Drought Signaling in Plants

G Sivakumar Swamy

Plants are firmly anchored in the soil, and they cannot evade the vagaries of nature by moving towards a convenient location as we normally encounter in case of animals. However, they are endowed with an ability to perceive the advent of an adverse environmental condition and also take adequate precaution to overcome such a condition. The current article deals with one such response of the plants when they face a condition of drought.

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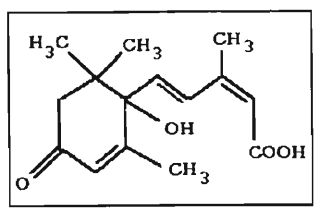
Plants can Communicate by Producing Signaling Molecules

Periodic droughts are of common occurrence these days which cause severe loss in crop productivity and upset agricultural economy. However, plants have evolved an inherent mechanism to overcome the effect of drought to a certain extent. This is accomplished by reducing the loss of water, and also by adjusting physiologically to the water deficit conditions. The occurrence of water deficit in the soil is first sensed by the plant roots, and a signal is generated which prepares the plants to sustain themselves in the drought condition. The signaling molecule in this case is a plant hormone, *abscisic acid* (ABA) (*Figure* 1)

What is Signaling?

A vast majority of plants and animals are multicellular in nature. Communication between cells is most essential in multicellular organisms for coordination of functions, so that the entire body of the organism functions as a single unit. Communication between neighbouring cells in the plant body can occur by direct cell-to-cell contact through intercellular cytoplasmic bridges known as plasmodesmata. Communication can be established between cells which are far apart by the secretion of

Figure 1. The chemical structure of abscisic acid (ABA)



chemical signals, and the perception of those signaling molecules by the target cells. In addition, environmental factors such as light, temperature, wind, water, minerals, gravity, and soil structures, would also act as signals and influence the structure and function of the plant body. Apart from the chemical communications between the cells of the plant body, and perception of the signals from the environment, communication also exists between different individuals of either the same species or of two different species.

Signaling in plants may be broadly classified into four categories depending upon the source and nature of signaling: (i) hormone signal, (ii) developmental signal, (iii) defence signal and (iv) environmental signal. It is also common that more than one of the above types of signaling may be involved in bringing about a response. There also occurs crosstalk in signaling.

Plant can Undergo Structural Adjustments to Reduce Water Loss

Anatomically, the plant leaf is surrounded by a layer of tissue of single cell thickness known as epidermis. Anatomical structure of a typical plant leaf and its position in the plant body in relation to water supply from the root system is diagramatically shown in *Figure* 2. The lower epidermis of the leaf is not continuous. It consists of pores called stomata. The stomata are involved in gaseous exchange between the inner tissues of leaf and the atmosphere. They are also the conduits for the escape of water in the form of vapour, the process being known as transpiration. The stomata are surrounded by a pair of curved cells (crescent shaped) called guard cells. The concave sides of the curved guard cells encircle the stomatal pore, and they consist of very thick walls. The convex sides of the guard cells are thin walled (*Figure* 3a).

The process of transpiration through stomata is an essential function of the plants to maintain the transport of water and inorganic nutrients from roots to leaves. But, under the condition of water deficit condition in the soil, continuous loss of water by

