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On the Potential of Flupyradifurone, a Butenolide Insecticide, for Drought Stress Attenuation in Spring Wheat (Triticum aestivum L.)
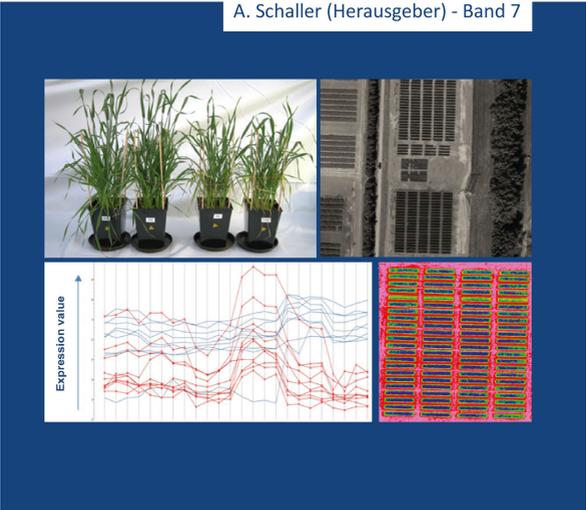

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1. Introduction

More than 10,000 years passed since mankind underwent a significant cultural shift from hunters and gatherers towards a sedentary agrarian society. This so called 'Neolithic Revolution' ended with the emergence of early human civilizations and a considerable technological progress (Salamini et al. 2002; Weisdorf, 2005). One driving force behind this process was the domestication of wild cereal grass species (*Poacae*) leading to increased kernel sizes and the loss of natural seed dispersal (Eckardt, 2010). A key step in cereal domestication was the cultivation of the early wheat species 'Einkorn' (*Triticum monococcum*) and 'Emmer' (*Triticum dicoccum*) due to their favorable harvesting properties and a superior climatic adaptation (Salamini et al. 2002). From that point on, another 1,000 years passed until the hexaploid bread wheat (*Triticum aestivum*) first appeared and evolved as one of today's most widely grown crops in the world (Lobell and Field, 2007; Shewry, 2009). In 2012, wheat[1] was cultivated on more than 215 million hectares representing 15 % of the global arable land (FAOSTAT, 2015). With an annual production of more than 700 million tons of grain yield worldwide, wheat forms an indispensable source for human nutrition and livestock production (Cordain, 1999; Shewry, 2009). The genetic diversity, high yields, grain energy density and unique dough properties for further processing have led to this great success of a single crop species (Song & Zheng, 2007; Wieser, 2007; Šramková et al. 2009). Although record grain yields were reported to be as high as 15 tons per ha, the average is usually in a range of 3 - 8 tons (Barker, 1999; Barber, 2014; IWYP, 2015).

The significant gap between potential and actual yields is caused by a combination of unfavorable agro-climatic conditions, biotic stress factors and the genetic adaptation of a crop variety (Rockström & Falkenmark, 2000). Despite crop protection measures, biotic stresses such as weeds, herbivory by animal pests, viral-, bacterial- or fungal-diseases can cause considerable yield losses of almost 30 % in wheat (Oerke, 2006). However, adverse agro-climatic conditions e.g. drought, salinity, extreme temperatures, excessive radiation, nutrient-deficient soils, etc. pose an even higher risk for crop productivity. Among the latter abiotic stress factors, drought is most devastating for agricultural production and expansion. Drought per se is defined as an extended period with below-average rainfall, and drought stress typically occurs when soil water is continuously lost by transpiration and evaporation (Jaleel et al. 2009). Depending on the seasonal timing, duration and severity of water deficits, average crop yields can be reduced by approximately 50 % (McWilliam 1986; Bray et al. 2000; Wang et al. 2003, Chaves & Oliveira, 2004; Tuberosa & Salvi, 2006; Passioura, 2007).

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[1] Wheat refers to *T. aestivum* and *T. durum* species whereas *T. aestivum* accounts for 95 % of the global wheat production.

It is predicted that world population will increase by 2.5 billion during the next 25 years and world agricultural production needs to rise by around 50 % until 2030 to meet future food demands (Somerville & Briscoe, 2001; Ronald, 2011). However, 70 % of the available water is already used for agriculture and the extension of area for food crop cultivation is restricted due to high environmental costs, urbanization, and adverse climatic conditions. The fact that drought and other climatic extremes are predicted to occur more frequently in the future as a result of global warming, makes this ambitious yield target even more challenging (Reyer et al. 2013, Trnka et al. 2014). In fact, the only sustainable option to increase agricultural productivity with the currently available land and resources is by closing the gap between potential and actual yields. It is therefore not surprising that much effort is put into basic research, breeding programs and crop-management to increase the resilience of the most important crop species to water scarcity and other stresses (CIMMYT, 2015, Fereres et al. 2011, Sinclair, 2011). In fact, a better physiological, biochemical and molecular understanding of plant drought stress responses could give rise to the identification of possible options helping to achieve this goal (Valliyodan & Nguyen, 2006).

1.1 Plant drought stress responses

1.1.1 The soil-plant-atmosphere continuum

Drought stress usually starts when the soil water content decreases to a point where the ability of roots to absorb water is insufficient to maintain an optimum steady state water flux between soil, plant and atmosphere to compensate evapotranspiration (Tezara et al. 1999). A common measure to characterize the water status of a specific system and its tendency of moisture flux is the water potential Ψ_w. At field capacity, when non-saline soil has been fully saturated with moisture and excess gravitational water drained away, the soil water potential ranges between -0.01 and -0.03 MPa (Veihmeyer & Hendrickson, 1949). In plants, the water potential varies among different compartments but decreases in the direction of stomatal-openings. Water movement from the soil through plants into the atmosphere follows the progressively decreasing water potential gradient. In hydrated plants, root xylem sap has a water potential Ψ_{root} of -0.1 MPa to -0.3 MPa. In leaves, Ψ_{leaf} displays values of -0.4 to -0.6 MPa and in the atmosphere Ψ_{atm} is in a range of around -90 MPa (Zwieniecki & Boersma 1997, McCully, 1999; Siddique et al. 2000; Tang & Boyer, 2002, Lambers et al. 2008). Stomata are essential and highly specialized cell types controlling the gas exchange of leaves. The dilemma of terrestrial plants is caused by the fact that CO_2 uptake for photosynthesis is associated with the inevitable loss of water due to transpiration. It is therefore not surprising that stomatal-openings are highly regulated, depending on the water status and other outside stimuli (Jia & Zhang, 2008; Arve et al. 2011; Wei et al. 2011).

