Review

Plant molecular stress responses face climate change

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Environmental stress factors such as drought, elevated temperature, salinity and rising CO_2 affect plant growth and pose a growing threat to sustainable agriculture. This has become a hot issue due to concerns about the effects of climate change on plant resources, biodiversity and global food security. Plant adaptation to stress involves key changes in the '-omic' architecture. Here, we present an overview of the physiological and molecular programs in stress adaptation focusing on how genes, proteins and metabolites change after individual and multiple environmental stresses. We address the role which '-omics' research, coupled to systems biology approaches, can play in future research on plants seemingly unable to adapt as well as those which can tolerate climatic change.

Plant reprogramming to survive in a changing climate

To survive, sessile plants must cope with climate change catastrophes or so-called environmental stress factors such as drought, elevated temperatures, elevated [CO_2] and salinity – both individually, or more commonly, in combination. Climate change catastrophes impact on all aspects of plant architecture and represent a serious challenge for developing sustainable agriculture at a time of significant growth in the global population [1–12]. To cope with climate change catastrophes, plants have evolved a wide spectrum of molecular programs to sense change rapidly and adapt accordingly [4–6,10,12–29]. Understanding these reprogramming events under constantly changing environmental conditions has been a subject of great interest for many decades. Nevertheless, there is still a significant knowledge gap and we are generally unable to predict how well plants will cope with these challenges. Specifically, such insight is required to breed crops or produce transgenic varieties with enhanced tolerance to multiple environmental stress factors, because in nature, plants are often simultaneously exposed to multiple environmental perturbations.

Here, we discuss some of the most recent physiological and molecular programs identified in plants which are of relevance to global climate change factors. We focus on the four major abiotic stresses, drought, elevated temperature, salt and elevated [CO_2] (Box 1), both individually and as multiple stresses. Looking to the future, we present the potential value of systems biology approaches to investigate biological networks in order to understand and improve plant responses to environmental stress (for a list of all gene and protein abbreviations used throughout this paper see Table 1).

Physiological and molecular programs: adaptive strategies

Plant adaptive strategies to stress are coordinated and fine-tuned by adjusting growth, development, cellular and molecular activities. Significant progress has been made in understanding the physiological, cellular and molecular mechanisms of plant responses to environmental stress factors. Responses to perturbations are usually accompanied by major changes in the plant transcriptome, proteome and metabolome [14,16,19,20,30–39]. Recent research has made efficient use of these 'omic' approaches to identify transcriptional, proteomic and metabolic networks linked to stress perception and response – not only in the model plant *Arabidopsis* (*Arabidopsis thaliana*) but also in crop, garden and woody species [16,18,20,30–34,40–45]. The wide range of genes, proteins and enzymes that impart resistance or are regulated in response to environmental stress factors have been summarised in Table 1 and Table S1 (see online supplementary material) with their descriptions and known or putative mechanisms of function. In addition, metabolites reported to increase or decrease during plant adaptations to these environmental stress factors have been summarised in Table 2.

Drought

Drought or continuous water deficit is one of the most important factors affecting plant growth, development, survival and crop productivity [1,6,8,29,32,38,39,46–49]. Physiological responses to drought include stomatal closure, decreased photosynthetic activity, altered cell wall elasticity, and even generation of toxic metabolites causing plant death. Concomitant molecular re-programming includes extensive changes in gene expression incurring alterations in the biochemical and proteomic machinery [1,6,10,13,32,33,38,46,47,49–51]. Here we discuss key molecular programs proposed to confer tolerance to drought stress along with their envisaged modes of action (Table 1, Table S1). Specific focus is given to abscisic acid (ABA)-dependent, ABA-independent (Figure 1a), *DREB2A* and ubiquitination-related mechanisms (Figure 1b).

ABA is a key signalling intermediate that controls the expression of many genes. It decreases water loss by

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Box 1. Elevated carbon dioxide e[CO_2] – the greenhouse gas story

e[CO_2] is the most publically recognised aspect of climate change. Continued human activity, deforestation, anthropogenic fossil fuel burning and industrialisation are the cause of e[CO_2]. [CO_2] (currently 390.09 ppm; July, 2010; http://co2now.org/), due to constant CO_2 emissions at year values, would lead to concentrations of about 520 ppm by 2100 [12], inevitably resulting in significant changes to vegetation and agricultural plant productivity [5].

Plants sense and respond to e[CO_2] through increased photosynthesis and decreased stomatal conductance in a broad range of species under different conditions [100]. e[CO_2] affects flowering time and is possibly as influential as temperature in determining future changes in plant developmental timing. e[CO_2] was shown to affect the expression of floral-initiation genes in *Arabidopsis* [18]. Moreover, delayed flowering under e[CO_2] was associated with sustained expression of FLC, in an e[CO_2]-adapted genotype.

In response to e[CO_2], *Arabidopsis* showed downregulation of transcripts related to photosynthesis, the Calvin cycle, photorespiration, photosystem (PS) I and II subunits, light harvesting and electron transport [19]. In contrast, upregulated transcripts included genes linked to carbon metabolism and utilisation, cellulose synthesis enzymes, cell wall proteins, glycolysis, trehalose metabolism, callose biosynthesis, and fructokinase involved in starch/sucrose degradation [19]. In contrast, in aspen (*Populus tremuloides*) long-term exposure to e[CO_2] caused upregulation of photosynthesis genes encoding chloroplast 30S ribosomal protein, PS II and PS q(b) proteins, and auxin-binding proteins, while aquaporin plasma membrane intrinsic protein PIPa2 showed downregulation [44]. In another transcriptomic study in aspen, it was suggested that a CO_2-responsive genotype partitions carbon into pathways associated with active defence and/or response to stress, carbohydrate and/or starch biosynthesis and subsequent growth, while a CO_2-unresponsive genotype partitions carbon into pathways associated with passive defence (e.g. lignin and phenylpropanoids) and cell wall thickening [31]. However, a proteomic response of rice to e[CO_2] showed differential expression of proteins belonging to photosynthesis, carbon metabolism, and energy pathways [20]. Several molecular chaperones and ascorbate peroxidase also responded to e[CO_2]. Furthermore, the combination of e[CO_2] and iron (Fe) limitation induced morphological, physiological, and molecular responses, enhancing the plant's capacity to access and utilise Fe from Fe(III)-oxide [5].

All these transcriptomic, proteomic and metabolomic studies show regulation of novel genes, proteins and metabolites emphasising changes in photosynthesis, carbon metabolism, growth, amino acids, sugars, starch and other metabolic processes. The current molecular data of plant adaptations to e[CO_2] still seem rudimentary and future studies should be oriented more towards finding genes that can impart resistance to e[CO_2] with specific focus on crop plants.

regulating stomatal aperture [1,6,47,52], and has a broad range of essential functions in plant adaptation to several stress factors, including drought resistance [1,6,13,28,47,50,52–55] (Figure 1a). Several abiotic stress-inducible genes are controlled by ABA, but others are not, indicating involvement of both ABA-dependent and ABA-independent regulatory systems [55]. Moreover, major *cis*-acting elements, such as the ABA-responsive element (ABRE) and the dehydration responsive element/C-repeat (DRE/CRT) have been shown to be important to ABA-dependent and ABA-independent gene expression in abiotic stress responses [55].

