1. Introduction

Among environmental factors, water availability is probably the most limiting for crop quality and productivity, comprising economical output and human food supply (Roche et al., 2009). Water deficit is a multidimensional stress affecting plants at various levels of their organization (Yordanov et al., 2000). Thus, the effects of stress are often manifested at morpho-physiological, biochemical and molecular level, such as inhibition of growth (Bahrani et al., 2010), accumulation of compatible organic solutes (Sánchez-Díaz et al., 2008; DaCosta and Huang 2009), changes in phytohormones endogenous contents (Perales et al., 2005; Seki et al., 2007; Huang, 2008; Dobra et al., 2010), modifications in expression of stress responsive-genes (Xiong and Yang 2003; Yamaguchi-Shinozaki and Shinozaki, 2005; Huang et al., 2008), among others. Some of these responses are directly triggered by the changing water status of the tissues while others are brought about by plant hormones (Chaves et al., 2003). In this sense, abscisic acid (ABA), jasmonic acid (JA) and salicylic acid (SA) are involved in a complex signal-transduction network that coordinates growth and development with plant responses to the environment (Agrawal et al., 2002; Jiang and Zhang 2002; Fujita et al., 2006; Szalai et al., 2010).

The aim of this chapter is to present the results of an actual progression of water stress tolerance, its associated hormones and the crosstalk between them in Panicum virgatum (switchgrass), a member of the Poaceae family intensively studied as a source of lignocellulosic biomass to produce renewable energy. In recent years, important research efforts have been focused on improving the yields of crop species under water stress. Advances in functional genomics have been a major contribution to both the study and manipulation of abiotic stress in cereals as well as in forage species. These have been possible, in part, because of the increasing success in methods of grass genetic manipulation which have facilitated basic and applied research. The top four agricultural commodities by quantity are grass crops (sugarcane, maize, rice, wheat). Cow’s milk, the sole animal product in the top 10 agricultural commodities by quantity, for the most part comes from animals fed by grasses. Primary production from agriculture, therefore, assumes an important role in the transition to increasingly sustainable food and industrial production.
methods. Grass crops are centrally important targets for biotechnological improvement for food and fuel production. In particular the exploitation of a currently untapped resource of grass biomass (primarily lignocellulosic cell walls) is of high interest for sustainable fuel production. Basic and applied research on the sequencing of the rice, sugarcane, maize and sorghum genome have provided an invaluable resource to infer gene localization in other grasses that have not been sequenced yet. In the mid term, increases in drought tolerance could be introgressed from tolerant genotypes using a marker-assisted breeding approach. The Poaceae is the fourth largest plant family in the world -with over 10000 species distributed widely across the earth- and has an extensive synteny among the genomes of its members. Hence, what we learn about one member of the family can enhance our understanding of the entire group and contribute to the improvement of grass crops in meeting the challenges of attaining a sustainable agriculture for feeding the world’s population and for developing renewable supplies of fuel and industrial products (Baven et al., 2010).

2. Phytohormones and drought stress

As sessile organisms, plants are only able to survive by their ability to build up fast and highly adapted responses to diverse environmental stresses, e.g., drought, high salinity, and low temperature. Perception of these stress signals often results in the production of a huge arsenal of chemical compounds, among which a variety of hormones to adapt and respond to environmental challenges are included. Some of these compounds, such as the phytohormones are in a prominent position, playing important regulatory roles in plant physiology (Wasternack and Hause 2002; Chen et al., 2006; Browse, 2009a) affecting both developmental processes and responses to a wide range of abiotic and biotic stresses.

The key role of ABA, JA and SA as primary signals in the regulation of plant defense has been well established (Bari and Jones 2009; Pieterse et al., 2009). These hormones generate a signal transduction network that leads to a cascade of events responsible for the physiological adaptation of the plant to stress. It should be noted that the degree of drought tolerance varies with developmental stages in most plant species (El-Far and Allan 1995; Reddy et al., 2004; Rassaa et al., 2008). Experiments conducted to identify highly drought sensitive growth stages of sunflower showed that maximum reduction in yield occurred when drought was imposed during flowering (Karaata, 1991). In addition, drought during the vegetative phase of sunflower plants affects both final biological and economic yields (Agele 2003; Turhan and Baser 2004). In maize water deficit in the late developmental stage tends to reduce kernel size rather than number (Saini and Westgate, 2000; Boyer and Westgate, 2004). Similarly, Barney et al. (2009) evaluated fitness under stressful growing conditions to characterize the agronomic and ecological traits related to environmental tolerance of switchgrass and found that drought treatments (-4.0 and -11.0 MPa) reduced tiller length and number, leaf area, and biomass production by up to 80%.

The final outcome of stress response indicates that there is no single response pattern that is highly correlated with yield under all drought environments.

2.1 Abscisic acid (ABA)

ABA is well known hormone for its regulatory role in integrating environmental adversity with the developmental programs of plants (Chow and McCourt 2004; Christmann et al., 2005). Thus, it affects a wide range of processes at different developmental stages such as
embryo and seed development, acquisition of desiccation tolerance and dormancy, flowering and organogenesis (Finkelstein et al., 2002; Barrero et al., 2005; De Smet et al., 2006; Liang et al., 2007). ABA also promotes plant growth under non stressful condition and has shown to be essential for vegetative growth in several organs (Sharp et al., 2000; Spollen et al., 2000; Cheng et al., 2002).

Continuous synthesis, transport and degradation dynamically maintain ABA levels in plant cells. Therefore, plants control their developmental programs and stresses responses by modulating endogenous ABA levels (Schwartz et al., 2003).

