Abscisic Acid

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Geetika Kalra and Satish C Bhatla

Some molecules produced by plants exert their effects as negative regulators of various plant responses. **Abscisic acid** is an inhibitory hormone that helps plants adapt to stress. It also maintains water balance, prevents seed embryos from germinating, and induces seed and bud dormancy. Initial attempts to identify abscisic acid were made by Fredrick T. Addicott and his coworkers in cotton fruits in 1963. It was earlier suspected that seed and bud dormancy are caused by some inhibitory compounds and attempts were made to extract these compounds from various plant tissues. Acidic compounds separated by paper chromatography from these tissues were tested for their ability to promote growth in oat coleoptile, but instead these compounds inhibited coleoptile elongation. This compound was referred as "β-inhibitor complex." Subsequently, high β-inhibitor levels were correlated with suppression of sprouting in *Solanum tuberosum* tubers, abortion of *Lupinus arboreus* pods, and bud dormancy in trees like *Betula pubescens*. This compound was subsequently named abscisin II, since it was identical to a substance that promotes abscission in cotton fruits (commercially important for mechanization of cotton picking). Another substance isolated a substance from *Betula pubescens*, a deciduous plant, inhibits growth and induces bud dormancy. It was named "**dormin**." "Dormin" was subsequently found to be structurally similar to "abscisin II." The compound was subsequently renamed as abscisic acid (ABA)—a compound which inhibits growth and stomatal opening when plants are under environmental stress.

ABA is ubiquitous in vascular plants, and in several genera of fungi, it is produced as a secondary metabolite. In higher plants, it is found in all living tissues from root cap to the apical bud. It is synthesized in all cells which contain chloroplasts or amyloplasts. ABA is a 15-carbon sesquiterpene characterized by a cyclohexane ring with a keto and hydroxyl group and a side chain with a terminal carboxyl group in its structure. The orientation of carboxyl group at C2 position determines the *cis* and *trans* isomers of ABA. ABA has an asymmetric carbon atom at position 1 in the ring resulting in S- and R-enantiomers. S-enantiomer is the active

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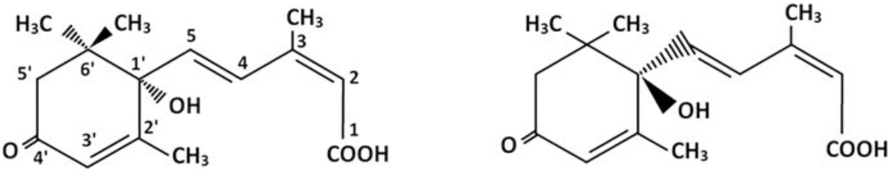
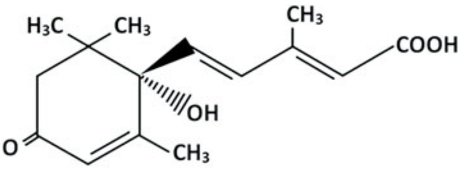


***(S)-cis*-ABA (naturally occurring active form)**

***(R)-cis*-ABA (inactive in stomatal closure)**



***(S)-2-trans*-ABA (inactive, but interconvertible with active [*cis*] form)**

Fig. 18.1 Structures of “S” and “R” forms of abscisic acid (ABA)

form leading to fast responses like stomatal closure. In long-term responses, both the enantiomers are active. (Fig. 18.1). Contrary to its name, ABA does not induce abscission. Accumulation of ABA during seed development has been associated with maturation of seeds, desiccation tolerance, and suppression of vivipary. ABA, when produced in terminal buds, slows plant growth and directs leaf primordia to develop scales to protect the dormant buds from low temperature during cold season. It also inhibits cell division in vascular cambium and acclimatizes plants to cold conditions by suspending primary and secondary growth. ABA is synthesized in green fruits at the beginning of the winter or in maturing seeds while establishing dormancy. It is rapidly translocated through xylem vessels from the roots to the leaves along the transpiration stream. ABA is also produced in response to environmental stresses, such as heat, water, and salt stress.