NFYA5 was strongly induced in an ABA-dependent manner and its induction occurred both at transcriptional and post-transcriptional levels [47]. Analysis of *nfya5* knockout or *NFYA5* overexpression lines showed *NFYA5* to be important in controlling stomatal aperture and drought resistance. *OCP3* also plays a pivotal role in the signal pathway controlling drought tolerance through modulation of ABA-mediated stomatal closure [6]. *MYB96* is proposed to function as a molecular link by integrating ABA and auxin signals [1]. Suppression of *FTA* in canola using the *AtHPR1* promoter to drive an RNAi construct, resulted in yield protection under drought stress in the field [53]. *SAL1* acts as a negative regulator of ABA-independent and ABA-dependent stress response pathways such that its inactivation results in altered osmoprotectants, higher relative leaf water content and maintenance of viable tissues during prolonged drought [50]. Upregulation of ABA-responsive genes in *msi1-cs* (*MSI1* co-suppression) lines suggests that *MSI1* plays a role in the negative regulation of drought-stress response [54] through the binding of *MSI1* to the chromatin of the drought-inducible downstream target *RD20*. *PLDα1*-mediated ABA effects, through interaction with PP2C and G protein, show a bifurcated signalling pathway [52]. However, high *PLDα1* affected the water deficit response by promoting early stomatal closure, but disrupted membranes after prolonged drought stress [28]. Moreover, a feedback mechanism was found to link the circadian clock with plant responses to drought [13]. *TOC1* is induced by ABA, and this induction is gated by the clock and determines the timing of *TOC1* binding to the *ABAR* promoter. Molecular-genetic studies showed the existence of a negative feedback loop in which *TOC1* negatively regulates the expression of *ABAR*, whose activity is in turn necessary for *TOC1* activation by ABA [13].

The studies above emphasise the regulational complexity of transcriptional and post-transcriptional cell programs in stomatal guard cell responses involving different action modes add new information on the role of ABA in mediating drought responses. The induction of *NFYA5* by ABA and drought at both transcriptional and post-transcriptional levels shows its critical importance in imparting drought resistance [47]. The functioning of *MYB96* as a molecular link mediating ABA–auxin crosstalk in both drought stress response and lateral root growth represents an adaptive strategy under drought stress conditions [1]. In this regard, the conditional and specific downregulation of *FTA* in canola shows significant progress towards engineering drought tolerance in this important crop [53]. To enhance our knowledge about the effect of chromatin modifications on plant stress responses, the authors aim to characterise *MSI1* function in regulating drought stress responses [54]. In addition, new information on *PLDα1* pathway-mediated drought responses may be exploited to produce plants with increased water-use efficiency and drought tolerance [28,52].

Ubiquitination plays a role in a variety of biological processes, but our understanding of its exact role in abiotic stress is still limited [9]. The transcription factors *DREB1A/CBF3* and *DREB2A* specifically interact with *cis*-acting DRE/CRT involved in cold and drought stress-responsive gene expression in *Arabidopsis* [10]. Recent insights suggest *DREB2A* and ubiquitination (post-translational attachment of ubiquitin) are related to the modulation of drought response through *DREB2A*-regulated gene expression, drought tolerance of *Arabidopsis* plants

Table 1. Reference table abbreviations of genes/proteins associated with abiotic stresses detailed in this article

Gene	Name	Refs	Gene	Name	Refs
ABAR	ABA-related	[13,91]	*ITN1*	Increased tolerance to NaCl1	[27]
ABI1	ABA Insensitive	[52,91]	LEA	Late embryogenesis abundant	[30,49,77]
AP37, 59	APETELA 37, 59	[46]	*LEW1*	Leaf Wilting 1	[22]
At1g09350	Galactinol synthase (*GolS3*)	[51]	*LOS2*	Enolase 2/Low expression of osmotically responsive genes 1	[35,91]
At1g22985	AP2 - DNA binding protein	[10]	*LTP3, 4*	Lipid transfer protein 3, 4	[54,91]
At3g50970	Dehydrin xero2	[51]	miR169	MicroRNA 169A	[47]
At1g52690	LEA protein	[10]	*MSI1*	Chromatin modifying protein	[54,91]
At1g56600	Galactinol synthase, (*GolS2*)	[51]	*MT2A*	Metallothionein 2A	[10]
At1g69870	Proton-dependent oligopeptide transport	[10,91]	*MYB96*	MYB transcription factor 96	[1]
At1g80160	Lactoylglutathione lyase/glyoxalase 1	[54]	*NCED3*	Nine-*cis*-epoxycarotenoid dioxygenase 3	[30,91]
At3g53990	Universal stress protein	[10]	*NFYA5*	Nuclear factor Y A5	[47,91]
At3g55940	Phosphoinositide-specific phospholipase C	[51]	*OCP3*	Overexpressor of cationic peroxidase 3	[6]
At4g36010	Thaumatin family protein	[51]	*OsABF1*	*O. sativa* ABA responsive element binding factor 1	[76]
ATHB-7	*A. thaliana* homeobox 7	[30,91]	*OsDREB2A*	*O. sativa* DREB protein 2A	[66]
BhHsf1	*Boea hygrometrica* heat shock factor	[62]	OsRMC	*O. sativa* root meander curling	[66]
BiP	Binding protein	[22]	PAO	Polyamine oxidase	[69]
BOB1	BOBBER1	[59]	*PF3-D, 5*	Prefoldins 3 and 5	[23]
bZIP60	Basic domain/leucine zipper60	[22]	PIP2	Phosphatidylinositol 4,5-bisphosphate	[25]
CBK3	Calmodulin-binding protein kinase 3	[61]	PIPK	Phosphatidylinositolphosphate kinase	[25]
CcHyPRP	*Cajanus cajan* hybrid-proline-rich protein	[75]	PLD	Phospholipase D	[25]
COR	Cold Regulated	[77]	*PLDα1*	Phospholipase Dα1	[28]
COR47	Cold Regulated 47	[22,91]	*POX22.3, 8.1*	Peroxidases 22.3 and 8.1	[29]
DDF1	Dwarf and delayed flowering 1	[65]	*PP2C*	Protein phosphatase 2C	[30,91]
DELLA	Gibberellic acid signal mediators	[65]	*PUB22, 23*	Plant U-Boxes 22 and 23	[2,91]
DHN	Dehydrin genes	[49]	*Rab16A*	Rice ABA responsive gene 16A	[66]
DREB2A	Dehydration-responsive element binding protein 2	[10,91]	*RBOHC* & *RBOHD*	Respiratory burst oxidase homologs C and D	[27,91]
DRIP1, 2	DREB2A-interacting proteins 1, 2	[56,91]	RCA	Rubisco activase	[24]
DSM1	Mitogen-activated protein kinase kinase kinase	[29]	*RCA1*	Short isoform of RCA	[24]
ERF	Ethylene response factor	[30,91]	*RD20*	Responsive to desiccation 20	[54,91]
FLC	Flowering locus C	[18]	*RD22*	Responsive to desiccation 22	[27]
FTA	α-farnesyltransferase	[53]	*RD29A, B*	Responsive to desiccation 29A	[22,30,91]
FtsH11	Filamentous temperature-sensitive	[4]	*Rma1H1*	RING membrane-anchor 1 E3 ubiquitin ligase	[9,91]
GA2ox7	Gibberellic Acid 2-oxidase 7	[65]	*ROF1*	Rotmase FK506 Binding Protein 1	[21,91]
GH3	auxin conjugating enzyme	[1]	RPN12a	Regulatory particle non-ATPase 12a	[2,91]
GmWRKY	*G. max* WRKY transcription factor	[45]	*SAL1*	3′(2′), 5′-biphosphate nucleocidase	[50,91]
G protein	GTP-binding protein	[25,52]	Ser 228	Autophosphorylation site of SOS2	[67]
GRP7	Glycine-rich protein 7	[72,91]	SODs	Superoxide dismutases	[57]
GSNOR	S-nitrosoglutathione reductase	[63]	SOR	Superoxide reductase	[64]
HKT1;1	High affinity potassium transporter 1	[71]	*SOS2*	Salt Overly Sensitive 2	[67]
HOS3	Hyper-osmotically sensitive gene	[73]	*TaSnRK2.4*	*T. aestivum* serine/threonine protein kinase	[74]
HOT5	Sensitive to hot temperatures	[63]	*TdDHN*	*T. durum* dehydrins	[49]
HPR1	Hydroxypyruvate reductase	[53]	*TOC1*	Timing of CAB expression 1	[13]
HSFA1a	Heat Shock Factor 1A	[61,91]	*TsVP*	*Thellungiella halophila* V-H^+-PPase	[8]
HsfA3	Heat Shock Transcription Factor A3	[26,91]	V-H^+-PPase	Vacuolar H^+-pyrophosphatase	[8]
HvCBF4	*Hordeum vulgare* C-repeat binding factor 4	[7]	V-ATPase	Vacuolar H^+-ATPase	[35]
InsP5-ptase	Inositol polyphosphate 5-phosphatase	[51]	*WLIP19*	Wheat low-temperature induced protein 19	[77]
ISPS	Isoprene synthase	[58]			

