# Role of the potassium nutritional status on drought stress-induced leaf senescence in barley

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# 1 Summary

Occurrence of drought during generative plant development may induce premature leaf senescence that leads to a decline in photosynthesis and grain yield. Persisting water scarcity also affects nutrient uptake and translocation to shoots. The nutritional status of plants plays a critical role in increasing plant tolerance to drought. In particular potassium (K) plays a protective role in alleviating drought stress. To reveal the role of K nutrition in carbohydrate metabolism and hormonal regulation during drought, 3 near-isogenic barley lines, representing an earlier senescing line with high remobilization capacity (LP104), a line with normal senescence (LP110) and a stay-green line (LP106) were subjected to drought stress under low, medium or high K supplies. Analysis of chlorophyll concentrations and of the gene expression levels of the senescence marker *HvS40* showed that the line LP104 developed later leaf senescence than the lines LP110 and LP106 when plants grew under drought stress and low K supply. This coincided with elevated leaf K levels, suggesting that higher tolerance to drought-induced leaf senescence was caused by superior K efficiency in the line LP104. Moreover, the depletion of starch in flag leaves was significantly lower in LP104, indicating a higher carbohydrate reserve under drought. Based on the higher carbohydrate and starch levels in LP104, it was further investigated whether the different behaviour of these lines under drought stress may be related to the stress hormone abscisic acid (ABA). For another experiment the two most contrasting lines, LP104 and LP106, were subjected to drought stress to determine changes in phytohormone concentrations. While ABA concentrations significantly increased under drought in both lines regardless of K supply, this increase was less pronounced in the line LP104. However, LP104 accumulated higher levels of the ABA degradation products phaseic acid (PA) and dehydro-phaseic acid (DPA), suggesting a higher turnover of ABA which may have contributed to the better metabolic performance of this line. Altogether, the results indicated that the ability of a genotype to retain more K in flag leaves is associated with a higher turnover of ABA. As indicated by elevated starch levels in the K-retaining genotype, superior tolerance to drought may benefit from a larger reserve of carbohydrates required for osmotic adjustment. Thus, this study indicates that the beneficial effect of K on drought-induced ABA signaling, which has been so far mainly described at the molecular level, is also of physiological relevance in drought-stressed crop plants.

**Key words**: Terminal drought stress, barley, potassium nutrition, primary metabolites, abscisic acid, nutrient efficiency

# 2 Zusammenfassung

Trockenheit während der generativen Pflanzenentwicklung kann zur Induktion einer vorzeitigen Blattseneszenz führen, die eine Verringerung der Photosynthese und des Kornertrags zur Folge hat. Anhaltender Wassermangel beeinflusst zudem die Aufnahme und Translokation von Nährstoffen zu den Blättern. Auch der Ernährungszustand von Pflanzen spielt eine entscheidende Rolle bei der Steigerung der pflanzlichen Toleranz gegenüber Trockenheit. Insbesondere trägt Kalium (K) zur Verringerung von Trockenstress bei. Um die Rolle von K im Kohlenhydratstoffwechsel und in der hormonellen Regulation während der Trockenheit zu untersuchen, wurden 3 nahezu isogene Gerstenlinien, eine Linie mit hoher Remobilisierungskapazität (LP104), eine natürlich seneszierende Linie (LP110) und eine “stay-green“-Linie (LP106) unter niedrigem, mittlerem oder hohem K-Angebot angezogen und einem terminalen Trockenstress ausgesetzt. Die Analyse der Chlorophyllkonzentrationen und der Genexpression des Seneszenzmarkers *HvS40* zeigte, dass die Linie LP104 im Vergleich zu den Linien LP110 und LP106 bei niedriger K-Konzentration und Trockenstress eine verzögerte Blattseneszenz aufwies. Dies ging mit einer erhöhten K-Konzentration im Blatt einher, was darauf hindeutete, dass eine höhere Toleranz gegenüber trocken-induzierter Blattseneszenz bei der Linie LP104 durch eine erhöhte K-Effizienz verursacht wurde. Darüber hinaus war der Abbau von Stärke in den Fahnenblättern der Linie LP104 deutlich niedriger, was auf eine höhere Kohlenhydratreserve unter Trockenheit hindeutete. Aufgrund der erhöhten Kohlenhydrat- und Stärke-Konzentrationen in LP104 wurde weiterhin untersucht, ob das unterschiedliche Verhalten der untersuchten Linien unter Trockenstress in Zusammenhang mit dem Stresshormon Abscisinsäure (ABA) steht. Dazu wurden die kontrastierenden Linien, LP104 und LP106, Trockenstress ausgesetzt, um Veränderungen in den Hormon-Konzentrationen zu bestimmen. Während die ABA-Konzentration unabhängig von der K-Versorgung unter Trockenstress in beiden Linien deutlich erhöht war, war dieser Anstieg bei der Linie LP104 weniger ausgeprägt. Allerdings akkumulierte die Linie LP104 höhere Mengen an den ABA-Abbauprodukten Phasein-Säure (PA) und Dehydro-Phasein-Säure (DPA), was auf einen höheren Umsatz von ABA zugunsten einer verbesserten Stoffwechselleistung hindeutete. Zusammenfassend zeigten die Ergebnisse, dass die Fähigkeit eines Genotyps mehr K in den Fahnenblätter zurückzubehalten mit

einem erhöhten Stoffwechsel von ABA einhergeht. Wie durch die erhöhte Stärkereserve im Genotyp mit höheren K-Gehalten angedeutet, könnte die erhöhte Trockentoleranz in LP104 von einem größeren Kohlenhydratpool profitieren, um osmotische Anpassungen zu begünstigen. Somit offenbaren diese Studien, dass die positive Wirkung von K auf die trockenstressbedingte ABA-Bildung, die bisher vor allem auf molekularer Ebene beschrieben wurde, auch eine wichtige physiologische Bedeutung bei Kulturpflanzen unter Trockenstress beigemessen warden kann.

**Schlüsselwörter:** terminaler Trockenstress, Gerste, Kaliumernährung, Primärmetabolismus, Abszissinsäure, Nährstoffeffizienz.

# 3 Introduction

## 3.1 Importance of drought stress for plant production

The world population is expanding rapidly and will pass from its current number of 7.0 billion to 9.4 billion by the year 2050. To provide enough food for an expanding world population, a massive increase in crop production is required to meet the food demands of future generations, while preserving the ecological and energy-related resources of our planet (Wang *et al.*, 2013). Agricultural production continues to be limited by a variety of biotic (e.g., pathogens, insects and weeds) and abiotic (e.g., drought, salinity, cold, frost and waterlogging) factors that can significantly reduce the quantity and quality of crop yield (Alegre, 2004). Among the abiotic stresses drought is one of the major stress factors limiting crop production worldwide (Helena and Carvalho, 2008). Drought occurs over most parts of the world, even in wet and humid regions like Asia, sub-Saharan Africa, and central and South America. Also in Europe, future climate change is expected to become problematic, resulting more often in dry periods during spring and rainy summers in Northern Europe and longer dry periods in the south (Marshall *et al.*, 2012). According to data provided by the Energy Information Administration (EIA), Unites States, Europe and Australia suffered crop losses of 20, 4.7 and 1.2 billion U.S. dollars, respectively. Therefore, improving biomass production and seed yield under suboptimal water availability or other abiotic stresses is now even more urgent (Marshall *et al.*, 2012)

The severity of drought stress for crop production not only depends on the duration and intensity (Samarah *et al.*, 2009) but also on the time point when drought stress sets in. Plants may experience drought stress as transient or terminal drought stress which leads to different physiological and developmental responses in plants (Alegre, 2004). If drought stress occurs during the vegetative growth phase, the stress is mostly transient as it holds on until rainfall comes back and restores plant growth. This is typical for pre-summer drought periods, which are seen more frequently in the continental climates of middle Europe. In this case, cereal crops like wheat slow down growth and may even start wilting, while yield formation is mainly limited by suppressed tillering, i.e. a lower number of ear-bearing tillers per plant (Sreenivasulu *et al.*, 2007).

Occurrence of drought during generative plant development, i.e. around flowering, may induce premature leaf senescence that leads to a decline in photosynthesis and assimilate allocation, ultimately causing an acceleration in the whole-plant maturation process (Gan, 2003). Under terminal drought crop yields are mostly restricted by plant reproductive failure, followed by a shortening of the grain filling period which ultimately reduces grain number and grain size (Sreenivasulu *et al.*, 2007). However, plant responses to drought stress differ among plant species, e.g. with barley being more tolerant than wheat, but strongly depend also on the plant genotype (Farooq *et al.*, 2009).

Another early consequence of the low water availability under drought is a decrease in total nutrient uptake and translocation to shoots. A lower absorption of inorganic nutrients results mainly from a reduced transpirational flow and hence a decreased bulk flow to the root surface of soil water containing soluble nutrients. This is supported by the observation that transient periods of drought often decrease the uptake of nitrate, whose transport to the root surface mainly depends on mass flow. Persisting water scarcity will affect nutrients whose transport depends more on diffusion, such as phosphate or potassium (K). Plant species and genotypes may partially counteract these unfavorable conditions by forming deeper roots and further expanding the root system (Römheld and Kirkby, 2007) .

## 3.2 Effects of drought on plants

### 3.2.1 Crop growth and yield

Under drought stress, cell elongation is inhibited by reduced turgor pressure which impairs cell expansion. As a result, turgor gets lost. However, drought stress also inhibits the photo-assimilation and processes involved in cell division. As a consequence, impaired cell division, cell elongation and expansion result in reduced growth (Farooq *et al.*, 2009). In addition, exposition of seedlings to drought resulted in poor germination and poor seedling establishment (Kaya *et al.*, 2006). In barley, drought stress during vegetative development decreased grain yield by reducing the number of tillers, spikes and number of grain per plant (Samarah *et al.*, 2009). These authors have shown that barley grain filling decreased by 40% under drought stress during the grain filling period. Grain filling in cereals is also affected in particular by terminal drought stress which reduces the activity of major enzymes involved in starch biosynthesis and results in a

decline of grain development (Taiz and Zeiger, 2006). During the grain filling period, drought stress negatively impacts grain weight and yield more than during late grain filling. Samarah (2004) reported that drought during the grain filling decreased the length of the grain filling period.

### 3.2.2 Water relations under drought

In the initial phases of drought, root water uptake and transpiration decrease. Then soil conductivity decreases. An impairment of the contact between roots and soil are limiting to water movement towards the root surface when drought becomes more pronounced (Aroca *et al.*, 2012). Root sensing of water deficit result in chemical and hydraulic signaling toward shoots that ultimately reduce leaf transpiration and leaf growth due to loss of turgor pressure. Consequently, plants close their stomata to prevent the transpirational water loss which results in a decrease in photosynthesis and water use efficiency (WUE). Such a reduction of photosynthesis and WUE has been reported in drought-stressed flag leaves of senescing barley plants under post-anthesis drought stress (Seiler *et al.*, 2014*b*).

### 3.2.3 Nutrient relations under drought

There is a close relation between the nutrient and water status in plants (Junjittakarn *et al.*, 2013). Decreasing water availability under drought generally results in limited total nutrient uptake and lower nutrient tissue concentrations in crop plants. An important effect of water deficit is a decrease in the diffusion rate of nutrients in the soil which decreases the acquisition of nutrients by the root and their transport to shoots. A negative influence of drought on plant nutrition may also be related to limited availability of energy for the assimilation of $NO_3^-$ and $SO_4^{2-}$ which must be reduced in highly energy-demanding processes before these elements can be used for growth and development of plants (Mcwilliams, 2003). During drought stress, root growth and the rates of K diffusion in the soil towards the roots are both restricted and limiting for K acquisition in root. The availability of K to the plant decreases with decreasing soil water content, due to the decreasing mobility of $K^+$ under these conditions (Hu and Schmidhalter, 2005). Therefore, the lower K availability can decreases plant tolerance to drought stress (Wang *et al.*, 2013).

### 3.2.4 Photosynthesis under drought

As the key process of primary metabolism, photosynthesis plays a central role in plant performance under drought (Pinheiro and Chaves, 2011). The decline observed in leaf net carbon uptake as a result of plant water deficits is followed by alterations in the partitioning of assimilates at the whole-plant level, corresponding in general to an increase in the root-to-shoot ratio. Such a response is mediated by hormonal control, namely by abscisic acid (ABA), ethylene, and their interactions. Upon reduction in the amount of available water, plants close their stomata via ABA signaling which decreases the $CO_2$ influx. Reduction in $CO_2$ influx not only reduces the carboxylation reaction of ribulose-1, 5 bisphosphate carboxylase/oxygenase (Rubisco) directly but also directs more electrons towards the formation of reactive oxygen species (ROS). Severe drought conditions limit photosynthesis due to a decrease in the activities of calvin cycle enzymes including rubisco and phosphoenolpyruvate carboxylase (PEPCase). Another important effect that inhibits the growth and photosynthetic abilities of plants is the loss of balance between the production of reactive oxygen species and the antioxidant defense (Fu J. and Huang 2001; Reddy et al. 2004), causing accumulation of ROS which cause oxidative damage to proteins, membrane lipids and other cellular components (Farooq *et al.*, 2009). Alterations in the level of photosynthetic pigments were also detected in water-stressed plants, showing a reduced or even no pigmentation. Both chlorophyll a and b declined in stressed plants and this directly affected plant biomass production. The reduction of photosynthetic pigments then resulted in a lower energy consumption and carbon demand for chlorophyll synthesis (Zingaretti and Inácio, 2013).

### 3.2.5 Oxidative damage under drought

The first plant organ to detect a limitation of the water supply is the root system. It has been shown that besides water and minerals, roots also send signals to the leaves through the xylem sap, and the phytohormone abscisic acid is considered to be one of the major root-to-shoot stress signals. When the stress signal reaches the leaves, it triggers stomatal closure and the plant shifts to a water-saving growth modus. However, by adjusting stomatal opening, plants are able to control water loss by reducing the transpiration flux, but they are concomitantly limiting the entrance of $CO_2$. This has direct and indirect effects on the reduction of net

photosynthesis and on the overall production of ROS by plants under drought stress. There are many studies that have reported an increased ROS accumulation and oxidative stress under drought stress. Production of ROS is common in all drought-stressed plant species (Chool Boo and Jung, 1999). In fact, under drought stress ROS production is enhanced through multiple ways. For instance, the limitation of $CO_2$ fixation will reduce nicotinamide adenine dinucleotide phosphate ($NADP^+$) regeneration through the Calvin cycle, hence provoking an over-reduction of the photosynthetic electron transport chain. Moreover, during photosynthesis and under drought stress there is a higher leakage of electrons to $O_2$ by the Mehler reaction. In fact, under drought stress the photorespiratory pathway is also enhanced, when RuBP oxygenation is favoured due to a limitation in $CO_2$ fixation. It has been estimated that photorespiration is likely to account for over 70% of total $H_2O_2$ production under drought stress conditions (Helena and Carvalho, 2008).

The chloroplast is a quite robust cellular compartment towards ROS, because the different scavenging enzymes and metabolites protect chloroplast constituents from oxidative damage (Chool Boo and Jung, 1999). However, under drought stress one of the real threats to the chloroplast is the production of hydroxyl radicals in the thylakoids through the 'iron-catalysed' reduction of $H_2O_2$. In this process, $O_2^{\bullet-}$ donate an electron to iron ($Fe^{3+}$) to yield a reduced form of iron ($Fe^{2+}$) which can then reduce $H_2O_2$, produced as a result of SOD led dismutation of $O_2^{\bullet-}$ to $OH^{\bullet}$ . This reactions is called the Habere Weiss reaction, whereas the final step which involves the oxidation of $Fe^{2+}$ by $H_2O_2$ is referred to as the Fenton's reaction (Helena and Carvalho, 2008).

## 3.3 Drought-induced senescence

Leaf senescence is a part of the developmental program of plants and its occurrence largely depends on the growth conditions to which plants have been adapted. Drought accelerates the time-course of senescence in several species, so that plant reproduction is subjected to stressful conditions (Alegre, 2004). Drought stress-induced senescence contributes to plant survival by an accelerated retranslocation of nutrients to the sink organs. It is well known that stress conditions might induce premature senescence of leaves that leads to a shortage of assimilates, ultimately causing an acceleration in the whole-plant maturation

process (Gan, 2003) and in the end strongly affecting crop productivity (Gregersen *et al.*, 2013). Leaf yellowing is the first visible symptom of drought-induced leaf senescence. This is a result of chlorophyll degradation and to lesser extent of carotenoid degradation (Alegre, 2004). Drought-induced leaf senescence leads to a progressive decline in photosynthesis such that the leaf ultimately reaches the compensation point, at which carbon assimilation and respiration are equal. At this point, the leaf will no longer contribute as a photosynthetic organ to the assimilatory needs of the rest of the plant. Nevertheless, senescent leaves still contain a significant pool of nutrients in the form of proteins, lipids and other macromolecules, which the plant may catabolize and export to other organs. During drought-induced leaf senescence, the chloroplast with the photosynthetic apparatus becomes dismantled and nutrients are exported from senescing leaves to young growing tissues (leaves, flowers and fruits) or storage organs. Accelerated leaf senescence in response to drought may be of advantage, considering that it reduces the water demand and consumption at the whole-plant level. In fact, one of the early responses during drought is stomatal closure, which is particularly pronounced in the oldest senescing leaves and reduces the total amount of water lost by transpiration (Alegre, 2004).

### 3.3.1 Regulation of drought-induced senescence

#### 3.3.1.1 Hormonal control of drought-induced leaf senescence

Drought changes the endogenous levels of plants hormones. Most of the studies demonstrated a role of ABA and cytokinins in the regulation of drought-induced leaf senescence (Yang *et al.*, 2003*b*). Indeed, all classical plant hormones, namely auxins, cytokinins, gibberellins, ABA and ethylene, have been described to play a role in the regulation of drought-induced leaf senescence (Gan, 2007). In general, leaf senescence is accelerated by ethylene, jasmonic acid (JA), abscisic acid (ABA) and salicylic acid (SA), but delayed by auxin, gibberellic acids (GAs) and cytokinins (Chen *et al.*, 2007; Guo and Gan, 2012). Several studies have directly analysed the influence of hormone levels on plant senescence. Yang et al. (2003) demonstrated that water stress enhanced leaf senescence and substantially increased ABA levels, but decreased cytokinin levels in wheat. Furthermore, ABA levels positively correlated with the remobilization of carbon reserves during grain filling. This study revealed that enhanced ABA levels increase carbon

remobilization from senescing leaves to grains in drought-stressed rice and wheat plants. In contrast, cytokinin levels, presumably preventing leaf senescence in drought-stressed plants, showed a positive correlation with the photosynthetic rate and chlorophyll content. Additional data indicated that, besides a role in drought stress-induced senescence, ABA is also involved in the control of developmental senescence. ABA levels increased in leaves of several species (including rice and maize) and transcript levels of genes associated with ABA synthesis and signalling also enhanced with increasing leaf age (Jibran *et al.*, 2013). Furthermore, Zhang and Gan (2012) have recently demonstrated in Arabidopsis that stomatal movement and water loss during senescence are controlled through a regulatory chain consisting of ABA, the transcription factor AtNAP and a type 2C protein phosphatase, suggesting a direct mechanism by which ABA is involved in the regulation of developmental leaf senescence. In line with this finding, Seiler et al. (2014) showed that the ABA level in drought-stressed flag leaves of an early senescing barley line increased in comparison with a stay-green line. They related this observation to an activation of ABA metabolism, affecting WUE and assimilation negatively in the early senescing line.

#### 3.3.1.2 Stress response pathways in drought-induced senescence

It is known that drought induces oxidative stress in plants and the expression of senescence-associated genes (SAGs) (Navabpour *et al.*, 2003). Among the ROS, in particular hydrogen peroxide has been proposed as a signal to regulate SAGs, since it has been shown to increase during drought-induced leaf senescence (Munné-Bosch and Alegre, 2002). ROS signals also interact, directly or indirectly, with other signalling pathways, such as those triggered by nitric oxide or ethylene (Jung *et al.*, 2009). These signalling compounds employ calcium (Ca) as a second messenger to subsequently change root morphology and the regulation of stomatal guard cells to enhance drought tolerance (Römheld and Kirkby, 2010). Several NAC and WRKY transcription factors have been identified among the genes involved in senescence regulation. In barley, a list of 48 NAC genes has recently been provided, which revealed that many of these genes are strongly expressed in senescing flag leaves (Distelfeld *et al.*, 2014).

## 3.4 Drought-induced senescence and nutrient remobilization during grain filling in cereals

The occurrence of drought during the late phase of plant development results in retarded growth and limits crop yield by affecting flowering and seed set. One of the major physiological constraints in yield formation and quality under terminal drought is reproductive failure followed by a shortening of the grain filling period. These events reduce grain number and grain size under severe drought stress (Sreenivasulu *et al.*, 2007). Strategies to improve yield stability under harsh environmental conditions need to address the synchrony between source (expanded leaves) and sink (reproductive tissue/developing seeds) organs by either maintaining the assimilation capacity in source leaves through optimized photosynthesis or by triggering the remobilization of assimilates from the stem and the leaves to the grains.

The re-translocation of nitrogen from senescing leaves to developing seeds has been shown to occur mainly in the form of amino acids. Amino acids can be delivered from leaves to grains either directly through phloem transport or in part via the roots and xylem transport. However, as the transpiration rate of developing seeds is typically low, xylem-to-phloem transfer in the peduncle has been demonstrated to be important for the delivery of reduced nitrogen compounds and other solutes to seeds (Feller and Fischer, 1994). Glutamate, aspartate as well as threonine and serine have been identified as the most abundant forms of reduced nitrogen which are exported from senescing barley or wheat leaves to the grains (Gregersen *et al.*, 2008).

## 3.5 An ideal senescence phenotype of cereals

There is evidence for genetic variation in the onset and progress of drought-induced senescence in barley. Therefore, the appearance of senescence symptoms has become an important trait in the breeding of drought-tolerant lines (Joshi *et al.*, 2006).

A delay in leaf senescence or a “stay-green” phenotype is in general believed to confer higher plant productivity due to an extended photosynthetic activity of the source leaves (Thomas, 2000). In hybrid “stay-green” winter wheat (Gong *et al.*, 2005), high grain yield was observed to correlate with delayed senescence (Zhang *et al.*, 2006*a*).

The protein content in barley grains is an important trait with regard to the production goal. While low protein content is desirable for the malting process, a high protein content is desired for feed production. Thus, different breeding strategies need to be developed that influence the protein content also by an altered senescence behaviour (Gregersen *et al.*, 2008).

Although previous studies have indicated that manipulation of the senescence process could result in higher yields, the approach is far from being clear. The overall dilemma is that delayed senescence increases the assimilation capacity and thereby promotes grain yield, but is coupled to inefficient nitrogen remobilization leading to a lower nitrogen harvest index (Gong *et al.*, 2005). In consequence, more nitrogen remaining in the residual crop may potentially decrease nitrogen use efficiency of applied fertilizers. In addition, if slow grain filling is associated with delayed senescence, the cultivar may be vulnerable to damage by heat stress and drought during the late stages of crop development. Delayed senescence may also be caused by management factors, in particular excess nitrogen input or irrigation, and can lead to heavy lodging problems (Zhang *et al.*, 2006*b*). On the other hand, accelerated senescence confers efficient nitrogen remobilization and high protein contents, but may associate with a lower total grain yield, presumably due to a shorter period of active photosynthesis (Mickelson, 2003). This reflects the overall strong negative correlation observed between total grain yield and grain protein concentration, which appears to be very difficult to break in the breeding process of cereals. Therefore, an ideal senescence phenotype should cope with this dilemma while being robust against the influence of environmental factors triggering senescence (Gregersen *et al.*, 2008).

## 3.6 Drought tolerance mechanisms

Plants adapt to and survive under drought stress by the induction of various morphological, biochemical and physiological responses. Drought tolerance is defined as the ability of a genotype to grow, flower and display economic yield under suboptimal water supply (Farooq *et al.*, 2009). The major drought tolerance mechanisms display at different levels:

### 3.6.1 Physiological level

#### 3.6.1.1 Cell and tissue water conservation

Osmoregulation and turgor pressure are two important processes modulated by plant cells subjected to drought stress. In response to drought stress plant cells increase the osmotic potential by a net accumulation of solutes (Turner and Kramer, 1980), which triggers water influx into the cell and supports turgor maintenance. This improves the tissue water status which is essential for maintaining physiological activities during long term drought stress (Farooq *et al.*, 2009). An osmotic adjustment is also seen in stomata, where it warrants stomatal conductance also at low leaf water potential and maintains photosynthesis under drought conditions. It has been shown that growth reduction under drought was lower in those barley plants that responded with osmotic adjustment (Blum, 1989). Also, drought-sensitive pea genotypes were more affected by a decline in relative water content under drought in comparison with tolerant ones (Upreti *et al.*, 2000).

#### 3.6.1.2 Antioxidant Defense

In higher plants photosynthesis takes place in chloroplasts, which contain a highly organized thylakoid membrane system that harbours all components of the light-capturing photosynthetic apparatus and provides all structural properties for optimal light harvesting. Oxygen generated in the chloroplasts during photosynthesis can accept electrons passing through the photosystems, thus forming superoxide ($O_2^{\bullet -}$). Under steady state conditions, ROS molecules are scavenged by various antioxidative defense mechanisms. Drought and nutrient deficiency may perturb the equilibrium between the production and the scavenging of ROS (Gill and Tuteja, 2010). The antioxidant defense system in plant cells constitutes of enzymatic and non-enzymatic components. Non-enzymatic components encompass cysteine, reduced glutathione and ascorbic acid (Gong *et al.*, 2005). Enzymatic components include superoxide dismutase (SOD), catalase, peroxidase, ascorbate peroxidase and glutathione reductase. The activity of these enzymes has been proposed to be in very crucial for plant drought stress. The first line in the detoxification of ROS is the dismutation of superoxide to hydrogen peroxide ($H_2O_2$) by superoxide dismutase. In a subsequent step, catalases dismutate $H_2O_2$ into $H_2O$ and $O_2$. APX is also is involved in scavenging of $H_2O_2$ in with higher affinity than catalase (Gill and Tuteja, 2010). An increased in SOD

activity under drought stress was reported in different plant species (Sharma and Shanker Dubey, 2005; Zlatev *et al.*, 2006; Wang *et al.*, 2008). Similarly, an increase in catalase activity has been reported in drought-stressed wheat where it was higher especially in sensitive varieties (Simova-Stoilova *et al., 2010*). Therefore, plants with the ability to scavenge or control the level of cellular ROS may be useful for plant breeding in future to combat harsh environmental conditions.

#### 3.6.1.3 Cell Membrane Stability

Biological membranes are the first target of drought-generated ROS. It is generally accepted that the maintenance of the integrity and stability of membranes under water stress is a major factor of drought tolerance in plants. The degree of cell membrane injury induced by water stress may be easily estimated through measurements of electrolyte leakage from the cells (Bajji *et al., 2001*). A broad range of compounds like proline, glutamate, glycine-betaine, carnitine, mannitol and sorbitol, has been identified that can prevent an increase of the viscosity of the cytoplasm and prevent protein denaturation and membrane fusion (Hoekstra *et al.*, 2001). Moreover, the cell membrane stability of the leaf segment has been suggested as a valuable trait to screen barley germplasm for drought tolerance (Dhanda *et al.*, 2004). Measures of cell membrane stability at different growth stages have already been used to map quantitative trait loci in drought-stressed rice (Tripathy *et al.*, 2000).

### 3.6.2 Molecular level

#### 3.6.2.1 Aquaporins

Aquaporins belong to the family of small integral membrane proteins (Maurel *et al.*, 2008). They have the ability to facilitate and regulate the transport of water across membranes (Tyerman *et al.*, 2002). Water stress induces primarily a loss of water, which can result in a marked drop in cell turgor and ultimately, but more rarely, in cell plasmolysis. In this context, the cytosol, which contributes to a minor fraction of the plant cell volume, may be very sensitive to a differential flow of water across the plasma membrane and the tonoplast. Abrupt changes in the cytosolic volume can theoretically be avoided if the mobilization of water from or into the vacuole is non-limiting. Higher water permeability and aquaporin abundance in the plasma

membrane has been found in membranes purified from wheat and tobacco under drought stress. In this sense, aquaporins increase root water conductivity and play a central role in cellular osmoregulation under drought stress (Maurel *et al.*, 2008).

#### 3.6.2.2 Stress Proteins

Under drought stress many stress proteins are synthesized which help overcoming low water availability. Most of these stress proteins are soluble in water and contribute to the hydration of cellular structures (Wahid *et al.*, 2007). For instance, drought stress induces the expression of late embryogenesis abundant dehydrin-type genes and molecular chaperones that protect cellular proteins from denaturation. In addition, many heat shock proteins which are induced by drought stress serve as molecular chaperones that participate in ATP-dependent protein unfolding or assembly/disassembly reactions and prevent the denaturation of sensitive proteins during stress (Gorantla *et al.*, 2007). These proteins are increased the water-binding capacity of other proteins or cellular structures by creating a protective environment, and are referred to as dehydrins. They also play a major role in the sequestration of ions that become more concentrated during cellular dehydration. These proteins help to protect their partner protein from degradation by proteinases that function to remove denatured and damaged proteins (Gorantla *et al.*, 2007). It is reported in rice that overexpression of the STRESS-RESPONSIVE NAC1-regulated protein (SNAC1) phosphatase, a stress-responsive NAC transcription factor, confers drought and oxidative stress tolerance by regulating ROS homeostasis through ABA-independent pathway (You *et al.*, 2014).