1.1.2 Morphological aspects of drought stress

When drought causes soil moisture levels to decline, soil pore water is progressively replaced by air leading to a dramatic decrease of hydraulic conductivity and soil water potential. The consequence of approximating soil- and root water potentials is a constraint for the bulk-flow driven movement of soil water towards the absorbing root surface (Taiz & Zeiger, 2006). Impaired water uptake by roots rapidly triggers a wide set of fundamental physiological, biochemical and molecular responses aiming at acclimation and survival during the stress situation (Seki et al. 2003). The primary response of established plants is growth arrest (Fig. 1), reduced transpiration by stomatal closure and a decline of relative water content (Lambers et al. 2008, Osakabe et al. 2014).
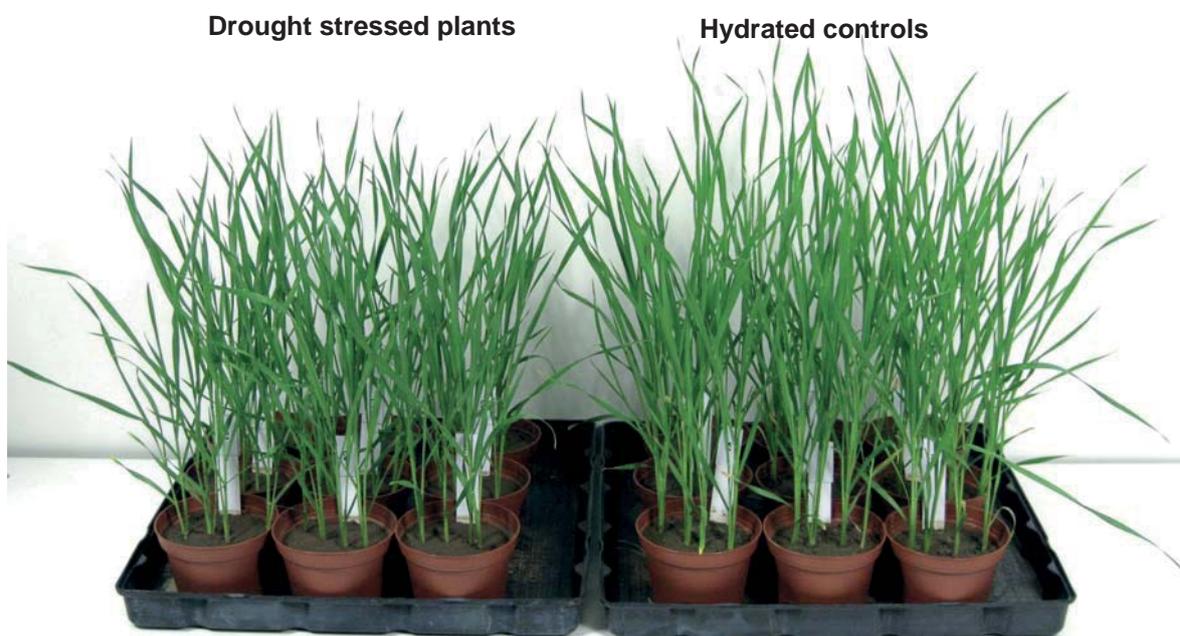

Fig. 1: Effect of moderate drought stress on shoot growth of spring wheat (AC® Harvest).

Drought stress which occurs directly at or after sowing, delays and reduces seed germination resulting in poor seedling establishment in the field (Murillo-Amador et al. 2002). Plant growth and cell expansion have been reported to be one of the most drought-susceptible physiological processes as they depend on the cellular turgor pressure (Jaleel et al. 2009). Drought-stressed plants also produce less canopy useable for photosynthesis. In addition, it has been observed that leaf temperature and the root/shoot ratio significantly increases as a consequence of water shortage (Siddique et al. 2000; Zhang et al. 2009, Anjum et al. 2011a). Another aspect of drought stress is its effect on plant reproductive processes and yield formation. In cereals, insufficient water availability during the transition from the vegetative to the generative development stage could cause a delay of anthesis or its complete inhibition (Barnabás et al. 2008).

When drought stress appears during the meiotic stage it can significantly reduce grain set by increased pollen sterility (Sheoran & Saini, 1996). Water deprivation during the final grain filling stage reduces endosperm cell division and photosynthesis. In addition, it promotes early leaf-senescence. However, the latter effect triggers the remobilization of assimilates from the ripening foliage into the grain kernels (Barnabás et al. 2008).

1.1.3 Physiological and biochemical aspects of drought stress

Nutrient uptake from soil

The ability of roots to absorb nutrients from soil solution depends on the water potential gradient between soil and rhizosphere and the level of leaf transpiration. Since drought stress decreases both parameters, nutrient uptake and transport become aggravated (Hu & Schmidhalter 2005). It has been reported that leaf concentrations of especially nitrogen (N), phosphorus (P) and potassium (K) decline under water scarcity (Farooq et al. 2009a). N is required in large amounts for plant growth and development as it incorporates into amino and nucleic acids, chlorophyll and many other cellular components. P is an important component of cellular energy equivalents, phospholipids and nucleic acids, and K plays an essential role in enzyme activation, osmoregulation and the maintenance of cellular ion homeostasis. Drought stress was also predicted to impair calcium (Ca) uptake and to increase symptoms of calcium deficiency (Rouphael et al. 2012). In brief, water deprivation adversely impacts on nutrient availability, absorption and usage leading to limitations in associated physiological pathways (Farooq et al. 2009a).