The molecular basis of ABA biosynthesis and catabolism were established by genetic and biochemical approaches (Seki, 2002; Yamaguchi-Shinozaki and Shinozaki, 2005). Based on these studies it has become clear that ABA is synthesized from zeaxanthin, a C_{40} carotenoid. The conversion of zeaxanthin to xanthoxin, which is the C15 intermediates, is catalyzed in plastids by distinct enzyme: zeaxanthin epoxidase (ZEP) (Agrawal et al., 2001; Xiong et al., 2002), neoxanthin synthase (North et al., 2007), an unidentified epoxycarotenoid isomerase, and 9-cis-epoxycarotenoid dioxygenase (NCED) (Schwartz et al., 1997; Qin and Zeevaart 1999; Iuchi et al., 2001). In cytosol, the oxidation of xanthoxin produces abscisic aldehyde, which can be converted to ABA by aldehyde oxidase 3 (AAO3) (Seo et al., 2000).

Catabolism of ABA can occur through different pathways, the nature of which often depends on the species, their developmental stage or tissue. There are at least two pathways for ABA catabolism, an oxidative pathway and conjugation (Kushiro et al., 2004; Nambara and Marion-Poll 2005). The most common oxidative pathway is initiated by oxidation of the 8′-hydroxy ABA (8′-OH ABA), which can reversibly cyclize to phaseic acid (PA) (Zaharia et al., 2005). This compound can then be reduced to the major product dihydrophaseic acid (DPA), with minor amounts of epi- dihydrophaseic acid (epi-DPA). The minor oxidation pathway includes the formation of 7′-hydroxy ABA (7′-OH ABA) and 9′-hydroxy ABA (9′-OH ABA). The latter can cyclize reversibly to neophaseic acid (neoPA) (Zhou et al., 2004). In addition, ABA and hydroxy ABA may be conjugated with glucose, thereby forming corresponding glucose esters at C-1 (ABA-GE) or glycosides at C-1′ or C-4′ (Zeevaart 1999; Oritani and Kiyota 2003).

ABA action is one of the most studied topics in abiotic stress response research (Hirayama and Shinozaki 2007; Wasilewska et al., 2008). An increase in ABA content in response to water-deficit stress may arise from an increase in ABA biosynthesis and/ or a decrease in ABA breakdown (reviewed by Cutler and Krochko, 1999; Zeevaart, 1999). In Arabidopsis thaliana seedlings, Huang et al. (2008) showed that drought enhanced both ABA biosynthesis and catabolism, resulting in an increase in ABA and catabolites. Likewise, drought-treated plants of Laurus azorica (Seub) showed an increase in leaf ABA concentrations respect to that of the control (Sánchez-Díaz et al., 2008). On the other hand, exogenous application of ABA enhances the tolerance of plants or plant cells to drought (Lu et al., 2009). In relation to endogenous ABA, different reports showed that drought tolerant cultivars have more ABA than susceptible ones (Perales et al., 2005; Veselov et al., 2008; Thameur et al., 2011). Nevertheless, the direct relation between stress tolerance and increased ABA contents does not always exist.

In addition to the well established model of Arabidopsis, increments in endogenous ABA level under water stress are also reported in cereals and forage crops. For instance, increment in ABA contents under water stress in diverse developmental stages was reported in maize (Xin et al., 1997; Wang et al., 2008; Nyysar 2005), sorghum (Kannangara et al.,
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Plants of wheat and maize, representatives of C3 and C4 plants, respectively, were subjected to mild (−0.4MPa), moderate (−0.8MPa) and high (−1.5MPa) water stress levels induced by PEG-6000 for 7 days under controlled conditions. No significant change occurred in ABA content in roots and leaves of both species at mild stress level. Moderate stress resulted in higher accumulation of ABA in roots and leaves of maize as compared to wheat roots and leaves. At high stress level, ABA content increased in maize whereas wheat did not show any significant change. The differences were more pronounced between the leaves of the two species. These findings suggest a differential sensitivity of C3 and C4 plants to water stress. Higher ABA content in maize may also impose greater stomatal restrictions on these species to reduce water loss more effectively compared with wheat having lower ABA content (Nayysar, 2005).

In maize seedlings, Wang et al. (2008) assessed the inhibitory effect of ABA on the grain growth and reported that, at early stages, the endogenous ABA contents increased dramatically in leaves after 24 h of exposure to water stress, and then it remained high till the end. On the other hand, ABA content in seeds of wheat plants subjected to water deficit during grain filling showed variations. Water status parameters, ABA levels in flag leaf and grains, and grain yield were investigated in two drought tolerant (i.e. cv. MV Emese and cv. Plainsman V) and two drought-sensitive (i.e. cvs. GK E’let and Cappelle Desprez) wheat genotypes. In flag leaves, endogenous ABA levels increased significantly after the suspension of irrigation in all genotypes and remained high during anthesis; afterwards, it decreased markedly. In grains, ABA increased significantly in all genotypes exposed to water stress at 9 days post anthesis (DPA). Tolerant cultivars had higher ABA levels at 9 DPA and then it decreased rapidly toward maturity. By contrast, in sensitive cultivars ABA levels remained high until the end of grain filling period, which affected more negatively the grain yield of sensitive cultivars (Guoth et al., 2009).

Water stress effect and ABA levels were studied in sorghum cv. CSH8. A gradient of water stress was created among sorghum plants with a line-source sprinkler irrigation system and it was observed that leaf ABA levels increased with decreasing irrigation. ABA was very sensitive to stress, ranging over the irrigation gradient from 50 to 800 ng g⁻¹ DW in the well irrigated and water stressed plants, respectively. This study shows that ABA synthesis in leaves begins with a water potential of -1.3 MPa. This threshold has been observed in several species in a variety of conditions. The increase in ABA levels also correlated with a marked decrease in plant height and leaf senescence (Kannangara et al., 1983).

In plants of *Festuca arundinacea* cv. Grasslands Roa drought was imposed through water deprivation. An increase in leaf ABA levels from a range of 5–30 ng g⁻¹ FW in leaf tissue from water sufficient plants (control) to up to 200 ng g⁻¹ FW in leaf tissue of stressed plant was observed. ABA concentration was correlated with soil moisture content and leaf water potential. The accumulation of ABA occurred after the soil moisture content had dropped below approx. 8% in pots of treatment. The maximum rate of ABA accumulation occurred between water potential values of -1.5 and -2.5 MPa. Under these conditions, leaf elongation ceased and there was an increase in proline levels (Abernethy et al., 1998).

