REVIEW ARTICLE

Role of abscisic acid in plant stress tolerance

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The phytohormone abscisic acid (ABA) plays a regulatory role in many physiological processes in plants. Different stress conditions such as water, drought, cold, light, and temperature result in increased amounts of ABA. The action of ABA involves modification of gene expression and analysis of responsive promoters revealed several potential *cis*- and *trans*-acting regulatory elements. The nature of ABA receptors is unknown. The precise role of ABA under both abiotic and biotic stress conditions, while unclear, is of current interest in many laboratories in the world. Molecular mechanisms underlying the role of ABA in stress tolerance in plants need to be understood.

UNDERSTANDING responses of plants to their environment in terms of adaptability and performance is of paramount importance. Apart from interest in factors important for ecological and evolutionary distribution, much present day interest is fueled by practical needs. Modern agriculture is affected by environmental factors such as water, drought, temperature, light, and salt stress. Sustainable agriculture in harsh environments requires an understanding of the ways that plant genes respond to both biotic and abiotic factors. Interest in low impact sustainable agriculture (LISA) has increased considerably, reflected by the burst of activity in genetic engineering.

Stress may induce common responses such as enhancement of plant hormones. For instance, wounding can induce the production of increased ethylene, auxin, and abscisic acid (ABA). Since many kinds of stresses including water, salt, and cold temperatures, induce ABA synthesis, ABA may be considered a plant stress hormone. It regulates several important aspects of plant growth and development. Recent studies have demonstrated a pivotal role for ABA in modulation at the gene level of adaptative responses for plants in adverse environmental conditions[1–6]. ABA is also involved in several other physiological processes such as stomatal closure, embryo morphogenesis, development of seeds, and synthesis of storage proteins and lipids[7–11], germination[12–17], leaf senescence[18], and defense against pathogens[19,20]. Nevertheless, ABA acts as a mediator in controlling adaptative plant responses to environmental stresses[21–23]. In several instances, it has been implicated in signal transduction at the single-cell level[24].

Abscisic acid biosynthesis

In 1960, ABA was isolated and identified from cotton bolls[25]. Mutants of ABA biosynthesis are known from a variety of plant species[26,27]. Characterization of these mutants together with their physicochemical studies has enabled the pathway of biosynthesis to be elucidated in higher plants. However, questions still remain. There is evidence that either of two different routes may be used (Figure 1). In both the cases, ABA is synthesized via the mevalonate pathway, as are all terpenoids. The hormone may be synthesized directly through the mevalonic acid pathway via the 15-carbon precursor, farnesylpyrophosphate. Alternatively, it may be formed indirectly by the cleavage of the 40-carbon carotenoid precursor, violaxanthin, also synthesized via the mevalonate pathway. Both pathways may be used for the synthesis of ABA, but in different tissues or different conditions[18,19,27,28,30,31]. Two genes involved in ABA biosynthesis have been cloned, and should provide insight into the regulation and site of ABA synthesis[29,32]. It is believed that the hormone is synthesized in mature leaves and transported in the phloem through the shoot system.

Increased ABA levels as a response to environmental stress

During vegetative growth, roots of many angiosperms synthesize ABA and transport it into the shoots under conditions of water stress. ABA is an essential mediator in triggering plant responses to adverse environmental stimuli[18,33,39]. This is known to occur in a number of crop plants which include rice[35], barley[36], soybean[37], tomato[38], cotton[39], and alfalfa[40]. Leaf ABA content in wild plants increased with water stress. Upon rehydration, the ABA level ceased to increase and returned to pre-stressed levels[34,36–38]. Substantial evidence suggests that increased ABA levels limit water loss by reducing stomatal aperture.

