Chapter 4
Morpho-Physiological Traits and Molecular Intricacies Associated with Tolerance to Combined Drought and Pathogen Stress in Plants

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Abstract Crops in field conditions are challenged by the simultaneous occurrence of drought and pathogen stress. In the past, research was primarily focused on studying the impact of individual stresses on plants and selection of crop varieties potentially tolerant to particular stress by yield-associated morpho-physiological traits. However, several molecular responses of crop plants underlying morpho-physiological features to concurrent stresses are not similar to that of individual stresses. Certain morpho-physiological traits such as cell membrane stability, leaf water potential, stomatal movement, and root length were shown to be altered distinctly under combined stress to combat the stress condition. However, the relevance of such traits under combined stress tolerance is not precisely known. In this chapter, from the extensive literature survey, we identified several morpho-physiological changes that could be cognate with better plant performance under combined stress and represented them as traits that have potential to impart combined stress tolerance. We have comprehensively explained physiological and molecular basis for each trait and, where possible, suggested the ways to exploit the information for identification of varieties with prospective traits. Also, we proposed the need for systematically studying the underlying regulatory traits under combined stress conditions in the future.

Keywords Combined stress · Drought · Pathogen infection · Morpho-physiological traits · Combined stress tolerance

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4.1 Introduction

In field condition, crop plants encounter a combination of drought and pathogen infection, which affects their growth and yield more than the respective individual stress conditions (Atkinson and Urwin 2012; Suzuki et al. 2014; Ramegowda and Senthil-Kumar 2015; Pandey et al. 2015). Plant responses to combined stress are quite complex and different from that of respective individual stress conditions (Atkinson and Urwin 2012; Suzuki et al. 2014; Gupta and Senthil-Kumar 2017; Pandey et al. 2017). Under combined drought and pathogen infection, drought influences pathogen infection by predisposing or enduring the plant to infection (Pandey et al. 2017). For instance, *Fusarium oxysporum* f. sp. *ciceris* (causal agent of fusarium wilt), *Rhizoctonia bataticola* (causal agent of dry root rot), and *F. solani* (causal agent of black root rot) infections occur in *Cicer arietinum* (chickpea) mainly under drought stress. In contrast, *R. solani* (causal agent of wet root rot) infection is less prevalent under drought stress (Cook and Papendick 1972; Blanco-Lopez 1983; Landa et al. 2004; Sharma et al. 2015; Jiménez-Díaz et al. 2015). Further, drought predisposes the plants to foliar pathogens such as *Pseudomonas syringe* (causal agent of bacterial speck) on *Arabidopsis thaliana* (thale cress or *Arabidopsis*) (Gupta et al. 2016). Gupta et al. (2016) demonstrated that *Arabidopsis* plants showed reduced *P. syringe* pathogen multiplication under drought stress compared to pathogen-only infected plants. Similarly, pathogen also influences drought-induced changes in plants under combined drought and pathogen infection (Dossa et al. 2017). For example, *Uromyces phaseoli* (causal agent of rust) infection disrupts cuticular layer and prevents stomatal closure in *Phaseolus vulgaris* (bean), which aggravates water loss under subsequent drought stress (Duniway and Durbin 1971).

Plant response to combined stress cannot be understood directly from individual stress studies. For example, stomatal closure is a plant response to individual drought and foliar pathogen infection to reduce transpirational water loss and stop pathogen entry, respectively (Melotto et al. 2006; Grimmer et al. 2012). However, under combined drought and foliar bacterial infection, plant response, in case of stomatal movement, may or may not be similar. Further, *Zea mays* (maize) subjected to drought stress increased its cuticular wax deposition to prevent water loss (Ristic and Jenks 2002). Also, it was shown that maize with reduced cuticular wax deposition was found susceptible to *Clavibacter michiganensis* subsp. *Nebraskensis* (causal agent of Goss's wilt) infection (Marcell and Beattie 2002). This study implies that when maize is subjected to combined stress, plants with high cuticular wax deposition might provide resistance to pathogen infection. However, regulation of wax deposition, as well as composition under combined drought and pathogen infection, is not known.