### 3.6.3 Eco-physiological strategies of drought-stressed plants

#### 3.6.3.1 Escape from drought

Plants can escape from drought by shortening the life cycle, allowing plants to produce seeds before drought arrests growth processes. Hence, an early flowering time is an important trait supporting drought adaptation, particularly when the growth season is restricted by terminal drought and high temperatures. Therefore, developing varieties with short life cycles have turned out to be an effective strategy for minimizing yield losses under terminal drought stress (Kumar and Abbo, 2001). The enhanced water consumption before the transpirational

became optimum and before observing a higher level of ABA in leaf, has been suggested as physiological mechanism allowing *Arabidopsis* plants to escape from drought stress (Meyre *et al.*, 2001).

#### 3.6.3.2 Drought avoidance

Drought avoidance describes the ability of a plant to maintain a high tissue water potential despite a soil water deficit. This strategy mostly reduces water loss by plants, due to an improved stomatal control of transpiration and a longer lasting water uptake through an extensive or prolific root system. In particular root characters such as root length, root length density and rooting depth are major drought avoidance traits that contribute to growth and yield formation under terminal drought (Turner and Kramer, 1980; Kavar *et al.*, 2007). At the shoot level, the formation of a thicker cuticula in leaves can contribute to drought stress avoidance. In addition, glaucousness or waxy bloom on leaves helps to maintain a higher tissue water potential and has been considered as a favorable trait for drought tolerance (Farooq *et al.*, 2009). It has been shown that a better performance of different creeping bentgrass cultivars under drought stress was mainly related to drought avoidance traits like water use efficiency, root viability, root length and root number (McCann and Huang, 2008).

#### 3.6.3.3 Phenotypic Flexibility

Many studies have examined the phenotypic plasticity of plant adaptation to environmental stresses like drought. The ability of a plant to keep its root system functional when water and other resources become limiting may be an important aspect of adaptive plasticity. In this context, root length and density as well as proliferation after re-watering of drought-stressed plants have been proposed as major traits for drought tolerance (Bell and Sultan, 1999). The drought tolerance of tea, onion and cotton was increased by improved root growth (Farooq *et al.*, 2009). Selection for a deep and extensive root system has been proposed to increase the productivity of food legumes under water deprivation by increasing the capacity to acquire water from soil (Subbarao *et al.*, 2010). In addition, to reduce the water consumption under drought stress, plants often decrease the number and area of their leaves. Moreover, leaf hairiness minimizes water loss under high temperature and radiation stress by increasing the boundary layer to reduce the evaporation of water from the leaf surface (Sandquist and Ehleringer,

2003). It has also been shown that hairy leaves reduce leaf temperatures and transpiration under drought conditions (Sandquist and Ehleringer, 2003).

## 3.7 Terminal drought stress and hormonal regulation

Several studies have indicated that the perception of drought increases the accumulation of the stress hormone ABA in plants. Synthesis of ABA in the roots and transport through the xylem to the shoot is one of the most rapid hormonal responses to drought. In leaves, ABA causes stomatal closure to reduce transpirational water loss and eventually restricting cellular growth (Albacete *et al.*, 2014). Studies in wheat have shown that an enhanced ABA level in the grains was consistent with an increase in the grain filling rate under drought stress (Yang *et al.*, 2000). The grain filling rate and grain weight in rice plants was significantly increased by spraying ABA at the initial grain filling stage (Yang *et al.*, 2003*c*). Also under terminal drought stress, a higher concentration of ABA has been reported to occur in barley (Seiler, 2011).

Several studies have reported increases or decreases in CK levels under drought stress that could have a positive impact on stress tolerance, since CK homeostasis and signalling play a key regulatory role in growth processes (Merewitz *et al.*, 2012; Le *et al.*, 2012; Ha *et al.*, 2012; Kuppu *et al.*, 2013). For instance, transgenic plants with higher expression of the isopentyl transferase (*IPT*) gene displayed a delay in drought-induced senescence and showed drought tolerance.

There is ample evidence that CKs and ABA act as hormonal antagonists under drought stress. During drought stress, the ABA content substantially increased in the leaves, stems, and grains of wheat plants, whereas the active CK content markedly decreased in leaves (Yang *et al.*, 2003*b*). Measurements of CK levels and expression analysis of genes involved in CK metabolism after plants were treated with exogenous ABA indicated that stress-induced ABA is involved in the down-regulation of CK levels through repression of CK biosynthesis genes (Nishiyama *et al.*, 2011). It has been reported that enhanced drought tolerance of CK-deficient plants was attributed to an improved capacity to maintain higher water content under stress (Nishiyama *et al.*, 2011). The same study also indicated that drought tolerance was not caused by differences in stomatal opening, since ABA responses and ABA concentrations were similar in both wild-

type and CK-deficient plants. Therefore, a threshold CK concentration in the regulation of plant growth and biomass partitioning seems to be critical for plant survival under stress (Albacete *et al.*, 2014).

## 3.8 Role of mineral nutrition in alleviating drought stress

In dry climates, nutrient availability in the topsoil declines during the growth season because the low soil water content becomes a limiting factor for nutrient delivery to the root surface. Nutrient uptake is further decreased by impaired root growth in dry soils. Plants have developed a wide range of adaptive responses to maintain productivity and to ensure survival under a variety of environmental stress conditions. There is ample evidence that the mineral nutrient status of plants plays a critical role in increasing plant tolerance to environmental stress factors (Marschner, 2012). There are several studies indicating an important role of K in the mitigation of different stress factors, such as drought, heat or frost (Römheld and Kirkby, 2010). This is partially due to the role of K in controlling cell turgor and stomata closure but also to its role in stress signaling. In fact, K deficiency elicits the same signalling pathway as drought stress. In K-deprived plants, ethylene is formed, which activates ROS production and triggers via cytosolic Ca signaling (as second messenger) the induction of K transporters in roots as well as in leaves (Figure 1). The induction of K transporters allows an increase in the K uptake capacity in roots while in leaves it confers a more tight regulation of stomatal opening. A prominent molecular target in this common regulation is the K channel AKT1 which is expressed in the rhizodermis as well as in stomata (Cheong *et al.*, 2007). On the other hand, ethylene production in K-deprived plants is also important for changes in root morphology and in the whole-plant tolerance to low K supply (Jung *et al.*, 2009). Besides K, also the application of P fertilizer can considerably improve plant growth under drought conditions. The positive effects of P on plant growth under drought have been attributed to an increase in stomatal conductance and photosynthesis, to a higher cell-membrane stability, and improved water relations under drought stress (Waraich *et al.*, 2011). The necessity of a sustained supply of calcium (Ca) for the recovery from drought stress has been explained by its prominent role in the maintenance of the structure of cell walls and of the integrity of the plasma membrane (Palta, 1990). Ca further activates plasma membrane-ATPases, which create the membrane potential

required for secondary active uptake of nutrients (Palta, 1990). Among the other divalent cations, magnesium (Mg) increases root growth and the root surface area, which helps to increase the uptake of water and nutrients. It also improves carbohydrate translocation by stimulating phloem loading and reduces ROS generation as well as photo-oxidative damage in chloroplasts under drought conditions (Waraich *et al.*, 2011).

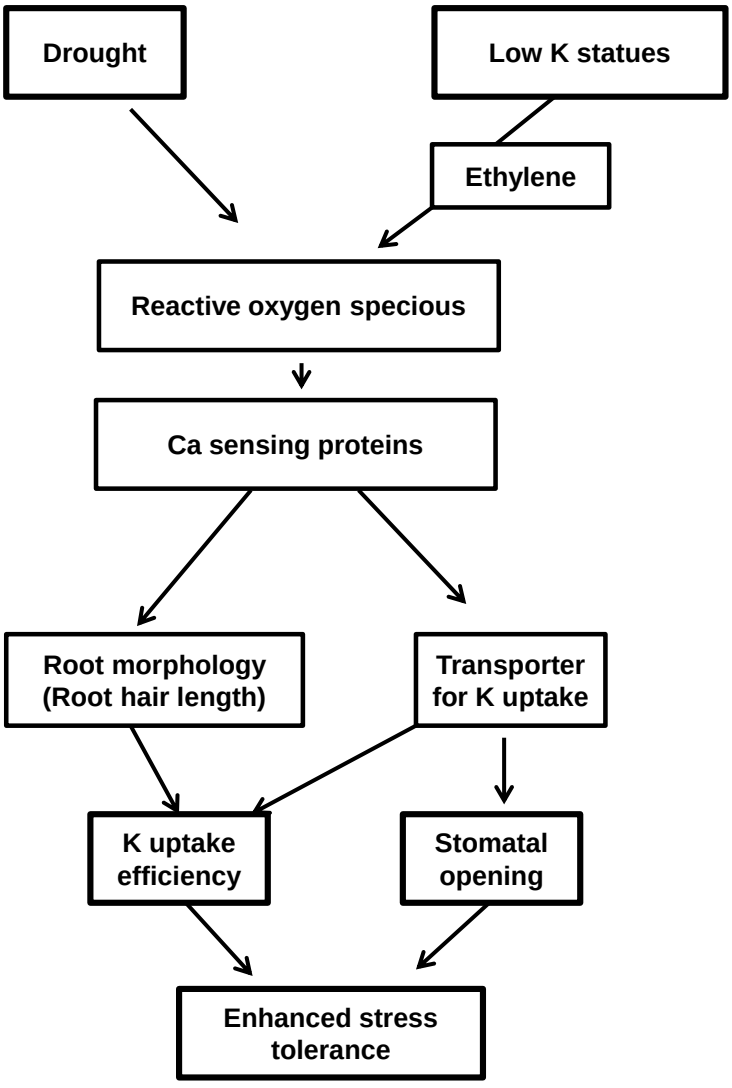


**Figure 1. Proposed common signalling pathway induced by drought and a low K nutritional status of plants in the regulation of K uptake and drought stress tolerance.** The work of Cheong and his colleagues (2007) indicates that K deficiency and drought employ the same signaling pathway for the induction of physiological and morphological responses. Drought induces ABA which leads to the generation of ROS, while under K deficiency ethylene formation enhances ROS levels. Then, ROS trigger Ca fluxes which lead to an enhanced K uptake capacity in roots as well as to an improved regulation of guard cells in leaves.

## 3.9 Potassium in soils and plants

Potassium (K) is the most abundant inorganic cation in plant tissues. In adequately supplied plants it may vary between 1 and 6% of the dry matter or reach concentrations of about 200 mM (Leigh, 2001). In contrast, vacuolar K concentrations may vary between 10 and 200 mM or even reach up to 500 mM in guard cells of stomata (Marschner, 2012). K is unique as a plant nutrient as it occurs exclusively in the form of the free ion. Highest concentrations of K are found in young developing tissues and reproductive organs indicative of its high activity in cell metabolism and growth. K activates numerous enzymes including those involved in energy metabolism, protein synthesis, and solute transport (Amtmann *et al.*, 2008). K is needed for cytoplasmic pH homeostasis, for the maintenance of electrochemical gradients across membranes and for the transport of inorganic anions and metabolites. In long distance transport, K is the dominant cation within the xylem and phloem saps neutralizing inorganic and organic anions. This also confers a high K mobility and cycling throughout the entire plant. Uptake and accumulation of K by plant cells is the primary driving force for their osmotically-driven expansion (Römheld and Kirkby, 2010).

For diffusion-dependent nutrients like K, it is important that plant roots have a large surface area which is in contact with soil constituents. In this sense, root hairs contribute effectively to the K uptake capacity by increasing the surface area and the extent of the K depletion zone in the rhizosphere. Thereby, root hairs help creating a steeper concentration gradient towards the root surface which drives K diffusion. Plants can also enhance the mobilization of mineral K by the release of organic acids from the roots. For instance, exposure of silicate-based rocks, like gneiss, to malic and tartaric acids resulted an enhanced release of mineral $K^+$ (Wang and Wu, 2010).

## 3.10 Potassium efficiency

The capacity of a genotype to grow and yield better in the soils with low K availability is termed K efficiency. K efficiency has been described as the proportion of yield that can be achieved under K deficiency relative to K-sufficient conditions (Damon *et al.*, 2007; Damon and Rengel, 2007). K-efficient genotypes can show a higher acquisition of K from the soil (uptake efficiency) and/or a higher dry matter production per unit of K taken up (utilization efficiency). The K uptake

and utilization efficiency are interlinked. The capacity of crop genotypes to take up relatively more K under low K availability (Trehan and Sharma, 2002; Chen *et al.*, 2007; Zhang *et al.*, 2007) have been associated to those that produce a relatively large biomass per unit of K (Bassam; Yang *et al.*, 2003*a*). In addition, a positive correlation has been found between K utilization efficiency and relative shoot dry weight at the tillering stage when the K availability was low (Yang *et al.*, 2003*a*). Also in sweet potato, K utilization efficiency was positively correlated with total plant biomass and root yield (George et al. 2002).

## 3.11 Genotypic differences in K efficiency

A considerable variation in K uptake and utilization efficiency has been found among existing genotypes of different crop species including barley (Pettersson and Jensén, 1983), wheat (Damon and Rengel, 2007) and canola (Damon *et al.*, 2007). Genotypic differences in K efficiency have not only been attributed to enhanced K uptake, e.g. as conferred by a higher expression of K transporters and a higher proton secretion, but also to an improved K utilization, i.e. a higher capacity to distribute K efficiently between cells and organs (Rengel and Damon, 2008). During the reproductive stage of crop development when there is a high demand for photo-assimilates by developing seeds, plants are highly susceptible to K deficiency due to the essential contribution of K to phloem loading. K-deficient source leaves may then accumulate sugars and develop leaf chlorosis and necrosis (Marschner, 2012). Under K deficiency, cytosolic K activity is first maintained at the expense of vacuolar K, even though vacuolar (but not cytosolic) K activity is regulated differently in root and leaf cells (Römheld and Kirkby, 2010). A high K utilization efficiency is based on an effective K translocation between organelles, cells and organs, the capacity to use other ions as substitutes for K, such as Na, and the capacity to direct assimilates to the sink organs under low K supplies. Moreover, a fast root turnover and the associated export of K to sink organs in the shoot may also contribute to K efficiency whenever plants are exposed to low K availabilities (Römheld and Kirkby, 2010).

## 3.12 The protective function of K under drought stress

The unique function of K as a plant nutrient relies on its exclusive occurrence in the form of the free ion $K^+$. Highest concentrations of K are found in young

developing tissues and reproductive organs, indicative of the great importance of K for cell metabolism and growth. $K^+$ activates numerous enzymes including those involved in energy metabolism, protein synthesis, and solute transport (Amtmann *et al.*, 2008). Among those, pyruvate kinase is particularly sensitive to cytoplasmic K (Ramírez-Silva *et al.*, 2001) and its activity in root cells can be rapidly inhibited after plants have been subjected to K deficiency (Armengaud *et al.*, 2009).

Consequently, plants have an even higher requirement for K under drought stress. Reducing water loss through stomatal closure is one strategy to minimize the adverse effects of drought (Riera *et al.*, 2005); therefore, K homeostasis may be considered as a key factor in drought adaptation. There are several studies indicating an important role of K for the mitigation of drought stress (Cakmak, 2005; Römheld and Kirkby, 2010). Furthermore, Arabidopsis roots respond to drought with an enhanced expression of the K deficiency-inducible high-affinity K transporter HAK5 and related KUP/HAK-type transporters (Gierth *et al.*, 2005) whose up-regulation under drought plays an important role in mitigating drought stress (Osakabe *et al.*, 2013). Induction of AtHAK5 orthologues at low external K supply has also been reported in barley, tomato and rice. On the other hand, ethylene production in K-deprived plants is also important for changes in root morphology and in the whole-plant tolerance to low K supply (Jung *et al.*, 2009).

In addition, an increased level of ABA in grains and flag leaves has been reported for K-deficient wheat plants, suggesting a cross-talk between ABA and K deficiency signaling (Haeder and Beringer, 1981). Drought stress triggers abscisic acid (ABA) and then employs the same signaling cascade as K deficiency (Figure 1), also leading to the induction of K transporters and channels in roots and guard cells (Cheong *et al.*, 2007). By this way, drought stress additionally stimulates the induction of K deficiency responses, which may be suppressed by an adequate K nutritional status.

Additionally, ABA may act as a signal under reduced nutrient supply (Peuke *et al.*, 2002). For example, an increased ABA concentration in grains as well as in flag leaves of K-deficient wheat plants (Haeder and Beringer, 1981) has been reported. Microarray analyses on Arabidopsis roots under K-deficiency have further unraveled the expression of Nuclear Protein X1 (*NPX1*), which is up-regulated by K deprivation (Shin and Schachtman, 2004; Kim et al., 2009).

## 3.13 Aim of present work

Previous studies have provided strong evidence for a role of phytohormones, in particular of ABA, in the adaptation of carbohydrate metabolism to drought stress (Seiler *et al.*, 2011; Nishiyama *et al.*, 2011). On the other hand, the plant nutritional status has been shown to strongly affect drought stress responses. Among all nutrients, K has turned out as a key nutrient in the modulation of drought stress responses due to its interference with the ABA signaling pathway (Pandey *et al.*, 2007; Cheong *et al.*, 2007). As the K nutritional status also modulates primary carbon metabolism (Armengaud *et al.*, 2009), the question arises to what extent the K nutritional status and K fertilization impact on the metabolic and hormonal regulation during drought stress. This question may be of special interest during post-anthesis drought when senescence in source leaves promotes K re-translocation and resembles a K-deficient nutritional status. Based on the hypothesis that the K nutritional status modulates senescence-related drought stress responses in source leaves via its impact on metabolic and phytohormonal adaptations, it was the aim of the present thesis:

1) to investigate the role of K nutrition in the mitigation of post-anthesis drought stress, and
2) to investigate whether K nutrition interferes with genetically determined differences among genotypes in the onset of drought-induced leaf senescence.

For this purpose, barley genotypes were chosen that show a different physiological behavior under drought stress. These lines were identified in a thorough screening of more than 150 barley genotypes for their expression of physiological and biochemical traits under drought. One line showed a functional stay-green phenotype with delayed leaf senescence, while another line became earlier senescent and showed an efficient nutrient remobilization and a third line showed an intermediate phenotype. Relative to the latter line, the early and late senescing lines showed a weaker yield loss under drought. These lines were grown in a greenhouse trial under sufficient water supply (control) or water limitation (stress) at low or high K supplies.

In this context, the first chapter of the present thesis (4.1) analyzes the impact of K nutrition on physiological and molecular responses to terminal drought stress in these three barley lines with different senescence behaviour. Here, a special

emphasis was laid on the impact of K nutrition on carbohydrate metabolism in drought-stressed flag leaves of barley.

For the second chapter of this thesis (4.2) the two most contrasting (early and late senescing) barley lines have been selected to investigate the influence of K nutrition on phytohormonal responses in drought-stressed flag leaves.

# 4 Materials and Methods

## 4.1 Origin of plant materials

In framework of the GABI-GRAIN project funded by the BMBF in Leibniz-Institute for Plant Genetics and Crop Plant Research (IPK), Dr. N. Sreenivasulu and his coworkers screened 150 barley lines (breeding lines, gene bank accessions and introgression lines) under terminal drought stress during grain filling in both green house and field conditions in two independent years. Based on physiological and biochemical analyses, they categorized three genotypes differing in the development of drought stress-induced leaf senescence into stay-green, senescing and early senescing with efficient nutrient remobilization (Figure 2). For the present experiments an earlier senescing line with high remobilization capacity (LP104), a line with normal senescence (LP110) and a stay-green line (LP106) were selected. These 3 lines were used for a physiological, biochemical and molecular characterization of drought-induced senescence under altered K supply.

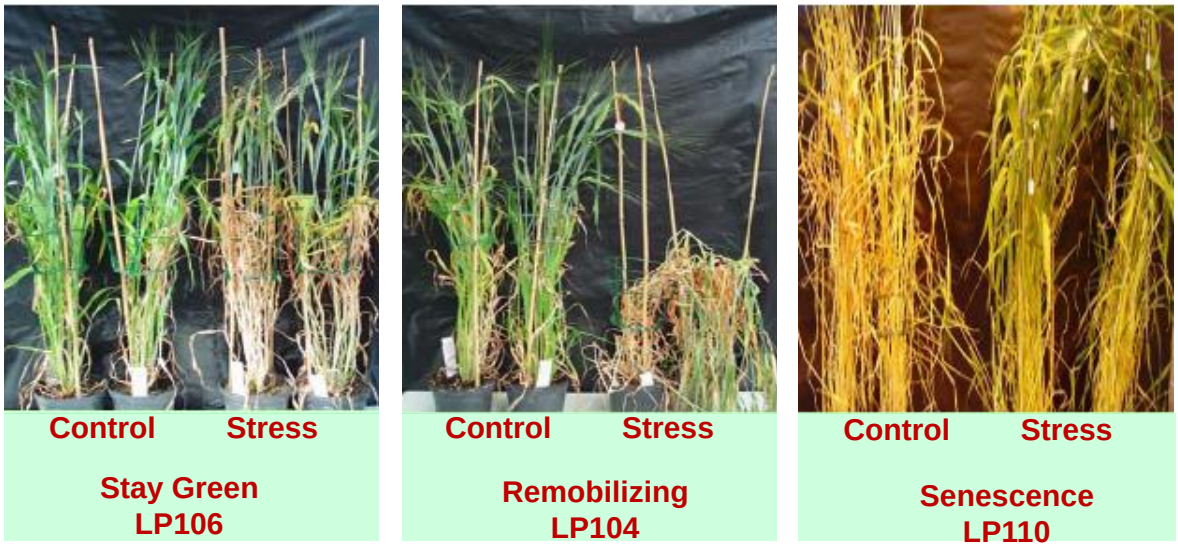


**Figure 2. Phenotypes of three barley lines differing in the progression of leaf senescence during post-anthesis drought stress.** Under drought stress the line LP106 maintains stay green (left), the line LP104 progresses early into senescence (middle) and the line LP110 shows an intermediate phenotype with normal senescence progression (right).

## 4.2 Growth conditions and stress treatments

Seeds of LP104, LP110 and LP106 were germinated separately in seed germination trays in a climate-controlled growth chamber for two weeks. Then,

germinated seed were transferred to the cold room for vernalization at 8°C for a period of two weeks. A commercial growth substrate (Substrate, Klasmann Deilmann GmbH, germany) was analyzed for mineral element contents (Eurofin Company, Germany), and the required amounts of nutrients were calculated that were needed to be added to the substrate to sustain plant growth until senescence. Thereafter, 4 weeks-old plants were sown in 5L pots filled with 2 kg of peat-based growth substrate fertilized with potassium (K). K was supplied at three levels to the substrate: low (no K), medium (2g $Kg^{-1}$ $K_2SO_4$) or high (4g $kg^{-1}$ $K_2SO_4$). In addition to that the substrate was supplemented with 9 g $kg^{-1}$ $CaCO_3$ and 4.05 g $kg^{-1}$ CaO in order to increase the pH of the acidic peat-based substrate from 5.5 to 6.5. The temperature in the greenhouse was approximately 15°C at night and 20°C during day with a 16 h/8 h light/dark cycle. When spikes emerged, all spikes were tagged manually with the date of the beginning of flowering, so that only spikes of the same developmental stage could be used for the collection of flag leaves and of grains for physiological and biochemical analyses (Figure 3).

When the plants started to flower (BBCH 60) drought stress was imposed by maintaining a batch of plants at 20 – 25% of field capacity (corresponding to 10% soil moisture content) (Lancashire *et al.*, 1991). Stress was maintained until maturity or seed harvest, while control plants were continuously held at 100% of field capacity (corresponding to 40% soil moisture content).

The soil moisture content was monitored using the moisture meter HH2 coupled with the soil moisture sensor SM200 (Delta T Devices Ltd., England). Plants were grown in 3 independent replications for yield and yield component analysis, while additional 6 replications were used for biochemical and physiological measurements. Flag leaves from 12 weeks-old plants that were exposed to drought stress 5 days after flowering (DAF) were harvested 12 days later. Harvested plant organs obtained from this experiment were used for measuring hormones, gene expression levels and various physiological and biochemical parameters.

**Figure 3. Growth of three different barley lines in a greenhouse under ambient conditions and labeling of spikes at flowering stage.** The barley lines were subjected to drought stress 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) under low, medium or high K supplies (left). Spikes of barley plants were tagged manually with the date of beginning of flowering in which the same developmental stage could be used for the collection of flag leaves and grains for physiological and biochemical analyses (right).

## 4.3 Photosynthesis measurements

Infra-red gas analysis was carried out on individual fully emerged flag leaves of all three barley lines at 12 days after flowering (DAF, corresponding to 17 DAS) using a LCpro+ device (ADC Bioscientific Ltd, Great Amwell, England). A constant supply of 400 ppm $CO_2$ (flow rate 200 µmol $s^{-1}$) was provided by a $CO_2$ cartridge at a photon flux density of 900 µmol $m^{-2}$ $s^{-1}$ by a mixed red/blue LED light source mounted above the leaf chamber head. The net assimilation rate (A), internal $CO_2$ concentration (Ci), stomatal conductance (gs) and transpiration rate (E) were all recorded from five individual plants growing under either well watered or drought stressed conditions, with four technical replications per measurement. All the parameters were recorded in the morning hours starting from 10:00 am to 1:00 pm. The instrument was stabilized for 30 min in the greenhouse where measurements were taken (Figure 4). The measurements were only taken once after the internal $CO_2$ concentration had stabilized (2-3 min after insertion of the leaf into the measuring chamber).

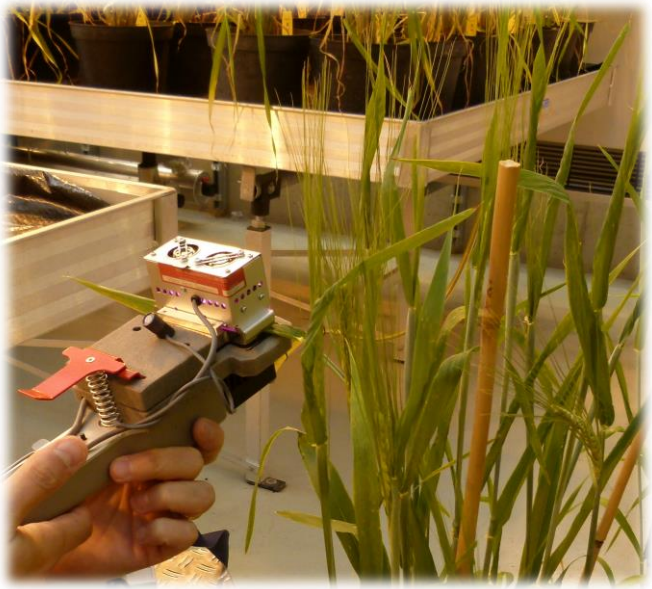

**Figure 4. Photosynthesis measurement of flag leaves in greenhouse.** The net assimilation rate (A), internal $CO_2$ concentration (Ci), stomatal conductance (gs) and transpiration rate (E) were all recorded 12 days after flowering (12 DAF) corresponding to 17 days after stress (DAS). A LCpro+ device (ADC Bioscientific Ltd, Great Amwell, England) was used for the measurements.

## 4.4 Chlorophyll determination

Chlorophyll concentrations were determined in flag leaves following the protocol of (Porra, 1989). 20 mg of fresh flag leaf material were incubated at 4°C for 24 h in N,N'-dimethyl formamide (Merck). The samples were centrifuged at 14000 rpm for 2 min and the absorbance at 647 nm and 664 nm was determined in a photometer (BIO-TEC, UVLKONxl, Germany).

## 4.5 Element determination

Flag leaf samples were dried for 48 h at 65°C and digested with $HNO_3$ in polytetrafluoroethylene vials in a pressurized microwave digestion system (UltraCLAVE IV; MLS GmbH, Leutkirch, Germany). Potassium (K), magnesium (Mg), calcium (Ca) and sodium (Na) concentrations were analyzed by inductively-coupled plasma optical emission spectrometry (ICP-OES iCAP 6500 dual OES spectrometer; Thermo Fischer Scientific, Waltham, U.S.A.).

## 4.6 Measurement of soluble and insoluble carbohydrates

Soluble sugars and starch were determined in flag leaves according to the method of Chen et al. (2005). 50 mg frozen flag leaf material was homogenized in

liquid nitrogen, dissolved in 0.75 ml of 80% (v/v) ethanol and incubated at 80°C for 60 min. Crude extracts were centrifuged at 14,000 rpm at 4ºC for 5 min and the upper phase was concentrated in a speed vacuum concentrator (Christ, Germany) at 45°C for 180 min. The pellet was re-suspended in 0.25 ml HPLC-grade water and shaken for 15 min at 4°C. The remaining insoluble material was kept for starch measurements.

