33 Tolerance to Environmental Stresses

Do Fungal Endophytes Mediate Plasticity in Solanum Dulcamara?

Sasirekha Munikumar, Karaba N. Nataraja, and Theo Elzenga

CONTENTS

33.1	Introduction	497
33.1.1	Conventional Breeding Approach.....	498
33.1.2	Transgenic Approach	498
33.1.3	Plasticity Induced by Endophytes Increasing Ecological Amplitude	499
33.1.3.1	Ecological Amplitude.....	499
33.1.3.2	Phenotypic Plasticity	499
33.1.3.3	Symbiont-Induced Stress Tolerance	499
33.1.3.4	S. Dulcamara – Microbiome – Existing Gap	500
33.1.3.5	Fungal Endophytes	500
33.1.3.6	Salinity – As a Case Study	501
33.1.4	Classification of Plants Based on Their Response to Salinity	501
33.1.5	Salinity Tolerance Mechanism in Plants.....	502
33.1.6	Endophytic Fungi-Mediated Salinity Stress Tolerance.....	502
33.1.7	Possible Mechanism of Salt Tolerance by Endophytes	504
33.1.8	Criteria to Select Potential Endophytic Fungal Isolate	506
33.2	Results	507
33.2.1	In Vitro Screening for Potential Isolates.....	507
33.3	Conclusion	511
	Acknowledgment	511
	References.....	512

33.1 INTRODUCTION

As the area of arable land for the optimal cultivation of food crops is decreasing due to inadequate irrigation management practices, land degradation, sea water level rise and global climate change, there is growing demand for developing stress-tolerant crops,

specifically crops that can withstand saline conditions. Various methods to develop salt-tolerant crops have been used, these include conventional breeding, transgenics, use of plant growth promoting (endophytic) bacteria, mycorrhizal or/and endophytic fungi.

Salt-tolerant crops could be developed by five possible ways: a) exploitation of existing variation present in crops; b) use of interspecific hybridization to improve the salt tolerance of existing crops; c) creating variation by using traditional breeding and genetic modification; d) use of halophytes as alternative crops; and e) breeding for stable yield instead of tolerance (Flowers and Yeo, 1989). While introduction of genes from wild salt-tolerant species has been explored for many plant species such as cereals, this did not lead to the development of many other salt-tolerant crops. As a wide range of halophytes is available, this approach is still being considered for generating salt-tolerant crops (Flowers, 2004).

33.1.1 CONVENTIONAL BREEDING APPROACH

Conventional breeding approaches have been explored to develop salt-tolerant varieties by integrating traits from wild parent species into superior crop varieties. The most notable results of this approach are some salt-tolerant cereals. Since, salinity tolerance is a complex and multigenic trait, this approach has had very limited success, as no commercial varieties have become available so far. The introduction of a gene decreasing Na⁺ uptake by durum wheat is a notable exception (Munns *et al.*, 2012). Identification and validation of important traits contributing to tolerance would be very crucial for trait introgression through conventional breeding. Conventional breeding has several disadvantages as the process is labor-intensive and time-consuming and depends on germplasm collections (Hanin *et al.*, 2016).

33.1.2 TRANSGENIC APPROACH

Genetic-modification techniques have been extensively used to introduce very specific genetic changes into commercial varieties, thereby maintaining the elite traits found in commercial crops. Expression of foreign genes improved the level of salinity tolerance in various plant species (Srivastava *et al.*, 2016). Physiological traits targeted by genetic modification include the plant cell metabolic pathway, for instance, to increase the accumulation level of osmoprotectants when plants are exposed to saline conditions (Carillo *et al.*, 2011), to quench reactive oxygen species (ROS) produced during salinity-induced oxidative stress (Roy *et al.*, 2014), to reduce lipid peroxidation of membranes (Alvarez Viveros *et al.*, 2013) and to protect protein structural integrity and their functioning (Jha, 2019). As Na⁺ uptake and compartmentalization into the vacuole is a critical mechanism for plant survival under salinity stress, most work on developing salt-tolerant crops has been concentrated on ion transporter genes and the genes controlling their activity. Overexpressing genes encoding membrane transporters improved salinity tolerance in many crops (Gupta and Huang, 2014). Also, for the genetic-modification approach, the complex nature of salt-stress tolerance and our limited understanding of the processes or traits involved has been an obstacle for the successful introduction of tolerant crop varieties (Shrivastava and Kumar, 2015).

33.1.3 PLASTICITY INDUCED BY ENDOPHYTES INCREASING ECOLOGICAL AMPLITUDE

33.1.3.1 Ecological Amplitude

The range of conditions a species can survive in is known as the ecological amplitude of that species. Often this range is defined by the limitations imposed by abiotic conditions affecting plant functioning, such as temperature, light and precipitation. The species belonging to a particular ecosystem will have adaptations and responses that have been shaped by the prevailing environmental factors for that ecosystem (Ogbemudia *et al.*, 2018). All land plants possess regulatory mechanisms to respond to suboptimal environmental conditions, such as salinity, high and low temperature and drought. The ability to effectively tolerate stress conditions can differ strongly between species and is associated with the genetic traits a species has (Smallwood *et al.*, 1999). However, inherent plasticity and the association with microbial symbionts can also confer stress tolerance and a wider ecological amplitude.

33.1.3.2 Phenotypic Plasticity

The phenotype of a plant is the result of the interactions between its genotype and environment. Phenotypic plasticity is defined as the variation in phenotypic expression of a genotype in response to different environmental conditions, where it enhances the fitness and reproduction of an individual under those conditions. Plasticity may increase the chances of a population's persistence through time by allowing certain reversible, environment-induced changes in morphology and metabolism, and, thus, increase the geographical range of a species. Most plant species are extremely plastic by nature. In plants, many phenotypic characteristics of a species are strongly influenced by different environmental factors, much more so than in most animal species (Sultan, 1987).

The semi-woody vine, Bittersweet nightshade (*Solanum dulcamara*), has a widespread occurrence in ecologically contrasting habitats, ranging from wetlands to coastal dunes (Dawood *et al.*, 2014). Environmental conditions have a strong influence on many phenotypic characteristics of *S. dulcamara*. It survives under contrasting environments, such as flood-prone and dry habitats, by producing adventitious roots from preformed dormant primordia, and having a high root to shoot ratio, respectively. The plant is being extensively investigated as a model system for adaptive mechanisms, such as phenotypic plasticity and local adaptation, to cope with the contrasting environmental factors (Zhang *et al.*, 2016).

33.1.3.3 Symbiont-Induced Stress Tolerance

To various degrees, plants form symbioses with endosymbionts, which means that they are inhabited internally by diverse microbial communities. These microorganisms with endophytic lifestyles can play crucial roles in plant development, growth, fitness, and diversification (Hardoim *et al.*, 2015). Plants provide a unique ecological niche for diverse communities of symbiotic microbes which, in turn, have various benefits for the plant, such as the promotion of growth, nutrient, water use and photosynthetic efficiency, and adaptation to biotic and abiotic stresses.

