



ADVANCEMENTS IN DEVELOPING ABIOTIC STRESS-RESILIENT PLANTS

Basic Mechanisms to Trait Improvements

EDITED BY

M. Iqbal R. Khan

Palakolanu Sudhakar Reddy

Ravi Gupta



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1 Physiological, Molecular, and Biochemical Responses of Rice to Drought Stress

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1.1 INTRODUCTION

Drought or water deficit is one of the major environmental constraints on rice productivity, particularly in rainfed ecosystems and largely in major rice -growing ecosystems, as groundwater, a valuable resource for irrigation during drought, is continuously declining (Ray *et al.*, 2015). Erratic rainfall across the globe may exacerbate droughts, with increasing frequency of water stress during the cropping season. Increased drought will increase water stress. Exposure of plants to drought adversely affects them at every stage from germination to reproduction and finally limits yield (Pandey and Shukla, 2015; Khan *et al.*, 2015). Rice is highly susceptible to water stress. Drought stress reduces accumulation of biomass, in general, and causes yield reduction. The magnitude of yield loss depends on timing, plant growth stage and duration, and the severity of drought stress. Drought stress affects rice plants throughout their lifecycle, but drought stress from the intermittent (during maximum tillering, flowering-reproductive growth) to the late (after panicle initiation) stage may greatly affect the yield. The drought-mediated yield losses in the reproductive stage in rice are given in Table 1.1.

TABLE 1.1
Yield Losses in Rice as a Result of Reproductive-Stage Drought

Severity of Reproductive Drought Stress	Yield Reduction (%)	Reference
Lowland moderate stress	45–60	Vikram <i>et al.</i> , 2011 Dixit <i>et al.</i> , 2012, Rajurkar <i>et al.</i> , 2019, 2021
Lowland severe stress	65–91	Vikram <i>et al.</i> , 2011 Ghimire <i>et al.</i> , 2012 Rajurkar <i>et al.</i> , 2019, 2021
Upland mild stress	18–39	Vikram <i>et al.</i> , 2011 Sandhu <i>et al.</i> , 2014
Upland moderate stress	70–75	Vikram <i>et al.</i> , 2011 Sandhu <i>et al.</i> , 2014
Upland severe stress	80–97	Bernier <i>et al.</i> , 2007 Lafitte <i>et al.</i> , 2007 Dixit <i>et al.</i> , 2012

Extreme climate change causing lower precipitation and drought has negative effects in many growing areas of the world (Lobell *et al.*, 2011). Drought is frequent in many parts of South and Southeast Asia, affecting 46 Mha rainfed lowland and 10 Mha upland rice area in Asia (Pandey *et al.*, 2007). In India alone, 14.4 and 6.0 Mha of the rainfed lowland and upland rice area, respectively, are affected by drought (Mahajan *et al.*, 2017). Recent predictions suggest further increased frequency and intensity of drought and increase concern over water deficit problems in the coming decades (Wassmann *et al.*, 2009). Given the increasing severity, it is necessary to develop cultivars with inbuilt mechanisms for drought stress tolerance and to deliver adapted varieties to improve productivity in drought-affected environments.

Plants adapt different strategies, such as drought escape, recovery and resistance, which can be further divided into drought avoidance and tolerance (Fukai and Cooper, 1995). Drought avoidance is usually associated with reduced water loss, extensive root system for water uptake and reduced leaf area to avoid evaporation. During drought tolerance, plants maintain their normal functioning even with low water potential within the tissues, and this is associated with accumulation of compatible solutes and protoplasmic resistance (Price *et al.*, 2002). Improving drought resistance is a complex and difficult task to achieve, because sometimes it comes with limitations such as short lifecycle, leading to reduced grain yield, and lower carbon assimilation with ultimate reduction in grain yield is seen in varieties using drought escape and avoidance strategies. In the case of drought tolerance mechanisms, increased solute concentration for osmotic adjustment may have a negative impact on plant growth. Therefore, adaptation of crops to drought stress must maintain a balance between the drought resistance mechanisms introduced to guarantee sustainable productivity (Mitra, 2001; Yang *et al.*, 2010). Plants sense their environments by adaptive morphological, anatomical, cellular, physiological and biochemical changes. And thus, multidisciplinary approaches are needed to understand responses and mechanisms and develop strategies to bridge the yield gaps under various environmental stresses. A detailed understanding of drought stress responses is desirable for the development of resilient cultivars suitable for drought conditions (Khan *et al.*, 2021; Ratnakumar *et al.*, 2016). In the past decades, our understanding of molecular and cellular mechanisms involved in drought stress resistance has strongly improved, enabling us to integrate drought avoidance and tolerance strategies into the desired crop cultivars for improving their performance under stress conditions. The current chapter reviews different physiological, molecular

and biochemical responses of plants to drought stress and also provides molecular insight into the adaptive mechanisms occurring in plants as a defensive plan to combat complex stress like drought.

1.2 PHYSIOLOGICAL RESPONSES AND MECHANISMS UNDER DROUGHT STRESS

Molecular, cellular, physiological, biochemical and developmental responses to abiotic stress involve several traits and the underlying genes controlling drought tolerance. To address the complexity of plant responses to drought, it is vital to understand drought stress and its effects on physiological processes or component traits, and their role in yield improvement. However, efforts to dissect drought resistance, by identifying and characterizing component traits and transferring them into cultivars with high-yielding genetic backgrounds, have had very limited success. Nevertheless, there are a few instances in which trait-based selection for drought resistance has resulted in actual yield improvement (Richards, 2006; Sinclair *et al.*, 2004).

Understanding the physiological basis of crop performance under drought conditions will contribute to the identification and manipulation of traits associated with improved water use efficiency and yield under water deficit conditions and will complement conventional and molecular breeding programs. Different traits may play important roles at different drought severity levels and at different crop developmental stages. The drought resistance traits are mainly divided into constitutive traits (e.g., root traits: root diameter, root thickness) and induced/adaptive traits (e.g., osmotic adjustment). In evaluating traits for improved adaptation to drought, both constitutive and adaptive (inducible) traits are considered important. Constitutive traits are expressed under normal conditions and do not require water stress for their expression, whereas adaptive traits are expressed only in response to water deficit (Kamoshita *et al.*, 2008). All the traits have either positive or negative influence on yield, depending on the existing drought situation (timing, severity and duration) and depending on whether a survival or production mechanism is necessary. The physiological traits whose importance in drought resistance in rice has been demonstrated are summarized in the following subsections.

1.2.1 LEAF ROLLING AND LEAF AREA INDEX

The turgidity of the cells is lost with severe drought, resulting in wilting of leaves, which is expressed as leaf rolling in rice and most of the cereals. Leaf rolling is the first visual symptom of drought reaction and occurs due to the inability of leaves to sustain the transpiration demand of the plant (Blum, 1988). This trait has been found to be a useful criterion in assessing levels of drought tolerance in large-scale screening (Chang and Loresto, 1986). Leaf rolling is an adaptive response to water deficit, which helps in maintaining favorable water balance within plant tissues and thus maintains cell growth and activity (Sellammal *et al.*, 2014). Bhattarai and Subudhi (2018) observed higher leaf rolling as a major phenotypic change in rice plants under drought stress. Leaf rolling reduces the transpiration rate and canopy temperature, thereby improving water use efficiency (WUE; Townley-Smith and Hurd 1979). Xu *et al.* (2002) reported better WUE in genotypes with partially rolled or folded leaves but reduced leaf area, while a recent study by Cal *et al.*, 2019 suggested that leaf rolling under drought was more affected by leaf morphology than by stomatal conductance, leaf water status, or maintenance of shoot biomass and grain yield. QTLs for leaf rolling have been identified in rice (Nguyen *et al.*, 2013; Subashri *et al.*, 2009). Subashri *et al.* (2009) also found collocation of the leaf rolling QTL region with QTLs for panicle exertion, panicle length, plant height and biological yield.

Evaporative demand is controlled by a decrease in leaf growth that affects leaf area either by reducing individual leaf growth or by reducing the number of leaves (Tardieu, 1996). A plant reduces its leaf area by drying older leaves and thereby reducing water loss, overall reducing WUE. Leaf area is

usually given in terms of Leaf Area Index (LAI), which is leaf surface area per unit soil surface area and affects plant transpiration. Plants with fewer leaves transpire less without significantly changing their net primary production. Also, the greater the LAI, the lower is the evapotranspiration from soil, and vice versa. Grain yield has been reported to be positively correlated with green leaf area under terminal drought, and negative relationships were observed between grain yield and leaf senescence (Borell *et al.*, 2000).