Chilling temperatures also increased ABA levels[21]. In crops such as winter wheat, potatoes, and alfalfa, a large

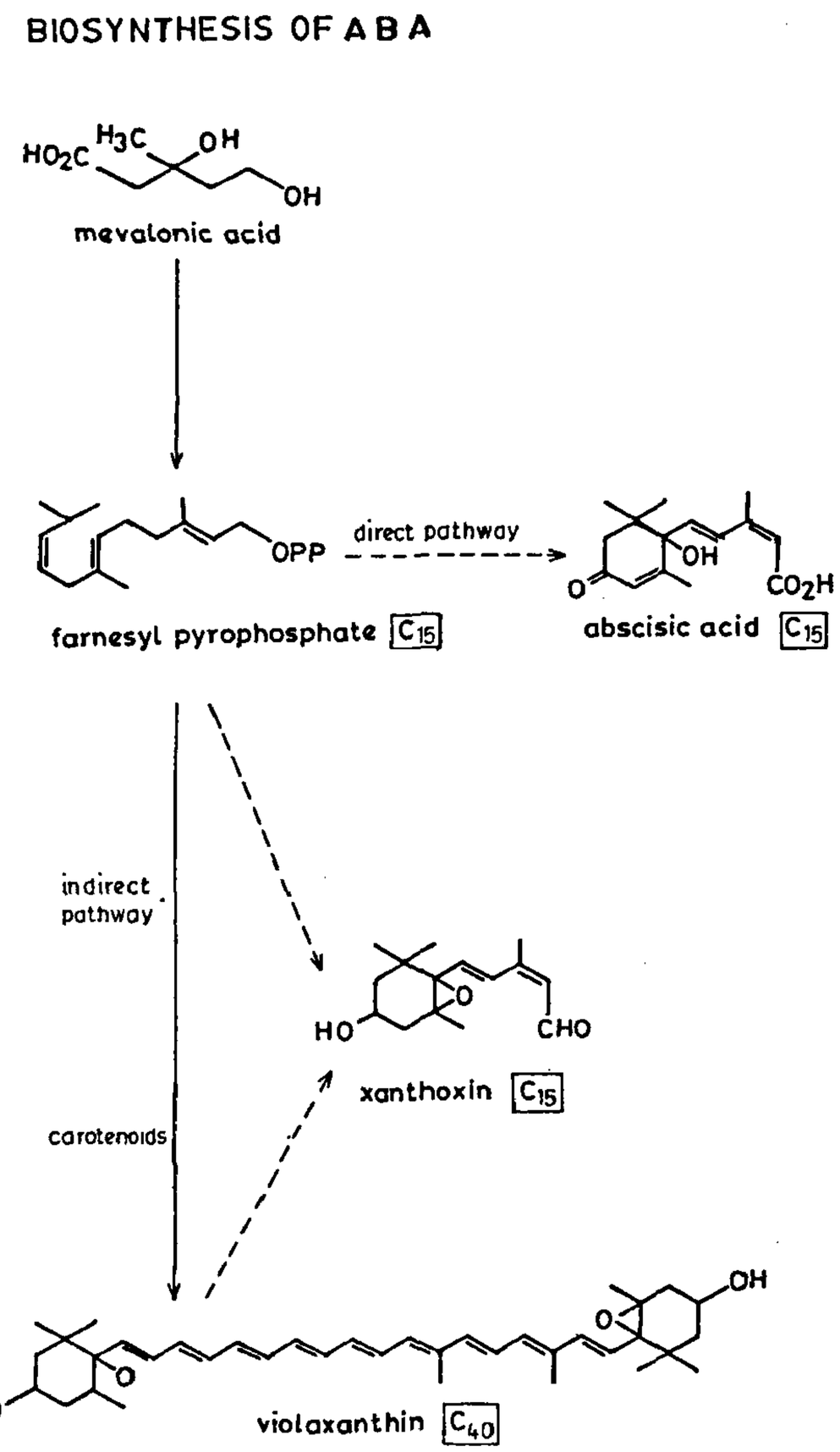

Figure 1. Schematic representation of biosynthetic pathways of abscisic acid. The hormone may be synthesized directly through the mevalonic acid pathway via the 15-carbon precursor, farnesylpyrophosphate. Alternatively, it may be formed indirectly by the cleavage of the 40-carbon carotenoid precursor, violaxanthin, also synthesized via the mevalonate pathway.

increase in the ABA content of leaves was observed during hardening and cold acclimation[1,40–42]. However the extent of the ABA response depends on varietal differences – particularly in winter wheat[41]. A freeze-resistant variety of wheat had a higher ABA level than a less resistant variety, although the difference was not significant[42]. Similarly for potato species, an increase in ABA was observed in *Solanum commersonii*, but not in *S. tuberosum*, which failed to acclimate at –3°C.

