoderma* spp., already used as BCAs against bacterial and fungal phytopathogens, could be advantageously used also against viruses. Considering the theoretical and practical importance of the broad range of mechanisms responsible for the growth response due to the direct (growth-promoting) and indirect (antipathogenic) actions of *Trichoderma*, these need to be investigated in more detail.

This chapter, based on the most significant and updated studies published in the last years, aims to contribute to a better understanding of the fundamental biochemical and physiological aspects of the antipathogenic and plant growth-promoting activities of *Trichoderma* on some important economically important crops. In particular, the strain T-22 of *T. harzianum* is of key importance because it is often used as active ingredient in many commercial biocontrol products. This could promote a rational and non-empirical inclusion of this important fungal species in modern agricultural sustainable practices.

14.2 The Genus *Trichoderma harzianum* Strain T-22

The filamentous ascomycetous fungi *Trichoderma* spp. are abundant and present in many soil types. These fungi are able to infect plant roots, invading the first or second layers of cells of the root epidermis (Harman *et al.*, 2004a). *Trichoderma* spp. show a number of different activities between strains (Harman *et al.*, 2004b). They are rarely associated with diseases of living plants (Gams and Bissett, 2002). On the contrary, many *Trichoderma* species (e.g. *T. harzianum*, *T. viride*) are used as BCAs by antagonizing many plant pathogenic fungi. Indeed, approximately 60% of all commercial biocontrol formulations are based on *Trichoderma* (Verma *et al.*, 2007).

By working as a deterrent, T-22 protects the roots from the assault of pathogenic fungi (e.g. *Fusarium*, *Pythium*, *Rhizoctonia* and *Sclerotinia*). Establishing itself in the rhizosphere, T-22 can grow on the root system, along which it establishes a barrier against pathogens. As long as the root system remains active, T-22 continues to grow, feeding on the root exudates and subtracting the nutrients that the pathogens use to feed (Tataranni *et al.*, 2012).

Biocontrol studies have confirmed the effectiveness of *Trichoderma* spp. in plant protection not only against many pathogenic fungi (Akrami *et al.*, 2011), but also bacteria (Segarra *et al.*, 2009) and viruses (Luo *et al.*, 2010), probably due to the induction of hypersensitive response (HR), systematic acquired resistance (SAR) and induced systematic resistance (ISR) (Kaewchai *et al.*, 2009). However, the antiviral effects of *Trichoderma* spp. and the associated biochemical and molecular mechanisms implicated are still scarcely known. Plant resistance induced by *Trichoderma* spp. at a molecular level is due to the release of specific defence metabolites and enzymes, such as: (i) phenyl-alanine ammonia-lyase (PAL) and chalcone synthase (CHS), involved in the biosynthesis of phytoalexins (HR response); (ii) chitinases and glucanases, that include pathogenesis-related proteins (PR) and SAR response; and (iii) other enzymes involved in the response to oxidative stress (Benítez *et al.*, 2004).

It was demonstrated that T-22 improves growth in maize plants, increasing root formation (size and area of main and secondary roots) and, at the same time, enhancing crop yields, tolerance to drought and resistance to compacted soils (Harman, 2000; Harman *et al.*, 2004c). This improved plant growth was probably due to direct effects on plants because of a better solubilization of soil nutrients or by a direct enhancing plant uptake of nutrients linked to the presence of T-22 in the agroecosystem (Yedidia *et al.*, 2001). More recently, it was demonstrated that plant overall morphology and metabolism of plant colonized by T-22 caused enhanced root growth and suberification (Sofo *et al.*, 2011, 2012) and the induction of the synthesis of antimicrobial phenolic compounds (Mathivanan *et al.*, 2008). The enhanced plant growth due to T-22 was confirmed also in terms of total biomass and root development, not only in herbaceous plants but also in tree species (Sofo *et al.*, 2010). Furthermore, the beneficial effects of T-22 application depend on the treated plant genotype, as recently demonstrated by Tucci *et al.* (2011) on tomato plants.

