FROM SIGNAL PERCEPTION TO GENE EXPRESSION: EARLY RESPONSES OF PLANTS TO DROUGHT.

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Abstract

Plants are continuously exposed to biotic and abiotic stresses, such as pathogens (fungi, bacteria, viroids, and virus), drought, extreme temperature, trace elements, or salinity. Drought stress can severely impair plant growth and production. However, as a function of stress intensity, various defense responses can take place in plants. The aim of this review is to present first responses of plants, meaning stress perception, signal production and amplification and expression of first genes involved in defenses. In order to counteract drought effect, plants developed two main defense strategies, meaning the abscisic acid dependent and independent pathways.

INTRODUCTION

Among the diverse stress factors plants have to face, drought severely impairs plant growth and development and limits crop yield. When water stress is a short period of a couple of hours, defense mechanisms are efficient. However, the capacity to tolerate a longer period of drought, such as a particularly warm summer, can severely affect plant metabolism. Available water resources for successful crop production have been decreasing in recent years in many countries. Furthermore, various climatic change models indicate that in several areas of world, crop losses due to increasing water shortage will be more important than actually. The consequences of drought on plants include a reduction of membrane integrity and photosynthetic pigment content as well as disturbs in osmotic adjustment, leading to a lower growth rate and a decrease in plant productivity [1-3]. The susceptibility of plants to drought stress depends on stress intensity and duration, plant species and its developmental stage when drought occurs [4]. It has become necessary to elucidate the responses (early and late) and adaptations of plants to water deficit in order to improve the drought resistance ability of plants and to ensure higher crop yields against unfavorable environmental conditions.

I. Drought perception and signal propagation

Soil water moves down a gradient of water potential from soil to roots (Fig. 1). As water evaporates from a leaf, it moves from higher water potential within the leaf to much lower water potential of surrounding air. Water evaporation from leaf surface reduces water potential of sap in the leaf and creates negative pressure. This negative pressure created by water evaporating pulls up water through water-conducting cells (xylem) in the stem.

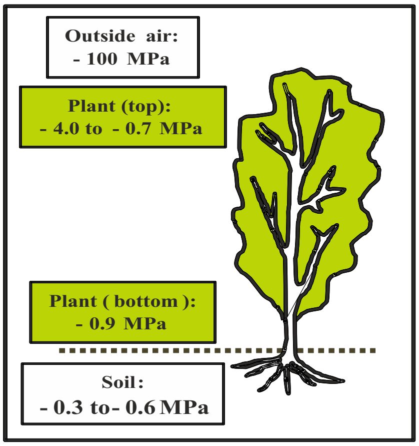

Figure 1.
Evolution of water potential in plant from bottom to top organs. Values are expressed in Mega Pascal (MPa) and water potential of pure water is set at 0 by convention.

Drought perception is due to a modification of soil and air water potentials: the reduction of water availability in soil generates a first signal in plant roots whereas the decrease of atmospheric humidity, which is often accompanied by high temperatures, induces another signal in leaves.

1. Membrane modifications and production of secondary messengers

The lack of water at root level causes a destabilization of membranes (Fig. 2) [5]. Indeed, membranes are mainly composed of phospholipids and the phosphate part is bound to water molecules *via* hydrogen atoms. The disappearance of water molecules leads to a loss of membrane integrity. This phenomenon also occurs at leaf level but is more due to high temperatures. Indeed, the lipid parts of phospholipids change their structure when temperature increases and tend to become fluidized, leading to a loss of membrane integrity similar to what is described in roots.

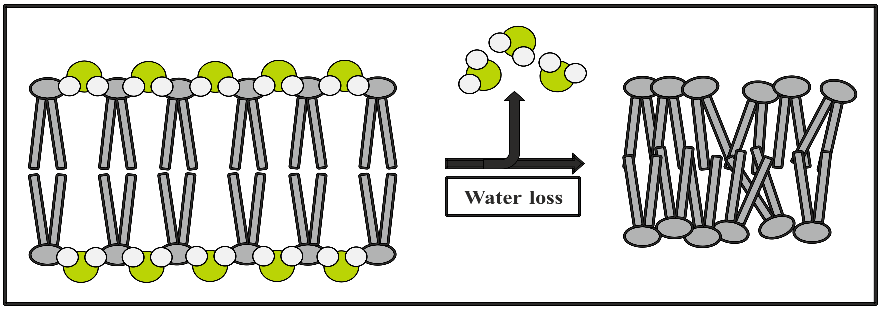

Figure 2. Effects of water loss on membrane integrity. The schema of a hydrated membrane is represented on left with phospholipids (in grey) and molecules of water (in green and white). The elimination of water molecules leads to a disorganization of phospholipids (schema on the right).