A buffer containing 100 mM imidazol-HCl (pH 6.9), 5 mM $MgCl_2$, 2.25 mM NAD, 1 mM ATP (as final concentrations) was used for the measurement of soluble sugars using an EL808 ultramicroplate reader (BioTeK Inc., Germany) at 340 nm. The sequential addition of auxiliary enzymes allowed detecting glucose (Glc), fructose (Fru) and sucrose (Suc). Glucose-6-phosphate dehydrogenase (G6PDH) was first added to remove endogenous hexose-phosphates. Subsequently, hexokinase (HK), phosphoglucoisomerase (PGI) and β-fructosidase were added successively to measure Glc, Fru and Suc as described in Hajirezaei et al. (2000).

The residue of sugar extraction was washed twice with 1 ml of 80% (v/v) ethanol. Starch was decomposed with 0.4 ml 0.2 N KOH for 16 h at 4°C and neutralized with 70 µl of 1 M acetic acid. Hydrolysis of starch was performed using a 1:1 ratio of sample and a buffer containing 50 mM sodium acetate, pH 5.2 and 7 units $mg^{-1}$ of amyloglucosidase (Roche, Germany). The cocktail was incubated at 37°C for 16 hours. Determination of produced Glc was performed according to Hajirezaei et al. (2000).

## 4.7 Measurement of sugar alcohols

The concentrations of sugar alcohols were determined using an ion chromatography system (Dionex, Idstein, Germany) consisting of a gradient pump (GS50), an ED50 electrochemical detector, and an autosampler (AS50). Anionic compounds were separated on a CarboPac MA1 column (4×250 mm) connected to a guard column (4×10 mm) and an ATC-1 anion trap column that was placed between the eluent and the separation column. The eluent (500 mM NaOH) was made from HPLC grade water (Millipore) and 50% NaOH (Merck, Germany). The column was equilibrated for one hour at a flow rate of 0.35 ml/min. The run time was 50 min and a linear gradient was accomplished with 52% water and 48% NaOH. The calibration and quantitative calculation of sugar alcohols was carried out using the Chromeleon client software 6.6 SP, Build 1566 (Dionex, Germany).

## 4.8 Measurement of free amino acids

Free amino acids were extracted as described in section 3.6. To detect primary and secondary amino acids, a fluorescing reagent AQC (6-aminoquinolyl-N-hydroxysuccinimidylcarbamate) was used. ACQ was dissolved in 3 mg $ml^{-1}$ of acetonitrile and incubated at 55$^{o}$C for 10 min. Twenty ml of sugar extract were derivatized in a cocktail containing 20 µl of the fluorescing reagent ACQ, 160 µl of a 0.2 M boric acid buffer (pH 8.8) in a final volume of 200 µl. The solution was incubated at 55$^{o}$C for 10 min.

The separation of derivatized samples was carried out with a reversed phase HPLC system (Waters, Germany) consisting of a gradient pump (Alliance 2795 HT, Waters, Germany), a degassing module, an autosampler and a fluorescence detector (Waters 2475, Germany). A reversed phase column (XBridge; 150 mm, 5 µm) was used for separation and detection of amino acids at an excitation wavelength of 300 nm and an emission wavelength of 400 nm. The gradient was accomplished with buffer A containing 140 mM sodium acetate, pH 5.8 (Suprapur, Merck) and 7 mM triethanolamine (Sigma, Germany). Acetonitrile (Roti C Solv HPLC, Roth) and purest HPLC water (Geyer, Germany) were used as eluents B and C. Chromatograms were recorded using the software program Empower Pro.

## 4.9 Metabolite determination

### 4.9.1 Extraction of primary metabolites

Primary metabolites were extracted from flag leaves of barley plants. Samples were harvested and frozen immediately in liquid nitrogen. Six independent biological replications were used for measurement. Plant tissue was ground in liquid nitrogen and 50 mg of finely powdered fresh material were extracted using 1 ml (v/v)(1:1) ice-cold methanol and chloroform. Subsequently, 0.4 ml of LC-MS water was added to each tube. The mixture was mixed and kept on ice for 20 min. The samples were centrifuged for 10 min at 14000 rpm and 4°C. Thereafter, the upper phase containing methanol/water was transferred to new Eppendorf tubes and concentrated at 45°C for 2 hours in a speed vacuum concentrator (Christ, Germany). The remaining pellet was re-suspended in 0.4 ml of LC-MS water and was kept at -80°C for metabolite analysis.

### 4.9.2 Targeted metabolite analysis by ion chromatography mass spectrometry (IC-MS-MS)

For quantification of primary metabolites, a targeted metabolite analysis was performed using external standards. 0.1 ml of sample volume extracted in section 3.9.1 was filtered at 2000g for 90 min using a multiscreen filter plate (multiscreen ultracel-10 ultra-filtration membrane 10000 NMWL). The IC-MS-MS instrumentation consisted of a Dionex ICS5000 (Dionex, Idstein, Germany) with a 6490 triple Quad LC-MS-MS (Agilent, USA). Anionic compounds were separated on a 250×2 mm AS11-HC column (Dionex) connected to a 10×2 mm AG 11-HC guard column (Dionex) and an ATC-1 anion trap column. The gradient was produced with $H_2O$ (buffer A; HPLC grade water) and KOH which was generated by an EGCIII KOH eluent generator cartridge. The column was equilibrated with a mixture of buffer A (96%) and 4% KOH at a flow rate of 0.38 ml $min^{-1}$ and heated to 37°C during the measurement. The gradient was produced by changes of KOH concentration as follows: 0–4 min: 4%; 4–15 min: 15%; 15–25 min: 25%; 25–28 min: 50%; 28–31 min: 80% and 31-40 min; 4%. Quantitative analysis of metabolites was performed using an Agilent 6490 triple quadruple mass spectrometer (Agilent, Germany). Electron spray ionization (ESI)-MS/MS was set as follows: gas temperature 350°C, drying gas flow rate 12 l $min^{-1}$, nebulizer pressure 35 psi, capillary voltage ± 3.5 kV. The fragmentor voltage and collision energy were optimized for each compound individually by tuning standards with a defined concentration. Primary metabolites were detected in the negative ion mode using multiple reactions monitoring (MRM) (Appendix Table 1). The data were extracted using the MassHunter software version B.03.01 (Agilent Technologies, Germany). Quantification of metabolites was performed by creating a batch for each sample sets using the Quantitative Analysis (QQQ) software (Agilent Germany). $^{13}C$-pyruvate was used to normalize the data and was added to each sample as internal standard before analysis.

## 4.10 Enzyme extraction and measurement

Enzyme extracts were prepared from 50 mg frozen flag leaf material with 0.5 ml of extraction buffer containing 50 mM Tris-HCl, pH 6.8, 5 mM $MgCl_2$, 5 mM mercaptoethanol, 15% glycerol, 1 mM EDTA, 1 mM EGTA and 0.1 mM pefablock phosphatase inhibitor. Pefablock phosphatase was always prepared freshly and

added prior to extraction. The homogenate was centrifuged at 14,000 rpm for 5 min, and the supernatant was used for the enzyme assay. The maximum enzyme activity was determined spectrometrically using an ultramicroplate reader (BioTeK Inc, Germany) in coupled reactions by monitoring NADH or NADPH oxidation or $NAD^+$ or $NADP^+$ reduction at 340 nm.

Pyruvate kinase activity was measured after slight modification of the protocol by Ireland et al., (1980) in 0.5 M TES (pH 7.9) buffer containing 10 mM $MgCl_2$, 40 mM KCl, 0.1 mM NADH, 2 mM dithiothreitol, 0.5 mM PEP and 4 units lactate dehydrogenase (LDH). The final reaction was started by the addition of 1 mM ADP in the assay medium.

## 4.11 Phytohormone measurements

### 4.11.1 Hormone extraction

Hormones were extracted from frozen flag leaf material in 6 independent biological replications for each measurement. The leaf material was ground in liquid nitrogen and 100 mg of finely powdered fresh material were extracted using 1 ml (v/v/v)(15:1:4) ice-cold methanol:formic acid:water. The homogenate was mixed thoroughly and stored at -20°C for 16 h and centrifuged after incubation at 13,000 rpm and 4ºC for 20 min. The supernatant was re-extracted using 300 µl of extraction buffer. Subsequently, samples were dried at 38ºC for 3h in a speed vacuum concentrator (Christ, Germany). Pellets were re-suspended in 100 µl 80% methanol.

### 4.11.2 Separation and elution of different hormones using a MCX column

Solid phase MCX columns were equilibrated first with 1 ml acetonitrile followed by 1 ml methanol (MS grade). Columns were further washed by 1 ml of 1 M formic acid and hydrogen chloride (HCl). Re-suspended samples (from section 3.11.1) were mixed with 900 µl of 1 M formic acid and added to the MCX columns. ABA and ABA degradation products were collected with 1 ml pure methanol (MeOH), whereas cytokinins were eluted with 1 ml 0.35 M ammonia ($NH_3$) dissolved in 60 % MeOH. The dried eluents were re-solved in 50-100 µl of 25% MeOH and used for LC-MS-MS analysis.

### 4.11.3 Determination of hormones by UPLC

All UPLC-ESI-MS/MS experiments were carried out using an Agilent 1290 infinity system connected to an Agilent triple quadruple mass spectrometer QQQ6490 (Agilent Germany). Separated compounds were ionized at atmospheric pressure via electrospray and directed to the mass spectrometer. The control of the complete system and recording of the spectra were performed with the MassHunter, software B.04.00 (B4038).

To separate the individual cytokinines and ABA, a UPLC system was used including a gradient pump, an autosampler, and a column compartment. Separation was carried out using a high capacity column (Eclipse Plus C18, RRHD 1.8 µm, 2.1x50 mm). A gradient was accomplished with LC-MS grade water (Chem. solute, Geyer, Germany) containing 0.1% formic acid (Fluka, Germany) as buffer A and LC-MS grade methanol (Chem. solute, Geyer, Germany) including 0.1% formic acid as buffer B. The column was equilibrated with a mixture of buffer A (86.5%) and buffer B (13.5%) at a flow rate of 0.4 ml per min and heated to 40°C during the whole measurement. The gradient was produced by changes of the buffer B as follows: 0 to 5 min at 18%, 5 to 6 min at 70 %, 6 to 7 min at 99%, 7 min at 13.5% and kept up to 9 min at 13.5%. The whole duration of the run was 9.0 min.

Phytohormone analysis was performed using a MS/MS triple quadruple mass spectrometer (Agilent 6490, USA). The following parameters were employed: dissolving temperature 350°C, desolation nitrogen gas of 720 l $h^{-1}$, capillary voltage 2.0 KV, detection in positive ion mode and different dwell times between 40 and 200 seconds. Collision energy (CE) differed among the compounds (see table below). Protonated ions [M-H]+ were monitored with a span of 1 amu. Multiple reactions monitoring (MRM) was performed to identify individual compounds accurately. This allows minimizing parallel monitoring and enhancing the sensitivity.

## 4.12 RNA isolation and cDNA synthesis

Total RNA was extracted from 100 mg leaves using TRIzol reagent (Invitrogen, USA) following the manufacturer's protocol. RNA was purified using the RNayes MinElute clean up kit (Qiagen, USA). The quality of RNA was checked on a 1% agarose gel according to the manufacturer's instructions. RNA concentration was

measured using a photometer (BIO-TEC, UVLKONxl, Germany). Removal of genomic DNA was achieved using RQ1 RNase-Free DNase (Promega). 2 µg of total RNA were taken for cDNA synthesis using the RevertAid First Strand cDNA Synthesis Kit of Fermentas (St. Leon-Rot, Germany) and oligo-dT-primers. To test cDNA yield, qPCR was performed using primers of barley ubiquitin gene as a housekeeping gene in parallel for each sample that is stably expressed under the experimental conditions tested.

## 4.13 PCR primer design and quantitative real-time PCR analysis

The primers for qPCR were designed using the primer3 software and were synthesized by Metabion (Germany); for details refer to Appendix Table 2. The following criteria were considered: Tm=60 ± 1ºC, 18–25 bp length, close to the 3'-end if possible, GC content between 40% and 60% to generate unique, short PCR products between 60 bp and 150 bp (Seiler *et al.*, 2011). The cDNA samples were then used to determine gene expression levels by quantitative real-time PCR with the Mastercycler ep realplex (Eppendorf, Hamburg, Germany) and the iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA).

The reaction was carried out in optical 96-well plates, each reaction well containing 7 µl of Power SYBR Green mastermix reagent, 2 µl of cDNA, 0.5 µl of each gene-specific primer and 4 µl distilled water in a final volume of 14 µl. The following standard thermal profile was used for all PCR reactions: 95°C for 5 min, 45 cycles of 95°C for 15 s and 60°C for 1 min. Amplicon dissociation curves, i.e. melting curves, were recorded after 40 cycles by heating from 60°C to 95°C with a ramp speed of 1.9°C per minute.

Expression levels of the genes of interest (GOI) relative to the reference gene (RG) were calculated using the equation $2^{-\Delta CT} = 2^{-(CT(GOI)- CT(RG))}$. To express the GOI in terms of fold-change $2^{-\Delta\Delta CT}$ was used, where $\Delta\Delta CT$ = Stress $^{(CT(GOI)- CT(RG))}$- Control $^{(CT(GOI)- CT(RG))}$.

### 4.13.1 Relative water content (RWC) measurement

The RWC was determined from the flag leaf of the main tiller of both control and stressed plants. The fresh weight (FW) of the flag leaf was recorded immediately after harvest and floated in a petri dish containing distilled water and the petri dish was kept at 4°C overnight. The turgid weight (TW) was recorded the next day after gently wiping the sample with tissue paper to remove the water adhering to the

sample. Finally, the samples were oven-dried at 70°C for 24 h and the dry weights (DW) were recorded. The RWC was calculated using the formula RWC (%) = [(FW-DW) / (TW-DW)] x 100.

### 4.13.2 Analysis of yield data

For the analysis of yield parameters 3 individual plants were combined into one biological replication. When kernels physically matured at BBCH 92, thousand grain weight (TGW), grain yield, seed area, seed length and seed breadth were analysed using a Marvin instrument.

# 5 Results

## 5.1 Influence of K supply on drought-induced leaf senescence in three different barley lines

In order to evaluate the influence of K nutrition on drought-induced senescence, three lines differing in their senescing behavior, LP104, LP110 and LP106 were grown in pots in the greenhouse for a period of 12 weeks. Plants were grown at 3 different levels of K supply. Five days after flowering (DAF) drought stress was imposed by reducing the soil moisture content to 10% (corresponding to 25% field capacity). After 12 days of drought stress flag leaves were harvested for the assessment of physiological and biochemical parameters induced by drought stress.

### 5.1.1 Relative water content (RWC) under drought stress and varied K supply

The relative water content (RWC) defines the hydration state of a plant sample by the amount of water lost after drying. The RWC was determined here by the fresh weight of a drought-stressed leaf relative to the fresh weight of a control leaf at full turgidity (Seiler *et al.*, 2014*b*). In this study, no particular decrease in RWC was observed in the lines when K was supplied at medium or high levels to the substrate. However, RWC decreased in plants suffering from concomitant drought and K deficiency (Figure 5). In contrast to LP110 and LP106, the RWC of LP104 was not significantly lower under drought and remained at a higher level than in the other two lines, indicating that LP104 lost less water under drought conditions.

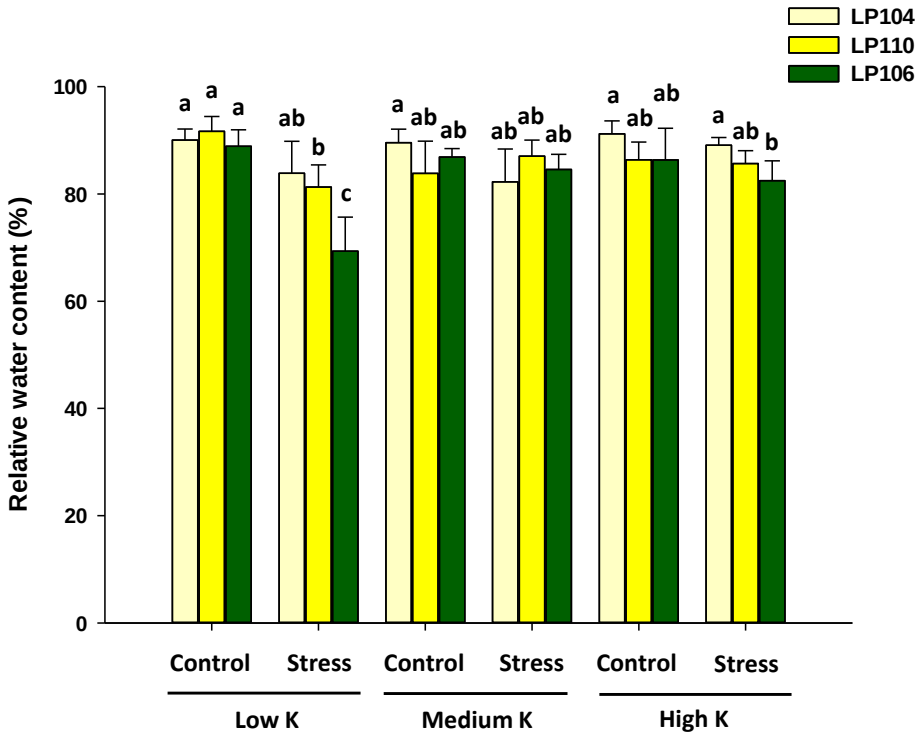


**Figure 5. Effect of drought stress and varied potassium supply on the relative water content (RWC) in flag leaves of barley.** RWC was measured in flag leaves of LP104, LP110 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low, medium and high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test in each K treatment ($p<0.05$; n=6)

### 5.1.2 Chlorophyll concentrations in flag leaves under drought stress and varied K supply

In a first approach to determine the influence of sufficient and insufficient K supply on senescence parameters in drought-stressed flag leaves, the chlorophyll concentration was measured in all three investigated lines. In general, chlorophyll concentrations decreased under drought stress regardless of K supply in all three lines. Differences in the chlorophyll concentration of flag leaves grown under medium or high K supply were relatively small and not significant. However, the chlorophyll concentration under drought was significantly higher in low K-fed LP104 plants compared to the other lines (Figure 6). Despite the initial categorization of LP104 as an early senescing line (3.1), this observation indicated that LP104 showed delayed drought-induced chlorophyll degradation in flag leaves under the present conditions.

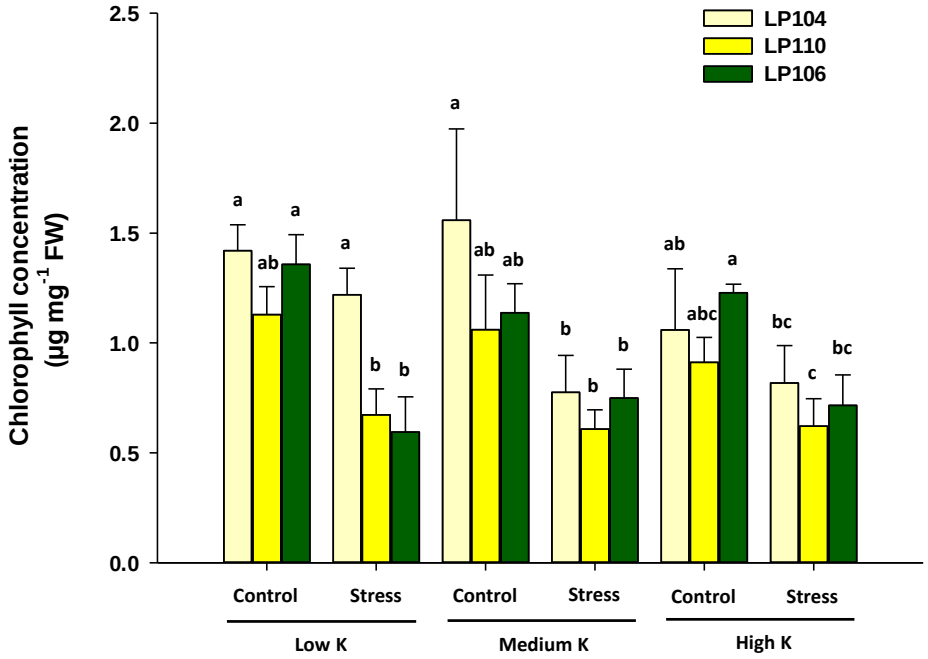


**Figure 6. Effect of drought stress and varied potassium supply on chlorophyll concentrations in flag leaves of barley.** Concentrations of chlorophyll were measured in flag leaves of LP104, LP110 and LP106 subjected to drought stress 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low, medium and high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test within each K treatment ($p<0.05$; n=4-6)

### 5.1.3 Expression analysis of the senescence-induced gene *HvS40* in flag leaves under drought stress and varied K supply

The induction of many senescence-associated genes has been reported under drought and nutrient deficiency (Buchanan-Wollaston *et al.*, 2003; Balazadeh et al., 2014). In order to evaluate the progress of drought-induced senescence under varied K supply, the expression level of the well characterized barley senescence marker gene *S40* (Krupinska *et al.*, 2002) was analyzed in all three lines. The expression level of *S40* gene was strongly up-regulated under drought stress at low K supply in the lines LP110 and LP106, while it was less pronounced in flag leaves of LP104 under the same conditions (Figure 7). This trend also held true in the line LP110 under drought at medium K supply. However, the expression level of *S40* declined under drought stress, when K supply to the substrate increased (Figure 7). Overall, the observed changes in gene expression levels of this

senescence marker supported the view that the additional K supply delayed drought-induced senescence in flag leaves of barley.

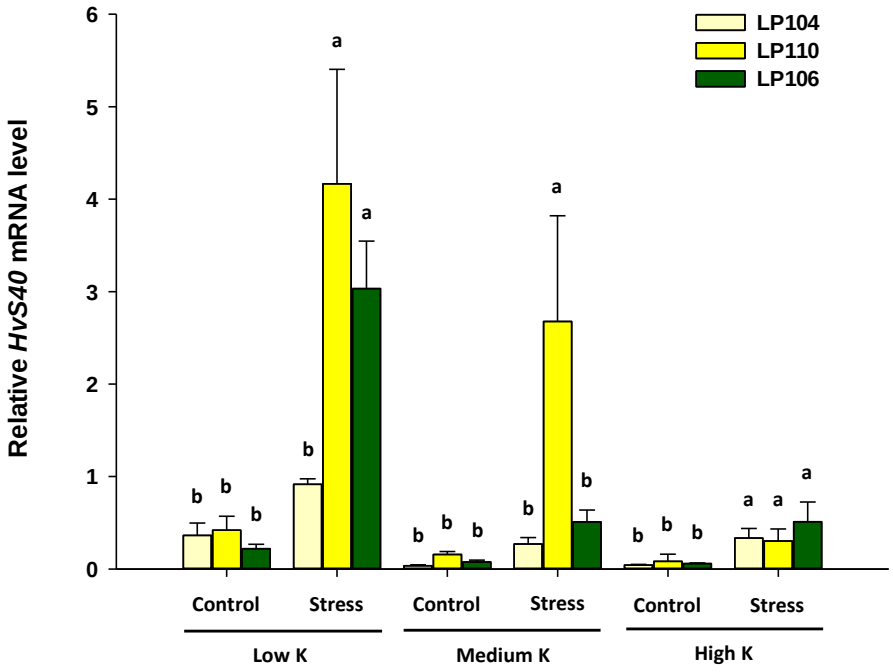


**Figure 7. Effect of drought stress and varied potassium supply on mRNA levels of *HvS40* in flag leaves of barley.** Relative *HvS40* mRNA levels were measured in flag leaves of LP104, LP110 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low, medium or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test within each potassium treatment ($p<0.05$; n=6). *Ubi E2-17* (ubiquitin-conjugating enzyme) was used as reference gene.

### 5.1.4 Element analysis in flag leaves under drought stress and varied K supply

For several mineral elements a role in the alleviation of drought stress in plants has been well characterized (Waraich *et al.*, 2011). Among the major mineral elements, K plays unique role to enhance drought tolerance due to its function related to the maintenance of stomatal regulation and cellular turgor (Römheld and Kirkby, 2010). To determine how an imbalance in K nutrition affects the nutrient status in all three lines, flag leaf concentrations of K and also of Mg and Ca were determined, as in particular Mg and Ca are often used by plants to counterbalance deficiencies in K (Marschner, 2012).

With increasing K supply to the substrate, the K concentration in the flag leaves increased in all three investigated lines (Figure 8a). However, compared to the treatment with medium K supply, there was only a small, non-significant increase in leaf K levels under high K supply. As flag leaves achieved a K concentration of 1-2% already at medium K supply, which is considered to be above critical deficiency levels, plants were already adequately supplied with K in this medium K treatment. Compared to LP104, the K concentration significantly decreased under drought stress at low K supply in the lines LP110 and LP106. Relative to control plants, the Mg concentration increased significantly under drought at low K supply in all three lines (Figure 8b). This was most likely a consequence of the lower K uptake under these conditions. Taken together, these results supported the view of an antagonistic relation between K and the other two divalent cations and confirmed an adequate Mg and Ca status in these plants irrespective of K supply and drought stress conditions.

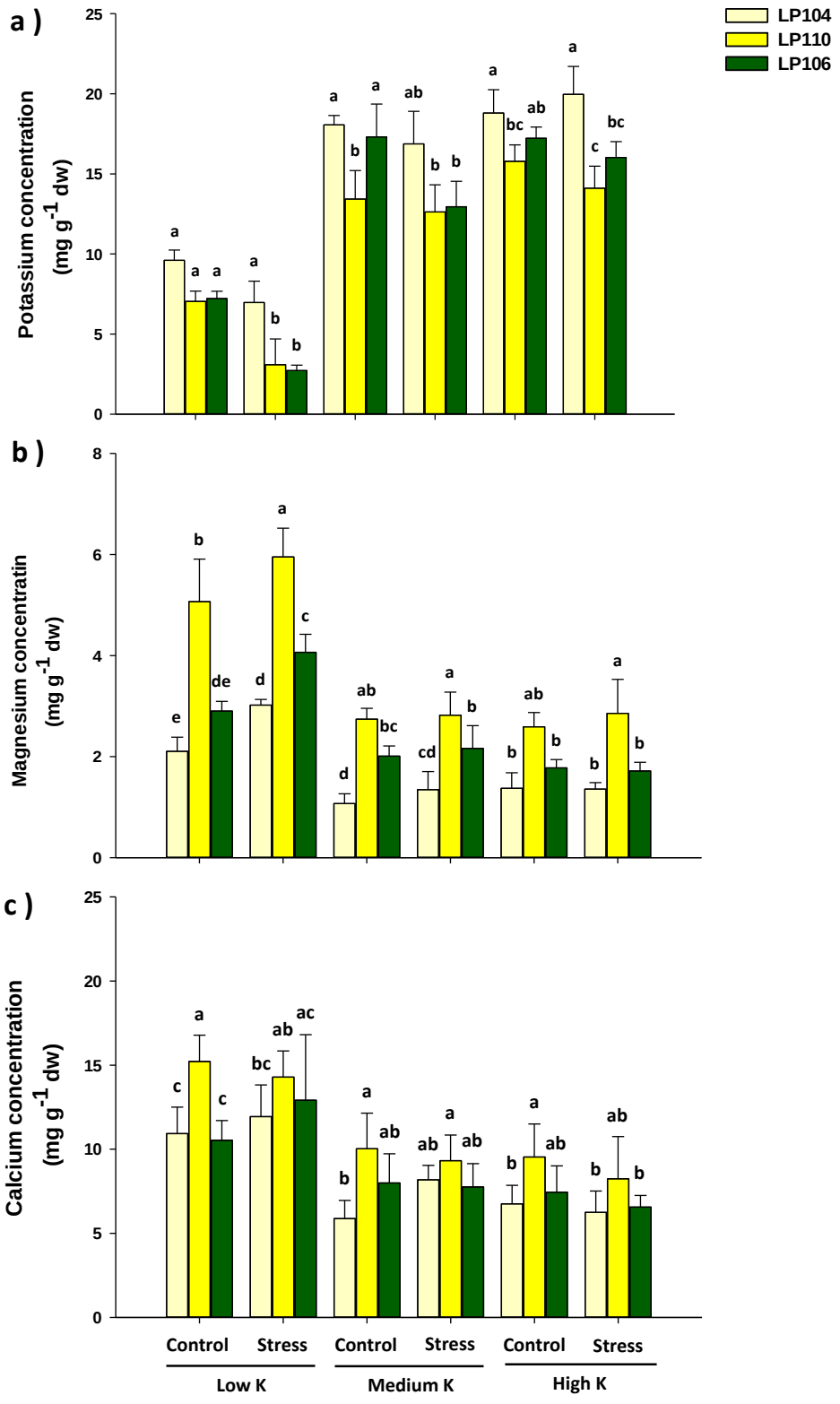


**Figure 8. Effect of drought stress and varied potassium supply on the concentrations of Mg and Ca in flag leaves of barley.** Concentrations of a) potassium (K), b) magnesium (Mg), and c) calcium (Ca) were measured in flag leaves of LP104, LP110 and LP106 lines subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low, medium or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test within each K treatment ($p<0.05$; n=4-6).

