Physiologia Plantarum 126: 45–51. 2006

REVIEW

Reactive oxygen species and temperature stresses: A delicate balance between signaling and destruction

Nobuhiro Suzuki and Ron Mittler*

Department of Biochemistry and Molecular Biology, University of Nevada, Mail Stop 200, Reno NV 89557, USA

Correspondence
*Corresponding author,
e-mail: ronm@unr.edu

Received 25 July 2005; revised 30 August 2005

doi: 10.1111/j.1399-3054.2005.00582.x

Temperature stress can have a devastating effect on plant metabolism, disrupting cellular homeostasis, and uncoupling major physiological processes. A direct result of stress-induced cellular changes is the enhanced accumulation of toxic compounds in cells that include reactive oxygen species (ROS). Although a considerable amount of work has shown a direct link between ROS scavenging and plant tolerance to temperature stress, recent studies have shown that ROS could also play a key role in mediating important signal transduction events. Thus, ROS, such as superoxide (O_2^-), are produced by NADPH oxidases during abiotic stress to activate stress-response pathways and induce defense mechanisms. The rates and cellular sites of ROS production during temperature stress could play a central role in stress perception and protection. ROS levels, as well as ROS signals, are thought to be controlled by the ROS gene network of plants. It is likely that in plants this network is interlinked with the different networks that control temperature stress acclimation and tolerance. In this review paper, we attempt to summarize some of the recent studies linking ROS and temperature stress in plants and propose a model for the involvement of ROS in temperature stress sensing and defense.

Introduction

Cellular homeostasis is achieved by a delicate balance between multiple pathways that reside in different organelles. This coordination may, however, be disrupted during temperature stress, because different pathways within cells have a different temperature optimum. For example, due to the physical properties of membranes, membrane-associated processes such as photosynthesis and respiration are more sensitive to temperature stress compared with pathways that are mainly carried out by soluble enzymes. When different pathways are uncoupled, electrons that have a high-energy state are transferred to molecular oxygen (O_2) to form reactive O_2 species (ROS; Asada and Takahashi 1987, Mittler 2002). ROS, such as $^1O_2$, H_2O_2, O_2^-, and $HO^\bullet$, are toxic molecules capable of causing oxidative damage to proteins, DNA, and lipids (Apel and Hirt 2004). Under optimal growth conditions, they are mainly produced at a low level in organelles such as chloroplasts, mitochondria, and peroxisomes. However, during stress, their rate of production is dramatically elevated. In chloroplasts, limitation of CO_2 fixation coupled with over-reduction of the electron transport chain is the main cause of ROS production. Over-reduction of the electron transport chain in mitochondria is also a major mechanism of ROS production during stress (Davidson and Schiestl 2001). In contrast, in peroxisomes, H_2O_2 is produced when glycolate is oxidized to glyoxylic acid

Abbreviations – ABA, abscisic acid; APX, ascorbate peroxidase; CAT, catalase, GPX, glutathione peroxidase; HSF, heat shock factor; HSP, heat shock protein; MAPK, mitogen-activated-protein kinase; PrxR, peroxiredoxin; Rboh, respiratory burst oxidative homolog; ROS, reactive oxygen species; SA, salicylic acid; SOD, superoxide dismutase.

during photorespiration (Mittler et al. 2004). Essential for ROS detoxification during normal metabolism, and particularly during stress, are antioxidants such as ascorbic acid and glutathione, and ROS-scavenging enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), and peroxiredoxin (PrxR) (Asada and Takahashi 1987, Iba 2002, Mittler et al. 2004). These have been found in almost all cellular compartments, demonstrating the importance of ROS detoxification for cellular survival (Mittler et al. 2004).

Temperature stress such as heat, cold or freezing is a principal cause for yield reduction in crops (Boyer 1982), and ROS generated by these stresses have been shown to injure cell membranes and proteins (Larkindale and Knight 2002, O'Kane et al. 1996). An additional contributor to cellular damage during temperature stress is high light. High-light stress has the potential to enhance the production of ROS in cells and cause oxidative damage to chloroplasts (Niyogi 1999). It was shown to enhance the production of ROS and ROS-associated injury during temperature stresses (Larkindale and Knight 2002). Previous studies demonstrated that ROS-scavenging mechanisms have an important role in protecting plants against temperature stresses and a combination of high light and temperature stress (Iba 2002, Larkindale and Knight 2002, Yabuta et al. 2002, Yoshimura et al. 2004). Controlling ROS production might therefore be a promising avenue of genetic engineering to enhance the tolerance of plants to temperature stress and a combination of temperature stress and high light (Allen 1995).