In barley, the differences in responses among five genotypes (i.e. Ardahoui, Pakistan, Rihane, Manel ad Roho) were evaluated. Water stress induced a reduction in relative water content, as well as an increase in proline content and endogenous ABA in all genotypes. Drought tolerant cv. Ardhaoui had the highest increase in endogenous ABA (5-fold) after
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water deprivation, while intermediate values were obtained in cvs. Rihane, Pakistan and Manel (Thameur et al., 2010).

In alfalfa cvs. Longdong (strong drought-resistance) and BL-02-329 (weak drought-resistance) ABA contents were evaluated. Under water stress, the ABA content increased in leaves. In response to severe drought stress, the drought-resistant cv. Longdong adjusted better to growth rate reduction to ensure surviving and avoid water deficit damage (Han et al., 2008).

In addition, exogenous ABA was demonstrated to increase drought tolerance in some forage crops. For example, Shaoyun et al. (2009) studied the effect of exogenous ABA added on plant of bermudagrass cv. TifEagle. They evaluated the protective effect of ABA based on relative water content and found that every ABA treatments (e.g. 19, 38 and 57 µM) significantly decreased the mortality rate in drought conditions compared to control. Treatment of 19 µM ABA showed the best protection against injury.

The increase in ABA endogenous level under drought induces the stomatal closure. This fact constitutes one of the first external symptoms of water deficit, and is recorded as the increase of stomatal resistance or the decrease of its inverse (stomatal conductance). Stomatal closure take place to minimize the water loss by transpiration, and ABA plays a fundamental role in this process. Thus, stomatal resistance is used as a reference to compare the intensity of water deficit in different species and growth conditions (Medrano et al., 2002). Guard cells continuously sense information from the surrounding environment, biotic and abiotic, as well as long distant signals coming from the roots. Stomatal closing under drought is a response to increasing levels of endogenous ABA synthesized in the roots as a result of water deprivation in the soil (Kim et al., 2010). Hence, decreasing of stomatal conductance under water stress is a wide-ranging response in plants. For instance, in kidney beam stomatal conductance diminishes rapidly after two days of drought, but it recovers the levels of well watered plants after two days of re-watering (Miyashita et al., 2005). In Brachiaria decumbens and brizantha, stomatal conductance significantly decreased after six days of water deprivation (Carmona et al., 2003).

Another symptom of water deficit is the reduction in cell turgency, which in turn, limits cell expansion and growth. Drought tolerance of grasses is associated closely with their morphological and physiological traits, with varying degrees of reduction of them among the species. For example, water stress decreases plant height in most grass species (Pennypacker et al., 1990; Jiang et al., 1995; Berg and Zeng 2006). On the contrary, this stress generally had no effects on the root: shoot ratio of the grasses. Bahrani et al. (2010) found that water stress constrained the total water use in ten forage species through a reduction in plant height, leaf water potential, leaf area and dry weight of roots. In corn, 160 lines (pure lines and hybrids) were evaluated in their tolerance to drought; one of the first detected symptoms was a reduction in plant height, with values ranging from 60 to 75 % (Dass et al., 2001). Similar effect was found in genotypes of wheat, where plant height showed a significant reduction under water stress (Shirazi et al., 2010).

In our laboratory we investigated the response of Panicum virgatum cv. Greenville to water stress. Plants of 55 days old were grown in a growth chamber; water was withheld at the same time as stomatal conductance was monitored. After water withholding, a consistent drop in the conductance was detected (drought treatment) and plants were re-watered to evaluate their recovering after 12 and 24 h. Plant height, stomatal conductance, content of stress related hormones (ABA, JA, SA) were evaluated. Water stress negatively affected the
plant height and, after watering was restored (i.e. 24 h after re-watering) plant growth reached the control height (Fig. 1.B). In addition, the stomatal resistance drastically increased during the stress period and it gradually decreased to the control level at 24 h of re-watering (Fig. 1.C).

After five days under stress, endogenous ABA content increased 4.5 fold compared to the control (Fig. 1.A). After 12 h of rehydration ABA content decreased to 1.5 fold the control and, after 24 h, ABA content in treated and control plants were similar. This increment in ABA content under stress is associated with the increase of stomatal resistance. Once plants recovered, both ABA content and stomatal resistance decreased to the control level. These results are in agreement with reports from other plant species as we discussed earlier.

The first steps of ABA sensing and signaling during stomatal closure under drought is related to the localization of ABA receptors in the guard cells. Two of these ABA receptors reside inside the cell but a third was found on the cell surface (Liu et al., 2007). Therefore, plant cell could sense both extracellular and intracellular ABA concentrations. Under drought, an increasing in stomata closure occurs because of an increasing in the pH of sap. This fact suggests that extracellular ABA is sensed by guard cells via receptors on the plasma membrane (Schachtman and Goodger, 2008). In the last decade, hydrogen peroxide (H$_2$O$_2$) and nitric oxide (NO) have also been involved in the ABA-induced stomatal closure (Assmann 2003; Desikan et al., 2004; Bright et al., 2006).

It is well documented that, in response to biotic and abiotic stimuli, there is an increment in the reactive oxygen species (ROS). ROS are short-lived molecules produced through diverse cellular mechanisms in different cell compartments, e.g. chloroplast, peroxisomes, mitochondria (Cho et al., 2009). This overproduction of ROS is highly controlled by a versatile oxidative system that establishes the redox balance inside the cell. On the other side, increase of ROS under stress conditions act as a signal of warning that activates responses of acclimation and/ or defense. Particularly, it activates specific pathways where H$_2$O$_2$ is involved as a second messenger. ROS signaling is connected to ABA, flux of Ca$^{2+}$ and sugars, and it is possible that they participate both up and downstream of pathways dependent of ABA in drought conditions (Kwak et al., 2006). In Panicum virgatum, ROS has been related to ABA signaling during germination (Sarath et al., 2007). Inhibition of germination imposed by ABA apparently requires both ROS and NO as intermediates in its action, where ROS produced by membrane-bound NADPH-oxidases responsive to ABA. In switchgrass seeds, externally supplied hydrogen peroxide restrain ABA-imposed inhibition of germination. Apart from this study on germination, no other report has involved ABA and ROS in switchgrass responses.