expressing InsP5-ptase and the roles of *DRIP1* and *DRIP2*, *Rma1H1*, *PUB22* and *PUB23* (Figure 1b) [2,9,10,51,56]. The overexpression of constitutively-active DREB2A resulted in significant drought stress tolerance by regulating drought-responsive gene expression [10]. Two novel proteins, *DRIP1* and *DRIP2*, were suggested to act as novel regulators in drought-responsive gene expression by targeting DREB2A protein to 26 s proteosome proteolysis [56]. *DREB2A* and a subset of *DREB2A*-regulated genes and drought tolerance of the InsP5-ptase plants to be mediated partly via a *DREB2A*-dependent pathway [51]. *PUB22* and *PUB23* were shown to function as negative regulators. *PUB22*- and *PUB23*-overexpressers were hypersensitive to drought stress while loss-of-function *pub22* and *pub23* mutants showed enhanced drought-tolerance [2]. The same was found for *Rma1H1* overexpression in transgenic *Arabidopsis* [9]. *Rma1H1* was proposed to play a critical role in the downregulation of plasma membrane aquaporin levels by inhibiting aquaporin trafficking to the plasma membrane and subsequent degradation as a response to dehydration in transgenic *Arabidopsis* plants.

These findings highlight the importance of the transcription factor *DREB2A* and ubiquitination in drought stress responses. DREB2A protein stability and its activation, regulate drought stress-responsive gene expression and leads to drought stress tolerance [10]. The InsP 5-ptase results indicate that coordinated regulation of *DREB2A* and a subset of *DREB2A*-regulated genes can help confer drought tolerance without adversely affecting plant growth [51]. The selective regulation of this gene subset may prove a promising target for enhancing drought tolerance in crop plants. Other findings [2,9,56] have shown the

Table 2. Plants metabolic adaptations to Climate Change Catastrophes/Environmental Stress Factors

Plant	Plant parts	Metabolites	Levels	Refs
Drought				
Arabidopsis (*Arabidopsis thaliana*) wt and mutants (*alx8*, *fry1-1*, *nc3-2*, *msi-cs*)	Leaves, aerial parts	ABA, amino acids, carbohydrate derivatives, organic acids, polyamine putrescine, sugar metabolites, carbon metabolites (starch, hexoses, sucrose, fumurate, malate, proline and total amino acids)	Accumulated/ increased	[38,50, 54,92]
Wheat (*Triticum aestivum* and *Aegilops*)	Seedlings, leaves, mature grains	ABA, fumaric acid, proline	Accumulated/ increased/higher	[11,49]
Maize (*Zea mays*)	Xylem sap	ABA, phaseic acid, p-coumaric acid, caffeic acid, 6-benzylaminopurine	Increased	[33]
		trans-zeatin, trans-zeatin riboside, ferulic acid	Decreased	[33]
Cotton (*Gossypium hirsutum*)	Leaves	Proline, free amino acids, total and reducing sugars, polyphenol contents	Increased	[93]
		Chlorophylls, carotenoids, protein, starch	Decreased	[93]
Pea (*Pisum sativum*)	Leaf	Proline, valine, threonine, homoserine, myoinositol, aminobutyrate, trigonelline (nicotinic acid betaine)	Higher	[39]
Transgenic P_{SARK}-IPT (*Nicotiana tabacum*)	Leaf	Glycerate	Increased	[94]
Alfalfa (*Medicago sativa*)	Nodules	Succinate, sucrose, chlorophylls, carotenoids, oxidised lipids, ABA	Accumulated	[57]
Brassica (*Brassica napus*)	Leaf	ABA	Highest	[95]
Black poplar (*Populus nigra*)	Saplings	Isoprene	Decreased	[58]
C_4 grasses (*Cynodon dactylon* and *Zoysia japonica*)	Leaves	5-hydroxyvaline	Increased	[48]
Elevated temperature				
Lettuce (*Lactuca sativa*)	Seedlings	Chicoric acid, chlorogenic acid	Increased	[43]
		Quercetin-3-O-glucoside, luteolin-7-O-glucoside	Accumulated	[43]
St. John's wort (*Hypericum perforatum*)	Shoots, flowers, flower buds	Secondary metabolites (hyperforin, pseudohypericin and hypericin)	Increased	[34]
Arabidopsis and rice	Seedlings	Phosphatidylinositol 4,5-bisphosphate, phosphatidic acid	Accumulated	[25]
Salinity				
Maize (*Z. mays*)	Leaf blade	Polyamines (apoplastic spermine and spermidine)	Increased	[69]
Brassica oleracea	Leaves, roots	Fatty acids (linoleic, linolenic and stigmasterol), aquaporins of PIP1 and PIP2 subfamilies, glucosinolates	Increased	[68]
		Fatty acids (palmitoleic, oleic and sitosterol)	Decreased	[68]
Elevated [CO_2]				
Arabidopsis	Leaves	Starch, glucose, galactose, maltose, malic acid, histidine, tryptophan, phenylanine	Increased	[19]
		other amino acids	Decreased	[19]
B. napus	Leaves	Chlorophyll a, chlorophyll b	Higher	[95]
		ABA, indolic glucosinolate	Decreased	[95,96]
Cassava (*Manihot esculenta*)	Leaves	Cyanogenic glycosides	Increased	[97]
Maritime (*Plantago maritima*)	Foliage, roots	Caffeic acid, p-coumaric acid, verbascoside	Increased	[37]
Sugarcane (*Saccharum* ssp.)	Leaves	Sucrose	Increased	[98]
Soybean (*Glycine max*)	Leaves	Hexose, sucrose, starch, ureides, amino acids	Increased	[36]
Multiple environmental stresses				
Arabidopsis	Leaf	Cuticular lipids	Increased	[99]
B. napus	Leaf	Chlorophylls a and b, carotenoids, ABA	Increased	[95]
Arabidopsis (*lew1*)	Seedlings	Dolichol	Reduced	[22]