While ROS have the potential to cause oxidative damage to cells during environmental stress, recent studies have shown that ROS play a key role in plants as signal transduction molecules involved in mediating responses to pathogen infection, environmental stresses, programmed cell death and developmental stimuli (Mittler et al. 2004, Torres and Dangl 2005). The rapid increase in ROS production, referred to as 'the oxidative burst', was shown to be essential for many of these processes, and genetic studies have shown that respiratory burst oxidase homolog (Rboh) genes, encoding NADPH oxidases, are the main producers of signal transduction-associated ROS in cells during these processes (Mittler et al. 2004, Torres and Dangl 2005).

The two, somewhat opposing 'faces' of ROS, i.e. on the one hand, the damaging toxic molecule, and on the other hand, the beneficial signal transduction molecule, underscore the need to control the steady-state level of ROS in cells. Elucidating the mechanisms that control ROS signaling in cells during cold, heat, or freezing stress could therefore provide an additional powerful strategy to enhance the tolerance of crops to these environmental stress conditions.

Heat stress and ROS

Heat stress was shown to cause impairments in mitochondrial functions and result in the induction of oxidative damage that manifested in lipid peroxidation (Davidson and Schiestl 2001, Larkindale and Knight 2002; Vacca et al. 2004). The steady-state transcript and protein level of many ROS-scavenging enzymes were found to be elevated by heat stress (Rainwater et al. 1996, Rizhsky et al. 2002, Sato et al. 2001, Vacca et al. 2004). In addition, acquired thermotolerance, i.e. the ability of plants to develop heat tolerance following a mild heat pretreatment, was shown to be mediated in part by enhancing cellular mechanisms that prevented oxidative damage under heat stress (Bergmüller et al. 2003, Larkindale and Huang 2004).

Heat stress-response signal transduction pathways and defense mechanisms, involving heat shock transcription factors (HSFs) and heat shock proteins (HSPs), are thought to be intimately associated with ROS (Pnueli et al. 2003). Several studies have indicated that HSFs are involved in the sensing of ROS. The works of Mittler and Zilinskas (1992) and Storozhenko et al. (1998) have revealed the presence of a HSF-binding sequence at the promoter region of the gene encoding the H_2O_2-scavenging enzyme APX1. Transgenic *Arabidopsis* overexpressing HSF3 showed higher activity of APX during postheat-stress recovery and had a much stronger induction of *Apx2* than wild type plants (Panchuk et al. 2002). HSF21 was elevated during early stages of light stress in knockout *Apx1* plants that accumulate H_2O_2 under moderate light stress (Davletova et al. 2005a, Pnueli et al. 2003). Transcripts encoding HSF21 were also found to accumulate in wild-type cells treated with H_2O_2 (Davletova et al. 2005b). Transgenic plants expressing a dominant negative variant of HSF21 showed suppressed expression of Zat12, a H_2O_2-responsive zinc finger protein required for expression of APX1, and APX1 (Davletova et al. 2005a). These results indicate a central role of HSFs in early sensing of H_2O_2 and expression of APX1, APX2, and Zat12.

Recent studies demonstrated that protection against heat stress-induced oxidative damage involves calcium, abscisic acid (ABA), ethylene, and salicylic acid (SA) (Larkindale and Knight 2002, Larkindale and Huang 2004). Calcium channel blockers and calmodulin inhibitors induced oxidative damage to membrane, and pretreatment with calcium, SA, ABA, and 1-aminocyclopropane-1-carboxylic acid (ethylene precursor) increased survival rate of plants following a lethal heat

stress (Larkindale and knight 2002). Calcium, ABA, SA, and ethylene were also shown to enhance the activities of different ROS-scavenging enzymes under heat stress (Larkindale and Huang 2004). In accordance, mutants deficient in ethylene, ABA, ROS, and SA-signaling pathways, including knockouts for the respiratory burst oxidase enzyme RbohD, showed strong defects in acquired heat tolerance, suggesting essential roles for these pathways in acquired heat tolerance (Larkindale et al. 2005, Suzuki et al. 2005).