Symbiotic fungi may contribute to plant adaptation to environmental stresses (Clay and Holah, 1999; Redman *et al.*, 2002) and thus are crucial to the overall

development of plant communities (Petrini, 1986; Bacon and Hill, 1996; Rodriguez and Redman, 1997). There are two major groups of fungal symbionts known to be associated with plants, mycorrhizal fungi which reside only in roots but extend out into the rhizosphere, and endophytic fungi which reside entirely within the plant tissues and are associated with leaves, stems and roots (Rodriguez *et al.*, 2004). Symbiotic association with mycorrhizal or endophytic fungi can provide fitness benefits to several crop species (Redman *et al.*, 2002; Waqas *et al.*, 2012; Hamayun *et al.*, 2017).

Endophytes isolated from grasses confer stress tolerance to genetically distant plants, such as tomato and rice. Although, the evolutionary divergence of these plants occurred already approximately 400 million years ago, still they depend on their symbionts (Rodriguez and Redman, 2008; Redman *et al.*, 2011). Fungal endophytes, the species that colonize the whole plant are known to confer stress tolerance through habitat adapted symbiosis (HAS) mechanism where plants are proposed to associate with particular endophytes that increase tolerance or resistance to the predominant biotic or abiotic stresses of their habitats. Rodriguez *et al.* (2008) defined this phenomenon by endophytic fungi as an intergenomic epigenetic mechanism for plant adaptation and survival in high-stress environments, indicating that the increased stress resistance and effect of epigenetic changes are induced by the symbiont. Utilizing endophytes to mitigate climate change impacts has been suggested as a potential strategy for expanding agricultural production on marginal lands (Rodriguez *et al.*, 2008).

33.1.3.4 *S. Dulcamara* – Microbiome – Existing Gap

Although *S. dulcamara* with its extremely wide ecological amplitude has been adopted as a model system for acclimation to changing environmental conditions, its associated microbiome and the interaction between plant and microbiome are hardly studied. *S. dulcamara* and its endophyte community were, therefore, chosen in this study as a model for utilization of endophytic services as an adaptive strategy of plants to survive under various abiotic stress conditions. If *S. dulcamara* plants indeed depend on their associated endophytic fungal community to maintain plant fitness under abiotic stress conditions, comparing the endophytic community found in plants from different habitats will provide a much sought-after test of the ‘habitat adapted symbiosis’ hypothesis.

33.1.3.5 Fungal Endophytes

Endophytes are a huge and diverse group of fungi that colonize healthy plant tissues (Wilson, 1995; Pawłowska *et al.*, 2014). They reside in the intercellular spaces beneath the epidermis (Petrini, 1986) and are an enigmatic component of every terrestrial plant community, presenting us with intriguing evolutionary questions, relating to the origin and nature of this symbiosis and to the widespread dependence on symbionts for resistance to quite common stresses. Four major classes of endophytes are being distinguished: 1) the clavicipitaceous endophytes, 2) the non-clavicipitaceous endophytes that colonize the whole plant, 3) the non-clavicipitaceous endophytes that colonize shoots and 4) the non-clavicipitaceous endophytes that colonize roots (Rodriguez *et al.*, 2009). All land plants are known to be colonized by class 3

endophytes which are transmitted horizontally (Davis *et al.*, 2003). The symbiosis of the fungi found as endophytes could be mutualistic, providing fitness benefits, be commensalistic, or be dormant pathogenic, residing in the plants without any positive or negative effects (Lewis, 1985) until the conditions are favorable for disease development (Dodd, 1980). Endophytes may act as pathogens or mutualists by indirectly affecting the host health (Fravel *et al.*, 2003). Endophyte lineages might have evolved from pathogens over time as commensals and as a result now lack the traits that confer pathogenicity.

33.1.3.6 Salinity – As a Case Study

Soil salinization is slowly developing into a major global threat. An increasing percentage of arable land is affected by salinization, due to a rise in sea level and irrigation practices (Sanders, 2020). It is estimated that 1125 million hectares of land are affected by salt, of which, 76 million hectares are affected by human-induced salinization (Hossain, 2019).

In general, salinity limits the productivity in several, economically important crop plants. Munns *et al.* (1995) proposed a two-phase model to describe the osmotic and ionic effects of salinity on plants. High salinity induces rapid osmotic stress and there is also a delayed ionic stress, especially due to a high Na^+ concentration (Gupta and Huang, 2014). Osmotic stress affects various physiological processes, such as a reduction in photosynthetic activity and the production of ROS, by decreasing the water absorption by roots (Munns and Tester, 2008). Ionic stress is caused by an increased accumulation of ions, which affects the metabolic homeostasis (Greenway and Munns, 1980; Zhu, 2001). The osmotic stress effect is characterized by a rapid inhibition of growth rate of young leaves, while the ionic effects typically result in an increased rate of senescence in older leaves (Sekmen *et al.*, 2013). Our knowledge on how a plant senses salt stress is meager. The rate at which the plant senses toxicity differs greatly between salt-tolerant and -sensitive plants. During the first, osmotically induced phase, growth is inhibited in both tolerant and sensitive plants. During the second, ionic toxicity phase, the photosynthetic capacity is reduced and older leaves start senescing in salt-sensitive plants.

Plants experience a wide range of constraints under salinity stress. These constraints include accumulation of sodium ions in the rhizosphere, reduced water availability due to hyperosmotic stress induced by salinity, a rapid accumulation of ROS in plant tissues and alterations in cytosolic Ca^{2+} and K^+ homeostasis. Every constraint could be sensed by either plasma membrane bound or cytosolic sensors, triggering a cascade of events that ultimately leads to acclimation to the stress condition (Shabala *et al.*, 2015).

33.1.4 CLASSIFICATION OF PLANTS BASED ON THEIR RESPONSE TO SALINITY

Based on their tolerance or sensitivity, plants are often classified as halophytes or glycophytes. Halophytes are species that can withstand a salt concentration of about 200mM, using specialized anatomical or morphological adaptations or avoidance mechanisms (Flowers *et al.*, 2010). These mechanisms help plants to survive and maintain growth under unfavorable conditions. Examples of traits found in these

specialized halophytic species are salt glands extruding salt on the leaves (for instance, in *Avicennia marina*, a mangrove species) and well developed Casparian strips with early vacuolation and suberization of the hypo- and endodermis in roots (for instance in *Suaeda maritima*, Seepweed) (Sekmen *et al.*, 2013).

In contrast, glycophytes, evolved in areas with low soil sodium levels, are highly sensitive to salinity and have to maintain low sodium levels in their aboveground tissues by restricting the upward movement of excess ions to the shoot (Cheeseman, 2015). In glycophytic plants, root and shoot growth are already inhibited when exposed to moderate levels of salinity (Greenway and Munns, 1980).