### 5.1.5 Soluble and insoluble sugar concentrations under drought and varied K supply

The role of sugar accumulation in leaf senescence resulting from abiotic stress like drought has been well evaluated (Wingler and Roitsch, 2008). For instance, it has been shown that many early senescence associated genes (SAGs) are sugar inducible (Paul and Pellny, 2003). To determine if the K nutritional status regulates drought-induced senescence through the accumulation of carbohydrates, the concentrations of soluble and insoluble sugars were measured in flag leaves. As expected, the concentrations of glucose and fructose significantly increased under drought stress irrespective of the K level in the soil (Figure 9a and b). However, there were no consistent differences among the lines. The increase in hexoses under drought was accompanied by a decrease in concentrations of sucrose (Figure 9c) and starch (Figure 10). However, the depletion in flag leaf starch concentrations was significantly lower in the line LP104, indicating a higher carbohydrate reserve under drought in this line. This lower reduction in starch was also observed at medium and high K supplies in drought-stressed flag leaves of LP104 plants but only under high K supply in the line LP106 (Figure 10). Taken together, these results suggested that genotypic differences in the K nutritional status and senescence behaviour under low K supply and drought were also related to the level of the starch reserve in flag leaves.

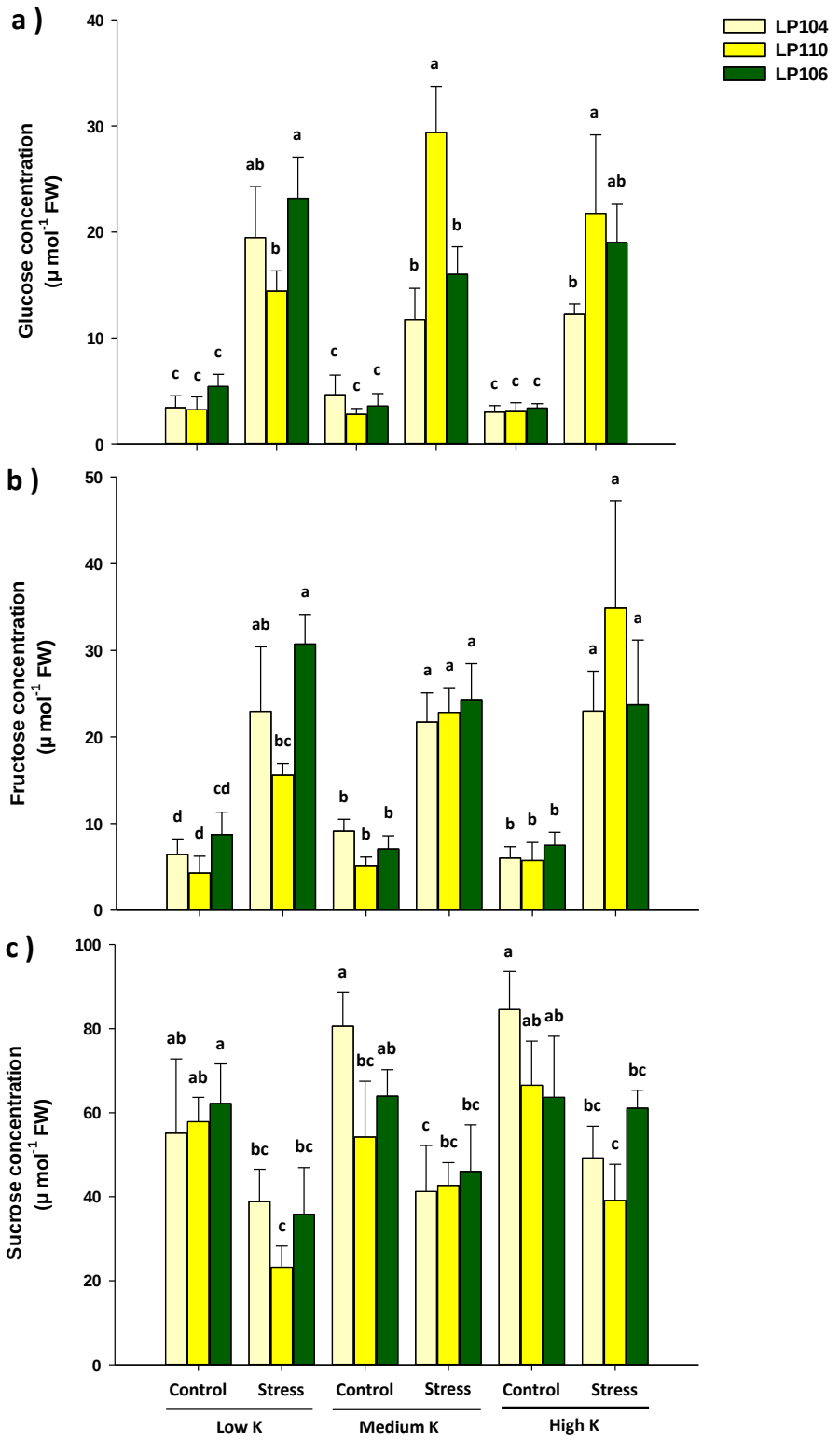


**Figure 9. Effect of drought stress and varied potassium supply on the concentrations of soluble sugars in flag leaves of barley.** Concentration of a) glucose, b) fructose and c) sucrose were measured in flag leaves of LP104, LP110 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low, medium or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test within each K treatment ($p<0.05$; n=4-6).

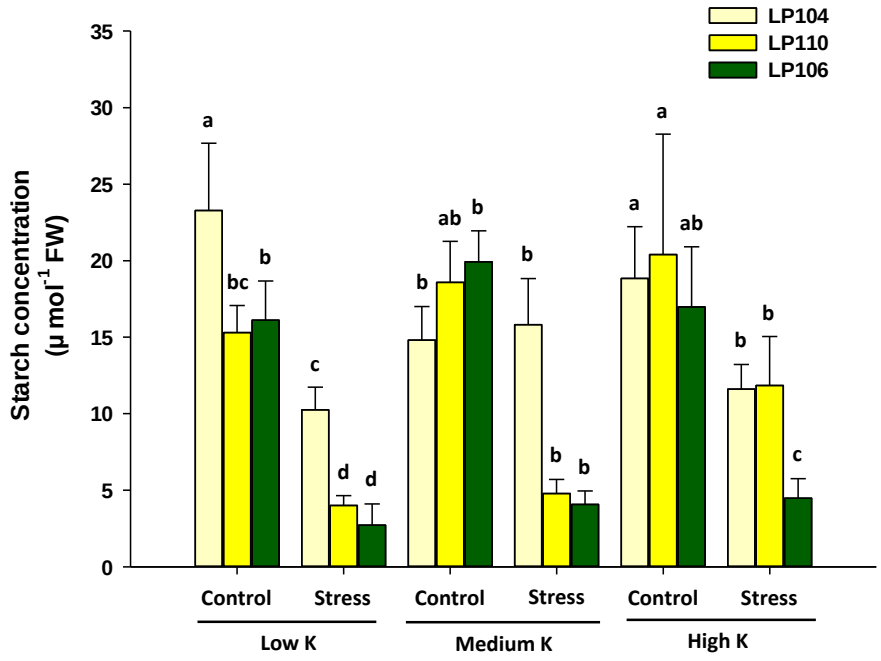


**Figure 10. Effect of drought stress and varied potassium supply on starch concentrations in flag leaves of barley.** Concentrations of starch were measured in flag leaves of LP104, LP110 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low, medium or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test within each K treatment ($p<0.05$; n=4-6).

### 5.1.6 Sugar alcohol concentrations under drought stress and varied K supply

Sugar alcohols act as compatible solutes and their accumulation under drought stress has been reported to increase drought tolerance in plants (Farooq *et al.*, 2009). To verify the influence of K on the amount and composition of sugar alcohols accumulating under drought stress, the concentrations of mannitol, inositol and erythritol were measured in flag leaves of all 3 lines. Irrespective of the K level in the substrate, the concentrations of mannitol were marked by a significant increase under drought stress in all 3 investigated lines. This increase was not seen, or only in tendency, for inositol and erythritol. Interestingly, under drought stress and K deprivation mannitol concentrations in the line LP104 were significantly higher than in the other two lines (Figure 11a). An influence of K supply was also seen on the concentrations of erythritol, which tended to decrease in all lines with increasing K provision (Figure 11c). Thus, mannitol turned out as the most responsive sugar alcohol to drought stress, and obviously, LP104 was

able to produce larger amounts of mannitol also under concomitant drought and K deficiency.

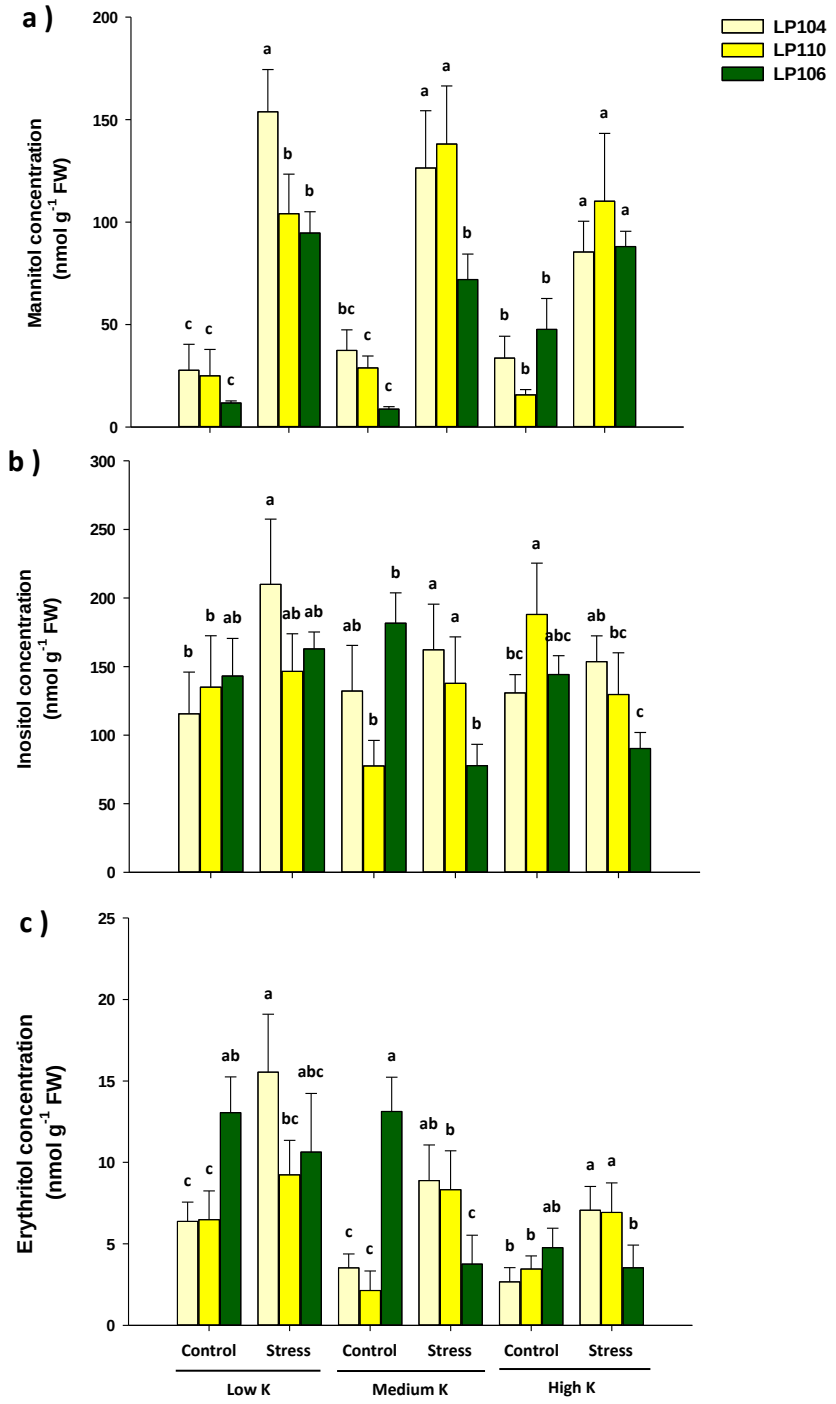


**Figure 11. Effect of drought stress and varied potassium supply on sugar alcohol concentrations in flag leaves of barley.** Concentrations of a) mannitol, b) inositol, and c) erythritol were measured in flag leaves of LP104, LP110 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test within each K treatment ($p<0.05$; n=4-6).

### 5.1.7 Amino acids measurement under drought stress and varied K supply

Drought stress is mostly linked with alterations in the metabolism of amino acids. In particular the synthesis of proline is increased, which takes over functions as a osmoprotectant and positively correlates with tolerance against leaf wilting under drought stress (Sayed *et al.*, 2012). To assess the role of K on amino acid changes under drought, the concentrations of essential amino acids were measured. In general, amino acid profiles strongly depended on the genotype, and there was no significant change under drought stress or by K supply that was consistent in all 3 lines. Only aspartate showed a slight tendency to decrease by drought stress in all lines. In contrast, K supply tended to increase aspartate levels. With regard to proline, LP106 showed a remarkably high level in K-deficient control plants, whereas LP104 showed highest levels under concomitant drought and K deficiency (Figure 12a). The line LP106 also showed a strong increase in phenylalanine and glutamine concentrations under drought conditions (Figure 14a and b). Irrespective of the K supply, the concentrations of asparagine significantly decreased under drought in LP110, while this trend was not observed in LP104 and LP106 (Figure 12c).

In the lines LP110 and LP106, gama-aminobutyric acid (GABA) concentrations slightly increased with increasing K supply to the soil (Figure 13a). The other amino acid levels did not show consistent responses to drought stress or K supply. Taken together, proline proved as a drought stress-induced amino acid only in K-deficient LP104, while the other amino acids showed little or no consistently significant responses to either stress factor.

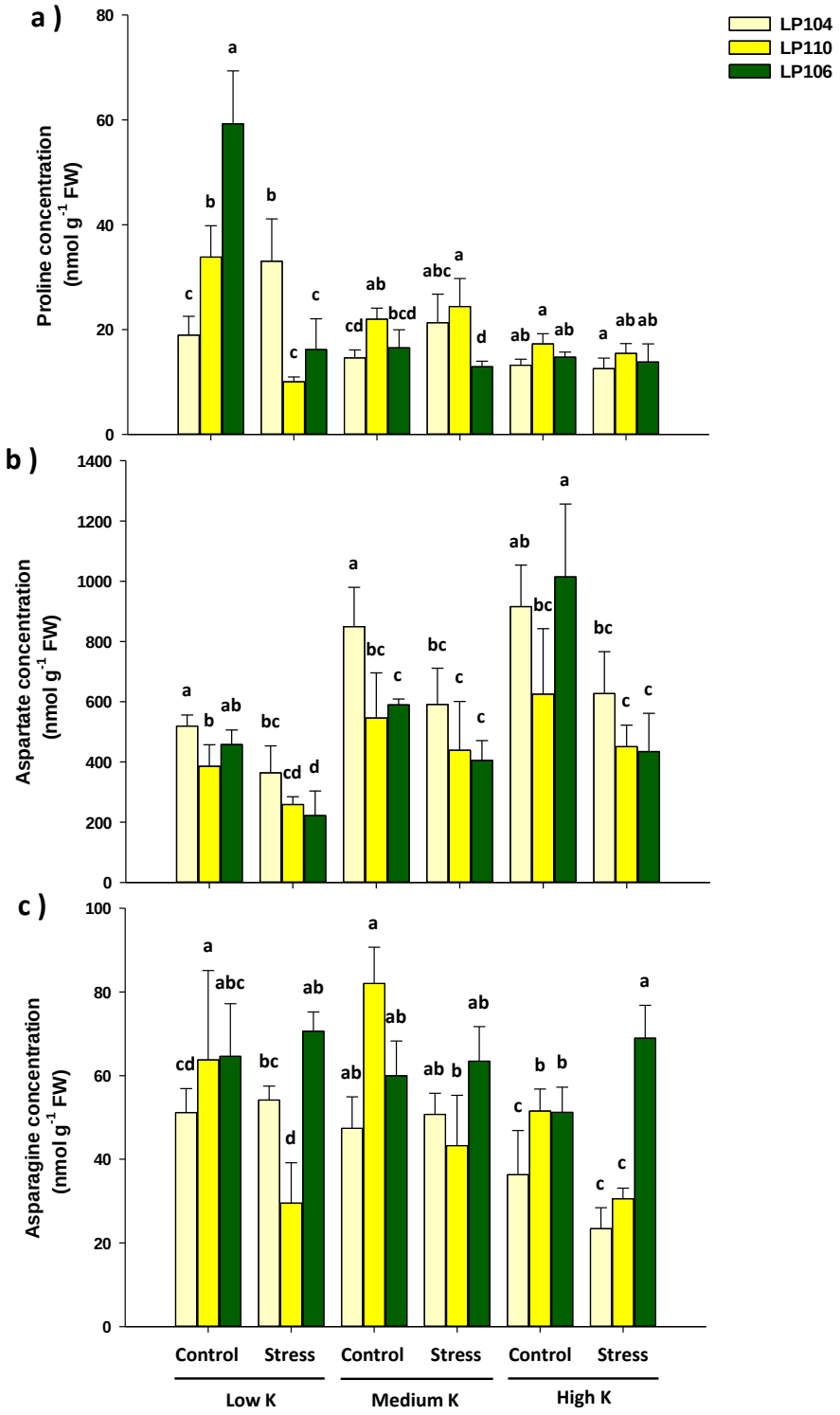


**Figure 12. Effect of drought stress on amino acid concentrations in flag leaves of barley.** Concentrations of a) proline, b) aspartate and c) asparagine were measured in flag leaves of LP104, LP110 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low, medium or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test within each K treatment ($p<0.05$; n=4-6).

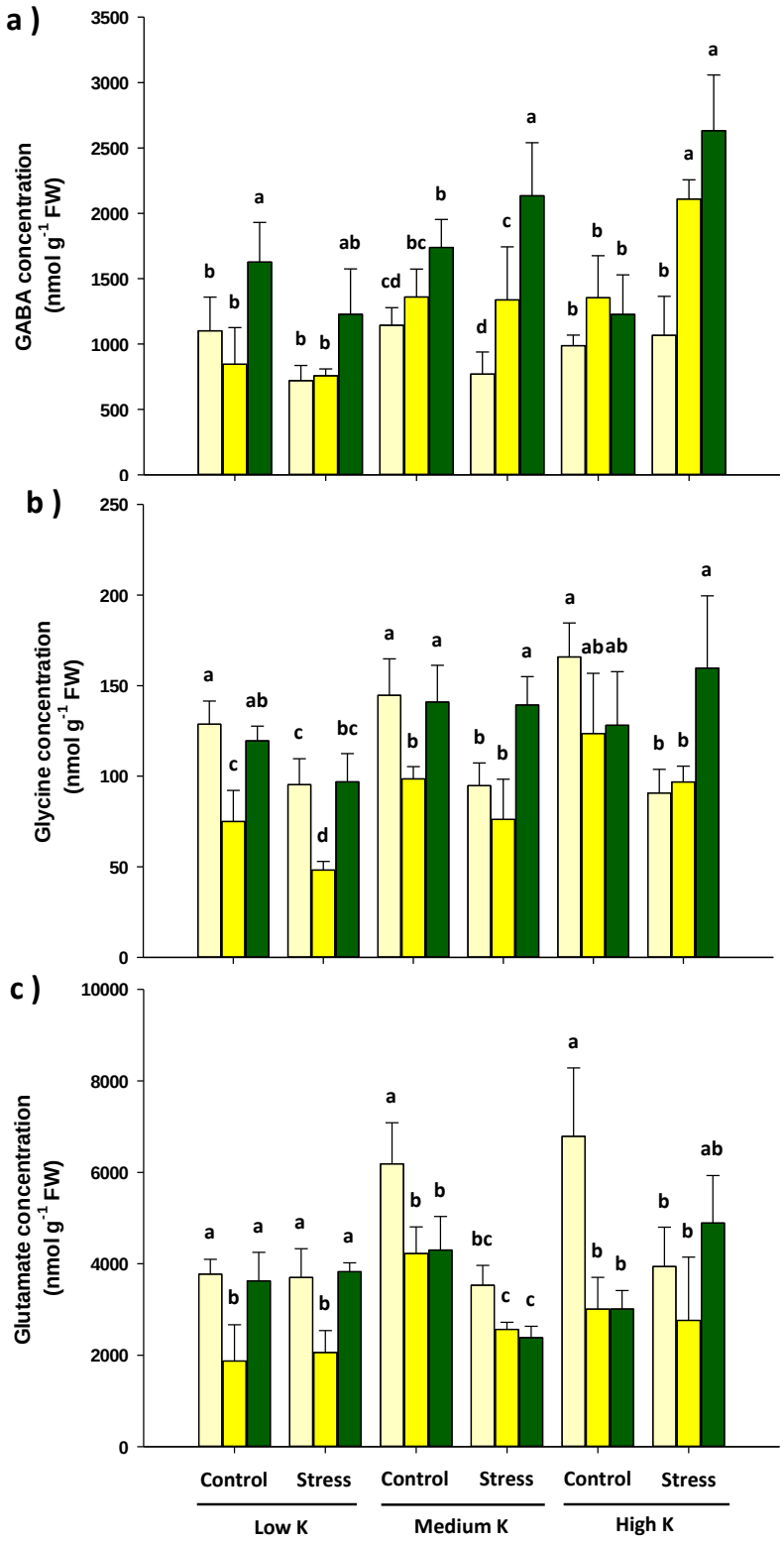


**Figure 13. Effect of drought stress on amino acid concentrations in flag leaves of barley.** Concentrations of a) GABA (gamma-aminobutyric acid), b) glycine and c) glutamate were measured in flag leaves of LP104, LP110 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low, medium or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test within each K treatment ($p<0.05$; n=4-6). Abbreviation is as follows: GABA: gamma aminobutyric acid.

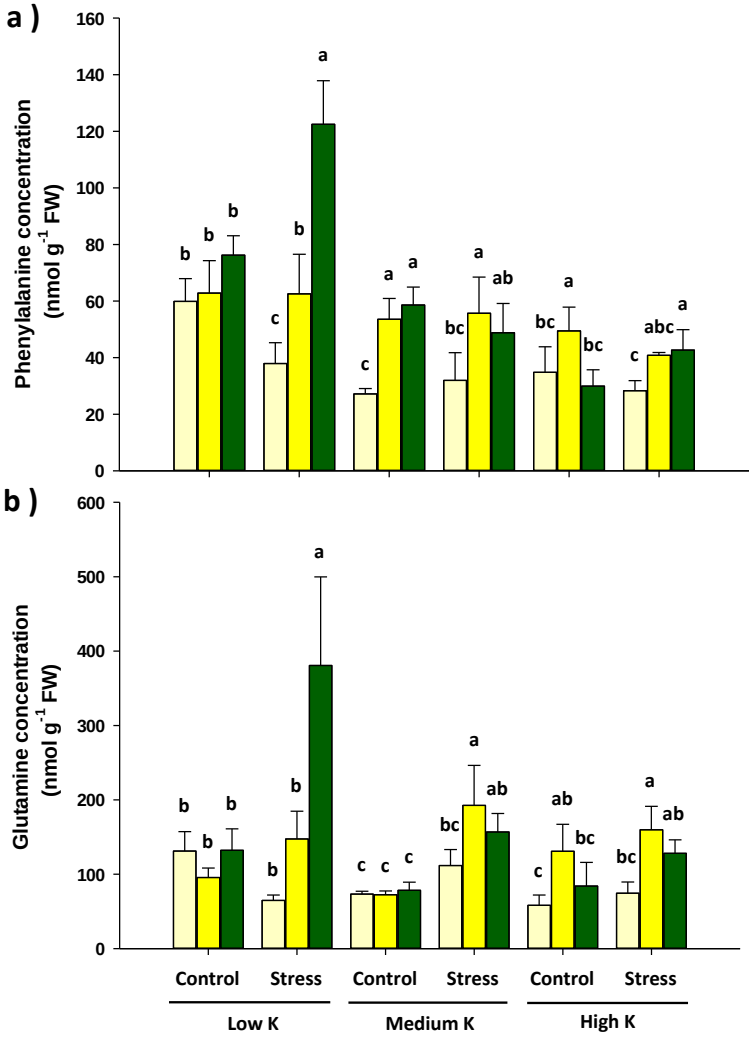


**Figure 14. Effect of drought stress and varied K supply on amino acid concentrations in flag leaves of barley.** Concentrations of a) phenylalanine and b) glutamine were measured in flag leaves of LP104, LP110 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low, medium or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test within each K treatment ($p<0.05$; n=4-6).

### 5.1.8 Primary metabolite changes under drought stress and varied K supply

To further investigate the role of K on primary carbon metabolism under water deprivation, targeted metabolite profiling of flag leaves were determined which go beyond Calvin cycle, glycolysis and TCA cycle. These metabolites covered the most important metabolic routes of primary carbon and nitrogen metabolism. Nine of these metabolites which showed significantly differences at least between the lines or one of the treatments were described. Similar to the control plant, the higher concentration of hexose phosphate was observed under drought and low K

supply in the line LP104 compared to other two lines (Figure 15a). In addition, the concentrations of ADP-glucose increased under drought at low K supply in LP104 in comparison with LP110 and LP106 (Figure 15b). Although, the concentration of oxoglutarate decreased under drought in all three investigated lines, however, this reduction was significantly less in the line LP104 compared to other two lines (Figure 15c). An increase in concentration of oxoglutarate was observed under drought stress in both LP110 and LP106 by additional K into the substrate (Figure 15c).

The concentrations of 3-PGA increased in all 3 investigated lines under normal watering regimes. In LP104, the concentration of 3-PGA increased under drought when the K level was high in the substrate (Figure 16c). The concentrations of NADPH, ATP and UDP-glucose were relatively high in the both LP104 and LP110 in comparison with LP106 (Figure 16c, 17a and b). Notably, the concentration of fumarate was also significantly higher under drought in the line LP104 compared to the line LP106 either at low or high K supplies (Figure 16b). Trehalose-6-P level also was increased with high amount of K in the soil in all 3 lines; however, this positive effect was significantly more pronounced in LP106 compared to LP104 and LP110 in spite of different watering regimes (Figure 17c).

In summary, the levels of primary metabolites, in particular those of hexoses were less affected in LP104 and remained at a more constant level under concomitant drought and K deficiency than in the two other lines.

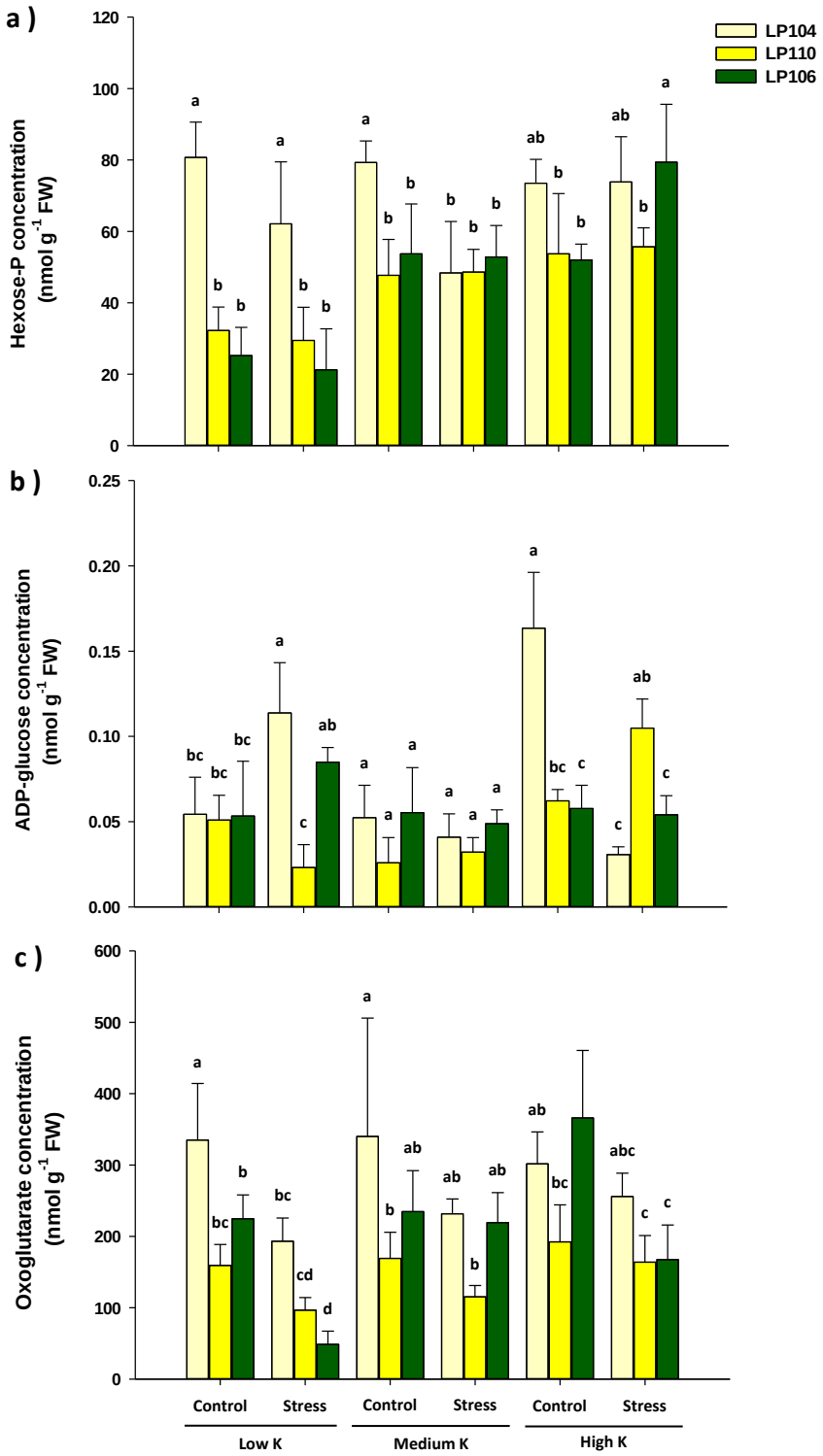


**Figure 15. Effect of drought stress and varied K supply on primary metabolite concentrations in flag leaves of barley.** Concentrations of a) hexose phosphate, b) ADP-Glucose and c) oxoglutarate were measured in flag leaves of LP104, LP110 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low, medium and high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test within each K treatment ($p<0.05$; n=4-6). Abbreviations are as follows: Hexose-P: hexose phosphate.