Cold stress and ROS

Cold stress was shown to enhance the transcript, protein, and activity of different ROS-scavenging enzymes (O'Kane et al. 1996, Prasad et al. 1994, Saruyama and Tanida 1995, Sato et al. 2001). Low temperature stress was also shown to induce H_2O_2 accumulation in cells (O'Kane et al. 1996).

In *Arabidopsis*, a number of cold responsive genes such as RD29A, KIN1, KIN2, COR15A, COR47, DREB1A, DREB2A, and ERD10 have been identified (Seki et al. 2002, Thomashow 1999). The contribution of some cold-responsive genes to controlling ROS under cold stress was suggested by Lee et al. (2002). *Arabidopsis* frostbite1 (fro1) mutant displayed reduced expression of cold-responsive genes such as RD29A, KIN1 COR15A, and COR47, and accumulated ROS constitutively. The *FRO1* gene was shown to encode a mitochondrial complex I protein, suggesting that expression of the cold-responsive genes and ROS accumulation might be modulated by the disruption of a mitochondrial function.

DNA regulatory elements in the promoters of cold-responsive genes such as C-repeat (CRT)-related – and dehydration responsive element (DRE) – motifs have been identified (Yamaguchi-Shinozaki and Shinozaki 1994). Hsieh et al. (2002) showed that transgenic expression of the transcriptional activator, CRT/DRE-binding factor 1 (CBF1), enhanced the cold tolerance of tomato plants. Enhanced expression and enzymatic activity of CAT were also detected in transgenic plants, and the level of H_2O_2 in transgenic plants was lower than that of wild-type plants. In *Arabidopsis*, overexpression of NDP kinase 2 (NDPK2) enhanced cold tolerance (Moon et al. 2003). NDP kinase 2 was shown to interact with two oxidative stress-related mitogen-activated protein kinases (MAPKs), AtMPK3, and AtMPK6 (Moon et al. 2003). Transgenic plants overexpressing NDPK2 showed lower levels of H_2O_2 compared with wildtype, and a mutant lacking AtNDPK2 displayed an enhanced accumulation of H_2O_2. Zat12, an ROS-response zinc finger protein (Davletova et al. 2005a, Rizhsky et al. 2004) was shown to regulate cold-induced genes. Microarray analysis demonstrated that cold-responsive genes were upregulated by overexpression of Zat12 (Vogel et al. 2005), and Zat12 downregulated CBF transcript expression suggesting a role for Zat12 in suppressing the CBF cold-response pathway. These studies demonstrate a close link between ROS, ROS signaling, and the cold stress response. However, to the best of our knowledge, the involvement of Rboh proteins in generating an oxidative burst during cold stress was not established.

Freezing stress and ROS

Plants are capable of acquiring freezing tolerance following a mild cold pretreatment, known as cold acclimation. In pine trees, elevated levels of ROS-scavenging enzymatic activities such as APX, glutathion reductase, monodehydroascorbate reductase, and dehydroascorbate reductase were found to be associated with increased freezing tolerance during cold acclimation (Tao et al. 1998). Cold-responsive genes are activated by cold acclimation, and these genes contribute to freezing tolerance of plants (Thomashow 1998). Transgenic *Arabidopsis* plants overexpressing COR15A showed higher freezing tolerance than wild-type plants, and COR15A expression increased chloroplast- and protoplast-freezing tolerance (Artus et al. 1996). The DRE-binding protein, DREB1A was shown to regulate the expression of DREB1A target genes such as RD29A and COR15A (Seki et al. 2002). Overexpression of DREB1A induced freezing tolerance in *Arabidopsis*, indicating that CRT/DRE regulation increases the freezing tolerance (Liu et al. 1998). Overexpression of *Arabidopsis* CBF1 induces expression of KIN2, COR15A, COR47, and RD29A genes and enhances freezing tolerance of nonacclimated plants (Jaglo-Ottosen et al. 1998). As described above, the regulation of many of these cold-response genes is associated with ROS signaling suggesting that ROS play a similar role in mediating cold or freezing tolerance in plants.