33.1.5 SALINITY TOLERANCE MECHANISM IN PLANTS

Salt tolerance is the complex mechanism which is often controlled by inter-correlated metabolic pathways. It is achieved by detoxification, ion homeostasis and maintenance of growth. First, the detoxification is based on the production of antioxidants and the enzymes responsible for the inactivation of radical oxygen species, such as superoxide dismutase and catalase (CAT). This is followed by the synthesis of compatible osmolytes such as glycine betaine, proline or sugar alcohols to maintain osmotic balance. Ionic homeostasis at the cellular level is accomplished through compartmentalization of excess ions in the vacuole. Finally, the expression of regulatory genes, like transcription factors, help to maintain plant growth at a high salt concentration (McCord, 2000; Zhu, 2001; Flowers and Colmer, 2008; Flowers *et al.*, 2010). Salt-tolerance mechanisms involve the complex interplay between the several genes associated with biochemical and cellular pathways. The up-regulation of several miRNAs is essential for alleviating salt stress (Goswami *et al.*, 2017).

Many salt responsive genes have been identified in plants, and several signaling cascades involved in the response to salinity have been predicted and validated in several model plants. In *Arabidopsis* Na⁺, homeostasis is controlled by the Salt Overly Sensitive pathway (Zhu, 2002; Jamil *et al.*, 2011; Zhang *et al.*, 2012). It is stated that 'salinity tolerance is multigenic and needs to be viewed in a wider context than just that of membrane transport of NaCl' (Sanders, 2020).

33.1.6 ENDOPHYTIC FUNGI-MEDIATED SALINITY STRESS TOLERANCE

Unlike halophytes, glycophytes does not produce any special anatomical structures such as salt glands, or well developed Casparian strips, or suberization of hypo- or endodermis as an adaptive strategy. In order to overcome the effects of salinity, glycophytes have to maintain low Na⁺ levels in the cytosol at the cellular level and low shoot Na⁺ concentrations at the whole plant level. Maintenance of high cytosolic K⁺/Na⁺ ratios in shoots is suggested to be crucial for salt tolerance in glycophytes (Horie *et al.*, 2012; Cheeseman, 2015). In addition to their inherent mechanisms to maintain low sodium levels, glycophytes may also depend on their endosymbionts under saline conditions.

Most of the domesticated crop plants are glycophytes. These might be improved for better adaptation to saline environments with the use of beneficial symbionts. The

microbial approach is considered as one of the environmental-friendly approaches to tackle the salinity issue in sustainable agriculture (Shokat and Großkinsky, 2019).

Studies have shown that inoculation with endophytic fungi enhances salinity tolerance in plants (Rodriguez *et al.*, 2004; Waller *et al.*, 2005; Baltruschat *et al.*, 2008; Khan *et al.*, 2011; 2012; 2013). Endophytes isolated from plants growing in a saline environment have the potential to confer salinity tolerance to other plants via habitat adapted mechanism (Rodriguez *et al.*, 2004). The endophyte *Fusarium culmorum*, FcRed1, isolated from a dune grass, *Leymus mollis* on coastal beach habitats of San Juan Island Archipelago, WA, shown to confer salinity tolerance to dune grass and tomato (Rodriguez *et al.*, 2008). Plants adapted to harsh environmental conditions like sand dunes and salt marshes in Mediterranean ecosystems harbor huge biodiversity of fungal root endophytes. These fungi could protect against the environmental stresses and reduce the symptoms caused by root pathogens (Lopez-Llorca and Macia-Vicente, 2009). Commercial rice varieties have had increased fitness benefits against several abiotic stress when inoculated with endophytes via HAS mechanism (Redman *et al.*, 2011). Tomato plants colonized by the endophytic fungi *Alternaria* spp. and *Trichoderma harzianum* had better water use efficiency, higher biomass and photosynthetic efficiency than the control plants when exposed to salt or drought stress (Azad and Kaminskyj, 2016).

The plant root colonizing, basidiomycete fungus *Piriformospora indica*, isolated from plants growing in the Indian Thar desert, is being one of the best-studied root endophytes so far, to significantly promote growth (30% increase in fresh weight of Chinese cabbage at 200 mM NaCl, Khalid *et al.*, 2018) as well as to improve salinity tolerance in symbiotic association with a wide variety of plant species such as barley, tomato, rice, soybean (Verma *et al.*, 1998; Waller *et al.*, 2005; Baltruschat *et al.*, 2008). Endophytic fungi, *Ampelomyces* sp. or *P. chrysogenum* improved growth and drought and salinity stress tolerance in tomato (Morsy *et al.*, 2020).

The endophytic fungus *Penicillium minioluteum* which was isolated from the roots of field-grown soybean is reported to synthesize gibberellins (GAs) and promote the growth of mutant rice line Waito-C and improved salinity stress tolerance in Soybean (Khan *et al.*, 2011). Pretreatment of soybean seeds with an endophytic fungus, *Fusarium verticillioides*, proved to be an effective method to improve growth under salinity stress conditions (Radhakrishnan *et al.*, 2013). An endophyte, *Aspergillus flavus* CSH1, confers salinity tolerance in Soybean, by modulating its phytohormone levels and antioxidant system (Lubna *et al.*, 2018). A similar effect was found in cucumber plants by an endophytic fungus, *Paecilomyces formosus* LHL10. In addition to GAs, endophytes also produced indole acetic acid to improve growth (Khan *et al.*, 2012). Another fungal endophyte, *Penicillium janthinellum* LK5 isolated from roots of tomato plants, improved the growth of abscisic acid (ABA)-deficient mutant *sitiens* plants under salinity (Khan *et al.*, 2013). *P. indica* alleviated salt stress in salt-sensitive barley cultivar Ingrid through decreasing lipid peroxidation, metabolic heat efflux and fatty acid desaturation in the leaves. Also, a significant increase in the amount of ascorbic acid and other antioxidant enzymes was recorded in barley root under salinity stress. It states that

antioxidants might play a role in both endophyte-mediated plant tolerance to salinity (Baltruschat *et al.*, 2008).

Tomato plants inoculated with *P. indica* had a higher level of photosynthetic pigments and osmoprotectants like proline and glycine betaine under different salinity gradient (Ghorbani *et al.*, 2018). Plants inoculated with endophytes had an enhanced expression NHX1 gene related to salt tolerance which, in turn, regulates iron homeostasis. Inoculated plants had better osmotic tolerance under salinity stress by maintaining a higher Na⁺ level in leaves (Molina-Montenegro *et al.*, 2018).

Functional characterization revealed that PiHOG1 plays a significant role in the salinity response of *P. indica* as well as helps the host to overcome salinity stress. PiHOG1, a stress regulator MAP kinase from *Piriformospora indica*, confers salinity stress tolerance in rice plants. HOG1 MAP kinase plays a vital role in the osmo-adaptation pathway in different fungi as it required to restoring osmotic pressure by increasing glycerol accumulation (Jogawat *et al.*, 2016). Random overexpression of *P. indica* genes (cDNA library) in *Escherichia coli* identified six genes that were upregulated in response to salinity. These are genes encoding cyclophilin, stearyl-CoA desaturase, thiamine pyrophosphate-binding domain-containing protein, BCL-2 associated athanogene 3-like protein, cytochrome P450 and 60S ribosomal protein genes upregulated in 400mM NaCl-treated *P. indica* compared to the control. These genes are involved in different cellular processes such as metabolism, biosynthetic processes, DNA repair, protein turnover, transport and salt tolerance (Gahlot *et al.*, 2015).