18.1 Bioassay

A number of bioassays have been developed to detect the presence of ABA in plant systems. These include stimulation of stomatal closure, inhibition of germination of isolated wheat embryos, inhibition of growth of duckweed-*Lemna minor*, acceleration of the abscission of cotyledons in cotton seedlings, inhibition of IAA-induced straight growth of *Avena* (oat) coleoptile, and inhibition of GA-induced α-amylase in the cells of aleurone layer in germinating barley seeds. These bioassays have now been replaced with more accurate and reliable methods for ABA detection and estimation. These include high-performance liquid chromatography (HPLC) followed by mass spectrometry (MS). These modern methods are useful for determination of the molecular structure. Highly sensitive and specific immunoassays are also applied for quantification and spatial distribution of intracellular ABA.

18.2 Biosynthesis, Catabolism, and Homeostasis

Plant responses due to ABA depend on its concentration within the tissue and on the sensitivity of the tissue to the hormone. ABA biosynthesis takes place in chloroplasts or amyloplasts. Complete ABA biosynthetic pathway has been elucidated with the help of ABA-deficient mutants which exhibit blockade of ABA biosynthesis at specific steps. Two pathways have been proposed for ABA biosynthesis:

1. *Isoprenoid pathway:*

   Mevalonate → Isopentanyl pyrophosphate → Geranyl pyrophosphate
   → Farnesyl pyrophosphate

   ABA

2. *Carotenoid pathway* (Fig. 18.2):

Carotenoid pathway begins with isopentenyl diphosphate (IPP), the biological isoprene unit which leads to the synthesis of C_{40} xanthophyll called violaxanthin. The reaction is catalyzed by zeaxanthin epoxidase (ZEP). Violaxanthin leads to the formation of C_{40} compound, 9-cis-neoxanthin, which is further cleaved to form the C_{15} compound called xanthoxin, by the action of NCED (9-*cis*-epoxycarotenoid dioxygenase). Xanthoxin is a natural growth inhibitor that has physiological properties similar to ABA. Synthesis of NCED is rapidly induced by water stress and constitutes the key regulatory step for ABA synthesis. Lastly, xanthoxin leads to the formation of ABA via oxidative steps involving the intermediates ABA-aldehyde or xanthoxic acid in the cytoplasm.

ABA catabolism is brought about by hydroxylation and conjugation steps. Its hydroxylation at C-8′ position is the predominant ABA catabolic route. The 8′-hydroxy ABA, however, contains substantial biological activity like ABA, but its spontaneous cyclization results in the formation of **phaseic acid (PA)** which does not exhibit any ABA-like activity. ABA-binding proteins from barley aleurone layer are also unable to bind to phaseic acid suggesting that PA is an inactive catabolite of ABA for some physiological processes. Very recently, however, PA has been observed to activate a subset of ABA receptors. Additionally, ABA homeostasis is maintained through glucosylation of ABA's hydroxylated catabolites.

ABA concentration in tissues is highly variable during development or in response to changing environmental conditions. ABA biosynthesis is precisely