Enhancement of oxidative damage by high-light stress during temperature stress

High light enhances the production of ROS and has the potential to damage the photosynthetic apparatus (Asada and Takahashi 1987, Niyogi 1999). High-light stress could therefore enhance ROS-mediated damage during temperature stresses. Compared with dark conditions, temperature stress-induced damage to cells was shown to be enhanced by light (Jeong et al. 2002, Larkindale and Knight 2002). In cucumber, the primary

target of cold stress combined with high light is Cu/Zn SOD, followed by inactivation of PSI by ROS (Choi et al. 2002). Transgenic plants over-expressing Cu/Zn SOD, APX, and glutathione reductase, GPX, or thylakoid-bound APX were found to be more tolerant than wild-type plants to a combination of temperature and high-light stress (Allen 1995, Payton et al. 2001, Yabuta et al. 2002, Yoshimura et al. 2004). Mutants deficient in ascorbate, zeaxanthinin or glutathione were subjected to a lethal heat stress in the presence or absence of high light (Larkindale et al. 2005). These mutants showed a dramatic decrease in survival rate under a combination of high light and temperature stress. In addition, transgenic plants overexpressing β-carotene hydroxylase, an enzyme catalyzing the conversion of β-carotene to zeaxanthin, showed enhanced tolerance to a combination of heat and high-light stress (Davison et al. 2002), indicating a role for zeaxanthin in enhancing the tolerance of plants to a combination of heat and high-light stress. These results indicate that ROS-scavenging enzymes play an important role in preventing photooxidative damage under a combination of temperature and high-light stress.

Conclusion and future challenges

A putative model for the role of ROS in temperature stress is shown in Fig. 1. Two converging pathways are depicted in the figure: the temperature stress-response pathway and the ROS-response pathway. Temperature stress is shown to result in the enhanced production of ROS in cells by the disruption of cellular homeostasis and the uncoupling of metabolic processes (stress-generated ROS box). In addition, the sensing of temperature stress by the temperature sensor could lead to the enhanced production of ROS by NADPH oxidases (ROS generation by Rboh box). The ROS sensor would sense ROS produced by these processes and activate the ROS defense mechanisms that include ROS-scavenging enzymes (ROS defense box) or further enhance ROS