At molecular level, many transcription factors (TFs), such as dehydration-responsive element binding protein 1 (DREB1)/C-repeat binding factor (CBF), DREB2 and ABA-responsive element (ABRE) binding protein (AREB)/ABRE binding factor (ABF) can be used to improve stress tolerance to abiotic stresses in various grasses. ABA is involved in transcriptional regulations of numerous drought responsive genes (Zhang et al., 2006). Some drought-inducible genes may be regulated by both the ABA-independent and the ABA-dependent regulatory systems. For example, the promoter of a drought-, high salinity-, and cold- inducible gene, RD29A/COR78/LTI7, contains two major cis-acting elements (ABRE) and DRE/ C-Repeat (CRT), both of which are involved in stress-inducible gene expression (Yamaguchi-Shinozaki and Shinozaki, 2005).
Fig. 1. A. Content of ABA in leaves of Panicum virgatum cv. Greenville grown under drought (Drought) and after 12 and 24 h of re-watering (RW 12 h and RW 24 h). Data are means and SEs of three replicates, P ≤ 0.05. B. Plant height. C. Stomatal resistance (S.R.). Measurements were made with porometer Delta-T on the abaxial side of leaves. Black circle: control conditions. Gray square: Drought, RW 12 h and RW 24 h of re-watering. Data are means of twenty-four replicates with SEs. Values with the same letter are not significantly different, P ≤ 0.05.
2.2 Salicylic acid (SA)

SA is an endogenous regulator of growth involved in a broad range of physiologic and metabolic responses in plants (Hayat, 2010). During the last years, SA has been intensively studied as a signal molecule mediating local and systemic defense responses against pathogens. Currently, it has been reported that this compound plays also a role in plants responses to abiotic stresses, such as drought, low and high temperatures, heavy metals, and osmotic stress (Janda et al., 1999; Rao and Davis 1999; Molina et al., 2002, Nemeth et al., 2002; Munne-Bosch and Peñuelas 2003; Shi et al., 2008; Rivas-San Vicente and Plasencia 2011). SA was also shown to influence a number of physiological processes, including seed germination, seedling growth, fruit ripening, flowering, ion uptake and transport, photosynthesis rate, stomata conductance, biogenesis of chloroplast (Fariduddin et al., 2003; Khodary 2004; Hayat et al., 2005; Shakirova 2007).

There are two main routes for SA biosynthesis in plants (Shah 2003). Earlier studies suggested that SA is synthesized from phenylalanine via cinnamic acid. The decarboxylation of the side chain of cinnamic acid may generate benzoic acid, which may then undergo hydroxylation at the C-2 position forming SA (Yalpani et al., 1993; Ribnicky et al., 1998). The other pathway for the SA biosynthesis involves a 2-hydroxylation of cinnamic acid to o-coumaric which is then decarboxylated to salycilic acid (Alibert and Ranjeva 1971; 1972). Recent studies in Arabidopsis plants showed that there is another main route for SA biosynthesis taking place in the chloroplast, where SA is synthesized from chorismate via isochorismate (Wildermuth 2006; Mustafa et al., 2009). SA may be conjugated with a variety of molecules either by glycosylation or by esterification (Popova et al., 1997), and may also be metabolized to 2,3 dihydrobenzoic acid or 2,5 dihydrobenzoic acid (Billek and Schmook, 1977).

Recent results show that most abiotic stresses altered in planta SA endogenous contents, which also point to its involvement in stress signaling (Horváth et al., 2007). For example, endogenous SA increased in roots of barley plants under water stress. In addition, when plants were treated with SA before stress, the damaging effect of water deficit on the cell membrane in the leaves decreased, and an increase in ABA content was observed. Also, the proline level increased only in the wild species of Hordeum spontaneum. These results suggest that ABA and proline may contribute to the development of the antistress reactions, induced by SA (Bandurska and Stroinski, 2005). Previously, Munne- Bosch and Peñuelas (2003) reported that in Phillyrea angustifolia L. plants exposed to drought the SA level increased progressively to as much as 5-fold, and showed a strong negative correlation with the relative water content. During recovery, SA levels decreased, but remained slightly higher than those observed before drought. SA levels were positively correlated with those of tocopherol -also known as vitamin E acetate- during drought, but not during recovery. This result also indicates the possible role of endogenous SA in the induction of a protective mechanism during water stress.

Application of exogenous SA improves the plant performance under water, as reported by several authors. Low concentrations of exogenous SA provided tolerance against the damaging effects of drought in tomato and bean plants, whereas, higher concentrations did not show the same positive results (Senaratna et al., 2000). Enhanced tolerance to drought and dry matter accumulation was also observed in plants of wheat raised from grains soaked in acetyl salicylic acid aqueous solution (Hamada 1998; Hamada and Al-Hakimi 2001). Wheat seedlings subjected to drought and treated with SA exhibited higher moisture content and dry matter accumulation, carboxylase activity of Rubisco, SOD and total...
chlorophyll content compared to untreated control. The SA treatment also provided a considerable protection to the enzyme nitrate reductase thereby maintaining the level of diverse proteins in leaves (Singh and Usha, 2003). In addition, the treatment of water stressed *Lycopersicum esculentum* plants with SA low concentrations significantly enhances the photosynthetic parameters, membrane stability index, leaf water potential, activities of the enzymes nitrate reductase and carbonic anhydrase; thus improving tolerance to drought (Hayat et al., 2008). SA is also involved in the promotion of drought-induced leaf senescence in *Salvia officinalis* plants grown under drought in Mediterranean field conditions (Abreu and Munne-Bosch 2008). In addition, SA applied exogenously was effective in providing resistance to the plants against the excessive water stress in cell suspensions from the fully turgid leaves of *Sporobcdus stapianus* (Ghasempour et al., 2001).