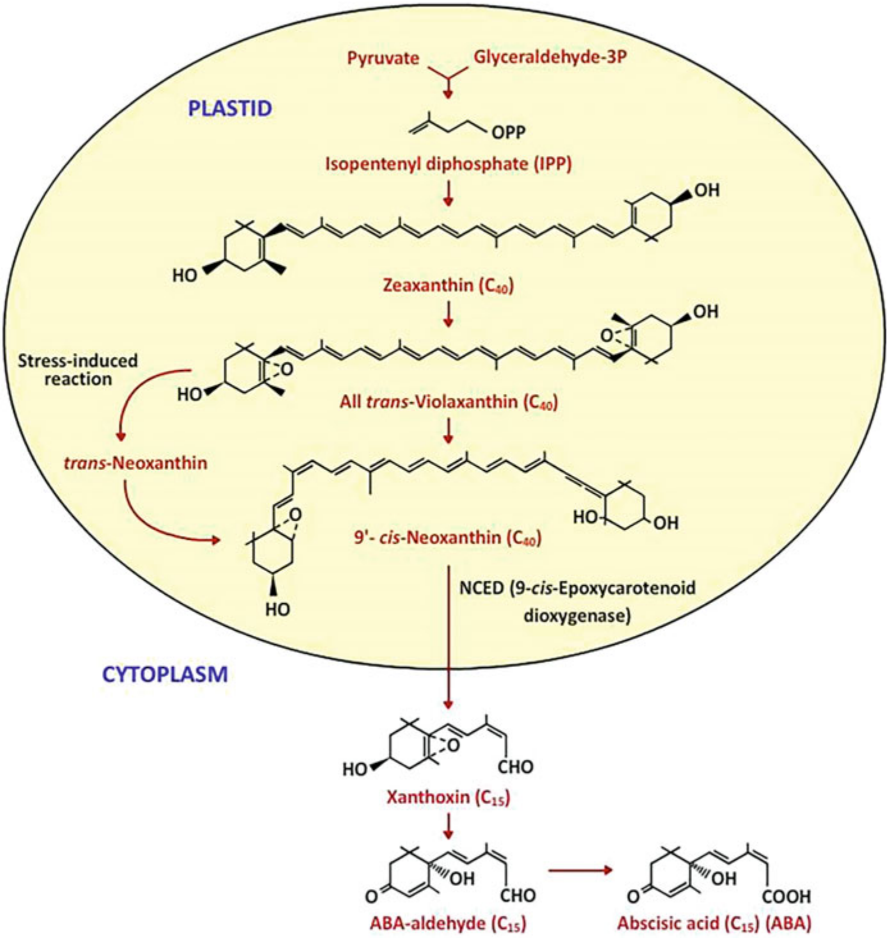


Fig. 18.2 ABA biosynthesis through carotenoid pathway

regulated with reference to its timing and location. Its availability is enhanced during desiccation of vegetative tissues. It is synthesized in green tissues at the beginning of winter period. ABA establishes dormancy when synthesized in maturing seeds. Under conditions of water stress, ABA concentration in the leaves increases up to 50-fold within 4–8 h. Later, its content declines upon rewatering.

18.3 Translocation of Abscisic Acid

ABA is transported through xylem and phloem, but it is much more abundant in the phloem sap. With the onset of water stress, ABA is synthesized in roots, and it gets transported to aerial parts before the low water potential of soil causes any measurable change in the water status of the leaves; ABA is believed to be a root signal which helps reduce transpiration rate by closing stomatal pores in the leaves. During early stages of water stress, pH of xylem sap becomes more alkaline. The dissociated anionic form of the weak acid accumulates in alkaline compartments and may be redistributed according to the steepness of the pH gradient across membrane. Also, specific uptake carriers contribute in maintaining a low apoplastic ABA concentration in unstressed plants. Stress-induced alkalinization of apoplast favors dissociation of ABA, which does not cross membranes. Hence, less ABA enters the mesophyll cells and more reaches the guard cells via transpiration stream.

18.4 Developmental and Physiological Effects of ABA

18.4.1 ABA Levels Increase in Response to Environmental Stress

ABA is an essential mediator in triggering plant responses to adverse environmental stimuli. Increase in the ABA content is observed during hardening and cold acclimation. The response also depends upon variety of the plant. For example, a freeze-resistant variety of wheat had a higher ABA level than a less resistant variety. Salt stress also results in intercellular accumulation of ABA during growth of tobacco cells and also increased ABA following infection by TMV. These observations indicate that ABA production is positively related to the degree of resistance to a given stress factor. Moreover, exogenous application of ABA increases plant adaptive response to various environmental conditions in variety of plants (Table 18.1).

18.4.2 Seed Development

Seed development in flowering plants proceeds in three discrete stages, i.e., early, mid-, and late embryogenesis. During late embryogenesis, seeds lose water and desiccate. They develop hard and resistant seed coat and have negligible metabolism and respiration rate. Four hormones play major role in seed development. IAA and cytokinin levels are high during early phase of embryogenesis when cell divisions occur at high frequency. IAA also plays a major role in patterning and polarity

Table 18.1 ABA-induced proteins in response to stress

Crop	Name of the protein	Nature of stress
Rice	RAB 16A, 16B	Salt and desiccation
Wheat	EM, RAB 15	Drought
Barley	Dehydrins B8, B9, B17, B18	Desiccation
Maize	pMAH9	Drought and wounding
Tobacco	Osmotin	Salt
Tomato	TAS 14	Salt and drought
Potato	Pt II	Wounding

establishment in the young embryos. Gibberellic acid is involved in the growth of embryo and endosperm, thus creating a sink for photoassimilates. ABA content rises when the levels of all other hormones decline. It plays a central role during mid to late embryogenesis transition. Once development is complete, ABA content falls.