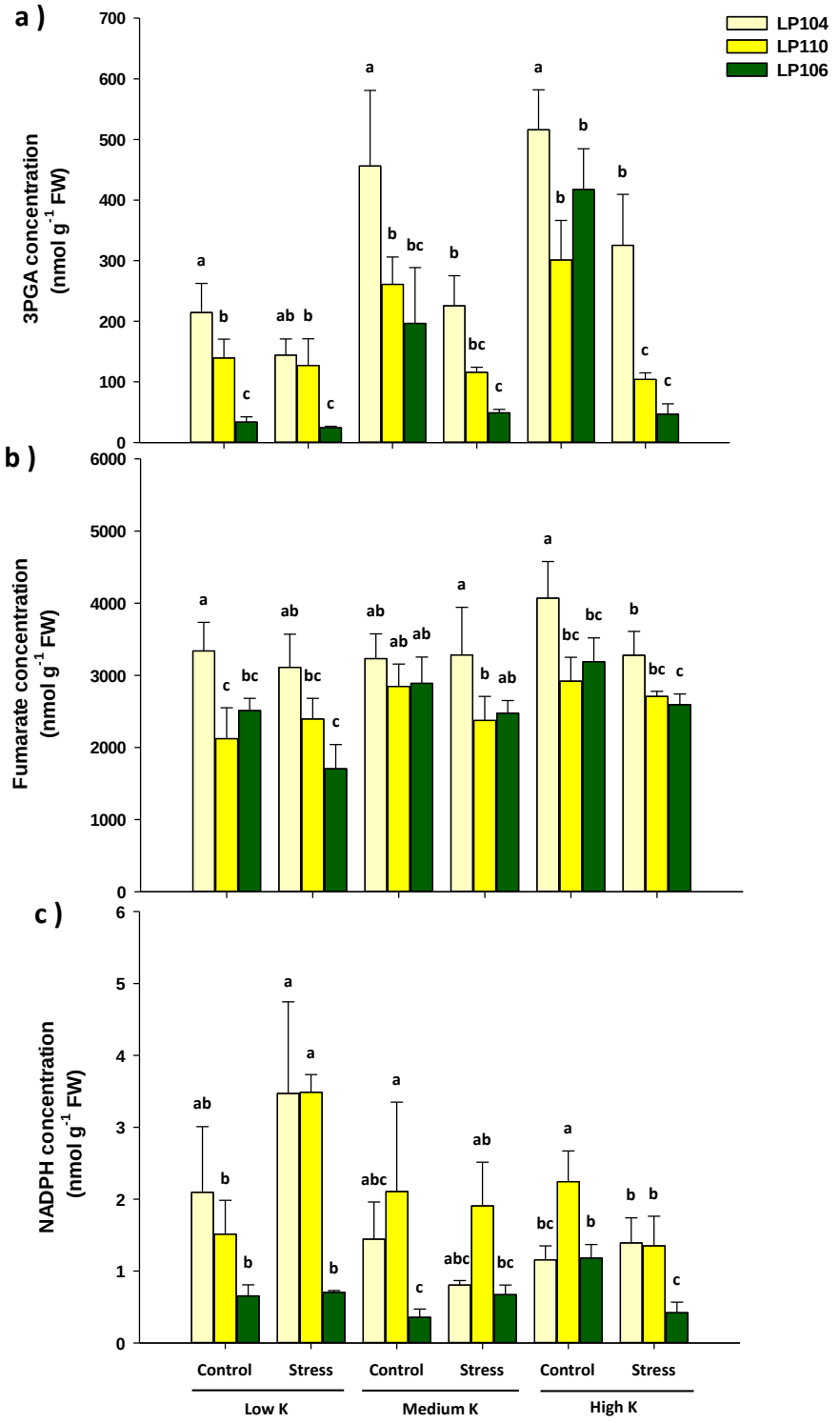


**Figure 16. Effect of the drought stress and varied K supply on primary metabolite concentrations in flag leaves of barley.** Concentrations of a) 3PGA, b) fumarate and c) NADPH were measured in flag leaves of LP104, LP110 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low, medium and high K supply. Flag leaves from 12 weeks-old plants that were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test within each K treatment ($p<0.05$; n=4-6). Abbreviations are as follows: 3PGA: 3-phosphoglycerate, NADPH: Nicotinamide adenine dinucleotide phosphate.

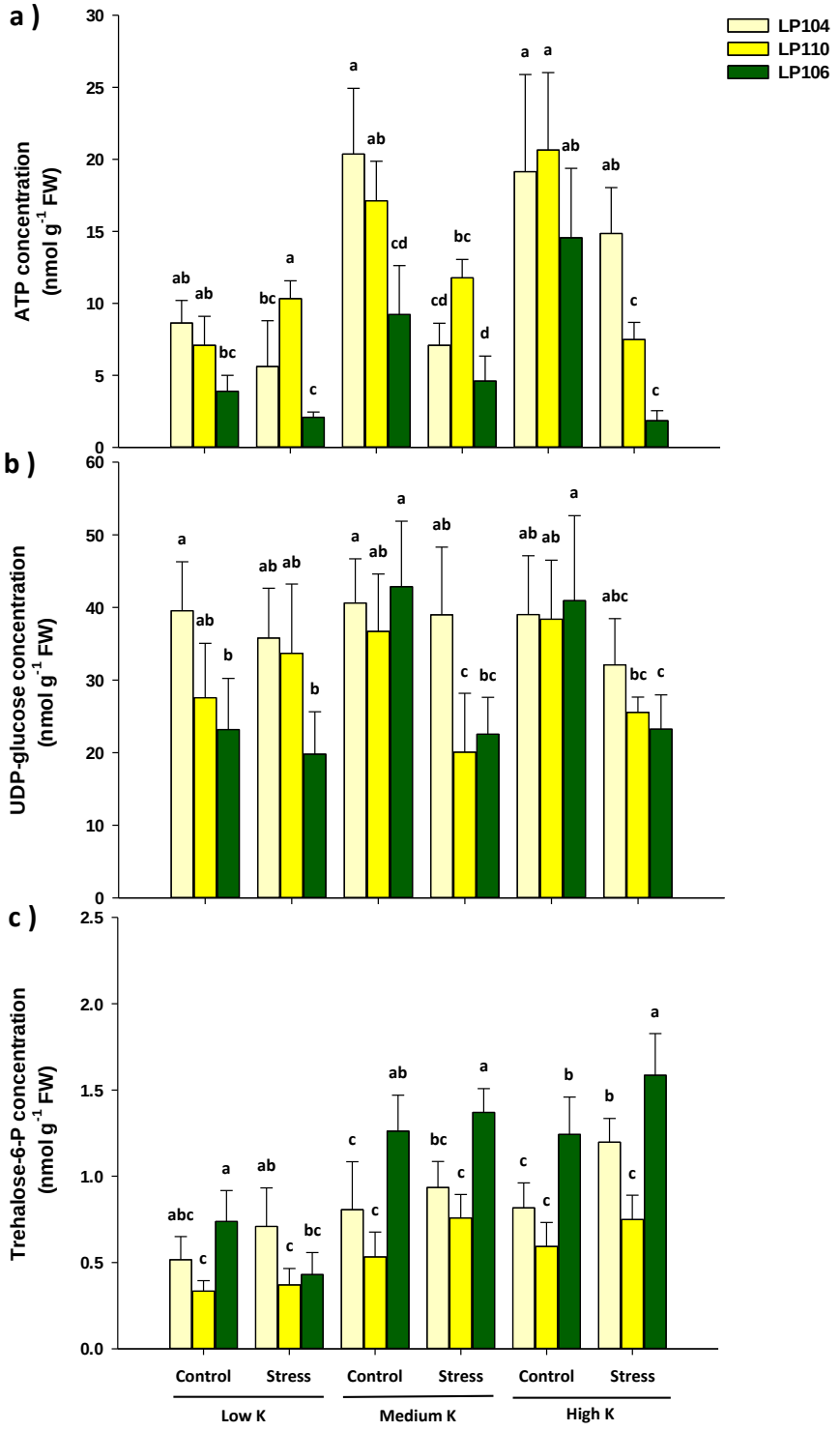


**Figure 17. Effect of drought stress and varied K supply on primary metabolite concentrations in flag leaves of barley.** Concentrations of a) ATP, b) UDP-glucose and c) trehalose-6-P were measured in flag leaves of LP104, LP110 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low, medium and high K supply. Flag leaves from 12 weeks-old plants that were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test within each K treatment ($p<0.05$; n=4-6). Abbreviations are as follows: Trehalose-6-p: trehalose-6-phosphate, ATP: Adenosine triphosphate.

## 5.2 Influence of K supply on the hormonal regulation of two barley lines with different behaviour under drought stress

Based on the higher carbohydrate and starch levels in LP104, the question arose whether the different behaviour of the investigated barley under drought stress may be related to the stress hormone ABA. Previously, a cross talk has been postulated between starch metabolism in barley leaves and the ABA signaling pathway (Fujii *et al.*, 2009; Seiler *et al.*, 2011). To investigate ABA-related responses in the two most contrasting lines LP104 and LP106, plants were grown in a pot experiment in the greenhouse for a period of 12 weeks and fertilized with an adequate (high K treatment, 4g/kg dry soil of $K_2SO_4$) or deficient (low K treatment, without adding $K_2SO_4$) amount of K. Five days after flowering (DAF) drought stress was imposed by reducing the soil moisture content to 10% (corresponding to 25% field capacity). After 12 days of drought stress flag leaves were harvested for the assessment of physiological and biochemical parameters induced by drought stress.

### 5.2.1 Chlorophyll concentrations in flag leaves under drought stress and different K regimes

In consistence with the findings in the previous experiment (Figure 5) drought stress decreased chlorophyll concentrations in LP106 at low and high K supply (Figure 18). In contrast, the line LP104 retained a higher concentration of chlorophyll under drought stress irrespective of the supplied K level confirming that LP104 is less sensitive to drought stress-induced chlorophyll degradation (Figure 18).

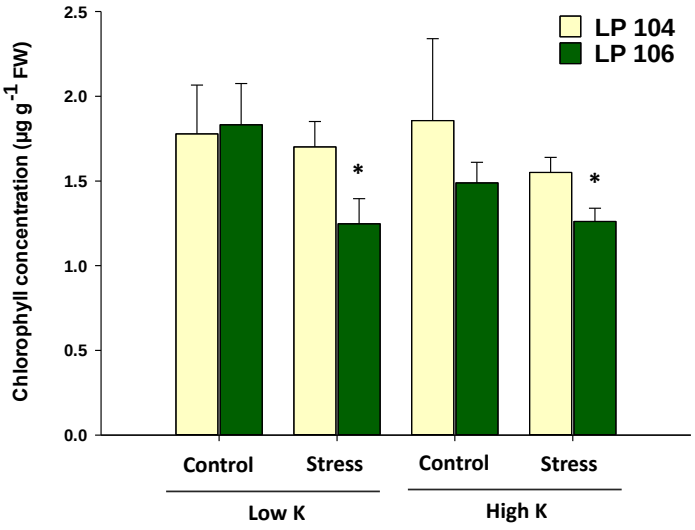


**Figure 18. Influence of different K regimes on chlorophyll concentrations in flag leaves of barley under terminal drought stress.** Chlorophyll concentrations were measured in flag leaves of LP104 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Asteriscs denote significant differences according to 3-way ANOVA and Tukey's test in each K treatment under drought stress between the two lines ($p<0.05$; n=4-6)

### 5.2.2 Expression analysis of the senescence-induced gene *HvS40* in flag leaves under drought stress and different K regimes

In line with the finding in Figure 7, mRNA expression of *HvS40* showed a contrasting regulation in LP104 and LP106 plants (Figure 19). Drought stress was lead to an up-regulation of *HvS40* mRNA levels in both lines. At low K supply, this increase was significantly higher in LP106 than in LP104 (Figure 19). Notably, this gene was also up-regulated in both lines when a sufficient amount of K was applied, but this change was less pronounced compared to K-deprived plants (Figure 19). This indicated that a higher K fertilization level delayed drought-induced senescence in flag leaves of barley.

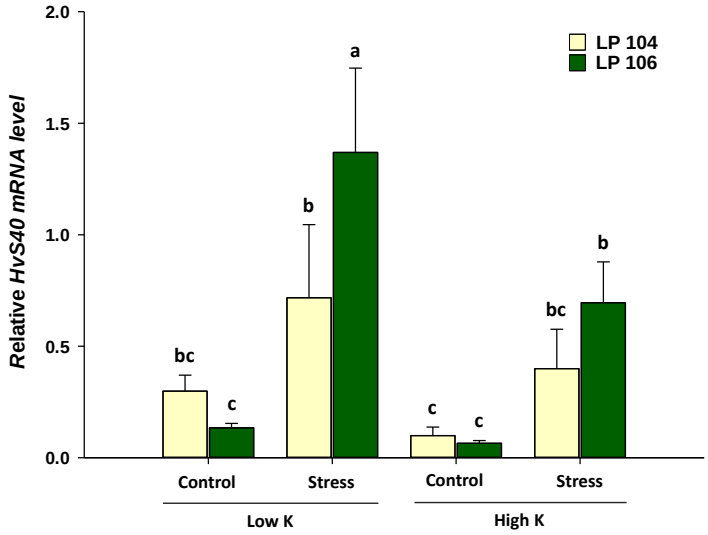


**Figure 19. Influence of different K regimes on transcript levels of the senescence marker gene *HvS40* in flag leaves of barley under terminal drought stress.** Relative *HvS40* mRNA levels were measured in flag leaves of LP104 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test ($p<0.05$; n=6). *Ubi E2-17* (ubiquitin-conjugating enzyme) was used as reference gene.

### 5.2.3 Element analysis in flag leaves under drought stress and different K regimes

In line with the results in 4.1.4, K concentrations increased in flag leaves of both lines when the K level in the substrate increased (Figure 20). While the concentration of K was in general lower under drought at either K supply level, LP104 maintained a significantly higher K concentration compared to the line LP106 (Figure 20). Moreover, the concentrations of magnesium (Mg), sodium (Na) and to a lesser extent also of calcium decreased by increasing K supply to the substrate , while drought stress had only a little impact on the accumulation of these elements (Figure 21a, b and c). Plant usually compensate for lower K uptake by an enhanced uptake of Na. This was also the case here and expressed in a narrow K-to-Na ratio at low K supply (Figure 21d). Interestingly, even at high K supply, when LP104 accumulated more K than LP106 (Figure 20), a higher K-to-Na ratio still maintained in LP104, indicating a superior uptake efficiency of LP104.

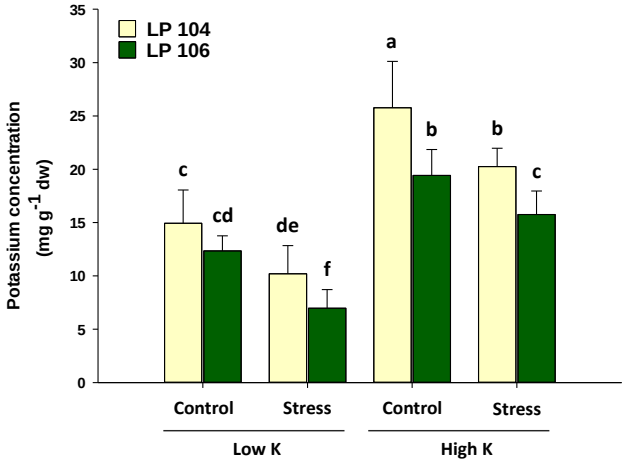


**Figure 20. Influence of different K regimes on K concentrations in flag leaves of barley under terminal drought stress.** Concentrations of K were measured in flag leaves of LP104 and LP106 lines subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test ($p<0.05$; n=4-6).

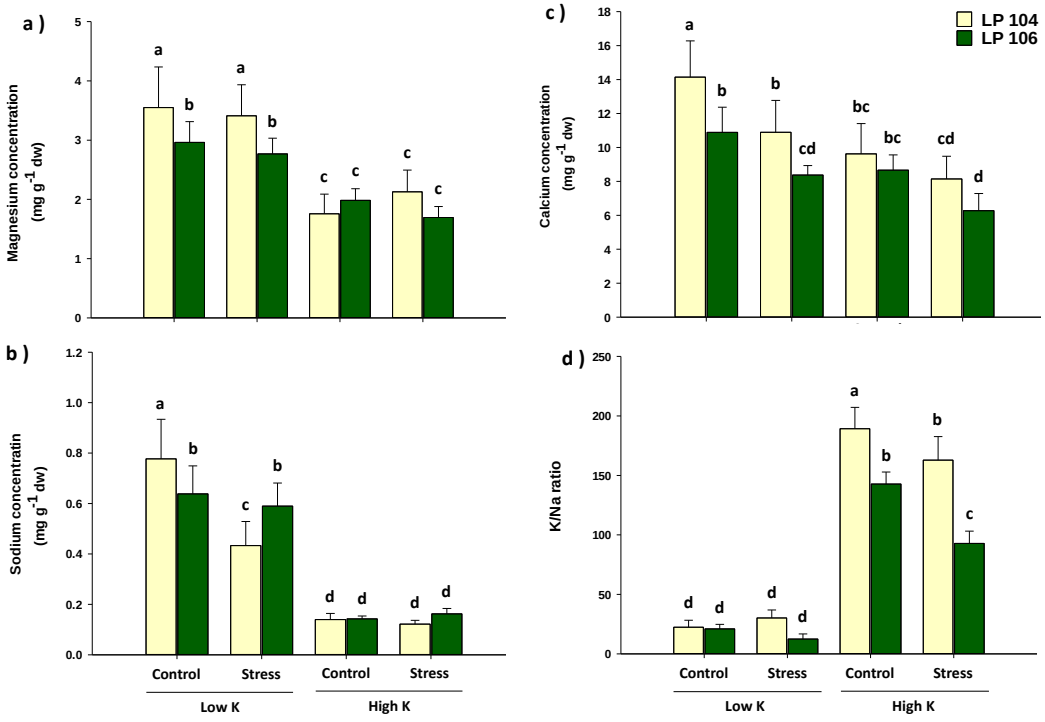


**Figure 21. Influence of different K regimes on element concentrations in flag leaves of barley under terminal drought stress.** Concentrations of a) magnesium, b) sodium, c) calcium and d) the ratio of potassium to sodium were determined in flag leaves of LP104 and LP106 lines subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test ($p<0.05$; n=4-6).

### 5.2.4 Expression analysis of genes involved in starch biosynthesis and degradation under drought and different K regimes

Based on the observation that LP104 maintained a larger starch reserve under drought stress and concomitant K deficiency (Figure 10), the present experiment attempted to compare between the two lines the transcriptional regulation of major genes involved in starch metabolism. Investigating expression levels of the two genes ADP-glucose pyrophosphorylase small subunit (*HvAGPS2*) and ADP-glucose pyrophosphorylase large subunit (*AvAGPL1*), which are involved in starch biosynthesis, showed little differences between the two lines (Figure 22a and b). However, monitoring beta-amylase (*AvBAM2*) and isomerase (*HvISA1*), which are key components in the starch degradation pathway (Radchuk *et al.*, 2009), showed a strong up-regulation of both genes under concomitant drought and K deficiency only in LP106 (Figure 22c and d). This observation suggested that LP106 induced a higher turnover of starch than LP104 under severe drought and K deprivation.

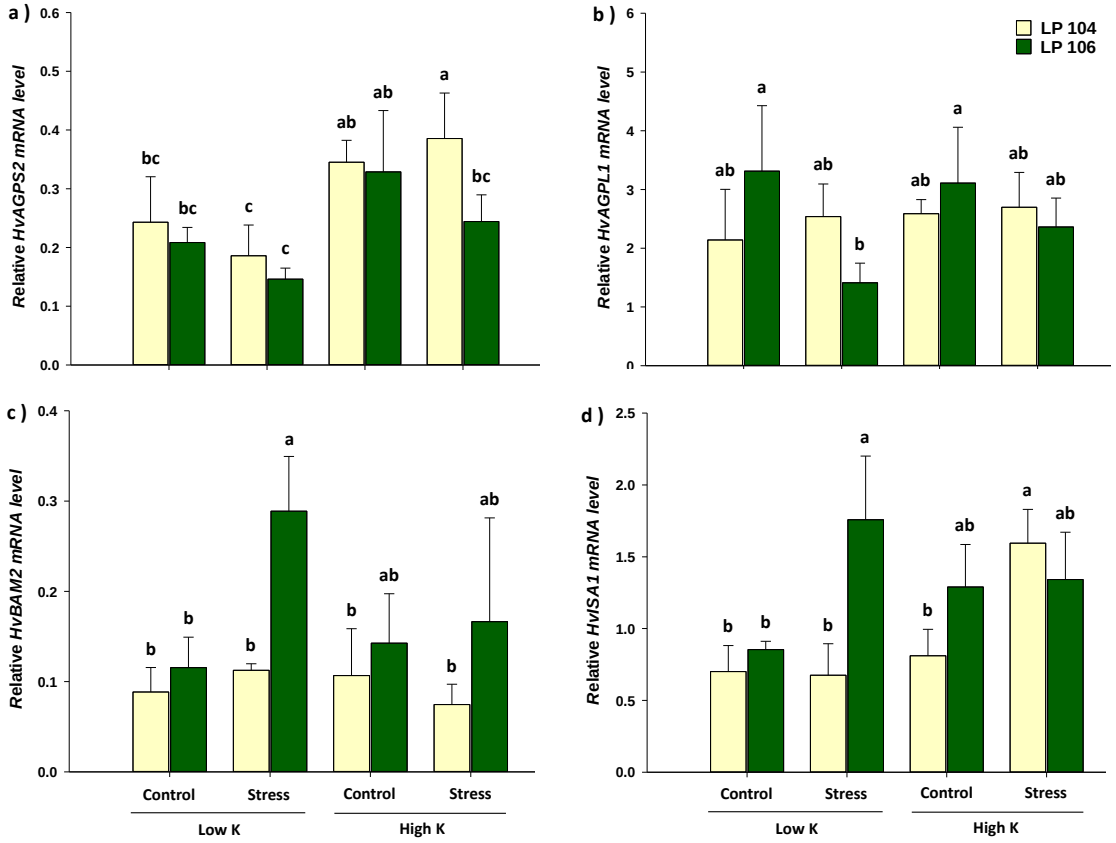


**Figure 22. Influence of different K regimes on the expression of genes involved in starch biosynthesis and degradation in flag leaves of barley under terminal drought stress.** Relative mRNA levels of a) ADP-glucose pyrophosphorylase small subunit (*HvAGPS2*), b) ADP-glucose pyrophosphorylase large subunit (*HvAGPL1*), c) ß-amylase (*HvBAM2*) and d) isoamylase (*HvISA1*) were analyzed in flag leaves of LP104 and LP106 subjected to drought stress 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test ($p<0.05$; n=4-6). *Ubi E2-17* (ubiquitin-conjugating enzyme) was used as reference gene.

In addition, monitoring of the expression level of sucrose non-fermenting related protein kinase *HvSnRK30-25* (Seiler *et al.*, 2011), which has been described as key regulator of starch biosynthesis genes in Arabidopsis (Tiessen *et al.*, 2003; Halford and Paul, 2003) showed that this gene was expressed at a significantly higher level in flag leaves of LP104 plants in comparison with LP106 (Figure 23). However, higher expression levels in the line LP104 were observed under control and stress conditions regardless of K fertilization (Figure 23).

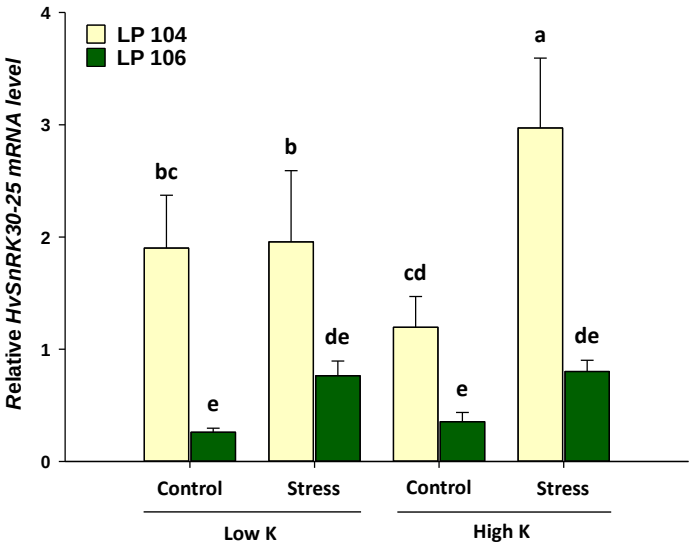


**Figure 23. Influence of different K regimes on the transcript level of sucrose non-fermenting related protein kinase *HvSnRK30-25* in flag leaves of barley under terminal drought stress.** Relative *HvSnRK30-25* mRNA levels were measured in flag leaves of LP104 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test ($p<0.05$; n=4-6). *Ubi E2-17* (ubiquitin-conjugating enzyme) was used as reference gene.

### 5.2.5 Enzyme activity of pyruvate kinase in flag leaves of barley under drought stress and different K regimes

A large number of enzymes are either completely dependent on or are stimulated by K (Marschner, 2012). In particular for pyruvate kinase (PK) which activates pyruvate and may determine the rate of incorporation of C into amino acid biosynthesis, a strong dependence on the K nutritional status has been reported (Smith *et al.*, 2000; Armengaud et al). Therefore, PK activity was measured in flag leaves of both lines. There was a weak trend of a reduced PK activity in drought-stressed flag leaves of both lines which was more pronounced under low K conditions (Figure 24) Whereas the PK activity was higher in LP104 than in LP106 when plants were under K deficiency, there was no consistent genotypical difference at high K supply (Figure 24).

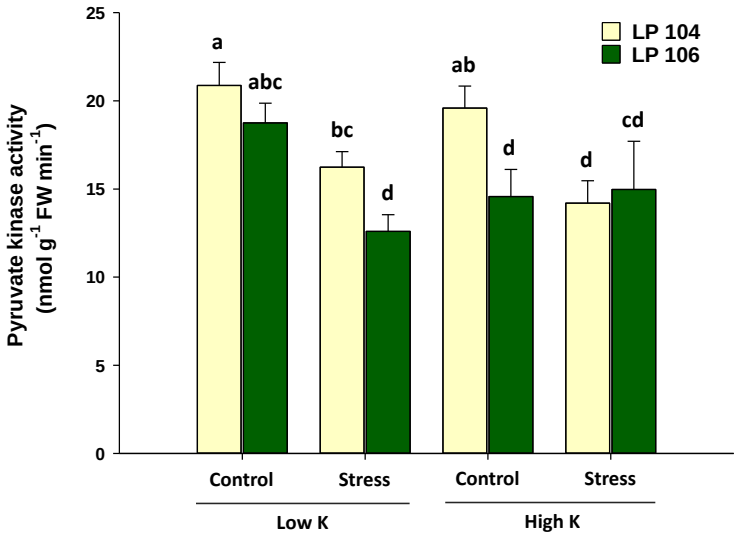


**Figure 24. Influence of different K regimes on pyruvate kinase activity in flag leaves of barley under terminal drought stress.** Pyruvate kinase activity was measured in flag leaves of LP104 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test ($p<0.05$; n=4-6).

## 5.2.6 Hormone concentrations in flag leaves under drought stress and different K regimes

### 5.2.6.1 Abscisic acid (ABA)

Terminal drought stress leads to an enhanced accumulation of the phytohormone ABA (Seiler *et al.*, 2011), and external K supply can effectively protect plants from drought injuries (Wei *et al.*, 2013). To test whether the K nutritional status influences drought tolerance via the regulation of phytohormone ABA and/or via its degradation products phaseic acid (PA) and dehydro-phaseic acid (DPA), all three compounds were measured in flag leaves of both lines. In both lines the ABA concentration was significantly increased under drought stress and this increase was even higher in K-supplied plants (Figure 25a). In particular under drought stress LP106 accumulated more ABA in flag eaves than LP104. In contrast, the degradation products PA and DPA significantly increased under drought irrespective of K supply only in the line LP104 (Figure 25b and c).