There are several reports suggesting a role of endophytic fungus in stress tolerance and growth promotion (Rodriguez *et al.*, 2004; Waqas *et al.*, 2012; Hamayun *et al.*, 2017; Dhanyalakshmi *et al.*, 2019). However, the mechanism by which both fungal and plant partners are interacting and how stress tolerance is conferred by endophytic fungi are poorly understood (Johnson *et al.*, 2018; Hyde *et al.*, 2019).

33.1.7 POSSIBLE MECHANISM OF SALT TOLERANCE BY ENDOPHYTES

The mechanism by which endophytic fungi improve stress tolerance in plants is still intriguing. Several attempts have been made to unravel the stress tolerance mechanism using *Piriformospora indica* and other endophytic fungi as model systems (Table 33.1). It is reported that *P. indica* colonization elevated the antioxidant capacity of plants to cope with oxidative stress induced by salinity in several plants, such as barley (Waller *et al.*, 2005; Baltruschat *et al.*, 2008), *Medicago truncatula* (Li *et al.*, 2017) and tomato (Abdelaziz *et al.*, 2019). Colonized plants had 1.5- and 8-fold higher levels of the osmoprotectant proline, compared to non-colonized rice and *Medicago truncatula*, respectively (Jogawat *et al.*, 2013; Li *et al.*, 2017). In symbiotic plants, the level of photosynthetic pigment like chlorophyll was higher, whereas the anthocyanin was lower in Rice, Arabidopsis and Tomato, growth was higher and ion homeostasis was better maintained when exposed to salt (Jogawat *et al.*, 2013, Abdelaziz *et al.*, 2017; 2019). In Maize, *P. indica* colonization improved stomatal regulation and improved K⁺ transport into the shoots (Yun *et al.*, 2018). The endophytic fungus, *Porostereum spadiceum*, modulated the level of phytohormones in Soybean,

TABLE 33.1**List of Fungal Endophytes Conferring Salinity Tolerance in Various Crops**

Fungal endophytes	Crops	Mechanisms	References
<i>Alternaria</i> spp. and <i>Trichoderma harzianum</i>	Tomato	Improved the water use efficiency, higher biomass and photosynthetic efficiency	Azad and Kaminskyj, 2016
<i>Fusarium culmorum</i> , FcRed1	Dune grass, Tomato	Habitat adapted symbiosis - Intergenomic epigenetic mechanism;	Rodriguez <i>et al.</i> , 2008
<i>Fusarium</i> sp.	Rice	Improved assimilation rate and chlorophyll stability index;	Sampangi-Ramaiah <i>et al.</i> , 2020
<i>Paecilomyces formosus</i> LHL10	Cucumber	Accumulated higher levels of phytohormone such as gibberellic acid and indole acetic acid	Khan <i>et al.</i> , 2012
<i>Penicillium brevicompactum</i> and <i>P. chrysogenum</i>	Tomato and lettuce	Improved ecophysiological performance; enhanced expression of NHX1 gene	Molina-Montenegro <i>et al.</i> , 2018
<i>Penicillium janthinellum</i> LK5	Tomato	Decreasing lipid peroxidation and significantly increasing antioxidant enzyme activities; higher amount of abscisic and reduced levels of jasmonic acid	Khan <i>et al.</i> , 2013
<i>Penicillium minioluteum</i> ; <i>Fusarium verticillioides</i> ; <i>Aspergillus flavus</i> CSH1, <i>Piriformospora indica</i>	Soybean	Production of phytohormones and activating defense mechanisms of the host;	Khan <i>et al.</i> , 2011; Radhakrishnan <i>et al.</i> , 2013; Lubna <i>et al.</i> , 2018
	Barley, <i>Medicago truncatula</i> , Tomato, Maize, Tomato	Antioxidant capacity; Higher levels of the osmoprotectant; upregulated expression of several defense related genes and transcription factors Improved stomatal regulation and the K ⁺ transport into the shoots By improving plant water status and gas exchange characteristics	Waller <i>et al.</i> , 2005; Baltruschat <i>et al.</i> , 2008; Li <i>et al.</i> , 2017; Abdelaziz <i>et al.</i> , 2019 Yun <i>et al.</i> , 2018 Ghorbani <i>et al.</i> , 2018
<i>Porostereum spadicum</i>	Soybean	Increasing the levels of GA and isoflavones, and reducing levels of ABA and JA	Hamayun <i>et al.</i> , 2017
<i>Ampelomyces</i> sp. or <i>P. chrysogenum</i>	Tomato	Unknown mechanism	Morsy <i>et al.</i> , 2020

increasing the levels of GA and isoflavones, and reducing levels of ABA and JA under salt stress (Hamayun *et al.*, 2017). In *M. truncatula*, it was shown that *P. indica* colonization upregulated expression of several defense-related genes and transcription factors (Li *et al.*, 2017). In summary, endophytic fungi help plants to cope with salt stress through the production of phytohormones and activating antioxidant defense mechanisms of the host (Khan *et al.*, 2011; 2012; 2013; Radhakrishnan *et al.*, 2013; Baltruschat *et al.*, 2008). It is also shown to improve the ecophysiological performance such as plant water status and gas exchange characteristics of the host under saline conditions (Ghorbani *et al.*, 2018; Molina-Montenegro *et al.*, 2018).

So far there are no indications that symbiotic fungi protect plants from the effect of abiotic stress through totally novel mechanisms, but rather through increased mobilization of inherent stress protection processes. Nevertheless, the information available on the mechanisms of the acclimation response conferred by endophytic fungi still cannot answer the questions of *why* plants do depend on the presence of EF for their full stress resistance response. These studies have provided a *proof of concept* that endophytic fungi could be harnessed to ameliorate plant growth under abiotic stresses and provide an additional and alternate approach toward management of these stresses in crop plants.

33.1.8 CRITERIA TO SELECT POTENTIAL ENDOPHYTIC FUNGAL ISOLATE

In the next paragraph, we examine the ecological significance of endophytic fungi-induced stress tolerance in *Solanaceae* plants and test the hypothesis (Figure 33.1)

FIGURE 33.1 A hypothetical model showing endophytic fungi may increase ecological amplitude of *Solanum dulcamara*. When the plant has no fungal symbiont, it has a restricted ecological amplitude, whereas the plant harboring fungal symbiont has the potential to expand into a wider range of environmental conditions.

that endophytic fungi are instrumental in the enormous phenotypic plasticity of *Solanum dulcamara* under contrasting habitats. If this hypothesis holds true, a potential candidate should fulfill the following criteria.

A potential isolate should be able to: (1) colonize the host/selected non-host plants, (2) grow on high concentrations of salt/under simulated home stress conditions, and (3) improve stress tolerance under simulated stress conditions when inoculated to plants. To test our hypothesis, we sampled the dry coastal dunes and permanent wet regions in the island, Schiermonnikoog. Five plants were collected from each location and brought to the laboratory and processed same day. [Figure 33.2](#) explains the methodology in brief.