Photosynthesis, CO_2 fixation and oxidative stress

Of great importance for plant growth and subsequent yield formation is the potential of leaves to perform photosynthesis. In fact, drought stress impairs photosynthesis in multiple ways. The biggest limitation comes from water stress-induced stomatal closure and the resulting depletion of internal CO_2 concentrations (Chaves, M. 1991; Lu & Zhang 1999). Reduced CO_2 pools restrict CO_2 assimilation catalyzed by ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). Under drought conditions, the expression of RuBisCO small subunits was found to be decreased and its activity reduced by binding of inhibitors. However, inhibitory proteins can also protect RuBisCO from protease degradation. RuBisCO activase involved in the removal of bound inhibitors is considerably less active as a consequence of low ATP concentrations under water shortage (Gutteridge & Gatenby, 1995; Farooq et al. 2009a). In addition, drought stress can increase photorespiration in C3 plants as relative O_2 conentration increases at low CO_2 and increasing temperatures (Brown & Morgan, 1980; Nocter et al. 2002). Under normal illumination, a decreasing cellular CO_2 concentration facilitates the accumulation of reduced components of the photosynthetic electron transport chain (Bhargava & Sawant, 2013).

Excited pigments in thylakoid membranes may transfer excitation energy or free electrons to molecular oxygen (O_2) forming reactive oxygen species (ROS) i.e. superoxide (O_2^-), singlet oxygen ($^lO_2$), hydrogen peroxide (H_2O_2) or the hydroxyl radical OH* (Carvalho, 2008). These short-living highly reactive molecules can damage photosystems (PS), associated pigments, membrane lipids, proteins and DNA (Reddy et al. 2004; Bhattacharjee, 2012). DNA damage such as single or double-strand breaks may activate poly-ADP-ribose polymerases (PARPs) catalyzing an extensive self-ADP-ribosylation under the consumption of NAD^+. Ribosylated PARPs carry a negative charge keeping the damaged DNA open and accessible for the recruited DNA-repairing machinery. Hence, increased PARP activities under drought-induced oxidative stress could lead to NAD^+ breakdown and subsequent ATP depletion (De Block et al. 2005; Karlberg et al. 2010; Ko & Ren 2012).

Respiration

The daily carbon balance of a specific plant is determined by the ratio between CO_2 assimilation (photosynthesis and Calvin cycle) and the release of CO_2 due to respiration. Acclimation to drought stress is cost-intensive reducing carbon resources for plant growth and yield production (Farooq et al. 2009a). However, the extent to which desiccation stress shifts the carbon balance by influencing respiration is not fully understood, yet (Gimeno et al. 2010; Zhao et al. 2013). Interactions between chloroplast photosynthesis and mitochondria exist since some important reactions of the photo-respiratory pathway are located in the mitochondria (Bhargava & Sawant, 2013). In addition, it has been shown that mitochondria can support the maintenance of the photosynthetic electron transport chain under drought and other adverse conditions (Bartoli et al. 2005). This protective function of mitochondria is based on the presence of an alternative NADP(H) oxidase and an alternative oxidase (AOX) pathway within the mitochondrial electron transport chain. Alternative NADP(H) oxidase allows the oxidation of excessive NADP(H) from photosystem I while AOX bypasses the mitochondrial complexes III and IV leading to a reduction of O_2 to H_2O. Hence, both processes can contribute to balance the cellular redox state by dissipating excess cellular reducing power (Rassmussen et al. 2004; Bartoli et al. 2005; Umbach et al. 2005; Vanlerberghe, 2013).

Accumulation of compatible solutes

The metabolic carbon balance under drought stress is not only affected by reduced CO_2 assimilation in the Calvin-cycle but also by a lower photosynthetic efficiency and a vast demand for soluble sugars and sugar alcohols acting as compatible solutes. "Compatible solutes are osmotically active, low-molecular-weight, highly soluble compounds that are not interfering with normal metabolism even at high cytosolic concentrations" (Bohnert & Shen 1999; Bartels & Sunkar 2005; Farooq et al. 2009a).

They include sugars and polyols such as sucrose, glucose, fructose, trehalose or pinitol that accumulate in the cytosol of drought-stressed leaves (Schubert et al. 1995; Farias-Rodriguez et al. 1998; Streeter et al. 2001; Garg et al. 2002; Xue et al. 2008). The accumulation of soluble sugars and pinitol can decrease the cellular water potential due to reduction of the cytosolic osmotic potential. This may contribute to maintain turgor pressure and to stabilize the water potential gradient between soil and plant, unless drought stress becomes too severe (Farooq et al. 2009a). Further, soluble sugars may act as signaling molecules (Xue et al. 2008). Beside carbohydrates, proline and glycine betaine exhibit comparable osmoprotective effects (Ashraf & Foolad 2007; Chen et al. 2007). Moreover, proline and other compatible solutes were reported to stabilize proteins and membranes and to alleviate oxidative stress by scavenging radicals (Jain et al. 2001; Bartels & Sunkar 2005; Jakab et al. 2005; Sorkeheh et al. 2012). The release and biosynthesis of these osmoprotectants require alterations in carbohydrate metabolism and the induction of specific biosynthetic pathways (Xue et al. 2008).