importance of plant E3 ubiquitin ligases in mediating cellular responses to drought stress. *DRIP1* and *DRIP2* interact with DREB2A in the nucleus and function as E3 ubiquitin ligases and mediate DREB2A ubiquitination [56]. Furthermore, the authors of [2] suggest that *PUB22* and *PUB23* (U-box-containing E3 ubiquitin ligases) coordinately control a drought-signalling pathway by ubiquitinating cytosolic RPN12a, while the authors of [9] showed that drought stress-induced *Rma1H1*, a RING membrane-anchor E3 ubiquitin ligase homolog, regulates aquaporin levels via ubiquitination. Both results were obtained in transgenic *Arabidopsis* plants.

Apart from ABA, *DREB2A* and ubiquitination-related adaptations, other systems imparting drought-resistance diversely involve vacuolar membrane transport, unfolded protein response (UPR) pathway genes and reactive oxygen species (ROS) signalling [8,22,29]. In maize (*Zea mays*), the heterologous expression of *TsVP* resulted in enhanced V-H^+-PPase activity [8]. *DSM1* is a novel nuclear protein kinase shown to play a critical role in drought and oxidative stress resistance in rice (*Oryza sativa*) by directly or indirectly regulating expression of *POX22.3* and *POX8.1* and ROS scavenging [29].

DHN genes (*TdDHN15.2*, *TdDHN15.1*, *TdDHN13*, *TdDHN15.3* and *TdDHN9.6*) were induced in drought-stressed wheat (*Triticum durum*). In alfalfa (*Medicago sativa*), genes encoding anti-oxidant enzymes, such as SODs (CuZn-SOD, plastid FeSOD and MnSOD) were upregulated [57], whereas isoprene synthase (ISPS) mRNA transcript level and protein concentration decreased during drought stress in black poplar (*Populus nigra*) plants [58]. Thirty-six protein spots identified by 2D-PAGE and