Various morpho-physiological traits have been attributed to impart individual drought or pathogen stress tolerance, but these traits are not sufficiently studied under combined stress. In this chapter, based on the preliminary evidence described in the literature, we describe a few exploitable morpho-physiological traits, namely, cell membrane stability, leaf water potential, stomatal movement, and root length, and explain the importance of these traits under combined drought and pathogen infection. Besides, we describe few traits which are affected by combined stress such as leaf area, leaf greenness, canopy temperature, and anthesis. Additionally, traits described in individual stress studies, which have the potential for consideration under combined stress are also explained.

4.2 Morpho–Physiological Traits that Likely Govern Plant Response to Combined Stress

4.2.1 Cell Membrane Stability

Plants undergo changes in cell membrane composition and structure under drought and pathogen infection (Tripathy et al. 2000; Bajji et al. 2002; Pétriacq et al. 2016). Measurement of electrolyte leakage is routinely employed as a useful tool to assess cell membrane stability of plant cells under drought and pathogen infection (Tripathy et al. 2000; Govrin and Levine 2000; Bajji et al. 2002). *Triticum aestivum* (wheat) subjected to drought stress had more electrolyte leakage compared to well-watered plants, and it was ascribed to cell membrane damage due to drought stress (Tripathy et al. 2000; Bajji et al. 2002). *Arabidopsis* plants infected with *Plectosphaerella cucumerina* (causal agent of blight) had more electrolyte leakage as compared to mock-inoculated plants. Also, electrolyte leakage was increased with inoculum size as cell death was positively correlated with the inoculum concentration (Pétriacq et al. 2016). These individual stress studies indicate that plants with high cell membrane integrity can be tolerant to individual drought or pathogen infection. Similarly, the role of cell membrane stability in combined stress has been hinted (Gupta et al. 2016; Ramu et al. 2016). Under combined drought stress and *P. syringae* infection, *Arabidopsis* plants showed increased electrolyte leakage as compared to the plants subjected to individual stress conditions. Additionally, *Arabidopsis* plants subjected to drought stress followed by *P. syringae* infection and vice versa had high and low electrolyte leakage, respectively. High electrolyte leakage was associated with reduced *in planta* bacterial number in plants subjected to combined stress as compared to only infected plants, (Gupta et al. 2016). These studies show that plant response varies with stress chronology under combined stress. Ramu et al. (2016) showed that *Helianthus annuus* (sunflower) subjected to combined drought and *Plasmopara halstedii* (causal agent of downy mildew) infection had increased malondialdehyde content, an indication of cell membrane damage, as compared to plants exposed to individual stresses (Ramu et al. 2016). These studies indicate that plant response vary with plant and pathogen type. Quantitative trait loci (QTL) governing cell membrane stability in wheat under drought stress is known (Tripathy et al. 2000; Loarce et al. 2016) (Table 4.1). However, QTL variation under combined stress condition is yet to be identified.

Table 4.1 Quantitative trait loci (QTL) linked with exploitable morpho-physiological traits under individual drought stress[b]

Sl. no	Trait	Plant name	QTL[a]	QTL flanking marker	Reference
1	Cell membrane stability	*Oryza sativa*	QCMS3.1	EM11_2 – RZ403	Tripathy et al. (2000)
2	Leaf water potential	*O. sativa*	qDTY 12.1	RM28048- RM511	Bernier et al. (2009) Shamsudin et al. (2016)
3	Stomatal frequency	*Triticum aestivum*	Qsl-5A.1	Xwmc410–Xwmc74	Wang et al. (2016)
4	Root length	*T. aestivum*	Qrls. Uwa.3AL	Xgwm391-Xbcd1431	Ayalew et al. (2017)

[a] Only QTLs with high phenotypic variation are listed

[b] Representative list with select examples that are suitable for combined stress only