Exogenous application of SA and glycin-betaine (GB, a compatible osmotic solute) enhanced the yield of sunflower hybrids under different degrees of water stress. Under stress, diameter of the head (inflorescence), number of achene and seed oil content was reduced. However, applications of SA and GB improved these parameters (Hussain, 2008). In plants exposed to abiotic stress (e.g. salinity and drought), the accumulation of ROS, such as superoxide radicals (O$_2^-$), hydroxyl (OH$^-$), and H$_2$O$_2$ is induced. The increasing ROS levels in plants produce oxidative stress of lipids, proteins and nucleic acids, which, in turn, alter the redox homeostasis (Smirnoff, 1993). SA increases the activity of the oxidative enzymatic system as is the case of CAT and SOD. In plants of *B. juncea*, exogenous application of SA increased CAT and SOD activity. In the same line of evidence, Kadioglu et al. (2010)
reported that exogenous application of SA induced the activity of antioxidant enzymes at the same time that alleviates the water stress damage in the long run in plants of *Ctenanthe setosa*. In seedlings of wheat under water stress and supplemented with SA (1 mM), ABA (0.5 mM), Ca\(^{2+}\) (5 mM) and H\(_2\)O\(_2\) (0.05 mM), the activity of SOD, CAT, ascorbate peroxidase (APX), and NADPH oxidase (Agarwal, 2005) was induced.

![Graph A](image1.png)

![Graph B](image2.png)

**Fig. 3.** A. Activity of superoxide dismutase (SOD) and B. catalase (CAT), on leaves of *Panicum virgatum* cv. Greenville grown under drought (Drought) and after 12 and 24 h of re-watering (RW 12 h and RW 24 h). Data are means of four replicates with SEs. Values with the same letter are not significantly different at *P* ≤ 0.05.
In *P. virgatum*, we found that endogenous SA contents decreased considerably during a moderate water stress treatment and after 24 h of rehydration the endogenous contents increased significantly (p ≤ 0.05, Figure 2). This decrease in SA is accompanied with important peak of ABA content (four-fold increase) during the stress treatment (Figure 1.C). It has been proposed that an antagonistic interaction between these two hormones in response to water stress naturally occurs in several species, probably as a result of sharing common intermediaries in the signaling cascade (Yasuda et al., 2008). In addition, the increase in SA content corresponds with a raise in SOD and CAT activities after plants were rehydrated (Figure 3 A and B).

Despite of SA participation in abiotic stress responses, its role is ambiguous. The stress tolerance imparted by SA appears to be dosis-dependent, since deficiency or very high SA contents increase the susceptibility. Hence, the role of SA under a certain level of moderate or severe stress might be different. It could possibly be a result of the interaction between ROS and SA down-stream signals, where redox regulations play a key role (Yuan and Lin, 2008).

### 2.3 Jasmonic acid (JA)

JA, and its cyclic precursors and derivatives constitute a family of bioactive oxylipins that regulate plant development and responses to environmental cues (Turner et al., 2002; Devoto and Turner, 2003). This family of compounds is form by 12-oxophytodienoic acid (OPDA), methyl jasmonate (Me-JA), JA hydroxylated (11-OH-JA and 12-OH-JA), JA conjugated to some amino acids such as leucine (JA-leucine) and isoleucine (JA-Ile) as well as the glucoside and sulfate of 12-OH-JA (12-O-Glc-JA, 12-HSO₄-JA), and collectively receive the name of jasmonates (JAs). These molecules are involved in a variety of processes related to plant development and survival, including direct and indirect defense responses (e.g., defense against insects and necrotrophic pathogens), secondary metabolism, reproductive processes (e.g., pollen maturation and anther dehiscence, ovule development), and fruit development, among others (Seo et al., 2001; Wasternack and Hause, 2002; Arimura et al., 2005; Liechti and Farmer, 2006; Wasternack, 2007). In addition, it is known that JA-related responses are directly associated with a reset downstream of gene expression in the biosynthesis pathway (Thines et al., 2007).

Vick and Zimmerman (1983) were the first authors to demonstrate the steps of the JA biosynthesis, and recently it was reviewed by Wasternack and Kombrink (2010). JA biosynthesis and signaling pathway have been extensively studied, mainly in dicots such as *Arabidopsis* and tomato, and to a lesser extent in some monocots (Kazan and Manners, 2008). JAs are produced from α-linolenic acid (α-LeA; C18:3) or hexadecatrienoic acid (C16:3) released from plastidial galactolipids by phospholipases. Following the oxidation of α-LeA by lipoxygenase (LOX) to 13(S)-hydroperoxyoctadecatrienoic acid (13(S)-HPOT), the first committed step of JA biosynthesis is conversion of the LOX product to the allene oxide 12,13(S)-epoxyoctadecatrienoic acid (12,13(S)-EOT) by allene oxide synthase (AOS). This unstable allylic epoxide can be enzymatically cyclized by allene oxide cyclase (AOC) to optically pure cis-(+)-12-oxophytodienoic acid (9S,13S)-OPDA), which is the last product of the plastid-localized part of the JA biosynthesis pathway. Translocation of OPDA into peroxisomes, where the subsequent part of the JA biosynthesis pathway occurs, is mediated by the ABC transporter COMATOSE and/or an ion-trapping mechanism (Theodoulou et al., 2005). The OPDA reduction is catalyzed by a peroxisomal OPDA reductase (OPR) to
produced 3-oxo-2(2[Z]-pentenyl) cyclopentane-1-octanoic acid (OPC-8:0). Then, three cycles of β-oxidation catalyzed by acyl-CoA oxidase (ACX), multifunctional protein (MFP), and L-3-ketoacyl-CoA thiolase (KAT) lead to jasmonoyl-CoA, from which a yet unknown thioesterase releases (+)-7-isoo-JA (3R,7S)-JA that equilibrates to the more stable (-)-JA (3R,7R)-JA.