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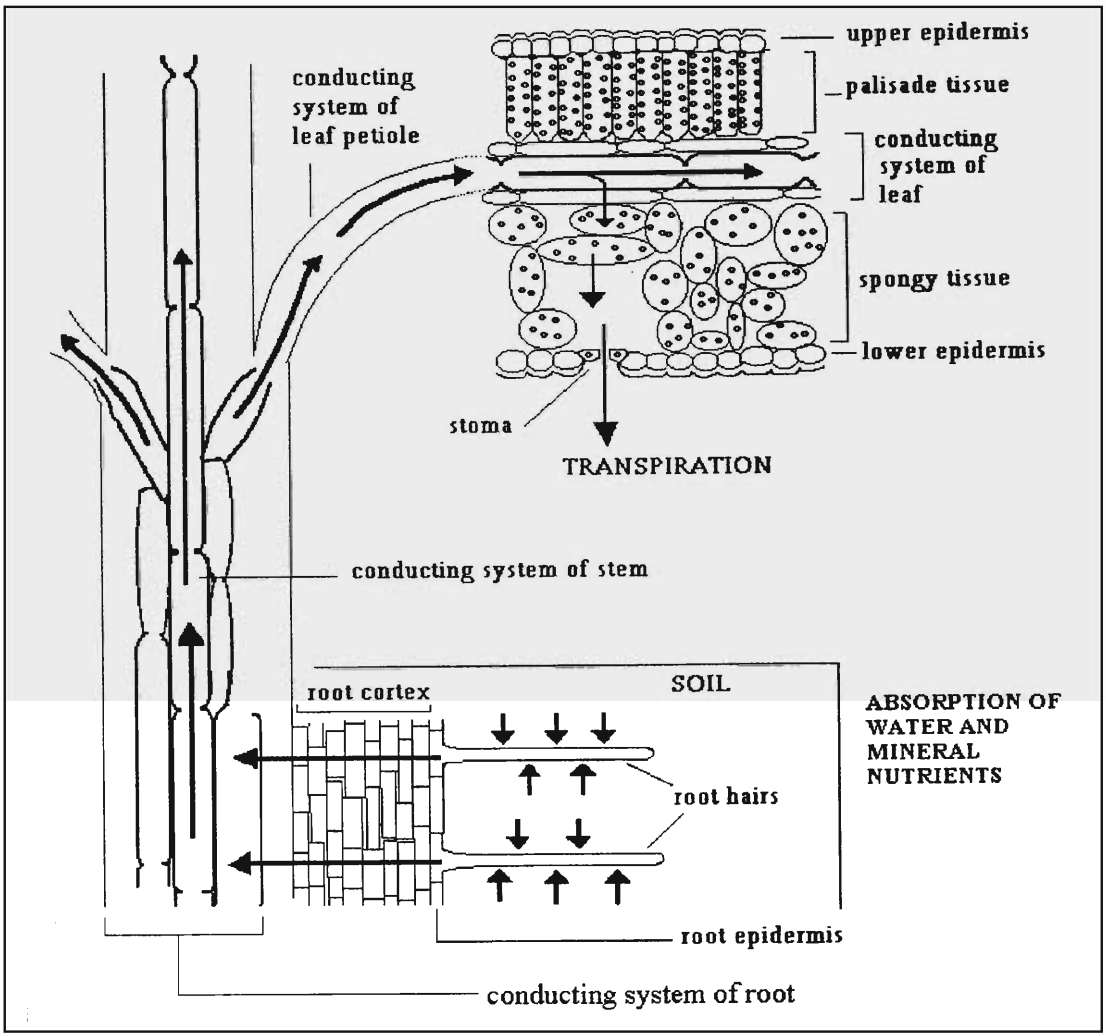
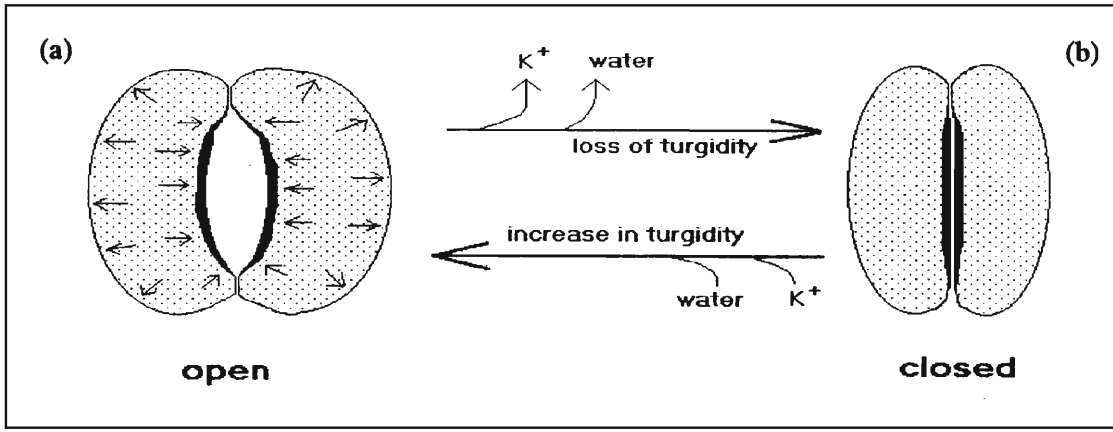