4.2.2 Leaf Water Potential

Under drought and pathogen infection, plants undergo changes in leaf water potential, which is a result of an increase in hydraulic resistance and cell turgor loss (Paul and Ayres 1984; Yan et al. 2017). Leaf water potential was exploited as a direct physiological parameter to estimate plant water status under combined stress (McElrone et al. 2003). Effect of individual drought and *Xylella fastidiosa* (causal agent of bacterial leaf scorch) infection and combined stress on leaf water potential of *Parthenocissus quinquefolia* (Virginia creeper) was demonstrated. Plants subjected to combined stress had low water potential as compared to plants exposed to individual stresses. The low water potential was attributed to the reduction in hydraulic conductance and increase in embolism in response to infection (McElrone et al. 2003). Similarly, *Senecio vulgaris* (groundsel) subjected to combined drought and *Puccinia lagenophorae* (causal agent of rust) infection had decreased leaf water potential as compared to plants under individual stresses. In this study, reduction in leaf water potential was attributed to the cuticle rupture instigated by infection and its subsequent sporulation (Paul and Ayres 1984). In contrast, *Medicago sativa* (alfalfa) subjected to combined drought and *Verticillium albo-atrum* (causal agent of wilt) infection had high leaf water potential as compared to drought-stressed plants (Pennypacker et al. 1991). These studies indicate that effect on leaf water potential varies with the type of plant and pathogen. QTL linked with leaf water potential was demonstrated in rice plants under drought stress (Table 4.1). Nonetheless, characterization of QTL under combined stress is yet to be demonstrated.

4.2.3 Stomatal Movement

Plants alter stomatal movement (closure or opening) in response to both drought and pathogen infection to impart tolerance (Ramos and Volin 1987; McElrone et al. 2003; Melotto et al. 2006). *Vitis vinifera* (grapevine) plants subjected to drought stress had less stomatal conductance than that of well-watered plants. Drought severity was negatively correlated with the stomatal conductance of plants (Tombesi et al. 2015). *Solanum lycopersicon* (tomato) with less stomatal frequency infected with *Xanthomonas campestris* (causal agent of bacterial spot disease) had less infection than the plants with high stomatal frequency. In addition, plants treated with antitranspirant (phenylmercuric acetate) or abscisic acid (ABA) had less infection under spray inoculation as compared to the control plants. Further, plants subjected to spray inoculation had less infection as compared to plants infected with infiltration mode of inoculation. Here, reduction in infection in spray inoculated plants was attributed to the stomatal closure in response to infection (Ramos and Volin 1987). Similarly, *Arabidopsis* plants infected with *P. syringae* had no infection at the initial hours as compared to mock-inoculated plants. However, infection was observed in the later hours, and the reason behind such plant response was pathogen-induced stomatal opening. The resistance observed during the initial period of infection was conferred by the stomatal closure (Melotto et al. 2006). Stomatal response under combined stress has been recently investigated. Grapevine subjected to combined drought and *X. fastidiosa* infection had low stomatal conductance as compared to plants under individual stresses. Reduction in stomatal conductance under combined stress was additive as compared to plants subjected to individual stresses. The additive effect was attributed to drought caused a reduction in hydraulic conductance and infection instigated vessel occlusion (McElrone et al. 2003; Choi et al. 2013). These studies indicate that stomatal frequency and stomatal conductance play a role in individual and combined stress in imparting tolerance. A hypothetical model depicting the primary underlying mechanism of stomatal movement under individual and combined drought and pathogen infection is provided (Fig. 4.1). QTLs linked with the stomatal movement were reported in wheat (Table 4.1). However, under combined stress, the role of these QTLs is yet to be studied.