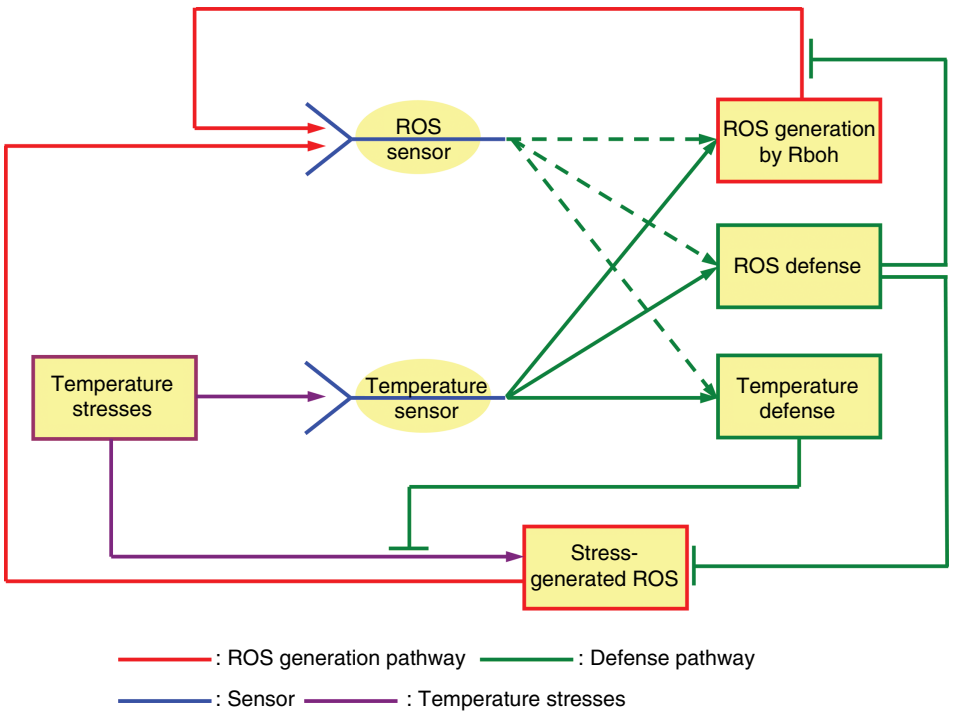

Fig. 1. A proposed model for the involvement of reactive oxygen species (ROS) in temperature stress sensing and protection. Temperature stress is shown to result in the enhanced production of ROS in cells by the disruption of cellular homeostasis and the uncoupling of metabolic processes (stress-generated ROS box). The sensing of temperature stress by the temperature stress sensor could also lead to the enhanced production of ROS by NADPH oxidases (ROS generation by Rboh box). The ROS sensor would sense ROS produced by these processes and activate the ROS defense mechanisms that include ROS-scavenging enzymes (ROS defense box) or further enhance ROS production by Rboh (ROS generation by Rboh box) to enhance the ROS signal. Both sensors, for ROS or temperature stress, could activate the temperature defense pathway that includes heat shock proteins and other protective mechanisms (temperature defense box) and/or the ROS-scavenging pathways (ROS defense box), resulting in the suppression of stress-associated ROS production. The pathways shown above would be activated upon temperature stress; however, their converging nature would cause them to suppress each other when the stress subsides or when the cell achieves a new state of homeostasis that enables it to survive the temperature stress and reduce the cellular rate of production of ROS.

production by Rboh (ROS generation by Rboh box) to enhance the ROS signal (Mittler et al. 2004). Both sensors, for ROS or temperature stress, could activate the temperature defense pathway that includes HSPs and other protective mechanisms (temperature defense box) and/or the ROS-scavenging pathways (ROS defense box), resulting in the suppression of stress-associated ROS production. The pathways shown in Fig. 1 would be activated upon temperature stress; however, their converging nature would cause them to suppress each other when the stress subsides or when the cell achieves a new state of homeostasis that enables it to survive the temperature stress. The latter could be achieved, for example, once the uncoupling of pathways and the enhanced production of ROS would be put under control by different adjustments to cellular homeostasis and the function of HSPs and/or other cellular protectants.

A major challenge to the study of abiotic stress in plants is to understand how different defense and signaling pathways are coordinated. Much of the work described above suggests that temperature stress and ROS signaling are interlinked. The model presented in Fig. 1 suggests that it would be hard to separate these pathways from each other and that further work is required to understand how much of the ROS produced during temperature stress can result in cellular damage and how much of the ROS produced during temperature stress is made for the purpose of signaling. The localization and timing of ROS production and ROS scavenging during temperature stress might also play a key role in these processes. For example, ROS for signaling might be produced at the apoplast, whereas ROS generated as a direct result of temperature stress might be produced in the chloroplast or mitochondria. Mutants deficient in key components of the ROS gene network, the ROS or temperature sensors, and/or the temperature signal transduction pathway would be essential to begin answering some of these questions.

The plant genome contains a large number of HSF genes (21 in *Arabidopsis*; Nover et al. 2001). As described above, these appear to play a key role in temperature stress and ROS sensing in plants (Davletova et al. 2005a, Mittler and Zilinskas 1992, Panchuk et al. 2002, Pnueli et al. 2003, Storozhenko et al. 1998). However, the complexity of the HSF gene network, and the high number of potential interactions between different HSF subunits (Czarnecka-Verner et al. 2000, Nover et al. 2001), makes the study of plant HSFs a challenge. It is possible that through the network of HSFs, ROS, and temperature signals are perceived and integrated. However, further studies using different knockouts and mutants are needed to address this question. The identification, purification, and characterization of signaling complexes involved in temperature and/or ROS signal transduction is also a major challenge. These complexes could be similar to the constitutively photomorphogenic 1 complex and function by integrating temperature, ROS, and high-light signals (Casal 2002).