18.4.3 Desiccation Tolerance

ABA induces desiccation tolerance in seeds. Desiccation can damage membranes and other cellular components. During mid- to late stages of seed development, ABA content is high, and numerous metabolic processes are activated that contribute to the acquisition of desiccation tolerance. One of them is synthesis of **late embryogenesis abundant (LEA) proteins**. These are hydrophilic and thermostable proteins which perform protective function against desiccation. These proteins have the ability to form hydrogen bonds with sucrose, which is known to accumulate during seed maturation. This interaction turns it (sucrose) into highly viscous liquid with slow molecular diffusion rate due to which it participates in limited chemical reactions, thereby rendering desiccation tolerance to the seeds. ABA affects the accumulation and composition of storage proteins. ABA-deficient mutants have reduced storage protein accumulation. It also maintains embryos in dormant state until the environmental conditions are optimal for growth. ABA induces changes in cellular metabolism by activating a network of transcription factors. For example, AB13 induces the synthesis of storage proteins and LEA proteins through interactions with bZIP transcription factors, such as AB15.

18.4.4 Inhibition of Precocious Germination and Vivipary

When immature embryos are removed from their seeds and placed in culture, midway through development before the onset of dormancy, they germinate precociously. ABA added to the culture medium inhibits precocious germination. This suggests that ABA is the natural constraint that maintains developing embryos in their embryogenic state. ABA levels are high during mid to late seed development phases. Further evidence was provided by ABA-deficient mutants of maize, which germinate directly on the cob. **Vivipary** in these mutants is prevented by treatment with exogenous ABA.

18.4.5 Counteraction of GA Action

ABA counters many effects of GA, such as induction of hydrolases and α-amylases in barley seedlings. ABA inhibits the synthesis of hydrolytic enzymes which are essential for the breakdown of storage reserves in seeds. It inhibits transcription of α-amylase mRNA via two mechanisms: (i) UP1, a protein which is an activator of ABA-induced gene expression, acts as a transcriptional repressor of some

GA-regulated genes; and (ii) ABA represses GA-induced expression of GA-MγB, a transcription factor that mediates the induction of α-amylase expression by GA.

18.4.6 Seed Dormancy

It has also been observed that ABA can be replaced by solution of high osmolarity. Many stress-related genes are induced by both ABA and high osmoticum. ABA-deficient *(aba)* mutants in *Arabidopsis* have been instrumental in demonstrating the role of ABA in seed dormancy. *Aba* mutants are nondormant at maturity. The reciprocal cross between *aba* and wild-type plants exhibits dormancy only when embryo itself produces ABA. The overall effect of ABA on seed dormancy is also influenced by other hormones. Interestingly, rise in ABA production in seeds coincides with decline in IAA and GA level. An experiment to demonstrate the effect of ABA/GA ratio was conducted on *Arabidopsis* seeds that could not germinate in the absence of exogenous GA. These seeds were mutagenized and grown in green house. The seeds produced by these mutagenized plants were screened for **revertants** (seeds that regain the ability to germinate). These revertants were found to be mutants of ABA synthesis, wherein dormancy could not be induced and hence subsequent synthesis of GA was not required to overcome it. This leads to a general conclusion that the balance of plant hormones is more critical than their absolute concentrations in regulating development.

18.4.7 Stomatal Closure During Water Stress

Accumulation of ABA in stressed leaves plays an important role in the reduction of water loss by transpiration under water-stressed conditions. Mutants that lack the ability to produce ABA exhibit permanent wilting due to their inability to close stomata. Application of exogenous ABA to such mutants causes stomatal closure and a restoration of turgor pressure. It has been suggested that the permeability of chloroplast membranes in mesophyll cells increases in response to ABA. As a result, ABA diffuses to cytoplasm and moves to guard cells through plasmodesmata. When water potential is restored, movement of ABA into the guard cells stops. ABA causes stomatal closure by inhibiting the ATP-mediated H^+/ K^+ exchange pumps in the guard cells. Figure 18.3 summarizes major biochemical routes of ABA action during regulation of stomatal opening and closing and in release of seed dormancy.