4.2.4 Root Length

Change in primary root length and lateral root number has been shown as a plant response to both individually occurring drought and pathogen infection and their combination (Blaker and MacDonald 1981; Dryden and Van Alfen 1984). Maize recombinant inbred lines (RIL) with fewer lateral roots but longer primary root length showed better performance as high leaf relative water content was demonstrated under drought stress than RIL with more lateral roots and shorter primary root length (Zhan et al. 2015; Gao and Lynch 2016). Oren et al. (2003) demonstrated that lateral roots served as the site of initial infection of *F. verticillioides* (causal agent of rot and wilt) in maize, whereas primary root was free of infection. Similarly, when maize plants were subjected to *F. verticillioides* infection, lateral roots were the vulnerable site of infection irrespective of resistant or susceptible genotype (Wu et al. 2013). These studies indicate that increase in lateral roots play a negative role in plant response to drought and pathogen infection as it did not aid in water absorption and increased the infection, respectively. Similarly, the role of

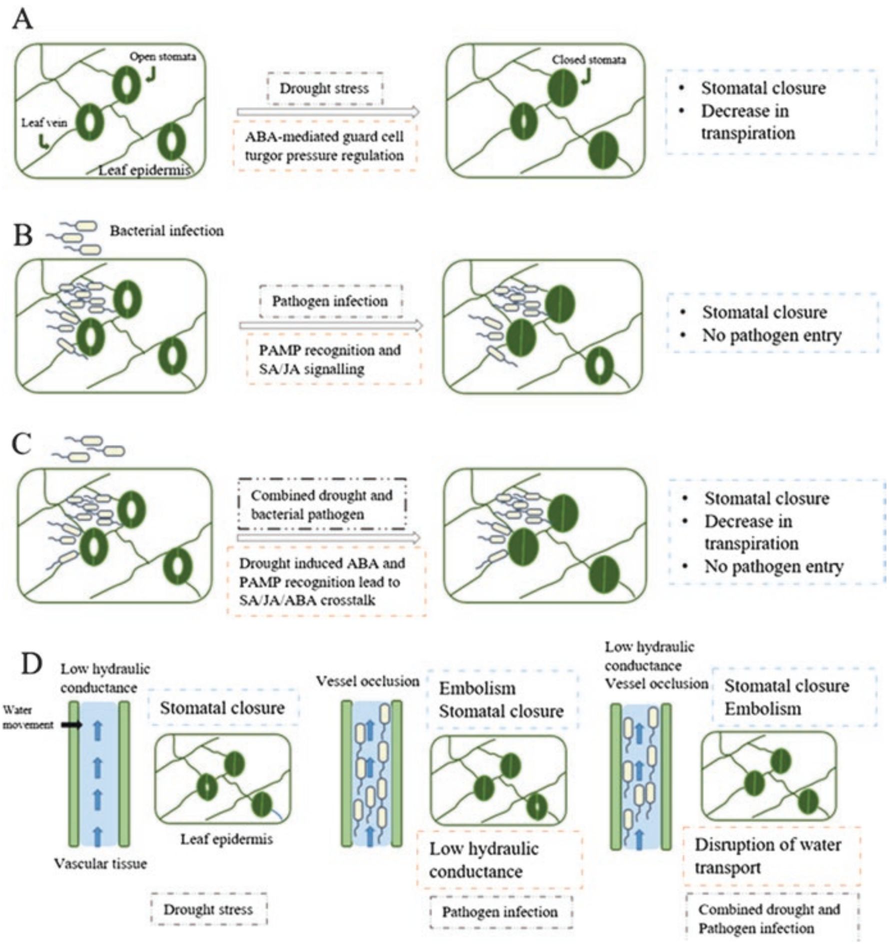

Fig. 4.1 Hypothetical model depicting the role of stomatal movement under combined drought and pathogen to impart stress tolerance. (**a**) Under drought stress, *A. thaliana* induces 9-cis-epoxycarotenoid dioxygenase (NCED3), which promotes ABA biosynthesis, and it regulates stomatal movement to avoid water loss (Iuchi et al. 2001). (**b**) *A. thaliana* activates salicylic acid (SA)/jasmonic acid (JA) as a plant response to pathogen-associated molecular pattern (PAMP) from foliar pathogen (e.g., *Pseudomonas syringae*) and regulates stomatal movement (Melotto et al. 2006). (**c**) Under combined drought and foliar bacterial pathogen infection, *A. thaliana* could upregulate ABA level, which could then interact with SA/JA and tune stomatal movement. This event is yet to be fully understood. (**d**) Under drought stress, *Parthenocissus quinquefolia* (vine) closes stomata, while under *Xylella fastidiosa* (vascular bacterial pathogen) infection, plant induces embolism as well as stomatal closure. Under combined stress, plant induces stomatal closure to prevent water loss. Additionally, embolism is induced as plant experiences low hydraulic conductance and vessel occlusion (McElrone et al. 2003; Choi et al. 2013). Blue box represents plant response to stress; orange box represents stress impact on plants; gray box represents stress imposed on plants