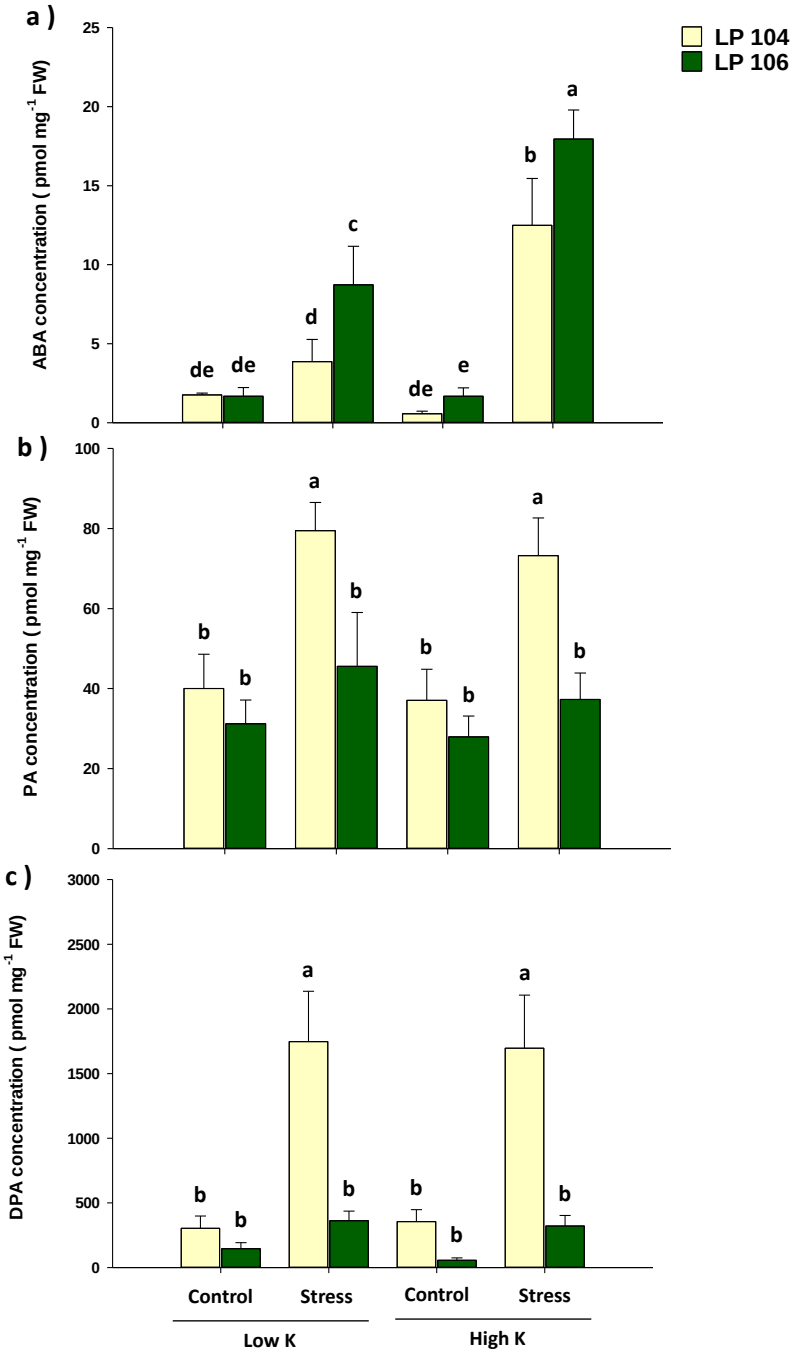


**Figure 25. Influence of different K regimes on ABA concentration in flag leaves of barley under drought stress.** Concentrations of a) abscisic acid (ABA), b) phaseic acid (PA), c) dehydro-phaseic acid (DPA) were measured in flag leaves of LP104 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Flag leaves from 12 weeks old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test ($p<0.05$; n=4-6).

### 5.2.6.2 Cytokinin (CK)

A number of studies postulated that CKs and ABA exert antagonistic activities during certain developmental and physiological processes, including the regulation

of plant growth, leaf senescence, and stomatal closure (Nam *et al.*, 2012). Therefore, cytokinin measurements were carried out in the investigated lines under low or high K supply to study the effect of K on a possible interaction between CKs and ABA. Most samples contained amounts of trans-zeatin and cis-zeatin-type CKs which were below the detection limit of the analytics used in the present study. Among the bioactive CKs, the levels of isopentenyladenine and its corresponding ribosides (Figure 26) tended to decrease in drought-stressed flag leaves of LP106 plants grown under drought stress at low K. Although the line LP104 showed slightly higher concentrations of iP and iPR in comparison with the line LP106, differences between the two lines were not significant, except at adequate K supply in control plant (Figure 26). These results indicated that neither drought nor K supplies changed the concentrations of bioactive cytokinins under the present growth conditions.

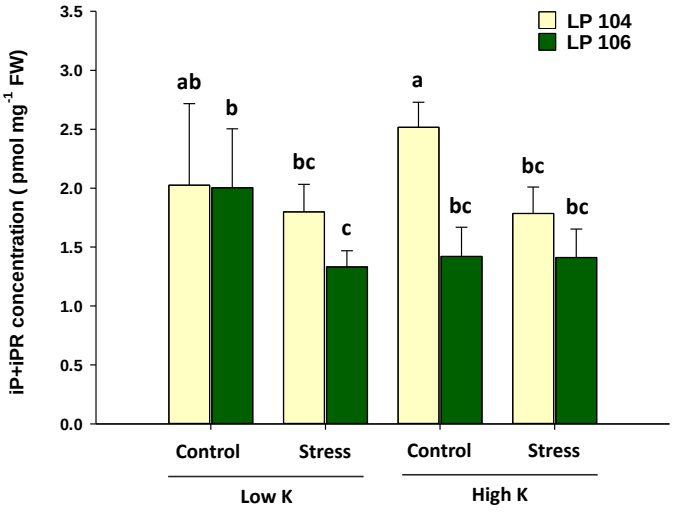


**Figure 26. Influence of different K regimes on cytokinin concentrations in flag leaves of barley under terminal drought stress.** Concentrations of isopentenyladenine and its ribosides (iP+iPR) were measured in flag leaves of LP104 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test ($p<0.05$; n=4-6).

### 5.2.6.3 Expression of genes involved in ABA biosynthesis, catabolism and signaling in flag leaves under drought and different K regimes

*HvNCED2* encodes a plastid-localized protein involved in ABA biosynthesis that has been proposed as a main regulator of ABA pathway (Cutler and Krochko,

1999). Transcript levels of *HvNCED2* significantly increased under drought stress regardless of the K supply to the substrate (Figure 27a). This increase was almost 2 times lower in LP104 in comparison with LP106. In contrast, *HvCCD3,* which encodes a protein from the ABA biosynthsis pathway down-stream of *HvNCED2*, showed no consistent transcriptional regulation either by K supply or by drought (Figure 27b).

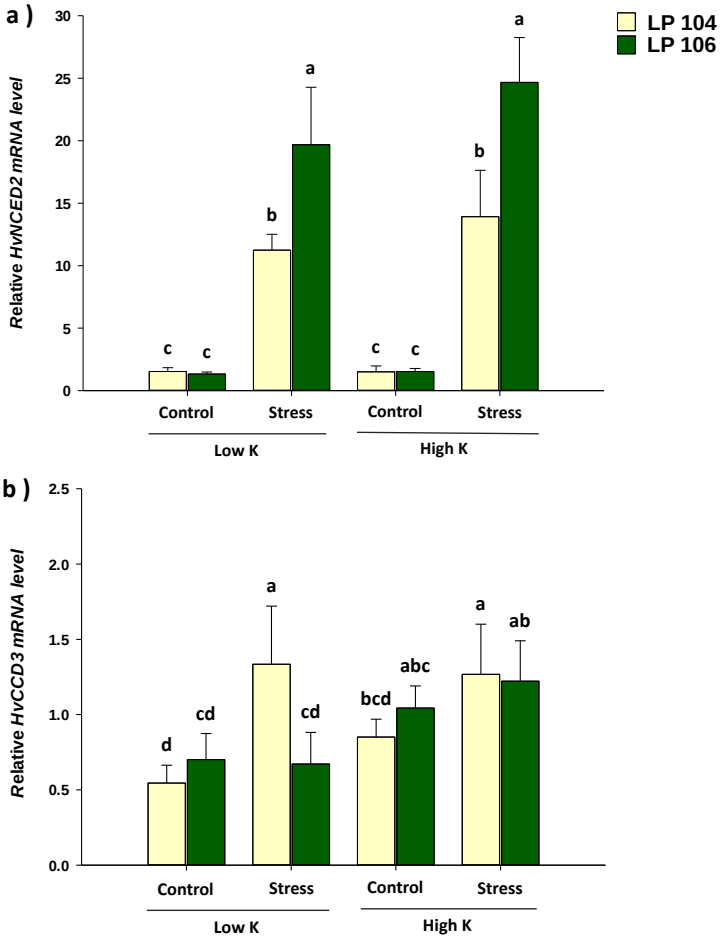


**Figure 27. Influence of different K regimes on the expression of genes involved in ABA biosynthesis in flag leaves of barley under terminal drought stress.** Relative mRNA levels of a) 9-cis-epoxycarotenoid dioxygenase (*HvNCED2*), and b) carotenoid cleavage dioxigenases (*HvCCD3*) were analyzed in flag leaves of LP104 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test ($p<0.05$; n=4-6). *Ubi E2-17* (ubiquitin-conjugating enzyme) was used as reference gene.

As components of the cytosolic pathway in ABA biosynthesis the expression levels of the two genes *HvAO4* and *HvSRD3* were determined (Xiong and Zhu, 2003). Besides the higher expression level of *HvAO4* in the line LP104 under drought and

K deficiency, there were no consistent changes due to the K or drought treatment (Figure 28a). Similarly, *HvSRD3* expression in both lines remained rather constant when K supply or water regimes were altered (Figure 28b). These results indicated that in particular the expression level of *HvNCED2* responded to drought, while there was no influence of the K treatment on the transcriptional regulation of the other tested genes of the ABA biosynthesis pathway.

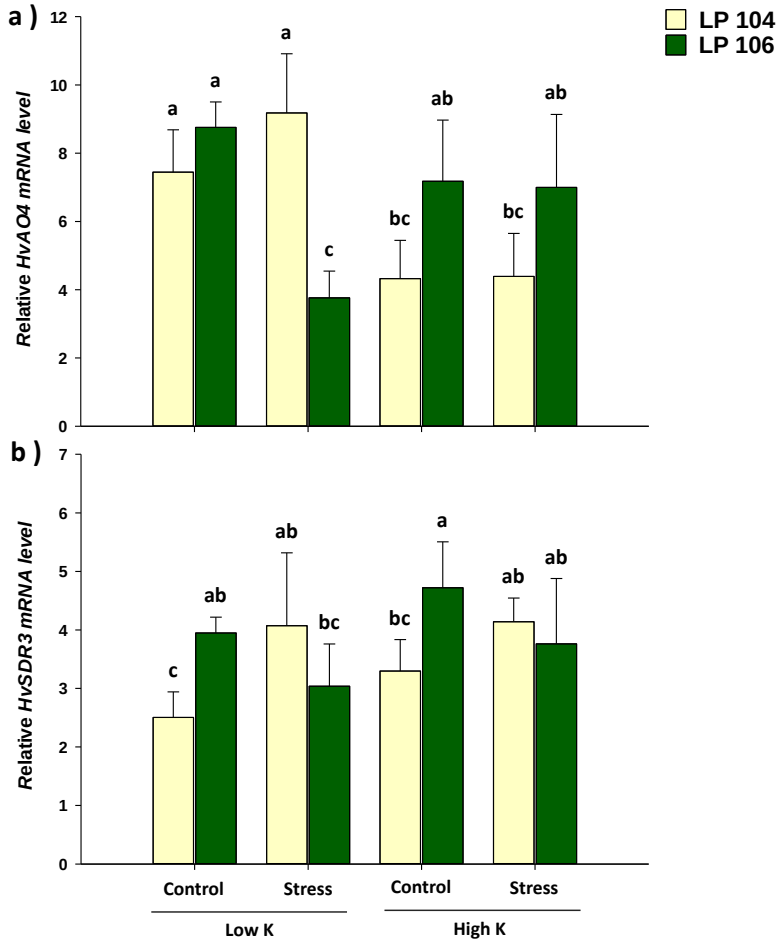


**Figure 28. Influence of different K regimes on the expression of genes involved in ABA biosynthesis in flag leaves of barley under terminal drought stress.** Relative mRNA levels of a) short-chain dehydrogenase/reductase (*HvSDR3*) and b) aldehyde oxidase (*HvAO4*) were analyzed in flag leaves of LP104 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test ($p<0.05$; n=4-6). *Ubi E2-17* (ubiquitin-conjugating enzyme) was used as reference gene.

Next, the transcript levels of *ABA-8'-hydroxylase* were assayed, since the corresponding gene product hydroxylates ABA to 8'-OH-ABA and has been characterized as a key enzyme in the ABA degradation pathway (Cutler and

Krochko, 1999). Transcript levels of *HvABA-8'-OH* did not differ between the two lines at high K supply but tended to be higher in LP104 under low K supply (Figure 29). In particular drought stress at low K supply induced a strong increase in mRNA levels but only in LP104. Interestingly, this increase went along with an increase in ABA degradation products in the line LP104 (Figure 25b and c).

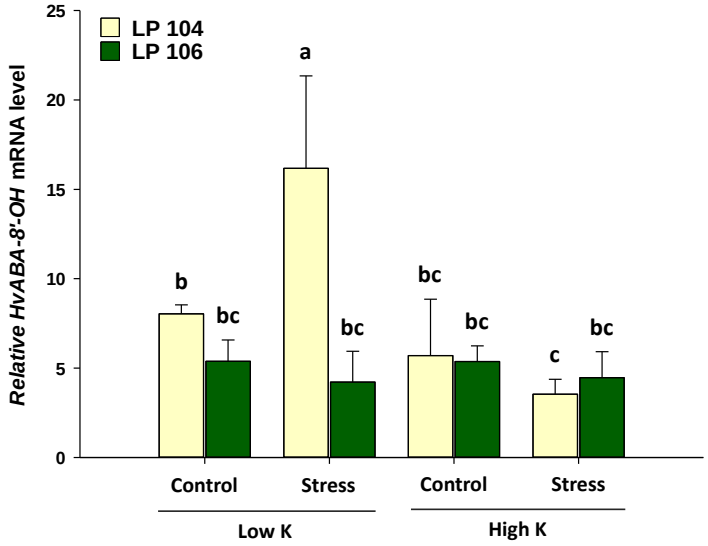


**Figure 29. Influence of different K regimes on the expression level of *ABA-8'-hydroxylase* in flag leaves of barley under terminal drought stress.** Relative mRNA levels of *ABA-8'-hydroxylase* were analyzed in flag leaves of LP104 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test ($p<0.05$; n=4-6). *Ubi E2-17* (ubiquitin-conjugating enzyme) was used as reference gene.

To further elucidate the influence of the K nutritional status on the regulatory network of ABA signaling during terminal drought stress, the expression level of the pyrabactin resistance 1/PYR1-like ABA receptors *HvRCAR35-25* and *HvRCAR35-27* were measured (Seiler *et al.*, 2011). In addition, the expression level of ABA-insensitive 1b and 1d (Seiler *et al.*, 2011) were measured, which may be seen as prototypes of type-2C serine-threonine phosphatases (PP2C) and play a major role in ABA signaling (Ma *et al.*, 2009). Q-PCR analysis showed that the mRNA levels of both *HvRCAR35-25* and *HvRCAR35-27* decreased under drought at low and high K supply (Figure 30a and b). In LP104, the expression level of *RCAR35-25* was slightly higher under drought than in LP106. This trend was also observed for *HvRCAR35-27* but only under K deficiency (Figure 30b).

The mRNA level of *HvABI1b* in LP104 was significantly higher under drought stress and concomitant K deficiency than in the line LP106, while the expression level of *HvABId* was significantly increased under drought in both lines (Figure 30c). Although *HvABId* mRNA levels responded to drought stress with a strong up-regulation, there were no significant differences between the two lines at either treatment (Figure 30d).

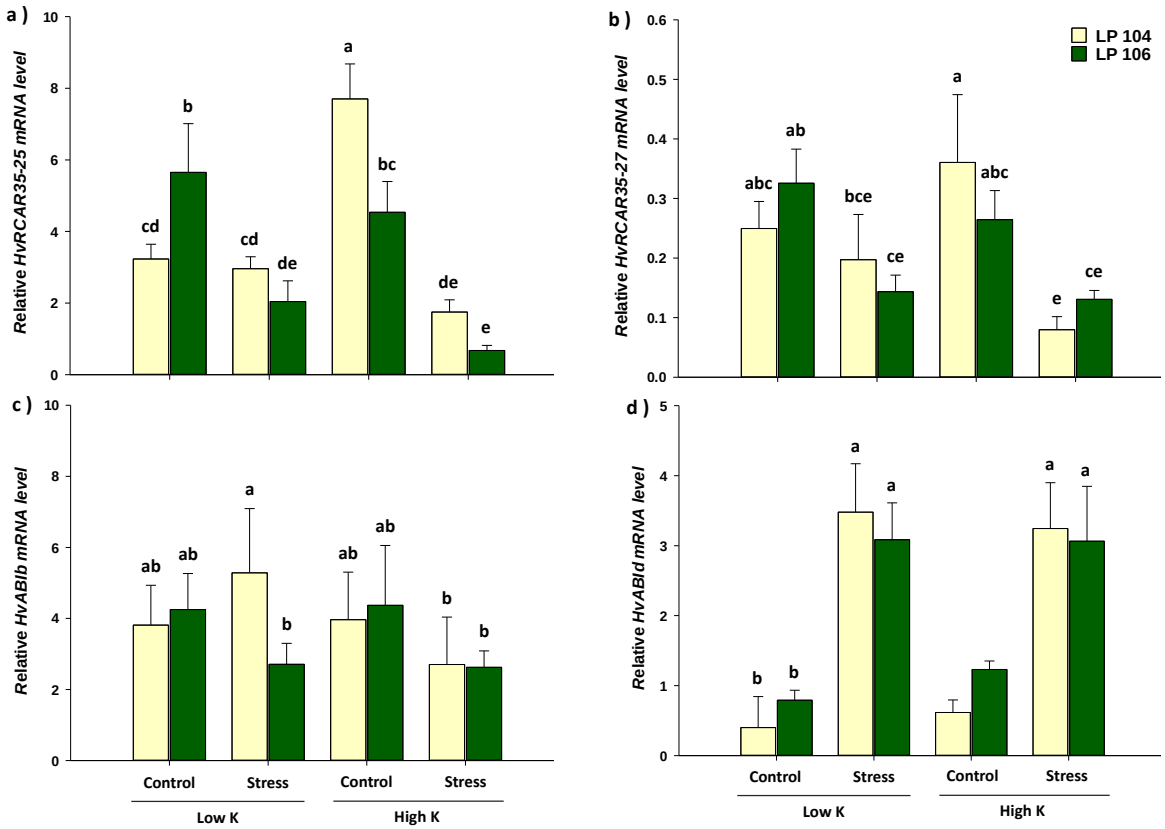


**Figure 30. Influence of different K regimes on the expression level of genes involved in ABA signaling in flag leaves of barley under terminal drought stress.** Relative mRNA levels of a) *ABA-insensitive1b* (*ABI1b*), b) *ABA-insensitive1b* (*ABI1d*), c) *ARCAR35-25* and d) *RCAR35-27* were analyzed in flag leaves of LP104 and LP106 subjected to drought stress 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test ($p<0.05$; n=4-6). *Ubi E2-17* (ubiquitin-conjugating enzyme) was used as reference gene.

### 5.2.6.4 Expression levels of a key gene involved in cytokinin biosynthesis

*Iso-pentenyl-transferases* (*IPTs*) are known to catalyze the final step in the biosynthesis of physiologically active cytokinins and were examined here for transcriptional responses to K supply and drought stress. In both investigated lines

there was no consistent change in the expression levels of *HvIPT* under drought or altered K supply. Only at high K supply and drought stress, the expression level of *HvIPT* was significantly up-regulated in the line LP106 and thus two fold higher than in the line LP104 (Figure 31).

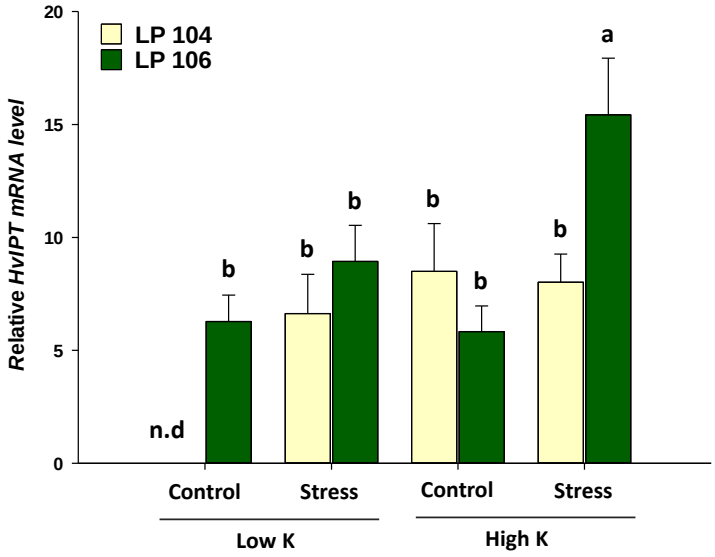


**Figure 31. Influence of different K regimes on the gene expression levels of *HvIPT* in flag leaves of barley under terminal drought stress.** Relative mRNA levels of *HvIPT* were analyzed in flag leaves of LP104 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test ($p<0.05$; n=4-6).

### 5.2.7 Photosynthesis rate and related parameters in flag leaves under drought stress and different K regimes

The assimilation rate, stomatal conductance and transpiration rate decreased under drought stress in flag leaves of both lines regardless of the K level in the substrate (Figure 32a, b and c). However, all these parameters tended to remain slightly higher under drought stress in the line LP104 compared to LP106 suggesting that in particular water loss in drought-stressed LP104 plants was slightly lower which may have promoted photosynthesis.

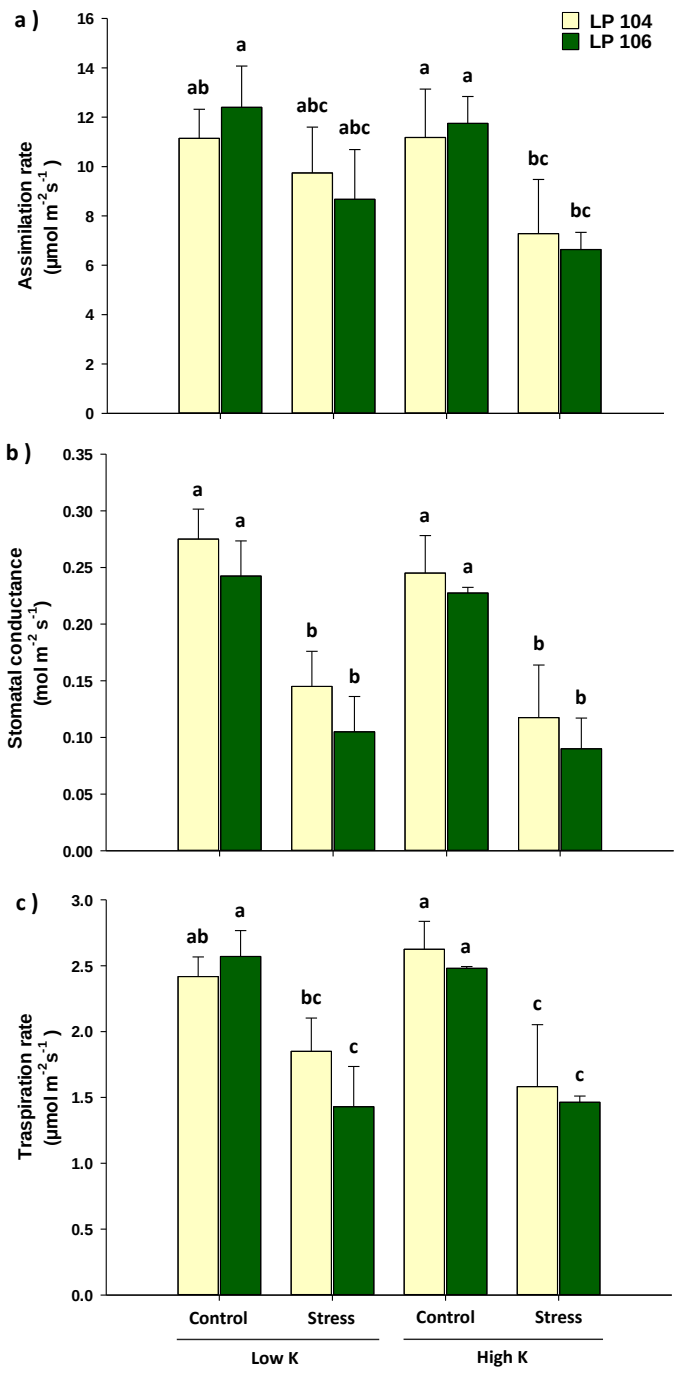


**Figure 32. Influence of different K regimes on photosynthesis in flag leaves of barley under terminal drought stress.** a) assimilation rate, b) stomatal conductance and c) transpiration rate were measured in flag leaves of LP104 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Flag leaves from 12 weeks-old plants were harvested 12 days after imposing drought stress. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test ($p<0.05$; n=8-12).

### 5.2.8 The effect of K supply on grain yield formation under drought stress

To determine whether K supply finally influenced grain yield in barley, another set of plants was grown until full maturity to harvest grains from all spikes. Relative to the control plants, grain yield significantly decreased under drought in LP106 at both low and high K supply. However, this decrease was observed for drought-stressed LP104 plants only at high K supply (Figure 33). Interestingly, LP104 plants had significantly higher yield compared to LP106 under drought either at low or high K supply. In general, the high K level in the soil enhanced grain yield in both lines which indicated the necessity of optimum K nutrition to achieve higher yield under drought stress.

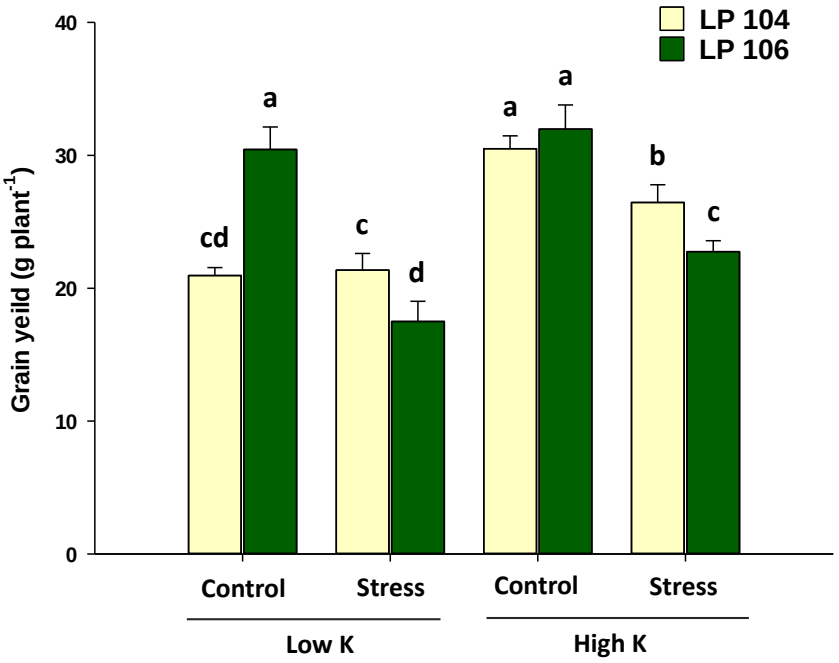


**Figure 33. Influence of different K regimes on grain yield of barley under drought stress.** Grain yield was analyzed in mature spikes of the lines LP104 and LP106 subjected to drought stress at 5 days after flowering. Plants were pre-cultured under sufficient water supply (control) or water limitation (stress) and under low or high K supply. Bars indicate means ± SD. Different letters denote significant differences according to 3-way ANOVA and Tukey's test ($p<0.05$; n=3-4).

# 6 Discussion

## 6.1 Monitoring the role of K in drought stress-induced leaf senescence

In contrast to pre-anthesis drought which triggers senescence processes in individual plant organs, post-anthesis drought triggers senescence at the level of whole plants with flag leaves being those vegetative organs that generally are senescing last (Simova-Stoilova *et al.*; Sreenivasulu and Schnurbusch, 2012). Developing wheat grains may obtain 50-95% of mobile mineral nutrients from senescing leaves, while the remainder derives from root uptake (Kichey *et al.*, 2006). Therefore, determining the potential contribution of assimilate and nutrient remobilization during leaf senescence is of great importance to understand processes limiting plant productivity under terminal drought stress (Gan, 2003; Distelfeld *et al.*, 2014). Since readily retranslocated nutrients such as N or K are part of the overall re-translocation process initiated during plant senescence, the question arises to what extent the nutritional status of source leaves can influence the progression of leaf senescence and the concomitant retranslocation of assimilates. Since K represents a readily retranslocated macronutrient (Marschner, 2012) and since K additionally takes over a protective role against drought stress (Cakmak, 2005), it was one objective of this thesis to work out the interaction between carbon assimilation in drought-stressed flag leaves and the K nutritional status in barley.

