An Insight into Functions and Signal Transduction of Abscisic Acid

Nahla Zakariya P.

PG Department of Botany, Korambayil Ahamed Haji Memorial Unity Women's College, Manjeri, Malappuram, 676122, Kerala, India.

Email: nahlazakariya8@gmail.com

Abstract

Abscisic acid is one of the most important plant growth inhibitors rather than a stimulatory hormone. ABA synthesis occurs in cells containing chloroplasts or amyloplasts. Despite its name reference to abscission, its role extends far beyond that function. The chemical structure of ABA consists of 15 carbon atoms, and its synthesis occurs through two pathways: direct synthesis from mevalonic acid and indirect synthesis from the oxidation of carotenoids within the chloroplast. ABA functions encompass diverse roles, including stomatal regulation during water stress, influencing root and shoot growth, regulating dormancy and germination in seeds, impacting fruit growth and ripening, and participating in senescence regulation. Understanding ABAs signal transduction involves challenges, with multiple putative receptors identified (ABAP1, CHLH, FCA, GCR2) and its involvement in gene transcription, Ca^{2+} signaling, and protein phosphorylation highlighted. Yet, the precise mechanism and interplay with various stress signals remains a subject of ongoing research.

Key words: Abscisic acid, Dormin, Stomatal closure, Stress hormone.

Chapter 5: An Insight into Functions and Signal Transduction of Abscisic Acid

1. Introduction

Abscisic acid is one of the major plant hormones that regulate many aspects of plant growth and development. ABA is ubiquitous in plants. It is also produced by some Phytopathogenic fungi, bacteria and metazoans (Nambara and Marion-Poll 2005). Despite of the name, ABA does not appear to control abscission directly; the presence of ABA in abscising organs reflects its role in promoting senescence or stress responses. This chapter explains the current understanding of ABA structure, biosynthesis, functions and signal transduction.

2. Discovery and occurrence

In 1940 Torsten Hemberg reported that dormant potato tubers and buds of ash (*Fraxinus excelsior*) contained inhibitor that hindered the affect of IAA (Hemberg 1949a, 1949b). Upon germination of the buds, the concentration of these inhibitors decreased. Eagles and Wareing (1963) isolated an inhibitor from the birch (*Betula pubescens*) leaves held under short day conditions. When this substance was reapplied to the leaves of birch seedlings, apical growth was completely arrested. As this substance induce dormancy, they named its as 'dormin' (Eagles and Wareing 1963).

During the same period, Frederick Addicott discovered a substance that regulated the abscission of cotton fruits, and named abscisin II (Ohkuma et al. 1963). Cornforth and his associates demonstrated that both Dormin and Abscissin II where chemically identical and given a common name Abscisic Acid (Cornforth et al. 1965; Addicott et al. 1968). Abscisic acid is a

misnomer for this compound, because it has little to do with abscission. It is mostly found in monocots, dicots, gymnosperms and some ferns. Various genera of fungi produce ABA as a secondary metabolite. In plants ABA is synthesized in almost all cells containing chloroplast and amyloplast, and its presence has been detected in every living tissue, ranging from root caps to apical buds.

3. Chemical structure of ABA

ABA is a sesquiterpene consisting of 15 carbon atoms (Fig. 1). It is unique among plant hormones in having an asymmetric carbon atom. Its structure comprises of six carbon rings with an attached side chain. Because of the asymmetric carbon atom (carbon- 1), it occurs in two enantiomorphic forms, R-abscisic acid and S-abscisic acid. The naturally occurring form is S-abscisic acid. In contrast to auxin, gibberllins and cytokinins, which are represented by various active derivatives, ABA is a single compound.

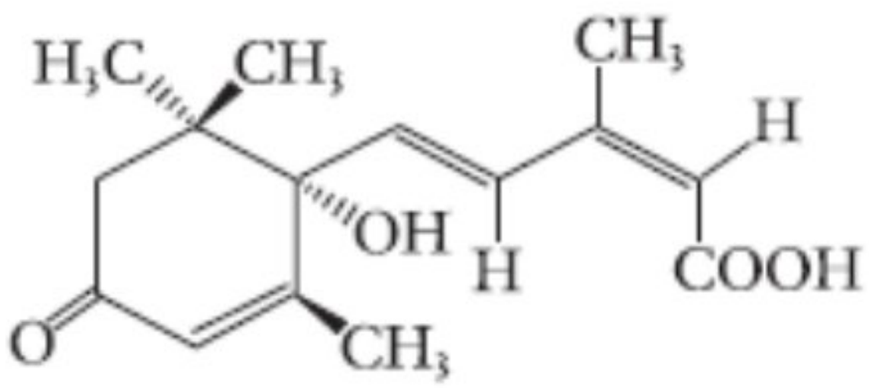

Figure 1. Chemical structure of abscisic acid

4. Biosynthesis of ABA

Two pathways for the biosynthesis of ABA have been identified:

4.1 By direct synthesis from Mevalonic acid

Direct synthesis of ABA from mevalonic acid through farnesyl pyrophosphate has been demonstrated in many cases, especially in water stressed tissues (Hirai et al. 2000; Izquierdo Bueno et al. 2018; Takino et al. 2018). The water stress increases the ABA formation.

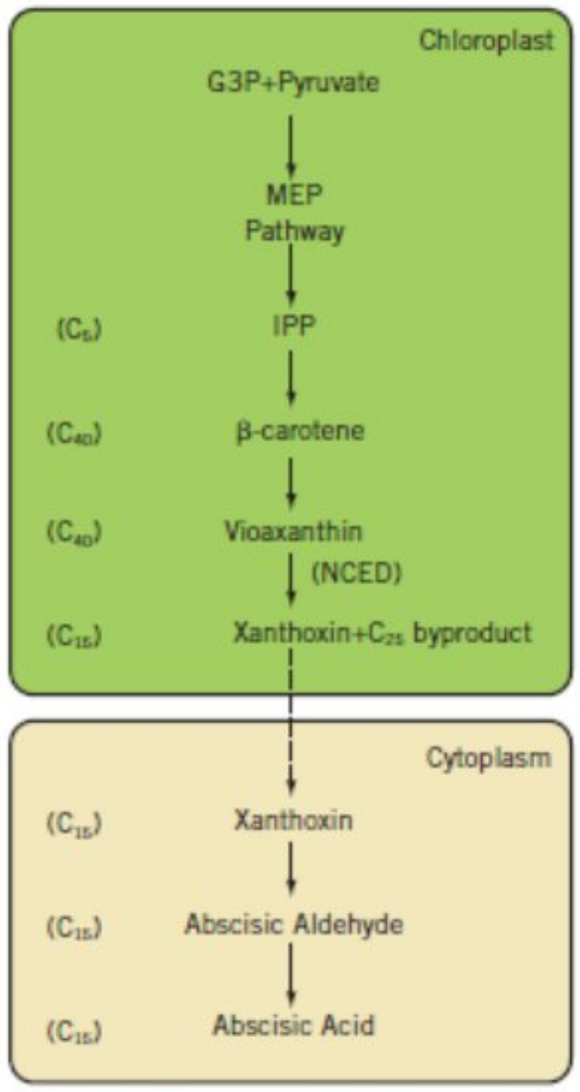

Figure 2. Biosynthesis of ABA from oxidation of carotenoids. G3P- glyceraldehyde 3 phosphate, MEP- methyl erythritol-4-phosphate, IPP- isopentyl diphosphate, NCED- 9'– cis – epoxycarotenoid dioxygenase.

4.2 By indirect synthesis from oxidation of carotenoids

In the chloroplast, isopentyl diphosphate (IPP) is synthesized from glyceraldehyde 3 phosphate and pyruvate via the methyl erythritol-4-phosphate (MEP) pathway. IPP is then converted in to zeaxanthin. Later violaxanthin is synthesized from zeaxanthin in a reaction that is catalysed by the enzyme zeaxanthin epoxidase (ZEP). Violaxanthin is converted in to 9' – cis – neoxanthin which is then converted in to xanthoxal (previously called xanthoxin) by the enzyme 9' – cis – epoxycarotenoid dioxygenase (NCED).

Xanthoxal is then carried to the cytoplasm and converted to ABA aldehyde by the enzyme short chain dehydrogenase (SDR). Finally, ABA aldehyde is converted in to ABA by the enzyme abscisic acid oxidase (AAO) (Nambara and Marion-Poll 2005; Arc et al. 2013) (Fig. 2).

5. Functions

5.1 Stomatal closure

Stomata are pivotal for gas exchange and transpiration of plants, and the closure of stomata can be induced by numerous environmental factors such as drought, pathogen attack, darkness, low humidity, high CO_2 concentrations and so on (Bauer et al. 2013; Assmann and Jegla 2016; Martin et al. 2017; Su et al. 2017). During water stress, ABA concentration increases dramatically in plant leaves, which leads to stomatal closure. Stomatal closure is driven by a reduction in guard cell turgor pressure caused by a large efflux of potassium ion and anions (such as chloride and malate ions) from the cells. Higher ABA concentration in cell increases the cytosolic calcium concentration. ABA stimulates the elevation in the concentration of cytosolic calcium in two ways:

1. By influx of calcium ions through plasma membrane.
2. By release of calcium ions into the cytosol from internal compartments such as Endoplasmic reticulum and vacuole.