Plant hormones such as SA, ABA, and ethylene have been shown to play an important role in mediating ROS and temperature stress signals (Larkindale and Knight 2002, Larkindale and Huang 2004). ABA, in particular, was shown to activate Ca^{2+} channels during drought stresses via the function of ROS and Rboh proteins (Torres and Dangl 2005). However, the cause-and-effect relationship(s) between ROS, SA, ABA, and ethylene during temperature stress is not clear. Further studies are needed to elucidate the role of ROS in mediating the action of different plant hormones during temperature stress.

The ROS gene network of plants includes over 150 genes that encode for different ROS-scavenging or ROS-producing enzymes (Mittler et al. 2004). Typically, each cellular compartment contains more than one enzymatic activity that scavenges a particular ROS. For example, the cytosol contains at least three different enzymatic activities that scavenge H_2O_2: APX, GPX, and PrxR. How these activities are coordinated within each compartment and between different compartments during temperature stress is an important question that requires future investigation? The answer to this question is likely to explain, at least in part, how the different ROS produced during temperature stress affect cellular functions and signaling (Fig. 1). Detailed time-course analysis of enzymatic activities, coupled with microarray assays and measurements of ROS, antioxidants, and protein oxidation in different compartments during temperature stress is needed to resolve this question.

Reducing the rate of ROS production in cells is likely to be as important as active scavenging of ROS during stress (Mittler 2002). This could be achieved, for example, by adjustments to cellular metabolism that reduce the rate of electron flow in particular compartments or by controlling the accumulation of particular compounds with a high redox potential. A good example for a defense enzyme that suppresses the potential of a charged electron transfer chain to form ROS is alternative oxidase (McIntosh 1994). Alternative oxidases are found in the mitochondria and chloroplast and reduce the formation of ROS during stress (Mittler 2002). Protection of different complexes and pathways using HSPs and/or other protective compounds such as sugars could also lower the rate of electron leakage from different apparatuses. To completely understand how

plants cope with temperature stress, we should include in future research the study of these mechanisms and the manner by which they are coordinated with other, more active, defenses.

Acknowledgements – Research in the laboratory of R. Mittler is supported by funding from The National Science Foundation (NSF-0431327; NSF-0420033).

References

Allen RD (1995) Dissection of oxidative stress tolerance using transgenic plants. Plant Physiol 107: 1049–1054

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55: 373–399

Artus NN, Uemura M, Steponkus PL, Gilmour SJ, Lin C, Thomashow MF (1996) Constitutive expression of the cold-regulated *Arabidopsis thaliana* COR15a gene affects both chloroplast and protoplast freezing tolerance. Proc Natl Acad Sci USA 93: 13404–13409

Asada K, Takahashi M (1987) Production and scavenging of active oxygen in photosynthesis. In: Kyle DJ, Osmond CB, Arntzen CJ (eds) Photoinhibition. Elsevier, Amsterdam, pp 227–287

Bergmüller E, Porfirova E, Dörmann P (2003) Characterization of an *Arabidopsis* mutant deficient in γ-tocopherol methyltransferase. Plant Mol Biol 52: 1181–1190

Boyer JS (1982) Plant productivity and environment. Science 218: 443–448

Casal JJ (2002) Environmental cues affecting development. Curr Opin Plant Biol 5: 37–42

Choi SM, Jeong SW, Jeong WJ, Kwon SY, Chow WS, Park YI (2002) Chloroplast Cu/Zn-superoxide dismutase is a highly sensitive site in cucumber leaves chilled in the light. Planta 216: 315–324

Czarnecka-Verner E, Yuan CX, Scharf KD, Englich G, Gurley WB (2000) Plants contain a novel multi-member class of heat shock factors without transcriptional activator potential. Plant Mol Biol 43: 459–471

Davidson JF, Schiestl RH (2001) Mitochondrial respiratory electron carriers are involved in oxidative stress during heat stress in *Saccharomyces cerevisiae*. Mol Cell Biol 21: 8483–8489

Davison PA, Hunter CN, Horton P (2002) Overexpression of beta-carotene hydroxylase enhances stress tolerance in *Arabidopsis*. Nature 418: 203–206