1.2.2 LEAF WATER POTENTIAL (LWP) AND RELATIVE WATER CONTENT (RWC)

Exposure of plants to drought stress substantially decreases the LWP, RWC and transpiration rate, with a concomitant increase in leaf temperature (Siddique *et al.*, 2001). RWC and LWP have long been associated with rice performance under water deficit (O'Toole and Moya, 1978) and were found to be correlated with yield at the flowering stage under drought stress (Lafitte, 2002; Jongdee *et al.*, 2006). RWC is also useful in better characterization of plant root and shoot physiology under drought stress (Anupama *et al.*, 2019). Drought-resistant varieties show higher LWP and maintain a higher RWC as compared with drought-sensitive varieties under moisture deficit conditions (Swamy *et al.*, 1983; Anupama *et al.*, 2019). Maintenance of higher LWP under drought is empirically related to better stem extension and panicle exertion (Jearakongman, 2005) as well as to reduced delay in flowering (Pantuwan *et al.*, 2002).

1.2.3 OSMOTIC ADJUSTMENT

Osmotic adjustment (OA) is a metabolic process associated with the synthesis of various compatible solutes within plant cells in response to water stress (Serraj and Sinclair, 2002) and is a prime drought stress adaptive engine in support of plant production and crop yield under water-limiting conditions (Blum, 2017). As soil moisture declines, OA causes a reduction in osmotic potential, favoring turgor maintenance and protecting the integrity of metabolic functions. The role of OA in maintaining yield under drought has been reported in rice (Praba *et al.*, 2009). Under severe water stress, higher OA capacity may help plants to withstand a prolonged drought spell and recover more promptly upon rehydration. Significant variations in OA were reported among rice lines (Babu *et al.*, 2001). Indica rice varieties are known to have higher OA capacity compared with japonica varieties (Kole, 2006). OA has a positive effect on leaf rolling, tissue death and retention of leaf area in rice under drought stress (Fukai and Cooper, 1995). OA is recognized as an effective cellular and metabolic mechanism that can be manipulated to produce water deficit-resistant crop varieties (Blum, 2017; Abdelrahman *et al.*, 2018). Swapna and Shylaraj (2017) identified rice varieties under polyethylene glycol (PEG)-mediated osmotic stress and drought stress using hydroponics and found that the identified varieties had less leaf rolling, better drought recovery ability and better RWC, increased membrane stability index with osmolyte accumulation, and antioxidant enzymatic activity. The role of different osmolytes in OA is detailed in Zivcak *et al.* (2016) and is briefly discussed in the later biochemical section. QTLs for OA have been detected by several studies (Lilley *et al.*, 1996; Zhang *et al.*, 2001; Robin *et al.*, 2003).

1.2.4 STOMATAL DENSITY, APERTURE SIZE AND STOMATAL CONDUCTANCE

Stomata play a central role in the pathways of both carbon uptake and water loss by plants (Chaves *et al.*, 2016). Stomata maintain leaf temperature through water loss in transpiration and regulate the gaseous flow needed in the process of photosynthesis. Stomata plays an adaptive role under drought by stomatal pore opening or closing, aperture size, stomatal density and distribution pattern (Hetherington and Woodward, 2003). Stomatal density and aperture size are the two main factors determining stomatal conductance and thus affect photosynthetic ability. Stomatal conductance,

by reducing transpiration and soil water absorption, plays an essential role in regulating the water balance of the plant (Sinclair *et al.*, 2011). Canopy temperature measured by thermal imaging is a proxy for stomatal conductance and has already proven to be a good indicator of drought stress in the field (Leinonen *et al.*, 2006; Munns *et al.*, 2010) and has been used in several studies (Melandri *et al.*, 2020). Reducing stomatal conductance under water-limited conditions may lead to a greater yield by improving intrinsic WUE (iWUE) (Leakey *et al.*, 2019). Lower stomatal density in drought-tolerant rice varieties showed a reduced transpiration rate (Anupama *et al.*, 2019), while Kulya *et al.* (2018) reported higher stomatal density and reduced stomatal length in chromosomal segment substitution lines (CSSLs) of rice cultivar KhaoDawk Mali 105 (KDML105) carrying drought-tolerant DT-QTL segments from drought-tolerant donors, DH103 and DH212. Henry *et al.* (2019) showed an interesting trend in a large effect drought yield QTL (qDTY12.1) near-isogenic lines (NILs) and the donor parent Way Rarem for conserving water through low and high stomatal conductance under high and low evapo-transpirational demand, respectively, compared with the recipient parent Vandana. Pantuwan *et al.* (2002) found an association of the process of plant water conservation through stomatal regulation with reduced spikelet sterility and increased grain yield under reproductive-stage drought.

1.2.5 ROOT TRAITS

Roots are the first organs exposed to water stress. A quick strategic response in root morphological traits is the important physiological parameter for drought adaptation (Ingram *et al.*, 1994; Niones *et al.*, 2012). Root growth and distribution are modulated by the stress and assist in providing drought resistance through avoidance mechanisms, either by growing deeper and exploring deep soil moisture or maintaining root growth. The possession of a deep, thick and larger root system is generally considered favorable, allowing the crop to maintain its water status even under water deficit conditions (Nguyen *et al.*, 1997), and is considered important in determining drought resistance in upland rice. Deep-rooting cultivars are more resistant to drought than shallow-rooting ones (Farooq *et al.*, 2009a).

Under different types of drought stress, plasticity in root density, total root length (Tran *et al.*, 2015), and lateral root length and/or branching (Kano-Nakata *et al.*, 2013) has been observed to improve shoot biomass, water uptake and photosynthesis under drought stress in rice (Sandhu *et al.*, 2016). Furthermore, root architecture is also considered to be a key trait for dissecting the genotypic differences in rice responses to water deficit cues (Henry *et al.*, 2011). The spatial distribution of roots largely determines the genotypic potential for extraction of water and influences productivity under drought stress. The deep root–shoot ratio is well correlated and affects the ability of a variety to absorb water from the deep soil layers, determining a variety's resistance to drought (Yoshida and Hasegawa, 1982), while thick roots persist longer and produce more and larger branch roots, thereby increasing root density and water uptake capacity (Ingram *et al.*, 1994). A deep and thick root system has been thought to be advantageous for improved drought tolerance in rainfed ecosystems (Fukai and Cooper, 1995; Comas *et al.*, 2013). Thick roots also exert greater penetration ability in hard soil under drought (Babu *et al.*, 2001). Jeong *et al.* (2013) observed increased yield (9–26%) in transgenic rice over-expressing *OsNAC5* through increased root diameter under drought. In a number of species, root characteristics such as increased root density, maximum root length, root thickness, rooting depth, number of nodal roots, root:shoot ratio and branching of root systems have all been associated with plant adaptation to water stress. A positive correlation between root traits and yield and its related components under drought stress was reported by Babu *et al.* (2003). Kumar *et al.* (2004) reported an association between higher root pulling resistance (RPR) and maintenance of higher LWP under severe drought stress, as well as a positive correlation with grain yield and RWC with RPR. Various QTLs for root length, root thickness and root penetration ability have been detected and incorporated into rice varieties to enhance drought tolerance; a yield advantage of 1 t ha⁻¹ was found in introgressed plants with longer root length QTLs compared with controls, with

60% less water consumption than traditional varieties (Steele *et al.*, 2006, 2007). Uga *et al.* (2013) identified, cloned and characterized the QTL *Deeper Rooting 1* (*DRO1*), controlling root growth angle. The NILs developed by introgression of *DRO1* into shallow-rooting IR64 exhibited enhanced drought tolerance by growing deeper roots, resulting in high-yield performance under drought (Uga *et al.*, 2013; Arai-Sanoh *et al.*, 2014).