Figure 2. Schematic representation of the entry of water into the plant roots from the soil, transportation through the conducting system of the stem, and evaporation (transpiration) through the stomata of the leaf. Water and inorganic nutrients are absorbed by the roots, mainly through the root hairs. Then, the water enters into the conducting tissue at the center, through the peripheral root cortex. An unbroken column of water is always maintained in the conducting tissue, from the root tip to the tip of the veins in the leaf. The water column in the conducting tissue is pushed upwards by the root pressure developed in the root, and also pulled upwards by the transpiration cohesion tension force. The transpiration cohesion tension force is established by the continuous evaporation of water into the intercellular spaces of leaf tissues which in turn draw up water from the water column present in the conducting system of leaf. The loss of water from the water column in the leaf veins results in the pulling up of water column in the conducting tissue. The water evaporated into the leaf intercellular spaces escapes out through stomata, the process being termed as transpiration. The arrows shown in thick lines represent the direction of water movement.



transpiration would lead to wilting of the plants which may eventually cause death. A mechanism has been evolved in plants to regulate the rate of transpiration through minor structural changes in the stomata. This is brought about by closing the stomata under the conditions of water deficit, and opening when there is enough water supply.

Figure 3. Diagram showing the guard cells as seen on the leaf surface, encircling the stomatal aperture. The stoma on the left side (a) is open because of the accumulation of K^+ followed by influx of water in the guard cells resulting in the increase of turgidity. The arrows facing the cell wall inside the guard cells represent the turgor pressure being exerted on the cell wall of the guard cell. The stoma on the right hand side (b) represents the closed one due to loss of turgidity as the result of efflux of K^+ and water from the guard cells.

Stomatal Opening is Controlled by Guard Cell Turgidity

The opening and closing of stomata depends on the turgidity of the guard cells. If the guard cells absorb more water and become turgid it creates a turgor pressure inside these cells. Due to this turgor pressure from within, the outer thin walls of the guard cells on the convex sides get stretched. This kind of stretching is comparatively very little in the inner thick walls surrounding the stomatal pore. As a result of unequal stretching of the outer and inner walls the guard cells become more curved. This results in the increase in the pore size of the stomata. If the guard cells lose their turgidity, the reverse process would occur. The inner thick walls would get straightened due to loss of turgidity, and the stomata would close. Opening and closing of stomata are shown diagramatically in *Figure* 3a and 3b.

Guard Cell Turgidity is Controlled by Solute Concentration in the Guard Cells

It is evident that guard cell turgidity is responsible for the

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opening and closing of the stomata. The turgidity of the guard cells in turn is maintained by the solutes in the cell sap of the guard cells. If the concentration of the cell sap increases due to increase in the solute concentration, water from the surrounding would naturally enter the guard cells due to osmosis, thereby increasing the turgidity. The decrease in solute concentration would naturally bring about loss of turgidity referred to as plasmolysis. Therefore, the regulation of solute concentration in the guard cells regulates the guard cell turgidity which in turn controls the opening and closing of stomata.

K^+ Influx and Efflux in the Guard Cells are Controlled by Membrane Potential

The guard cell plasma membrane has all the major transport systems present in plants [1]. The plasma membrane bound ATPase acts as a proton pump, which hydrolyzes ATP and transports protons from the cytoplasm to the cell exterior, thereby generating a transmembrane potential. There are channels for the transport of K^+, Cl^-, Ca^{2+}, etc., in the plasma membrane which would specifically allow the respective ions to pass through the membrane. Increase or decrease in turgidity in the guard cell is mainly brought about by the increase or decrease in K^+ concentration.

K^+ transport across the guard cell plasma membrane is mediated by two kinds of channels, (i) inward rectifying $K^+_{(in)}$ channels and (ii) outward rectifying $K^+_{(out)}$ channels. The $K^+_{(in)}$ channel functions at maximal capacity when the transmembrane potential of guard cell is relatively high (hyperpolarized). The half maximal activity of this channel occurs at the membrane potential of –190 mV. On the other hand, the half maximal activity of $K^+_{(out)}$ channel is at 75 mV, and therefore, it functions at the maximal rate when the guard cell membrane potential is low (depolarized). The $K^+_{(in)}$ and $K^+_{(out)}$ channels are involved in transport of K^+ into the cell, and efflux of K^+ from the cell, respectively. ABA-induced membrane depolarization activates the opening of $K^+_{(out)}$ channel and inactivate $K^+_{(in)}$ channel (*Figure* 4). This results in