primary root length has been studied under combined stress. Dryden and Van Alfen (1984) demonstrated that under combined drought and *F. solani* f. sp. *phaseoli* (causal agent of root rot) infection, lateral bean roots present at topsoil were infected more, whereas primary roots were infection-free. This susceptibility was ascribed to the variation in pathogen concentration along the soil depth, and plants with long primary root showed tolerance to the combined stress (Dryden and Van Alfen 1984). Cotton plants with fewer lateral roots experienced less infection than a plant with more lateral roots which sustained higher infection. Additionally, plants with increased primary root length manifested better plant growth under combined drought stress and *V. albo-atrum* infection as it had access to water present at greater soil depth. Moreover, the plants had escaped from the infection, which was prominent at topsoil (Cook and Papendick 1972). We conclude that increased primary root length and reduction in lateral root number might attribute to the tolerance of plants under combined stress. However, the role of primary root length in conferring tolerance to combined stress varies with the type of plants and their age. Additionally, QTL associated with root length was determined in wheat crops under individual drought stress (Table 4.1). However, the effect of QTL under combined stress is yet to be elucidated.

4.3 Impact of Combined Stress on Other Morpho–Physiological Parameters

4.3.1 Leaf Area

Plants respond to drought stress and pathogen infection by altering leaf area (Lopez et al. 1997; Williams and Ayres 1981; McElrone et al. 2001; Bradley et al. 2003). Under drought stress, *Cajanus cajan* (pigeon pea) had decreased leaf area, which resulted in low yield as compared to well-watered plants (Lopez et al. 1997). Bradley et al. (2003) demonstrated that under pathogen stress, leaf area of *Trifolium repens* (clover) was negatively correlated with susceptibility to *Stemphylium* spp. (causal agent of Stemphylium leaf spot) infection. Under combined drought and *Erysiphe graminis* infection (causal agent of powdery mildew), *Hordeum vulgare* (barley) had altered its leaf area in response to drought but not to pathogen infection. No difference was observed in transpiration rate and photosynthesis in plants subjected to combined stress and individual pathogen infection (Williams and Ayres 1981). Similarly, under combined drought and *X. fastidiosa* infection on Virginia creeper, a significant reduction in leaf area was demonstrated, while infection alone did not affect the leaf area (McElrone et al. 2001). These studies demonstrate that under combined stress condition, infection did not contribute to the plant susceptibility. On the other hand, groundsel subjected to combined drought and *P. lagenophorae* infection had reduced leaf area, and reduction in leaf areas was aggravated in an additive manner under combined stress (Paul and Ayres 1984).

Similarly, bean plants exposed to combined drought and *Macrophomina phaseolina* (causal agent of charcoal rot) infection had reduced leaf area as compared to plants subjected to infection alone (Mayek-PÉrez et al. 2002). As a consequence, photosynthesis was compromised more in combined stressed plants as compared to infected plants. These studies indicate that under combined stress, the effect of drought on leaf area resulted in a reduction in infection.