1.2.6 MOLECULAR BREEDING FOR PHYSIOLOGICAL AND SECONDARY TRAITS

Over the last decade, the drought breeding program at the International Rice Research Institute (IRRI, Philippines) has made significant progress in developing abiotic stress-tolerant lines in rice, targeting grain yield, and led to the development of more than 20 high-yielding, drought-tolerant lines with release of varieties across South and Southeast Asia and Africa since 2009 (Sandhu and Kumar, 2017). There are reports of several grain yield QTLs, i.e., *qDTY1.1*, *qDTY2.1*, *qDTY2.2*, *qDTY3.1*, *qDTY3.2*, *qDTY9.1* and *qDTY12.1*, showing large effects under drought (Khan *et al.*, 2021). However, studies targeting secondary traits are scarce. Though a number of secondary traits have been determined to be associated with drought resistance, very few have been used in rice breeding programs to improve yield under water limited conditions, which is still in progress. This is because selection for secondary traits in breeding programs needs extensive investment in phenotyping these traits and is prone to problems of repeatability due to high genotype $\times$ environment ($G \times E$) interaction. Progress in high-throughput precision phenotyping (phenomics) and genomics technologies is now overcoming the phenotyping and genotyping bottlenecks, enabling a more precise detection of QTLs for “hard-to phenotype” complex secondary/physiological traits, a problem that is particularly relevant for the selection of drought-resistant genotypes. Molecular marker technology or marker assisted breeding has been identified as a powerful tool for selection of traits that are otherwise difficult to screen. Molecular markers allow breeders to track the genetic loci controlling drought resistance without measuring the phenotype, thus reducing the need for extensive field testing over space and time (Nguyen *et al.*, 1997). With this advancement, recent mapping approaches have already provided closely linked markers for complex traits such as stay-green (Borrell *et al.*, 2014), OA (Babu *et al.*, 2001; Robin *et al.*, 2003), root traits (Champoux *et al.*, 1995; Uga *et al.*, 2013; Wade *et al.*, 2015), and QTLs controlling drought avoidance mechanisms (such as leaf rolling, leaf drying, RWC of leaves, LWP, stomatal conductance and relative growth rate under stress) in rice (Courtois *et al.*, 2000; Babu *et al.*, 2003; Khowaja and Price, 2008; Barik *et al.*, 2018, 2019). The QTLs identified for various physiological and secondary traits using different mapping populations are summarized in Table 1.2.

1.3 MOLECULAR RESPONSES AND MECHANISMS UNDER DROUGHT STRESS

Tolerance to water stress is a quantitatively controlled trait in plants. Drought stress induces multiple molecular and biochemical changes within plants, leading to alterations in morpho-physiological characters that are important in order to survive. Drought changes the expression of genes regulating water transport, oxidative damage, osmotic balance and damage repair mechanisms. Recently developed molecular tools, e.g., RNA-Seq and bioinformatics, have accelerated the discovery of stress-responsive genes and TFs in many plant species (Khan *et al.*, 2018; Kaur and Asthir, 2017; Xiong *et al.*, 2005).

Some of the changes that are transduced are in the form of signals such as protein phosphorylation/de-phosphorylation, calcium signaling, reactive oxygen species, abscisic acid (ABA) biosynthesis and cross-talk with other phytohormones (Ali *et al.*, 2020; Tiwari *et al.*, 2017; Khan and Khan, 2017). Phytohormones play a central role in regulating plant growth and development in response to environmental changes (Cutler *et al.*, 2010; Khan *et al.*, 2015). Phytohormonal cross-talk occurs

TABLE 1.2
Recent QTLs Identified for Physiological and Secondary Traits under Drought Stress in Rice

Population	Environment	Physiological/Secondary Traits	References
IR64/Azucena DH (Double Haploid) population	Upland fields	Leaf rolling, leaf drying, relative water content	Courtois <i>et al.</i> , 2000
	PVC cylinders	Leaf rolling, root length, root number, root volume, root weight, root thickness, drought score	Hemamalini <i>et al.</i> , 2000
CT9993/IR62266 DH population	Greenhouse	Cell membrane stability	Tripathy <i>et al.</i> , 2000
	Field conditions	Osmotic adjustment, root thickness, root weight, root length, root pulling force	Zhang <i>et al.</i> , 2001
Bala/Azucena Recombinant Inbred lines (RILs)	Field conditions	Relative water content, leaf rolling, canopy temperature, leaf drying, root morphology	Babu <i>et al.</i> , 2003
	Field conditions	Leaf rolling, leaf drying and leaf relative water content	Price <i>et al.</i> , 2002a
	Growth room with controlled environment conditions	Leaf morphological traits, leaf area, leaf dry weight, leaf water relations and rolling	Khawaja and Price, 2008
IAC165/Co39	Greenhouse	Root traits, root thickness, root weight, maximum root length	Courtois <i>et al.</i> , 2003
IR1552/Azucena RIL Population	Controlled environment and PVC pots	Seminal root length, adventitious root number, lateral root length and number	Zheng <i>et al.</i> , 2003
IR62266/IR60080 IRAT109/ Yuefu DH Lines	Greenhouse	Osmotic adjustment	Robin <i>et al.</i> , 2003
	Field, PVC pipes, aerobic conditions	Root traits, basal root thickness, total root number, maximum root length, root weight	Li <i>et al.</i> , 2005
Zhenshan 97/IRAT109	Field conditions	Leaf rolling time and leaf drying score, canopy temperature, root traits	Yue <i>et al.</i> , 2005, Yue <i>et al.</i> , 2006
	Rainproof drought screen facility	Canopy temperature, leaf water potential	Liu <i>et al.</i> , 2005
Akiahikari × IRAT109	Hydroponics drought stress using PEG	Relative growth rate and specific water use	Kato <i>et al.</i> , 2008
Teqing/Lemont BC introgression lines (ILs)	Field conditions	Photosynthetic rate, stomatal conductance, transpiration rate, stomata frequency, chlorophyll content	Zhao <i>et al.</i> , 2008
IR20/Nootripathu RILs	Rainfed fields	Leaf rolling and leaf drying, canopy temperature, relative water content, drought score	Gomez <i>et al.</i> , 2010, Salunkhe <i>et al.</i> , 2011, Prince <i>et al.</i> , 2015, Rajurkar <i>et al.</i> , 2019
Zhenshan 97/IRAT109 NILs	PVC pipes	Root growth rate, root volume, deep root volume	Ding <i>et al.</i> , 2011

(continued)

TABLE 1.2 (Continued)
Recent QTLs Identified for Physiological and Secondary Traits under Drought Stress in Rice

Population	Environment	Physiological/Secondary Traits	References
IR64/INRC10192 RILs, Backcross population	Hydroponics drought stress using PEG, field condition	Root length, root dry weight	Srividya <i>et al.</i> , 2011, Patil <i>et al.</i> , 2017
Haogelao/Shennong265	Field conditions	Photosynthesis parameters, Net photosynthesis rate, Stomatal conductance, Transpiration rate	Gu <i>et al.</i> , 2012
Khaunoongmo/Q5 F2 population	Field conditions	Leaf rolling, leaf drying, plant recovery	Nguyen <i>et al.</i> , 2013
IR64/Cabacu RILs	Field conditions	Leaf rolling, root pulling force	Trijatmiko <i>et al.</i> , 2014
Swarna/WAB450	Field conditions	Canopy temperature, grain yield under drought	Saikumar <i>et al.</i> , 2014
Association mapping population, collections of RILs from Zhenshan97/IRAT109 and Chinese landraces	Field conditions	Ratio of deep rooting	Lou <i>et al.</i> , 2015
Dular/IR64 RILs	Upland fields and greenhouse	Leaf rolling, root growth angle, seedling-stage root length, root dry weight and crown root number	Catolos <i>et al.</i> , 2017
Samgang/Nagdong DH population	Field and greenhouse conditions	Visual drought tolerance and relative water content	Kim <i>et al.</i> , 2017
Cocodrie/N-22 RILs	Greenhouse, pots	Leaf rolling score	Bhattarai and Subudhi, 2018
CR 143-2-2/Krishnahamsa RILs	Rain out shelter	Relative water content	Barik <i>et al.</i> , 2018
IAPAR-9/Akihikari and IAPAR-9/Liaoyan241	Hydroponics drought stress using PEG, field managed drought	leaf rolling index, rooty number and root length	Han <i>et al.</i> , 2018
Dongnong422/Kongyu131, Xiaobaijingzi/Kongyu131 RIL population	Field conditions	Leaf area, chlorophyll content	Yang <i>et al.</i> , 2018
Koshihikari/Takanari//Koshihikari, or Koshihikari/Takanari//Takanari, BC1F1 plants	Field condition	Canopy temperature difference	Fukuda <i>et al.</i> , 2018
CR 143-2-2/Krishnahamsa	Field condition, rain out shelter	Leaf rolling, leaf drying	Barik <i>et al.</i> , 2019
GWAS, Vietnamese landraces panel	Net house, plastic trays	Leaf relative water content, drought sensitivity score, recovery ability	Hoang <i>et al.</i> , 2019
IR55419- 04/Super Basmati F2 population	Greenhouse, PVC pipes	Leaf drying score, leaf dry weight, leaf area, root dry weight, deep root length, volume, surface area and deep root diameter	Sabar <i>et al.</i> , 2019

TABLE 1.2 (Continued)
Recent QTLs Identified for Physiological and Secondary Traits under Drought Stress in Rice

Population	Environment	Physiological/Secondary Traits	References
KDML105/ DH212 or DH103	Field condition	Traits relevant to drought tolerance and avoidance	Shearman 2020
CSSL population Rice diversity panel consisting of 293 <i>indica</i> accessions	Field condition	Canopy temperature	Melandri <i>et al.</i> , 2020

at the biosynthesis and degradation which are triggered after the occurrence of drought and acts as a signaling pathway to trigger adaptive responses. The role of ABA in drought stress response is well known, and several ABA-responsive genes controlling drought stress responses have already been reported elsewhere (Khan *et al.*, 2021). ABA is a major player in cellular growth reduction, stomatal closure and reduced transpiration rate under drought stress (Shinozaki and Yamaguchi-Shinozaki, 2007). However, several studies report that many drought-related genes do not respond to ABA, suggesting the existence of ABA-independent signal transduction pathways and cross-talk or involvement of other hormones like salicylic acid (SA), jasmonic acid (JA), cytokinins (CK) and ethylene (ET) in drought stress responses (Khan *et al.*, 2021; Per *et al.*, 2018). Li *et al.* (2017) isolated the *ABA stress* and *ripening* (*ASR*) gene from the upland rice variety IRAT 109 and demonstrated that over-expression of *OsASR5* enhanced osmotic and drought tolerance in rice by regulating leaf water status and ABA biosynthesis and promoting stomatal closure under drought stress conditions.