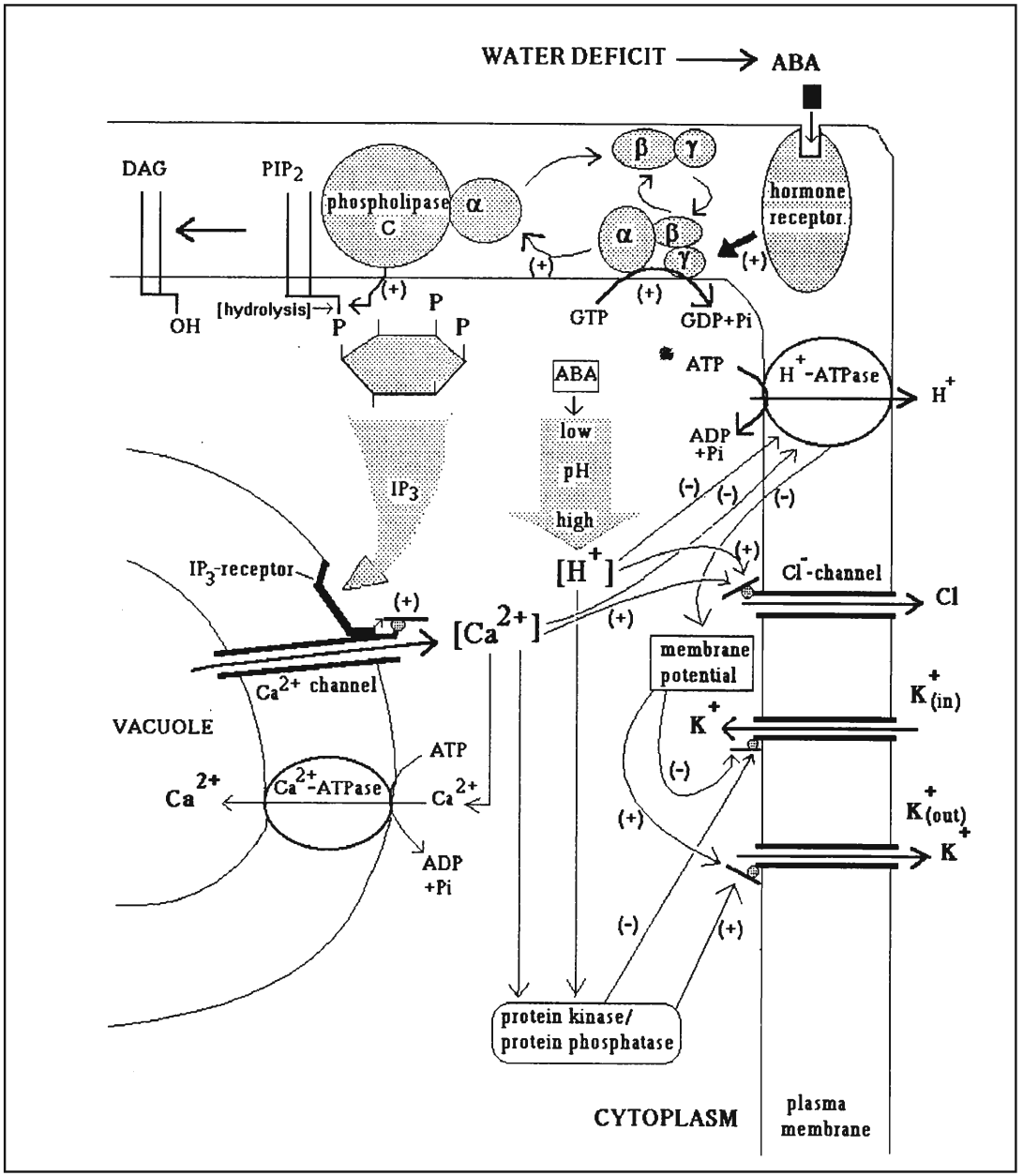

Figure 4. Diagram representing the signal transduction cascade system induced by water deficit resulting in the opening of $K^+_{(out)}$ channels and closing of $K^+_{(in)}$ channels. The detailed mechanism is described in the text.

Note: The (+) and (-) signs shown on the lines indicate the ABA-induced stimulation or inhibition of the respective process; the same signs shown on the ion channels represent the opening and closing of the gated ion channels, respectively.

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the loss of K^+ and turgidity in the guard cells leading to stomatal closure.

