9

Transgenic Implications for Biotic and Abiotic Stress Tolerance in Agricultural Crops

Shabnam Sircaik, Karuna Dhiman, Geetika Gambhir, Pankaj Kumar, and Dinesh Kumar Srivastava

Abstract

Plants encounter variable stresses in the environment which lead to huge crop losses worldwide. Environmental stresses that a plant can undergo are categorized into two categories as (a) biotic and (b) abiotic stress. Biotic stresses include attacks by different insects, nematodes, and microbial pathogens like fungi, bacteria, and viruses. While on the other hand, abiotic stresses include high salinity, heat, cold, drought, osmotic stress, and heavy metal. Plants are quite susceptible to both kinds of stressful situations and have adopted different mechanisms to encounter these situations. Plants sense these stresses and stimulated specific stress responses thereby activating different stress response signaling pathways and generating appropriate cellular responses helping in combating these stresses. This chapter gives an overview of the major stresses, plants encounter during growth and transgenic implications that have been made to modify these stress-tolerant properties to produce crops with improved crop yield and minimize crop losses.

S. Sircaik
School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

K. Dhiman · D. K. Srivastava
Dr Y.S. Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India

G. Gambhir
ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India

P. Kumar (✉)
Dr Y.S. Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India

CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India
e-mail: pksharmabiotech@yspuniversity.ac.in

D. Kumar Srivastava et al. (eds.), *Agricultural Biotechnology: Latest Research and Trends*, https://doi.org/10.1007/978-981-16-2339-4_9

Keywords

Transgenics · Biotic · Abiotic · Stress · Environment · Crops

9.1 Introduction

The plant undergoes different kinds of stresses in nature that contribute to adverse growth and compromised plant productivity. Any kind of stress induces a series of adaptive responsive in plant-like alteration in gene expression and induction of defensive cell metabolism. Plant stress could be divided into two major categories termed as biotic (microbial pathogens, nematode, insects, and weed) and abiotic caused through various environmental cues (heat, cold). Both kinds of stressors contribute to both pre- and post-harvest losses. Furthermore, it is also noticeable that with increasing global warming and changing climate situations, agricultural crops significantly encounter an increased event incidence of both abiotic and biotic stresses compromising plant yield (Mahalingam 2015; Ramegowda and Senthil-Kumar 2015; Kumar and Srivastava 2016, 2020a, 2020b; Srivastava et al. 2016; Parmar et al. 2017; Gambhir et al. 2020; Kumar et al. 2018a, 2018b, 2018c). Both kinds of stresses concurrently cause more destruction when compared to either of the stress occurring solely. For instance, abiotic stress directly influences the occurrence, survival, and dissemination of the different pathogens, insects, and weed growth. Additionally, these stress situations also influence plant physiology and host-defense responses to promote plant–pest interactions. However, this effect is not always additive as the outcome could be influenced by the nature of the interaction between these stress factors (Pandey et al. 2015; Ramu et al. 2016).

Tackling any kind of stress is a bigger problem for gradually increasing world population, estimated to reach ten billion by 2050 (Bengtsson et al. 2006; Secretariat UNIS for DR 2015). All this needs a significant increase in crop production by minimizing crop losses in any kind of stressful situation. The improvement could either be achieved by a traditional breeding method like wide-cross hybridization, mutation breeding or by modern technology including transgenics. Despite several attempts, the conventional plant breeding methods have failed in combating this issue. Current studies propose that the tolerance to any kind of stress is multigenic and quantitative QTL (quantitative trait locus) in nature (Collins et al. 2008), which could be a possible reason for this failure. Modern transgenics serve as an alternative to tackle this issue as this includes the introduction of exogenous genes into the host or an alteration in the expression of a host's gene that can help in improving stress tolerance (Roy and Basu 2009). Besides, modern transgenics require comparably less time than conventional plant breeding methods with a benefit of transfer of only desired gene(s) to the host plant, it always has an upper hand over the conventional crop breeding methods (Yamaguchi and Blumwald 2005). Due to ease in the use of transgenics for crop improvement, the technology has been used extensively world-wide (Wani et al. 2016a). However, the employment of genetic engineering needs the identification of the key genes underlying these processes of plant stress tolerance.

It is well known that any kind of stress induces a series of adaptive responses in the cells contributing to changes in the physiology and molecular makeup of the plants that could be dangerous to the cellular machinery. If we talk at the molecular level, any kind of stress leads to different molecular responses like the production of reactive oxygen species causing damages to cellular molecules like protein, nucleic acids, and lipids by the process of oxidation or peroxidation. In severe conditions, these stress situations may also lead to programmed cell death and thus cause the death of the entire plant (Sharma and Dubey 2007). Hence, exploring the underlying molecular mechanisms and key molecules contributing to stress signaling could be an alternative strategy for crop engineering for stress-tolerant properties, which could be done by application of the modern transgenic approaches like sequencing and functional genomics (Heidarvand and Amiri 2010). Usually, a cell signaling cascade is triggered upon any kind of stress upregulating the different transcription factors inducing various stress-responsive genes coding for proteins helping in stress-tolerant phenotypes. As stress-sensitive plants are compromised in synthesizing these proteins, they are susceptible to all these stressors impeding their overall growth. Several genes have been identified that code for stress-protective compounds and proteins in the different organisms (plants, animals, or microbes). The ideal genes for this kind of targeted insertion have been classified into three major categories; (a) associated with osmolyte synthesis like mannitol, glycine betaine, proline, and heat shock proteins, (b) associated with the uptake of ion and water, and (C) associated to transcriptional control and different signaling pathways. Progress in understanding the underlying mechanism of these stress responses has undergone major development in recent years (Jaspers and Kangasjärvi 2010). The transgenics have been successfully employed to produce plants with different attributes like an increase in grain yield, increase tolerance to high salt and drought stress in rice, barley, maize, etc. (Wani et al. 2016b). Following is a brief introduction of the different kinds of stressors contributing to significant crop losses and the genetic developments created to combat these losses associated with stressful situations. As losses due to both kinds of stressors cause severe crop losses worldwide, it is conceivable that the development of the plant with improved traits for abiotic and biotic stress requires identification and improvement of stress adaptive traits in these plants. This book chapter is a brief introduction about these stresses (biotic and abiotic) and the genetic improvement approaches that have been implicated for improving stress-responsive properties in plants.

9.2 Biotic Stresses

9.2.1 Insect Pest and Nematodes Resistance

The control of insect pests in commercially grown agricultural crops is predominantly based on the indiscriminate use of synthetic insecticides which is deleterious to human health and the environment. Transgenic crops have revolutionized modern agriculture and have become a major tool of integrated pest management leading to a reduction in insecticide use, protecting the environment and human health. Insect-resistant plants were also developed about two decades ago in other crops (Ansari et al. 2015). The search for novel useful genes encoding insecticidal proteins is still in progress and information deriving from the prolonged cultivation of commercialized GM products confirms the efficacy of this biotechnological tool. The new research area includes the use of novel transgenes and improved transformation protocols especially for the development of insect-resistant cultivars in economically grown agricultural crops.

The soil bacterium (*B. Thuringiensis, Bt*) produces a wide range of proteins (δ-endotoxins) that are included in crystals formed during sporulation and characterized by distinct insecticidal spectra (de Maagd et al. 2001; Sharma and Srivastava 2013). *Bt* spores contain high levels of δ-endotoxins harmful to specific insects of Lepidopteran, Dipteran, and Coleopteran orders which are the major pests of agricultural crops and perennial tree species. The *Bt* spores and the crystal (Cry) proteins are ingested by the insect and solubilized within the alkaline midgut. The protoxins are then activated by proteinases and finally, the active *Bt* toxin binds to specific molecular receptors causing the irreversible damage of the midgut epithelium by colloid osmotic lysis. *B. thuringiensis* has been used as a commercial insecticide for more than 50 years and to date, an extensive number of reports have demonstrated that *Bt* proteins have negligible potential adverse effects against humans, animals, and non-target invertebrates. More than 130 *Bt* genes encoding different δ-endotoxins have been isolated and, among this extremely large gene array, those coding for the *CryIA*(a) and *CryIA*(c) proteins have been used to develop transgenic crops resistant to Lepidoptera. Also, the *CryIIIA*(a) protein has been chosen by different research groups to specifically target Coleopteran pests. Insect resistance was firstly reported in tomato using *Bt.* gene in 1987. Cotton was the first commercially successful crop in which *cry* genes were incorporated to provide resistance against lepidopteron insect pest (Perlak et al. 1991). After the success of transgenic cotton, cry genes have been incorporated in many crops, viz., potato rice, canola, soybean, maize, chickpea, alfalfa, and tomato. Insect-resistant transgenic crops have the second largest area under cultivation which is 23.3 million hectares in 2017 (ISAAA 2017), 304 events have been approved in different crops worldwide for commercial cultivation. Out of these events, 208 events comprising various insect resistance (IR) genes in maize have been approved for cultivation. The commercialized crops having various IR genes are cotton (49 events), potato (30 events), soybean (6), rice (3), sugarcane (3), poplar (2), brinjal (1), and tomato (1).

Apart from *cry* genes, other insecticidal genes such as Proteinase inhibitors (PIs) are also used to develop insect-resistant GM crops. PIs are natural compounds abundantly found in seeds and storage organs of a wide range of plant species and contributing to the plant defense system against insect pests and pathogens (Schuler et al. 1998). Proteinase inhibitor families are specific for each of the four classes of proteolytic enzymes (cysteine, serine, aspartic, and metalloproteinases). Serpins and Cysteine are the most explored plant PIs against insect pests. Green and Ryan proposed this concept in a pioneer study reporting rapid accumulation of protease inhibitors in potato and tomato leaves attacked by Colorado potato beetles, both locally as well as systemically (Green and Ryan 1972). A few years later, a seminal study by Hilder et al. (1987) reported PI-expressing transgenic tobacco lines by utilizing the potentials of plant genetic transformation. With the advancement of gene transfer technology, this agronomically useful gene was introduced in rice cultivars that enhanced protection to stem borers (Xu et al. 1996) and wheat (Altpeter et al. 1999) to protect from leaf-feeding and storage pest. Since then, various research groups have reported numerous studies of enhanced herbivore protection in multiple plants involving bioassays, PI-expressing transgenic plants, and insect feeding assays. The inhibiting activity of PIs is due to the ability to form stable complexes with proteinases, blocking, altering, or preventing the access to the substrate-binding region of their catalytic site. To develop effective strategies for plant protection against insect pests based on PIs transgenesis, it is imperative to know the class of proteolytic enzymes present in the insect guts, which ultimately results in the extended developmental period, reduce fecundity and increase mortality due to amino acid deficiencies. Different proteinases predominate in different insects. Most of the Lepidopteran species have serine proteinases as the major digestive enzymes. Coleoptera has a wider range of dominant gut proteinases (Schuler et al. 1998). Broadway and Duffey (1986) suggested that the PI mediated inhibition of proteinases is responsible for hyperproduction of digestive enzymes, enhanced loss of essential amino acids, and finally inhibition of insect growth rates. However, some insect species seem to be able to modify dynamically the spectra of their digestive enzymes by the production of insensitive proteinases. Besides several reports of successful PIs-transformed plants enhanced protection to insect pests, this promising strategy of crop protection could not be successfully commercialized.

9.2.2 Nematodes Resistance

Plant-nematode parasitism is one of the most damaging uncontrollable biotic stresses on crops, and the cumulative effect on agriculture is severe. The majority of these losses are inflicted by relatively few species. The most damaging are root-knot nematodes (*Meloidogyne* species) and cyst nematodes (*Heterodera* and *Globodera* species), with root-knot nematodes the major contributors to yield losses (Koenning et al. 1999). Management of nematode parasitism is therefore imperative. Integrated use of chemicals, resistant varieties, and cultural and biological practices provide the most successful management strategy. These approaches are, however, becoming increasingly unsatisfactory. Although conventional intensive farming methods rely largely on the use of nematicides, dependence on this approach must diminish as environmental and health concerns around these toxic chemicals increase. Crop rotation as a strategy to limit nematode infestation has limited utility against those species with cosmopolitan host ranges, such as *M. incognita*, which may potentially parasitize up to 3000 plant species (Abad et al. 2003). Resistance in plants is therefore an attractive approach for controlling nematode populations. This may be either naturally occurring or transferred to crop cultivars from wild relatives or breeding lines through conventional breeding methods or engineered through molecular techniques. Biotechnology offers several benefits for nematode control in integrated management strategies such as reducing risks to the environment and human health, accessibility for food producers in the developing world, and the possibility of achieving durable, broad-spectrum nematode resistance (Thomas et al. 2006).

Several nematode resistance (Nem-R) genes have been isolated from plants which confer resistance against sedentary endoparasites. The first nematode resistance gene to be cloned was Hs1pro-1 from sugar beet, which confers resistance against the sugar beet cyst nematode (Cai et al. 1997). The encoded protein does not have obvious similarities to known plant genes. However, other cloned Nem-R genes closely resemble known plant R-genes in their domain structure. Four of these genes, *Mi-1*, *Hero A*, *Gpa2*, and *Gro1–4*, all cloned from tomato or potato relatives, fall into the NBS-LRR class of R-genes. The tomato genes Mi-1 and Hero A confer broad-spectrum resistance against several root-knot nematode species (Milligan et al. 1998) and against several pathotypes of two potato cyst nematode species (Ernst et al. 2002), respectively. By contrast, the potato genes *Gpa2* and *Gro1–4* confer resistance to a narrow range of pathotypes of a single potato cyst nematode species. *Mi-1*, *Gpa2,* and *Hero A* are members of the NBS-LRR class of plant R-genes that does not contain an N-terminal toll-interleukin receptor-like (TIR) domain. The *Hero A* gene product is 32% identical to *Mi-1* and w22% identical to *Gpa2* at the amino acid level (Williamson and Kumar 2006).

9.2.3 Antifungal Resistance

Antifungal proteins like chitinase, glucanase, defensin, thaumatin-like proteins, osmotin-like protein, phytoalexins, RIPs, etc. are produced by different flowering plants (Vigers et al. 1991), fungi (Guo et al. 2008), invertebrates and vertebrates (Raj and Dentino 2002) to combat the fungal pathogens. Some of this plant protein comes under the pathogenesis-related protein group (PR). Genes of these antifungal proteins were transferred to different plants to increase fungal resistance against fungal pathogens. Below is a summarized different antifungal protein which was used to produce transgenic plant against fungi.