In the last decades, hundreds of drought-inducible genes in rice have been identified using microarrays, expressed sequence tags and quantitative reverse transcription polymerase chain reaction (qRT-PCR) (Rabbani *et al.*, 2003; Degenkolbe *et al.*, 2009; Todaka *et al.*, 2012; Borah *et al.*, 2017; Sharma *et al.*, 2019). Among these identified genes, some are involved in the protection and repair mechanism of cells, while other genes are involved in regulation of signal transduction, activation of TFs and biosynthesis of signaling molecules (regulatory proteins) (Shinozaki and Yamaguchi-Shinozaki, 2007). The expression of stress-induced genes is largely regulated by specific TFs, and it has been estimated that the rice genome contains 1,611 TF genes that belong to 37 gene families (Xiong *et al.*, 2005). Stress-related TF families exhibit distinctive binding domains such as dehydration-responsive element binding (DREB)/C-repeat-binding factor (CBF), ethylene-responsive factor (ERF), basic-leucine zipper (bZip), ABA-responsive element binding (AREB)/ABRE binding factor (ABF), NAM (no apical meristem), and CUC (cup-shaped cotyledon) (NAC) conserved domain and homeodomain-leucine zipper (HD-Zip) (Xiong *et al.*, 2005). Some of the important TFs identified and characterized in rice are listed in Table 1.3.

TF binding to a DNA sequence can result in activation or repression of transcription. TFs bind to cis-regulatory elements, which are mainly found within the promoter region of stress-inducible genes. The most common cis-regulatory element in ABA-regulated genes is the ABRE, which is recognized by the bZip family (Hossain *et al.*, 2010). The DRE/C-repeat (CRT) element, which is recognized by the DREB/ERF family, regulates function in ABA-independent gene expression (Nakashima *et al.*, 2009). One particular large group of TFs involved in stress responses in several plant species is that of the HD-Zip genes (Bhattacharjee *et al.*, 2016), which are a subgroup of the homeobox genes. There are several reports of drought-tolerant genes that have been characterized in rice using genetic engineering technology, and their exact roles and phenotypes have been listed earlier (Yoo *et al.*, 2017; Oladosu *et al.*, 2019); furthermore, recently, a network-based supervised

TABLE 1.3
Transcription Factors Functionally Characterized under Stresses in Rice

Transcription Factor (TF)	Stress Tolerance	Reference
OsRab7	Drought and heat	El-Esawi and Alayafi, 2019
SNAC1	Drought and salinity	Hu <i>et al.</i> , 2006
OsNAC5	Drought	Jeong <i>et al.</i> , 2013
OsNAC6	Abiotic and biotic	Nakashima <i>et al.</i> , 2007
OsNAC10	Drought	Jeong <i>et al.</i> , 2010
OsNAC14	Drought	Shim <i>et al.</i> , 2018
OsNAC045	Drought and salinity	Zheng <i>et al.</i> , 2009
OsERF71	Drought	Lee <i>et al.</i> , 2016
SUB1A	Drought and flooding	Fukao <i>et al.</i> , 2011
OsbZIP23	Drought and salinity	Xiang <i>et al.</i> , 2008
OsbZIP16	Drought	Chen <i>et al.</i> , 2012
OsbZIP71	Drought and salinity	Liu <i>et al.</i> , 2014
OsbZIP46	Drought	Tang <i>et al.</i> , 2012
OsbZIP72	Drought	Lu <i>et al.</i> , 2009
OsMYB2	Dehydration, salinity and cold	Yang <i>et al.</i> , 2012
OaMYB55	Drought and heat	Casaretto <i>et al.</i> , 2016
OsDREB1F	Drought, salinity and low temperature	Wang <i>et al.</i> , 2008
OsDREB2A	Drought	Cui <i>et al.</i> , 2011
OsABF2	Drought, salinity and cold	Hossain <i>et al.</i> , 2010
Oshox4	Drought	Agalou <i>et al.</i> , 2008
ZFP245	Drought, cold and oxidative stress	Huang <i>et al.</i> , 2009
ZFP252	Drought and salinity	Xu <i>et al.</i> , 2008
OsCDPK7	Drought, salinity and cold	Saijo <i>et al.</i> , 2000
AP37	Drought	Oh <i>et al.</i> , 2009
OsbHLH148	Drought	Seo <i>et al.</i> , 2011
ARAG1	Drought	Zhao <i>et al.</i> , 2010
OsWR1	Drought	Wang <i>et al.</i> , 2012
OsTZF1	Drought and salinity	Jan <i>et al.</i> , 2013

machine learning framework that accurately predicts and ranks all rice TFs in the genome according to their potential association with drought tolerance has been reported (Gupta *et al.*, 2020).

1.4 BIOCHEMICAL RESPONSES AND MECHANISMS UNDER DROUGHT STRESS

Diversified groups of compatible solutes and biochemicals have been identified in various crop species under stress. They mainly act as osmoprotectants and go through a biochemical process of osmotic adjustment under stress conditions. Several solutes can be accumulated for osmoprotection, such as free amino acids like proline, nitrogenous compounds like glycine betaine, and sugars and other solutes, to balance osmotic pressure in plant cells. Among all the solutes, proline takes the lead when it comes to drought tolerance in plants, with others including the sugars and antioxidants, which are known as reactive oxygen species (ROS) scavengers (Hasanuzzaman *et al.*, 2018; Hanif *et al.*, 2020).

1.4.1 PROLINE

The role of proline in stress tolerance was first reported in rye grasses (Kemble and Macpherson, 1954). Changes in the concentration of proline compared with other amino acids have been

observed in rice under water stress (Mansour and Salama, 2020), and it is used as a biochemical marker to select resistance in such conditions (Fahramand *et al.*, 2014). Proline is proposed to screen germplasm for drought adaptation in various crop species. It plays a highly beneficial role by acting as an osmolyte (Verbruggen and Hermans, 2008; Mansour and Salama, 2020; Per *et al.*, 2017), a metal chelator, an anti-oxidative defense molecule and a signaling molecule during stress conditions (Dar *et al.*, 2016). Earlier studies have reported the role of proline as a detoxifier or osmoprotectant by scavenging ROS (Liang *et al.*, 2013; Per *et al.*, 2017). The accumulation of proline is predicted to be involved in plant damage repair ability by boosting antioxidant activity during drought stress (Lum *et al.*, 2014; Mansour and Salama, 2020).

1.4.2 POLYAMINES

Polyamines (PAs) are positively charged molecules involved in drought tolerance response (Takahashi and Kakehi, 2010). The most ubiquitous PAs in plants are putrescine (Put), spermidine (Spd) and spermine (Spm). They can regulate osmotic and ionic homeostasis, stabilize membranes and act as antioxidants along with interaction among other signaling molecules (Pandey and Shukla, 2015; Asgher *et al.*, 2018). Under drought stress conditions, higher PA contents in plants are related to increased photosynthetic capacity, reduced water loss, improved OA and detoxification. Other roles of polyamines include enhancing the DNA-binding activity of TFs, prevention of senescence (Bouchereau *et al.*, 1999; Panagiotidis *et al.*, 1995), protein phosphorylation and conformational transition of DNA (Martin-Tanguy, 2001). PA accumulation is the immediate response observed after exposure to drought conditions in rice leaves (Yang *et al.*, 2007). The exogenous application of PAs alleviates drought stress by improving the WUE, production of proline, anthocyanin, maintenance of LWC and reduction in oxidative damage in rice (Farooq *et al.*, 2009b). Studies indicate that foliar application is more effective than seed priming, and among PAs, Spm is the most effective in improving drought tolerance in rice (Farooq *et al.*, 2009a; Do *et al.*, 2013; Pandey and Shukla, 2015).