Abscisic Acid Elevates Cytoplasmic Ca^{2+} Level by Opening Ca^{2+} Channels

In plants, the cytoplasmic Ca^{2+} level is always maintained at a very low concentration (<10mM), while the Ca^{2+} concentration in the vacuole and the cell exterior (apoplast) is very high (often exceeding 1mM). The concentration of the plant hormone, ABA, is increased in the plant body when the plant is subjected to drought. This hormone in turn is capable of increasing cytoplasmic Ca^{2+} level by stimulating the opening of the gated channels in the vacuolar membrane. Since there always occurs a steep concentration gradient in Ca^{2+} concentration between the cytoplasm and the vacuole, Ca^{2+} would enter into the cytoplasm driven by an electrochemical gradient Ca^{2+} when the channels are open. The opening of Ca^{2+} channels by ABA is caused by a cascade of signal transducing events. The cascading events and the consequent changes in the cellular functions are shown in *Figure* 4.

The signaling cascade begins with the possible binding of ABA to a receptor protein present in the plasma membrane. Although an ABA receptor protein has not yet been characterized in the guard cell plasma membrane there is evidence to believe the existence of an ABA receptor. The resulting receptor-hormone complex appears to stimulate GTPase (GTP hydrolyzing) activity through a regulatory protein molecule known as G-protein. This regulatory protein consists of three subunits, viz., α, β, and γ. As we find in a parallel situation in animal cells, the G-protein seems to be inactive when all the three subunits are associated together forming a complex, and becomes active when α-subunit is separated from this complex followed by GTPase activity.

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Now, there is enough evidence to show that heteromeric (protein with subunits of different sizes) G-protein is involved in guard cell signaling in plants. The GTPase activity mediated by G-

protein is triggered when ABA binds to its putative (supposed) receptor protein molecule in the plasma membrane. The putative receptor-ABA complex may act as a catalyst to separate the α-subunit from the G-protein heteromeric complex. In animal system, the α-subunit of G-protein stimulates a membrane-bound enzyme, phospholipase C. This enzyme catalyzes the hydrolysis of the membrane lipid, phosphoinositol-bis-phosphate (PIP-2) into diacylglycerol (DAG), and releases its inositol triphosphate (IP-3) moiety into the cytoplasm. IP-3, thus released from the plasma membrane, acts as a ligand to open the gated Ca^{2+} channel. This process would result in the release of stored Ca^{2+} into the cytoplasm. In plants, ABA is shown to trigger both IP-3 production and the elevation of cytoplasmic Ca^{2+} level in the guard cells. The pathway for the elevation of cytoplasmic Ca^{2+} level in the guard cells of the plants appears similar to that of animal cells. This cascade of events would eventually lead to an increase in the Ca^{2+} concentration in the guard cell cytoplasm. Ca^{2+}, now takes over the role of a second messenger mediating the action of ABA. The Ca^{2+} level in the cytoplasm is subsequently brought down to the normal level by a calcium pump (Ca^{2+}-ATPase) present in the vacuolar membrane. This enzyme utilizes cellular energy (ATP) and pumps Ca^{2+} against a concentration gradient, back into the vacuole. Ca^{2+} pump is also present in the plasma membrane which pumps Ca^{2+} into the apoplast (outside the cell). In fact, elevated cytoplasmic Ca^{2+} concentration is thus balanced. It is also evident that the cell has to keep spending energy (in the form of ATP) to maintain a low cytoplasmic Ca^{2+} concentration.

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Ca^{2+} Acts as Second Messenger and Inhibits K^+ Intake by Guard Cells

Ca^{2+} is known to regulate the K^+ channels in the plasma membrane of the guard cell. Increased cytoplasmic Ca^{2+} generated by the ABA signaling via IP-3 release induces the closure of $K^+_{(in)}$ channel, and stimulates the opening of Cl^- channel. The closure of $K^+_{(in)}$ and opening of Cl^- channel would prevent the K^+ intake by the guard cells, and in addition, causes

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the loss of Cl^-. As a result, the guard cells cannot acquire the required solutes for developing turgidity, and consequently, the stomata remain closed. In fact, it has been experimentally shown that by microinjecting IP-3 into the guard cell with a microsyringe, cytoplasmic Ca^{2+} concentration increased, and K^+ intake decreased in the guard cell. These results add support to the view that ABA-induces signal cascading system which is mediated by IP-3 and Ca^{2+}, decreases K^+ level in the guard cell which in turn reduces guard cell turgidity causing stomatal closure.

Abscisic Acid Causes Alkalinization of the Guard Cell Cytoplasm. Does H^+ Also Act as Second Messenger?