9.3 Chitinase and Glucanase

Most of the fungi contain chitin and glucan in their cell wall as major components. Chitinase and glucanases are hydrolytic enzymes that can degrade chitin and glucan, thus leads to the degradation of the cell wall of fungi. Chitinase comes under the glycosyl hydrolase family and it hydrolyzes glycosidic bond in chitin. On the basis of isoelectric pH, the sequence at N-terminal, enzyme localization, and signal peptides chitinase enzyme was found to belong to 18 and 19 families of glycosyl hydrolases. Classes III and V of chitinase come under glycosyl hydrolase 18 and I, II, and IV under family 19. These enzymes are PR-3 group members (PR-Pathogen-related proteins) are first described in the orchid bulb by Bernard as an antifungal factor (Sharma et al. 2011). These are probably the most frequently studied and described PR proteins. These hydrolytic enzymes can cause lysis of fungal hyphae, inhibition of fungal growth, and exhibit in vitro antifungal activity (Boller 1993; Neuhaus 1999). Chitinase was reported to be present in plants, animals, microbes, and human beings. But the most preferred one is microbial chitinase because they can be easily produced in bulk and also available is more. But the first group of PR families is plant chitinases which are abundantly present in the plant kingdom and found to be effective against many phytopathogenic fungi like Ascomycota and Basidiomycota phyla (Punja 2004). It was also reported that these hydrolytic enzymes can also be produced in plants in response to abiotic stress as well as growth conditions. The number of chitinase gene isolated from different sources has been studied, sequenced, cloned as well as transformed into plants to develop fungal resistance. It was found out that when glucanase and chitinase genes were expressed in transgenic plants, then it results in more resistance to fungal pathogens (Nishizawa et al. 1999). But the expression of these genes in transgenic plants at a low level is a key issue. Lee and Raikel (1995) reported that in rice and tobacco, the expression of chitinase genes increased the plant's resistance to phytopathogenic fungi. Jabeen et al. (2015) studied for the first time that transgenic tomato plants showed resistance to two major fungal pathogens, i.e. *Fusarium oxysporum* f. sp. lycopersici (Fol) causing fusarium wilt and *Alternaria solani* causing early blight (EB) when rice chitinase (*RCG3*) was expressed in tomato. *Agrobacterium*-mediated transformation of cotyledonary petioles with an endochitinase gene (*chit33*-cDNA) isolated from *Trichoderma atroviride* under CaMV35S constitutive promoter showed increased resistance against *Sclerotinia sclerotiorum* in canola (R line Hyola 308) (Solgi et al. 2015). *EuCHIT2*, a new chitinase gene that was isolated from *Eucommia ulmoides* Oliver was overexpressed in tobacco plants showed resistance to *Erysiphe cichoracearum* DC (Dong et al. 2017). Khan et al. (2017) also developed transgenic potatoes using the *Agrobacterium*-mediated method that overexpressed endochitinase gene and showed resistance against *Alternaria solani.* Novel chitinase gene *LOC_Os11g47510* from indica rice Tetep provides enhanced resistance against sheath blight pathogen *Rhizoctonia solani* in rice (Kamboj et al. 2017). Chitinase enzyme gained attention towards biocontrol of fungal pathogen, but glucanase enzymes are less studied as compared to chitinase. Only a few reports are available on glucanase gene transformation in plants, but some transgenic plants overexpressing glucanase gene was successfully produced. Glucanase gene of tobacco was overexpressed in groundnut which showed tolerance to *Cercospora arachidicola* and *Aspergillus flavus* (Sundaresha et al. 2010). It was found out that transgenic groundnut was not only resistant to fungi but also produced less aflatoxin. In another report, grapevine, b-1,3-glucanase (*VvGHF17*) gene was overexpressed in *Arabidopsis* plants, showed resistance to *Colletotrichum higginsianum* and *Botrytis cinerea* (Fujimori et al. 2016). However, the synergistic action of these hydrolytic enzymes with each other as well as with other antifungal proteins has resulted in excellent in vitro and in vivo antifungal action (Melchers and Stuiver 2000). For example, when chitinase (*chi11*) and osmotin (*ap 24*) antifungal proteins encoding genes isolated from rice and tobacco, respectively, were expressed in tobacco, then this synergistic action can cause enhancement of sheath blight tolerance in transgenic rice (Sripriya et al. 2017).

9.4 Defensin

Defensin is a small antimicrobial cationic peptide that is present in various living organisms such as plants, microbes, and mammals. It contains about 45–54 amino acid residues which form a highly conserved structure scaffold with cysteine amino acid to forms αβ conformation. In plants, γ-thionin of wheat and barley was renamed as defensin based on structure and function similarity with insect defensin. The tertiary structure of plant defensin is formed of 3 antiparallel strands and 1 α- helix strand stabilized with disulfide bridges is highly conserved to form CSα/β (Cysteine stabilized α-helix β sheet motif) (Zhu et al. 2005). 3D Structures of different plant defensins are almost similar instead of low-level amino acid identity. Plant defensin is secreted in extracellular space in plant cells except for some floral defensin which is targeted to the vacuole. Plant defensin is naturally synthesized and present in every organ of plants. In plants defensing, major role is in the inhibition of phytopathogenic fungal growth at a very less concentration (Lay and Anderson 2005). Main mechanism of how defensin prevents fungal growth is not clear but it was found out that defensin bound to fungal cell membrane because of some electrostatic or hydrophobic interactions and at a very high concentration it causes membrane permeabilization which leads to the death of fungi (Sagaram et al. 2011; Thevissen et al. 2003; Valente et al. 2013; Hayes et al. 2013). The transgenic expression of defensins has enhanced plant resistance to phytopathogenic fungi. However, so far, there are no reports of enhanced resistance through the transgenic overexpression of defensin genes in those plants from which it was initially originated. Defensin gene isolated from plants was overexpressed in many plant species. For example, a defensin gene *Rs-AFP2* was isolated from *Raphanus sativus* and overexpressed in transgenic rice showed antifungal ability against *Rhizoctonia solani* and *Magnaporthe oryzae* (Jha and Chattoo 2010). This gene causes direct inhibition of theses pathogen (Lacerda et al. 2016). Spore germination and growth of obligate biotrophic fungi *Fusarium tucumaniae* and *Colletotrichum gossypii* var. cephalosporioides was inhibited in transgenic *Pichia pastoris expressing* rDrr230a defensin protein gene. This gene also showed inhibition of the Asian soybean rust pathogen *Phakopsora pachyrhizi* and was used against cotton and soybean fungal diseases. A defensin gene J1–1 was overexpressed in transgenic pepper showed increased resistance against *Colletotrichum gloeosporioides* fungi which is the causal agent of fruit-specific anthracnose fungus (Seo et al. 2014). Transgenic poplar plant expressing a putative defensin gene showed enhanced resistance against *Septotis populiperda* (Wei et al. 2020).

9.5 Thaumatin-like Proteins

Thaumatin-like proteins are present in plants such as Kalemfe which is a tropical flowering plant. It is a very sweet tasting protein that is almost about 100,000 times sweeter than sucrose. These proteins also come under the PR-5 Proteins family. These are low molecular weight proteins of about 20–24 kDa with 200 residues and 16 conserved cysteine which are involved in 8 disulfide bond formation which gives stability to this protein (Fierens et al. 2009). TLPs are present in different kingdoms such as plants (angiosperms, gymnosperms), animals, and fungi also (Liu et al. 2010). These proteins also expressed in plants against biotic and abiotic stress (Muoki et al. 2012; Singh et al. 2013). TLPs are also found to be antifungal proteins when overexpressed in transgenic plants (Singh et al. 2013; Wang et al. 2011a, b; Liu et al. 2012; Mahdavi et al. 2012; Acharya et al. 2013). It's unclear how these thaumatin-like antifungal proteins interact with the fungal pathogen, and more research is needed. Thaumatin-like proteins possess the antifungal activity and overexpression of these proteins showed tolerance to fungal pathogens (Wang et al. 2011a, b; Liu et al. 2012; Mahdavi et al. 2012; Acharya et al. 2013; Singh et al. 2013). Thirty-three putative TLPs gene of grape was studied for grape disease resistance and it was found that overexpression of *TLP29 in Arabidopsis thaliana* causes powdery mildew resistance (Yan et al. 2017). ObTLP1 which is an ocimum thaumatin-like protein was found to be an antifungal protein and was reported to inhibit the growth of *Ceratonia sclerotiorum* and *Botrytis cinerea.* When this gene was overexpressed in transgenic Arabidopsis, then it led to resistance against these fungi and also against dehydration and salt stress; thus suggesting their role in abiotic stress also (Misra et al. 2016). *Agrobacterium*-mediated transformation of *Ostlp*, a thaumatin-like protein in cassava inhibits *Colletotrichum gloeosporioides* f. sp. *Manihotis* growth (Ojola et al. 2018).

9.6 Osmotin-like Proteins

Osmotin or osmotin like proteins is a multifunctional protein that comes under the PR-5 protein family because they are homologous to thaumatin. Osmotin structure shows three motifs with similar folding as thaumatin and other PR-5 proteins. It consists of three domains. Singh et al. (1987) characterized osmotin from salt adapted cultures tobacco (*Nicotiana tobaccum*) cells. Osmotin is a multifunctional stress-responsive protein that enhances biotic and abiotic stress resistance in plants (Anu et al. 2015; Le et al. 2018; Su et al. 2017). Osmotin gene expression and protein formation is induced by any biotic stress such as fungal attack and its overexpression in transgenic plants leads to less disease symptoms (LaRosa et al. 1992; Liu et al. 1994; Zhu et al. 1996). Osmotin protein attacks specifically the plasma membrane of the pathogen which leads to signaling for cell death. Cell wall composition also determines osmotin toxicity because it governs osmotin protein access to the plasma membrane (Ibeas et al. 2000, 2001; Narasimhan et al. 2001, 2005). *Oryza sativa, Glycine max, Capsicum chinense, Vitis vinifera,* and *Sesamum indicum* are examples of plants in which osmotin gene was being transformed and showed increased resistance to fungal growth (Kim et al. 2004; Elvira et al. 2008; Weber et al. 2014; Katam et al. 2015; Chowdhury et al. 2017). *Ob*TLP1, which showed similarity to stress-responsive osmotin protein as well as to thaumatin-like protein, was isolated from *Nicotiana tabacum* and showed resistance to *Botrytis cinerea*, *Sclerotinia sclerotiorum,* and to salt stress and dehydration when expressed in *Arabidopsis* (Misra et al. 2016). Chowdhury et al. (2017) reported that *SindOLP* when overexpressed in sesame showed resistance against biotic as well as abiotic stresses. Transgenic lines of potato cultivar "Kufri Chipsona 1" were developed containing *OsmWS* osmotin gene isolated from *Withania somnifera*. These transgenic lines showed 22 fold expression of this gene within 3 days and inhibit *Alternaria solani* growth (Kaur et al. 2020).

9.7 Plant Ribosome-Inactivating Proteins

RIPs are RNA N-glycosidase which causes depurination of the highly conserved region, i.e. α-sarcin loop of 28s rRNA and thus inactivates ribosome by inhibiting the eF-Ia to bind with the ribosome. This inhibition leads to blockage of translation on the ribosome (de Virgilio et al. 2010). RIPs are widespread in nature and are distributed among different plant genera within different tissues. A number of RIPs are found to possess different antimicrobial activities in nature such as antifungal, antitumoural, antibacterial, and antiviral activities (Stirpe 2004; Puri et al. 2009; Bian et al. 2010). In agriculture, it is demonstrated in vitro and in transgenic plants that RIPs have been connected to defense by antifungal, antibacterial, antiviral, and insecticidal activities (Akkouh et al. 2015). For example, transgenic tobacco plant containing maize proRIP antifungal protein showed increased resistance against *R. solani* (Maddaloni et al. 1997). Yuan et al. (2002) reported that blast disease in transgenic rice was found to be inhibited by a type I RIP TCS (Yuan et al. 2002). Curcin-2 isolated from *Jatropha curcas* leaves was expressed in tobacco plants showed antifungal activity against R. *solani*, this protein was found to exhibit activity against different other stresses also (Huang et al. 2008). *Agrobacterium*-mediated transformation of the potato cultivar "Desirée" with Ribosome-Inactivating Protein (*rip30*) gene of barley produced a transgenic which showed enhanced resistance to *Rhizoctonia solani* in greenhouse condition (M'Hamdi et al. 2013). Plant RIPs also showed enhanced resistance when co-expressed with other antifungal proteins, e.g. when rice basic chitinase (*RCH10*) and modified maize RIP (*MOD1*) were co-expressed in rice, it showed good resistance against *R. solani* (Kim et al. 2003). Transgenic plants of blackgram co-expressing chitinase gene from barley and RIP showed Corynespora leaf spot fungal growth inhibition (Chopra and Saini 2014). Transformation of RIP α-MMC gene into rice showed increased resistance to blast fungus (Qian et al. 2014). A transgenic potato lines expressing *PhRIP I* gene of *Phytolacca heterotepala* coding for a ribosome-inactivating protein was found to possess more resistance to *Botrytis cinerea* and *Rhizoctonia solani* fungal pathogens (Gonzales-Salazar et al. 2017).

9.8 Phytoalexin

Phytoalexin term was originally coined by Müller (1958) and they come under low molecular weight plant antibiotic group. These are naturally produced secondary metabolites that possess antimicrobial activity. These are produced naturally in plant cells as normal growth metabolites or can be induced in the presence of pathogen attack or other stress. They can inhibit bacteria, fungi, insects, nematodes, toxic against animals or plants itself. About 350 phytoalexins have been identified and characterized from 30 plant families, *Leguminosae* plant family produces maximum 130 phytoalexins. These phytoalexins are well diversified in the plant kingdom and are characterized among different classes of chemical compounds such as coumarins, diterpenes, flavonoids, alkaloids, phenolic compounds, luteolinidin, apigenidin, and apigeninidin. Pisatin was the first phytoalexin isolated and characterized from garden pea, *Pisum sativum* (Cruickshank and Perrin 1960). The molecules that signal plants to begin the process of phytoalexin synthesis are called elicitors. Elicitors of biotic origin may be involved in the interaction of plants and potential pathogens, whereas abiotic elicitors are not involved in normal host–pathogen interactions. Phytoalexins only showed resistance in a sufficient concentration which will be produced by one or more phytoalexins along with another component. Phytoalexins were biosynthesized by phenylpropanoid pathways mainly around resistant tissue and also in necrotic lesions. Also, the acetate-mevalonate and shikimate pathways are involved in flavonoid biosynthesis. These all pathways are interconnected and are involved in the synthesis of some important enzymes which play a crucial role in resistance such as chalcone isomerase (CHI), chalcone synthase (CHS), phenyl-alanine ammonia lyase (PAL) CoA ligase, and stilbene synthase. Overexpression of these potential enzymes shows resistance against different diseases. Stark-Lorenzen et al. (1997) expressed the stilbene synthase gene of grapevine in rice and found that disease resistance was increased. Resveratrol synthase and isoflavone methyltransferase gene was also expressed in alfalfa plants to increase disease resistance. Similarly, isoflavone reductase (*GmIFR*) isolated from soybean enhanced resistance against *Phytophthora sojae* in Soybean (Cheng et al. 2015).