Salt stress resulted in intercellular accumulation of ABA during growth of tobacco cells[4] and elevated endogenous levels of ABA in seedlings of alfalfa[40]. Mechanical injury to potato leaves resulted in an increase in ABA levels[6], and increased ABA expression of transcripts coding for protease inhibitor. Similar treatment of an ABA-deficient mutant had little effect. Young tobacco leaves had elevated levels of ABA following infection by tobacco mosaic virus (TMV).

From the above observations, it is evident that the extent of ABA production is positively related to the degree of resistance to a given stress factor. However these observations alone do not establish that ABA is a necessary intermediary for acquisition of stress tolerance.

Exogenous application of ABA and stress tolerance

Exogenous application of ABA was able to increase plant adaptive response to various environmental conditions. The resurrection plant, *Craterostigma plantaginem* can tolerate extreme dehydration. However, *in vitro* propagated callus derived from this plant has a strict requirement for exogenously applied ABA in order to survive severe dehydration. Therefore, addition of ABA (5 mg/l) to the callus of *Craterostigma plantaginem* (resurrection plant) induced tolerance to dessication[43].

Abscisic acid, when added to suspension cultures of tobacco (*Nicotiana tabacum* L. cv. Wisconsin) induced adaptation to salt stress[44,45]. The role of ABA in wound-induced expression of the proteinase inhibitor II gene is well demonstrated[6,46]. These plants have been shown to accumulate inhibitors when the leaves are wounded. These inhibitors appear to play a significant role in preventing damage by insects. In addition, several studies have convincingly demonstrated that exogenously applied ABA can indeed increase cold tolerance. Application of ABA at room temperature increased cold resistance in callus explants of tobacco[47], cucumber, winter wheat[48], and alfalfa[49]. However, in some cases, exogenous application of ABA did not increase stress tolerance[50]. The failure may be attributed to lack of ABA uptake or its degradation by microbes. Sterile suspension cultures overcame these problems[2,44,51–53]. Cell suspension cultures of winter wheat, winter rye, and Bermuda grass, on being treated with ABA, could tolerate extremely cold temperatures (–30 to –60°C).

Most reports have demonstrated that the application of exogenous ABA provides tolerance to various stress conditions. However, endogenously produced ABA may not show the same effects as elicited by exogenously applied hormones; and, of course, correlated variations may not reflect the cause.

ABA-deficient and ABA-insensitive mutants

Mutants are powerful tools for investigating the role of ABA in stress tolerance[22]. Mutants have been obtained for *Arabidopsis*, tomato, and potato that have been ei-

ther unable to respond to the hormone or have been unable to synthesize ABA. The two different types of ABA regulatory mechanisms in these plants[54–56] are *aba* (ABA deficient) mutations which affect synthesis of ABA (ref. 56), resulting in lower ABA levels; and *abi* (ABA-insensitive) mutations which impair ABA sensitivity. Three ABA-insensitive mutants of *Arabidopsis*; *abi1*, *abi2*, and *abi3* have been found. The product of the *abi3* gene acts primarily in seed development[13]. The role of ABA in signaling stress has been extensively documented by molecular studies showing that ABA-deficient mutants are affected in the regulation of many genes by drought, salt, and cold[22,26,57]. ABA has also been shown to be involved in the induction of gene expression by mechanical damage[58].