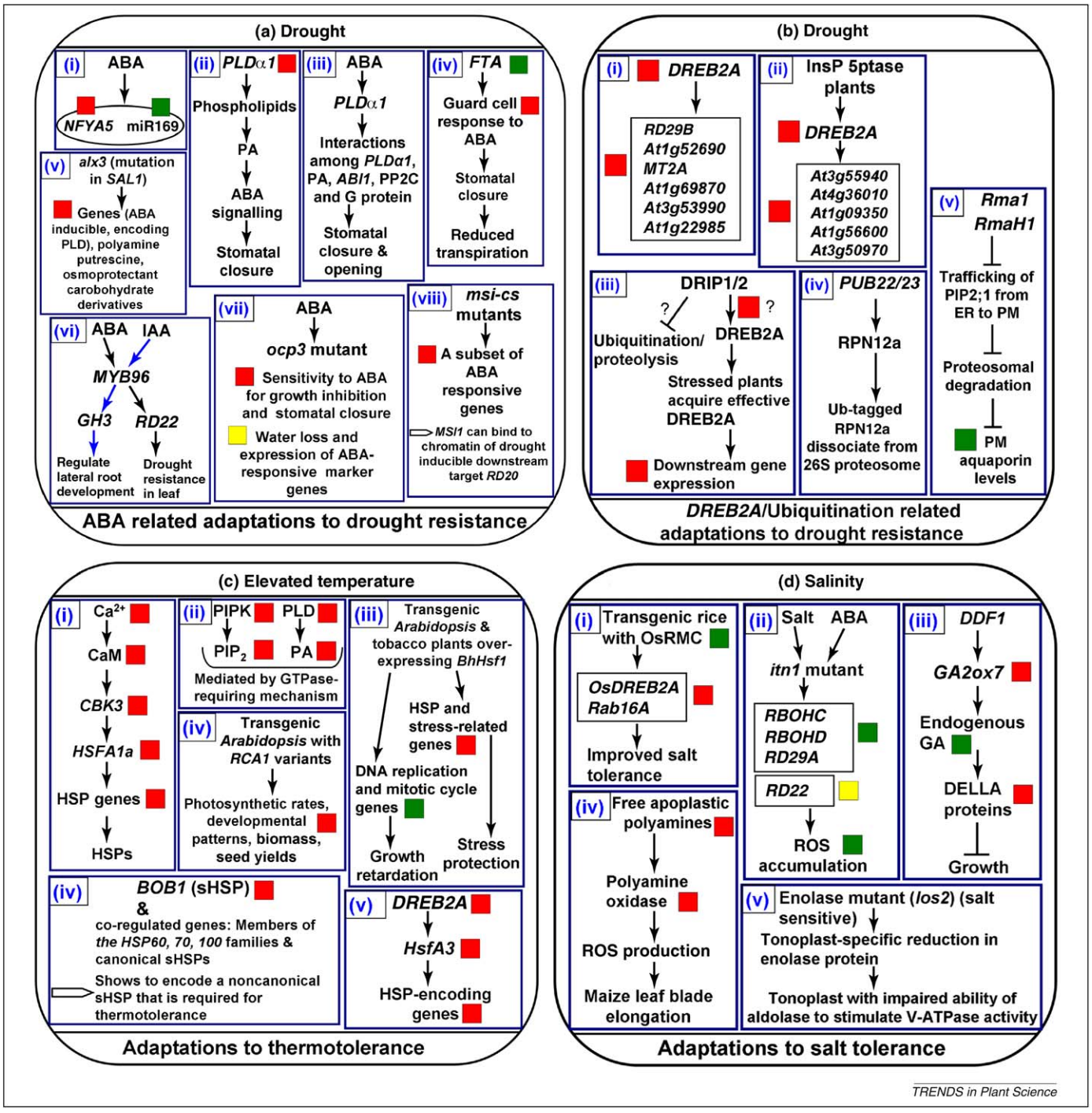

Figure 1. Diagrammatic representation of plant adaptations to drought, elevated temperature and salinity stress factors. **(a)** ABA-related adaptations to drought resistance. **(i)** *NFYA5* is induced and miR169 is suppressed in an ABA-dependent manner. **(ii)** Activated *PLDα1* mediates ABA signalling by a rapid stomatal closure. **(iii)** *PLDα1* mediates ABA effects by interacting with PP2C and G protein. **(iv)** *FTA* downregulation enhances guard cell response to ABA, and leads to stomatal closure and reduced transpiration. **(v)** Drought tolerant mutant (*alx8*) has mutation in *SAL1*, such that its inactivation maintains viable tissues during prolonged water stress. **(vi)** *MYB96* regulates drought resistance by mediating ABA/auxin-signalling through *RD22* and *GH3*, respectively. **(vii)** *ocp3* mutant is associated with increased ABA sensitivity in growth and stomatal closure, however no effect on water loss and ABA-responsive marker genes. **(viii)** Upregulation of ABA-responsive genes in *msi-cs* mutant and binding of *MSI1* to chromatin suggests role of *MSI1* in negative regulation of response to drought stress. **(b)** *DREB2A* and ubiquitination-related adaptations to drought resistance. **(i)** Overexpression of *DREB2A* regulates drought-responsive gene expression. **(ii)** Plants expressing InsP 5-ptase revealed *DREB2A* and *DREB2A*-regulated genes to be upregulated. **(iii)** DRIP1 and DRIP 2 proteins possibly function in stress signalling by blocking ubiquitination or proteolysis and plants under stress acquire sufficient DREB2A. Under normal growth conditions, DREB2A protein is expressed at low levels and to prevent activation, the translated DREB2A protein is recognised and ubiquitinated by the constitutively expressed DRIP1 and DRIP2 proteins and subjected to 26S proteosome proteolysis **(iv)** *PUB22* and *PUB23* co-ordinately control a drought signalling pathway by ubiquitinating cytosolic RPN12a. **(v)** *Rma1H1* and *Rma1* play a critical role in the downregulation of plasma membrane aquaporin levels. **(c)** Plant adaptations towards thermotolerance. **(i)** Increase in calcium ion (Ca^{2+}) levels activates calmodulin (CaM), which regulates activity of *CBK3* and that in turn promotes *HSFA1a* activity, HSP genes and HSPs. **(ii)** A sudden temperature increase activates PIPK and PLD, which leads to accumulation of PIP_2 and PA. **(iii)** *BhHsf1* may play dual roles in mediating heat stress tolerance and growth retardation. **(v)** *BOB1*, a noncanonical small HSP that is required for thermotolerance **(vi)** *HsfA3* is induced by *DREB2A*, and regulates the expression of HSP-encoding genes. **(d)** Plant adaptations to tolerate salt. **(i)** Knockdown of OsRMC results in upregulation of *OsDREB2A* and *Rab16A*. **(ii)** The ABA-induced suppression of *RBOHC*, *RBOHD* and *RD29A* genes, while non-regulation of *RD22* shows that *itn1* mutation partially impairs ABA signalling pathways. **(iii)** *GA2ox7* is induced by *DDF1*, which reduces endogenous GA, causes accumulation of DELLA proteins and represses growth for stress adaptation. **(iv)** Under salinity, oxidation of free apoplast polyamine levels is possibly the main source of ROS in the elongation zone of maize leaf blades. **(v)** The decreased abundance of enolase at the tonoplast results in a reduction in the ability of aldolase to stimulate vacuolar H^+-ATPase (V-ATPase) activity, and reveals a role of glycolytic enzymes in salt tolerance. Red

matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry, discriminating control and water-stressed wheat samples, were shown to be involved in glycolysis and gluconeogenesis [32]. These proteins have been proposed as putative biomarkers to define physiological effects at the molecular level and as targets for improving drought resistance in wheat. Furthermore, 2D-electrophoresis and Liquid chromatography–Mass Spectrometry/Mass Spectrometry (LC–MS/MS) analysis identified the differential occurrence of a highly diverse range of proteins between well-watered and water-stressed maize plants [33].

Elevated temperature

Plants regularly face elevated temperature throughout their multi-seasonal life cycle [17,59]. Elevated global temperature in the future will impact ecology and agriculture and may prove to be a major factor limiting crop production [24,59,60]. A plant's ability to tolerate elevated temperatures, without prior conditioning, is referred to as basal thermotolerance, whereas a plant's adaptive capacity to survive lethal high temperatures after pre-exposure to sub-lethal temperatures is known as acquired thermotolerance [4,60]. How plants cope with elevated temperatures is ultimately determined by both basal and acquired thermotolerance [4]. Elevated temperatures can induce a dramatic re-setting of physiological and molecular mechanisms in order to facilitate continued homeostasis and survival [4,25,59]. Of these different mechanisms, one involves transcriptional regulators, i.e. heat shock factors (HSFs) activating expression of heat shock proteins (HSPs) [26,61,62]. HSPs are of particular importance in thermotolerance reactions and act as molecular chaperones to prevent denaturation or aggregation of target proteins as well as facilitating protein refolding [17,59,61].