4.3.2 Leaf Greenness

As a plant response, change in chlorophyll content has been observed under combined drought and pathogen infection (Gupta et al. 2016; Sinha et al. 2016), and this can be associated with leaf greenness. Leaf greenness was employed as a parameter to assess drought stress effect in *S. tuberosum* (potato) and grapevine (Fanizza et al. 1991). Potato plants subjected to drought stress showed increased leaf greenness as compared to the well-watered plants, and the increment in greenness was accompanied by a reduction in leaf growth and delayed leaf senescence (Rolando et al. 2015). In contrast, *Saccharum officinarum* (sugar cane) infected with *P. kuehnii* (causal agent of orange rust) display decreased SPAD index, an indicator of chlorophyll content, and low photosynthetic rate (Zhao et al. 2011). These studies indicate that drought stress induced the increase in chlorophyll content, which was associated with tolerance, whereas pathogen infection reduced it, which led to susceptibility. Similarly, the effect of combined stress has been investigated on chlorophyll content. For example, chickpea plants subjected to combined drought and *Ralstonia solanacearum* (causal agent of wilt) infection had high chlorophyll content as compared to the infected plants. Additionally, infection level was found less in plants under combined stress as compared to plants subjected to infection alone and which was associated with tolerance under combined stress (Sinha et al. 2016). Similarly, chickpea plants subjected to combined drought and *P. syringae* infection had more chlorophyll content than the plants under infection alone. Further, low infection level was demonstrated in plants under combined stress as compared to the plants under infection alone (Sinha et al. 2016). Similarly, *Arabidopsis* plants subjected to combined drought and *P. syringae* infection had increased chlorophyll content as compared to only infected plants (Gupta et al. 2016). These studies specify that despite an increase in chlorophyll content which was observed under combined stress, its role in imparting tolerance is yet to be explained.

4.3.3 Canopy Temperature

Plants display altered transpiration that changes canopy temperature in response to drought stress and pathogen infection to sustain growth. Canopy temperature has been used to determine plant water status in many crops (Stark et al. 1991; González-Dugo et al. 2006). Wheat plants subjected to drought stress showed higher canopy temperature as compared to the well-watered plants. It was found that plants with higher canopy temperature were susceptible to drought stress, whereas plants with less canopy temperature were tolerant (Blum et al. 1989). Wheat plants showed high canopy temperature when infected with *Mycosphaerella graminicola* (causal agent of tritici blotch) as compared to non-infected plants. The canopy temperature was positively correlated with disease severity (Eyal and Blum 1989). Similarly, *Cucumis sativus* (cucumber) infected with *Pseudoperonospora cubensis* (causal agent of downy mildew) pathogen had higher canopy temperature as compared to non-infected plants (Oerke et al. 2006). Similarly, the impact of combined stress on canopy temperature has been studied. For example, wheat plants subjected to combined drought and *P. striiformis* (causal agent of stripe rust) infection had high canopy temperature as compared to pathogen-only infected plants (Smith et al. 1986). Similarly, *Beta vulgaris* (beet) under combined drought and *Pythium aphanidermatum* (causal agent of root rot) infection manifested increased canopy temperature as compared to only infected plants (Pinter et al. 1979). Likewise, under combined drought and *Phymatotrichum omnivorum* infection, cotton plants had high canopy temperature as compared to only infected plants. Also, plants moderately infected did not show wilting under drought stress (Pinter et al. 1979). These studies indicate that changes in canopy temperature influence combined stress tolerance.

4.3.4 Time to Anthesis

Plants undergo changes in anthesis time and post-anthesis developments such as grain filling that influence grain number and size under drought stress and pathogen infection (Mahalakshmi and Bidinger 1985; Manjarrez-Sandoval et al. 1989; Winkel et al. 1997). *Sorghum bicolor* (sorghum) plants subjected to drought stress before and after anthesis had less grain number and reduced grain size, respectively, as compared to the well-watered plants. This effect was attributed to the delay in anthesis (Manjarrez-Sandoval et al. 1989). Similarly, *Pennisetum glaucum* (pearl millet) subjected to drought stress before anthesis had less grain number as compared to the control plants (Mahalakshmi and Bidinger 1985; Winkel et al. 1997). In contrast, *Arabidopsis* plants infected with *P. syringae* before anthesis showed accelerated anthesis as compared to the mock-treated plants. Also, infection increased the basal branches, which resulted in more number of primary inflorescence (Korves and Bergelson 2003). Likewise, wheat plants were more susceptible to *Gibberella zeae* (causal agent of Fusarium head blight) infection during the time of anthesis. This susceptibility was ascribed to the presence of partially exserted anthers. Plants with fully exserted anthers had dehisced along with inoculum as compared to the plants with partially exserted anthers, which acted as a source of inoculum (Reis et al. 2016). Under combined drought and *Verticillium albo-atrum* (causal agent of wilt) infection, Medicago plants had experienced a delay in anthesis compared to drought-stressed plants (Pennypacker et al. 1991). These studies imply that anthesis is affected by both individual and combined stress conditions.