1.4.3 ALLANTOIN

Allantoin is a major purine metabolite in plants under drought stress conditions (Silvente *et al.*, 2012). Recent studies on knockout mutants in the allantoinase gene have shown an accumulation of allantoin and enhanced seed survivability and growth in drought and osmotic stress conditions (Watanabe *et al.*, 2014; Takagi *et al.*, 2016). Allantoin plays a role in activating ABA metabolism, which is known as a stress hormone and regulates various drought stress responses in plants (Sah *et al.*, 2016). A metabolomics study on drought-tolerant rice shoots and roots revealed the accumulation of more allantoin in shoots compared with the roots (Casartelli *et al.*, 2018). Recently, scientists at IRRI, Philippines have discovered the first plant guanine deaminase (OsGDA1) gene in rice and shown its importance for plant survival under drought stress by maintaining the xanthine pull, which is also required for accumulation of the allantoin that will induce ABA synthesis during stress conditions (unpublished data).

1.5 CONCLUSION AND FUTURE PERSPECTIVES

Rice improvement for environmental stresses such as drought is complex and challenging. Considering the future global climate change, increased intensity and frequency of drought, and its effects on cereal crops, development of crop cultivars resilient to specific environmental stress is needed for future food security. Genetically, drought tolerance of rice is a complex trait under polygenic control and involves complex morpho-physiological mechanisms. Drought tolerance involves various aspects such as OA, plant signaling to control growth, transpiration, shoot and root system architectures, and several phytohormonal feedbacks and/or cross-talks. The responses of plants to tissue water deficit

determine their level of drought tolerance. To address the complexity of plant responses to drought, it is vital to understand the physiological and genetic basis of this response with comprehensive information and a better understanding of mechanisms with a multi-disciplinary approach, i.e., physiology, molecular biology, genomics and breeding, which are believed to address the multigenic nature of abiotic stresses, including drought tolerance. Considerable work has been undertaken to understand the genetic basis of putative drought-adaptive traits in rice. However, breeding for drought tolerance is extremely challenging due to the complexity and responses associated with various stress-adaptive mechanisms, uncertainty in onset of stress, large $G \times E$ interactions, and various molecular, biochemical and physiological phenomena affecting plant growth and development. Large effect QTLs for grain yield under drought have been identified in rice. However, the use of these QTLs in molecular breeding is limited, with a lack of repeatability across environments and genetic background. Comprehensive understanding of these QTLs is needed in order to improve their efficacy in marker assisted breeding (MAB). Understanding the physiological and molecular mechanisms associated with these yield QTLs will hasten MAB for drought resistance. Also, the majority of responses are mediated by cellular and biochemical mechanisms, involving cross-talk between phytohormones, which in turn triggers the stress-responsive mechanisms. Understanding phytohormonal cross-talk and hormonal dynamics and identifying gene regulatory networks of complex traits will help in developing a realistic framework to uncover the drought responses that will make progress in understanding how rice productivity can be maximized under limited water conditions. The increased access to rice genome information and transcriptomic datasets has provided a path to identify the gene regulatory networks of complex traits like drought tolerance. The QTLs, genes, TFs and hormones that modulate the physiology and morphology of plants under stress are indicative developers of next-generation “drought-proof” rice varieties.

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2 Coordinated Functions of Reactive Oxygen Species Metabolism and Defense Systems in Abiotic Stress Tolerance

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2.1 INTRODUCTION

Maintaining plant growth and productivity to sustain the needs of a growing population under perturbed environmental scenarios is a major challenge for agricultural and plant scientists. Morphological, physiological, and metabolic activities of plants during development are highly influenced by abiotic factors. The aberrant changes in climatic phenomena have altered the abiotic factors and aggravated the duration and strength of stresses (Raza et al. 2019). Incidences of abiotic stresses, including drought, waterlogging, heat, chilling/freezing, salinity, UV radiation, light, and pollutants (heavy metals, organic compounds), diminish plant yield and increase economic losses (Ahanger et al. 2017; Bhuyan et al. 2020; Iqbal et al. 2021). In normal field conditions, a number of stresses often occur in combination, which may be due to interrelated pathways (Bulgari et al. 2019; Sharma et al. 2019; Zandalinas et al. 2020). For instance, drought elevates salt stress and encourages over-production of reactive oxygen species (ROS) in plant cells, which react with biomolecules and modify their structure and function, causing oxidative stress (Sachdev et al. 2021). Multiple stresses acting together as a consortium on plants severely damage their growth and development (Bulgari et al. 2019; Sharma et al. 2019).

Members of the ROS family comprise radical and non-radical forms of molecular oxygen (O_2) (Mittler 2017). Plants under normal circumstances convert 1–2% of consumed O_2 into ROS as a by-product of aerobic processes (Roychowdhury et al. 2019; Shah et al. 2019). However, unfavorable conditions exacerbate ROS production, resulting in an ROS burst in a plant cell. ROS are considered as toxic molecules, and their excess accumulation affects cellular and molecular components of plants (Kerchev et al. 2020). Generally, plants contain enzymatic and non-enzymatic antioxidant-based defense systems that scavenge or detoxify ROS. Nevertheless, if the ROS level remains unchecked or persists higher than the antioxidants' quenching threshold, this triggers oxidative stress and may lead to programmed cell death (PCD) (Kerchev et al. 2020). Due to their severe impacts on plant cells, ROS in the past have always been referred to as “bad” or toxic molecules; however, in the last few decades, studies have established the role of ROS in the regulation of developmental processes and facilitation of defense response against abiotic stresses, designating them as “good” molecules also (Mittler 2017; Kerchev et al. 2020). Therefore, ROS are addressed as a double-edged sword.

ROS, on the one hand, incite cell death, on the other hand, the transient increase upregulates genes and causes a surge in concentration of proteins and metabolites, enhancing the plant's tolerance against stress (Kerchev et al. 2020). As ROS perform a dual role within the plant cell, understanding the pathways responsible for triggering oxidative stress or switching tolerance can be employed as a strategic approach to reduce oxidative damage and reinforce their defense mechanism under projected climatic scenarios. The present chapter attempts to summarize the knowledge about the metabolism of ROS within the plant cell, the activity of different antioxidants in maintaining their level under equilibrium, and the mechanism of stimulating plant defense systems under abiotic stresses.

2.2 DIFFERENT KINDS OF ROS GENERATED UNDER ABIOTIC STRESSES

ROS primarily include superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($\cdot OH$), hydrogen peroxide (H_2O_2), and singlet oxygen (1O_2) generated through partial reduction or excitation of O_2 (Figure 2.1) in different cell organelles like chloroplasts, mitochondria, peroxisomes, and others (Mittler 2017; Jalil and Ansari 2018; Maurya 2020). Some of the ROS species are very toxic and highly reactive, while others are less toxic. However, less toxic or reactive species undergo conversion leading to the production of reactive ROS, which finally affect cellular components such as cellular and organelle membranes, photosynthetic activity, etc. (Das and Roychowdhury 2014).

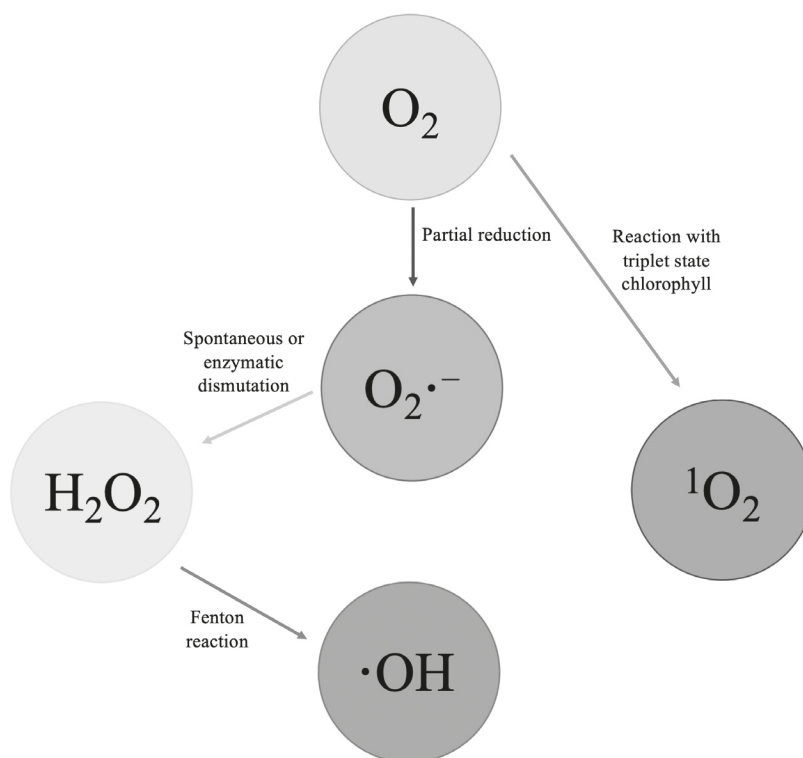


FIGURE 2.1 Different types of ROS generated within plant cell from molecular oxygen (O_2). O_2 on partial reduction forms superoxide radical ($O_2^{\cdot-}$), which undergoes spontaneous or enzymatic dismutation by superoxide dismutase, resulting in the formation of hydrogen peroxide (H_2O_2). H_2O_2 in the presence of redox metals produces hydroxyl radical via Fenton reaction. Singlet oxygen (1O_2) is generated on reaction of O_2 with triplet state chlorophyll.