9.9 Antibacterial Proteins

These are small-sized lytic peptides which are having amphipathic α-helical structure. These proteins produce pores in the bacterial cell membrane causing lysis of bacterial cells (Boman 1991). These antimicrobial proteins are produced by the different living organisms from bacteria to animals as defense proteins. Some of the antibacterial proteins which are transferred to plants to increase resistance against bacteria are summarized below;

9.9.1 Cecropins

These are positively charged antimicrobial peptides which are isolated from giant silk moth (*Hyalophora cecropia*) hemolymph. Cecropin term was given because of its source of isolation. They are proteinaceous in nature consisting of 31–39 amino acid residues and synthesized as lipid bodies in cells. Cecropin mainly lyses the cell membrane of bacteria, on interaction with bacterial membrane, it forms an α-helical structure interaction which causes ion channel formation. It also inhibits proline uptake and leads to leaky membranes. It acts as the main constituent of insects immune system of bacteria and at low concentration (0.1–5μM) inhibits many gram-positive bacteria as well as some gram-negative bacteria (Chen et al. 1997). Antibacterial activity of cecropins isolated from *Antheraea pernyi, Hyalophora cecropia*, and *Bombyx mori* has been demonstrated towards different genes (Jaynes et al. 1993; Sharma et al. 2000). *Agrobacterium*-mediated transformation of antimicrobial peptide cecropin P1 (cecP1) in rapeseed (*Brassica napus* L.) was done and it has shown that these transgenic plants showed resistance to the bacterial and fungal pathogens *Erwinia carotovora* and *Fusarium sporotrichioides* (Zakharchenkoa et al. 2020). Cecropin B isolated from Chinese tasar moth (*Antheraea pernyi*) has been expressed in transgenic citrus to eliminate the effect of Huanglongbing (HLB), associated with *Candidatus liberibacter asiaticus* bacteria (Zou et al. 2017).

9.9.2 Attacins

Attacins are also another type of antibacterial proteins that are much larger than cecropins, i.e. about 180–190 aminoacids. There are about six different types of attacins (A-F) that have been isolated from a moth, i.e. *H. cecropia*. These proteins, i.e. A-F attacins differ from each other because of the processing step during synthesis, protein from A-D constitute a basic group whereas E and F are acidic. Attacins are found to attack gram-negative bacteria but these proteins do not cause lysis but disrupt outer membrane structure. Attacin proteins are not broad spectrum like cecropins but it can inhibit the growth of some bacteria like *E. coli*, *Acinetobacter calcoaceticus*, and *Pseudomonas maltophilia* (Hultmark et al. 1983). Apples were transformed using cDNAs coding for attachin E which were coupled to plant promoters (Norelli et al. 1994). Transformed plants of a susceptible apple rootstock N.26 possessed increased resistance to the fire blight pathogen *Erwinia amylovora* compared to the untransformed control, but were still more susceptible than the naturally resistant rootstock Liberty. Attacin expressed in transgenic potato enhanced its resistance to bacterial infection by *E. carotovora* subsp. *atroseptica* (Arce et al. 1999). Transgenic pear and apple expressing attacin genes have significantly enhanced resistance to *E. amylovora* in in vitro and growth chamber tests (Ko et al. 2000). Transgenic apple expressing attacin targeted to the intercellular space, where *E. amylovora* multiplies before infection, has significantly reduced fire blight, even in apple plants with low attacin production levels (Ko et al. 2000). Attacin A gene was transferred to citrus under the control of a phloem-specific promoter to control Huanglongbing disease (Tavano et al. 2019).

9.9.3 Lysozyme

This is a low molecular weight self-defense enzyme that was discovered in 1922 by Alexander Fleming. These enzymes come under antimicrobial proteins because they are hydrolytic in nature and attack the peptidoglycan layer of bacteria. It specifically cleaves between N-acetylmuramic acid and N-acetylglucosamine of cell wall peptidoglycan (Wohlkönig et al. 2010). The number of reports suggested that lysozyme can kill gram +ve and gram –ve bacteria. T4 lysozyme (T4L), human lysozyme, and Hen egg-white lysozyme (HEWL) are some of the classes of this antimicrobial protein gene, which have been cloned and transformed to different plants. Transgenic tobacco plants expressing these lysozyme genes were found to be more resistant against plant pathogenic bacteria (Trudel et al. 1995; Kato et al. 1998). *E. carotovora* causes soft rot disease in potato, resistance against this disease has been conferred in potato by expressing *T4L* gene from T4-bacteriophage (Düring et al. 1993). Fungal and bacterial growth was inhibited in transgenic tobacco expressing human lysozyme gene suggesting its potential use for controlling plant disease (Nakajima et al. 1997). A plant lysozyme was isolated from *Momordica charantia* L., which can be used further to increase bacterial resistance in plants. Resistance to these diseases could also be achieved by engineering potato with lysozyme gene (*chly*) from chicken (Serrano et al. 2000), and complete resistance was achieved by expression of the phage T4 lysozyme (Ahrenholtz et al. 2000).

9.10 Herbicide Resistance

Weeds are a major constraint to crop production because they compete for nutrients and other resources with the main crops, posing a serious threat to crops (Fartyal et al. 2018). Herbicide resistance is the most predominant trait that has been adopted for cultivating GM crops. In the early 1990s, GM crops resistant to broad-spectrum herbicides such as glyphosate and glufosinate have first been cultivated commercially. These GM crops are highly valued worldwide and have shown economic, social, and ecological benefits (Green and Castle 2010; Heap and Duke 2018). The herbicide glyphosate is known to inhibit 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS), a key enzyme in the shikimate and phenylpropanoid pathway that is responsible for the biosynthesis of aromatic amino acids and several secondary metabolites. In 1996, the first glyphosate-tolerant soybean ("Roundup Ready") harboring *cp4epsps* gene was commercialized and many other commercialized crops harbor this gene. Glyphosate-resistant maize was introduced in the year 1998. Till now, maximum events, i.e. 210 have been approved for maize for herbicide resistance (ISAAA database 2020). Another important herbicide glufosinate also known as phosphinothricin inhibits glutamine synthetase (GS) enzyme that catalyzes the assimilation of ammonia with glutamate to form glutamine. The inhibition of glutamine synthetase leads to the assimilation of ammonia that inhibits photosystem I and II reactions and indirectly inhibits photosynthesis (Wang et al. 2018). Two glufosinate resistance genes *bar* and *pat*, were isolated from soil bacteria. Out of these two, pat (phosphinothricin N-acetyltransferase) gene was isolated from *Streptomyces viridochromeogenes* and *bar* gene from *S. hygroscopicus*. These genes encode resistance to phosphinothricin (PAT) and bialaphos (L-Alanyl-L-alanyl-phosphinothricin; Bar). Thus, researchers primarily focused on these two genes in basic research for the development of herbicide-resistant genetically engineered crops. In the past few years, the glyphosate and glufosinate resistant genes (*EPSPS* and *bar*) have been introduced into pepper, soybean, maize, millet, potato, and other crops (Zhao et al. 2020). Besides these two above-mentioned herbicides, transgenic crops specific to other herbicide groups such as 2,4-D (*aad*-1 and *aad*-12 genes), dicamba (*dmo* gene), isoxaflutole, mesotrione, oxynil, and sulfonylurea, have been commercialized recently. Currently, different multinational companies such as Monsanto, Dow, Bayer, Syngenta, and BASF are developing new herbicide-resistant traits in different crops. *Agrobacterium*-mediated gene transfer technology has been used to introduce the *EPSPS* and *bar* genes, which confers resistance to glyphosate, and glufosinate into castor (Zhao et al. 2020). Maize, soybean, cotton, and canola are among the most widely grown commercial crops that confer herbicide resistance (Brookes and Barfoot 2015).

9.11 Virus Resistance

Plant viruses have been a major threat to agricultural production and with the advent of transgenic technology, there has been a revolutionary enhancement in the production of GM crops resistant to various plant viruses through various strategies. Significant resistance to a variety of plant viral diseases has been accomplished by gene silencing techniques. Bonfim and co-workers in 2007 explored the concept of using an RNA interference construct to silence the sequence region of the *AC1* viral gene and generated highly resistant transgenic common bean plants (Bonfim et al. 2007). This method has also been adopted for the engineering of plum pox virus resistance (Scorza et al. 2013). Exceptional progress has been perceived for transgenic alfalfa, melon, potato, rice, tomato, and tobacco against a broad spectrum of plant viruses, including alfalfa mosaic virus, cucumber mosaic virus, potato virus X

(PVX), potato virus Y (PVY), and potato leaf roll virus (Parray et al. 2019). In recent years, non-coding RNAs (ncRNAs) have gained unprecedented attention for regulating cellular processes and engineering plant virus resistance (Taliansky et al. 2021).

9.12 Abiotic Stress Tolerance

Variable photoperiod, nutrient deprivation, starvation, drought, high salt conditions, temperature, and osmotic changes are some of the situations that contribute to abiotic stress (Hirayama & Shinozaki 2010; Zhu 2016). These are of critical importance as studies have estimated the loss caused to reach around 50% by 2050 by this kind of stress situation (Bengtsson et al. 2006; Ahuja et al. 2010; Thakur et al. 2010; Lobell and Gourdji 2012). Furthermore, it is also estimated that the frequency of drought, salinity, and heat will increase in the coming years (Easterling et al. 2000; Bernstein et al. 2008), standing as a challenge to agriculture production. Plant encounters various kinds of abiotic stresses that contribute to a significant crop loss worldwide. All the abiotic stresses are interconnected and include the following.

9.12.1 Salinity

High salt situations in the soil and drought are two main abiotic stresses affecting around 20% of the crop losses in irrigated fields (Qadir et al. 2014). The ions that contribute to high salinity situations are Na^+, Ca^{2+}, Mg^{2+}, K^+, Cl^-, $SO4^{2-}$, $HCO3^-$, $CO3^{2-}$, and NO3 (Flowers and Flowers 2005). The accumulation of either a single type of ions or more than one type of ion may result in a condition that is termed salination. High saline conditions can be caused due to various reasons like mineral weathering, precipitation followed by the movement of salt towards land surfaces are some primary causes of the salinity. High salt has a variable effect on crop plants, though the primary effect includes osmotic stress and toxicity. These primary effects may lead to another secondary effect on the cell-like compromised cell and membrane expansion thereby a compromised metabolism. It is quite difficult to assess which factor contributes to salinity tolerance in crops. It is conceivable that the proper correlation studies between a trait and salinity tolerance could help in easing out the direct relationship between the effects of any salt on crop productivity. In past, many studies have been proposed to characterize the response of any biological molecule, e.g. transcription factors and biomolecules (Negrão et al. 2017) towards any kind of salt stress, however, these studies are still very limited. We propose that engineering the biomolecules to produce stress-resistant crops could be a promising approach because they all play a significant role in the plant's adaptation to variable salt conditions. Some of the development in major food crops has been described in the coming sections.

9.12.2 Heat

Global warming has led to a significant increase in temperature. It has been estimated that average temperature rise by 3–4 °C has estimated to increase the crop losses to 15–35% in tropical regions like Africa and Asia and by 25–35% in the Middle East (Ortiz et al. 2008). Despite a significant increase in global food production, a food deficit still survives as global cereal production is declining because of increasing temperature (Fischer and Edmeades 2010). The most common effect of high temperature on any crop is an effect on their overall reproductive development due to pollen infertility (Zinn et al. 2010). Heat stress is a major abiotic stress that plant gets exposed in nature and affects every stage of plants life, however, the variability could be seen in the patter they affect the plant that differs from species to species (Sakata and Higashitani 2008). The noticeable effects of the increase of temperature on plants are leaf and stem scorching, abscission, and senescence, inhibition of root and shoot growth, finally causing the fruit damage and results in a decrease in plant productivity (Vollenweider and Günthardt-Goerg 2005). In certain cases, high temperature may also result in changes in plant architecture like elongated hypocotyls and petioles (Hua 2009). Like any other stress, in response to heat stress, plants undergo a series of cellular and metabolic responses that are necessary to survive in these high-temperature situations. This includes changes in a cellular organization like changes in cytoskeleton and membrane functions. These structural changes are also accompanied by production transcription faction producing biomolecules like heat shock proteins (HSPs) (Bray et al. 2000), and the production of biomolecules like phytohormones (abscisic acid; ABA) and antioxidants (Maestri et al. 2002).

9.12.3 Drought

Drought is a situation where the plant does not have a sufficient amount of water which is necessary for the optimum growth of the plant. Various reasons responsible for this kind of stress are low rainfall and compromised irrigation conditions. Drought has a variety of effects on plant growth. The first is a lack of germination and seedling establishment in various crops (Ashraf and Harris 2004; Kaya et al. 2006). It has been reported that in rice, drought during a vegetative state severely compromises overall plant growth (Manickavelu et al. 2006). Drought situations are often associated with compromised photosynthetic activity and finally wilting, thereby compromising plant yield which could be followed by plant death.

9.12.4 Cold

Sensitivity to low temperature is another important abiotic stress that is of critical importance contributing towards the significant yield loss in crops. Cold affects plant life in different aspects, however, an ability to tackle this kind of stress may lead to cell death. Plant encounters temperature fluctuations in the natural environment and thus need a different mechanism to different responses to minimize cellular damages. Response to cold may involve an initiation of a signaling cascade to cause metabolic changes significantly adding to the increased tolerance to chilling temperatures (Chinnusamy et al. 2003). These changes are quite beneficial to plant because these changes ease a plant to cope up with cold stress. These changes/ processes induce upon stress are collectively termed as "low temperature-induced signal transduction (LTST)." LTST leads to the upregulation of certain genes that produces specific proteins that help is an adaptation to freezing temperatures. From the above sections, it is clear that any kind of abiotic stress on plants induces a series of adaptive responses in the plant that are quite common in every kind of stress.