33.2 RESULTS

The results showed that almost all plant parts were colonized by endophytic fungi ([Figure 33.3](#)). In total, 242 isolates were isolated from surface sterilized plant tissues, out of which 153 were identified based on Internal Transcribed Spacer sequencing. Colonization frequency was higher in leaves and roots, followed by stems. The identified fungi were divided into 21 genera from dry and 17 genera from wet habitats.

The endophytic fungal community differed between both habitat and plant parts ([Figure 33.4](#)). Based on the occurrence in the two habitats, the isolates were classified as common (occurring in both habitats) or specific (exclusively occurring in one of the habitats) ([Figure 33.5](#)). In both habitats, the genus *Alternaria* was dominant in leaves. From dry habitats, the isolate *Boeremia exigua* from stem and *Harzia velata* from roots were more abundant. From the wet habitat, the isolate unknown fungus_4 and *Colletotrichum coccodes* were recovered more frequently. In general, the genus, *Alternaria* accounted for highest proportion in dry and wet habitat (38% from the dry and 40% from the wet habitat).

33.2.1 IN VITRO SCREENING FOR POTENTIAL ISOLATES

Based on their occurrence in common and specific habitats, twenty-one isolates were selected and tested for their salt-stress tolerance. Our assumption for this experiment is, if the isolate itself is able to tolerance high-stress condition, it could potentially also provide stress tolerance to plants upon inoculation and since most endophytic species have to infect every plant individually, it needs to be able to survive in saline soil to be effective. Almost all the isolates tested were grown up to 300 mM NaCl concentration and a few were grown at 600 and 1200 mM ([Figure 33.6](#)). The morphology of the endophytic fungi isolates changes with the salt treatment. The change in morphology is probably due to different metabolic reactions activated by different salt concentrations.

Based on *in vitro* screening, a few species were identified as salt tolerant. Recently, a method has been developed to test our isolates of interest using *P. indica*, as a positive control in Chinese cabbage under different water level. In brief, the experimental setup is as indicated in [Figure 33.7](#). The results showed that plants inoculated with

FIGURE 33.2 Overview of the approach used to identify the potential isolate. 1. Endophytic fungi were isolated from surface sterilized leaves, stem and root segments of *S. dulcamara* and cultured on potato dextrose agar media and purified into a single isolate. 2. DNA was extracted from each isolate and their ITS region were amplified using ITS1F and four primers. Samples were subjected to Sanger sequencing. The species were identified using UNITE database based on sequence homology. 3. The isolates were taxonomically classified and grouped into common and specific isolates based on their habitats. Screening was done to assess the salt-tolerance ability of selected fungal isolates, in which the fungal isolates were cultured under different salt concentrations and growth patterns were observed. 4. After selecting the potential candidates, plants were inoculated with those isolates and their ability to impart their abiotic stress tolerance ability to plants was assessed.

FIGURE 33.3 Isolation of endophytic fungi from *Solanum dulcamara*. Arrow heads indicate the emergence of endophytic fungi from the surface sterilized tissue segments. A-leaf, B-stem and C-root.

FIGURE 33.4 Diversity of endophytic fungi from *Solanum dulcamara* collected from contrasting habitats. From left to right: Row I: 1. *Gibberella avenacea* (W2); 2. *Alternaria arborescens* (W8); 3. *Epicoccum nigrum* (W36); 4. *Alternaria rosae* (W55II); 5. *Colletotrichum coccodes* (W46I); 6. *Alternaria tenuissima* (W12). Row II: 1. *Fusarium* sp. (W60); 2. *Epicoccum nigrum* (W74); 3. *Phomopsis velata* (D27); 4. W83; 5. *Diaporthe cotoneastri* (W118); 6. *Alternaria alternata* (D23). Row III: 1. *Neofabraea vagabunda* (D32); 2. *Boeremia exigua* (D74); 3. *Aureobasidium pullulans* (D107); 4. *Harzia velata* (D36I); 5. *Colletotrichum godetiae* (D83); 6. Pleosporaceae (D88). W and D indicate wet and dry habitats, respectively.

FIGURE 33.5 A Venn diagram represent the common (occurring in both habitats) and specific (exclusively occurring in one of the habitats) isolates recovered from *Solanum dulcamara* in contrasting habitats. In total, 24 and 17 isolates were specific to dry and wet habitat, respectively. 13 isolates were found in both dry and wet habitat.

P. indica accumulated higher photosynthates compared to the non-inoculated plants under different water levels. This was more evident in the biomass of the plants. This setup could be used further to test the abilities of selected isolates to confer salt/drought stress tolerance.

As mentioned previously, the potential isolate has to fulfill three criteria. From the results, it is evident that the isolates fulfilled the first two criteria, that is, they are able to colonize the plant tissues and grow under high salt concentrations, as well as osmotic and drought stress conditions. The third criteria, the ability to impart stress tolerance upon inoculation with plants, is presently being tested in tomato a model for use in other Solanaceous crops.

FIGURE 33.6 *In vitro* screening of endophytic fungus from *Solanum dulcamara* against salinity stress. Growth pattern of endophytic fungus, *Phomopsis velata* under different NaCl concentrations. (a) 0, (b) 300, (c) 600, and (d) 1200 mM NaCl. *P. velata* grew better under both control and 300 mM NaCl concentration. Under 600 and 1200 mM NaCl concentration, *P. velata* showed the mycelial growth inhibition of about 33 and 75%, respectively, compared to control.

P. indica mediated responses in Chinese cabbage under different water level

FIGURE 33.7 Experimental setup to test the potential isolate. *P. indica* mediated responses in Chinese cabbage under different water level. Seeds of Chinese cabbage were surface sterilized and germinated on a filter paper. After 4 days, the germinated seedlings were transferred to hydroponic system for better acclimatization. Uniform seedlings were transplanted onto the pots containing sterile sand. Before transplanting, one group of plants was treated with the 1% mycelial suspension and the other group of seedlings was mock treated with broth without fungus. Additionally, 2 ml of mycelial suspension/broth without fungus were pipetted out near the root zone. These seedlings were left for 4–6 days to establish a successful colonization of *P. indica*. Later, the plants were subjected to stress conditions; in this case, we imposed drought stress by maintaining certain water level (100, 70 and 30% water holding capacity-WHC). Once the required water level was achieved, these pots were maintained under stress for 10 days. Eco physiological measurements were done before and after imposing stress.

33.3 CONCLUSION

Recent studies have shown that symbioses with endophytes are of vital importance in the distribution of plant communities worldwide. These symbioses in many cases are responsible for adaptation of the plant to the biological and environmental stresses. Exploitation of such plant symbiotic organisms could be a potential approach leading to sustainable development of the agricultural production and ecosystem management. The novel endophytes identified from this study will be evaluated for their potential to improve crop productivity under suboptimal conditions such as salinity.