The modification of membrane organization has rapid consequences on other membrane compounds such as channels and transporters. Among them, two elements play an important role in the initiation of drought responses:

- The first element is **calcium channels** which increase calcium influx when membranes are modified. The cytosolic accumulation of calcium ions is considered as an essential secondary messenger in drought signaling.
- The second element is related to **nitrate uptake**: the lack of water in soil limits the influx of minerals and more particularly nitrate ions by the low and high affinity transport systems [6]. As a consequence, nitrate content rapidly decreases in cytosol and nitrate reductase activity becomes lower and lower. In addition to the reduction of amino acid synthesis (via the GS/GOGAT pathway), the reduction of nitrate concentration causes an increase in pH in cytosol and xylem sap. This variation of pH participates to the amplification of stress signal. However, the variation of pH is not registered in all plants in response to drought and soil composition may influence plant responses in terms of pH modification [7].

The loss of membrane integrity caused by the lack of water and high temperatures initiates a third concomitant signal in cells. Indeed, it was often shown that membrane alteration is accompanied by the production of **reactive oxygen species (ROS)**, which are considered as efficient secondary messengers (as well as calcium ions) in drought stress signaling. The most important source of ROS is related to electron transport chains in mitochondria (for respiration) and chloroplasts (photosystems for photosynthesis) [8].

2. Secondary messengers and signaling

Calcium ions which accumulated as a response of drought stress are bound to proteins called sensors. These sensors change their conformation in a Ca^{2+}-dependent manner. Most of them bind Ca^{2+} using a helix-loop-helix motif termed the "EF hand" (corresponding to the E and F regions of parvalbumin), which binds a single Ca^{2+} ion with high affinity. The major families of Ca^{2+} sensors include calmodulin (CaM), CaM-like proteins, calcium-dependent protein kinases (CDPKs), mitogen-activated protein kinases (MAPKs), calcineurin B-like proteins, and calreticulin [9, 10]. Several CDPKs are activated directly by Ca^{2+} whereas others are regulated by CaM or concomitantly activated by Ca^{2+} and CaM [11].

Calcium accumulation in cytosol is not only caused by an influx but also by a release of calcium stored in various organelles such as vacuoles or endoplasmic reticulum [10]. For example, Ca^{2+} activates a membrane phospholipase C which converts phosphatidyl 4,5-bisphosphate (PIP2) into inositol 1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol (DAG) (Fig. 3). IP3 activates channels of endoplasmic reticulum, leading to a release of Ca^{2+} to cytosol whereas DAG activates a sensor (protein kinase PKC) previously modified by Ca^{2+}.

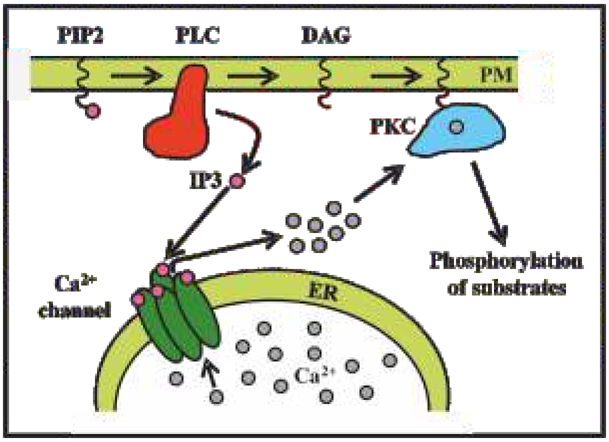

Figure 3.
Contribution of intracellular calcium to the amplification of drought signal. See the text for abbreviations.

ROS interact directly with kinases such as mitogen-activated protein kinases (MAPKs) or bind first with sensors (not well identified), which then activates kinases. Moreover, it was shown that ROS can concomitantly inhibit several phosphatases.

Kinases directly or indirectly activated by Ca^{2+} and ROS phosphorylates other kinases to amplify the signal. At the end of this cascade of phosphorylation, several constitutive transcription factors (TFs) are activated [12]. Moreover, CaM and ROS can directly bind several of these TFs. Some TFs are more or less specific to drought stress whereas others are regulated in response to various stresses such as soil salinity or cold. However, these constitutive TFs are still poorly known.

II. Early expression of genes

Plant responses to drought involve two periods. Indeed, constitutive TFs activate genes considered as the "early response" after few minutes, whereas the "delayed response" needs sometimes several hours to be efficient. The early response corresponds to transcription of genes encoding other TFs which can be divided into two main categories.

The first category is called the abscisic acid (ABA)-independent pathway. The main involved TFs contain a DREB domain (Dehydration Responsive Element Binding) which binds to gene promoters containing a conserved sequence DRE (5'-TACCGACAT-3') and another conserved sequence called CRT for C-repeat Responsive Element (5'-CCGAC-3') [13]. Other TFs are involved in the ABA-independent pathway such as ZF-HD (Zinc-Finger Homedomain) but literature is not well documented for them.