9.13 Genetic Engineering for Stress-Tolerant Properties

It is conceivable that the introduction of these alien/novel stress-responsive genes into plants to bring a stress-tolerant property is a promising approach to tackle these stress-sensitive phenotypes (Mittler 2002; Mittler and Blumwald 2010). Therefore, an extensive part of the current genetic research has been dedicated to producing stress-resistant plants by this kind of gene introduction. With the advancement of genetic engineering technology, the cloning and overexpression of stress-resistant genes has become an easy task. The conventional *Agrobacterium*-mediated gene introduction is the common method for gene introduction into the host plant. Besides, the *Agrobacterium*-mediated transformation other non-agrobacterium species that have been identified for genetic transformation in plants are *Rhizobium* sp. NGR234, *Sinorhizobium meliloti,* and *Mesorhizobium loti*. Herein, we describe some important genes/proteins that have been identified and modified in plants for their stress-responsive roles. Depending upon the type of response they belong to; these genes can be divided into two major groups; ones involved in cellular protection (osmoprotectants, membrane stabilization, detoxification), transcription factors, and signaling molecules (Vendruscolo et al. 2007).

9.14 Abscisic Acid (ABA) Response Genes

Abscisic acid is one of the most important plant hormones that serves variable functions in the plant. ABA is an important messenger that is involved in different adaptive responses like regulation of accumulation of osmolytes, LEA (Late Embryogenesis Abundant) protein synthesis, and antioxidant enzymes (Chaves et al. 2003; Verslues et al. 2006). ABA levels in plants increase in response to different stressors and result in stomatal closure to minimize the water loss occurring due to the process of transpiration from leaves. Different cellular responses are largely dependent on the ABA levels (Sreenivasulu et al. 2012), thus conceivable that engineering this trait for crop improvement could be a promising approach. *ERA1* is one such gene that has been identified in *Arabidopsis*, and ß-subunit of a farnesyltransferase. It has been shown that plants that do not have this gene are shown to have increased drought tolerance. This has been also shown that downregulation of *ERA1* by expressing under a drought inducible promoter through antisense expression of *ERA1* in *Arabidopsis* and canola (Jalakas et al. 2017). Similarly, the mutants for ABA receptors like pyrabactin resistance 1-like 1 (*pyl1*), *pyl4*, and *pyl6* in rice have been shown to improve plant improvement for drought resistance (Miao et al. 2018). Following a similar strategy, a Canadian company is developing new transgenic plants under the name Yield Protection Technology™ and it has developed transgenic plants for maize, soybean, and cotton since 2011.

9.15 Gene Encoding Compatible Solute

Stress-induced biosynthesis and accumulation of various organic metabolites is the common and most effective defense mechanism plant display in response to any kind of stress. Osmoprotective adaptation to a stressful situation is a widespread response that is conserved in all kinds of living organisms (Saxena et al. 2013). These solutes also act as scavengers for free radicals and stabilize the plant proteins during stress (Nahar et al. 2016). These compounds have low molecular weight and do not inhibit normal cellular functions and are termed as compatible osmolytes. These chemicals are fundamental to all organisms from bacteria to plants and can be characterized into different categories depending on their biochemical nature (Khan et al. 2009; Jewell et al. 2010) (Table 9.1). The majority of these proteins are hydrophilic and uncharged in nature and function in a vast variety of functions including scavenging of the ROS (reactive oxygen species), as osmoprotectants, pH stabilizers, proteins, enzymes, and membrane. The first report of this type of gene to plant lies in the early 90s, where the introduction of these osmolytes has shown to confer the cold and salt resistance properties to the host plants. Glycine betaine, β-alanine, proline, and mannitol are some common compatible solutes that are conventionally used for metabolic engineering for stress-resistant properties. However, glycine betaine is regularly used for this purpose in different crops. Various transgenic plants that have been produced by this kind of gene introduction are listed in Table 9.2.

Table 9.1 Categories of plant protectants based upon their biochemical nature

S. no.	Categories	Sub-groups
1.	Amino acids	Proline, glutamate, glutamine, alanine
2.	Amino acid derivatives	Ectoine, hydroxyectoine
3.	Quaternary amines	Glycine betaine, polyamines, dimethyl sulfonioproprionate, DMSP
4.	Sugars	Trehalose, sucrose
5.	Polyols including sugar alcohols	Mannitol, sorbitol, galactinol

Table 9.2 Recent examples of the plant development through gene modification in agriculturally important crops

S. no.	Gene	Type of gene	Improved tolerance	References
Rice (*Oryza sativa*)				
1.	*Si*MYB56	R2R3-MYB transcription factor	Drought	(Xu et al. 2020)
2.	PheASR2	Transcription factor	Drought	(Wu et al. 2020, p 2)
3.	*Jc*MADS40	MADS-box family genes	Drought and salt	(Tang et al. 2020)
4.	*Os*ARD1	Acireductone dioxygenase (ARD) metal-binding protein family	Drought and salt	(Liang et al. 2019)
5.	*Os*ZFP350	Zinc finger protein	Heat, salt and drought	(Kang et al. 2019)
6.	*Os*MYB6	MYB family gene	Drought and salt	(Tang et al. 2019)
7.	*Os*JMJ703	Rice histone demethylase gene	Drought	(Song et al. 2018)
8.	*Os*CTZFP8	Zinc finger transcription factor	Cold	(Jin et al. 2018)
9.	*Zm*PIF3	Phytochrome-interacting factors (PIFs)	Drought	(Gao et al. 2018)
10.	PYL3	Pyrabactin resistance-like (PYL) gene family	Cold and drought	(Lenka et al. 2018)
11.	*Os*JAZ1	JAZ (JASMONATE ZIM-domain) proteins	Drought	(Fu et al. 2017)
12.	*Os*MAPK3	MAPK family	Cold	(Zhang et al. 2017)
13.	*Os*bZIP46	bZIP transcription factor	Drought and temperature stress	(Chang et al. 2017)
14.	*Os*LOL5	Zinc finger proteins (ZFPs)	Alkaline and salt	(Guan et al. 2016)
Wheat (*Triticum aestivum*)				
15.	*Ta*HsfA6f	Heat shock factors (Hsfs)	Salt	(Bi et al. 2020)
16.	*Ta*OAT	Ornithine amino transferase	Salt	(Anwar et al. 2020)
17.	*Ta*DREB3	DREB transcription factors	Heat, cold and salt	(Niu et al. 2020)
18.	*At*WRKY30	Transcription factor	Heat and drought	(El-Esawi et al. 2019)
19.	ERF1-V	AP2/ERF transcription factor	Salt and drought	(Xing et al. 2017)
Barley (*Hordeum vulgare)*				
20.	*Ta*HsfA6bT	Heat shock factor	Heat	(Poonia et al. 2020)

(continued)

Table 9.2 (continued)

S. no.	Gene	Type of gene	Improved tolerance	References
21.	*Hv*MYB1	Transcription factor	Drought	(Alexander et al. 2019)
22.	OSM	Osmotic response gene	Salt	(Viktorova et al. 2019)
23.	*Hv*SHN1	Ethylene responsive transcription factor	Salt and drought	(Djemal et al. 2018)
24.	*At*VHA-C	Vacuolar ATPase subunit C	Salt	(Adem et al. 2017)
25.	CPK2a	Calcium-dependent protein kinase	Drought	(Cieśla et al. 2016)

9.16 Free Radicle Scavengers

Exposure to any kind of stress situation finally leads to the production of reactive oxygen species, production of which negatively affects different processes like enzyme and biochemical activities and thus affects the biosynthesis of DNA, protein and carbohydrates, thus exceeding the oxidative stress in a cell. The exposure of plants to these environmental stresses leads to reactive oxygen species (ROS) (Verslues et al. 2006; Jewell et al. 2010). Production of the free radicle upon stress situation is another phenomenon of the stress response that is seen in plants upon waterlogging, drought, high salinity, and high temperatures. It is evident that ROS influences the expression of different genes that influence growth, cell cycle, response to pathogens, plant development, and even cell death (Gill and Tuteja 2010). Thus, modification and expression of the gene helping in scavenging these reactive oxygen species could be an alternative approach to produce stress-tolerant plants. Some of these genes that have already been shown to be successfully introduced in plants for imparting stress-tolerant properties are; ascorbate peroxidase, superoxide dismutase, and glutathione reductase.

9.17 Genetic Engineering of the LEA Proteins Coding Genes

The late embryogenesis abundant (LEA) protein is a stress-induced protein produced in vegetative tissues of the plants. Although the exact functions of these stress-induced proteins are not known, it has been known that these proteins are water-binding molecules that are dehydration and cold-responsive. These are the proteins that help in protecting the desiccation and protect the seed development during salt stress, dehydration, and cold. These proteins are encoded by different genes in different plant species, for instance, responsive to dehydration (RD), early responsive to dehydration (ERD), inducible to cold (KIN), regulated by cold (COR), and responsive to abscisic acid (RAB). Two members of this class of proteins are HVA1 (protein from barley) and LE25 (protein from tomato). The reports have shown that the introduction of the *LEA* gene from barley into rice imparts tolerance to salinity and water stress (Xu et al. 1996). A similar observation was made in yeast transformed with *LE24* from tomato (Imai et al. 1996). Similarly, expression of the *HVA* gene in wheat has also been reported to improve the quality of plants to grow in water-deficient situations with an ability to produce high biomass. A recent example of this kind of gene introduction for stress resistance was achieved by expressing Melon *Y3SK2*-Type *LEA* gene in tobacco. A recent study has shown that the introduction of this gene increases resistance to drought and salt (Aduse Poku et al. 2020).

9.18 Molecular Chaperones

Heat shock proteins (HSPs) are the to-date known molecular chaperons that help incorrect folding of the proteins. Besides these, conventions protein folding molecules other protein folding molecules that have been extensively studied are known as peptidyl-prolyl-isomerases. It has been reported that HSP is produced in response to rapid heat stress. Besides HSP's are also known to get produced under different stages of plant development like embryogenesis, seed germination, development of pollen, and fruit maturation (Prasinos et al. 2005). The modification of these molecules for developing stress-tolerant plants lies early in 1987, when the transgenic tobacco expressing HSP17 were produced, though no conclusion was made on the stress-tolerant property as a promoter was incompetent (Schöffl et al. 1987).

9.19 Proteins for Ion Homeostasis Across the Membrane

A high salt situation causes osmotic stress in the cell and leads to an increase in the high salt situations in the cytoplasm. To counter this, plant has to develop a mechanism where a plant can utilize these ions to minimize the adverse effects of these ions in the cell. This is regulated by genes that regulate the ion channels like Na^+/H^+ antiports and stress signaling through calcium- and calmodulin-dependent protein phosphatase calcineurin. The first successful example of this kind of gene introduction was achieved by overexpressing a single endogenous gene (*AtNHX1*) encoding a vacuolar Na^+/H^+ antiport protein in *Arabidopsis*. The transgenic Arabidopsis was found to be thriving well in the high salt situation, i.e. 200 mM sodium chloride, which correlated well with higher levels of the *AtNHX1* transcripts and protein and vacuolar Na^+/H^+ antiport activity. Similarly, expressing catalytic and regulatory subunit of yeast calcineurin in tobacco has also been shown to generate transgenic tobacco with salt-tolerant properties.

9.20 Transcription Factors

A number of genes that are activated upon abiotic stress are controlled by a complex network of transcription factors (Yamaguchi-Shinozaki and Shinozaki 2006). The stress-responsive genes can generally be classified into two groups; regulatory and functional (Shinozaki et al. 2003). Functional proteins include different enzymes, membrane proteins (water channel and transporters), heat shock proteins, all these proteins have a direct role in stress response. Regulatory proteins include different transcription factors, kinases, and phosphatases that regulate various stress responses. Furthermore, a response to any kind of stress on the plant is multigenic in nature, involving the role of two or more genes in a stress response mechanism. Thus, the introduction of only a single gene is not sufficient to induce a series of changes that may be necessary for a specific stress adaptation. Thus, engineering the different transcription factors involved in controlling specific traits together may be a promising alternative approach to achieve a specific stress-tolerant trait. Some successful initial examples of this kind of gene modification have been achieved by the overexpression of the HSPs have known to confer thermotolerance in *Arabidopsis* (Lee et al. 1995). Similarly, overexpression of the *CBF1* ("C-repeat binding factor"), a transcriptional activator has been shown to confer tolerance to freezing in *Arabidopsis* inducing the expression of four *COR* ("cold-regulated") genes (Jaglo-Ottosen 1998). Another transcription factor is the dehydration-responsive Element (*DREB1A*), this TF is also known to upregulate different stress-responsive genes. Both DRE and CBF factors are known as a cis-acting element that regulates the gene expression in response to variable dehydrating stressors (salt, cold, and drought) (van Rensburg and Krüger 1994). A member of the DRE family, *DREB1A*, is reported to impart drought resistance in *Arabidopsis thaliana* via inducing the expression of different stress-responsive genes (Pellegrineschi et al. 2003). Another family of a transcription factor that is involved in stress tolerance is the NAC gene family members. These transcription factors are known to get expressed in different stages of growth and response to the environment. SNAC1 is a member of this family, expression of which is known to improve drought resistance in rice (Hu et al. 2006). Other transcription factors like *bHLH*, *bZIP*, *NAC*, *AP2/ERF*, *MYB*, Zinc finger, *WRKY*, and kinases are associated with increase crop yield in rice (Dubouzet et al. 2003; Hu et al. 2006; Hossain et al. 2010).

9.21 Recent Advances in Plant Improvement for Abiotic Stress Tolerance

In the above section, we have successfully described the traditional transgenic methods to crop improvement. The current section focuses on the recent advancement that has been made in crop development programs to improve plant for the desired traits.

9.22 Development of Abiotic Stress-Tolerant Crops by miRNA

MicroRNA (miRNA) can be described as the single-stranded RNAs that are approximately 21–24 base pairs in length (Zhao et al. 2011). Studies have shown an involvement of these microRNAs in imparting stress resistance to variable stress on plants (Xia et al. 2012). These miRNAs inhibit the expression of target RNA by binding to the 3′ end of the RNA, thus inhibiting the translation (Meng et al. 2010; Li et al. 2011; Ding et al. 2011). These miRNAs are involved in different cellular processes like transcription, protein stability, and degradation (Shen et al. 2010; Ding et al. 2011). Several studies have shown that 11 miRNAs exert tissue-specific expression towards major abiotic stresses in *Arabidopsis thaliana*. According to the reports in *Arabidopsis thaliana*, miRNA-169 has been shown to contribute towards drought resistance. Similarly, other miRNA's like 159, 396, and 393 also showed to contribute to other abiotic stressors (salinity, cold, and heat) (Table 9.3).