2.2.1 SUPEROXIDE RADICAL ($O_2^{\cdot-}$)

Superoxide radical ($O_2^{\cdot-}$) is the first or primary ROS generated within the plant cell under stress (Das and Roychowdhury 2014). $O_2^{\cdot-}$ is generated at photosystem (PS) I in chloroplasts through partial reduction of O_2 by the photosynthetic electron transport chain (ETC) (Kohli et al. 2019). In addition to the chloroplast, $O_2^{\cdot-}$ is also produced in mitochondria, peroxisomes, apoplast, and cell wall (Kohli et al. 2019). It is a moderately reactive species with a half-life time of 2–4 μs . $O_2^{\cdot-}$ can migrate to a distance of 30 nm and is present in a concentration lower than 0.5% ($\sim 0.3\%$) in the cytosol (De Grey 2002; Mittler 2017; Kohli et al. 2019). Due to moderate toxicity, these molecules do not induce extensive damage; however, they undergo chemical or enzymatic dismutation and generate H_2O_2 , which further transforms to yield highly toxic $\cdot OH$ radicals, which cause oxidative damage (Das and Roychowdhury 2014). The dismutation reaction is catalyzed by the enzymatic antioxidant superoxide dismutase (SOD); it occurs at the rate $\sim 2 \times 10^9 M^{-1} s^{-1}$, or 10^4 times the rate constant for spontaneous dismutation (Kohli et al. 2019). Moreover, the protonated form of $O_2^{\cdot-}$, known as hydroperoxyl radical ($HOO\cdot$ or $HO_2\cdot$), is comparatively more toxic and induces oxidative stress in the plant cell (Kohli et al. 2019).

2.2.2 SINGLET OXYGEN ($^1\text{O}_2$)

These ROS molecules are produced by the reaction of triplet state chlorophyll (^3Chl) with O_2 . Insufficient availability of carbon dioxide (CO_2) under stress due to the closure of stomata favors the formation of $^1\text{O}_2$. The half-time of $^1\text{O}_2$ is 3 μs , but it possesses the ability to diffuse to the distance of 100 nm and thus causes damage to a wide range of biomolecules, such as proteins, lipids, etc. $^1\text{O}_2$ can easily oxidize the C-C double bond; thus, it mainly targets the double bonds of amino acid residues of proteins, polyunsaturated fatty acids (PUFA) of lipid membranes, guanine bases in nucleic bases, and thiol groups (Dmitrieva et al. 2020). This leads to the formation of hydroperoxides, which are responsible for initiating a free radical chain reaction (Dmitrieva et al. 2020). $^1\text{O}_2$ induces severe damage to photosynthetic machinery, affecting PS I and PS II, causing loss of activity and sometimes leading to cell death (Das and Roychowdhury 2014). An *Arabidopsis* mutant with enhanced $^1\text{O}_2$ production ability shows increased lipid peroxidation under photo-oxidative stress and induced cell death (Triantaphylides et al. 2008). Apart from damaging effects, $^1\text{O}_2$ also has been identified as instrumental in the upregulation of genes that protect against photo-oxidative stress (Das and Roychowdhury 2014).

2.2.3 HYDROGEN PEROXIDE (H_2O_2)

H_2O_2 is a moderately reactive species. Enzymatic or spontaneous dismutation of $\text{O}_2^{\cdot-}$ results in the production of H_2O_2 . Spontaneous dismutation of $\text{O}_2^{\cdot-}$ is favored by low pH, whereas enzymatic dismutation reaction is catalyzed by the enzymatic antioxidant SOD. Under stress conditions, due to limited availability of CO_2 , the ribulose 1,5-bisphosphate (RuBP) oxygenation process is favored, leading to photorespiration and production of H_2O_2 . Apart from photorespiration, photo-oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase (XOD) result in H_2O_2 production. The major organelles that participate in H_2O_2 formation include chloroplasts and mitochondria via ETC, cytosol, apoplast, plasma membrane, and peroxisomes (Mittler 2017; Smirnov and Arnaud 2019). H_2O_2 is moderately reactive and displays both toxicity and defense activity. At low concentration, it regulates signaling pathways for processes like photorespiration, stomatal movement, cell cycle, growth, and development (Das and Roychowdhury 2014). H_2O_2 as compared with other ROS has a significantly long half-life time of 1 ms; hence, it can travel to a longer distance (more than 1 μm) and can cross plant cell membranes through aquaporins (Das and Roychowdhury 2014). On the contrary, at a high concentration, H_2O_2 oxidizes cysteine (Cys) and methionine (Met) residues of amino acids, inactivates Calvin cycle enzymes and SOD enzymes by oxidizing their thiol group, and can even trigger PCD (Das and Roychowdhury 2014).

2.2.4 HYDROXYL RADICAL ($\cdot\text{OH}$)

$\cdot\text{OH}$ radicals are the most potent form of ROS. They have a single unpaired electron, which enhances their reactivity with triplet ground state oxygen (Sharma et al. 2012). The Haber–Weiss reaction and the Fenton reaction are major pathways/reactions that mediate $\cdot\text{OH}$ formation inside plant cells under stress. In the presence of transition metals, H_2O_2 and $\text{O}_2^{\cdot-}$ are ultimately converted into $\cdot\text{OH}$ radical via the Haber–Weiss reaction (Richards et al. 2015). In the Fenton reaction, reduced forms of transition metals catalyze the formation of $\cdot\text{OH}$ radical from H_2O_2 (Richards et al. 2015). Under dark conditions, production of $\cdot\text{OH}$ radical, possibly via the Fenton reaction, triggered wilting in the epicotyl of pea seedlings (Hideg et al. 2010). Cells do not recruit any enzymatic mechanism to scavenge these radicals; therefore, their excess accumulation leads to cell death (Sharma et al. 2012). $\cdot\text{OH}$ radicals are short-lived ROS with a half-life time of 1 ns; they have strong positive redox potential and can migrate to a distance of 1 nm (Mittler 2017), and thus often display reactivity near sites of production (Sharma et al. 2012). Due to their highly toxic and reactive nature, $\cdot\text{OH}$ radicals

interact with all biomolecules and subsequently damage cellular proteins, induce lipid peroxidation, and disrupt membranes (Sharma et al. 2012).

2.3 ABIOTIC STRESS AS A PRECURSOR OF ROS OVER-PRODUCTION

Plant growth is determined by their genetic makeup and persisting environmental conditions such as temperature, water, light, radiation, etc., collectively known as abiotic factors. Slight variations in environmental conditions induce mild stress that temporarily restricts plant growth (Gull et al. 2019). However, extreme fluctuations in abiotic factors trigger lasting damage that not only impinges on plants' physiological performance but also alters plants' genomics to an extent that reduces plant growth and development (Raza et al. 2019; Saijo and Loo 2020). Abiotic stress(es) trigger production of ROS within plant cells, which hinders vital processes including transpiration, respiration, and photosynthesis and disrupts enzymatic activities that correspond to ROS over-production (Figure 2.2). Abiotic stress-induced ROS production in important crops, resulting in cellular damage, is summarized in Table 2.1.