To investigate the role of K in terminal drought stress, 3 near-isogenic barley lines, which differed in the progression of leaf senescence under terminal drought stress were subjected to drought under three different levels of K supply. As expected, K supply modulated the relative water content under drought stress: While drought-stressed flag leaves from plants grown under high or medium K supply showed a similar water content than the corresponding control plants, there was a significant decrease in the relative water content of LP110 and LP106 plants under low K supply (Figure 5). This observation indicated that LP106 responded more sensitively to drought than LP110 and especially LP104, which actually maintained the same water content as under continuous water supply. Next, chlorophyll concentrations were determined as a physiological marker and *HvS40* transcript levels as a molecular marker for drought stress-induced leaf senescence

(Krupinska *et al.*, 2002; Shi *et al.*, 2012). In the drought treatment chlorophyll degradation set in earlier and progressed faster than during age-dependent plant senescence that occurred under adequate water supply (Figure 6). Considering the average response in the three lines, however, drought stress-induced chlorophyll concentration occurred at all three levels of K supply, although K concentrations in flag leaves were more than two-fold lower under low K supply and thus below the critical deficiency level of approx. 1% (Figure 8a; Marschner, 2012). By contrast, *HvS40* transcript levels increased under drought stress only when plants were subjected to K deficiency except in the line LP110 which also showed elevated *HvS40* mRNA levels at medium K supply (Figure 7). A similar observation was made in a follow-up experiment, in which drought stress was reflected by decreasing chlorophyll levels only in one of the two assessed barley lines irrespective of the level of K supply (Figure 18). Although low K supply resulted in a significant decrease in K concentrations of the flag leaves (Figure 20), K concentrations were still between 8 and 15 mg $g^{-1}$ DW, which may not yet be sufficiently low to induce a K-deficient status. However, *HvS40* transcript levels revealed more distinct changes in the senescence status by reporting a stronger induction under drought stress when plants were K deficient (Figure 19, 20). Thus, *HvS40* transcript levels appeared to represent a more sensitive marker for drought-induced leaf senescence than the chlorophyll concentration of the flag leaf, especially when additional abiotic stress factors interfere with drought-induced senescence, such as K nutrition in the present case.

## 6.2 Genotypic variability in drought-induced leaf senescence under varied K supply

There is a large difference among plant species and among genotypes within a plant species in their response to K deficiency (Römheld and Kirkby, 2007; Rengel and Damon, 2008; Zeng *et al.*, 2014*a*). In particular the diversity within a plant species promises that it may be successful to breed for K-efficient genotypes. An interesting question is then whether such K-efficient genotypes will also be more tolerant to drought and drought stress-induced senescence. Vice versa, the hypothesis can be raised whether genotypes with higher tolerance to drought-induced senescence are characterized by higher leaf concentrations of K. The present study took an approach to evaluate this hypothesis by comparing near-

isogenic barley genotypes which derived from a cross between a stay-green line and a normally senescing line. Among the progenies of this cross, LP106 showed a stay-green phenotype while LP110 was senescing normally and LP104 most rapidly (Figure 2). When these three lines were subjected to drought stress at varied K supply, the line LP104 performed better than LP110 and LP106 under drought and low K. This was not only documented by the higher relative water content of LP104 but also by its higher chlorophyll concentrations and lower *HvS40* transcript levels in the flag leaves, which clearly indicated a delay in the onset of drought-induced leaf senescence. This observation was unexpected with regard to the initial characterization of these lines, in which LP106 was found to behave as a stay-green and LP104 as an early senescing line (Figure 2; Dr. Nese Sreenivasulu, pers. comm.). The reason for this opposite behaviour is not yet clear. However, in the present growth experiment, the nutrient demand of the barley plants was calculated for the whole growth period, and considering the nutrient content of the growth substrate a sufficient amount of all nutrients was provided right from the start of germination. In the case of K, either a sufficient amount (high K treatment), a slightly limiting amount (medium K) or an insufficient amount was provided (low K) that was adjusted in a way to induce K deficiency when plants came to flowering. While there was no difference in the onset of K deficiency or in K concentrations observed between the high and medium K treatment (Figure 8a), plants grown at low K indeed developed K deficiency symptoms at the flowering stage. This way of plant culture thus circumvented a repeated supply of nutrients during the course of plant development. It may thus be, that the repeated supply of nutrients during the initial characterization of the lines (Figure 2) had an impact on senescence development. In particular the supplementation of N is known to promote the re-greening of plants, even though plant senescence processes may have already started (Balazadeh *et al.*, 2014). A differential genotypic responsiveness to repeated nutrient supplies, as conducted in the previous experiments (Dr. Nese Sreenivasulu, pers. comm.) may thus have altered the senescence phenotypes.

Nevertheless, in the present experiments the lines LP104 and LP106 showed a consistent response to drought and K treatments. Compared to the other two lines, LP104 performed better under concomitant K and drought stress and turned later into senescence (Figures 7 and 19). This delayed senescence coincided not only

with a higher relative water content (Figure 5) but also with higher K concentrations in the flag leaves (Figures 8a and 20). It can thus be concluded that in the flag leaves K took over a protective role against drought stress and that the higher K retention in LP104 is a determinant of drought stress tolerance.

## 6.3 The contribution of an altered carbohydrate metabolism at varied K nutrition to genotypic differences in drought-induced senescence

An adequate K nutritional status is known to prevent the formation of reactive oxygen species (ROS), as ROS are known to be generated also under drought (Kar, 2011; Zeng *et al.*, 2014*b*). This may be partially related to a decrease of phloem loading with assimilates in K-deficient plants, because sugars accumulating in source leaves can provoke photo-inhibition or even photo-oxidation (Marschner, 2012). On the other hand, K exhibits multiple interactions with other steps in primary carbon metabolism (Armengaud et al., 2009) as well as an interference with ABA signaling (Ren *et al.*, 2013). Therefore, it was further investigated whether genotypical differences in K retention in the flag leaves and the associated higher tolerance to drought stress-induced senescence may also be reflected by an altered carbohydrate metabolism and ABA homeostasis.

Primary metabolites are highly responsive to drought stress and known to play critical roles in a plant's metabolic adaptation to drought stress, because these primary metabolites may serve as energy reserves, osmolytes to maintain cell turgor, antioxidants, byproducts of stress, or as signal transduction molecules (Bartels and Sunkar, 2005; Seki *et al.*, 2007; Krasensky and Jonak, 2012; Bhargava and Sawant, 2013; Seiler *et al.*, 2014*a*). In previous studies a tight link has been shown between drought tolerance in cereals and carbohydrate metabolism (Bowne *et al.*, 2012; Broyart *et al*). In these studies, the accumulation of soluble sugars turned out as a major determinant in drought stress responses. In particular the drought stress-induced increase in those primary metabolites that act as compatible solutes, such as mannitol or proline, have been found to be associated with drought tolerance (Barnabás *et al.*, 2008; Naser *et al.*, 2010). Moreover, there are also indications on an impact of K nutrition on carbohydrate metabolism in leaves (Marschner, 2012). In Arabidopsis roots, K deficiency has been shown to deplete pyruvate and to increase soluble sugars, which was mainly

attributed to an inhibition of pyruvate kinase activity. In consequence, K was proposed to maintain carbon flux into amino acids and proteins and to increase the nitrogen-carbon ratio in amino acids (Armengaud *et al.*, 2009). In order to verify whether K-dependent metabolic changes may account for the genotypical differences found in drought stress-induced senescence, more than 40 primary metabolites were measured (Appndix Table 3 and 4). Those metabolites which were significantly different between LP104, LP110 and LP106 under drought stress at low K supply are shown in Figure 34. Most interestingly, in the line LP104, which retained most K in the flag leaves under low K supply, several metabolites accumulated to higher levels than in the other two lines, which were hexose-P, ADP-glucose, mannitol, oxoglutarate, fumarate and trehalose-6-P. In addition, the well-known compatible solute proline also accumulated to higher levels in LP104 (Figure 34). Some of these metabolites, such as malate and 2-oxoglutarate, have also been reported to be depleted by low K supply in Arabidopsis plants (Armengaud *et al.*, 2009). Other than reported in Armengaud et al. (2009), no particular changes in pyruvate kinase (PK) activity were measured (Figure 24). Thus, the reported K sensitivity of pyruvate kinase was obviously not expressed in the present experiment and not responsible for the genotypic differences in K-dependent leaf senescence.

Moreover, concentrations of ATP and NADPH were examined, because these metabolites may be seen as indicators for the energy status of the leaf metabolism. Interestingly, both metabolite levels were significantly higher in LP104 than in LP106, while in LP110 they tended to be intermediate (Figure 34). This suggested that the higher K retention in LP104 may have increased the photosynthesis rate to provide more ATP and NADPH under drought stress. Higher ATP and NADPH levels can provide redox equivalents and energy for starch biosynthesis which requires hexose-P and ADP-glucose. Moreover, the higher availability of energy and reduced carbon metabolites in the chloroplast may also have fueled glycolysis and the TCA cycle to synthesize organic acids which are necessary for assimilation of amino acids like proline under drought conditions. Therefore, the higher tolerance to drought-induced leaf senescence may have been associated with the superior K efficiency in the line LP104.

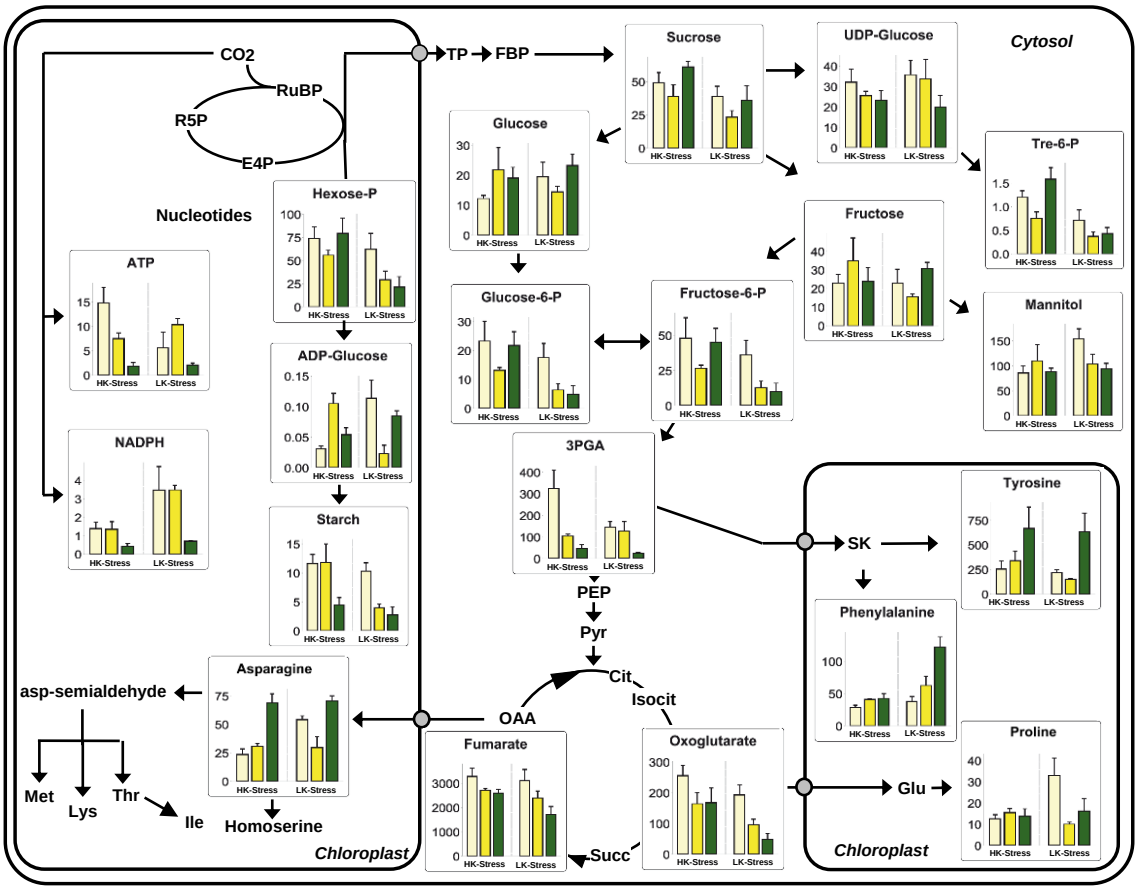


**Figure 25. Schematic representation of the influence of K nutrition on changes in metabolite concentrations of barley leaves under terminal drought stress**. Metabolite levels at high (left set of bars) and low K supply (right set of bars) are compared between flag leaves of LP104, LP110 and LP106 plants subjected to drought stress. Abbreviations are as follows: OAA: oxaloacetate, R5P: ribulose-5-phosphate, TP, triose-phosphate, RuBP:ribulose-1,5-bisphosphate, E4P: erythrose-4-phosphate, ATP: adenosine triphosphate, NADPH: nicotinamide adenine dinucleotide phosphate, 3PGA: 3-phosphoglycerate, Pyr: Pyruvate, PEP: phosphoenolpyruvate, Succ: succinate, Glu: glutamate, Asn: asparagine, Cit: citrate, Isocit: isocitrate, Glu: glutamate, SK: shikimate pathway, Thr: threonine, Lys: lysine, Met: methionine. Concentrations are expressed in (nmol $g^{-1}$ FW).

A highly remarkable observation in this metabolite analysis was the elevated concentration of starch in LP104, which was not decreased under K deficiency in drought-stressed leaves (Figure 19 and 34). This was in sharp contrast to the other two lines which showed severely depleted starch reserves under K deficiency and drought stress. Starch is a primary product of photosynthesis in leaves. In most plants, a large fraction of the carbon assimilated during the day is stored transiently in the chloroplast in the form of starch for the use of metabolic and growth processes during the subsequent night. Photosynthetic partitioning into starch is finely regulated, and the amount of carbohydrate stored is dependent on environmental conditions, particularly day length. These diurnal changes are

regulated at several levels to control the flux of carbon from the Calvin cycle into starch biosynthesis (Neill and Gould, 2003). As samples for RNA extraction could not be taken in the first experiment, transcript levels of key enzymes involved in starch metabolism were monitored in the second experiment by comparing drought-stressed LP104 with LP106 plants. Transcript levels of the two genes ADP-glucose pyrophosphorylase small subunit (*HvAGPS2*) and ADP-glucose pyrophosphorylase large subunit (*AvAGPL1*), both involved in starch biosynthesis, showed little differences between the two lines (Figure 22a and b). In addition, the sucrose non-fermenting-related protein kinase gene *HvSnRK30-25* has been monitored, as its paralog in Arabidopsis has been described as a key regulator for starch biosynthesis (Tiessen *et al.*, 2003; Halford and Paul, 2003). In fact transcript levels of *HvSnRK30-25* were higher in LP104 than in LP106 but there was no consistent influence of the drought or K treatment (Figure 22). Thus, genotypic differences between the two lines appeared not to be primarily related to starch biosynthesis. However, beta-amylase (*AvBAM2*) and isomerase (*HvISA1*), which are key components of the starch degradation pathway (Radchuk *et al.*, 2009), showed a strong up-regulation of mRNA levels under drought and K deficiency only in LP106 (Figure 22c and d). In line with this finding, Seiler et al. (2011) reported a decrease in starch content and an increase in starch degradation genes in developing barley seeds under terminal drought stress. It is worth noting that transcriptome profiling of two Tibetan wild barley genotypes subjected to low K supply also identified a marked alteration of 6 genes involved in starch and sucrose metabolism (Zeng *et al.*, 2014*a*). In line with these observations, it is suggested that a superior K level in the flag leaves of LP104 suppressed starch degradation genes under concomitant drought and K deficiency and thus resulted in a higher starch level which acted as a carbohydrate reserve and promoted a better performance of LP104 under drought conditions.

Several studies have proposed a sugar-signaling function of the metabolite trehalose-6-P in regulating the accumulation and turnover of transitory starch in Arabidopsis leaves. In these studies, trehalose-6-P regulated starch synthesis either via posttranslational redox activation of ADP-glucose pyrophosphorylase or by feedback inhibition of starch degradetion (Kolbe *et al.*, 2005; Martins *et al.*, 2013). The information provided by these reports indicate that trehalose-6-P does have some influence over the rate of starch synthesis and photoassimilate

partitioning. In addition, a strong accumulation of trehalose-6-P was found in senescing leaves of Arabidopsis, in parallel with a rise in sugar content (Oa *et al.*, 2012) indicating that trehalose-6-P is required for the initiation of senescence in response to high carbon availability. In a study in rice, overexpression of trehalose biosynthetic genes improved drought stress responses (Garg *et al.*, 2002). Considering the higher level of trehalose-6-P and starch in the line LP104 under concomitant drought and K deprivation (Figure 34) which coincided with a weaker turnover of starch in this line (Figure 22c and d), allows concluding that trehalose-6-P plays a crucial role under drought conditions by regulating sugar metabolism and in particular starch synthesis.

It has also been shown that the trehalose pathway and glucose phosphate act as a regulator of the potassium transporter TRK in *Saccharomyces cerevisiae* (Mulet *et al.*, 2004). Assuming a similar function in plants, a higher level of hexoses-P under drought and K deficiency, which is associated here with higher trehalose-6-P levels in the line LP104, might explain to some extent how a cross-talk between the K nutritional status and trehalose/hexose pathways help to maintain a favorable carbon pool under drought stress. However, this mechanisms remains to be further explored in plants.

## 6.4 Genotypic differences in the phytohormonal regulation in response to varied K nutrition and drought stress

Terminal drought is characterized by an excess production of the phytohormone abscisic acid (ABA) which plays an important role not only for developmental senescence but also for stress-induced senescence (Distelfeld *et al.*, 2014). Consistent with the observation that ABA concentrations in barley leaves increase under drought stress (Seiler *et al.*, 2014), ABA concentrations increased under drought in both lines (Figure 24a). Unexpectedly, this increase under drought was even higher in K-sufficient than in K-deficient plants. Regardless of K supply, ABA levels in drought-stressed flag leaves were lower in LP104 than in LP106, which coincided with the delayed induction of the senescence marker gene *HvS40* in LP104 (Figure 18). In contrast to the observation that ABA levels increased earlier and were higher in an early senescing barley line relative to a line with stay-green phenotype (Seiler et al., 2014), there was no correlation between *HvS40* and ABA levels over drought and K treatments in the present experiment (Figures 18 and

24a). This may in part be a consequence of the shortcoming in the present approach that hormone levels were only determined at one time point after the stress treatment, so that time-dependent changes in hormonal concentrations may have been missed. To verify whether ABA levels were primarily a result of enhanced ABA biosynthesis, quantitative measurements of the transcript levels of two genes involved in ABA biosynthesis were conducted (Figure 26). While transcript levels of *HvCCD3* did not show consistently different responses of the two lines to K supply and only a weak increase in response to drought (Figure 26b), mRNA levels of *9-cis-Epoxycarotenoid Dioxygenase2* (*HvNCED2*), which regulates ABA biosynthesis further upstream correlated very closely with ABA concentrations in both lines (Figure 26a). It was therefore concluded that the genotypic differences in ABA levels in response to drought and K supply were primarily due to the transcriptional regulation of *HvNCED2*.

ABA homeostasis results from a balance between de-novo synthesis and degradation (Cutler and Krochko, 1999). There are several pathways by which ABA can be removed from plant cells. In the simplest case, ABA is exported by passive or carrier-mediated efflux out of the cells. In the majority of plant tissues, catabolic inactivation of ABA proceeds via 8'-hydroxy-ABA, which is spontaneously converted to phaseic acid (PA). Then, PA is further reduced to form dihydrophaseic acid (DPA) (Cutler and Krochko, 1999). In this study, the concentrations of both, PA and DPA, strongly increased in drought-stressed LP104 plants regardless of the amount of K added to the substrate (Figure 24b and c). This suggested that the lower ABA levels in drought-stressed LP104 relative to LP106 plants may have been due to an accelerated degradation of ABA in LP104. Therefore, mRNA levels of *HvABA8'-OH* were determined, which takes in a central role in ABA catabolism (Seiler *et al.*, 2011; Cai *et al.*, 2015). Notably, the mRNA level of *HvABA8'-OH* in the line LP104 was up-regulated under water scarcity only when K supply was low (Figure 28). Presuming that these higher transcript levels also resulted in a higher activity of *HvABA8'-OH*, the activation of this catabolic enzyme may have contributed to the observed decrease of ABA in K-deficient versus K-sufficient LP104 plants under drought stress (Figure 24a). A similar observation has been made by Seiler et al. (2011) in flag leaves of senescing barley, where PA and DPA accumulated to several-fold higher levels than ABA which also coincided with elevated mRNA levels of *HvABA8'-OH* under

terminal drought stress. Such a regulatory link may be triggered by regulatory proteins like the nuclear protein X1 (*NPX1*), which is induced by K deficiency in Arabidopsis and modulates genes involved in ABA homeostasis (Kim *et al.*, 2009). However, the paralog to *AtNPX1* in barley has not yet been identified. Taken together, the higher turnover of ABA in K-deficient and drought-stressed LP104 may have profited from the high retention of K in the flag leaves.

Drought stress responses are not regulated at the hormonal level by ABA alone. In fact, antagonistic activities between ABA and cytokinins (CKs) have been reported under drought conditions (Yang et al., 2003; Distelfeld and Fischer, 2014). On the other hand, there are also studies showing that a decrease in CK contents do not necessarily lead to an increase in the ABA content, for example in CK-deficient *Arabidopsis* plants (Vankova *et al.*, 2011). Drought stress typically causes an inhibition of the synthesis and transport of cytokinins (Qin *et al.*, 2011). Furthermore, a reduction of the CK content under K-deficient conditions was observed in Arabidopsis shoots and roots (Nam *et al.*, 2012). To verify such a possibly antagonistic relationship between ABA and CKs, cis-/trans-zeatin, isopentenyladenine and their corresponding ribosides were measured. However, zeatins were too low to be detected. Although iP and iPR concentrations tended to decrease under drought stress in both lines, LP104 had slightly higher levels of bioactive isopentenyladenine and their corresponding ribosides in comparison to the line LP106 (Figure 25). This is in agreement with previous studies on field-grown wheat under drought, where a similar antagonistic relation has been found (Yang *et al.*, 2003*b*). This supported the view of an antagonistic relation between these two hormone classes, i.e. that an increase in ABA concentrations was associated with a decrease in CK concentrations under drought. Taken together, the present study supports the notion of a negative interaction between ABA and bioactive CKs under terminal drought conditions but not in dependence of the K nutritional status in barley.

## 6.5 Interaction between carbohydrate metabolism and ABA homeostasis in response to K supply and drought stress

In a genetic approach to identify sugar-response mutants, an interaction has been found between ABA and carbohydrate metabolism, in a way that ABA increases the ability of plant tissues to respond to sugar signals (Rook *et al.*, 2001). This has

been explained by the binding of ABA-dependent transcriptional regulators, such as ABI4, to promoter elements of genes involved in starch biosynthesis (Rook *et al.*, 2006*b*). Furthermore, members of the SnRK gene family are known to regulate key genes in starch biosynthesis such as sucrose synthase and ADP-glucose pyrophosphorylase (Tiessen *et al.*, 2003; Halford and Paul, 2003), while they are at the same time also responsive to drought stress (Seiler et al., 2014).

In this study, the expression of *SnRK30-25* was strongly up-regulated in LP104 under control conditions and drought stress irrespective of the K supply (Figure 23). The mRNA levels of two RCAR receptors (*RCAR35-25* and *RCAR 35-27*) tended to be down-regulated under drought stress in both lines (Figure 29a and b). Under K deficiency this reduction was less pronounced in LP104 plants. Despite the difference in ABA levels in the flag leaves of these two lines, two members of the protein phosphatase 2C family, which are supposedly involved in ABA signaling, were also induced under drought. One of the two genes, *HvABI1d,* showed a strongly enhanced expression level in both lines under drought, whereas the expression level of *HvABI1b* increased under drought only in low K-supplied LP104 plants (Figure 29c and d). Seiler et al. (2011) made a similar observation in seeds as well as flag leaves of barley under terminal drought stress, which was a contrasting regulation of transcript levels of ABA receptor/signalling genes in stay-green and senescing barley lines (Seiler *et al.*, 2014*b*). In addition, a similar situation has been reported in Arabidopsis, where the expression of *PP2C* genes was elevated, while the majority of *PYR/PYL* (*RCARs*) members was down-regulated upon exogenous ABA treatment (Santiago *et al.*, 2009; Szostkiewicz *et al.*, 2010). These authors also reported that ABA-related stress conditions or treatments alter the ratio of PP2C to PYR/PYL (RCARs) at both, transcript and protein levels. Recently, it was postulated that an increase in this ratio between PP2C and PYR/PYL might be necessary for activation of the downstream ABA signaling cascade under stress conditions (Chan, 2012). Although it can be postulated that the same ABA signalling components as reported above were also active in this experiment, i.e. by responding to drought stress, none of them responded to drought and K supply in a way that could explain the superior performance of LP104 under these conditions.

Studies on the role of ABA in K uptake and starch formation have shown that K uptake decreased while starch increased when the ABA concentration increased

(Mansfield and Jones, 1971; Reed and Bonner, 1974). The present study confirms a relation between K, starch and ABA in a way that a higher retention of K in the flag leaves, as found in LP104, results in a reduction of ABA levels and inhibits starch degradation genes which increases starch reserves, thus, improving drought stress tolerance.

## 6.6 Prospective

The various roles taken in by K in plant growth and metabolism, such as in stress signaling and adaptation to drought stress, indicate a beneficial role of K in stress mitigation at the physiological and molecular level. This beneficial action of K may not yet be fully exploited in crop production. Plant breeding of cereal crops is focusing more and more on an improved uptake and utilization efficiency of fertilized nutrients. So far, K efficiency has rarely been formulated as a breeding goal. However, considering the beneficial association between K and carbohydrate metabolism or phytohormone homeostasis as reported here, it is suggested that K-efficient genotypes may perform better under drought stress. In line with this suggestion, a first step may be to consider the measurement of flag leaf K levels as a possible nutritional marker for the selection of drought-tolerant barley cultivars.