4.4 More Potential Traits: Analysis from Individual Stress Studies

4.4.1 Trichome Type and Density

Plants alter trichome number in response to drought or pathogen infection (Ehleringer et al. 1976; Wagner 1991; Wagner et al. 2004). *Cbp20* (cap binding protein 20) mutant of *Arabidopsis* was shown to be drought tolerant. Mutant plants subjected to drought stress had more trichomes and low stomatal conductance as compared to the wild-type plant (Papp et al. 2004; Jäger et al. 2011). *Phlomis fruticosa* (Jerusalem sage) and *Hedera helix* (ivy) with non-glandular trichomes had entrapped dew and facilitated water absorption under drought stress as compared to plants without trichomes (Grammatikopoulos and Manetas 1994). Glandular trichomes of *Nicotiana tabbacum* (tobacco) infected with *Peronospora tabacina* (causal agent of blue mold disease) secreted potent inhibitors, namely, T-phylloplanins, which inhibited the infection, as compared to mock-inoculated plants. In addition, enrichment of T-phylloplanins secreted by glandular trichomes was observed with increase in trichome number (Kroumova et al. 2007; Nguyen et al. 2016). Similarly, glandular trichomes of potato infected with *Phytophthora infestans* (causal agent of late blight) secreted oxidative enzymes, which reduced pathogen infection as compared to plants without trichome (Lai et al. 2000). Barley plants subjected to drought and pathogen infection had increased non-glandular trichome number as compared to control plants. However, fungal biomass was increased with trichome number under pathogen infection as compared to mock-inoculated plants (Liu and Liu 2016). These studies indicate that tolerance is associated with trichome number and the type of trichome. Glandular and non-glandular trichomes serve as a physical defense against herbivory as they synthesize and secrete antimicrobial molecules (Ehleringer et al. 1976).However,under combined stress, the significance of trichome number is yet to be understood. It is evident that plants which possess both glandular and non-glandular trichomes might resist drought as well as pathogen infection by absorbing water and resisting infection. So it is noteworthy to study trichome as a trait under combined stress.

4.4.2 Cuticular Wax Composition

Plants alter cuticular wax composition in response to drought and pathogen infection (Marcell and Beattie 2002; Kosma et al. 2009). *Sesamum indicum* (sesame) subjected to drought stress had a high density of cuticular wax as compared to well-watered plants (Kim et al. 2007). Similarly, *Arabidopsis* plants subjected to drought had increased cuticular wax deposition as compared to well-watered plants (Kosma et al. 2009). Glossy mutants of maize plants infected with *Clavibacter michiganensis* (causal agent of Goss's wilt) pathogen retained more bacteria on its leaf surface, whereas wild-type plants had retained fewer bacteria and resisted infection. Susceptibility in the glossy mutant was attributed to the reduced density of cuticular wax crystals as compared to the wild-type plants (Marcell and Beattie 2002). A bloomless mutant of sorghum was used to study water loss and susceptibility to *Setosphaeria turcica* (causal agent of leaf blight) infection. Bloomless mutants had lost more water as compared to wild-type plants. In addition, bloomless mutants sustained more infection as compared to wild-type. Increased water loss and susceptibility to infection were ascribed to the reduced cuticular wax deposition in mutants (Jenks et al. 1994). This evidence indicates the cuticular wax composition play a role in drought and disease susceptibility to drought and pathogen infection. However, the significance of cuticular wax under combined stress is yet to be studied.