ABA induces closure of stomata. Water stress in the roots can lead to a 40-fold increase in the ABA levels in the plant. An ABA-deficient tomato mutant, *flacca*, is unable to close stomata during drought stress[59–61]. The *flacca* mutant had only 20–26% of the ABA content of wild-type tomatoes when well-watered. Water stress did not result in additional amounts of ABA (ref. 61). Moreover, the wilted character of the *flacca* mutant could be phenotypically reversed with ABA application[62]. Comparative analysis of proteins from wild-type and *flacca* mutants with 2-dimensional electrophoresis indicated that several polypeptides are specifically synthesized during adaptation to drought stress[38,63,64]. Synthesis of new polypeptides occurred when ABA was applied to the *flacca* mutant, indicating regulation by ABA concentrations during drought stress.

The relationship between ABA and cold tolerance has been well documented. While no apparent effect on tolerance to freezing was observed in *abi* mutant, cold tolerance was markedly impaired in the *aba* mutant compared with wild-type[65]. This observation supports the idea that the level of ABA during cold acclimation appears to be important for the development of tolerance to freezing. Expression of ABA-regulated *cor* (cold regulated) genes was unaffected by *abi2* and *abi3* mutants, but was greatly reduced in the *abi1* mutant. Cold-regulated expression of all three *cor* genes was unaffected in *abi1* mutant plants. Thus it is concluded that cold-regulated and ABA-regulated expression of the three *cor* genes may be mediated through independent mechanisms. Similar observations were made in alfalfa[49,66]. Likewise, the role of ABA in the wound-induced expression of proteinase inhibitors I and II has been well documented using ABA-deficient potato and tomato mutants[6]. Proteinase inhibitors I and II are regulated by PI and PII genes. Local wounding of potato and tomato plants resulted in accumulation of proteinase inhibitors PI-I and PI-II in the aerial parts of the plants[67]. On the other hand, ABA-deficient mutants of potato (*droopy*) and tomato (*sitiens*) showed considerable reduction in expression of these genes in response to plant wounding when compared to wild-type plants. External application of ABA completely abolished the proteinase inhibitor II (PI-II) induction of wild-type and mutant plants.

Stress and ABA-inducible proteins

Plants synthesize a spectrum of new proteins on exposure to different environmental stresses such as water stress[38,43,63,64,68–72], salt stress[3,73–78], dehydration and desiccation stress[79–81], cold stress[5,49,53,56,82–89], and wounding[6,40]. Proteins induced by stress fall into three categories: (i) those inducible by stress and ABA; (ii) specifically induced by stress but not by ABA; and (iii) inducible by ABA. Therefore, it is quite evident that many of these stress-responsive proteins are also induced by ABA (Table 1) and several groups of proteins listed are homologous.

Late embryogenesis abundant proteins

Genes encoding late embryogenesis abundant (LEA) proteins were consistantly represented in differential screens for transcripts with increased levels during drought. These proteins were first described in research on genes abundantly expressed during the final desiccation stage of seed development. Circumstantial evidence for their involvement in dehydration tolerance is strong. The genes are similar to many of those expressed in vegetative tissues of drought-stressed plants (Table 2). ABA can also induce *lea* genes in seeds and vegetative tissue[8,22]. The LEA proteins are divided into six groups based on deduced amino acid sequence homologies and dot matrix analysis with proteins from cotton. These groups were D19-LEA (group 1), D11-LEA (group 2 – also called dehydrins), D7-LEA (group 3), D113-LEA (group 4), D95-LEA (group 5), and D29-LEA (group 6 – may be similar to group 3). This system will remain useful until clear functions can be assigned to each gene. Each group has a stretch of amino acid sequences which is highly conserved among the proteins belonging to that group. These proteins are extremely, hydrophilic, remain heat stable at high temperatures, are basic and contain no cysteine and tryptophan. Two exceptions are the proteins from *Creterostigma plantagineum* and D11-LEA from cotton plants. Proteins of this group, including most of the *rab* genes[90], and some of the dehydrins and *lea* genes are also inducible by environmental stresses like salinity[91,92].