The second category corresponds to the ABA-dependent pathway. TFs are much more important than ABA-independent TFs. For example, it was shown than several hundred or thousand TFs are regulated by ABA in *Arabidopsis thaliana* depending on the intensity of drought stress [1]. These TFs contain an AREB/ABF (ABA-Responsive Element Binding / ABA-responsive element Binding Factor) domain interacting with promoters containing ABRE (ABA-Responsive promoter Element) sequences (C/TACGTGGC). Other TFs were identified as regulated by ABA such as MYC / MYB (myelocytomatosis / myeloblastosis oncogene) or bZIP (basic leucine zipper) factors [14].

III. Later expression of genes

TFs previously indicated up- or down-regulate the transcription of many genes.

1. ABA dependent pathway

This pathway is probably the most important for plant responses against drought since it stimulates various defense mechanisms.

1.1. Signal amplification

Several genes involved in ABA synthesis are among the first genes stimulated as reported on the figure 4. Indeed, the increase of ABA content is required to amplify and accelerate many synthesis pathways.

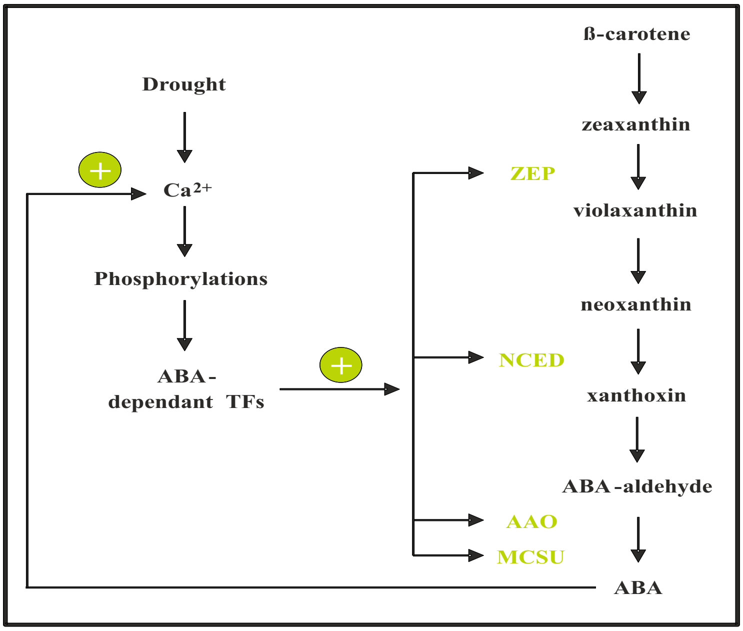

Figure 4.
Induction of ABA synthesis as a response of drought. Drought induces the accumulation of Ca^{2+} and a subsequent cascade of phosphorylations. The TFs which are synthesized up-regulate the expression of genes encoding enzymes involved in ABA synthesis. Then the accumulation of ABA can stimulate the accumulation of cytosolic Ca^{2+} via the activation of calcium channels. ZEP, zeaxanthin epoxidase; NCED, 9-*cis*-epoxycarotenoid dioxygenase; AAO, ABA-aldehyde oxidase; MCSU, MoCo sulfurase.

ABA-dependent accumulation of PAP (3'-phosphoadenosine 5'-phosphate) in chloroplasts was shown in response to drought [15]. Although the regulation is not well known, the hypothesis is an inhibition of SAL1, a nucleotidase involved in the transformation of PAP into AMP (Adenosine MonoPhosphate). PAP is exported to the nuclear compartment *via* an unidentified transporter and inhibits exoribonucleases. Then, several RNA can be transcribed into TFs and participate to signal amplification.

1.1. Reduction of water flux

The accumulation of ABA stimulates Ca^{2+} accumulation and modifies pH. The consequences are an inhibition of H^{+}-ATPase activity which reduces membrane potential. This modification leads to a closure of channels responsible of K+ entry and an opening of channels allowing the efflux of K^{+} and anions such as Cl^{-} [16, 17]. An example of channel regulation is presented on figure 5 according to [17]. In guard cells, this modification of ion influx and efflux causes a turgor decrease and leads to the stomata closure. This closure plays an important role in plant defense against drought since it limits the evapotranspiration.