Antioxidant defense

Plants have developed an interdependent antioxidant defense system to cope with ROS evolution from enzymatic reactions and drought or other environmental stresses. Antioxidant defense (AOD) in plants comprises both low-molecular antioxidants and an enzymatic system capable to rapidly decompose ROS to less toxic intermediates (Ahmad et al. 2010). The enzymatic system includes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and other classes of peroxidases (POX) shown to increase under drought stress. SOD catalyzes the reaction from O_2^- to H_2O_2 which is then further metabolized to H_2O and O_2 by catalase. Additional H_2O_2 not accessible for CAT can be detoxified by APX and POX using specific electron donors such as ascorbate or secondary phenolic metabolites (Almeselmani et al. 2006; Apostolova & Yaneva 2006, Ahmad et al. 2010). Glutathione reductase (GR) is another important member of the AOD-system since it reduces oxidized glutathione (GSSG) under consumption of NADPH. In turn, reduced glutathion (GSH) can then act as an electron donor for ROS-reduction (Meyer & Hell 2005; Ahmad et al. 2010). Another role in the AOD in plants is discussed for glutathione-S-transferases (GST). GSTs are required for the detoxification of xenobiotics (e.g. pesticides) but there is also evidence for a significant involvement in the oxidative stress response (Marrs, 1996; Moons, 2003). Besides discussed enzymes, additional antioxidants, radical scavengers and small molecular redox buffers play an important role in the cellular AOD-machinery. E.g. polyamines, α-tocopherol and carotenoids display molecular-scavenging and membrane-stabilizing features preventing lipid-peroxidation and thus, the formation of highly cytotoxic 4-hydroxyalkenals. Moreover, thioredoxins or (monothiol) glutaredoxins can buffer stress-induced disturbances in the cellular redox homeostasis (Marrs, 1996; Velikova et al. 2000; Foyer & Nocter 2005; McNulty et al. 2007; Groppa & Benavides 2008).

1.1.4 Molecular aspects of drought stress

Drought stress perception

Although much research has been conducted on drought stress-induced signaling and the regulation of associated genes, the exact mechanism of stress perception in roots still remains elusive (Zhao et al. 2001; Okasabe et al. 2013). According to Schachtman & Goodger (2008) and Haswell & Verslues (2015), there are three generalized models for sensing osmotic imbalances, a decreasing water potential or turgor loss:

1. Mechanosensitive ion channels may sense stress-induced modifications of plasma membrane tension. The change in membrane stretching could lead to an opening of mechanosensitive ion channels promoting the influx of e.g. Ca^{2+}. The subsequent membrane depolarization could be used as a signal for triggering a stress-dependent down-stream signaling cascade.

2. Plasma membrane receptors may sense extracellular fluctuations in the osmotic potential leading to a conformational change and subsequent protein phosphorylation for further signal transduction.

3. Drought causes the loss of cell wall integrity leading to a release of wall-associated carbohydrates, glycoproteins and pectins which in turn may be perceived by direct binding to receptor-like protein kinases. Another hypothesis is the potential existence of trans-membrane receptors capable to monitor drought-induced modifications in the spatial distance between the cell wall and plasma membrane.

Abscisic acid signaling and response

The phytohormone abscisic acid (ABA) has multiple functions in plants. Under favorable conditions ABA is not only important for seed maturation and dormancy but also involved in the development of young seedlings and lateral roots (Koornneef et al. 2002; Xiong & Zhu 2003). It also participates in a complex network of hormonal cross talk. Furthermore, ABA has a pivotal function in abiotic stress signaling and the subsequent regulation of gene expression (Danquah et al. 2014). After the initial step of drought stress perception, water shortage rapidly leads to the formation and accumulation of ABA in roots and leaf tissue (Davies et al. 2005). ABA originates from carotenoids and its biosynthesis starts with the conversion of zeaxanthin to violaxanthin, catalyzed by zeaxanthin epoxigenase (ZEP). Zeaxanthin is converted to 9'-*cis*-neoxanthin or 9-*cis*-violaxanthin and used as a substrate for the rate-limiting formation of xanthoxin catalyzed by 9-*cis*-epoxycarotenoid dioxygenase (NCED) in plastids (Qin & Zeevaart 1999). Xanthoxin is then released into the cytosol and oxidatively converted to abscisic aldehyde (Seiler et al. 2011). The final step in ABA biosynthesis involves abscisic acid aldehyde oxidase (AAO) and a molybdenum cofactor (Seiler et al. 2011).

Following biosynthesis and accumulation, ABA can trigger downstream responses at the cellular level or act as a long-distance signal between roots and shoots. When initial ABA becomes distributed via the xylem, it regulates stomatal aperture and leaf growth in shoots (Zhang et al. 2006). Although initial ABA levels in the xylem sap increase 50-fold as a result of drought stress, these concentrations are insufficient to induce stomatal closure in wheat (Munns & King 1988). It is thus believed that other factors may act synergistically to amplify the effect of root-derived ABA. Water shortage often leads to an alkalinization of the xylem sap due to changes in nitrate availability, ionic composition and malate concentrations (Schachtmann & Goodger 2008). It was demonstrated that a more alkaline xylem sap could act as a signal for reduced transpiration, itself (Wilkinson & Davies 1997). In addition, an alkalinization of the xylem sap and the apoplast can impair the passive uptake of ABA into the mesophyll cells because it acts as a weak acid (Wilkinson & Davies 1997). Increased ABA concentrations in the apoplast of guard cells subsequently lead to the regulation of stomatal aperture (Hartung et al. 1998, Wilkinson, 1999). Another factor which may contribute to increased ABA concentrations is the reduced water flux through the xylem when soil water potential decreases (Davies et al. 2005). Next to free ABA, its degradation products or conjugates such as phaseic acid, diphaseic acid and ABA-glucose ester may also play an important role in root-to-shoot stress signaling (Sauter et al. 2002; Schachtman & Goodger 2008; Finkelstein, 2013).

The primary response of increasing ABA concentrations in the apoplast of guard cells is stomatal closure. In *Arabidopsis*, ABA is perceived through binding to a family of 14 specific PYR/PYL/RCAR[2] receptors (Ma et al. 2009; Park et al. 2009; Melcher et al. 2010). Binding of ABA causes a conformational change of the ABA-receptor-complex enabling a physical interaction with specific PP2Cs[3] such as ABI1[4] or ABI2 (Rodriguez, P. 1998; Raghavendra et al. 2010; Miyakawa et al. 2013). The PP2Cs act as negative ABA regulators by repressing the autophosphorylation of Snf1-like kinases (SnRK2s)[5], (Gosti et al. 1999; Joshi-Saha et al. 2011). Upon ABA-receptor binding, PP2C phosphatase activity is inhibited, thus enabling SnRK2-dependent phosphorylation of multiple targets involved in stress acclimation and response (Finkelstein, 2013). In guard cells, the activation of a specific SnRK2 facilitates phosphorylation of a NADPH oxidase and phosphorylation of certain ion channels (Raghavendra et al. 2010; Kulik et al. 2011). Phosphorylation of NADPH oxidase triggers ROS formation which in turn leads to the activation of Ca^{2+} channels in the guard cell membrane (Kimura et al. 2012). Enhanced cytosolic Ca^{2+} concentrations, together with the SnRK2-based phosphorylation of a specific anion channel, mediates anion and water efflux from guard cells (Danquah et al. 2014).