Another family of genes induced by multiple stresses and ABA-encoded proteins are rich in glycine (35–40%) (ref. 40). They also contain histidine, asparagine, and tyrosine in lesser quantities than glycine. All encoded proteins contained tandom repeats comprising glycine residues interrupted with histidine or tyrosine. Interest-

Table 1. Stress-responsive and ABA-inducible proteins

Crop	Clone name	Nature of stress	Organ specificity	Reference
Rice	RAB 16 A, 16 B,	Salt and desiccation	No	74
	16 C, 16 D	Salt and desiccation	No	106
	–	Salt and desiccation	Shoot and root	75
	GRP	Water	Doubtful	14
Wheat	EM	Drought	No	105
	RAB 15	Drought	No	72
Barley	dehydrins B8, B9, B17 and B18	Desiccation	No	80
	PH VAI	Cold	Doubtful	39, 89, 128
	GRP	Not known	Doubtful	126
Maize	pMAH9	Drought and wounding	No	69
	dehydrin M3	Desiccation	No	80
	RAB-17	Not known	No	136
	HS 70	Heat, desiccation and wounding	No	68
Cotton	LEA D7, D11, D19, D29 and D34	Not known	No	79
Tobacco	Osmotin	Salt	No	73, 128
Tomato	TAS 14	Salt and drought	Not known	76
	PLE 4	Salt; drought; cold; heat	Shoot and seed	64
	PLE 16	Salt; drought; cold; heat	Shoot and seed	71
	PI-II	Wounding	Leaves	6
Potato	PI-II	Wounding	Leaves	6

The data is adapted from compilations of Ma Luo *et al.* 1993.

Table 2. Genes encoding late embryogenesis abundant (LEA) proteins during drought stress

Plant name	cDNA[a]	Relationship of encoded polypeptide type	Reference
Helianthus annus (sunflower)	Hads10	D19-LEA	129
Triticum aestivum (wheat)	Em	D19-LEA	130
Hordeum vulgare (barley)	B19.1; B19.3; B19.4	D19-LEA	131
Lycopersicum esculentum (tomato)	PLE25	D113-LEA	132
Helianthus annus	Hads11	D113-LEA	129
Arabidopsis thaliana	pRABAT1	D11-LEA	133
Craterostigma plantageneum (resurrection plant)	pc C27-04	D11-LEA	70
Zea mays (maize)	M3 (RAB-17)	D11-LEA	80
Hordeum vulgare	B8; B9; B17	D11-LEA	80
Lycopersicum esculentum	PLE4	D11-LEA	132
L. esculentum	TAS14	D11-LEA	70
L. chilense	PLC 30–15	D11-LEA	76
L. plantageneum	pcC 6–19	D11-LEA	134
Stellaria longipes	H26	D11-LEA	135
Orzya sativa (rice)	pRAB16	D11-LEA	120
Daucus carota	pcECP40	D11-LEA	136
A. thaliana	ERD10; ERD14	D11-LEA	136
T. aestivum	pMA2005	D7-LEA	80
T. aestivum	pMA1949	D7-LEA	80
C. plantageneum	pcC3-06	D7-LEA	90
C. plantageneum	pcC27–45	D95-LEA	90

[a]The best characterized plant genes from which cDNA clones have been demonstrated to show increased mRNA expression in response to drought stress and also absisic acid.

ingly, these proteins showed striking homologies with other ABA-responsive proteins[40,69]. Therefore, it is quite clear that a wide spectrum of proteins are synthesized by the induction of ABA or environmental stresses (Tables 1 and 2). Most of the proteins are cloned to clarify their identities, but their function in stress tolerance is yet to be elucidated.

Regulation of stress and ABA-inducible gene expression

Drought and low temperatures are adverse environmental conditions that affect plant growth and crop productivity. Plants may have similar mechanisms to tolerate both. For example, ABA is produced under both

drought and low temperature stress. Also, plants grown under water stress show a higher tolerance to low temperature stress than do well-watered plants.

A number of genes have been described that respond to drought and low temperature stress at the transcription level. The functions of some gene products have been predicted from sequence homology with known proteins and are thought to play a role in protecting cells from water deficits and low temperatures[57,93,94].

Most of the drought and cold-stress-inducible genes that have been studied to date are also induced by ABA. Dehydration appears to trigger the production of ABA which in turn expresses various genes. *Cis*- and *trans*-factors involved in ABA-induced gene expression have been analysed extensively[22,26,95,96].