Increase in cytosolic calcium causes opening of calcium-activated anion channels on the plasma membrane. The opening of anion channels permits large quantities chloride and malate ion to escape from the cells, moving down their electrochemical gradients. The outward flow of negatively charged chloride and malate ions depolarizes the membrane,

triggering the opening of the voltage-gated K^+ efflux channels and closing of voltage-gated K^+ influx channels. ABA also inhibits the activity of plasma membrane H^+ ATP ase, resulting in additional membrane depolarization. The sustained large efflux of both anions and potassium ions from guard cells contributes to loss of guard cell turgor, which leads to stomatal closing (Pei et al. 2000). The effect of ABA on stomatal apertures under red and blue light varies. Increasing ABA concentration inhibits blue light –stimulated stomatal opening in a concentration dependant fashion, but there is no effect on red light stimulated opening. These contrasting responses to blue and red light can be explained by the effect of ABA on guard cell osmoregulation. ABA concentration has been shown to inhibit proton pumping and potassium uptake, which are central to blue light-stimulated opening. Red light, on the other hand, stimulates guard cell photosynthesis and sucrose accumulation and this osmoregulatory pathway appears to be insensitive to ABA.

5.2 Root and shoot growth

ABA has different effect on root and shoot growth, and the effects are strongly dependent on water status of the plant. Under low water potential, when ABA levels are high, the endogenous hormone exerts a strong positive effect on root growth by suppressing ethylene production, and a negative effect on shoot growth (Watts et al. 1981). Endogenous ABA act as a signal to reduce shoot growth only under water stress conditions.

5.3 Dormancy and germination

ABA is required for the development of desiccation tolerance in the developing embryo, the synthesis of storage proteins and the acquisition of dormancy. The high levels of ABA in maturing seeds inhibit germination.

Many types of dormant seeds germinate when ABA is removed or inactivated. Often, the ratio of ABA to gibberllins determines whether the seed remains dormant or germinates. ABA inhibits the GA-dependant hydrolytic enzyme synthesis that is essential for the breakdown of storage reserves in seeds. Although less is known about the role of ABA in bud dormancy, ABA is one of the inhibitors that accumulate in dormant buds (Yan and Chen 2017).

5.4 Vivipary

ABA-deficient embryos may exhibit precocious germination and vivipary. Vivipary is the germination of mature seed within the fruit on maternal plant prior to dispersal. It is rare in angiosperms and is largely restricted to mangroves where seeds germinate while attached to the mother plants and seedlings are shed, stick in to the mud below, and continue to grow (Farnsworth and Farrant 1998). The phenomenon of seedling formation without completing normal embryonic development is called precocious germination. Inactivated ABA or low levels of ABA can lead to precocious germination and vivipary.

5.5 Fruit growth and ripening

The role of ABA in fruit growth and ripening has been described in several studies. ABA seems to help in fruit ripening of tomato where high ABA where high ABA concentrations coincided with stoppage of fruit growth and initiation of coloring (Mou et al. 2016). Some workers believe that ABA might be inducing ethylene production or vice versa. Citrus fruits treated with ethylene had high amount of ABA. Similarly unripe tomato fruits sprayed with ABA underwent quick ripening. Possibly ABA synthesis

takes place in the pericarp of seeds of the fruit. There are also reports available in other succulent fruits that the ABA level increases during the last phase of development. In some ferns there is high ABA accumulation during spore ripening.

5.6 Senescence

ABA plays a multifaceted role in regulating senescence in plants. Its accumulation often coincides with the onset of senescence. It triggers the expression of genes involved in senescence, leading to the breakdown of Chlorophylls, proteins and other macromolecules in Plants tissues (Gao et al. 2016; Zhao et al. 2016). ABA helps in efficient remobilization of nutrients from senescing tissues to other parts of the plant, ensuring essential elements are redistributed before leaf deterioration. Moreover, ABA interacts with other plant hormones like ethylene and cytokinin to regulate senescence. It often works antagonistically with cytokinin, which delay senescence, while interacting synergistically with ethylene to promote senescence.