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[2] PYR: pyrabactin resistant, PYL: PYR-like; RCAR: regulatory component of ABA receptor.
[3] PP2C: protein phosphatase 2C (serine/threonine phosphatases)
[4] ABI1: ABA insensitive1
[5] SnRK2: sucrose non-fermenting-1 related protein kinase 2.

H^{+} -ATPase, which is required for ion uptake and stomatal opening, is also inhibited by ABA and SnRK2 (Danquah et al. 2014). A simplified scheme of ABA-dependent control of stomatal aperture is shown in Fig. 2. In addition, other SnRK2s control the expression of several bZIP (basic leucine zipper) transcription factors such as ABFs (ABA responsive element binding factors)[6] controlling a wide set of genes involved in stress response and acclimation (Miyakawa et al. 2013). Besides bZIPs, ABA-controlled gene expression also affects the activity of various members of other classes of transcription factors (TFs) such as MYBs, MYCs, NACs and WRKYs (Nakashima et al. 2007; Finkelstein, 2013; Li et al. 2015). Moreover, drought signaling networks highly depend on additional second messengers (Ca^{2+}, sugars, ROS, PA, IP3, NO etc.)[7], phytohormonal crosstalk, the MAPK cascade and multiple other calcium-dependent or independent protein kinases / phosphatases (Bartels & Sunkar 2005; Golldack et al. 2014).
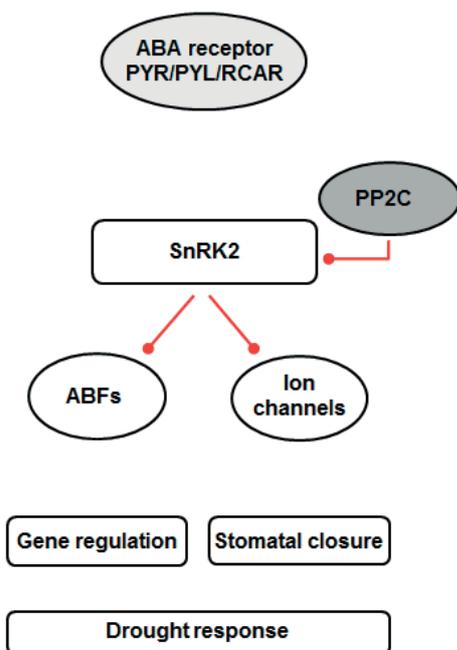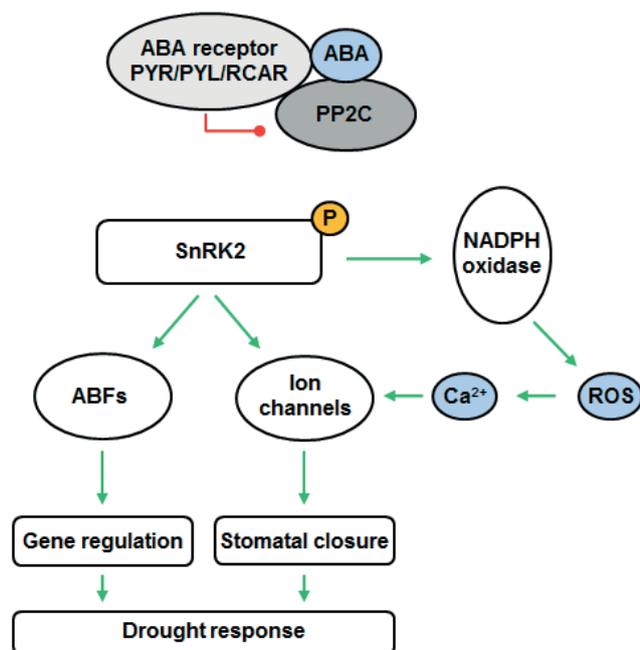

Fig. 2: Simplified scheme of ABA-dependent stomatal closure. ABA-binding to the PYR/PYL/RCAR receptor class inhibits PP2C protein phosphatase activity. Autophosphorylation of SnRK2 leads to the activation of associated ABFs triggering the expression of target genes. Stomatal closure is induced by the SnRK2 dependent phosphorylation of guard cell ion channels and NADPH oxidase. The release of ROS and Ca^{2+} function as additional second messengers in modifying stomatal aperture. Red arrows indicate suppressing activities, green arrows represent inducing activities, respectively (modified, according to Danquah et al. 2014.)

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[6] ABA responsive element: specific sequence (CACGTG) in the promotor region of ABA responsive target genes.
[7] IP3: inositol 1,4,5-triphosphate, NO: nitric oxide.