18.4.8 Promotion of Root Growth and Inhibition of Shoot Growth at Low Water Potential

Under dehydrating conditions, when ABA levels are high, the endogenous hormone exerts a strong positive effect on root growth by suppressing ethylene production and negative effect on shoot growth. This increases the root:shoot ratio at low water

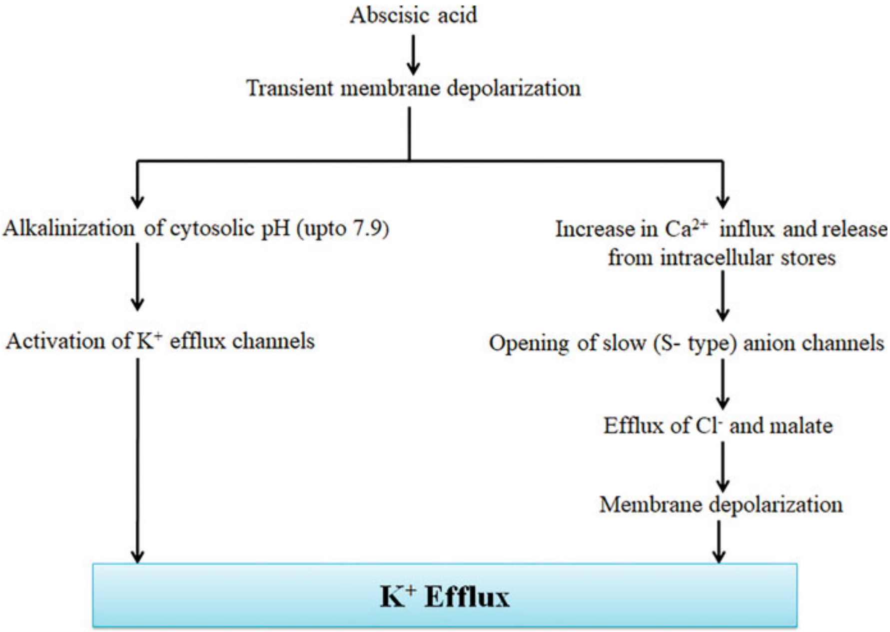


Fig. 18.3 ABA induces depolarization of plasma membrane and increase in cytosolic calcium which are required for activation of S-type anion channels leading to opening of K^+ efflux channels on the plasma membrane of guard cells

potential which, along with the effect of ABA on stomatal closure, helps the plant to cope up with water stress.

18.5 Mode of Action

ABA influences both short-term physiological effects, like stomatal closure, and long-term developmental processes, like seed maturation, and combats drought stress. Rapid responses involve alterations in the fluxes of ions across membranes and may also involve gene regulation. Long-term responses involve major changes in the pattern of gene expression. ABA receptor is a protein, apparently located on the outer surface of the membrane. Stomatal closure is driven by reduction in guard cell turgor pressure caused by efflux of K^+ ions. Extracellular application of ABA is effective in inhibiting stomatal opening at pH 6. At this pH, ABA is protonated and is readily taken up by guard cells. The first change detected, after exposure of guard cells to ABA, is transient membrane depolarization caused by the net influx of hydrogen ions and increase in cytosolic Ca^{2+} concentration. ABA stimulates this influx of calcium through the release of calcium from plasma membrane channels and also from internal compartments like vacuoles. Calcium release can also be induced by second messengers like inositol 1,4,5-trisphosphate (IP3) and cyclic

ADP-ribose (cADPR). ABA also causes alkalinization of cytosol from pH 7.6 to 7.9. This activates K^+ efflux channels on the plasma membrane by increasing the number of channels available for activation. The rapid, transient depolarization induced by ABA is insufficient to open the K^+ efflux channels. They require long-term membrane depolarization to open K^+ channels. ABA activates slow ion channels leading to long-term membrane depolarization. Long-term depolarization is triggered by two factors: (1) ABA-induced transient depolarization of plasma membrane and (2) increase in cytosolic calcium. Both of these conditions are prerequisites for opening of calcium-activated slow (S-type) anion channels on the plasma membrane. Prolonged opening of these slow anion channels permits large quantities of chloride and malate ions to escape from the cell, moving down their electrochemical gradients. The outward flow of these negatively charged ions depolarizes the membrane and triggers the opening of voltage-gated K^+ efflux channels (Fig. 18.3).