Davletova S, Rizhsky L, Liang H, Shengqiang Z, Oliver DJ, Coutu J, Shulaev V, Schlauch K, Mittler R (2005a) Cytosolic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of *Arabidopsis*. Plant Cell 17: 268–281

Davletova S, Schlauch K, Coutu J, Mittler R (2005b) The zinc-finger protein Zat12 plays a central role in reactive oxygen and abiotic stress signaling in *Arabidopsis*. Plant Physiol 133: 847–856

Hsieh TH, Lee JT, Yang PT, Chiu LH, Charng YY, Wang YC, Chan MT (2002) Heterology expression of the Arabidopsis C-repeat/dehydration response element binding factor 1 gene confers elevated tolerance to chilling and oxidative stresses in transgenic tomato. Plant Physiol 129: 1086–1094

Iba K (2002) Acclimative response to temperature stress in higher plants: approaches of gene engineering for temperature tolerance. Annu Rev Plant Biol 53: 225–245

Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF (1998) *Arabidopsis* CBF1 overexpression induces COR genes and enhances freezing tolerance. Science 280: 104–106

Jeong SW, Choi SM, Lee DS, Ahn SN, Hur Y, Chow WS, Park YI (2002) Differential susceptibility of photosynthesis to light-chilling stress in rice (*Oryza sativa* L.) depends on the capacity for photochemical dissipation of light. Mol Cells 13: 419–428

Larkindale J, Knight MR (2002) Protection against heat stress-induced oxidative damage in *Arabidopsis* involves calcium, abscisic acid, ethylene, and salicylic acid. Plant Physiol 128: 682–695

Larkindale J, Huang B (2004) Thermotolerance and antioxidant systems in *Agrostis stolonifera*: involvement of salicylic acid, abscisic acid, calcium, hydrogen peroxide, and ethylene. J Plant Physiol 161: 405–413

Larkindale J, Hall JD, Knight MR, Vierling E (2005) Heat stress phenotypes of *Arabidopsis* mutants implicate multiple signaling pathways in the acquisition of thermotolerance. Plant Physiol 138: 882–897

Lee BH, Lee H, Xiong L, Zhu JK (2002) A mitochondrial complex I defect impairs cold-regulated nuclear gene expression. Plant Cell 14: 1235–1251

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively. *Arabidopsis*. Plant Cell 10: 1391–1406

McIntosh L (1994) Molecular biology of the alternative oxidase. Plant Physiol 105: 781–786

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7: 405–410

Mittler R, Zilinskas BA (1992) Molecular cloning and characterization of a gene encoding pea cytosolic ascorbate peroxidase. J Biol Chem 267: 21802–21807

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9: 490–498

Moon H, Lee B, Choi G, Shin D, Prasad DT, Lee O, Kwak SS, Kim DH, Nam J, Bahk J, Hong JC, Lee SY, Cho MJ, Lim CO, Yun DJ (2003) NDP kinase 2 interacts with two oxidative stress-activated MAPKs to regulate cellular redox