5.7 Other ABA responses

Recent findings suggest ABA might play a role in lateral or secondary root development. Although auxin predominantly controls the initiation and growth of lateral roots, ABA can impede lateral root development if applied during early stages before the lateral root meristem organization. Previous studies hinted at ABA's impact on flower formation under specific conditions, but the data lacked clarity, failing to establish a direct relationship between endogenous ABA levels and flowering behavior. However, the idea of ABA's involvement in flowering gained renewed attention when ABA-deficient mutants of Arabidopsis displayed earlier

flowering under conditions that typically delay it in wildtype plants. This observation implies that endogenous ABA might typically restrain or postpone flowering in Arabidopsis. Additionally, the discovery that the FCA gene, known for regulating flowering time, also possesses characteristics of an abscisic acid receptor provides further support for ABA's role in flowering (Rai et al., 2024).

6. Signal transduction

The comprehension of ABA perception and signaling remains intricate despite years of studying its metabolism and physiology. ABA, being a weak acid, is likely present in both protonated and unprotonated forms within the relatively acidic apoplast. It might diffuse across the plasma membrane in its protonated state to interact with intracellular receptors, or in its unprotonated form, it could remain external to the cell and be detected by a site on the plasma membrane. Multiple experiments employing impermeable ABA derivatives or microinjections of ABA into cells suggest the presence of several ABA receptors at various locations. Traditional methods for identifying hormone receptors have been largely ineffective in locating ABA receptors. Recently, anti-idiotypic antibodies, created from antibodies raised against ABA, have been employed. These antibodies mimic ABA's binding characteristics, potentially identifying proteins binding with them as putative ABA receptors. ABAP1, found in the plasma membrane of barley aleurone cells, was identified through this method for its specific and reversible ABA binding in vitro. Following ABAP1's discovery, three other putative ABA receptors have been recognized: CHLH (ABAR), FCA, and GCR2. Despite their in vitro ABA binding abilities, confirming them as true receptors requires demonstrating alterations in ABA functions through loss-of-function

or gain-of-function mutants. The intricate signal chain of ABA effects, upstream and downstream of the hormone, undergoes intensive study (Lim et al., 2022). Understanding the interactions between abiotic signals, receptors, second messengers, and ABA-induced gene transcription poses challenges in creating a definitive scheme. Most of the recent progress has been made through newly discovered ABA-insensitive gene mutations and can be summarized in the following points.

- Events sensing abiotic stress and initiating ABA accumulation remain unknown despite rapid ABA turnover in both stressed and unstressed plants.
- Ca^{2+} plays a significant role in the ABA signal chain, especially in stomatal guard cells, mediating ABA-induced turgor adjustments by activating plasma membrane anion channels.
- The promoter region of certain genes contains an ABA response element (ABRE), where transcription factors (ABFs) regulate ABA-induced genes, aiding the plant in adapting to water stress.
- ABA-insensitive mutants like abi3, abi4, and abi5 hinder seed germination and early seedling development, suggesting ABA's role in seed development requires gene transcription.
- ABA-activated protein kinases positively regulate ABA responses, while protein phosphatases like ABI1 and ABI2 negatively regulate them, highlighting the importance of protein phosphorylation in ABA signaling.

Constructing a comprehensive model of the signaling chains for diverse ABA-mediated responses will likely require time, considering the numerous components yet to be discovered.

7. Conclusion

Abscisic acid (ABA) stands out as a pivotal regulator in the realm of plant growth and development. Despite its misleading name, significance of ABA extends far beyond abscission control, influencing various physiological processes across different organisms, including plants, fungi, bacteria, and metazoans. Originally discovered as a growth inhibitor in dormant potato tubers and ash buds, ABA's chemical structure, biosynthesis pathways, and multifaceted functions have been elucidated over the years. With its unique sesquiterpene structure, ABA exists in two enantiomorphic forms, primarily as S-abscisic acid. Its biosynthesis involves both direct synthesis from mevalonic acid and indirect synthesis from carotenoid oxidation. Functionally, ABA plays a central role in stomatal closure, roots and shoots growth modulation, dormancy induction, and germination regulation. Additionally, ABA influences fruit growth and ripening, senescence, and potentially lateral root development and flowering. Understanding its signal transduction mechanisms remains complex, with ongoing research exploring its perception and signaling pathways. In essence, pervasive presence and diverse roles of ABA underscore its importance as a fundamental regulator in plant physiology, offering potential applications in agriculture and biotechnology. Continued research promises deeper insights into its complexities, paving the way for innovative strategies to harness its beneficial effects in plant science and beyond.

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