ABA-independent regulation of gene expression

Besides the ABA-dependent drought response, gene expression studies in ABA-deficient *Arabidopsis* mutants revealed an additional ABA-independent signaling network. Genes identified in mutant screens share a conserved motif within their promotor regions (A/G)CCGAC), termed the C-repeat/dehydration-responsive-element (CRT/DRE), (Bartels & Sunkar 2005; Shinozaki & Yamaguchi-Shinozaki 2007). DREs are cis-acting elements targeted by C-repeat binding factors/dehydration-responsive-element binding proteins (CBFs/DREBs). CBFs/DREBs belong to the family of AP2/ERF[8] DNA-binding domain transcription factors and can be divided into two structurally different groups (Chaves et al. 2003). Group 1 DREBs (CBF3/DREB1) were found to be predominantly induced by low temperatures and cold while group 2 DREBs (DREB2) are activated by drought and salinity stress (Nakashima et al. 2000; Oakenfull et al. 2013; Kidokoro et al. 2015). Overexpression of various CBF/DREB transcription factors confers enhanced tolerance to cold, drought, salinity or oxidative stress in *Arabidopsis*, rice, wheat, tobacco and soybean (Wang et al. 2009; Zhao et al. 2010; Hwang et al. 2013; Jiang et al. 2014). However, it is worth to mention that some stress responsive genes harbor both a CRT/DRE and an ABRE cis acting element in their promoter regions indicating a regulatory crosstalk between ABA-dependent and ABA-independent pathways (Zhao et al. 2010).

Calcium signaling in drought-stress responses

Ca^{2+} is one of the most important second messengers in plants as it is involved in a wide range of physiological responses including the regulatory signaling network induced by drought and other stresses (Knight et al. 1997; Batistič & Kudla 2009; Tanaka et al. 2010). An outstanding feature of Ca^{2+} signaling is its specificity achieved by a combination of differentially expressed and localized calcium channels, pumps and transporters generating a highly variable and distinct calcium signature (Boudsocq & Sheen 2010). Usually, cytosolic Ca^{2+} concentrations are maintained low but can rapidly increase upon an internal or external stimulus activating specific Ca^{2+} transport systems. Depending on the stimulus and localization of activated channels, Ca^{2+} signals can vary in duration, amplitude and frequency, allowing the precise and cue-specific regulation of downstream responses (Boudsocq & Sheen 2010). To activate cellular responses, Ca^{2+} signals have to be perceived and decoded. Calmodulin (CaM), calmodulin-like proteins (CMLs) and calcium-dependent protein kinases (CDPKs) function as calcium sensors capturing spatial and temporal fluctuations in Ca^{2+} levels (Luan et al. 2002). Calcium sensors comprise typical EF-hand[9] domains and Ca^{2+} binding results in a conformational change of the sensor (Luan et al. 2002; Tuteja & Mahajan 2007).

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[8] AP2/ERF: Apetala2/ethylene responsive element binding factor.
[9] EF-hand: elongation factor hand moti: A helix-loop-helix motif specific for Ca^{2+} binding proteins with a 12 amino acid consensus sequence.

This enables the interaction with effector proteins such as CaM-binding proteins or kinases (Boudsocq & Sheen 2010). A special class of Ca^{2+} sensors is represented by the CDPK family carrying both, a Ca^{2+} binding- and a protein kinase domain (Asano et al 2012). Under stress conditions, CMLs and CaMs were reported to regulate the expression of heat shock proteins (HSPs) or to maintain H_2O_2 homeostasis by activating wheat catalase (Yang & Poovaiah 2002; Liu et al. 2003). In *Arabidopsis*, several CDPKs were shown to modulate stomatal aperture or to function as conversion point between biotic and abiotic stress signaling. Furthermore, specific CDPKs were found to act as positive regulators of ABA mediated gene expression (e.g. by phosphorylation of ABFs), (Li et al. 2008; Asano et al 2012). Calcineurin B-like proteins (CBLs) represent another group of calcium sensor proteins. CBLs are structurally comparable to CaM but display distinct Ca^{2+} binding capacities and affinities (Batistič & Kudla 2009). As for CaM, binding of Ca^{2+} leads to a conformational shift facilitating subsequent interactions with effector proteins. Among these, the calcineurin B-like interacting protein kinases (CIPKs) are most important (Boudsocq & Sheen 2010). CIPKs belong to the SnRK3 family of serine/threonine protein kinases and were identified in several plant species e.g. in *Arabidopsis*, wheat, rice and maize. Expression of CIPKs is altered during abiotic stresses and functional analysis of some CIPKs revealed a positive role in freezing, salt and drought tolerance (Xiang et al. 2007; Deng et al. 2013; Chaves-Sanjuan et al. 2014; Chen et al. 2014).

Proteins for stress alleviation

Drought-induced transcriptional changes not only affect the expression of numerous regulatory proteins but also functional proteins involved in stress acclimation and alleviation (Harb et al. 2010). Of such, aquaporins (AQPs), late embryogenesis abundant proteins (LEAs) and heat shock proteins (HSPs) are representative. Aquaporins belong to large family of conserved and hydrophobic membrane pore-forming proteins called major intrinsic proteins (MIPs), (Kruse et al. 2006; Vendeleur et al. 2009). Depending on the cellular location in plants, AQPs are either referred to as tonoplast intrinsic proteins (TIPs) or termed as plasma membrane intrinsic proteins (PIPs). AQPs play a central role in cellular water relations as they form specific pores facilitating membrane water permeability and osmosis (Bartels & Sunkar 2005). The over-expression of specific AQPs has been shown to improve plant efficiency under both, abiotic stresses and favorable conditions (Li et al. 2015). However, in drought-stressed *Arabidopsis,* the majority of PIPs were found to be down-regulated and the authors concluded that down-regulation of PIPs could minimize water flux to maintain leaf turgor during water deprivation (Alexandersson et al. 2005).

The family of LEA proteins comprises seven groups of sequence-related highly hydrophilic proteins. LEAs were shown to accumulate during late seed maturation and under water deficit in many plant species (Hong-Bo et al. 2005; Battaglia et al. 2008).