Although the capability of *Trichoderma* spp. to alleviate the effects of various abiotic stresses on plants is recognized, an understanding of the mechanisms that control the factors implied in the specific plant stress are still missing. Using T-22 in organic management systems can surely improve plant physiological status using a holistic approach that adopts specific practices for promoting plant defence mechanisms, such as tolerance and/or resistance to pathogens (Woo *et al*., 2006).

14.3 Abiotic and Biotic Stresses in Plants

Plant growth and productivity are affected by various environmental stresses to which plants are subjected during their lifespan. Due to their sessile conditions, plants cannot avoid these stresses and must have strong defences to face them. Indeed, molecular, biochemical, physiological and morphological characteristics of plants are markedly affected by the exposure to abiotic and biotic stresses. The activation of induced defence in plants is mediated through the synthesis of molecules with signal functions acting as hormones or stimulators of plant growth and development (Vitti *et al*., 2013). Among phytohormones, a prevailing role in biotic stress signalling is played by SA, JA and ET, while abscisic acid (ABA) plays a role in the response to some abiotic stresses such as drought, low temperature and osmotic stress (Fraire-Velázquez *et al*., 2011).

For example, it is known that SA-induced resistance to viruses in tobacco and *Arabidopsis thaliana* is partly mediated by a pathway involving signals transduced through changes in reactive oxygen species (ROS) in the mitochondria (Singh *et al*., 2004). In fact, SA impedes electron flow through the respiratory electron transport chain and enhances ROS levels in the mitochondria (Mayers *et al*., 2005). Resistance to TMV is altered in transgenic tobacco plants with altered levels of alternative oxidase (AOX), an enzyme that negatively regulates mitochondrial ROS levels (Gilliland *et al*., 2003). In *A. thaliana*, as in tobacco, SA treatments inhibited the systemic movement of another virus, cucumber mosaic virus (CMV). At the same time, in squash, SA induced resistance to CMV and this was most likely due to inhibition of viral cell-to-cell movement. This means that the mechanisms of SA-induced resistance may differ markedly between host species (Mayers *et al*., 2005). ROS are important second messengers in the responses of plants to various other biotic and abiotic stresses (Kwon *et al*., 2007; Wahid *et al*., 2007; Miller *et al*., 2010; Torres, 2010).

Recently, Vitti and co-workers (2013) demonstrated that changes in root morphology observed in *A. thaliana* seedlings subjected to both biotic (CMV) and abiotic (excess cadmium) stressors are probably due to modifications in hormonal balances. As shown in Fig. 14.1, in our experience, evident variations

Fig. 14.1. *Arabidopsis thaliana* Columbia ecotype control plants (left), inoculated with CMV (centre) and treated with cadmium (right) observed 12 days after the viral infection or the exposure to cadmium.

occurred in plant growth, in terms of both shoot and root development and also in leaf colour (from green of the control plants to brownish violet of inoculated and, overall, treated plants). Molecular, biochemical, physiological and morphological characteristics of plants are markedly affected also by the exposure to some heavy metals. Indeed, treatments of plants with some metals induce changes in root morphology, caused by a hormonal inbalance, mainly governed by the auxin/cytokinin ratio (Sofo *et al*., 2013).

14.4 Benefits of the Use of *Trichoderma harzianum* Strain T-22 in Stressed Crops

A broad range of genetic traits and environmental conditions are able to affect the complex phenotype of mycorrhizal fungi, as in *Trichoderma* spp., as well as their ecological performance (Buée *et al*., 2009). The interactions between microbes and plant roots are known to have significant effects on plant nutrient condition and tolerance to pathogens (Altomare *et al*., 1999). Many studies included the use of proteomic (Grinyer *et al*., 2005) and functional genomic analysis in the attempt to obtain more information on the changes that occur in the *Trichoderma* spp., plant and pathogen expressomes when they interact with each other, especially when an increase in disease resistance is generated (Woo *et al*., 2006). In a recent study, the dynamics of gene expression in the roots of *Arabidopsis* colonized by *Trichoderma* were investigated, demonstrating that this colonization has induced deep changes in plant transcripts, through plant gene modulation, together with resistance to both biotic and abiotic stresses (Brotman *et al*., 2013).