2.3.1 TEMPERATURE STRESS

Variation from the optimum growth temperature induces heat or chilling/freezing stress, resulting in impaired plant architecture, reproduction, and fruit setting (Zaidi et al. 2014; Martinez 2016). An increase in greenhouse gases (GHG), high light intensities, and heat waves have subjected plants to heat stress, which triggers morphological, cellular, and metabolic changes such as elongation of petioles and hypocotyls, diminishes photosynthetic and respiratory performance, reduces enzymatic activity, up-regulates transcription and translation of heat shock proteins (HSP), elevates calcium influx, and increases ROS production (Bita and Gerats 2013). High temperature increases ground-level ozone (O_3) production, which can also trigger oxidative stress in plants (Coates et al. 2016). In contrast to heat stress, chilling stress is characterized by low-temperature events that increase solubility and accumulation of O_2 , and promote electron leakage from the photosynthetic and respiratory ETC, aggravating ROS production (Coates et al. 2016). Over-production of ROS under chilling stress affects protein and lipid molecules, resulting in increased membrane fluidity and reduced enzyme activity (Jalil et al. 2017). Due to chilling stress in cucumber seedlings, increased electrolyte leakage along with reduced tissue water and chlorophyll content has been reported by Zhang et al. (2012). Similarly, under low temperature, over-production of H_2O_2 and $O_2^{\cdot-}$ in tomato leaves has been reported with enhanced malondialdehyde (MDA) level and *RBOH1* (*respiratory burst oxidase homolog 1*) expression. Stress has been found to reduce net photosynthesis rate and chlorophyll fluorescence (Liu et al. 2018).

2.3.2 WATER STRESS

Change in climatic scenarios in the last decades has tremendously affected rainfall patterns, causing erratic precipitation with altered magnitude and seasonal variations (Feng et al. 2013). These extreme changes have resulted in water deficit (drought) or waterlogging (flooding) stress. It has been estimated that the proportion of the world's agricultural land engulfed by drought will be increased two-fold by 2050, which will considerably reduce agricultural productivity (Kumar et al. 2019; Khan et al. 2020a). The effect of water deficit stress greatly varies with the severity and time-length of the stress. Water deficit conditions have been reported to accelerate the rate of stomatal closure, decrease CO_2 fixation, and elevate photoreduction of O_2 in the chloroplast as well as photorespiration, resulting in ROS accumulation triggering oxidative stress in plants (Jalil et al. 2017). Accumulation of ROS in white clover leaves grown under water deficit conditions has been reported along with an increase in MDA content and decrease in dry mass (Lee et al. 2009).

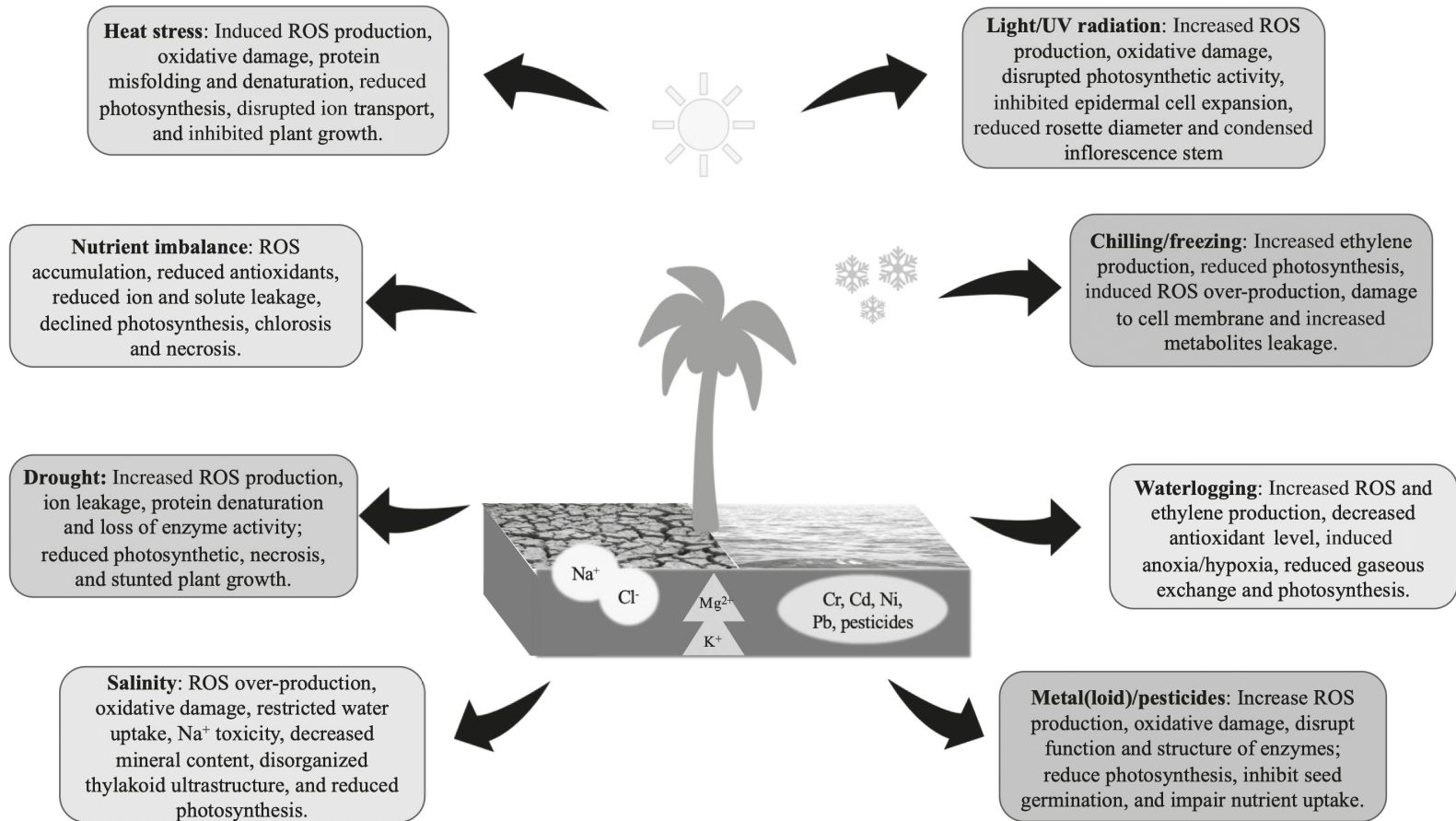


FIGURE 2.2 Abiotic stress-induced over-production of ROS and reduced plant activities.

TABLE 2.1
Production of Reactive Oxygen Species in Major Crops under Abiotic Stress(es)

Stress	Crops	ROS Production and Oxidative Damage	References
Chilling	Tomato (<i>Lycopersicon esculentum</i> Mill.)	H ₂ O ₂ content increased in roots and leaves	Diao et al. 2017
	Tomato (<i>L. esculentum</i> cv. C.H. Falat)	H ₂ O ₂ content increased two-fold and electrolyte leakage increased by 20%	Ghanbari and Sayyari 2018
Drought	Rice (<i>Oryza sativa</i> L.)	Increased production of H ₂ O ₂ and lipid oxidation	Sohag et al. 2020
	Wheat (<i>Triticum aestivum</i>)	Increased H ₂ O ₂ production and MDA content	Habib et al. 2020
	Maize (<i>Zea mays</i>)	Increased accumulation of ROS, membrane damage, electrolyte leakage. Elevation of lipoxigenase and thiobarbituric acid reactive substance (TBARS)	Anjum et al. 2017
	Tomato (<i>L. esculentum</i> Mill.)	Increased MDA content and electrolyte leakage	Malhotra et al. 2017
	Chinese crab apple (<i>Malus hupehensis</i>)	Increased lipid peroxidation and accumulation of H ₂ O ₂ and O ₂ ^{·-}	Wang et al. 2012
Heat stress	<i>Arabidopsis thaliana</i>	Increased ROS level and elevated TBARS content by 68.30%	Kipp and Boyle 2013
Heavy metal (chromium)	Rice (<i>Oryza sativa</i> L.)	Increased H ₂ O ₂ accumulation	Liu et al. 2019
	Mustard (<i>Brassica juncea</i>)	Increased TBARS content, H ₂ O ₂ production and lipoxigenase activity	Kabala et al. 2019
Heavy metal (cadmium)	Mung bean (<i>Vigna radiata</i>)	Increased H ₂ O ₂ and O ₂ ^{·-} production and increased MDA content	Nahar et al. 2017
Ground-level ozone (O ₃)	Rice (<i>Oryza sativa</i> L.)	Increased production of H ₂ O ₂ and lipid peroxidation	Ueda et al. 2013
	Wheat (<i>Triticum aestivum</i> L.)	Increased production of H ₂ O ₂ and O ₂ ^{·-} , and enhanced lipid peroxidation	Yadav et al. 2019
Salinity	Sweet pepper (<i>Capsicum annuum</i>)	Increased ROS and MDA content, and electrolyte leakage	Abdelaal et al. 2020
UV-B radiation	Cucumber (<i>Cucumis sativus</i>)	Increased H ₂ O ₂ accumulation in cotyledons	Rybus-Zajac and Kubis 2010
Water-logging	Barley (<i>Hordeum vulgare</i>)	Increased production of O ₂ ^{·-} and MDA content	Luan et al. 2018
	Millet (<i>Sorghum bicolor</i>)	Increased MDA content in sensitive cultivar	Zhang et al. 2019