Table 9.3 Recent examples of the plant development through miRNA technology

S. no.	Targeted miRNAs	Transgenic plant	Response	Reference
1.	miR398	Wheat	Cold tolerance	(Lu et al. 2020)
2.	MiR319	Rice	Salt stress tolerance	(Liu et al. 2019)
3.	miR393a	Creeping bentgrass	Salt, drought and heat tolerance	(Zhao et al. 2019)
4.	miR166	Rice	Drought tolerance in knocked-down mutants	(Zhang et al. 2018)
5.	miR5144	Rice	Salinity and mercury stress tolerance	(Xia et al. 2018)
6.	miR827	Maize	Drought tolerance	(Ferdous et al. 2017)
7.	miR159	Rice	Increased drought resistance	(Zhao et al. 2017)
8.	miR156	Rice	Reduced cold tolerance	(Cui et al. 2015)
9.	miR408	Chick pea	Enhanced drought tolerance	(Hajyzadeh et al. 2015)
10.	miR319	Bentgrass	Enhanced salt and drought tolerance	(Zhou et al. 2013)
11.	miR319	Rice	Enhanced tolerance to chilling stress	(Yang et al. 2013)
12.	miR395	Rapeseed	Enhanced tolerance to oxidative stress and heavy metal stress	(Zhang et al. 2013a, b)
13.	miR828	Sweet potato	Oxidative stress tolerance	(Lin et al. 2012)
14.	miR169	Tomato	Enhanced drought tolerance	(Zhang et al. 2011)

9.23 Development of Abiotic Stress-Tolerant Crops by CRISPR (Lustered Regularly Interspaced Short Palindromic Repeats)/Cas9

CRISPR-Cas9 is a recent development developed for crop improvement in recent years. This system depends on an RNA-DNA recognition system that employs a double-strand break in the host genome. The technology has an upper hand over the other crop improvement methods as this is a comparably fast, and efficient gene-editing method for crop improvement (Mao et al. 2013). Besides, it is preferred over the other gene-editing tools as this is simple in designing and efficiently introduces mutation with a targeted introduction at desired locations (Ma et al. 2015; Malzahn et al. 2017). The technique is preferred over other genetic improvement techniques as this is comparable to less tricky and avoids the tedious screening of the desired clone. Besides mutation, this technique can also be used to induce or repress the expression of the particular gene using modified CRISPR where an inactive form of Cas9 (dCas9) is fused with a transcriptional activator or a repressor (Bortesi and Fischer 2015). Due to its vast potential in genome improvement, it has the potential to replace other gene improvement methods. While the system is employed for the improvement of animal cells (Gilbert et al. 2013; La Russa and Qi 2015); the system has limited reports of successful use of this method in plant improvement (Piatek et al. 2015). Some recent examples of CRISPR-Cas mediated plant improvement are summarized in Table 9.4.

9.24 Conclusion

Overall, we have summarized the major biotic and abiotic stress mechanisms that can be engineered for imparting stress-resistant properties to different plants. Though, a huge effort has been already made in this area, there is a considerable challenge that remains to be addressed. As the plant undergoes multiple combinations of stress in field conditions, this area needs further addressing. We propose that the plant's response to multiple different stressors cannot be inferred from assessing the plant's response to individual stress. Thus, it is essential to test different improved varieties to multiple stressors in field conditions where all stressors occur at once. Another challenge to getting these improved varieties to farmers is their development expenses and the approvals these GM plants require for

Table 9.4 Some recent examples of crop improvement via employing CRISPR/Cas9 system

S. no.	TFs	Species	Response	Reference
1.	SST	Rice	Seedling salt-tolerant gene	(Lian et al. 2020)
2.	*Os*MYB30	Rice	Cold tolerance gene	(Zeng et al. 2020)
3.	*Os*GA20ox2	Rice	Lodging resistance	(Nawaz et al. 2020)
4.	ANAC069	Arabidopsis	Salt and osmotic sensitivity	(He et al. 2017)
5.	*Zm*WRKY17	Maize	Salt sensitivity	(Cai et al. 2017)

their field trials. As multiple precautions are already in the guideline to ensure the safety associated with the GM crops, precautions to ensure this safety should not become a barrier for future crop development programs.

References

Abad P, Favery B, Rosso M-N, Castagnone-Sereno P (2003) Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. Mol Plant Pathol 4:217–224

Acharya K, Pal AK, Gulati A, Kumar S, Singh AK, Ahuja PS (2013) Overexpression of Camellia sinensis thaumatin-like protein, CsTLP in potato confers enhanced resistance to Macrophomina phaseolina and Phytophthora infestans infection. Mol Biotechnol 54:609–622

Adem GD, Roy SJ, Huang Y et al (2017) Expressing Arabidopsis thaliana V-ATPase subunit C in barley (Hordeum vulgare) improves plant performance under saline condition by enabling better osmotic adjustment. Funct Plant Biol 44:1147–1159. https://doi.org/10.1071/FP17133

Aduse Poku S, Nkachukwu Chukwurah P, Aung HH, Nakamura I (2020) Over-expression of a melon Y3SK2-type LEA gene confers drought and salt tolerance in transgenic tobacco plants. Plants (Basel) 9. https://doi.org/10.3390/plants9121749

Ahrenholtz I, Harms K, De Vries J, Wackernagel W (2000) Increased killing of bacillus subtilison the hair roots of transgenic T4 lysozyme-producing potatoes. Appl Environ Microbiol 66:1862–1865

Ahuja I, de Vos RC, Bones AM, Hall RD (2010) Plant molecular stress responses face climate change. Trends Plant Sci 15:664–674

Akkouh O, Ng TB, Cheung RC, Wong JH, Pan W, Ng CC et al (2015) Biological activities of ribosome-inactivating proteins and their possible applications as antimicrobial, anticancer, and anti-pest agents and in neuroscience research. Appl Microbiol Biotechnol 99:9847–9863

Alexander RD, Wendelboe-Nelson C, Morris PC (2019) The barley transcription factor HvMYB1 is a positive regulator of drought tolerance. Plant Physiol Biochem 142:246–253. https://doi.org/10.1016/j.plaphy.2019.07.014

Altpeter F, Diaz I, McAuslane H et al (1999) Increased insect resistance in transgenic wheat stably expressing trypsin inhibitor CMe. Mol Breed 5:53–63

Ansari F, Naderi R, Dehghanian C (2015) Study on the protective function of cloisite incorporated silane sol–gel coatings cured at different conditions. Appl Clay Sci 114:93–102

Anu K, Jessymol KK, Chidambareswaren M, Gayathri GS, Manjula S (2015) Downregulation of osmotin (PR5) gene by virus-induced gene silencing (VIGS) leads to susceptibility of resistant piper colubrinum link. To the oomycete pathogen *Phytophthora capsici* Leonian. Indian J Exp Biol 53(6):329–334

Anwar A, She M, Wang K, Ye X (2020) Cloning and molecular characterization of Triticum aestivum ornithine amino transferase (TaOAT) encoding genes. BMC Plant Biol 20:187. https://doi.org/10.1186/s12870-020-02396-2

Arce P, Moreno M, Gutierrez M, Gebauer M, Dell'Orto P, Torres H, Acuna I, Oliger P, Venegas A, Jordana X, Kalazich J, Holuigue L (1999) Enhanced resistance to bacterial infection by *Erwinia carotovora* subsp. *atroseptica* in transgenic potato plants expressing the attacin or the cecropin SB-37 genes. Am J Potato Res 76:169–177

Ashraf M, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Sci 166:3–16

Bengtsson L, Hodges KI, Roeckner E (2006) Storm tracks and climate change. J Clim 19:3518–3543

Bernstein L, Bosch P, Canziani O et al (2008) IPCC, 2007: climate change 2007: synthesis report. IPCC, Geneva

Bi H, Zhao Y, Li H, Liu W (2020) Wheat heat shock factor TaHsfA6f increases ABA levels and enhances tolerance to multiple abiotic stresses in transgenic plants. Int J Mol Sci 21. https://doi.org/10.3390/ijms21093121

Bian XX, Shen FB, Chen YW, Wang BN, Deng M, Meng YF (2010) PEGylation of alpha-momorcharin: synthesis and characterization of novel anti-tumor conjugates with therapeutic potential. Biotechnol Lett 32:883–890

Boller T (1993) Antimicrobial functions of the plant hydrolases, chitinases and β-1,3-glucanases. In: Fritig B, Legrand M (eds) Mechanisms of plant defense responses. Kluwer Academic Press, Dordrecht, pp 391–400

Boman HG (1991) Antibacterial peptides: key components needed in immunity. Cell 65:205–207

Bonfim K, Faria JC, Nogueira EO et al (2007) RNAi-mediated resistance to bean golden mosaic virus in genetically engineered common bean (Phaseolus vulgaris). Mol Plant-Microbe Interact 20:717–726

Bortesi L, Fischer R (2015) The CRISPR/Cas9 system for plant genome editing and beyond. Biotechnol Adv 33:41–52

Bray EA, Bailey-Serres J, Weretilnyk E et al (2000) Responses to abiotic stresses. In: Biochemistry and molecular biology of plants. American Society of Plant Biologists, Rockville, MD, pp 149–158

Broadway RM, Duffey SS (1986) The effect of dietary protein on the growth and digestive physiology of larval *Heliothis zea* and *Spodoptera exigua*. J Insect Physiol 32(8):673–680

Brookes G, Barfoot P (2015) Global income and production impacts of using GM crop technology 1996–2013. GM Crops Food 6:13–46

Cai D et al (1997) Positional cloning of a gene for nematode resistance in sugar beet. Science 275:832–834

Cai R, Dai W, Zhang C et al (2017) The maize WRKY transcription factor ZmWRKY17 negatively regulates salt stress tolerance in transgenic Arabidopsis plants. Planta 246:1215–1231

Chang Y, Nguyen BH, Xie Y et al (2017) Co-overexpression of the constitutively active form of OsbZIP46 and ABA-activated protein kinase SAPK6 improves drought and temperature stress resistance in rice. Front Plant Sci 8:1102. https://doi.org/10.3389/fpls.2017.01102

Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought—from genes to the whole plant. Funct Plant Biol 30:239–264

Chen HM, Wang W, Smith D, Chan SC (1997) Effects of the anti-bacterial peptide cecropin B and its analogs, cecropins B-1 and B-2, on liposomes, bacteria, and cancer cells. Biochim Biophys Acta 1336:171–179

Cheng Q, Li N, Dong L, Zhang D, Fan S, Jiang L, Wang X, Xu P, Zhang S (2015) Overexpression of soybean isoflavone reductase (*GmIFR*) enhances resistance to *Phytophthora sojae* in soybean. Front Plant Sci. https://doi.org/10.3389/fpls.2015.01024

Chinnusamy V, Ohta M, Kanrar S et al (2003) ICE1: a regulator of cold-induced transcriptome and freezing tolerance in Arabidopsis. Genes Dev 17:1043–1054. https://doi.org/10.1101/gad.1077503

Chopra R, Saini R (2014) Transformation of blackgram (Vigna mungo (L.) Hepper) by barley chitinase and ribosome-inactivating protein genes towards improving resistance to Corynespora leaf spot fungal disease. Appl Biochem Biotechnol 174:2791–2800

Chowdhury S, Basu A, Kundu S (2017) Overexpression of a new osmotin-like protein gene (*SindOLP*) confers tolerance against biotic and abiotic stresses in sesame. Front Plant Sci 8:410

Cieśla A, Mituła F, Misztal L et al (2016) A role for barley calcium-dependent protein kinase CPK2a in the response to drought. Front Plant Sci 7. https://doi.org/10.3389/fpls.2016.01550

Collins NC, Tardieu F, Tuberosa R (2008) Quantitative trait loci and crop performance under abiotic stress: where do we stand? Plant Physiol 147:469–486

Cruickshank IAM, Perrin DR (1960) Isolation of a phytoalexin from Pisum sativum L. Nature 187:799–800

Cui N, Sun X, Sun M et al (2015) Overexpression of OsmiR156k leads to reduced tolerance to cold stress in rice (Oryza Sativa). Mol Breed 35:214

de Maagd RA, Bravo A, Crickmore N (2001) How Bacillus thuringiensis has evolved specific toxins to colonize the insect world. Trends Genet 17:193–199

de Virgilio M, Lombardi A, Caliandro R, Fabbrini MS (2010) Ribosome inactivating proteins: from plant defense to tumor attack. Toxins 2:2699–2737

Ding Y, Chen Z, Zhu C (2011) Microarray-based analysis of cadmium-responsive microRNAs in rice (Oryza sativa). J Exp Bot 62:3563–3573. https://doi.org/10.1093/jxb/err046

Djemal R, Mila I, Bouzayen M et al (2018) Molecular cloning and characterization of novel WIN1/SHN1 ethylene responsive transcription factor HvSHN1 in barley (Hordeum vulgare L.). J Plant Physiol 228:39–46. https://doi.org/10.1016/j.jplph.2018.04.019

Dong X, Zhao Y, Ran X, Guo L, Zhao D (2017) Overexpression of a new chitinase gene *EuCHIT2* enhances resistance to *Erysiphe cichoracearum* DC. In tobacco plants. Int J Mol Sci 18 (11):2361

Dubouzet JG, Sakuma Y, Ito Y et al (2003) OsDREB genes in rice, Oryza sativa L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. Plant J 33:751–763. https://doi.org/10.1046/j.1365-313X.2003.01661.x

Düring K, Porsch P, Fladung M, Lörz H (1993) Transgenic potato plants resistant to the phytopathogenic bacterium *Erwinia carotovora*. Plant J 3:587–598

Easterling DR, Evans JL, Groisman PY et al (2000) Observed variability and trends in extreme climate events: a brief review. Bull Am Meteorol Soc 81:417–426

El-Esawi MA, Al-Ghamdi AA, Ali HM, Ahmad M (2019) Overexpression of AtWRKY30 transcription factor enhances heat and drought stress tolerance in wheat (Triticum aestivum L.). Genes (Basel) 10. https://doi.org/10.3390/genes10020163

Elvira MI, Galdeano MM, Gilardi P, García-Luque I, Serra MT (2008) Proteomic analysis of pathogenesis related pro-tein (PRs) induced by compatible and incompatible interactions of pepper mild mottle virus (PMMoV) in *Capsicum chinense* L3 plants. J Exp Bot 59:1253–1265