ACKNOWLEDGMENT

This research has been carried out in the Laboratory of Plant Physiology (GREEN) at the Groningen Institute for Evolutionary Life Sciences (GELIFES) according to the requirements of the Graduate School of Science and Engineering (Faculty of Science and Engineering, University of Groningen; Groningen, the Netherlands). This research was supported by an Adaptive Life Programme grant awarded to

Sasirekha Munikumar, J.T(Theo). M. Elzenga, J. D(Dick) van Elsas and Nataraja N. Karaba made possible by the Board of the University of Groningen, the Faculty of Science and Engineering and the Groningen Institute for Evolutionary Life Sciences (GELIFES).

REFERENCES

- Abdelaziz, M. E., Kim, D., Ali, S., Fedoroff, N. V. and Al-Babili, S. (2017). The endophytic fungus *Piriformospora indica* enhances *Arabidopsis thaliana* growth and modulates Na^+/K^+ homeostasis under salt stress conditions. *Plant Science*. 263, 107–115. <https://doi.org/10.1016/j.plantsci.2017.07.006>.
- Abdelaziz, M. E., Abdelsattar, M., Abdeldaym, E. A., Atia, M. A. M., Mahmoud, A. W. M., Saad, M. M. and Hirt, H. (2019). *Piriformospora indica* alters Na^+/K^+ homeostasis, antioxidant enzymes and LeNHX1 expression of greenhouse tomato grown under salt stress. *Scientia Horticulturae*, 256, 108532. <https://doi.org/10.1016/j.scientia.2019.05.059>.
- Alvarez Viveros M. F., Inostroza-Blancheteau, C., Timmermann, T., González, M. and Arce-Johnson, P. (2013) Overexpression of *GlyI* and *GlyII* genes in transgenic tomato (*Solanum lycopersicum* Mill.) plants confers salt tolerance by decreasing oxidative stress. *Molecular Biology Report*, 40, 3281–3290.
- Azad, K. and Kaminskyj, S. (2016). A fungal endophyte strategy for mitigating the effect of salt and drought stress on plant growth. *Symbiosis*, 1, 73–78.
- Bacon, C. W. and Hill, N. S. (1996). Symptomless grass endophytes: Products of coevolutionary symbioses and their role in the ecological adaptations of grasses, in S.C. Redkin and L.M. Carris (eds.), *Endophytic Fungi in Grasses and Woody Plants*, St. Paul, MN, APS Press, pp. 155–178.
- Baltruschat, H., Fodor, J., Harrach, B. D., Niemczyk, E., Barna, B., Gullner, G. and Skoczowski, A. (2008). Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. *New Phytologist*, 180(2), 501–510.
- Carillo, P., Annunziata, M. G., Pontecorvo, G., Fuggi, A. and Woodrow, P. (2011). Salinity stress and salt tolerance, in A. Shanker and B. Venkateswarlu (eds.), *Abiotic Stress in Plants - Mechanisms and Adaptations*, IntechOpen, pp. 21–38. <https://doi.org/10.5772/22331>.
- Cheeseman, J. M. (2015). The evolution of halophytes, glycophytes and crops, and its implications for food security under saline conditions. *New Phytologist*, 206(2), 557–570. <https://doi.org/10.1111/nph.13217>.
- Clay, K. and Holah, J. (1999). Fungal endophyte symbiosis and plant diversity in successional fields. *Science*, 285, 1742–1744.
- Davis, E. C., Franklin, J. B., Shaw, A. J. and Vilgalys, R. (2003). Endophytic *Xylaria* (Xylariaceae) among liverworts and angiosperms: Phylogenetics, distribution, and symbiosis. *American Journal of Botany*, 90(11): 1661–1667.
- Dawood, T., Rieu, I., Wolters-Arts, M., Derksen, E.B., Mariani, C. and Visser, E. J. W. (2014). Rapid flooding induced adventitious root development from preformed primordia in *Solanum dulcamara*. *AoB Plants*, 6, plt058v1.
- Dhanyalakshmi, K. H., Mounashree, D. C., Vidyashree, D. N., Earanna, N. and Nataraja, K. N. (2019). Options and opportunities for manipulation of drought traits using endophytes in crops. *Plant Physiology Reports*, 24(4): 555–562.
- Dodd, J. (1980). The role of plant stresses in development of corn stalk rots. *Plant Disease*, 64, 533–537.
- Flowers T.S. and Yeo A.R. (1989). Effects of salinity on plant growth and crop yields, in J.H. Cherry (eds.), *Environmental Stress in Plants*. NATO ASI Series (Series G:

- Ecological Sciences), Berlin, Heidelberg, Springer, 19: 101–119. https://doi.org/10.1007/978-3-642-73163-1_11.
- Flowers, T. J. (2004). Improving crop salt tolerance. *Journal of Experimental Botany*, 55(396), 307–319. <https://doi.org/10.1093/jxb/erh003>.
- Flowers, T. J. and Colmer, T. D. (2008). Salinity tolerance in halophytes. *New Phytologist*, 179, 945–963.
- Flowers, T. J., Galal, H. K. and Bromham, D. L. (2010). Evolution of halophytes: Multiple origins of salt tolerance in land plants. *Functional Plant Biology*, 37, 604–612.
- Fravel, D., Olivain, C. and Alabouvette, C. (2003). *Fusarium oxysporum* and its biocontrol. *New Phytologist*, 157, 493–502.
- Gahlot, S., Joshi, A., Singh, P., Tuteja, R., Dua, M., Jogawat, A., and Tuteja, N. (2015). Isolation of genes conferring salt tolerance from *Piriformospora indica* by random overexpression in *Escherichia coli*. *World Journal of Microbiology & Biotechnology*, 31(8), 1195–1209. <https://doi.org/10.1007/s11274-015-1867-5>.
- Ghorbani, A., Razavi, S. M., Ghasemi Omran, V. O. and Pirdashti, H. (2018). *Piriformospora indica* inoculation alleviates the adverse effect of NaCl stress on growth, gas exchange and chlorophyll fluorescence in tomato (*Solanum lycopersicum* L.). *Plant Biology*, 20(4), 729–736. <https://doi.org/10.1111/plb.12717>.
- Goswami, K., Tripathi, A. and Sanan-Mishra, N. (2017). Comparative miRomics of salt-tolerant and salt-sensitive rice. *Journal of Integrative Bioinformatics*, 14(1), 1–18. doi: [10.1515/jib-2017-0002](https://doi.org/10.1515/jib-2017-0002).
- Greenway, H. and Munns, R. (1980). Mechanisms of salt tolerance in nonhalophytes. *Annual Review of Plant Physiology*, 31, 149–190.
- Gupta, B. and Huang, B.. (2014). Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. *International Journal of Genomics*, 2014, 1–18. <https://doi.org/10.1155/2014/701596>.
- Hamayun, M., Hussain, A., Khan, S. A., Kim, H. Y., Khan, A. L., Waqas, M., Irshad, M., Iqbal, A., Rehman, G., Jan, S. and Lee, I. J. (2017). Gibberellins producing endophytic fungus *Porostereum spadiceum* AGH786 rescues growth of salt affected soybean. *Frontiers in Microbiology*, 8, 686. doi: [10.3389/fmicb.2017.00686](https://doi.org/10.3389/fmicb.2017.00686).
- Hanin, M., Ebel, C., Ngom, M., Laplaze, L. and Masmoudi, K. (2016). New insights on plant salt tolerance mechanisms and their potential use for breeding. *Frontiers in Plant Science*, 7, 1–17. <https://doi.org/10.3389/fpls.2016.01787>.
- Hardoim, P. R., van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., and Sessitsch, A. (2015). The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews*, 79(3), 293–320.
- Horie, T., Karahara, I. and Katsuhara, M. (2012). Salinity tolerance mechanisms in glycophytes: An overview with the central focus on rice plants, *Rice*, 5, 11.
- Hossain, S. M. (2019). Present scenario of global salt affected soils, its management and importance of salinity research. *International Research Journal of Biological Sciences Perspective*, 1, 2663–5976.
- Hyde, K. D., Xu, J., Rapior, S., Jeewon, R., Lumyon, S., Niego, A. G. T...Nataraja, K. N., et al (2019). The amazing potential of fungi: 50 ways we can exploit fungi industrially. *Fungal Diversity* 97(1):1–136.
- Jamil, A., Riaz, S., Ashraf, M. and Foolad, M. R. (2011). Gene expression profiling of plants under salt stress. *Critical Reviews in Plant Sciences*, 30, 435–458. doi:[10.1080/07352689.2011.605739](https://doi.org/10.1080/07352689.2011.605739).
- Jha, S. (2019). Transgenic approaches for enhancement of salinity stress tolerance in plants. in S. Singh, S. Upadhyay, A. Pandey, S. Kumar (eds.), *Molecular Approaches in Plant Biology and Environmental Challenges. Energy, Environment, and Sustainability*, Singapore, Springer, pp. 265–322. https://doi.org/10.1007/978-981-15-0690-1_14.