The participation of JA in response to abiotic stress, such as drought and salinity, has been reported in several species. For instance, the treatment of barley leaves with sorbitol or mannitol (compatibles solutes to simulate water stress) increased JAs endogenous contents, followed by synthesis of jasmonate-induced proteins (JIPs, Lehmann et al., 1995). Other study showed that sorbitol treatment enhanced octadecanoids and JAs content, and this threshold was necessary and sufficient to initiate JA-responsive gene expression (Kramell et al., 2000). In addition, under water stress, endogenous JA content increased in maize root cells (Xin et al., 1997) and this compound was able to elicit betaine accumulation in pear leaves (Gao et al., 2004). Pedranzani et al. (2003) showed that tomato cultivars differing in salt tolerance differed in basal JA content. Steady-state amounts of JA and related compounds were higher in salt-tolerant cv. Pera compared to the salt-sensitive cv. Hellfrucht frühstamm. Moreover, studies in contrasting environments showed different basal JAs contents and patterns of response to water stress in two populations of Pinus pinaster Ait., perhaps as an adaptation to diverse ecological conditions (Pedranzani et al., 2007).

Studies performed in our laboratory with Panicum virgatum showed that, during the drought treatments, JA levels did not increase significantly compared to the control level. However, after watering was restored, contents of JA consistently increased and overcome the control (Figure 4).

![Content of JA in leaves of Panicum virgatum cv. Greenville grown under drought (Drought) and after 12 and 24 h of re-watering (RW 12 h and RW 24 h). Data are means of three replicates with SEs. Values with the same letter are not significantly different at P ≤ 0.05](www.intechopen.com)
Exogenous application of JA or Me-JA increased antioxidative ability of plants under water stress (Wang, 1999; Bandurska et al., 2003). Along the same line, other studies also showed that JAs play an important role in signaling drought-induced antioxidant responses, including ascorbate metabolism (Li et al., 1998; Ai et al., 2008). For instance, in shoots and roots of maize seedlings treated with Paraquat, an herbicide, and exogenous concentrations of Me-JA (50 and 100 μM) the expression of genes corresponding to the anti-oxidative defense system was detected. Certainly, Me-JA promoted increased production of several anti-oxidative enzymes, including glutathione reductase, guaiacol peroxidase and ascorbate peroxidase, and it has been suggested that this increase may be due to up-regulation of genes controlling the synthesis of these enzymes, or by activation of diverse constitutive genes (Norastehnia and Asghari, 2006).

### 3. ABA, SA, JA and cross talk between each other

To survive under various biotic and abiotic stresses, plants have developed complex mechanisms to perceive external signals, allowing them optimal response to the environment. ABA, SA, JA, and ethylene (ET) regulate protective responses of plants against both abiotic and biotic stresses via synergistic and antagonistic actions, which are referred to as signaling crosstalk (Fujita et al., 2006). Furthermore, ROS generation has been proposed as a pivotal process that is shared between abiotic and biotic responses (Apel and Hirt, 2004; Torres and Dangl, 2005).

ABA has been extensively involved in responses to various abiotic stresses (e.g., drought, salinity, low temperature) and, on the other side, SA, JA and ET play a key role in responses to biotic stress upon pathogen infection. Several studies have indicated that plant responses to environmental stresses have some effects on their response to pathogens. In many cases, ABA acts as a negative regulator of disease resistance (Narusaka et al., 2004). For instance, the ABA-deficient tomato mutant sitiens has increased resistance to pathogens and application of exogenous ABA restored the susceptibility of sitiens mutants. The sitiens mutant has greater SA-mediated responses, suggesting that high ABA concentrations inhibit the SA-dependent defense response in tomato (Fujita et al., 2006). It has also been reported that ABA treatment suppresses the induction of SAR in Arabidopsis. The use of several mutants in combination with chemicals that inhibit and/or stimulate SA revealed that ABA suppressed the SAR induction by inhibiting the pathway both upstream and downstream of SA, independently of the JA/ET mediated signaling pathway. These data strongly suggest that an antagonistic crosstalk might occur at multiple steps between the SA-mediated signaling of SAR induction and the ABA-mediated signaling of environmental stress responses (Yasuda et al., 2008). This antagonistic interaction between ABA mediated abiotic stress signaling and disease resistance might simply suggest that plants developed strategies to simultaneously producing proteins that are involved in abiotic stress and disease resistance (Anderson et al., 2004). Since pathogen infection requires relatively humid conditions, a simultaneous exposure of plants to drought and necrotrophic pathogens attack is actually rare in nature. In fact, high humidity and temperature weaken the plant resistance to pathogen attack. Thus, the view that the ABA-mediated abiotic stress signaling potentially takes precedence over biotic stress signaling (Anderson et al., 2004) supports the notion that water stress threatens plant survival more significant than pathogen infection does (Fujita et al., 2006).
Likewise, a positive interaction between SA and ABA might occur in abiotic stress. The roles playing by free SA, conjugated SA, and ABA in thermo-tolerance induced by heat acclimation (38°C) were investigated. To evaluate their potential functions, three inhibitors of synthesis or activity were infiltrated into pea leaves prior to heat acclimation treatment. The results showed that the burst of free SA in response to heat acclimation could be attributed to the conversion of SA 2-O-D-glucose, the main conjugated form of SA, to free SA. Inhibition of ABA biosynthesis also resulted in a defect in the free SA peak during heat acclimation. Overall, these results suggest that exogenous SA and ABA may lead to the enhancement of thermo-tolerance (Liu et al., 2006).