Ernst K et al (2002) The broad-spectrum potato cyst nematode resistance gene (Hero) from tomato is the only member of a large gene family of NBS-LRR genes with an unusual amino acid repeat in the LRR region. Plant J 31:127–136

Fartyal D, Agarwal A, James D et al (2018) Developing dual herbicide tolerant transgenic rice plants for sustainable weed management. Sci Rep 8:1–12

Ferdous J, Whitford R, Nguyen M et al (2017) Drought-inducible expression of Hv-miR827 enhances drought tolerance in transgenic barley. Funct Integr Genomics 17:279–292. https://doi.org/10.1007/s10142-016-0526-8

Fierens E, Gebruers K, Voet AR, De Maeyer M, Courtin CM et al (2009) Biochemical and structural characterization of TLXI, the *Triticum aestivum* L. thaumatin-like xylanase inhibitor. J Enzyme Inhib Med Chem 24:646–654

Fischer RAT, Edmeades GO (2010) Breeding and cereal yield Progress. Crop Sci 50:S-85–S-98. https://doi.org/10.2135/cropsci2009.10.0564

Flowers TJ, Flowers SA (2005) Why does salinity pose such a difficult problem for plant breeders? Agric Water Manag 78:15–24

Fu J, Wu H, Ma S et al (2017) OsJAZ1 attenuates drought resistance by regulating JA and ABA signaling in rice. Front Plant Sci 8:2108. https://doi.org/10.3389/fpls.2017.02108

Fujimori N, Enoki S, Suzuki A, Naznine HA, Shimizub M, Suzuki S (2016) Grape apoplasmic −1,3-glucanase confers fungal disease resistance in Arabidopsis. Sci Hortic 200:105–110

Gambhir G, Kumar P, Aggarwal G, Srivastava DK, Thakur AK (2020) Expression of *cry1Aa* gene in cabbage imparts resistance against diamondback moth (*Plutella xylostella*). Biologia Futura 71(1):165–173

Gao Y, Wu M, Zhang M et al (2018) Roles of a maize phytochrome-interacting factors protein ZmPIF3 in regulation of drought stress responses by controlling stomatal closure in transgenic rice without yield penalty. Plant Mol Biol 97:311–323. https://doi.org/10.1007/s11103-018-0739-4

Gilbert LA, Larson MH, Morsut L et al (2013) CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. Cell 154:442–451. https://doi.org/10.1016/j.cell.2013.06.044

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Gonzales-Salazar R, Cecere B, Ruocco M, Rao R, Corrado G (2017) A comparison between constitutive and inducible transgenic expression of the PhRIP I gene for broad-spectrum resistance against phytopathogens in potato. Biotechnol Lett 39:1049–1058

Green JM, Castle LA (2010) Transitioning from single to multiple herbicide-resistant crops. In: Glyphosate resistance in crops and weeds: history, development, and management. Wiley, New York, pp 67–91

Green TR, Ryan CA (1972) Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. Science 175:776–777

Guan QJ, Ma HY, Wang ZJ et al (2016) A rice LSD1-like-type ZFP gene OsLOL5 enhances saline-alkaline tolerance in transgenic Arabidopsis thaliana, yeast and rice. BMC Genomics 17:142. https://doi.org/10.1186/s12864-016-2460-5

Guo Z, Xing R, Liu S et al (2008) The influence of molecular weight of quaternized chitosan on antifungal activity. Carbohydr Polym 71:694–697

Hajyzadeh M, Turktas M, Khawar KM, Unver T (2015) miR408 overexpression causes increased drought tolerance in chickpea. Gene 555:186–193

Hayes BM, Bleackley MR, Wiltshire JL, Anderson MA, Traven A et al (2013) Identification and mechanism of action of the plant defensin NaD1 as a new member of the antifungal drug arsenal against *Candida albicans*. Antimicrob Agents Chemother 57:3667–3675

He L, Shi X, Wang Y et al (2017) Arabidopsis ANAC069 binds to C [A/G] CG [T/G] sequences to negatively regulate salt and osmotic stress tolerance. Plant Mol Biol 93:369–387

Heap I, Duke SO (2018) Overview of glyphosate-resistant weeds worldwide. Pest Manag Sci 74:1040–1049

Heidarvand L, Amiri RM (2010) What happens in plant molecular responses to cold stress? Acta Physiol Plant 32:419–431

Hilder VA, Gatehouse AM, Sheerman SE, Barker RF, Boulter D (1987) A novel mechanism of insect resistance engineered into tobacco. Nature 330(6144):160–163

Hirayama T, Shinozaki K (2010) Research on plant abiotic stress responses in the post-genome era: past, present and future. Plant J 61:1041–1052

Hossain MA, Cho J-I, Han M et al (2010) The ABRE-binding bZIP transcription factor OsABF2 is a positive regulator of abiotic stress and ABA signaling in rice. J Plant Physiol 167:1512–1520. https://doi.org/10.1016/j.jplph.2010.05.008

Hu H, Dai M, Yao J et al (2006) Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proc Natl Acad Sci 103:12987–12992. https://doi.org/10.1073/pnas.0604882103

Hua J (2009) From freezing to scorching, transcriptional responses to temperature variations in plants. Curr Opin Plant Biol 12:568–573

Huang MX, Hou P, Wei Q, Xu Y, Chen F (2008) A ribosome-inactivating protein (curcin 2) induced from *Jatropha curcas* can reduce viral and fungal infection in transgenic tobacco. Plant Growth Regul 54:115–123

Hultmark D, Engström A, Andersson K et al (1983) Insect immunity. Attacins, a family of antibacterial proteins from Hyalophora cecropia. EMBO J 2:571–576

Ibeas JI, Lee H, Damsz B, Prasad DT, Pardo JM, Hasegawa PM, Bressan RA, Narasimhan ML (2000) Fungal cell wall phosphomannans facilitate the toxic activity of a plant PR-5 protein. Plant J 23:375–383

Ibeas JI, Yun DJ, Damsz B, Narasimhan ML, Uesono Y, Ribas JC et al (2001) Resistance to the plant PR-5 protein osmotin in the model fungus Saccharomyces cerevisiae is mediated by the regulatory effects of SSD1 on cell wall composition. Plant J 25:271–280

Imai R, Chang L, Ohta A et al (1996) A lea-class gene of tomato confers salt and freezing tolerance when expressed in Saccharomyces cerevisiae. Gene 170:243–248

Jabeen N, Chaudhary Z, Gulfraz M, Rashid H, Mirza B (2015) Expression of rice chitinase gene in genetically engineered tomato confers enhanced resistance to fusarium wilt and early blight. Plant Pathol J 31(3):252–258

Jaglo-Ottosen KR (1998) Arabidopsis CBF1 overexpression induces COR genes and enhances freezing tolerance. Science 280:104–106. https://doi.org/10.1126/science.280.5360.104

Jalakas P, Huang Y-C, Yeh Y-H et al (2017) The role of enhanced responses to ABA1 (ERA1) in arabidopsis stomatal responses is beyond ABA signaling. Plant Physiol 174:665–671. https://doi.org/10.1104/pp.17.00220

Jaspers P, Kangasjärvi J (2010) Reactive oxygen species in abiotic stress signaling. Physiol Plant 138:405–413

Jaynes JM, Nagpala P, Destefanobeltran L, Huang JH, Kim JH, Denny T, Cetiner S (1993) Expression of a cecropin-B lytic peptide analog in transgenic tobacco confers enhanced resistance to bacterial wilt caused by *Pseudomonas Solanacearum*. Plant Sci 89:43–53

Jewell MC, Campbell BC, Godwin ID (2010) Transgenic plants for abiotic stress resistance. In: Kole C, Michler CH, Abbott AG, Hall TC (eds) Transgenic crop plants. Springer, Berlin, Heidelberg, pp 67–132

Jha S, Chattoo BB (2010) Expression of a plant defensin in rice confers resistance to fungal phytopathogens. Transgenic Res 19:373–384

Jin Y-M, Piao R, Yan Y-F et al (2018) Overexpression of a new zinc finger protein transcription factor OsCTZFP8 improves cold tolerance in rice. Int J Genom 2018:5480617. https://doi.org/10.1155/2018/5480617

Kamboj R, Tiwari IM, Devanna BN, Botella JR, Sharma V, Sharma TR (2017) Novel Chitinase gene LOC_Os11g47510 from Indica Rice Tetep provides enhanced resistance against sheath blight pathogen *Rhizoctonia solani* in rice. Front Plant Sci. https://doi.org/10.3389/fpls.2017.00596

Kang Z, Qin T, Zhao Z (2019) Overexpression of the zinc finger protein gene OsZFP350 improves root development by increasing resistance to abiotic stress in rice. Acta Biochim Pol 66:183–190. https://doi.org/10.18388/abp.2018_2765

Katam R, Chibanguza K, Latinwo LM, Smith D (2015) Proteome biomarkers in xylem reveal pierce's disease tolerance in grape. J Proteom Bioinform 8:217–224

Kato A, Nakamura S, Ibrahim H, Matsumi T, Tsumiyama C, Kato M (1998) Production of genetically modified lysozymes having extreme heat stability and antimicrobial activity against gram negative bacteria in yeast and in plants. Nahrung 42:128–130

Kaur A, Sudhakara Reddy M, Pati PK et al (2020) Over-expression of Osmotin (*OsmWS*) gene of *Withania somnifera* in potato cultivar 'Kufri Chipsona 1' imparts resistance to *Alternaria solani*. Plant Cell Tissue Organ Cult 142:131–142

Kaya MD, Okçu G, Atak M et al (2006) Seed treatments to overcome salt and drought stress during germination in sunflower (Helianthus annuus L.). Eur J Agron 24:291–295

Khan MS, Yu X, Kikuchi A et al (2009) Genetic engineering of glycine betaine biosynthesis to enhance abiotic stress tolerance in plants. Plant Biotechnol 26:125–134

Khan A, Nasir IA, Tabassum B, Aaliya K, Tariq M, Rao AQ (2017) Expression studies of chitinase gene in transgenic potato against *Alternaria solani*. Plant Cell Tissue Organ Culture, 128:563–576

Kim JK, Jang IC, Wu R, Zuo WN, Boston RS, Lee YH et al (2003) Co-expression of a modified maize ribosome-inactivating protein and a rice basic chitinase gene in transgenic rice plants confers enhanced resistance to sheath blight. Transgenic Res 12:475–484

Kim ST, Kim SG, Hwang DH, Kang SY, Kim HJ, Lee BH, Lee JJ, Kang KY (2004) Proteomic analysis of pathogen-responsive proteins from rice leaves induced by rice blast fungus, *Magnaporthe grisea*. Proteomics 4:3569–3578

Ko K, Norelli JL, Reynoird JP, Boresjza-Wysocka E, Brown SK, Aldwinckle S (2000) Effect of untranslated leader sequence of AMV RNA 4 and signal peptide of pathogenesis-related protein 1b on attacin gene expression, and resistance to fire blight in transgenic apple. Biotechnol Lett 22:373–381

Koenning SR, Overstreet C, Noling JW et al (1999) Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. J Nematol 31:587

Kumar P, Srivastava DK (2016) Biotechnological advancement in genetic improvement of broccoli (*Brassica oleracea* L. var. *italica*), an important vegetable crop. Biotechnol Lett 38 (7):1049–1063

Kumar P, Srivastava D (2020a) Molecular and biotechnological interventions for improving Brassicaceae crops for abiotic stress tolerance. In: The plant family Brassicaceae. Springer, Singapore, pp 437–450

Kumar P, Srivastava DK (2020b) Insight to biotechnological advances in the study of beneficial plant-microbe interaction with special reference to agrobacterium tumefaciens. In: Plant-microbe symbiosis. Springer, Cham, pp 287–302

Kumar P, Gambhir G, Gaur A, Thakur AK, Sharma KC, Srivastava DK (2018a) Development of transgenic broccoli with *cryIAa* gene for resistance against diamondback moth (*Plutella xylostella*). 3 Biotech 8(7):299

Kumar P, Thakur AK, Srivastava DK (2018b) Genetic engineering approaches for abiotic stress tolerance in broccoli: recent progress. In: Akula R, Gill SS (eds) Metabolic adaptations in plants during abiotic stress. Taylor & Francis (CRC Press), Boca Raton, FL, pp 363–367

Kumar P, Dhiman K, Srivastava DK (2018c) Morphogenic potential of different explants of broccoli (*Brassica oleracea* L. var. *italica*): important "nutrient rich" vegetable, using Thidiazuron. In: Ahmad N, Faisal M (eds) Thidiazuron: from urea derivative to plant growth regulator. Springer, Berlin, pp 373–392

La Russa MF, Qi LS (2015) The new state of the art: Cas9 for gene activation and repression. Mol Cell Biol 35:3800–3809

Lacerda AF, Del Sarto RP, Silva MS, de Vasconcelos EA, Coelho RR, dos Santos VO, Godoy CV, Seixas CD, da Silva MC, Grossi-de-Sa MF (2016) The recombinant pea defensin Drr230a is active against impacting soybean and cotton pathogenic fungi from the genera Fusarium, Colletotrichum and Phakopsora. Biotechnology 6:59

LaRosa PC, Chen Z, Nelson DE, Singh NK, Hasegawa PM, Bressan RA (1992) Osmotin gene expression is post transcriptionally regulated. Plant Physiol 100:409–415

Lay FT, Anderson MA (2005) Defensins - components of the innate immune system in plants. Curr Protein Pept Sci 6:85–101

Le TTT, Williams B, Mundree S (2018) An osmotin from the resurrection plant Tripogon loliiformis (TlOsm) confers tolerance to multiple abiotic stresses in transgenic rice. Physiol Plant 162(1):13–34

Lee HI, Raikel NV (1995) Prohevein is poorly processed but shows enhanced resistance to a chitin-binding fungus in transgenic tomato plants. Braz J Med Biol Res 28:743–750

Lee JH, Hubel A, Schoffl F (1995) Derepression of the activity of genetically engineered heat shock factor causes constitutive synthesis of heat shock proteins and increased thermotolerance in transgenic Arabidopsis. Plant J 8:603–612. https://doi.org/10.1046/j.1365-313X.1995.8040603.x

Lenka SK, Muthusamy SK, Chinnusamy V, Bansal KC (2018) Ectopic expression of rice PYL3 enhances cold and drought tolerance in Arabidopsis thaliana. Mol Biotechnol 60:350–361. https://doi.org/10.1007/s12033-018-0076-5