Flooding is another severe abiotic stress that creates a waterlogging condition, characterized by low light, impaired gas exchange, and hypoxia/anoxia, which reduces the diffusion of oxygen, thereby suppressing aerobic activity in the soil such as root respiration (Sasidharan et al. 2018; Khan et al. 2020b). During waterlogging due to deficient O₂ conditions, ROS and volatile gases like ethylene are produced. The anoxic state results in inhibition of photosynthetic and mitochondrial ETC, which consequently ends up in over-production of ROS (Chang et al. 2012; Sasidharan et al. 2018). Waterlogging conditions result in leaching of essential nutrients from the soil, accumulation

of salts, and increase in heavy metal availability to the plant due to alteration in soil pH. These changes aggravate stress conditions and trigger nutrient deficiency, salinity, heavy metal, and oxidative stress in plants (Steffen 2014). The onset of oxidative stress in sesame plants subjected to waterlogging conditions has been described by Anee et al. (2019), who reported accumulation of ROS and methylglyoxal content and increase in lipid peroxidation.

2.3.3 SALINITY

Soil salinity is one of the most stubborn abiotic stresses affecting agricultural produce (20–50%) and soil fertility worldwide (Yuan et al. 2015; He et al. 2018). It has been estimated that around 20% of the world's total arable land is affected by salinity (Etesami and Noori, 2019), and by the year 2050 more than 50% of cultivable land will be salinized (Etesami and Noori 2019). Climate change has resulted in sea level rise and intrusion of saline water into arable lands, thereby increasing salt content in soil and imposing stress on plant cultivation (Chima et al. 2015). Water scarcity (due to uneven precipitation and low groundwater level) and high temperature resulting in excess water evaporation also aggravate soil saline concentration (Zhang et al. 2018). Salinity stress occurs due to the presence of excessive Na^+ and Cl^- ions, which establishes hypertonic conditions and induces osmotic stress (Ilangumaran and Smith 2017). Induced osmotic stress hinders plant homeostasis and affects physiological processes such as photosynthesis, uptake of nutrients (causing nutrient deficiency) and their translocation, hormonal balance, etc. (Ilangumaran and Smith 2017). Salt stress reduces plants' capacity to absorb water and induces drought-like conditions (Jalil and Ansari 2020). This reduces stomatal conductance, disrupts PS, and affects photosynthetic enzymes, leading to ROS production in plants (Hasanuzzaman et al. 2018). Wheat plants subjected to salinity stress have been reported to accumulate excess ROS, which triggered lipid peroxidation and reduced membrane stability, leading to electrolyte leakage (Kaur et al. 2017).

2.3.4 HEAVY METAL STRESS

Anthropogenic activities have increased the load of heavy metals, chemicals, and other xenobiotic compounds in the environment, causing a stressful situation. Heavy metals include both essential and non-essential plant nutrients. Accumulation of non-essential metals is known to cause toxicity in plants via ROS generation; however, unrestricted uptake of essential nutrients also induces ROS generation. Heavy metals like iron (Fe), chromium (Cr), and copper (Cu) are major redox-active metals that result in oxidative stress in plants due to their high concentration in soil (Schutzendubel and Polle 2002; Steffens 2014; Khan et al., 2021). Heavy metals trigger ROS production in chloroplasts, mitochondria, apoplast, and peroxisomes (Pandey et al. 2009; Steffens 2014; Trinh et al. 2014). Cadmium (Cd) is a non-essential metal and is known to cause toxicity in plants. It induces over-production in plant cells indirectly by replacing Cu or Fe ions in metalloenzyme antioxidants, thus impairing the respiratory ETC and disturbing redox status (Gupta et al. 2017). Fe is an essential micronutrient, but when it accumulates in excess in plants, it induces over-production of ROS through a chain of reactions (Becana et al. 1998), causing damage to lipid membranes and chlorophyll (Hajiboland 2012). Fe present in reduced form oxidizes and generates H_2O_2 and $\text{O}_2^{\cdot-}$ in the cell via a process called auto-oxidation (Schutzendubel and Polle 2002). The H_2O_2 formed oxidizes another reduced Fe molecule to produce a highly toxic $\cdot\text{OH}$ radical (Hajiboland 2012).

2.3.5 XENOBIOTIC COMPOUND STRESS

Similarly to heavy metals, certain xenobiotic compounds, including pesticides, induce oxidative stress by triggering over-production of ROS within plant cells (Sharma et al. 2019). Only 1% of the total applied pesticides reach the target organisms, and the rest contaminate the ecosystem, resulting

in pesticide-induced stress (Sachdev and Singh 2018). Pesticides have been well documented to possess the ability to retard growth and photosynthetic efficiency of the plant, cause molecular alterations, induce over-production of ROS, and reduce plants' intrinsic antioxidant-based scavenging activity (Sharma et al. 2019; Yuzbasioglu et al. 2019). Application of pesticide (thiram) to tomato plants has been reported to increase the production of H_2O_2 , elevate MDA level, and degrade leaves' chlorophyll content (Yuzbasioglu et al. 2019). Similar effects were reported in *Brassica juncea* treated with the pesticide imidacloprid (Sharma et al. 2019).

2.4 ROS-INDUCED DAMAGE TO CELLULAR BIOMOLECULES

Oxidative bursts caused by over-production of ROS under adverse environmental conditions attack biomolecules, primarily DNA, RNA, proteins, and lipids, cause protein oxidation, enzyme inactivation, lipid peroxidation, disruption of membrane integrity, chlorophyll degradation, and nucleic acid damage, and instigate the apoptosis pathway, leading to PCD under severe conditions (Roychowdhury et al. 2019; Shah et al. 2019). This damage affect growths, development, and ultimately plant survival.

2.4.1 LIPID MEMBRANES

Cell and organelle membranes composed of lipids are a prime target for ROS. The PUFA of lipids are primarily attacked by ROS due to the presence of unsaturated C-C double bonds in fatty acids. The $\cdot OH$ radical attacks the methylene group of fatty acids and abstracts a hydrogen (H) atom, forming a carbon-centered lipid radical (Anjum et al. 2015). ROS also attack the ester linkage between glycerol and fatty acids and cause membrane phospholipids to disintegrate (Sharma et al. 2012; Das and Roychowdhary 2014). The PUFA of the plasma membrane and mitochondrial membrane-like arachidonic acid, linolenic acid, and linoleic acid are the most susceptible targets of ROS. The $\cdot OH$ radical initiates a cyclic reaction resulting in peroxidation of PUFA (Das and Roychowdhary 2014). The process of lipid peroxidation involves three stages: Initiation, propagation, and termination (cleavage) (Anjum et al. 2015). Initiation is the first stage, which involves the production of ROS by reduction of O_2 . The ROS generated in this way triggers a cascade of reaction leading to the production of lipid radicals (lipid peroxyl radicals, hydroperoxides, etc.), constituting the second or propagation stage. Finally, lipid radicals end up in lipid dimers, resulting in the culmination of the chain reaction and marking the termination of the process (Das and Roychowdhary 2014). The formation of lipid radicals by peroxidation causes membrane destabilization, increases membrane permeability and electrolyte leakage, deactivates membrane-located enzymes and receptors, and enhances the oxidation of other biomolecules such as nucleic acids and proteins (Sharma et al. 2012; Das and Roychowdhary 2014; Anjum et al. 2015).

Oxidative stress induced by stresses like salinity (Katsuhara et al. 2005), drought (Hameed et al. 2011; 2013), high temperature (Ali et al. 2005), metal(loid)s (Singh et al. 2006), pesticides (Majid et al. 2014), etc. has been found to be associated with increased cellular and organelle lipid peroxidation. Arsenic toxicity has been found to induce lipid peroxidation, electrolyte leakage, and oxidative damage in common bean seedlings due to the accumulation of H_2O_2 (Talukdar 2013). MDA, a lipid peroxidation product, indicates the degree of oxidative damage in the cell and is thus considered as a marker for lipid peroxidation (Sharma et al. 2012; Das and Roychowdhary 2014). Increased accumulation of H_2O_2 along with MDA content and lipid peroxidation in tomato plants has been found to be linked with salinity, heat stress, and the combination of these stresses (Martinez et al. 2016).

2.4.2 PROTEINS

Proteins are important functional and structural components of a plant cell that play a crucial role in facilitating tolerance to abiotic stress by adjusting the physiological characters of plants (Kosova