Recent findings on plant adaptation to thermotolerance point not only to HSP-based mechanisms [17,21,42,59–61], but also to other components such as phospholipids, *Pyrococcus furiosus* superoxide SOR, *DREB2A*, GSNOR and RCA [4,24–26,63,64] (Tables 1, 2 and S1; Figure 1c). *CBK3* is an important component of the Ca^{2+}-regulated heat-stress signal transduction pathway, downstream of calmodulin (CaM), that regulates expression of HSPs [61]. *HsfA1a* is believed to be an *in vivo* target of *CBK3* and phosphorylation of *HsfA1a* by *CBK3* influences HSPs and thermotolerance in *Arabidopsis* seedlings. *ROF1* was shown to bind HSP90.1 and localise in the cytoplasm under normal conditions. Exposure to heat stress induces nuclear localisation of the ROF1–HSP90.1 complex in the presence of the transcription factor *HsfA2* that interacts with HSP90.1 but not with *ROF1* [21]. Moreover, in a study by Schramm, *HsfA3* was demonstrated to be important for thermotolerance and transcriptionally controlled by *DREB2A* [26]. This, in turn, regulates the expression of HSP-encoding genes. Overexpression of *BhHsf1* induced growth retardation and thermotolerance in both *Arabidopsis* and tobacco (*Nicotiana tabacum*) [62]. *BOBBER 1* (*BOB1*) is cytoplasmic at basal temperatures, forms heat-shock granules containing canonical small HSPs at high temperatures. This has been proposed to be necessary for thermotolerance [59]. sHSP transcripts were highly upregulated in response to high temperatures in rice [17]. In both skin and phelloderm of potato (*Solanum tuberosum*), exposure to 37 °C resulted in differentially regulated stress-related genes. Most of the genes upregulated in the phelloderm encoded HSPs [42]. In wheat, a total of 6560 probe sets displayed a twofold or higher change in expression following a heat treatment of 34 and/or 40 °C [60].

Overall, these studies have advanced our molecular understanding of complex HS responses mediated through HS signal transduction pathways, HS factors (*HsfA1a*, *HsfA2*, *HsfA3* and *BhHsf1*) and HSPs in a wide range of species. In addition, recent results implicate the involvement of Ca^{2+} or CaM in the HS signal transduction pathway [61]. However, Meiri and Breiman showed *ROF1* to play a role in the prolongation of thermotolerance by sustaining the levels of sHSPs necessary for survival at high temperatures [21]. *BOB1*, was demonstrated to be a sHSP with a developmental role at basal temperatures and a thermotolerance role at elevated temperatures [59]. Consequently, in future, studies with *BOB1* should be aimed at identifying the targets of its chaperone activity and understanding how this unique sHSP regulates growth and development in plants.

FtsH11 of *Arabidopsis* contributed to overall tolerance to high temperatures [4]. Following heat stress, *Arabidopsis* seedlings and also rice leaves showed dramatic increases in PIP2 and phosphatidic acid (PA), mediated by PIPK and PLD [25]. Within minutes of a sudden rise in temperature, plants deploy phospholipids to specific intracellular locations: PLD and a PIPK are activated, and PA and PIP2 rapidly accumulate. For this transduction of heat-initiated signal, required for PIP2 and PA accumulation, active cycling of a G protein appears necessary. This adaptation response is somewhat similar to that observed during drought stress where *PLDα1* mediates ABA stomatal effects through interactions with PP2C and G protein [52]. RCA was identified as a major limiting factor in plant photosynthesis under moderately elevated temperatures and is thus a potential target for genetic manipulation to improve crop plant productivity under heat stress [24]. *HOT5* which encodes GSNOR, is required for thermotolerance and uncovers a role of nitric oxide (NO) in thermotolerance and plant development [63]. GSNOR function is necessary for acclimation to high temperature and for normal plant growth. GSNOR regulates nitrosation levels by metabolising S-nitroglutathione (GSNO), which is a mobile reservoir of NO in plant cells. This finding emphasises the need to understand the mechanism that regulates GSNOR activity,

square symbolises activation/upregulation/induction/accumulation/elevated, green square symbolises knockdown/downregulation/suppression/reduced, and yellow square symbolises unaffected/not-impaired. This figure is based on the findings and models presented/proposed in [1,2,6,9,10,24–28,35,47,50–54,56,59,61,62,65,66,69]. For abbreviations, please refer to Table S1 in online supplementary material. We acknowledge the copyright permission given by the publisher of The Plant Journal (John Wiley & Sons Ltd.) to reproduce Figure 9 [61], Figure 8 [65], and Figure 6 [26].

considered as a critical aspect in analysing the overall regulation of NO-related signalling and nitrosative stress in plants [63].

Salinity

High salinity is a critical environmental factor that inimically affects large areas of cultivated land. Plant growth, physiological and metabolic processes are affected, resulting in significant reductions in global crop productivity [27,65,66]. During salt stress, Na^+ enters the cells and its over-accumulation induces ionic and osmotic stress in plants [66]. Salt accumulation can modify plant cell plasma membrane lipid and protein composition, cause ion imbalance and hyperosmotic stress and eventually disturb normal growth and development [23,67,68]. Plant molecular adaptations to salt stress involve, e.g. components of the salt overly sensitive (SOS) pathway; salt and ABA induced accumulation of ROS; salt-inducible transcription factors; cytoskeleton, peroxisomes, apoplastic proteins and glycolytic enzymes [23,27,35,66–71]. Some significant advances are found in Tables 1 and S1, and Figure 1d.

The SOS pathway regulates Na^+/K^+ ion homeostasis when plants are cultivated at high salt conditions and operates to maintain low cytoplasmic concentrations of sodium by sequestering Na^+ in vacuoles. Autophosphorylation of Ser 228 of *SOS2* is considered to be important for *SOS2* functioning under salt stress [67]. Null mutations in the *Arabidopsis* genes *PFD3* or *PFD5*, encoding PFD subunits, resulted in decreased overall levels of α- and β-tubulin, and eventually, alterations in microtubule structure [23]. The *pfd3* and *pfd5* mutants showed high sensitivity to high NaCl concentrations. Transient overexpression of *DDF1* activated the promoter of *GA2ox7* resulting in repressed growth and stress adaptation [65]. Furthermore, under *in vivo* salinity stress, peroxisomes were shown to be necessary for NO accumulation in the cytosol [70]. NO accumulation participates in the generation of peroxynitrite ($ONOO^-$) and in enhancing protein tyrosine nitration, is a marker of nitrosative stress. *ITN1* modulates salt tolerance by affecting ABA-mediated production of ROS [27].

High salinity stress markedly modified the lipid composition of the plasma membrane in broccoli (*Brassica oleracea*) [68]. It was suggested that this modification could influence membrane stability or the activity of plasma membrane proteins such as aquaporins or H^+-ATPase, to provide a mechanism controlling water permeability and acclimation to salinity stress [68]. Moreover, under salinity, PAO activity was shown to provide ROS production in the apoplast, sustaining maize leaf elongation [69]. An apoplastic protein, OsRMC showed drastic abundance in response to salt stress, highlighting an important role for apoplastic proteins in salt tolerance [66]. Cell type-specific expression of *HKT1;1* in the mature root stele of *Arabidopsis* showed an efficient means of decreasing shoot Na^+ accumulation and increasing salinity tolerance [71]. Quantitative proteomics of *Mesembryanthemum crystallinum* plants showed membrane association of the glycolytic enzymes aldolase and enolase, along with subunits of the vacuolar H+-ATPase V-ATPase, revealing the importance of these enzymes in salt tolerance [35].