Our study in Panicum virgatum adds evidence to ABA/SA association. Results in our laboratory show that under drought, the content of endogenous SA is lower than that of the control. However, after 12 h of re-watering SA content reach the control value, and after 24 h the contents are significantly higher than that of the control (p ≤ 0.05, Figure 2). On the other hand, an opposite trend is described in ABA (Figure 1.C), showing that when ABA reach its maximum, SA content is minimum. By the time that ABA recovers the control value, SA content significantly increases over the well-watered control.

Interaction between ABA and JA has been reported in salt stress response. Moons et al. (1997) compared the effects of exogenous ABA and JA in the rice seedlings response to salt stress. In view of the proposed roles for JA and Me-JA in plants exposed to water-limiting stresses, changes in endogenous jasmonates -in particular MeJA content- were compared with the well established increase in endogenous ABA in plants subjected to salt stress. Salt shock (150 mM NaCl) induced a rapid increase in ABA content in roots of 10-day-old seedlings, reaching a maximum at 8 h of stress and decreasing to near control values after 12 h. On the contrary, Me-JA concentrations, showed a delayed and gradual increase of approximately 4-fold after 12 h of stress. This accumulation occurred when ABA levels were decreasing. In the same study, eight stress- induced proteins were compared for their ABA and/or JA response. In addition, the effect of JA, ABA, and salt stress on the transcript levels of three genes encoding pathogenic related proteins, a salt stress-responsive protein, and a group three LEA protein were analyzed. ABA and JA were found to exert antagonistic effects on the transcript and/or protein accumulation of two classes of salt stress-responsive genes.

In addition, in Arabidopsis it has been proposed that both ABA and JA participate in the responses to moderate drought (30% field capacity). Nevertheless, ABA and JA would be involved in different stages of the response, driving an acclimation process during growth through an extensive genetic reprogramming to finally reach a new homeostasis (Harb et al., 2010). These authors suggest that, during early stages of moderate drought, endogenous JA in combination with high ABA level is enough to stimulate the preparatory response needed for drought acclimation (e.g. stomatal closure and cell wall modification). JA is probably not required at high concentration under drought stress, and an increase in its concentration might negatively affect plant response to growth. Under moderate drought treatment, the response of Arabidopsis mutants coi1 and jin1 (both JA-insensitive) were found to be significantly resistant (or insensitive to drought stress). Compared to the wild type, biomass accumulation under drought did not differ from the well-watered control. These results are in agreement with studies showing that in coi1 mutant the JA-mediated inhibition of seedling and root growth is suppressed (Xie et al., 1998). Harb et al. (2010)
suggest that the reduced growth in response to drought stress, as a developmental program for acclimation, is not switched on in the absence of JA signal perception. Thus, the down-regulation of JA biosynthesis to minimize the inhibitory effect of JA on plant growth as well as signaling pathways under prolonged drought can establish new homeostasis during the acclimation process.

The crosstalk between JA and ABA might occur as they utilize a similar cascade of events to stimulate some responses (Harb et al., 2010; Fujita et al., 2006). Recent studies have revealed several molecules, including transcription factors and kinases, as promising candidates for common players that are involved in this crosstalk. The convergence points in JA and ABA stress signaling occurs, in part, by sharing some transcription factors. Transcription factor AtMYC2 plays a role in multiple hormone signaling pathways. Genetic analysis of the jasmonate-insensitive jin1 mutant revealed that JIN1 is allelic to AtMYC2, which was first identified as a transcriptional activator that is involved in the ABA mediated drought stress signaling pathway (Abe et al., 2003). The dehydration-inducible RD22 gene (involved in response to salt stress and response to desiccation) respond to both AtMYC2 and the R2R3MYB-type transcription factor. RD26 expression is induced by JA, hydrogen peroxide and pathogen infections, as well as by drought, high salinity and ABA treatment (Fujita et al., 2004; Harb et al., 2010; Fujita et al., 2010). In addition, protein phosphorylation and dephosphorylation significantly influence both the regulation of physiological morphology and gene expression associated with basic cellular activities in JA-dependent root growth and in AtMYC2 gene expression. The gene expression and kinase activity of OsMPK5 is also induced by ABA, various abiotic stresses and pathogen infection (Xiong et al., 2003).

Participation of ABA and JAs in stomatal closing was studied in Arabidopsis wild type and mutants, ABA-insensitive (ost1-2), and Me-JA-insensitive mutants (jar1-1), in order to examine a crosstalk between ABA and Me-JA signal transduction. In that study, cytoplasmic pH changes and ROS production in response to ABA or Me-JA were used to assess the respective roles of these genes in ABA or Me-JA signaling pathways, leading to stomatal closure. The modulation of Ca\textsuperscript{2+} mediates the response, and it appears to be a common effect of ABA and Me-JA. The primary actions of ABA and Me-JA at the plasma membrane level appear to be different: while Me-JA targets the Ca\textsuperscript{2+} channels, ABA activates effectors in the plasma membrane (i.e. phospholipase C, D). However, both signal transduction pathways converge at level of intracellular Ca\textsuperscript{2+}. The regulation of intracellular Ca\textsuperscript{2+} level, indeed, has a much greater dependence of Me-JA action than that of ABA (Blatt et al., 1993; McAinsh et al., 1995; Suhita et al., 2004).

Similar interaction between ABA and JA signaling pathways has been observed in seed germination in Arabidopsis. In this case, seed germination of the JA-resistant1 (jar1) and JA-insensitive4 (jin4) mutants were more sensitive to ABA than its wild type (Staswick et al., 1992; Berger et al., 1996).