Although ABA-induced increase Ca^{2+} concentration in the guard cell cytoplasm is found to be mainly responsible for K^+ channel regulation leading to stomatal closure, this phenomenon appears to be still more complex. It has been experimentally shown that by artificially manipulating guard cell cytoplasmic pH (H^+ ion concentration) with weak acids and bases, K^+ currents (which represent the opening of K^+ channels) could be regulated. At low cytoplasmic pH, $K^+_{(in)}$ channels were largely open, and $K^+_{(out)}$ channels were closed. The opposite situation was observed when the cytoplasmic pH was manipulated to increase, resulting in the opening of $K^+_{(out)}$ channels, and closing of $K^+_{(in)}$ channels. Interestingly, it has also been demonstrated that ABA causes guard cell cytoplasmic alkalanization. The molecular mechanism and the substrate source for ABA-regulated cytoplasmic alkalanization are not yet known. However, there is now considerable evidence for the existence of ABA binding sites in the plasma membrane as well as in internal sites. These results would suggest the regulation of K^+ channels by the alteration of cytoplasmic pH caused by ABA. Therefore, H^+ also acts as a second messenger of hormone action in plants.

Guard cell cytoplasmic pH and Ca^{2+} levels may be mutually exclusive in their action in the regulation of K^+ channels. It was

experimentally shown that changes in cytoplasmic pH, the resulting K^+ channel gating, and the concomitant stomatal regulation do occur without any significant changes in cytoplasmic Ca^{2+} levels in the guard cells. These results indicate that cytoplasmic pH is independent of Ca^{2+} in stomatal regulation. On the other hand, Ca^{2+} dependent inactivation of $K^+_{(in)}$ channels and its effect on the closing of stomata were also observed when the cytoplasm was artificially buffered preventing the pH to increase. Therefore, the action of these two messengers appears to be independent of each other. It is not yet clear whether under natural conditions the cytoplasmic pH would affect the binding of ligands in the calcium signaling system due to changes in ionization of the binding proteins. This may trigger calcium dependent events even in the absence of elevated cytoplasmic Ca^{2+} levels. Further, there is also evidence for the involvement of cytoplasmic pH in the regulation of protein phosphorylation and dephosphorylation. Phosphorylation and dephosphorylation of channel proteins have been found to regulate the channel opening and closing. In fact, catalytic functions of proteins are known to be altered by the addition of a phosphate group to the functional protein (by protein kinase

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Box 1. Protein Phosphorylation and Dephosphorylation are Involved in the Regulation of Stomatal Movement

Living cells contain a large number of enzymes and other functional proteins. The role of individual enzymes and proteins in the regulation of cellular metabolism can be determined by either inactivating that particular protein or stimulating its activity. This is normally achieved by using specific chemical compounds of extraneous source which either inhibit or stimulate the activity of the required enzyme or protein when those compounds are supplied to cells or tissues.

Okadaic acid and cyclosporin A inhibit protein phosphatases. It has been found that these two compounds enhance the closing of stomata. Similarly, the protein kinase inhibitor, K-252a, an inhibitor of serine/threonine protein kinases, inhibited stomatal opening. The chemical compound, phorbol myristate acetate is known as a stimulator (agonist) of protein kinase C. This compound was found to enhance stomatal opening. All these results indicate that protein phosphorylation and dephosphorylation are the controlling factors in stomatal movement. It is now believed that multiple protein kinases and phosphatases are involved in guard cell responses to ABA.

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activity), or the removal of the phosphate group (by protein phosphatase activity) from that protein. This process is regulated by the signaling cascade involving Ca^{2+} and H^+ as second messengers (*Figure* 4).

Conclusions

The ABA-induced changes in Ca^{2+} and H^+ concentrations inhibit H^+-ATPase activity resulting in the reduction of membrane potential. Under reduced membrane potential, $K^+_{(in)}$ channels are closed and $K^+_{(out)}$ channels are open. Therefore, this results in the loss of K^+ from the guard cell leading to stomatal closure. In addition, it is more likely that changes in ABA-regulated cytoplasmic H^+ and Ca^{2+} levels may act together in phosphorylation and dephosphorylation of channel proteins, and bring out the response of ABA in closing the $K^+_{(in)}$, and opening the $K^+_{(out)}$ channels (*Figure* 4). Thus, a clear picture of signal-induced stomatal regulation in response to water deficit is now beginning to unfold. Here, we find the increased ABA concentration induced by water deficit acting as a signal for stomatal closure which would reduce the loss of water from the plant body.

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