state and enhances multiple stress tolerance in transgenic plants. Proc Natl Acad Sci USA 100: 358–363
Niyogi KK (1999) Photoprotection reviseed: genetic and molecular approaches. Annu Rev Plant Physiol Plant Mol Biol 50: 333–359
Nover L, Bharti K, Doring P, Mishra SK, Ganguli A, Scharf KD (2001) *Arabidopsis* and the heat stress transcription factor world: how many heat stress transcription factors do we need? Cell Stress Chaperones 6: 177–189
O'Kane D, Gill V, Boyd P, Burdon R (1996) Chilling, oxidative stress and antioxidant responses in *Arabidopsis thaliana* callus. Planta 198: 371–377
Panchuk II, Volkov RA, Schoffl F (2002) Heat stress- and heat shock transcription factor-dependent expression and activity of ascorbate peroxidase in *Arabidopsis*. Plant Physiol 129: 838–853
Payton P, Webb R, Kornyeyev D, Allen R, Holaday AS (2001) Protecting cotton photosynthesis during moderate chilling at high light intensity by increasing chloroplastic antioxidant enzyme activity. J Exp Bot 52: 2345–2354
Pnueli L, Liang H, Rozenberg M, Mittler R (2003) Growth suppression, altered stomatal responses, and augmented induction of heat shock proteins in cytosolic ascorbate peroxidase (Apx1) -deficient *Arabidopsis* plants. Plant J 34: 187–203
Prasad TK, Anderson MD, Martin BA, Stewart CR (1994) Evidence for chilling-induced oxidative stress in Maize seedlings and a regulatory role for hydrogen peroxide. Plant Cell 6: 65–74
Rainwater DT, Gossett DR, Millhollon EP, Hanna HY, Banks SW, Lucas MC (1996) The relationship between yield and the antioxidant defense system in tomatoes grown under heat stress. Free Radic Res 25: 421–435
Rizhsky L, Davletova S, Liang H, Mittler R (2004) The zinc finger protein Zat12 is required for cytosolic ascorbate peroxidase 1 expression during oxidative stress in *Arabidopsis*. J Biol Chem 279: 11736–11743
Rizhsky L, Liang H, Mittler R (2002) The combined effect of drought stress and heat shock on gene expression in tobacco. Plant Physiol 130: 1143–1151
Saruyama H, Tanida M (1995) Effect of chilling on activated oxygen-scavenging enzymes in low temperature-sensitive and – tolerant cultivars of rice (*Oryza sativa* L.). Plant Sci 109: 105–113
Sato Y, Murakami T, Funatsuki H, Matsuba S, Saruyama H, Tanida M (2001) Heat shock-mediated APX gene expression and protection against chilling injury in rice seedlings. J Exp Bot 52: 145–151
Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M, Oono Y, Kamiya A, Nakajima M, Enju A, Sakurai T, Satou M, Akiyama K, Taji T, Yamaguchi-Shinozaki K, Carninci P, Kawai J, Hayashizaki Y, Shinozaki K (2002) Monitoring the expression profiles of 7000 *Arabidopsis* genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J 31: 279–292
Storozhenko S, Pauw PD, Montagu MV, Inzé D, Kushnir S (1998) The heat-shock element is a functional component of the *Arabidopsis* APX1 gene promoter. Plant Physiol 118: 1005–1014
Suzuki N, Rizhsky L, Liang H, Shuman J, Shulaev V, Mittler R (2005) Enhanced tolerance environmental stresses in transgenic plants expressing the transcriptional co-activator MBF1. Plant Physiol (In press)
Tao DL, Oquist G, Wingsle G (1998) Active oxygen scavengers during cold acclimation of scots pine seedlings in relation to freezing tolerance. Cryobiology 37: 38–45
Thomashow MF (1998) Role of cold-responsive genes in plant freezing tolerance. Plant Physiol 118: 1–8
Thomashow MF (1999) Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Physiol Plant Mol Biol 50: 571–599
Torres MA, Dangl JL (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. Curr Opin Plant Biol 8: 397–403
Vacca RA, de Pinto MC, Valenti D, Passarella S, Marra E, De Gara L (2004) Production of reactive oxygen species, alteration of cytosolic ascorbate peroxidase, and impairment of mitochondrial metabolism are early events in heat shock-induced programmed cell death in tobacco Bright-Yellow 2 cells. Plant Physiol 134: 1100–1112
Vogel JT, Zarka DG, Van Buskirk HA, Fowler SG, Thomashow MF (2005) Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of *Arabidopsis*. Plant J 41: 195–211
Yabuta Y, Motoki T, Yoshimura K, Takeda T, Ishikawa T, Shigeoka S (2002) Thylakoid membrane-bound ascorbate peroxidase is a limiting factor of antioxidative systems under photo-oxidative stress. Plant J 32: 915–925
Yamaguchi-Shinozaki K, Shinozaki K (1994) A novel cis-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cell 6: 251–264
Yoshimura K, Miyao K, Gaber A, Takeda T, Kanaboshi H, Miyasaka H, Shigeoka S (2004) Enhancement of stress tolerance in transgenic tobacco plants overexpressing *Chlamydomonas* glutathione peroxidase in chloroplasts or cytosol. Plant J 37: 21–33

Edited by C. Guy