In addition to causing stomatal closure, ABA prevents light-induced stomatal opening. In this case, ABA acts by inhibiting the inward K^+ channels which open when the membrane is hyperpolarized by the proton pump. Inhibition of the inward K^+ channels is mediated by ABA-induced increase in cytosolic calcium concentration. Thus, calcium and pH affect guard cell plasma membrane channels in two ways: (1) They prevent stomatal opening by inhibiting inward K^+ channels and plasma membrane proton pumps; (2) They promote stomatal closing by activating outward anion channels, thus leading to activation of K^+ efflux channels. It is thus evident that ABA elicits two types of responses in plants—rapid responses like stomatal closure and more gradual responses like effect on seed dormancy and reactions to abiotic stresses. It is also likely that there are multiple ABA receptors influencing these responses.

18.6 Mechanism of ABA Signaling

Three classes of ABA receptors have been identified till date. These include (i) the *plasma membrane-localized G-proteins* GTG1 and GTG2, (ii) a *plastid-localized enzyme* that coordinates nucleus to plastid signaling, and (iii) *cytosolic ligand-binding proteins* of START (steroidogenic acute regulatory protein-related lipid transfer) domain superfamily. Soluble START domain proteins are the principal ABA receptors which function in short-term as well as long-term responses. A well-worked out pathway for ABA signaling has been designated as PYR/PYL/RCAR pathway. It constitutes members of protein superfamily with a predicted hydrophobic ligand-binding pocket. Their nomenclature reflects their discoveries as PYRABACTIN RESISTANCE 1 (PYR 1) shows resistance to the synthetic sulphonamide compound pyrabactin which mimics ABA action and PYR 1-like (PYL) and regulatory components of ABA receptors (RCARs). This protein subfamily is conserved among plants. They interact with protein phosphatase PP2C in an ABA-dependent manner to regulate the downstream activity of serine/threonine protein kinases of the sucrose non-fermenting related kinase 2 (SnRK2) family. In the absence of ABA, the protein phosphatase (PP2C) keeps the protein kinase

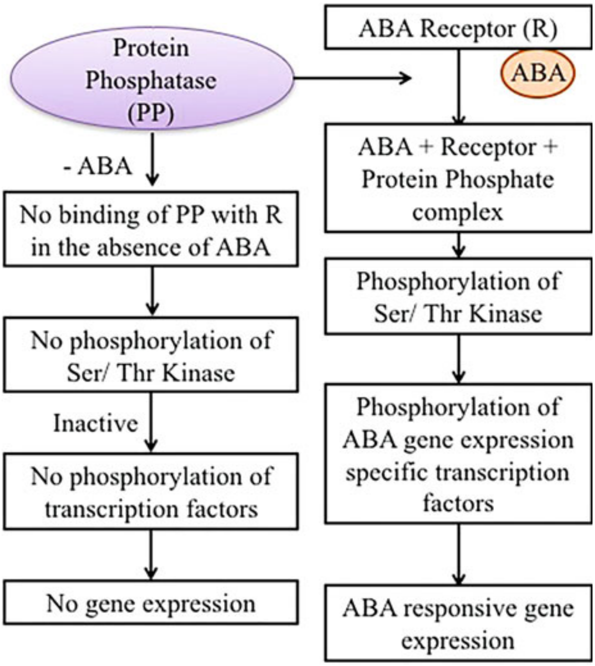


Fig. 18.4 Mechanism of ABA signaling

SnRK2 dephosphorylated and thereby inactivated. For inactivation, PP2Cs bind to the C-termini of SnRK2s and block its activity by removing phosphate group from a region within the kinase domain, termed the activation loop. ABA binding changes the conformation of PYR/PYL/RCAR receptors to permit or enhance interaction with PP2C and thereby repress PP2C phosphatase activity. This releases SnRK2 kinases from inhibition. SnRK2 proteins now activated phosphorylate ABA-responsive transcription factors, thereby inducing ABA responses (Fig. 18.4).

To sum up, ABA action on gene expression operates through a cascade of phosphorylation of transcription factors for ABA-sensitive genes. This phosphorylation remains blocked by keeping a serine/threonine protein kinase in non-phosphorylated state by a protein phosphatase. But with the availability of ABA receptor activated by ABA, this phosphatase activity is reduced, thereby allowing phosphorylation of serine/threonine protein kinase, which then phosphorylates the transcription factors for ABA-responsive genes, resulting in its activation.

Summary

- ABA is ubiquitous in vascular plants and is found in all living tissues. It is a 15-carbon sesquiterpene characterized by a cyclohexane ring with a keto and hydroxyl group and a side chain with a terminal carboxyl group in its structure.