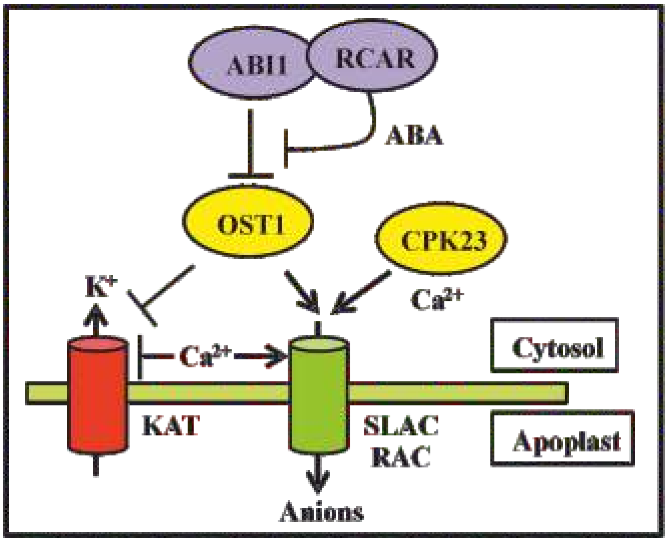

Figure 5.

Regulation of ion flux in guard cells by ABA. The ABA receptor is formed by a phosphatase ABI1(ABA Insensitive) and an ABA-binding element RCAR (Regulatory Component of ABA Receptor). The phosphatase activity of ABI1 inhibits the action of the protein kinases OST1 (Open STomata protein kinase) and CPK23 (Ca^{2+}-dependent Protein Kinase). When ABA binds to RCAR, the change of conformation blocks ABI1. As a consequence, OST1 and CPK can be active and activate SLAC/RAC (SLow and Rapid Anion Channels) and inhibit the potassium channel KAT.

In parallel, several genes encoding PIPs (Plasma membrane Intrinsic Proteins) are regulated in response to water stress. PIPs are a subfamily of plant aquaporins that facilitate the diffusion of water and small uncharged solutes through the cell membrane. The gene regulation and the post-translational mechanisms of PIPs regulation are complex and new regulatory processes are still being discovered [18]. These mechanisms are related to the conformation of PIPs monomers, their phosphorylation, their stability in the membrane, and their trafficking or subcellular localization. It is difficult to generalize the regulation of PIPS as a response to drought since several PIPS can be stimulated whereas others are down-regulated in drought conditions [11, 18, 19]. Moreover, it was shown that several PIPs can be regulated in an ABA-independent manner. The PIPs regulation is probably related to the intensity of drought and the length of stress period.

1.1.Antioxidative responses

As previously indicated, water stress generates the accumulation of ROS which are involved in signalization. However, a high concentration of ROS can be dangerous for cells since it generates membrane and protein oxidations. Genes encoding anti-oxidative molecules are up-regulated as a response to drought such as those involved in glutathione and ascorbate synthesis [20]. Moreover, many ROS-scavenging enzymes are up-regulated such as superoxide dismutase, catalase or various peroxidases [3, 20].

2. ABA independent pathway

Several studies showed that this pathway up-regulate genes encoding phospholipase C, LEA proteins (Late Embryogenesis Abundant), HSP (Heat-Shock Proteins), sugar transport proteins, desaturase, carbohydrate metabolism related proteins, osmoprotectant biosynthesis proteins, and protease inhibitors [14].

LEA proteins are constitutive but their content increases as a response of water or cold stress in tissues containing high ABA levels [21]. Their role is not fully understood but it was shown that several LEA proteins can be involved in chloroplast membrane or cytoplasm protection [21, 22].

HSP and protease inhibitors are involved in mechanisms of protein reparation and/or recycling [23]. These functions are important as a response of stress since they required less energy than *de novo* synthesis of proteins. Thus plant can mobilize energetic metabolites to other defense pathways.

Osmoprotectant synthesis is another main defense pathway to limit water loss. Indeed, osmoprotectants increase solute concentration in cells and thus facilitate turgor pressure and limit water loss. Several studies showed that the more frequent and more efficient osmoprotectants are nitrogenous compounds such a free proline and soluble carbohydrates such as sucrose, glucose and fructose [3, 11, 24]. Moreover, other osmoprotectants were identified such as polyamines, raffinose, galactinol or myo-inositol but in less plant species or in a lower concentration than molecules previously mentioned [3, 25].

CONCLUSIONS

Drought perception, signaling pathways and regulation of gene expression are complex mechanisms and lots of studies are still required to understand precisely how plants respond to this stress. The global response of plant tolerance to drought can be resumed on the figure 6.

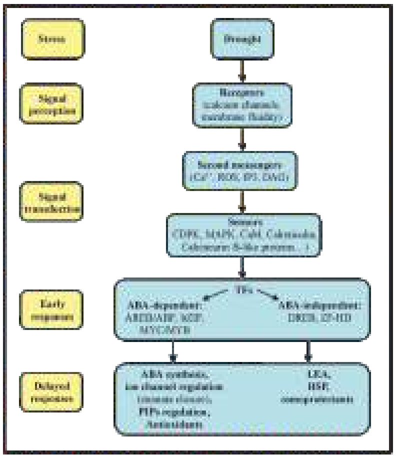

Figure 6.
Chronology of the main steps involved in responses to drought.

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