Li T, Li H, Zhang Y-X, Liu J-Y (2011) Identification and analysis of seven H2O2-responsive miRNAs and 32 new miRNAs in the seedlings of rice (Oryza sativa L. ssp. indica). Nucleic Acids Res 39:2821–2833. https://doi.org/10.1093/nar/gkq1047

Lian T, Huang Y, Xie X et al (2020) Rice SST variation shapes the rhizosphere bacterial community, conferring tolerance to salt stress through regulating soil metabolites. mSystems 5. https://doi.org/10.1128/mSystems.00721-20

Liang S, Xiong W, Yin C et al (2019) Overexpression of OsARD1 improves submergence, drought, and salt tolerances of seedling through the enhancement of ethylene synthesis in Rice. Front Plant Sci 10. https://doi.org/10.3389/fpls.2019.01088

Lin J-S, Lin C-C, Lin H-H et al (2012) Micro R 828 regulates lignin and H 2 O 2 accumulation in sweet potato on wounding. New Phytol 196:427–440
Liu D, Raghothama KG, Hasegawa PM, Bressan RA (1994) Osmotin overexpression in potato delays development of disease symptoms. Proc Natl Acad Sci U S A 91:1888–1892
Liu JJ, Sturrock R, Ekramoddoullah AK (2010) The superfamily of thaumatin-like proteins: its origin, evolution, and expression towards biological function. Plant Cell Rep 29:419–436
Liu D, He X, Li W, Chen C, Ge F (2012) Molecular cloning of a thaumatin-like protein gene from *Pyrus pyrifolia* and overexpression of this gene in tobacco increased resistance to pathogenic fungi. Plant Cell Tissue Organ Culture 111:29–39
Liu Y, Li D, Yan J et al (2019) MiR319 mediated salt tolerance by ethylene. Plant Biotechnol J 17:2370–2383. https://doi.org/10.1111/pbi.13154
Lobell DB, Gourdji SM (2012) The influence of climate change on global crop productivity. Plant Physiol 160:1686–1697. https://doi.org/10.1104/pp.112.208298
Lu Q, Guo F, Xu Q, Cang J (2020) LncRNA improves cold resistance of winter wheat by interacting with miR398. Funct Plant Biol 47:544–557. https://doi.org/10.1071/FP19267
M'Hamdi M, Chikh-rouhou H, Boughalleb N, Galarreta JR (2013) Ribosome inactivating protein of barley enhanced resistance to *Rhizoctonia solani* in transgenic potato cultivar 'Desirée' in greenhouse conditions. Biotechnol Agron Soc Environ 17(1):20–26
Ma X, Zhang Q, Zhu Q et al (2015) A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. Mol Plant 8:1274–1284
Maddaloni M, Forlani F, Balmas V, Donini G, Stasse L, Corazza L et al (1997) Tolerance to the fungal pathogen *Rhizoctonia solani* AG4 of transgenic tobacco expressing the maize ribosome inactivating protein b-32. Transgenic Res 6:393–402
Maestri E, Klueva N, Perrotta C et al (2002) Molecular genetics of heat tolerance and heat shock proteins in cereals. Plant Mol Biol 48:667–681
Mahalingam R (2015) Consideration of combined stress: a crucial paradigm for improving multiple stress tolerance in plants. In: Combined stresses in plants. Springer, Cham, pp 1–25
Mahdavi F, Sariah M, Maziah M (2012) Expression of rice thaumatin-like protein gene in transgenic banana plants enhances resistance to Fusarium wilt. Appl Biochem Biotechnol 166:1008–1019
Malzahn A, Lowder L, Qi Y (2017) Plant genome editing with TALEN and CRISPR. Cell Biosci 7:21
Manickavelu A, Nadarajan N, Ganesh SK et al (2006) Drought tolerance in rice: morphological and molecular genetic consideration. Plant Growth Regul 50:121–138
Mao Y, Zhang H, Xu N et al (2013) Application of the CRISPR–Cas system for efficient genome engineering in plants. Mol Plant 6:2008–2011
Melchers LS, Stuiver MH (2000) Novel genes for disease resistance breeding. Curr Opin Plant Biol 3:147–152
Meng Y, Chen D, Ma X et al (2010) Mechanisms of microRNA-mediated auxin signaling inferred from the rice mutant osaxr. Plant Signal Behav 5:252–254
Miao C, Xiao L, Hua K et al (2018) Mutations in a subfamily of abscisic acid receptor genes promote rice growth and productivity. Proc Natl Acad Sci 115:6058–6063
Milligan SB et al (1998) The root knot nematode resistance gene Mi from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. Plant Cell 10:1307–1319
Misra RC, Kamthan SM, Kumar S, Ghosh S (2016) A thaumatin-like protein of *Ocimum basilicum* confers tolerance to fungal pathogen and abiotic stress in transgenic *Arabidopsis*. Sci Rep 6:25340
Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410
Mittler R, Blumwald E (2010) Genetic engineering for modern agriculture: challenges and perspectives. Annu Rev Plant Biol 61:443–462. https://doi.org/10.1146/annurev-arplant-042809-112116

Müller KO (1958) Studies on phytoalexins I. The formation and the immunological significance of phytoalexin produced by Phaseolus vulgaris in response to infections with Sclerotinia fructicola and Phytophthora infestans. Aust J Biol Sci 11:275–300

Muoki RC, Paul A, Kumar S (2012) A shared response of thaumatin like protein, chitinase, and late embryogenesis abundant protein3 to environmental stresses in tea [*Camellia sinensis* (L.) O. Kuntze]. Funct Integr Genomics 12:565–571

Nahar K, Hasanuzzaman M, Fujita M (2016) Roles of osmolytes in plant adaptation to drought and salinity. In: Osmolytes and plants acclimation to changing environment: emerging omics technologies. Springer, New Delhi, pp 37–68

Nakajima H, Muranaka T, Ishige F, Akutsu K, Oeda K (1997) Fungal and bacterial disease resistance in transgenic plants expressing human lysozyme. Plant Cell Rep 16:674–679

Narasimhan ML, Damsz B, Coca MA, Ibeas JI, Yun DJ, Pardo JM, Hasegawa PM, Bressan RA (2001) A plant defense response effector induces microbial apoptosis. Mol Cell 8:921–930

Narasimhan ML, Coca MA, Jin J, Yamauchi T, Ito Y, Kadowaki T et al (2005) Osmotin is a homolog of mammalian adiponectin and controls apoptosis in yeast through a homolog of mammalian adiponectin receptor. Mol Cell 17:171–180

Nawaz G, Usman B, Zhao N et al (2020) CRISPR/Cas9 directed mutagenesis of OsGA20ox2 in high yielding basmati rice (Oryza sativa L.) line and comparative proteome profiling of unveiled changes triggered by mutations. Int J Mol Sci 21. https://doi.org/10.3390/ijms21176170

Negrão S, Schmöckel SM, Tester M (2017) Evaluating physiological responses of plants to salinity stress. Ann Bot 119:1–11. https://doi.org/10.1093/aob/mcw191

Neuhaus JM (1999) Plant chitinases (PR-3, PR-4, PR-8, PR-11). In: Datta SK, Muthukrishnan S (eds) Pathogenesis-related proteins in plants. CRC Press, Boca Raton, FL, pp 77–105

Nishizawa Y, Nishio Z, Nakazono K, Soma M, Nakajima E, Ugaki M, Hibi T (1999) Enhanced resistance to blast (Magnaporthe grisea) in transgenic rice by constitutive expression of rice chitinase. Theor Appl Genet 99:383–390

Niu X, Luo T, Zhao H et al (2020) Identification of wheat DREB genes and functional characterization of TaDREB3 in response to abiotic stresses. Gene 740:144514. https://doi.org/10.1016/j.gene.2020.144514

Norelli JL, Aldwinckle HS, Destéfano-Beltrán L, Jaynes JM (1994) Transgenic 'Mailing 26'apple expressing the attacin E gene has increased resistance to Erwinia amylovora. Euphytica 77:123–128

Ojola PO, Nyaboga EN, Njiru PN, Orinda G (2018) Overexpression of rice thaumatin-like protein (*Ostlp*) gene in transgenic cassava results in enhanced tolerance to *Colletotrichum gloeosporioides* f. sp. *Manihotis*. J Genet Eng Biotechnology 16(1):125–131

Ortiz R, Braun H-J, Crossa J et al (2008) Wheat genetic resources enhancement by the international maize and wheat improvement center (CIMMYT). Genet Resour Crop Evol 55:1095–1140. https://doi.org/10.1007/s10722-008-9372-4

Pandey P, Ramegowda V, Senthil-Kumar M (2015) Shared and unique responses of plants to multiple individual stresses and stress combinations: physiological and molecular mechanisms. Front Plant Sci 6:723. https://doi.org/10.3389/fpls.2015.00723

Parmar N, Singh KH, Sharma P, Singh L, Kumar P, Nanjundan J, Khan YJ, Chauhan DK, Thakur AK (2017) Genetic engineering strategies for biotic and abiotic stress tolerance and quality enhancement in horticultural crops: a comprehensive review. 3 Biotech 7:239

Parray JA, Mir MY, Shameem N (2019) Plant genetic engineering and GM crops: merits and demerits. In: Sustainable agriculture: biotechniques in plant biology. Springer, Singapore, pp 155–229

Pellegrineschi A, Ribaut JM, Thretowan R et al (2003) Preliminary characterization of the DREB genes in transgenic wheat. In: Vasil IK (ed) Plant biotechnology 2002 and beyond: proceedings of the 10th IAPTC&B congress June 23–28, 2002 Orlando, Florida, U.S.A. Springer, Dordrecht, pp 183–187

Perlak FJ, Fuchs RL, Dean DA et al (1991) Modification of the coding sequence enhances plant expression of insect control protein genes. Proc Natl Acad Sci 88:3324–3328

Piatek A, Ali Z, Baazim H et al (2015) RNA-guided transcriptional regulation in planta via synthetic dC as9-based transcription factors. Plant Biotechnol J 13:578–589

Poonia AK, Mishra SK, Sirohi P et al (2020) Overexpression of wheat transcription factor (TaHsfA6b) provides thermotolerance in barley. Planta 252:53. https://doi.org/10.1007/s00425-020-03457-4

Prasinos C, Krampis K, Samakovli D, Hatzopoulos P (2005) Tight regulation of expression of two Arabidopsis cytosolic Hsp90 genes during embryo development. J Exp Bot 56:633–644

Punja ZK (2004) Genetic engineering of plants to enhance resistance to fungal pathogens. In: Punja ZK (ed) Fungal disease resistance in plants. Food Products Press, New York, pp 207–258

Puri M, Kaur I, Kanwar RK, Gupta RC, Chauhan A, Kanwar JR (2009) Ribosome inactivating proteins (RIPs) from Momordicacharantiafor antiviral therapy. Curr Mol Med 9:1080–1094

Qadir M, Quillérou E, Nangia V et al (2014) Economics of salt-induced land degradation and restoration. In: Natural resources forum. Wiley Online Library, pp 282–295

Qian Q, Huang L, Yi R, Wang SZ, Ding Y (2014) Enhanced resistance to blast fungus in rice (*Oryza sativa* L.) by expressing the ribosome-inactivating protein alpha-momorcharin. Plant Sci 217–218:1–7

Raj PA, Dentino AR (2002) Current status of defensins and their role in innate and adaptive immunity. FEMS Microbiol Lett 206:9–18

Ramegowda V, Senthil-Kumar M (2015) The interactive effects of simultaneous biotic and abiotic stresses on plants: mechanistic understanding from drought and pathogen combination. J Plant Physiol 176:47–54. https://doi.org/10.1016/j.jplph.2014.11.008

Ramu VS, Paramanantham A, Ramegowda V et al (2016) Transcriptome analysis of sunflower genotypes with contrasting oxidative stress tolerance reveals individual- and combined- biotic and abiotic stress tolerance mechanisms. PLoS One 11:e0157522. https://doi.org/10.1371/journal.pone.0157522

Roy B, Basu AK (2009) Abiotic stress tolerance in crop plants: breeding and biotechnology. New India Publishing, New Delhi

Sagaram US, Pandurangi R, Kaur J, Smith TJ, Shah DM (2011) Structure-activity determinants in antifungal plant defensins MsDef1 and MtDef4 with different modes of action against *Fusarium graminearum*. PLoS One 6:e18550

Sakata T, Higashitani A (2008) Male sterility accompanied with abnormal anther development in plants–genes and environmental stresses with special reference to high temperature injury. Int J Plant Dev Biol 2:42–51

Saxena SC, Kaur H, Verma P et al (2013) Osmoprotectants: potential for crop improvement under adverse conditions. In: Plant acclimation to environmental stress. Springer, New York, pp 197–232

Schöffl F, Rossol I, Angermüller S (1987) Regulation of the transcription of heat shock genes in nuclei from soybean (Glycine max) seedlings. Plant Cell Environ 10:113–119

Schuler TH, Poppy GM, Kerry BR, Denholm I (1998) Insect-resistant transgenic plants. Trends Biotechnol 16:168–175

Scorza R, Callahan A, Dardick C et al (2013) Genetic engineering of plum pox virus resistance:'HoneySweet'plum—from concept to product. Plant Cell Tissue Organ Culture 115:1–12

Secretariat UNIS for DR (2015) Global assessment report on disaster risk reduction 2015: making development sustainable: the future of disaster risk management. UNDRR, Geeneva

Seo HH, Park S, Park S, Oh BJ, Back K, Han O, Kim J, Kim YS (2014) Overexpression of a defensin enhances resistance to a fruit-specific anthracnose fungus in pepper. PLoS One 9(5): e97936

Serrano C, Arce-Johnson P, Torres H et al (2000) Expression of the chicken lysozyme gene in potato enhances resistance to infection by Erwinia carotovora subsp. atroseptica. Am J Potato Res 77:191–199

Sharma P, Dubey RS (2007) Involvement of oxidative stress and role of antioxidative defense system in growing rice seedlings exposed to toxic concentrations of aluminum. Plant Cell Rep 26:2027–2038. https://doi.org/10.1007/s00299-007-0416-6

Sharma C, Srivastava DK (2013) Efficient *Agrobacterium*-mediated genetic transformation of tomato using petiole explant. Crop Improv 40(1):44–49

Sharma A, Sharma R, Imamura M, Yamakawa M, Machii H (2000) Transgenic expression of cecropin B, an antibacterial peptide from Bombyx mori, confers enhanced resistance to bacterial leaf blight in rice. FEBS Lett 484:7–11