The mechanism of the interaction *Trichoderma*–plant–pathogen is very complex and includes not only the colonization of rhizosphere and phyllosphere and mycoparasitism, but also antagonism against nematodes, production of extracellular hydrolytic enzymes and secondary metabolites that could be toxic to plant pathogens, as well as induction of systemic resistance against different pathogens' promotion. These *Trichoderma*–plant interactions can also result in better plant growth and root development (Harman *et al*., 2004c; Mathivanan *et al*., 2008). In particular, T-22 is adapted for facing many fungal or bacterial pathogens in a broad range of plant species (Sofo *et al*., 2010; Tataranni *et al*., 2012). Therefore, T-22 is considered a very efficacious BCA for the control of plant diseases.

14.4.1 Benefits of T-22 against abiotic stresses

It has been established recently that the change in phytohormone levels, particularly auxins and cytokinins, is one of the direct mechanisms by which T-22 acts for promotion of plant growth in fruit rootstocks (Sofo *et al*., 2011). Thus, T-22 seems to promote plant growth and development, so acting as a plant growth-promoting microorganism, that in turn determines a higher tolerance of the plants against abiotic stresses (Sofo *et al*., 2011). It was also discovered that soil colonization by T-22 enhances plant growth in terms of total biomass and root development by about 20% and 30%, respectively (Sofo *et al*., 2010).

The cross-talk between the different plant hormones, whose levels change after plant inoculation with T-22, results in synergetic or antagonistic interactions that play crucial roles in the response of plants to abiotic stresses, such as drought, salinity and toxic metals (Baroni *et al*., 2004; Peleg and Blumwald, 2011). An example of this is depicted in Fig. 14.2, where cherry seedlings inoculated with T-22 and subjected to water deficit appear to be more developed than control un-inoculated plants. Thus, it is possible that plant hormones play central roles in the ability of plants to adapt to changing environments by mediating growth, development, nutrient allocation and source/sink transitions.

In a study by Mastouri *et al*. (2010) it was shown that under either biotic stress caused by *Pythium ultimum* or different abiotic stresses

Fig. 14.2. Cherry seedlings (*Prunus cerasus* x *P. canescens*) inoculated with T-22, grown in sterile perlite and subjected to drought stress (right) and control un-inoculated plants subjected to the same degree of drought (left).

such as drought, salinity, elevated or low temperature, treatment of tomato seeds with T-22 led to more rapid and uniform germination in comparison to no treatment.

More recently, the same authors demonstrated that the application of T-22 to tomato seedlings enhanced the tolerance to water deficit by improving the antioxidant defence mechanism (e.g. higher activity of ascorbate and glutathione-recycling enzymes) (Mastouri *et al*., 2012). It is proposed that in addition to the hormonal factors, T-22 allows plants to tolerate abiotic stresses more efficiently by increased root suberification and hardening, as well as acidification of the soil, which would favour the diffusion of cations from the soil to the root against the concentration gradient and an increased availability of some inorganic compounds indispensable for plants (Sofo *et al*., 2012).

14.4.2 Benefits of T-22 against biotic stresses

Biocontrol by T-22 is related to its ability to compete with soil pathogens rather than to its control of plant diseases. Therefore, T-22 does not act by producing compounds that are toxic to the pathogens, but rather by inducing change in the physiology and metabolism of the plants leading to development of resistance to the disease (Harman *et al*., 2008). For that reason, various mechanisms are involved, foremost mycoparasitism and antibiosis (Howell, 2003; Vitale *et al*., 2012). In the case of mycoparasitism, recognition, binding and enzymatic disruption of the target cell wall take place (Woo and Lorito, 2007). On the other hand, inhibition or destruction of the microorganism target by metabolites or by the production of antibiotics able to inhibit their growth (antibiosis) were also observed.