- The asymmetric carbon atom at position 1 in the ring structure of ABA results in formation of S- and R-enantiomers. S-enantiomer is the active form leading to fast responses like stomatal closure. In long-term responses, both the enantiomers are active.
- Various bioassays have been developed to detect and estimate ABA in plant systems. Modern methods such as high-performance liquid chromatography (HPLC) followed by mass spectrometry (MS) are useful for determination of the molecular structure. Highly sensitive and specific immunoassays are also applied for quantification and spatial distribution of intracellular ABA.
- ABA biosynthesis takes place in chloroplasts or amyloplasts via two pathways, namely, isoprenoid pathway and carotenoid pathway.
- The catabolism of ABA is brought about by its hydroxylation at C-8′ position resulting in the formation of 8′-hydroxy ABA, which contains substantial biological activity like ABA. However, phaseic acid (PA) formed as a result of spontaneous cyclization of 8′-hydroxy ABA does not exhibit any ABA-like activity. Very recently, PA has been observed to activate a subset of ABA receptors. ABA is transported through xylem and phloem, but it is much more abundant in the phloem sap.
- ABA plays a central role in triggering plant responses to adverse environmental stimuli and during transition from mid to late embryogenesis. It induces desiccation tolerance in seeds through the synthesis of late embryogenesis abundant (LEA) proteins which are hydrophilic and thermostable proteins that perform protective function against desiccation.
- ABA counters many effects of GA, such as inhibiting the synthesis of hydrolases and α-amylases which are essential for the breakdown of storage reserves in barley seedlings. It is also responsible for maintaining the embryos in their embryogenic state, thereby preventing precocious germination and vivipary. The overall effect of ABA on seed dormancy is influenced by other hormones. Interestingly, rise in ABA production in seeds coincides with decline in IAA and GA level.
- Accumulation of ABA in stressed leaves plays an important role in the reduction of water loss by transpiration under water-stressed conditions. The endogenous hormone, under such stressed conditions, exerts a strong positive effect on root growth by suppressing ethylene production and negative effect on shoot growth. This increases the root:shoot ratio at low water potential which, along with the effect of ABA on stomatal closure, helps the plant to cope up with water stress.
- ABA influences both short-term physiological effects, like stomatal closure, and long-term developmental processes, like seed maturation, and combats drought stress. Rapid responses involve alterations in the fluxes of ions across membranes and may also involve gene regulation. Long-term responses involve major changes in the pattern of gene expression.
- Three classes of ABA receptors have been identified till date. These include (i) the *plasma membrane-localized G-proteins* GTG1 and GTG2, (ii) a *plastid-localized enzyme* that coordinates nucleus to plastid signaling, and (iii) *cytosolic ligand-*

binding proteins of START (steroidogenic acute regulatory protein-related lipid transfer) domain superfamily.
- ABA action on gene expression operates through a cascade of phosphorylation of transcription factors for ABA-sensitive genes.

Multiple-Choice Questions

1. "Dormin" was isolated from which of the following plant species?
   - (a) *Solanum tuberosum*
   - (b) *Lupinus arboreus*
   - (c) *Betula pubescens*
   - (d) *Lemna minor*
2. The cyclohexane ring of ABA has an asymmetric carbon at which position?
   - (a) 1
   - (b) 2
   - (c) 3
   - (d) 4
3. Which of the following is not an ABA-mediated response in plants?
   - (a) Stomatal closure
   - (b) Desiccation tolerance
   - (c) Seed dormancy
   - (d) Promotion of seed germination
4. ABA is synthesized in which of the following cellular organelle?
   - (a) Mitochondria
   - (b) Endoplasmic reticulum
   - (c) Chloroplast
   - (d) Golgi bodies
5. Which of the following is a natural growth inhibitor which has physiological properties similar to ABA?
   - (a) Xanthoxin
   - (b) Violaxanthin
   - (c) Phaseic acid
   - (d) Isopentenyl diphosphate
6. During ABA-mediated stomatal closure, membrane depolarization is caused by net influx of which of the following ions?
   - (a) Chloride
   - (b) Potassium
   - (c) Hydrogen
   - (d) Calcium

Answers

1. c 2. a 3. d 4. c 5. a 6. c

Suggested Further Readings

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