Depending on the specific group, LEAs can exhibit distinct molecular functions. Group 1 LEA proteins are characterized by high glycine content and expression occurs mainly during late seed maturation in response to ABA. Group 1 LEA proteins are thought to play a role in water binding and replacement (Bartels & Sunkar 2005). The second group of LEA proteins is denoted as dehydrins (DHNs) or "responsive to abscisic acid" (RABs), (Soulages et al. 2003). DHNs interact with partly dehydrated protein or membrane surfaces acting as chaperones for protein stabilization and folding (Hanin et al. 2011). Moreover, DHNs can maintain the intracellular space keeping incompatible reactions apart and preventing drought-induced cellular collapse. Some DHNs exhibit additional radical scavenging activity due a comparably large amount of reactive amino acid residues (Hanin et al. 2011). Other LEA proteins assigned to groups 3 and 5 are thought to function in ion-sequestering under drought stress (Bartels & Sunkar 2005). It has been observed that the secondary structure of some group 3a LEAs increased as a result of progressive dehydration forming similar structures than cytoskeleton associated proteins (Wise & Tunnacliffe 2004). It was thus suggested that these LEAs may function as cell-stabilizing proteins under water deficit (Wise & Tunnacliffe 2004). Heat shock proteins belong to a class of molecular chaperones which include "any protein that interacts with, stabilizes or helps another protein to acquire its functionally active conformation" (Hartl et al. 2011). HSPs are classified into 5 major groups according to their specific molecular weight: HSP60, HSP70, HSP90, HSP100 and low-molecular mass HSPs (small HSPs), (Schlesinger, M. 1990). HSP-mediated protein folding is promoted by ATP and / or interaction with small HSPs. Enhanced expression occurs usually under stress conditions such as drought or high temperatures (Sun et al. 2002; Wang et al. 2004). At the molecular level, HSPs have a major function in protein stabilization, folding and assembly. Moreover HSPs are involved in the disposal of denatured, aggregated and misfolded proteins. Thus, HSPs play an essential role in cellular proteome homeostasis and enhanced expression is associated with increased tolerance to multiple abiotic stresses (Wang et al. 2004; Hartl et al. 2011).

1.2 Options to increase crop yield under water scarcity

1.2.1 Farming approaches

Options to sustainably improve crop yield in areas with limited water availability include water saving farming practices and the development of drought-tolerant crop varieties. The advantages and disadvantages of alternative irrigation systems or farming practices to increase crop water use efficiency[10] and harvest index[11] were extensively reviewed by Tilman et al. (2002), Lamm & Trooien (2003), Chaves & Oliveira (2004), Trethowan et al. (2005), De Vita et al. (2007), Deng et al. (2006) and Sadras (2009).

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[10] ratio between biomass accumulation and water use (Steward & Steiner 1990)
[11] ratio between grain yield and total plant biomass (Sinclair, 1998)

In corn, subsurface drip irrigation significantly reduced (up to 55 %) seasonal water consumption without a negative impact on yield production. However, the initial costs for setting up a subsurface drip irrigation system in corn were high and required a life-time of at least 15 years to compete with standard irrigation systems such as center pivots (Lamm & Trooien, 2003). Another strategy relies on the adaptation of tilling practices to local water availabilities. Reduced soil cultivation, e.g. conservation tillage can restrict water loss from evaporation or run-off. In rice / wheat rotations, water savings were estimated to be as high as 1,000,000 L of water per ha of land. Although of great significance, reduced tillage practices could entail new risks for crop productivity such as an increased disease or pest occurrence due to remaining stover from previous crops (Trehowan et al. 2005).

1.2.2 Remote Sensing

Remote sensing can be used to monitor early drought stress, nutrient deficiency or other factors influencing crop performance. This may allow to precisely schedule plant irrigation or to apply fertilizer when needed by a crop (Penuelas & Inoue, 1999; Tilling et al. 2007; Linke, 2012; Shimada et al. 2012). Remote sensing techniques use the vegetation-specific spectrum of canopy reflectance at wavebands between 400 and 2,400 nm. Canopy reflectance is highly influenced by the amount of green biomass, ground coverage, leaf architecture, physiological state and leaf chemistry (Penuelas, 1998; Huber et al. 2008). In the visible part of the light spectrum (400 to 700 nm), canopy reflectance is usually low due to absorption by leaf pigments such as chlorophylls and carotenoids (Fig. 3), (Curran, 1989; Penuelas, 1998).
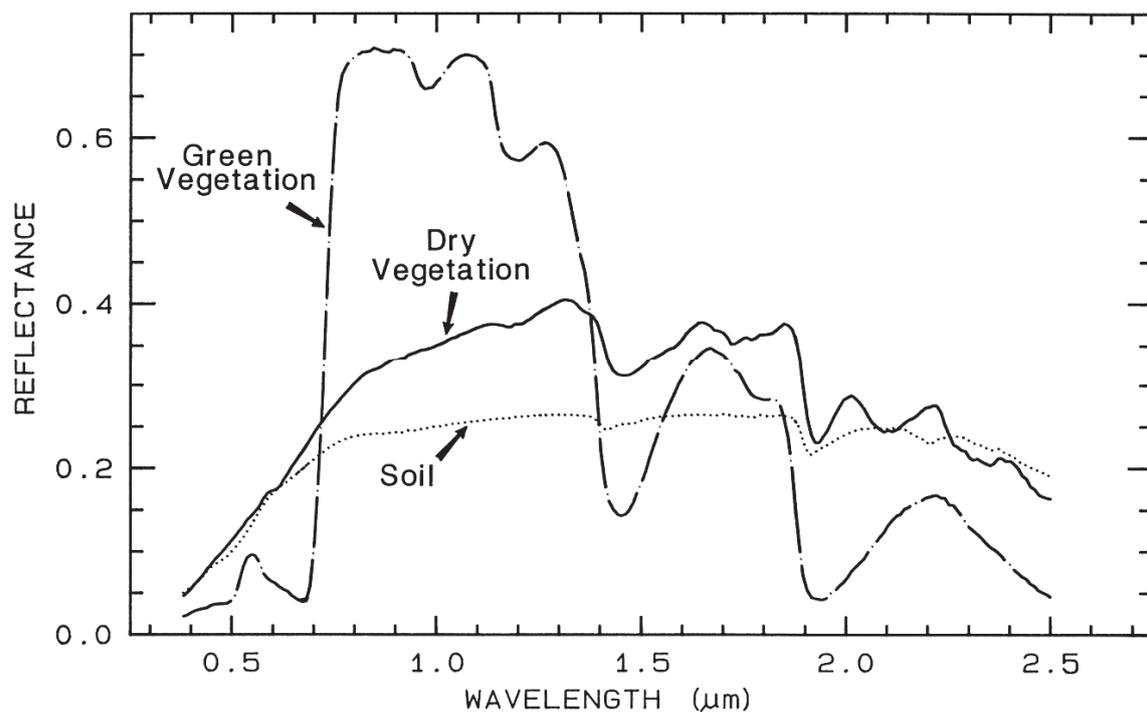

Fig. 3: Reflectance spectrum of different surfaces (Clark et al. 2003, modified).