Evidence of antagonistic interactions of ABA/JA was also found at the level of gene expression in Arabidopsis (Balbi and Devoto, 2007). Wild type and coi1 plants were wounded or treated with Me-JA, and changes in the expression of 8200 genes were examined using microarrays. A survey of the genes that were repressed by Me-JA identified many genes that have been implicated in ABA and drought stress response. These include the ATHB-12 transcription factor, the bZIP-transcription factor ABF3, COR47 and LEA D113. The nitrate transporter NTP2 and three members of the aquaporin family of transporters were also repressed by Me-JA in a COI1-independent manner. These findings reinforce the role of JA
in osmotic homeostasis and are complementary to the study of Armengaud et al. (2004). This author shows that transcript levels for the JA biosynthetic enzymes (i.e. lipoxygenase, allene oxide synthase, and allene oxide cyclase) as well as JA responsive genes (i.e. genes involved in storage of amino acids -VSP-, glucosinolate production -CYP79-, polyamine biosynthesis -ADC2-, and defense -PDF1.2) strongly increase during potassium starvation and quickly decreased after potassium resupply. These finding highlight the role of JA in nutrient signaling and stress management through a variety of physiological processes such as nutrient storage, recycling, and reallocation.

In our work, the experiments with Panicum virgatum show that endogenous JA content is not affected by a moderate water deficit, but such contents increase significantly after 24 h of re-watering. This trend is similar to the response observed in Arabidopsis during early stages of water and salt stress, where the contents of JA remain constant under drought and gradually recover after re-watering (Moons et al., 1997; Harb et al., 2010). Conversely, there is an increment in ABA levels under a moderate stress that corresponds with an increase in SOD and CAT activities (Figure 5). At the same time, the SA contents decreased, resembling an antagonistic interaction ABA/SA. After re-watering, ABA contents decreases at the same time as SA and JA endogenous contents display an increase. This last trend is accompanied by a rising in SOD and CAT activity during 24 h of plant recovering. Thus, recovered plants
reached a new homeostasis status where SA-JA-ABA balance is different from the well-watered control. Humidity produced by re-watering after a water stress could trigger a defense response to pathogen facing a potential attack. Or, it could reorganize the endogenous levels of plant hormones to reach a new homeostasis in acclimation to new environmental conditions (Fujita et al., 2006). Overall, this new hormonal status suggests the interplay among SA-JA-ABA in water stress responses in *P. virgatum*.

### 4. Conclusions and perspectives

Forage crops, which are grown to be utilized by grazing or harvesting as a whole crop, are essential for the successful operation of animal production systems. This fact is more relevant for ruminants which heavily depend upon forages for their health and for a cost-effective and sustainable production. While forages are an economical source of nutrients for animal production, they also help conserve the soil integrity, water supply and air quality (Chaudhry, 2008). In the last years, forage species have been widely studied for non-forage purposes—especially for bioenergy. Grasses are a source of lignocellulosic biomass to generate biofuels and they belong to a group of plant species considered as second generation crops. Nowadays, second generation of biofuels have gained relevance since they do not directly compete with human nutrition, unlike first generation of biofuel crops. The incorporation of forage species to the production of bioenergy is expected to expand the amount of biofuel that can be produced sustainably by using biomass of non-food crops such as switchgrass, whole crop maize, miscanthus and cereals that bear little grain, among others (Inderwildi and King, 2009). However, one of the major concerns about these crops is the environmental impact. It is likely that the expansion of crops for bioenergy utilization occurs with greater intensity in natural ecosystems, often characterized by their fragility in soil stability and water content. Global climate change intensifies these challenges as current crops are poorly adapted to more uncertain and extreme climatic conditions. In this context, the study of plant responses to water deficit as a strategy for the optimization in the use of water is of remarkable importance to increase production without further damage to the environment. In this chapter, we presented our contribution to this topic through the study of drought tolerance in *Panicum virgatum*, a member of the Poaceae family intensively studied as a source of lignocellulosic biomass to produce renewable energy. The Poaceae, a family with numerous species important to human nutrition, shares an extensive similarity among its members; hence, the comprehension of the bases of water stress tolerance in *Panicum virgatum* will improve our understanding of the entire group. Providing food and energy in conditions that maintain the sustainability of resources is a challenge that must be addressed. Faced with a global energy crisis and the steadily growing world population, forage crops are a suitable alternative to meet current and future demands of food and energy.

The biological significance of crosstalk between signaling pathways operating under stress conditions as well as the mechanism that underlie this crosstalk are still unclear. At present, these pathways have become better resolved due to the development of new tools that allow for the exploration of the physiological, genetic, and biochemical foundation of such processes. The genomic, proteomic and metabolomic approach is now widely used in model plants and, to a lesser extent, in crop and forage plants. The growing interest in forage crops has promoted its study at the molecular level, making it promising to research the improvement of these species. To date, the complete genome sequences of four grass species
(i.e. maize, sorghum, rice and brachypodium) representing the three most economically important grass subfamilies have been analyzed. In the same line, the first pooid grass, *Brachypodium distachyon* (*Brachypodium*), has recently been sequenced completely and proposed as a new model that can contribute to grass crop improvement (Bevan et al., 2010). This knowledge can be directly applied to accelerate the domestication of wild grasses (e.g. Switchgrass and Miscanthus) that are promising biomass crops. Genomics and functional genomics resources are centrally important for this research as they also directly facilitate biotechnological and genetic improvement through plant breeding. This information along with a system-level approach will significantly increase our knowledge of grass biology in order to understand how biotic and abiotic environments influence crop yield. In the near future, the combination of these new technologies will help to unravel the complex interactions between plant hormones in forage crops.

5. References


Plants and Environment


Drought Tolerance and Stress Hormones: From Model Organisms to Forage Crops


Changing environmental condition and global population demands understanding the plant responses to hostile environment. Significant progress has been made over the past few decades through amalgamation of molecular breeding with non-conventional breeding. Understanding the cellular and molecular mechanisms to stress tolerance has received considerable scientific scrutiny because of the uniqueness of such processes to plant biology, and also its importance in the campaign “Freedom From Hunger”. The main intention of this publication is to provide a state-of-the-art and up-to-date knowledge of recent developments in understanding of plant responses to major abiotic stresses, limitations and the current status of crop improvement. A better insight will help in taking a multidisciplinary approach to address the issues affecting plant development and performance under adverse conditions. I trust this book will act as a platform to excel in the field of stress biology.

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