Several genes in *Arabidopsis* are induced by both cold and drought in ABA-deficient (*aba*) and ABA-insensitive (*abi*) mutants. This suggests that these plants under conditions of cold and drought do not require ABA but do respond to it[51,93]. The genes include *rd29A/ilti78*, *kin1*, *cor6.6/kin*, and *cor47/rd17* (refs 30, 97–100). The promoter region of the *rd29A* gene was analysed, and a novel *cis*-acting element responsible for dehydration and cold-induced expression was identified at the nucleotide sequence level, using transgenic plants. A 9-bp conserved sequence, TACGACAT, termed dehydration responsive element (DRE), is essential for regulation of dehydration-responsive gene expression. The DRE has been demonstrated to function as a *cis*-acting element involved in the induction of *rd29A* expression by low temperature stress[30].

DRE-related motifs have been reported in promoter regions of cold- and drought-inducible genes such as *kin1* and *cor6.6*, and *rab17* (refs 100, 101). A similar motif was also reported (C-repeat TGGCCGAC) in the promoter region of the cold-inducible rape oil-seed gene *BN115* and in a low temperature-responsive element[102]. These results suggest that DRE-related motifs are involved in drought- and cold-responsive but ABA-independent gene expression. Recently, two independent families of DREB proteins, DREB1 and DREB2, have been reported to function as *trans*-acting factors in two separate signal transaction pathways under low temperature and dehydration[103,104].

Many of the changes in mRNA levels observed during various types of stresses reflect a transcriptional activation. Exogenous application of ABA can also induce these changes and this treatment has been utilized for setting up experimental systems to define *cis*- and *trans*-acting elements. To obtain a clear understanding of the transcriptional control mechanisms of ABA and stress induction by identifying *cis*-acting regulatory sequences, the corresponding genomic clones of some ABA- and stress-inducible proteins have been isolated and their nucleotide sequences analysed[71,74,77,105,112].

A functional analysis of the S′ upstream sequences for the *Em* gene of wheat identified a 50-bp ABA-responsive element (ABRE) that was capable of conferring ABA-inducibility in either orientation in a minimal cauliflower mosaic virus (CAMV) promoter[91,105]. Two elements, *Em1* and *Em2*, within this 50-bp ABRE were in the promoter of the other ABA-responsive genes, such as the *ita* and *rab* gene family[74,77,106,107]. A DNA-binding protein (EM bp-1) that interacted specifically with an 8-bp sequence in the *Em1* element (ACGT) with the mutation (CCCGGGGC) in the core (ACGT) of this sequence (CACGTGGC) prevented binding of EM bp-1 and reduced the ability of ABRE to confer ABA responsiveness to a CAMV promoter. These findings clearly suggest that the sequence CACGTGGC in one of the responsive elements and the DNA-binding protein EM bp-1 are involved in ABA response.

In contrast, ABRE motifs are not involved in ABA regulation of other stress-inducible genes such as in the *Arabidopsis thaliana* Rd (ref. 108) and the *Craterostigma plantagineum* CdeT27-45 genes[109]. The distinct sequence motif is essential for the ABA response to CdeT27-45. In this case the corresponding physiological *trans*-acting factors are presumably unknown. Genes that are induced by ABA and encode other potential transcription factors, include the box gene *ATHB-7* and several *myb* homologues from *A. thaliana*[110] and *C. plantagineum*[111]. Comparison of the available promoter sequences of ABA and stress-inducible genes revealed that ACGT cores were conserved in many promoter elements of these genes[112–114]. The existence of the ACGT core sequence in the promoter region of these genes suggests that the genes may be mediated by ABA (refs 115, 116). Previous work has shown that a 50-bp ABA responsive element (ABRE) is capable of conferring ABA inducibility. Cauliflower mosaic virus (CAMV) promoter and the sequence CACGTGGC is included in the 50-bp ABRE element containing a TGCA core[117,118]. It should be noted that promoter regions of many ABA-responsive genes contain more than one sequence element with an ACGT core. Whether these are involved in the ABA or stress response remains to be tested[105,107,119,120].