These recent and diverse investigations, through identification of molecular components of salt stress tolerance, widen our knowledge of the cellular mechanisms underlying plant adaptation. Characterisation of a novel *Arabidopsis* mutant *itn1*, has shown the possible roles of *ITN1* in the ABA-mediated regulation of ROS levels under salt-stress conditions [27]. However, more research is required to gain more information about this gene in salt-stress specific signal transduction. The dramatic abundance of apoplastic protein, OsRMC in response to salt stress, also highlights a significant role for these proteins in salt tolerance [66]. Recent results suggest that the cytoskeleton plays an essential role in *Arabidopsis* salt tolerance [23]. Further findings provide evidence on enolase as a multifunctional protein across species [35], implying that it may also play a regulatory or sensory role in multiple stresses at diverse cellular locations. The data on the necessity of peroxisomes for NO accumulation in the cytosol constitute a significant knowledge advancement regarding NO metabolism in plant peroxisomes and their involvement in abiotic stress responses [70]. Attempts to enhance salinity tolerance are currently being applied in cell type-specific manipulation of transport processes in commercially relevant plants such as rice and barley (*Hordeum vulgare*) [71].

Multiple stresses

There have been few studies investigating plant responses to environmental stresses applied in combination. Such research is particularly important as, in nature, simultaneous abiotic stresses are commonplace. For example, heat stress is often accompanied by a water deficiency and drought by salinity. *GRP7* is expressed abundantly in guard cells, and influences stomatal movement in accordance with the existing stress conditions [72]. In *Arabidopsis*, *GRP7* affected growth and stress tolerance under high salt and dehydrating conditions. It also conferred freezing tolerance, particularly via the regulation of stomatal opening [72]. *HOS3*, which encodes an elongase-like protein, inhibited ABA-mediated stress responses implicating the very long chain fatty acids (VLCFA) pathway as a control point for abiotic stress signalling and response [73]. These results provide further support for a role for ceramide in controlling stomatal behaviour. Transgenic *Arabidopsis* overexpressing *TaSnRK2.4* showed enhanced tolerance to drought, salt and freezing stresses, supported by decreased water loss, enhanced higher relative water content, greater cell membrane stability, improved photosynthetic potential and increased osmotic potential [74]. In rice, the overexpression of *AP37* and *AP59* increased tolerance to drought and high salinity [46] and overexpression of *HvCBF4* resulted in increased tolerance to drought, high-salinity and low-temperature [7]. Expression of the *CcHyPRP* gene from pigeonpea (*Cajanus cajan*) in *Arabidopsis* conferred tolerance against drought, salinity and heat stress and thus may be considered as a candidate gene for enhanced abiotic stress tolerance in crops [75]. The *lew1* mutant, after exposure to drought, exhibited increased expression of UPR pathway genes (*BiP* and *biZIP60*) and earlier expression of stress-responsive genes (*RD29A*, *COR47*) [22]. *LEW1* is implicated to play a crucial role in UPR pathway and abiotic stress responses in

Arabidopsis. Overexpression of soybean *GmWRKY13*, *GmWRKY21* and *GmWRKY54* genes conferred differential tolerance to abiotic stresses in *Arabidopsis* plants [45]. *OsABF1* enhanced abiotic-stress signalling in rice and is proposed to be a positive regulator of ABA-dependent abiotic signalling [76]. *WLIP19* was shown to act as a transcriptional regulator of *COR* or *LEA* genes in the development of abiotic stress tolerance [77]. *Pyrococcus furiosus* SOR can be produced as a functional enzyme *in planta* and plants producing SOR have enhanced tolerance to heat, light and chemically induced ROS [64]. This finding supports reducing cytosolic ROS as a promising approach to improve stress tolerance in crop plants.

The outcome from these comprehensive investigations on multiple stresses, where the molecular mechanisms imparting tolerance have been dissected revealing new roles for genes and proteins, can be a boon to strengthen future stress tolerance in crops. Our new knowledge about the functional roles of *GRP7* in environmental stress responses [72], the involvement of *HOS3* in abiotic stress signalling through the VLCFA pathway [73], conferring of multiple stress tolerance via *TaSnRK2.4* [74] and uncovering of the role of WRKY-type transcription factors in abiotic stresses [45] are of particular relevance. Moreover, barley *HvCBF4*, which is induced by low-temperature stress, on overexpression in rice also resulted in tolerance to other abiotic stresses. This also suggests that barley *CBF/DREBs* act differently in transgenic rice than *Arabidopsis CBF/DREBs* [7]. Furthermore, characterisation of (bZIP) transcription factors; *OsABF1* from rice [76] and *WLIP19* from wheat [77] emphasise their significance in general abiotic stress responses. In *Arabidopsis*, many known stress-responsive genes, such as *ERF/AP2*, *NCED3*, *ATHB-7*, *RD29B*, *PP2C* and diverse *LEA* genes, were strongly affected by individual stresses like salt, osmotic, ABA and temperature after 1–12 h [30]. Transcriptome datasets from *msi1-cs* plants (with highly reduced levels of *MSI1*) showed upregulation of a subset of ABA-responsive genes, which is an indicator for the response to drought and salt stress [54]. *PUB22*, *PUB23*, *TsVP* and *DSM1* play a role in modulating drought stress responses but have also been reported to impart resistance to oxidative and other abiotic stresses [2,8,29], Moreover, *DREB2A*, detailed here in relation to drought and high temperature stress, is also known for its role in salt stress.

Here, we have addressed some of the combined climate-change catastrophes that plants are facing and in the current decade, considerable progress has been made towards understanding molecular stress responses against different environmental stress factors. Different approaches, based on genetic and molecular studies have shown that a myriad of genes, proteins and metabolites, and their corresponding metabolic pathways or biological networks, modulate plant adaptation to environmental stresses. However, continued research is essential to show to what extent these adaptations are species and/or situation specific. Furthermore, the exact mechanisms involved and response and/or crosstalk relationships need further investigation.

Considering the most vulnerable farmers are often confronted with multiple stress factors, there is a clear desire to identify potential, more generally – applicable 'crop protectors' which may confer broader plant protection to combined abiotic stresses. However, much work is still needed although recent results show great promise. Several genes have already been identified, which are linked to plant responses to more than one abiotic stress (Figure 2).