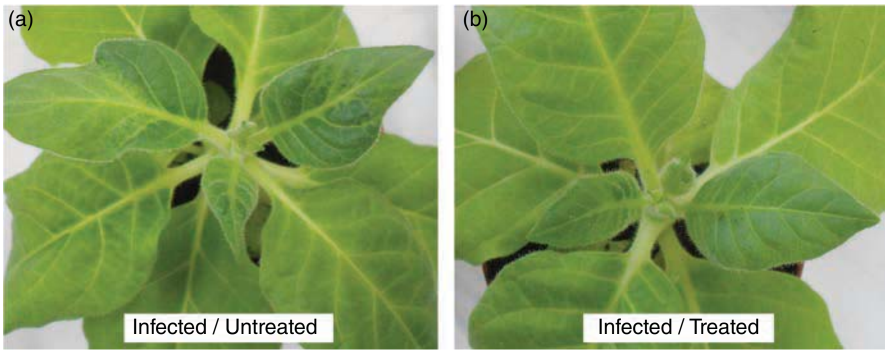

Fig. 14.3. (a) *Nicotiana tabacum* cv. Xanthi infected with CMV (left); (b) the same plant infected with CMV and also treated with *Trichoderma harzianum* T-22 (right).

In such case, antibiotics can stop spore germination (action known as fungistasis) or alternatively destroy the cells (veritable antibiosis) (Benítez *et al*., 2004).

The range of pathogens controlled by T-22 is broad and includes fungi, bacteria and viruses (Harman *et al*., 2004a). Among plant-pathogenic fungi, the following are the most represented: *Botrytis cinerea*, *Fusarium*, *Pythium* and *Rhizoctonia* (Kaewchai *et al*., 2009). The efficacy of *Trichoderma* spp. action is obviously related to the specific interaction between plant–pathogen–antagonist. For example, Vitale and co-authors (2012) demonstrated that T-22 was able to act as a BCA of collar and root rot caused by different *Calonectria pauciramosa* isolates on red clover (*Triflolium pratense*) and, specifically, that the degree of virulence and T-22 effects in controlling infections were highly variable among the isolates tested. In our experience, preliminary results of current studies conducted in our laboratories seem to indicate a potential antiviral activity of T-22 against the infection of CMV, strain Fny, on tobacco plants, as shown in Fig. 14.3, where the plant treated with the fungus does not show the symptoms induced by the virus.

14.5 Conclusion

In T-22-inoculated plants subjected to different types of adverse environmental conditions, comparative proteomics experiments should be carried out to identify specific proteins involved in plant resistance against specific stresses. For this kind of analysis, 2D-electrophoretic cells, protein fractionation and isoelectrofocusing techniques and MALDI-TOF MS are commonly used. Accurate microscopic analyses should be carried out through electron (SEM and ESEM), epifluorescence and light microscopes in order to ascertain T-22 persistence and evaluate their colonization. Finally, comparative proteomics experiments could be of primary importance to identify specific proteins involved in the common response of T-22-inoculated plants to face abiotic stresses.

Plant stresses contribute significantly to crop damage and yield loss. In agriculture, annual crop losses by phytopathogenic microorganisms in the field and also during postharvest exceed €500 billion (Tataranni *et al*., 2012). The balance of beneficial and detrimental effects is reflected in many other areas of agriculture and horticulture. In such a scenario, in modern agro-industry, fungi such as *T. harzianum* strain T-22 offer many established beneficial roles, particularly as biofertilizers, mycorrhizae, and BCAs of pathogens, pests and weeds. In addition to their biocontrol characteristics, T-22 also exhibits plant growth-promoting activity, acting as powerful biostimulants. The utilization of T-22 or other microorganisms as biostimulants can cause a reduction in the use of fertilizers and fungicides in agricultural production, with consequent benefits for the environment. This is necessary to help maintain ecosystems and to develop sustainable agriculture.

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