4.5 Molecular Mechanism Governing Traits Imparting Combined Stress Tolerance in Plants

Recently, several molecular and physiological studies have been undertaken to comprehend the gene expression patterns associated with tolerance to combined drought and pathogen infection. Marked change in expression of genes between the plants subjected to individual and combined stress was observed (Rizhsky et al. 2004; Prasch and Sonnewald 2013; Rasmussen et al. 2013). Majorly, under combined stress, gene expression pattern was categorized into two major divisions, viz., unique (idiosyncratic) and tailored or shared. Under combined stress, genes which are expressed uniquely are named as tailored, whereas genes which are expressed in both individual and combined stress conditions are termed as shared or common (Pandey et al. 2015; Gupta et al. 2016). Molecular basis of some of the morpho-physiological traits associated with tolerance in response to combined stress has been studied. For instance, stomatal behavior under combined drought and pathogen stress may result in either plant tolerance or susceptibility. A study conducted by Mantri et al. (2010) reported the transcriptional changes in *A. rabiei*-infected chickpea as compared to control. In this study, it was found that the genes involved in metabolism and photosynthesis were suppressed under individual drought stress treatment. Importantly, pathogenesis-related proteins were repressed under drought stress. In contrast, under pathogen stress, pathogenesis-related proteins were induced in chickpea. In *A. thaliana*, Gupta et al. (2016) reported that the genes such as *NTM1* (NAC with transmembrane motif1 (NTM1) and *PNP* (plant natriuretic peptide) were upregulated only under combined drought and *P. syringae* infection but not under individual stress conditions. Importantly, PNP protein has been reported to be involved in regulation of stomatal conductance (Turek et al. 2014). In addition, genes encoding JAZ proteins were also upregulated under combined drought and pathogen stress but downregulated in individual pathogen stress (Gupta et al. 2016). JAZ-interacting domains are found in bHLH protein family, which has been reported to be involved in trichome development and stomatal patterning (Pauwels and Goossens 2011). Silencing of genes encoding JAZ proteins in *S. lycopersicum* resulted in rapid disease development and also delayed the PAMP-mediated hypersensitive reaction upon *P. Syringae* infection as compared to control (Ishiga et al. 2013). Similarly, *Arabidopsis* mutants such as *ssi2* (stearoyl desaturase) and *fad7/fad8* (fatty acid desaturase 7 and 8) were demonstrated in defense manifesting resistance to oomycetes and *P. Syringae* infections. Genes governing lipid metabolism were also upregulated in drought-stressed rice as compared to control. Cell membrane stability is regulated by lipid content of the plants. Therefore change in lipid content or alteration in lipid metabolism results in plant tolerance (Borah et al. 2017; Rojas et al. 2014). Identification and functional characterization of genes governing morpho-physiological traits will assist in plant tolerance to combined stresses.

4.6 Future Perspectives

Crop plants alter morpho-physiological features to survive in the stressful environment. In plant breeding, researchers exploited morpho-physiological response-based traits such as root length for crop improvement under individual drought stress. However, as plants encounter simultaneous stresses in field conditions, for example, combined drought and pathogen infection, studies should be undertaken to evaluate the extent of the contribution of several of already known traits to combined stress tolerance. Additionally, understanding of the underlying molecular mechanism of the known traits, with reference to combined stress, is important. Although studies have shown the changes in traits such as cell membrane stability, leaf water potential, stomatal conductance, and root length in response to combined stress, a direct correlation about the contribution of these traits to combined stress tolerance is yet to be studied. Additionally, few papers have pointed out the plausible mechanisms such as drought-activated hydraulic signals, which alter stomatal movement under drought stress (Yan et al. 2017), exogenous and endogenous ABA-mediated changes in stomatal movement under drought and pathogen infection (Mohr and Cahill 2003), and drought-induced ROS-mediated resistance to bacterial infection which was demonstrated in *N. benthamiana* (Ramegowda et al. 2013). In a nutshell, it is essential to undertake a systematic investigation to study the molecular mechanism responsible for tolerance or susceptibility of plants to combined stress.

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