The best characterized *cis*-element in the context of drought stress is the ABA-responsive element (ABRE) which contains the palindromic motif CACGTC with the G-box ACGT core element[113]. ACGT elements have been observed in many plant genes regulated by diverse environmental and physiological factors. Systematic binding studies have shown that nucleotides flanking the ACGT core specify the DNA–protein interactions and subsequent gene interaction[100]. G-box-related ABREs have been observed in ABA-responsive genes, although their functions have not been proven experimentally. However, for several of these genes, the G-box type of ABREs, already described for ABA induction, both in

seeds and in vegetative tissues have been found[107,116,118,119].

Most research has focused on understanding how relevant genes are up-regulated during different types of environmental stresses. However, the abiotic stress factors also involve down-regulation of several genes. For example, studies of *C. plantagineum* have shown that transcripts encoding proteins relevant to photosynthesis are down-regulated during dehydration and thus reduce photoxidative stress. It has been shown that promoter regions of storage protein genes contain information for their down-regulation during seed desiccation[90,121]. The role of cold-responsive genes in freeze tolerance has been discussed recently[122].

There is a viviparous mutant (*vpl*) of maize in which the embryos do not become dormant[107]. This mutation reduces the sensitivity of the embryos to ABA without affecting ABA synthesis. The wild-type *vpl* gene product probably is involved in either the receptor mechanism for ABA or the signal transduction pathway. The *vpl* mutation also has pleiotropic effects on anthocyanin synthesis during seed development. In the wild-type, anthocyanin pigments are synthesized and accumulate in the aleurone layer of seed, but in the *vpl* mutant, this synthesis is blocked because the gene product is required for the expression of the *cl* gene. The *cl* gene encodes a transcriptional factor that regulates the genes encoding enzymes required for anthocyanin synthesis[9,123,124]. The *vpl* gene has been cloned, the transposor tagged, and sequenced. The *vpl* gene most likely produces a transcription factor involved in regulation of the expression of other genes. The *vpl* protein appears to activate the transcription of the *Em* gene which is promoted by ABA, but direct binding of *vpl* protein to regulatory elements in promoters of the *Em* or the *cc1* gene has not yet been observed. The *vpl*/AB13 proteins thus appear to be multifunctional transcription factors that integrate ABA and other regulatory signals in seed maturation, most likely by interacting with distinct *trans*-acting factors that remain to be identified[125]. It is quite evident from the above studies that environmental stresses induce an increase in the ABA content of plants in a species-dependent manner.

Conclusions and perspectives

Environmental stresses like cold, drought, desiccation, salt, and mechanical wounding induce the synthesis of ABA. Despite genetic differences, exogenous application of ABA accelerates the rate of plant acclimation to given stress conditions, while studies with ABA-insensitive and ABA-deficient mutants clearly reveal that ABA plays a cardinal role in plant adaptation to stresses. At the molecular genetic level, ABA-responsive genes have been identified and their expression studied. However, the precise role of ABA in stress tolerance has not been pinpointed. ABA regulates the process of adaptation into two interacting steps. First, ABA acts via differential signal transduction pathways on cells which are the least and most affected by the imposed stress. Second, ABA may regulate through some genes/gene products, which control the expression of stress or adaptative-specific genes. Some genes are up-regulated and others are down-regulated, resulting in overall synthesis of genomic products which may play a role in plant survival under diffferent environmental conditions. Despite the rapid progress in analysis of several ABA-responsive genes, key questions remain to be answered. For instance, does ABA interact with these genes directly? Do ABA and stress-specific genes coordinate regulation by *cis/trans*-acting factors? What are the functions of the ABA-responsive genes? Current research carried out in various laboratories worldwide is expected to provide answers to the above questions and also unravel the molecular mechanism(s) underlying stress tolerance in plants.

The level of understanding at the molecular level of stress-induced ABA synthesis has increased greatly in recent years. With a bit more knowledge, it might be possible to control levels of ABA and consequently plant-response to stress. Certainly exciting times lie ahead in this rapidly-changing field.

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