- Jogawat, A., Saha, S., Bakshi, M., Dayaman, V., Kumar, M., Dua, M., ... Johri, A. K. (2013). *Piriformospora indica* rescues growth diminution of rice seedlings during high salt stress. *Plant Signaling and Behavior*, 8(10): e26891. <https://doi.org/10.4161/psb.26891>.
- Jogawat, A., Vadassery, J., Verma, N., Oelmüller, R., Dua, M., Nevo, E. and Johri, A. K. (2016). PiHOG1, a stress regulator MAP kinase from the root endophyte fungus *Piriformospora indica*, confers salinity stress tolerance in rice plants. *Scientific Reports*, 6(1), 36765. <https://doi.org/10.1038/srep36765>.
- Johnson, J.M., Thürich, J., Petutschnig, E.K., Altschmied, L., Meichsner, D... Oelmüller, R. (2018). A poly(A) ribonuclease controls the cellotriose-based interaction between *Piriformospora indica* and its host Arabidopsis. *Plant Physiology*, 176: 2496–2514.
- Khalid, M., Hassani, D., Liao, J., Xiong, X., Bilal, M. and Huang, D. (2018). An endosymbiont *Piriformospora indica* reduces adverse effects of salinity by regulating cation transporter genes, phytohormones, and antioxidants in *Brassica campestris* ssp. *Chinensis*. *Environmental and Experimental Botany*, 153, 89–99. <https://doi.org/10.1016/j.envexpbot.2018.05.007>.
- Khan, A. L., Hamayun, M., Ahmad, N., Hussain, J., Kang, S. M., Kim, Y. H., ... Lee, I. J. (2011). Salinity stress resistance offered by endophytic fungal interaction between *Penicillium minioluteum* LHL09 and glycine max. L. *Journal of Microbiology and Biotechnology*, 21(9), 893–902. <https://doi.org/10.4014/jmb.1103.03012>.
- Khan, A.L., Hamayun, M., Kang, S. M., Kim, Y. H., Jung, H. Y., Lee, J. H. and Lee, I. J. (2012). Endophytic fungal association via gibberellins and indole acetic acid can improve plant growth under abiotic stress: An example of *Paecilomyces formosus* LHL10. *BMC Microbiology*, 12, 1–14. <https://doi.org/10.1186/1471-2180-12-3>.
- Khan, A. L., Waqas, M., Khan, A. R., Hussain, J., Kang, S.-M., Gilani, S. A., ... Lee, I.-J. (2013). Fungal endophyte *Penicillium janthinellum* LK5 improves growth of ABA-deficient tomato under salinity. *World Journal of Microbiology & Biotechnology*, 29(11), 2133–2144. <https://doi.org/10.1007/s11274-013-1378>.
- Lewis, D. H. (1985). Symbiosis and mutualism: Crisp concepts and soggy semantics, in D.H. Boucher (ed.), *The Biology of Mutualism*, London, Croom Helm Ltd, pp. 29–39.
- Li, L., Li, L., Wang, X., Zhu, P., Wu, H., Qi, S. (2017). Plant growth-promoting endophyte *Piriformospora indica* alleviates salinity stress in *Medicago truncatula*, *Plant Physiology and Biochemistry*, 119, 211–223.
- Lopez-Llorca, L. V. and Macia-Vicente, J. G. (2009). In. Plant symbioses with fungal endophytes: perspectives on conservation and sustainable exploitation of Mediterranean ecosystems. I.S.S.N.: 0210-5004.
- Lubna, Asaf, S., Hamayun, M., Latif, A., Waqas, M., Aaqil, M., Jan, R., ... Hussain, A. (2018). Salt tolerance of *Glycine max.* L induced by endophytic fungus *Aspergillus flavus* CSH1, via regulating its endogenous hormones and antioxidative system. *Plant Physiology and Biochemistry*, 128, 13–23. <https://doi.org/10.1016/j.plaphy.2018.05.007>.
- McCord, J. M. (2000). The evolution of free radicals and oxidative stress. *The American Journal of Medicine*, 108, 652–659.
- Molina-Montenegro, M. A., Acuña-Rodríguez, I. S., Torres-Díaz, C., and Gundel, P. E. (2018). Root endophytes improve physiological performance and yield in crops under salt stress by up-regulating the foliar sodium concentration. *BioRxiv*, preprint, 1–24.
- Morsy, M., Cleckler, B. and Armuelles-Millican, H. (2020). Fungal endophytes promote tomato growth and enhance drought and salt tolerance. *Plants*, 9, 877; <https://doi.org/10.3390/plants9070877>.
- Munns, R., Schachtman, D. and Condon, A. (1995). The significance of a two-phase growth response to salinity in wheat and barley. *Functional Plant Biology*, 22(4), 561–569.