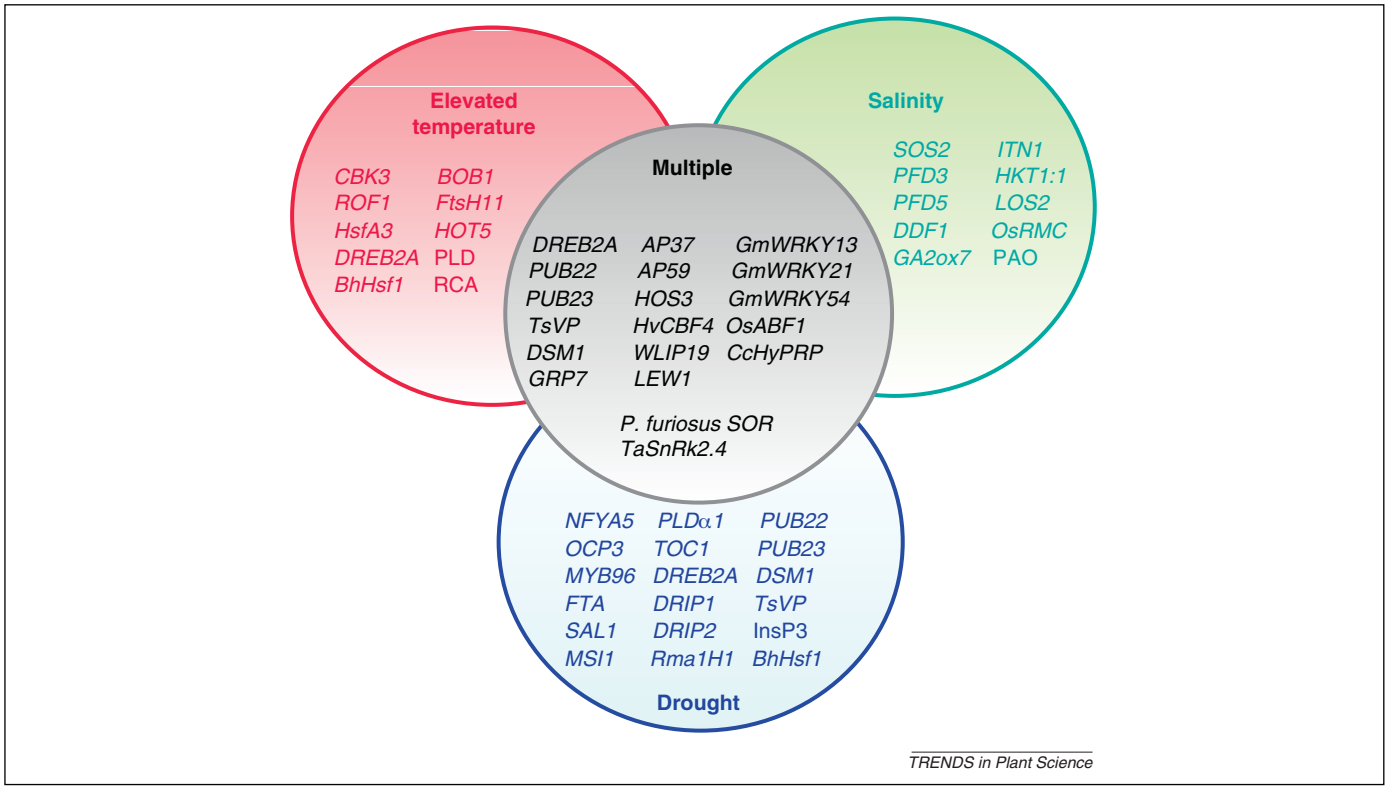

Figure 2. Recent research continues to identify gene responses induced by more than one environmental stress. Such genes or gene networks provide potentially valuable starting points to develop broader crop protection strategies.

It may therefore indeed be possible to identify common protection denominators based on complementary genes or molecular networks which could, through targeted breeding or GM approaches, form the molecular basis for a more global crop-stress protection strategy and more robust varieties for high-risk environments.

What are the future pathways to take?

From this huge diversity of functional genomic studies relating to climate change catastrophes, much knowledge has been acquired on the modulation of regulatory networks and metabolic pathways associated with or determinant for plant stress responses. Nevertheless, applicable knowledge relevant to crop cultivation remains scarce. Faced with the challenge of sustainable global food security we ultimately require more generic solutions for crop protection. Systems biology approaches could prove beneficial and may finally generate models showing the contribution of different signalling pathways defining plant '-omic' architectural responses in relation to climate change catastrophes. In order to achieve a holistic view of plant responses to climate change catastrophes, and to develop molecular engineering strategies to enhance plant tolerance to different stresses, it will be important to integrate '-omic' data with bioinformatics based systems-biology/systems-level modelling and to develop computational models. Some recent breakthroughs represent a promising start but are not yet the accomplishments we require [16,78–90].

Through a systems biology analysis, the photosynthetic metabolism of C_3 plants has been shown to be under highly cooperative regulation in changing environments, and systems-level modelling has been reviewed as a timely method to explore options for enhanced photosynthesis in the context of global climate change [83]. By performing bioinformatics analysis of *Arabidopsis* microarray data, a novel regulatory program was proposed [79]. This program combines transcriptional and post-translational controls and participates in modulating fluxes of amino acid metabolism in response to abiotic stresses. Simulations of stomatal response through a guard cell ABA-signalling model provided an efficient tool for the identification of candidate manipulations [88]. This may offer the best possibility of conferring increased drought stress tolerance and prioritising future wet-bench analyses. Weston *et al.* showed that a compendium of genomic signatures can be used to classify the plant abiotic stress phenotype in *Arabidopsis* according to transcriptome architecture, and then be linked to gene coexpression network analysis to determine the underlying genes governing the phenotypic response [90]. By applying this approach, the existence of known stress responsive pathways and marker genes was confirmed, and a common abiotic stress responsive transcriptome and related phenotypic classification to stress duration was presented. Furthermore, to dissect the transcriptional control of *Arabidopsis*, Carrera *et al.* presented a network analysis of genome-wide expression data combined with reverse-engineering network modelling [89]. The results suggested that *Arabidopsis* has evolved a high connectivity in terms of transcriptional regulation among cellular functions involved in responses and adaptation to changing environments, while gene networks constitutively expressed or less related to stress responses are characterised by a lower connectivity. A computational model AraNet (http://www.sciencedaily.com/releases/2010/01/100131142436.htm#) was recently presented as a source for predicting gene function of uncharacterised plant genes with unprecedented speed and accuracy [78]. For example, by using AraNet (http://www.functionalnet.org/aranet/), *At1g80710* (now DROUGHT SENSITIVE 1; *DRS1*) has been identified as a regulator of drought sensitivity [84].

In today's '-omics' era, which is already moving towards a more systems biology type approach, we have come some way to answering and modelling some of the plant stress responses. However, future directions for research seem to be more challenging because our global climate is changing unpredictably. We are still far behind establishing a comprehensive predictive model, in which we are just one click away from seeing the diverse biological networks in plant responses to combined climate change catastrophes. Such a model would be highly useful and could be exploited to help us improve and strengthen plant fitness to changing climates. Eventually this could bring us closer to a sustainable agriculture with food, fibre, oil, fuel and other crop plants that are more adaptable to changing climates.

Disclosure Statement

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tplants.2010.08.002.

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