Canopy reflectance peaks in the near infrared (NIR) part of the electromagnetic spectrum (700 - 1300 nm), with the greatest slope between 690 to 750 nm ("red edge") because live vegetation strongly scatters NIR energy (Knipling, 1970; Ollinger, 2011, Wang et al. 2015). Measuring the slope of spectral reflectance in the 'red edge' can give information on the overall physiological status of a canopy since it is tightly correlated with ground coverage, canopy architecture and leaf chemistry (Govaerts & Verhulst, 2010). Common vegetation indices are derived from ratios between absorbed and reflected parts of the red (550 – 700 nm) and NIR fraction of the electromagnetic spectrum. Among these the Normalized Difference Vegetation Index (NDVI) is commonly used to survey green biomass (Santin-Janin et al. 2009). Stressed canopies display increased reflectance in the visible light bands and increased absorption in the NIR light fraction, respectively. For this reason, NDVI values decrease with increasing stress intensities (Sims et al. 2014).

1.2.3 Breeding approaches

Breeding for improved crop yields under drought conditions aim at selecting varieties which can either, escape from, avoid or tolerate drought stress (Barnabás et al. 2008). Escaping from drought can be achieved by traits such as early crop maturity favorable for regions with terminal water scarcity. Cultivars producing deep and extensive root systems may avoid drought stress by a superior exploitation of available soil water resources (Champoux et al. 1995). The ability of a specific cultivar to better tolerate drought stress implies faster and enhanced defense mechanisms (accumulation of compatible solutes, expression of HSPs etc.) towards the stress situation (Chaves & Oliveira 2004). However, any breeding strategy must be adapted to local requirements since the spatial and temporal occurrence of drought stress greatly varies (Mir et al. 2012). During the last 80 years of breeding, crop yield consistently increased and selection based on phenotypic traits indirectly led to the appearance of varieties harboring higher yield potentials even under unfavorable conditions (Cattivelli et al. 2008).

To date, the principal understanding of drought stress and its impact on physiological and molecular processes in plants has led to the identification of multiple stress-relevant traits and hundreds of associated genes (Chaves et al. 2003). Current methods for introducing these traits in crops have been discussed comprehensively by Campos et al. (2004), Tuberosa & Salvi (2006), Cattivelli et al. (2008), Moose & Mumm (2008), Agbicodo et al. (2009) and Mir et al. (2012). However, despite the availability of molecular breeding approaches or genetic engineering by transferring single genes, the release of drought-tolerant varieties has only been marginal due to the high genetic complexity of this trait (Wang et al. 2003).

1.2.4 Chemical induction of drought tolerance

Tolerance to drought and other abiotic or biotic stresses was shown to be effectively induced by exogenous application of various chemicals and plant growth regulators (Beckers and Conrath, 2007; Farooq et al. 2009a). In *Arabidopsis* drought tolerance was achieved by pre-treating soils with *β*-aminobutyric acid (BABA). Plants showed significantly lower amounts of water loss and delayed symptoms of wilting. Further investigations revealed a BABA-dependent induction of salicylic acid (SA)- and ABA-dependent pathways. However, for the induction of drought stress, only ABA biosynthesis and signaling were required since *aba1* and *abi4* mutants were not positively affected by BABA pre-treatments (Jakab et al. 2005). Studies performed by Horn et al. (2013) indicated a superior drought stress-reducing effect after simultaneous application of the fungicide tebuconazole and ABA. Although the mode of action behind this synergistic effect was not fully uncovered, degradation of proteins involved in PSII activity, N- and S-assimilation and CO_2 fixation was reduced in plants treated with tebuconanzole and ABA. In addition, the strobilurin fungicide, kresoxim-methyl was shown to inhibit ethylene biosynthesis in drought-stressed wheat leading to delayed leaf senescence and a prolonged grain filling period (Grossmann et al. 1999). Foliar applications of the commercial herbicide safener[12] cyprosulfamide resulted in improved salt tolerance of rice (*Oryza sativa*). Dashevskaya et al. (2013) reported early flowering and a significantly increased tiller formation after foliar applications of cyprosulfamide. Rice seedlings sprayed with a mixture of cyprosulfamide and ABA showed even higher levels of salt tolerance suggesting a synergistic effect between ABA and the safener. Moreover, defense and detoxification-related proteins were found to be induced in cyprosulfamide treated rice seedlings, suggesting a potential mechanism for salinity tolerance (Dashevskaya et al. 2013).

The central role of SA in defense against biotic stresses is well described but there is increasing evidence of an additional involvement in abiotic stress responses, as well (Durner et al. 1997; Vlot et al. 2009; Gautam & Singh 2009; Tripathi et al. 2010). Exogenous application of the phytohormone SA was shown to enhance drought tolerance in multiple crops. Wheat plants grown in SA-containing nutrient solution exhibited improved plant growth parameters (biomass production, root length, plant height) under PEG (polyethylene glycol)-induced drought stress. Biochemical analysis of SA-treated plants indicated higher levels of GSH and ascorbate and decreased levels of lipid peroxidation. Consequently, subsequent gene expression profiling revealed significant alterations in transcript levels of GSH-cycle-associated genes such as GR and GSTs (Kang et al. 2013). There are many other studies reporting on the chemical induction of drought tolerance and a selection is given in Table 1.

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[12] Safener: A chemical which induces plant detoxification mechanisms to prevent crops from herbicide damage.