- Munns, R. and Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, 59, 651–681. PMID: 18444910.
- Munns, R., James, R. and Xu, B. *et al.* (2012). Wheat grain yield on saline soils is improved by an ancestral Na⁺ transporter gene. *Nature Biotechnology*, 30, 360–364. <https://doi.org/10.1038/nbt.2120>.
- Ogbemudia, F., Ita, R. and Philips, T. (2018). Geospatial variability and ecological amplitudes of plants along nutrient gradients in Imo River wetland. *Asian Journal of Environment & Ecology*, 7(2), 1–10. <https://doi.org/10.9734/ajee/2018/42881>.
- Pawłowska, J. and Wilk, M., Śliwińska-Wyrzychowska, A., *et al.* (2014). The diversity of endophytic fungi in the above-ground tissue of two *Lycopodium* species in Poland. *Symbiosis*, 63, 87–97.
- Petrini, O. (1986). Taxonomy of endophytic fungi of aerial plant tissues', in N.J. Fokkema and J. van den Heuvel (eds.), *Microbiology of the Phyllosphere*, Cambridge, Cambridge University Press, pp. 175–187.
- Radhakrishnan, R., Khan, A. L. and Lee, I. J. (2013). Endophytic fungal pre-treatments of seeds alleviate salinity stress effects in soybean plants. *Journal of Microbiology*, 51(6), 850–857. <https://doi.org/10.1007/s12275-013-3168-8>.
- Redman, R. S., Sheehan, K. B., Stout, R. G., Rodriguez, R. J. and Henson, J. M. (2002). Thermotolerance conferred to plant host and fungal endophyte during mutualistic symbiosis. *Science*, 298, 1581.
- Redman, R. S., Dunigan, D. D. and Rodriguez, R. J. (2011). Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: A strategy for mitigating impacts of climate change. *PLoS ONE*, 6(7), 1423–1428.
- Rodriguez, R. J. and Redman, R. S. (1997). Fungal life-styles and ecosystem dynamics: biological aspects of plant pathogens, plant endophytes and saprophytes. *Advances in Botanical Research*, 24, 169–193.
- Rodriguez, R. J., Redman, R. S. and Henson, J. M. (2004). The role of fungal symbioses in the adaptation of plants to high stress environments. *Mitigation and Adaptation Strategies for Global Change*, 9, 261–272.
- Rodriguez, R. and Redman, R. (2008). More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. *Journal of Experimental Botany*, 59(5), 1109–1114.
- Rodriguez, R. J., Henson, J., Van, Volkenburgh, E., Hoy, M., Wright, L. and Beckwith, F. (2008). Stress tolerance in plants via habitat adapted symbiosis. *ISME Journal*, 2, 404–416.
- Rodriguez, R. J., White Jr, J. F., Arnold, A. E. and Redman, R. S. (2009). Fungal endophytes: diversity and functional roles. *New Phytologist*, 182, 314–330. doi:10.1111/j.1469-8137.2009.02773.x.
- Roy, S.J., Negrao, S. and Tester, M. (2014). Salt resistant crop plants. *Current Opinion in Biotechnology*. 26, 115–124.
- Sampangi-Ramaiah, M. H., Jagadheesh, Dey, P., Jambagi, S., Vasantha Kumari, M. M., Oelmüller, R., ... Uma Shaanker, R. (2020). An endophyte from salt-adapted Pokkali rice confers salt-tolerance to a salt-sensitive rice variety and targets a unique pattern of genes in its new host. *Scientific Reports*, 10(1), 1–14. <https://doi.org/10.1038/s41598-020-59998-x>
- Sanders, D. (2020). The salinity challenge. *New Phytologist*, 225(3), 1047–1048. <https://doi.org/10.1111/nph.16357>.
- Sekmen, A. H., Bor, M., Ozdemir, F. and Turkan, I. (2013). Current concepts about salinity and salinity tolerance in plants, in N. Tuteja and Gill, S. S. (ed.) *Climate Change and Plant Abiotic Stress Tolerance*, Hoboken, NJ, Wiley-Blackwell, pp. 163–188.
- Shabala, S., Wu, H. H. and Bose, J. (2015). Salt stress sensing and early signalling events in plant roots: current knowledge and hypothesis. *Plant Science*, 241, 109–119.

- Shokat, S. and Großkinsky, D. K. (2019). Tackling salinity in sustainable agriculture – What Developing Countries May Learn from Approaches of the Developed World, *Sustainability*, 11, 4558. doi:[10.3390/su11174558](https://doi.org/10.3390/su11174558).
- Shrivastava, P. and Kumar, R. (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Sciences*, 22(2), 123–131. <https://doi.org/10.1016/j.sjbs.2014.12.001>
- Smallwood, M. F., Calvert, C. M. and Bowles, D. J. (1999). *Plant Responses to Environmental Stress*, Oxford, BIOS Scientific Publishers Limited, pp. 224.
- Srivastava, V. K., Raikwar S, Tuteja, R. and Tuteja, N. (2016). Ectopic expression of phloem motor protein pea forisome *PsSEO-F1* enhances salinity stress tolerance in tobacco. *Plant Cell Rep* 35(5):1021–1041.
- Sultan, S. E. (1987). Evolutionary Implications of Phenotypic Plasticity in Plants, in M.K. Hecht, B. Wallace, G.T. Prance (eds.), *Evolutionary Biology*, Boston, MA, Springer, pp. 127–178. https://doi.org/10.1007/978-1-4615-6986-2_7.
- Verma, S., Varma, A., Rexer, K. H., Hassel, A., Kost, G., Sarbhoy, A., Bisen, P., Bütehorn, B. and Franken, P. (1998). *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. *Mycologia*, 90 (5), 896–903. doi:[10.2307/3761331](https://doi.org/10.2307/3761331).
- Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., ... Kogel, K.-H. (2005). The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proceedings of the National Academy of Sciences of the United States of America*, 102(38), 13386–13391. <https://doi.org/10.1073/pnas.0504423102>.
- Waqas, M., Khan, A. L., Kamran, M., Hamayun, M., Kang, S. M., Kim, Y. H. and Lee, I. J. (2012). Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress, *Molecules*, 17, 10754–10773.
- Wilson, D. (1995). Endophyte – The evolution of a term, and clarification of its use and definition. *Oikos*, 73, 274–276.
- Yun, P., Xu, L., Wang, S. S., Shabala, L., Shabala, S. and Zhang, W. Y. (2018). *Piriformospora indica* improves salinity stress tolerance in *Zea mays* L. plants by regulating Na⁺ and K⁺ loading in root and allocating K⁺ in shoot. *Plant Growth Regulation*, 86(2), 323–331. <https://doi.org/10.1007/s10725-018-0431-3>
- Zhang, H., Han, B., Wang, T., Chen, S., Li, H., Zhang, Y. and Dai, S. (2012). Mechanisms of Plant Salt Response: Insights from Proteomics. *Journal of Proteome Research*, 11, 49–67.
- Zhang, Q., Peters, J. L., Visser, E. J. W., de Kroon, H. and Huber, H. (2016). Hydrologically contrasting environments induce genetic but not phenotypic differentiation in *Solanum dulcamara*. *Journal of Ecology*, 104: 1649–1661. doi:[10.1111/1365-2745.12648](https://doi.org/10.1111/1365-2745.12648).
- Zhu, J. K. (2001). Plant salt tolerance. *Trends in Plant Science*, 6: 66–71.
- Zhu, J. K. (2002). Salt and drought stress signal transduction in plants. *Annual Review of Plant Biology*, 53, 247–273.