Sharma N, Sharma KP, Gaur RK, Gupta VK (2011) Role of chitinase in plant defense. Asian J Biochem 6:29–37

Shen J, Xie K, Xiong L (2010) Global expression profiling of rice microRNAs by one-tube stem-loop reverse transcription quantitative PCR revealed important roles of microRNAs in abiotic stress responses. Mol Gen Genomics 284:477–488. https://doi.org/10.1007/s00438-010-0581-0

Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003) Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol 6:410–417. https://doi.org/10.1016/S1369-5266(03)00092-X

Singh NK, Bracker CA, Hasegawa PM, Handa AK, Buckel S, Hermodson MA et al (1987) Characterization of osmotin: a thaumatin-like protein associated with osmotic adaptation in plant cells. Plant Physiol 85:529–536

Singh NK, Kumar KRR, Kumar D, Shukla P, Kirti PB (2013) Characterization of a pathogen induced thaumatin-like protein gene AdTLP from Arachis diogoi, a wild peanut. PLoS One 8: e83963

Solgi T, Moradyar M, Zamani MR, Motallebi M (2015) Transformation of canola by Chit33 gene towards improving resistance to *Sclerotinia sclerotiorum*. Plant Prot Sci 51(1):1–5

Song T, Zhang Q, Wang H et al (2018) OsJMJ703, a rice histone demethylase gene, plays key roles in plant development and responds to drought stress. Plant Physiol Biochem 132:183–188. https://doi.org/10.1016/j.plaphy.2018.09.007

Sreenivasulu N, Harshavardhan VT, Govind G et al (2012) Contrapuntal role of ABA: does it mediate stress tolerance or plant growth retardation under long-term drought stress? Gene 506:265–273

Sripriya R, Parameswari C, Veluthambi K (2017) Enhancement of sheath blight tolerance in transgenic rice by combined expression of tobacco osmotin (*ap*24) and rice chitinase (*chi*11) genes. In Vitro Cell Dev Biol Plant 53:12–21

Srivastava DK, Kumar P, Sharma S, Gaur A, Gambhir G (2016) Genetic engineering for insect resistance in economically important vegetable crops. In: Ahmad N, Anis M (eds) Plant tissue culture: propagation, conservation and crop. Springer, Singapore, pp 343–378

Stark-Lorenzen P, Nelke B, Hanbler G, Muhlbach HP, Thomzik JE (1997) Transfer of a grapevine stilbene synthase gene to rice (*Oryza sativa* L.). Plant Cell Rep 16:668–673

Stirpe F (2004) Ribosome-inactivating proteins. Toxicon 44:371–383

Su HY, Chou HH, Chow TJ, Lee TM, Chang JS, Huang WL et al (2017) Improvement of outdoor culture efficiency of cyanobacteria by over-expression of stress tolerance genes and its implication as bio-refinery feedstock. Bioresour Technol 244:1294–1303

Sundaresha S, Kumar AM, Rohini S, Math SA, Keshamma E, Chandrashekar SC, Udayakumar M (2010) Enhanced protection against two major fungal pathogens of groundnut, *Cercospora arachidicola* and *Aspergillus flavus* in transgenic groundnut over-expressing a tobacco β 1–3 glucanase. Eur J Plant Pathol 126(4):497–508

Taliansky M, Samarskaya V, Zavriev SK et al (2021) RNA-based Technologies for Engineering Plant Virus Resistance. Plan Theory 10:82

Tang Y, Bao X, Zhi Y et al (2019) Overexpression of a MYB family gene, OsMYB6, increases drought and salinity stress tolerance in transgenic rice. Front Plant Sci 10:168. https://doi.org/10.3389/fpls.2019.00168

Tang Y, Wang J, Bao X et al (2020) Genome-wide analysis of Jatropha curcas MADS-box gene family and functional characterization of the JcMADS40 gene in transgenic rice. BMC Genomics 21:325. https://doi.org/10.1186/s12864-020-6741-7

Tavano ECD, Erpen L, Aluisi B, Harakava R, Lopes JRS, Vieira MLC, Piedade SMD, Mendes BMJ, Filho FDAM (2019) Sweet orange genetic transformation with the *attacin* A gene under the control of phloem-specific promoters and inoculation with *Candidatus* Liberibacter asiaticus. J Hortic Sci Biotechnol 94(2):210–219

Thakur P, Kumar S, Malik JA et al (2010) Cold stress effects on reproductive development in grain crops: an overview. Environ Exp Bot 67:429–443

Thevissen K, Ferket KK, Francois IE, Cammue BP (2003) Interactions of antifungal plant defensins with fungal membrane components. Peptides 24:1705–1712

Thomas A, Lallo CHO, Badrie N (2006) Microbiological evaluation of broiler carcasses, wash and rinse water from pluck shops (cottage poultry processors) in the county Nariva/Mayaro, Trinidad and Tobago, West Indies. Tropicultura 24:135

Trudel J, Potvin C, Asselin A (1995) Secreted hen lysozyme in transgenic tobacco: recovery of bound enzyme and in vitro growth inhibition of plant pathogens. Plant Sci 106:55–62

Valente AP, de Paula VS, Almeida FC (2013) Revealing the properties of plant defensins through dynamics. Molecules 18:11311–11326

van Rensburg L, Krüger GHJ (1994) Evaluation of components of oxidative stress metabolism for use in selection of drought tolerant cultivars of Nicotiana tabacum L. J Plant Physiol 143:730–737. https://doi.org/10.1016/S0176-1617(11)81166-1

Vendruscolo ECG, Schuster I, Pileggi M et al (2007) Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. J Plant Physiol 164:1367–1376

Verslues PE, Agarwal M, Katiyar-Agarwal S et al (2006) Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. Plant J 45:523–539

Vigers AJ, Roberts WK, Selitrennikoff CP (1991) A new family of plant antifungal proteins. Mol Plant-Microbe Interact 4:315–323

Viktorova J, Klcova B, Rehorova K et al (2019) Recombinant expression of osmotin in barley improves stress resistance and food safety during adverse growing conditions. PLoS One 14. https://doi.org/10.1371/journal.pone.0212718

Vollenweider P, Günthardt-Goerg MS (2005) Diagnosis of abiotic and biotic stress factors using the visible symptoms in foliage. Environ Pollut 137:455–465

Wang Q, Li F, Zhang X, Zhang Y, Hou Y, Zhang S, Wu Z (2011a) Purification and characterization of a CkTLP protein from *Cynanchum komarovii* seeds that confers antifungal activity. PLoS One 6:e16930

Wang S, Shao B, Chang J, Rao P (2011b) Isolation and identification of a plant lysozyme from *Momordica charantia* L. Eur Food Res Technol 232:613–619

Wang S, Zhang H, Geng B et al (2018) 2-arachidonyl glycerol modulates astrocytic glutamine synthetase via p38 and ERK1/2 pathways. J Neuroinflammation 15:220

Wani SH, Kumar V, Shriram V, Sah SK (2016a) Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. Crop J 4:162–176

Wani SH, Sah SK, Hossain MA et al (2016b) Transgenic approaches for abiotic stress tolerance in crop plants. In: Advances in plant breeding strategies: agronomic, abiotic and biotic stress traits. Springer, Cham, pp 345–396

Weber RL, Wiebke-Strohm B, Bredemeier C, Margis-Pinheiro M, de Brito GG, Rechenmacher C, Bertagnolli PF, de Sa ME, Campos Mde A, de Amorim RM, Beneventi MA, Margis R, Grossi-de-Sa MF, Bodanese-Zanettini MH (2014) Expression of an osmotin-like protein from *Solanum nigrum* confers drought tolerance in transgenic soybean. BMC Plant Biol 14:343

Wei H, Movahedi A, Xu C, Sun W, Wang X, Li D, Zhuge Q (2020) Overexpression o*f PtDefensin* enhances resistance t*o Septotis populiperda* in transgenic poplar. Plant Sci 292:110379

Williamson VM, Kumar A (2006) Nematode resistance in plants: the battle underground. Trends Genet 22:396–403

Wohlkönig AH, Looze J, Y Wintjens R, and Uversky V N. (2010) Structural relationships in the lysozyme superfamily: significant evidence for glycoside hydrolase signature motifs. PLoS One 5:e15388

Wu M, Liu R, Gao Y et al (2020) PheASR2, a novel stress-responsive transcription factor from moso bamboo (Phyllostachys edulis), enhances drought tolerance in transgenic rice via increased sensitivity to abscisic acid. Plant Physiol Biochem 154:184–194. https://doi.org/10.1016/j.plaphy.2020.06.014

Xia K, Wang R, Ou X et al (2012) OsTIR1 and OsAFB2 downregulation via OsmiR393 overexpression leads to more tillers, early flowering and less tolerance to salt and drought in rice. PLoS One 7:e30039. https://doi.org/10.1371/journal.pone.0030039

Xia K, Zeng X, Jiao Z et al (2018) Formation of protein disulfide bonds catalyzed by OsPDIL1;1 is mediated by microRNA5144-3p in rice. Plant Cell Physiol 59:331–342. https://doi.org/10.1093/pcp/pcx189

Xing L, Di Z, Yang W et al (2017) Overexpression of ERF1-V from Haynaldia villosa can enhance the resistance of wheat to powdery mildew and increase the tolerance to salt and drought stresses. Front Plant Sci 8:1948. https://doi.org/10.3389/fpls.2017.01948

Xu D, Duan X, Wang B et al (1996) Expression of a late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol 110:249–257

Xu W, Tang W, Wang C et al (2020) SiMYB56 confers drought stress tolerance in transgenic rice by regulating lignin biosynthesis and ABA signaling pathway. Front Plant Sci 11:785. https://doi.org/10.3389/fpls.2020.00785

Yamaguchi T, Blumwald E (2005) Developing salt-tolerant crop plants: challenges and opportunities. Trends Plant Sci 10:615–620

Yamaguchi-Shinozaki K, Shinozaki K (2006) Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annu Rev Plant Biol 57:781–803. https://doi.org/10.1146/annurev.arplant.57.032905.105444

Yan X, Qiao H, Zhang X et al (2017) Analysis of the grape (*Vitis vinifera* L.) thaumatin-like protein (TLP) gene family and demonstration that *TLP29* contributes to disease resistance. Sci Rep 7:4269

Yang C, Li D, Mao D et al (2013) Overexpression of microRNA319 impacts leaf morphogenesis and leads to enhanced cold tolerance in rice (*O ryza sativa* L.): Rice miR319 and cold response. Plant Cell Environ 36:2207–2218. https://doi.org/10.1111/pce.12130

Yuan H, Ming X, Wang L et al (2002) Expression of a gene encoding trichosanthin in transgenic rice plants enhances resistance to fungus blast disease. Plant Cell Rep 20:992–998

Zakharchenkoa NS, Buryanova YI, Lebedevaa AA, Pigolevaa SV, Vetoshkinab DV, Loktyushovc EV, Chepurnovab MA, Kreslavskid VD, Kosobryukhov AA (2020) Physiological features of rapeseed plants expressing the gene for an antimicrobial peptide cecropin P1. Russ J Plant Physiol 60(3):411–419

Zeng Y, Wen J, Zhao W et al (2020) Rational improvement of rice yield and cold tolerance by editing the three genes OsPIN5b, GS3, and OsMYB30 with the CRISPR–Cas9 system. Front Plant Sci 10. https://doi.org/10.3389/fpls.2019.01663

Zhang X, Zou Z, Gong P et al (2011) Over-expression of microRNA169 confers enhanced drought tolerance to tomato. Biotechnol Lett 33:403–409

Zhang LW, Song JB, Shu XX et al (2013a) miR395 is involved in detoxification of cadmium in Brassica napus. J Hazard Mater 250:204–211

Zhang M, Zhuo Q, Tian Y, Piao J, Yang X (2013b) Long-term toxicity study on transgenic rice with Cry1Ac and sck genes. Food Chem Toxicol 63:76–83

Zhang Z, Li J, Li F et al (2017) OsMAPK3 phosphorylates OsbHLH002/OsICE1 and inhibits its ubiquitination to activate OsTPP1 and enhances rice chilling tolerance. Dev Cell 43:731–743.e5. https://doi.org/10.1016/j.devcel.2017.11.016

Zhang J, Zhang H, Srivastava AK et al (2018) Knockdown of rice microRNA166 confers drought resistance by causing leaf rolling and altering stem xylem development. Plant Physiol 176:2082–2094

Zhao H, Zheng J, Li QQ (2011) A novel plant in vitro assay system for pre-mRNA cleavage during 3′-end formation. Plant Physiol 157:1546–1554. https://doi.org/10.1104/pp.111.179465

Zhao Y, Wen H, Teotia S et al (2017) Suppression of microRNA159 impacts multiple agronomic traits in rice (Oryza sativa L.). BMC Plant Biol 17:215

Zhao J, Yuan S, Zhou M et al (2019) Transgenic creeping bentgrass overexpressing Osa-miR393a exhibits altered plant development and improved multiple stress tolerance. Plant Biotechnol J 17:233–251

Zhao Y, Kong H, Guo Y, Zou Z (2020) Light-harvesting chlorophyll a/b-binding protein-coding genes in jatropha and the comparison with castor, cassava and arabidopsis. PeerJ 8:e8465

Zhou M, Li D, Li Z et al (2013) Constitutive expression of a *miR319* gene alters plant development and enhances salt and drought tolerance in transgenic creeping bentgrass. Plant Physiol 161:1375–1391. https://doi.org/10.1104/pp.112.208702

Zhu J-K (2016) Abiotic stress signaling and responses in plants. Cell 167:313–324. https://doi.org/10.1016/j.cell.2016.08.029

Zhu B, Chen TH, Li PH (1996) Analysis of late-blight disease resistance and freezing tolerance in transgenic potato plants expressing sense and antisense genes for an osmotin-like protein. Planta 198:70–77

Zhu S, Gao B, Tytgat J (2005) Phylogenetic distribution, functional epitopes and evolution of the CSab superfamily. Cell Mol Life Sci 62:2257–2269

Zinn KE, Tunc-Ozdemir M, Harper JF (2010) Temperature stress and plant sexual reproduction: uncovering the weakest links. J Exp Bot 61:1959–1968

Zou X, Jiang X, Xu L et al (2017) Transgenic citrus expressing synthesized *cecropin* B genes in the phloem exhibits decreased susceptibility to Huanglongbing. Plant Mol Biol 93:341–353