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# 8 Abbreviations

| Full Name | Abbreviation |
|---|---|
| 2-oxoglutarate | 2-oxo |
| 3-phosphoglycerate | 3PGA |
| Alanine | Ala |
| Arginine | Arg |
| Asparagin | Asn |
| Aspartate | Asp |
| Cis-aconitate | Cacnt |
| Citrate | Cit |
| Day after flowering | DAF |
| Fructose-1,6-bisphosphatase | FBPase |
| Fructose | Fru |
| Fructose-1,6-bis phosphate | Fru1,6BP |
| Fructose-6-phosphate | Fru6P |
| Fucose | Fuc |
| Fumarate | Fum |
| Gamma aminobutyric acid | GABA |
| Glucose | Glc |
| Glucose 1,6-bisphosphate | Glc1,6BP |
| Glucose-1-phosphate | Glc1P |
| Glucose-6-phosphate | Glc6P |
| Glucuronic acid | Glucua |
| Glutamate | Glu |
| Glutamine | Gln |
| Glycerate | Glyct |
| Glycine | Gly |
| Hexadecanoic acid | Hxda |
| Hexokinase | HK |
| Histidine | His |
| Indole acetic acid | IAA |
| Inositiol | Inost |

| | |
|---|---|
| Isocitrate | Isocit |
| Isoleucine | Ile |
| Leucine | Leu |
| Lysine | Lys |
| Malate | Mal |
| Methionine | Met |
| Oxaloacetate | OAA |
| Phenylalanine | Phe |
| Pyruvate kinase | PK |
| Ribose-5-phosphate | Rib5P |
| Ribulose-1,5-bisphosphate | RuBP |
| Ribulose-5-phosphate | R5P |
| Serine | Ser |
| Sorbitol | Sbt |
| Sorbose-6-phosphate | Sor6P |
| Starch synthase | SS |
| Succinate | Succ |
| Sucrose | Suc |
| Sucrose-6-phosphate | Suc6P |
| Thousands grain weight | TKW |
| Threonine | Thr |
| Trans-aconitate | Tacnt |
| Trehalose-6-phosphate | Tre6P |
| Tricarboxylic acid | TCA |
| Tyrosine | Tyr |
| UDP-glucose | UDPGlc |
| Valine | Val |

# 9 Appendix

**Table 1. Multiple reaction monitoring (MRM) of potentials of MS/MS transitions used in negative mode.** Nitrogen was used as carrier gas, nebulizer gas, heater gas and collision gas. Ion spray voltage and the capillary temperature were -3500 V and 350°C, respectively. Pre ion=precursor ion, Pro ion=product ion, Dwell=dwell time, Frag=fragmentor, CE= collision energy, CAV= Cell accelerator voltage.

| Compound Name | Prec Ion | Pro Ion | Dwell | Frag | CE | CAV | Polarity |
|---|---|---|---|---|---|---|---|
| **Acetyl-CoA** | 808.1 | 460.9 | 20 | 380 | 33 | 5 | Negative |
| **Acetyl-CoA** | 808.1 | 403.9 | 20 | 380 | 20 | 5 | Negative |
| **NADPH** | 744 | 159 | 20 | 380 | 53 | 5 | Negative |
| **NADPH** | 744 | 79 | 20 | 380 | 80 | 5 | Negative |
| **NADH** | 664 | 408 | 20 | 380 | 33 | 5 | Negative |
| **NADH** | 664 | 346 | 20 | 380 | 29 | 5 | Negative |
| **ADPGlc** | 588 | 346 | 20 | 380 | 29 | 5 | Negative |
| **ADPGlc** | 588 | 241 | 20 | 380 | 29 | 5 | Negative |
| **ADPGlc** | 565 | 322.9 | 20 | 380 | 25 | 5 | Negative |
| **ADPGlc** | 565 | 158.9 | 20 | 380 | 53 | 5 | Negative |
| **ATP** | 505.9 | 408 | 20 | 380 | 21 | 5 | Negative |
| **ATP** | 505.9 | 159 | 20 | 380 | 33 | 5 | Negative |
| **UTP** | 483 | 402.9 | 20 | 380 | 21 | 5 | Negative |
| **UTP** | 483 | 384.2 | 20 | 380 | 17 | 5 | Negative |
| **ADP** | 426 | 328 | 20 | 380 | 17 | 5 | Negative |
| **ADP** | 426 | 159 | 20 | 380 | 21 | 5 | Negative |
| **Tre6P** | 421 | 240.9 | 20 | 380 | 25 | 5 | Negative |
| **Tre6P** | 421 | 139 | 20 | 380 | 29 | 5 | Negative |
| **Suc6P** | 421 | 96.8 | 20 | 380 | 33 | 5 | Negative |
| **Suc6P** | 421 | 79.1 | 20 | 380 | 53 | 5 | Negative |
| **UDP** | 403 | 272.8 | 20 | 380 | 21 | 5 | Negative |
| **UDP** | 403 | 110.9 | 20 | 380 | 17 | 5 | Negative |
| **AMP** | 346 | 134.2 | 20 | 380 | 37 | 5 | Negative |
| **AMP** | 346 | 106.9 | 20 | 380 | 61 | 5 | Negative |
| **Fru 1.6BP** | 339 | 97 | 20 | 380 | 21 | 5 | Negative |
| **Fru1.6BP** | 339 | 79 | 20 | 380 | 57 | 5 | Negative |
| **Glc1P** | 259 | 96.8 | 20 | 380 | 9 | 5 | Negative |
| **Glc1P** | 259 | 78.9.8 | 20 | 380 | 44 | 5 | Negative |
| **Fru6P** | 259 | 96.8 | 20 | 380 | 9 | 5 | Negative |
| **Fru6P** | 259 | 78.9 | 20 | 380 | 44 | 5 | Negative |
| **Glc6P** | 259 | 78.9 | 20 | 380 | 44 | 5 | Negative |
| **Glc6P** | 259 | 96.8 | 20 | 380 | 44 | 5 | Negative |
| **Rib5P** | 229 | 97 | 20 | 380 | 5 | 5 | Negative |
| **Rib5P** | 229 | 79 | 20 | 380 | 44 | 5 | Negative |
| **Gluca** | 193 | 113 | 20 | 380 | 5 | 5 | Negative |
| **Gluca** | 193 | 85 | 20 | 380 | 13 | 5 | Negative |
| **Isocit** | 191 | 110.8 | 20 | 380 | 9 | 5 | Negative |
| **Isocit** | 191 | 87 | 20 | 380 | 13 | 5 | Negative |

**Continue**

| Compound Name | Prec Ion | Pro Ion | Dwell | Frag | CE | CAV | Polarity |
|---|---|---|---|---|---|---|---|
| **3PGA** | 185.1 | 97 | 20 | 380 | 13 | 5 | Negative |
| **3PGA** | 185.1 | 79 | 20 | 380 | 41 | 5 | Negative |
| **Cacnt** | 173.1 | 128.9 | 20 | 380 | 1 | 5 | Negative |
| **Cacnt** | 173.1 | 85 | 20 | 380 | 9 | 5 | Negative |
| **Tacnt** | 173.1 | 111 | 20 | 380 | 5 | 5 | Negative |
| **Tacnt** | 173.1 | 128.9 | 20 | 380 | 1 | 5 | Negative |
| **PEP** | 167 | 78.9 | 20 | 380 | 13 | 5 | Negative |
| **PEP** | 167 | 63.2 | 20 | 380 | 77 | 5 | Negative |
| **2-oxo** | 145.1 | 101 | 20 | 380 | 5 | 5 | Negative |
| **Mal** | 133 | 115 | 20 | 380 | 5 | 5 | Negative |
| **Mal** | 133 | 71 | 20 | 380 | 9 | 5 | Negative |
| **Succ** | 116.9 | 73 | 20 | 380 | 5 | 5 | Negative |
| **Fum** | 115 | 70.9 | 20 | 380 | 1 | 5 | Negative |
| **13C-Pyr** | 89.9 | 89.9 | 20 | 380 | 1 | 5 | Negative |
| **13C-Pyr** | 89.9 | 45.1 | 20 | 380 | 5 | 5 | Negative |
| **Pyr** | 87.1 | 87 | 20 | 380 | 5 | 5 | Negative |

**Table 2. List of primers used for qRT-PCR in this study.**

| Gene | Forward primer | Reverse primer | Reference |
|---|---|---|---|
| ***HvNCED2*** | CATGGAAAGAGGAAGTTG | GAAGCAAGTGTGAGCTAAC | Millar et al., 2006 |
| ***HvCCD3*** | CGGCTCGGCTGTCGCTACCA | CACCGCCACCGCCTCTCC | Seiler et al, 2011 |
| ***HvSDR3*** | CAATCCGGTGAAAGTCGCTCTC | CGCCACGCCATCCCTATCAAAA | Seiler et al, 2011 |
| ***HvAO4*** | CCGACGGGCTGGTGGTGAAC | CGCCGGACGCCTTGGATG | Seiler et al, 2011 |
| ***HvABA8'OH-1*** | AGCACGGACCGTCAAAGTC | TGAGAATGCCTACGTAGTG | Millar et al., 2006 |
| ***HvS40*** | ATGTACCAACGACGCGAAG | ATGAATCCGGTCATCCTGAG | |
| ***HvAGP-L2*** | GCCGGCGCAAGAAGCACA | ATCGCGCAACCAATGAATCC | Seiler et al, 2011 |
| ***HvSSIIIa*** | ATCCTCTTGCCTCCTCCATCTG | CGTCACTGCGGTTCTTATCTCG | Seiler et al, 2011 |
| ***HvGBSS1a*** | TGCCGGTGGACAGGAAGGT | CCCGGTGCCAAGGAGAATGA | Seiler et al, 2011 |
| ***HvBAM1*** | ATGGGTGGGCAGGCTGAAGG | GAGCTCCCCACCCATGCCACTA | Seiler et al, 2011 |
| ***HvBAM2*** | GCGCTCCTTGCCCCTGTGG | CTTGGCGGCTTATTTCCTGTGC | Seiler et al, 2011 |
| ***HvSucSyn*** | GGCGCATTCATCGAGCAGGAG | ACCCCACGACAGACGGACCAA | Seiler et al, 2011 |
| ***HvSucSyn1*** | GCGCCCTGAGCCGTGTCC | GGCTGCAGCATCCCCTTTCCTT | Seiler et al, 2011 |
| ***HvGBSS1a*** | TGCCGGTGGACAGGAAGGT | CCCGGTGCCAAGGAGAATGA | Seiler et al, 2011 |

**Continue**

| | | | |
|---|---|---|---|
| ***HvISA1*** | CACAAAAGGGGGCAACAACAAT | GGCCAAGACCCTCGCACTCC | Seiler et al, 2011 |
| ***HvISA2*** | AGGGGACCGGCATTTTCTTTCA | GGACTGCACTGTTCTCGGATGG | Seiler et al, 2011 |
| ***HvSSI*** | CCGCCGAGCAGTACGAGCAGA | GGCCGCGGAAGGATGAGGAA | Seiler et al, 2011 |
| ***HvPP2C1*** | TCGCCAGCAGCCGTGAGC | CCGCCGTGCCCATCGTAGA | Seiler et al, 2014 |
| ***HvPP2C2*** | GCACGTCGGCAAATCCAGCAGT | TTTCCTCCGGGGTTTCAAGTCG | Seiler et al, 2014 |
| ***SnRK35_3036*** | GCAGAAACCGGGCGATAACG | GCTCCCCAGGCAGGCAACC | Seiler et al, 2011 |
| ***SnRK35_15990b*** | CTTGCAGCCAGCTACGGTGAG | CTGAGAGCGGAACGGGTGAAAC | Seiler et al, 2011 |
| ***HvABI5*** | CCGGTCCCTGTTGCCCCTAAAG | CGCCGCCCATACCGAGTG | Seiler et al, 2011 |
| ***RCAR35_2538*** | GTTTCTTCGGCGCGTGAGCAT | TTGGACAAGCAGGGAGGAGAGG | Seiler et al, 2011 |
| ***RCAR35_27243*** | GAGGGCAACACCGAGGAGGACA | CGATGGCGGCGAGTTTCTGG | Seiler et al, 2011 |
| ***HvABI1b*** | GCACGTCGGCAAATCCAGCAGT | TTTCCTCCGGGGTTTCAAGTCG | Seiler et al, 2011 |
| ***HvABI1d*** | ACGCGGCAGCAAGGACAACATC | ATCCCCATCCAGCCAGCCACTC | Seiler et al, 2011 |

**Table 3.** Analysis of amino acids using three different barley lines under **low K supply** and terminal drought stress.

| | Low K- Control | | | Low K- Stress | | |
|---|---|---|---|---|---|---|
| | **LP104** | **LP110** | **LP106** | **LP104** | **LP110** | **LP106** |
| **Asp** | 518 ± 36 | 406.55 ± 55 | 439 ± 61 | 394 ± 52 | 264 ± 26 | 221 ± 81 |
| **Glu** | 3568 ± 108 | 2488 ± 434 | 2936 ± 366 | 3009 ± 404 | 986 ± 59 | 1005 ± 608 |
| **Ser** | 438 ± 21 | 291 ± 50 | 527 ± 58 | 287 ± 69 | 213 ± 36 | 430 ± 4.4 |
| **Asn** | 51.19 ± 5.72 | 72 ± 7.91 | 57.31 ± 9.57 | 54.17 ± 3.32 | 24.62 ± 7.7 | 48.95 ± 35.54 |
| **Gly** | 128.7± 12.7 | 74.96 ± 17 | 118.01 ± 8.04 | 77.59 ± 20.1 | 48.18 ± 4.7 | 96.83 ± 15.7 |
| **Gln** | 131 ± 26 | 90.88 ± 8.2 | 120.5 ± 17.94 | 64.78 ± 7.3 | 147.41 ± 37.2 | 376.46 ± 168 |
| **His** | 19.35 ± 0.57 | 11.98 ± 1.79 | 10.09 ± 1.74 | 12.23 ± 3.9 | 31.91 ± 1.53 | 46.17 ± 6.51 |
| **Thr** | 256 ± 36 | 166 ± 4 | 252 ± 21 | 187.43 ± 55 | 110 ± 10 | 168 ± 60 |
| **Arg** | 13.37 ± 1.44 | 11.63 ± 3.28 | 14.49 ± 5.4 | 13.14 ± 4.71 | 15.44 ± 3.20 | 18.11 ± 3.57 |
| **GABA** | 941 ± 93 | 942 ± 166 | 1501 ± 200 | 751.85 ± 105 | 756 ± 52 | 1271 ± 479 |
| **Pro** | 18.93 ± 3.58 | 33.8 ± 6.01 | 56.23 ± 11.6 | 33.02 ± 8.11 | 27.66 ± 5.01 | 16.17 ± 5.88 |
| **Tyr** | 275 ± 15 | 205 ± 21 | 269 ± 40 | 227 ± 19 | 149 ± 10 | 613 ± 260 |
| **Val** | 78.56 ± 9.07 | 20.82 ± 4.66 | 32.38 ± 50 | 49.34 ± 6.05 | 34.75 ± 2.91 | 47.46 ± 7.75 |
| **Met** | 344 ± 65 | 197 ± 26 | 274 ± 30 | 186 ± 46 | 170 ± 13 | 309 ± 40 |
| **Ile** | 6.6 ± 0.86 | 8.06 ± 3.8 | 8.43 ± 1.17 | 6.41 ± 0.64 | 8.44 ± 1.09 | 10.51 ± 7.2 |
| **Leu** | 76.46 ± 9.37 | 58.47 ± 7.2 | 67.59 ± 9.8 | 50.85 ± 8.29 | 45.14 ± 2.99 | 113.95 ± 17.42 |
| **Lys** | 54.11 ± 18.47 | 24.52 ± 4.13 | 22.39 ± 2.6 | 25.07 ± 1.7 | 21.5 ± 4.61 | 36.03 ± 0.5 |
| **Phe** | 54.98 ± 3.6 | 71.49 ± 2.01 | 75.27 ± 7.2 | 49.16 ± 20.4 | 50.18 ± 5.1 | 114.28 ± 8.5 |
| **Asp** | 518 ± 36 | 406.55 ± 55 | 439 ± 61 | 394 ± 52 | 264 ± 26 | 221 ± 81 |

**Continue of table 3.** Analysis of amino acids using three different barley lines under **medium K supply** and terminal drought stress.

| | Medium K- Control | | | Medium K- Stress | | |
|---|---|---|---|---|---|---|
| | **LP104** | **LP110** | **LP106** | **LP104** | **LP110** | **LP106** |
| **Asp** | 906 ± 34 | 545 ± 84 | 589 ± 19 | 657 ± 73 | 413 ± 69 | 405 ± 65 |
| **Glu** | 5879 ± 556 | 3833 ± 420 | 4393 ± 332 | 3311 ± 432 | 2472 ± 422 | 3035 ± 817 |
| **Ser** | 349 ± 40 | 318 ± 9.6 | 609 ± 111 | 217 ± 25 | 392 ± 33 | 524 ± 63 |
| **Asn** | 49.82 ± 5.17 | 80.08 ± 8.12 | 60 ± 8.3 | 50.75 ± 5.03 | 32.84 ± 4.31 | 57.73 ± 3.49 |
| **Gly** | 149 ± 18 | 97.36 ± 7.3 | 137.41 ± 21 | 94.69 ± 12.5 | 86.35 ± 9.7 | 140.71 ± 13 |
| **Gln** | 73.24 ± 3.87 | 73.17 ± 5.44 | 85.77 ± 17.46 | 102.87 ± 10.11 | 184.19 ± 46.91 | 156.71 ± 24.9 |
| **His** | 19.33 ± 4.8 | 13.48 ± 2.12 | 12.74 ± 2.23 | 6.48 ± 1.77 | 38.55 ± 7.86 | 33.85 ± 2.17 |
| **Thr** | 314 ± 28 | 229 ± 12 | 317 ± 49 | 148 ± 32 | 221 ± 29 | 231 ± 26 |
| **Arg** | 8.65 ± 1.2 | 16.06 ± 2.91 | 10.15 ± 4.14 | 7.86 ± 0.78 | 10 ± 1.73 | 10.65 ± 2.01 |
| **GABA** | 1143 ± 134 | 1314 ± 206 | 1738 ± 214 | 815 ± 142 | 2088 ± 222 | 1646 ± 287 |
| **Pro** | 14.59 ± 1.52 | 22.39 ± 2.18 | 16.52 ± 2.97 | 10.43 ± 3.93 | 19.9 ± 6.6 | 12.9 ± 1.06 |
| **Tyr** | 237 ± 12 | 208 ± 19 | 304. ± 18 | 231 ± 68 | 1188 ± 205 | 795 ± 121 |
| **Val** | 67.58 ± 5.19 | 29.95 ± 1.38 | 32.07 ± 7.65 | 42.11 ± 9.85 | 41.81 ± 8.79 | 41.89 ± 10.5 |
| **Met** | 300 ± 41 | 246 ± 15 | 319 ± 31 | 206 ± 27 | 317 ± 5 | 247± 28 |
| **Ile** | 6.04 ± 0.78 | 9.12 ± 0.73 | 6.26 ± 0.73 | 7.11 ± 0.66 | 7 ± 0.53 | 10.32 ± 1.95 |
| **Leu** | 73.23 ± 4.92 | 76.72 ± 8.97 | 75.73 ± 13.6 | 83.05 ± 8.56 | 75.03 ± 10.43 | 82.15 ± 14.73 |
| **Lys** | 30.12 ± 5.5 | 21.75 ± 1.22 | 17.56 ± 2.76 | 16.7 ± 2.1 | 21.69 ± 5.66 | 14.23 ± 1.36 |
| **Phe** | 40.34 ± 16.1 | 51.72 ± 6.54 | 58.6 ± 6.32 | 37.09 ± 14.29 | 47.06 ± 4.51 | 45.32 ± 7.86 |
| **Asp** | 906 ± 34 | 545 ± 84 | 589 ± 19 | 657 ± 73 | 413 ± 69 | 405 ± 65 |

**Continue of table 3.** Analysis of amino acids using three different barley lines under **high K supply** and terminal drought stress.

| | High K- Control | | | High K- Stress | | |
|---|---|---|---|---|---|---|
| | **LP104** | **LP110** | **LP106** | **LP104** | **LP110** | **LP106** |
| **Asp** | 828 ± 98 | 552 ± 15 | 1014 ± 241 | 710 ± 88 | 448.03 ± 79 | 477 ± 96 |
| **Glu** | 5771 ± 659 | 4527 ± 652 | 3933 ± 743 | 3795 ± 633 | 4054 ± 203 | 5084 ± 929 |
| **Ser** | 364 ± 34 | 340 ± 48 | 497 ± 71 | 289 ± 49 | 380 ± 42 | 739 ± 121 |
| **Asn** | 36.3 ± 10.58 | 52.87 ± 5.01 | 51.24 ± 6 | 23.4 ± 5.01 | 37.55 ± 11.87 | 63.58 ± 1.49 |
| **Gly** | 165.86 ± 18.7 | 125.51 ± 31.2 | 115.71 ± 19.2 | 81.19 ± 3.8 | 102.75 ± 16.6 | 141.25 ± 16.3 |
| **Gln** | 61.72 ± 13.19 | 79.37 ± 22.88 | 73.11 ± 28.41 | 75.8 ± 17.71 | 223.66 ± 50.53 | 128.08 ± 18.16 |
| **His** | 31.42 ± 6.11 | 14.74 ± 3.71 | 8.24 ± 1.15 | 38.34 ± 5.16 | 40.79 ± 2.44 | 38.62 ± 6.99 |
| **Thr** | 384 ± 59 | 236 ± 50 | 274 ± 54 | 254 ± 49 | 277 ± 48 | 234 ± 17 |
| **Arg** | 7.35 ± 0.69 | 11.55 ± 4.46 | 8.5 ± 1.79 | 8.56 ± 0.72 | 11.15 ± 1.43 | 9.39 ± 1.45 |
| **GABA** | 952 ± 20 | 1399.11 ± 337 | 1226 ± 302 | 1387 ± 312 | 2156 ± 222 | 2492 ± 331 |
| **Pro** | 13.21 ± 1.15 | 17.87 ± 2.18 | 14.72 ± 0.97 | 12.33 ± 1.86 | 16.56 ± 3.65 | 13.81 ± 3.46 |
| **Tyr** | 240 ± 30 | 220 ± 20 | 212 ± 34 | 218 ± 19 | 452 ± 30 | 745 ± 187 |
| **Val** | 60.02 ± 7.53 | 33.71 ± 9.88 | 31.54 ± 9.49 | 34.76 ± 8.25 | 53 ± 11.48 | 32.68 ± 4.21 |
| **Met** | 382 ± 54 | 361 ± 98 | 252 ± 9 | 203 ± 44 | 282 ± 32 | 245 ± 9 |
| **Ile** | 6.05 ± 0.72 | 7.59 ± 1.87 | 5.73 ± 0.56 | 7.37 ± 0.64 | 7.8 ± 0.58 | 9.98 ± 1.38 |
| **Leu** | 78.75 ± 9.97 | 74.39 ± 5.84 | 62.13 ± 6.89 | 63.03 ± 17.33 | 74.87 ± 13.95 | 97.1 ± 8.20 |
| **Lys** | 29.21 ± 6.43 | 22.57 ± 3.34 | 10.27 ± 0.38 | 13.14 ± 2.86 | 21.39 ± 3.83 | 13.47 ± 2.84 |
| **Phe** | 28.67 ± 4.62 | 52.22 ± 4.72 | 29.91 ± 5.79 | 28.8 ± 3.43 | 40.01 ± 2.38 | 42.67 ± 7.21 |
| **Asp** | 828 ± 98 | 552 ± 15 | 1014 ± 241 | 710 ± 88 | 448.03 ± 79 | 477 ± 96 |

**Table 4.** Primary metabolites profiling, using three different barley lines under **low K supply** and terminal drought stress.

| | Low K- Control | | | Low K- Stress | | |
|---|---|---|---|---|---|---|
| | **LP104** | **LP110** | **LP106** | **LP104** | **LP110** | **LP106** |
| **Trehalose-6-P** | 0.51 ± 0.13 | 0.33 ± 0.06 | 0.73 ± 0.18 | 0.71 ± 0.22 | 0.36 ± 0.1 | 0.42 ± 0.16 |
| **Sucrose-6-P** | 1.22 ± 0.19 | 0.69 ± 0.38 | 0.79 ± 0.31 | 1.34 ± 0.25 | 1.93 ± 0.86 | 0.86 ± 0.63 |
| **Succinate** | 2.39 ± 0.73 | 1.73 ± 0.53 | 6.01 ± 1.18 | 2.31 ± 0.96 | 1.9 ± 0.37 | 3.31 ± 1.9 |
| **Oxoglutarate** | 335 ± 79 | 147 ± 39 | 224.71 ± 33 | 193.06 ± 32.53 | 84.35 ± 31.29 | 48.67 ± 22.56 |
| **ADP-Glucose** | 0.05 ± 0.02 | 0.05 ± 0.01 | 0.05 ± 0.03 | 0.11 ± 0.03 | 0.02 ± 0.01 | 0.08 ± 0.001 |
| **NADH** | 0.54 ± 0.21 | 0.75 ± 0.24 | 0.94 ± 0.39 | 1.56 ± 0.41 | 0.78 ± 0.28 | 0.81 ± 0.17 |
| **3PGA** | 214 ± 47 | 139.41 ± 30.92 | 33.62 ± 8.8 | 116.11 ± 42.93 | 106.56 ± 59.58 | 23.43 ± 2.35 |
| **Isocitrate** | 257 ± 73 | 136 ± 31 | 182 ± 37 | 176.1 ± 30.37 | 159.64 ± 13.4 | 149.95 ± 14.85 |
| **UDP-Glucose** | 39.55 ± 6.4 | 27.57 ± 7.49 | 23.18 ± 7.06 | 35.79 ± 6.87 | 33.69 ± 9.53 | 16.5 ± 1.84 |
| **ADP** | 8.5 ± 2.37 | 6.13 ± 1.91 | 1.69 ± 0.72 | 4.62 ± 1.88 | 6.18 ± 2.62 | 2.26 ± 0.53 |
| **Fru1,6bisP** | 1.2 ± 0.3 | 0.63 ± 0.32 | 0.47 ± 0.14 | 3.67 ± 1.44 | 3.02 ± 1.29 | 3.1 ± 1.05 |
| **NADPH** | 2.09 ± 0.91 | 1.37 ± 0.52 | 0.44 ± 0.28 | 3.47 ± 1.28 | 3.08 ± 0.83 | 0.71 ± 0.04 |
| **UDP** | 2.94 ± 1.24 | 1.82 ± 0.52 | 0.74 ± 0.45 | 1.65 ± 0.43 | 1.62 ± 0.41 | 1.45 ± 0.63 |
| **ATP** | 10.88 ± 5.22 | 7.08 ± 2.01 | 2.95 ± 0.44 | 5.61 ± 3.18 | 10.32 ± 1.24 | 2.15 ± 0.49 |
| **Malate** | 5324 ± 1603 | 6641.3 ± 1889 | 2312 ± 50 | 4433.68 ± 705 | 8929.67 ± 1329 | 4805.8 ± 2087 |
| **Fumarate** | 3082 ± 720 | 2121 ± 428 | 2510± 169 | 3112± 458 | 2197± 503 | 1677± 469 |
| **Citrate** | 3990± 1040 | 5380± 21 | 3319.1 ± 358 | 3892.87 ± 658 | 5902.56 ± 644 | 4710.02 ± 935 |
| **Isocitrate** | 3440.6 ± 819 | 3838.92 ± 703 | 4788.95 ± 700 | 3735 ± 1184 | 3628.56 ± 683 | 3072.74 ± 228 |
| **Pyruvate** | 381.59 ± 105 | 529.18 ± 128 | 759± 65 | 329.24 ± 76.8 | 588.3 ± 111.67 | 458.65 ± 110 |
| **PEP** | 19.16 ± 8.92 | 3.38 ± 1.67 | 0.68 ± 0.07 | 4.88 ± 1.25 | 6.3 ± 2.62 | 0.51 ± 0.05 |
| **Hexose-P** | 80.72 ± 9.88 | 32.23 ± 6.56 | 31.23 ± 16.37 | 61.61 ± 15.55 | 34.28 ± 14.42 | 56.14 ± 34.53 |

**Continue, table 4.** Primary metabolites profiling, using three different barley lines under **medium K supply** and terminal drought stress.

| | Medium K- Control | | | Medium K- Stress | | |
|---|---|---|---|---|---|---|
| | **LP104** | **LP110** | **LP106** | **LP104** | **LP110** | **LP106** |
| **Trehalose-6-P** | 0.8 ± 0.28 | 0.53 ± 0.14 | 1.26 ± 0.21 | 0.93 ± 0.15 | 0.7 ± 0.2 | 1.27 ± 0.22 |
| **Sucrose-6-P** | 1.1 ± 0.05 | 1.37 ± 0.19 | 1.17 ± 0.14 | 1.43 ± 0.44 | 1.44 ± 0.89 | 1.77 ± 0.38 |
| **Succinate** | 2.66 ± 0.51 | 2.73 ± 1.03 | 2.53 ± 0.9 | 1.47 ± 0.26 | 2.64 ± 1.3 | 3.07 ± 1.14 |
| **Oxoglutarate** | 340.9 ± 165 | 169.06 ± 36 | 234.7 ± 57.57 | 231.53 ± 20 | 96.27 ± 27 | 219.26 ± 41 |
| **ADP-Glucose** | 0.05 ± 0.02 | 0.03 ± 0.01 | 0.05 ± 0.03 | 0.04 ± 0.02 | 0.03 ± 0.01 | 0.03 ± 0.02 |
| **NADH** | 0.25 ± 0.14 | 0.19 ± 0.03 | 0.44 ± 0.13 | 0.67 ± 0.28 | 0.72 ± 0.23 | 0.98 ± 0.52 |
| **3PGA** | 456.29 ± 124 | 260.96 ± 44.94 | 196.41 ± 92.16 | 225.46 ± 49 | 93.69 ± 31 | 56.14 ± 12.1 |
| **Isocitrate** | 208.48 ± 166 | 84.92 ± 31.62 | 108.73 ± 44 | 88.29 ± 33.7 | 159.35 ± 14 | 117.56 ± 24 |
| **UDP-Glucose** | 40.62 ± 6.08 | 36.71 ± 7.92 | 42.87 ± 9.01 | 38.98 ± 9.32 | 20.05 ± 8.13 | 26.74 ± 9.14 |
| **ADP** | 16.13 ± 1.69 | 9.56 ± 1.29 | 4.47 ± 1.37 | 7.62 ± 1.68 | 7.17 ± 2.34 | 3.14 ± 1.49 |
| **Fru1,6bisP** | 3.26 ± 1.73 | 2.14 ± 0.11 | 1.57 ± 0.62 | 3.28 ± 1.99 | 4.47 ± 1.36 | 5.45 ± 2.51 |
| **NADPH** | 1.44 ± 0.52 | 2.11 ± 1.24 | 0.32 ± 0.13 | 0.6 ± 0.41 | 2.13 ± 0.73 | 0.53 ± 0.23 |
| **UDP** | 3.2 ± 0.96 | 2.67 ± 0.77 | 2.78 ± 0.75 | 3.02 ± 0.39 | 1.86 ± 0.61 | 1.41 ± 0.64 |
| **ATP** | 20.36 ± 4.57 | 17.13 ± 2.74 | 12.24 ± 6.66 | 9.54 ± 5.49 | 9.64 ± 3.07 | 3.465 ± 2.22 |
| **Malate** | 8019± 482 | 7300± 227 | 7025± 1018 | 7626± 2130 | 6716± 2217 | 5575± 1400 |
| **Fumarate** | 3232± 343 | 2846± 310 | 2889± 364 | 3283.01 ± 660 | 2349± 554 | 2575± 439 |
| **Citrate** | 7486± 1322 | 5961 ± 567 | 7300± 1680 | 5023.35 ± 934 | 3682.55 ± 770 | 5254.17 ± 696 |
| **Isocitrate** | 8017± 2694 | 7010.39 ± 1280 | 11276± 2682 | 6493± 957 | 4319.04 ± 1057 | 8209.74 ± 1501 |
| **Pyruvate** | 434± 179 | 558.48 ± 93.32 | 754.55 ± 138 | 523.67 ± 68.71 | 437± 110.85 | 952± 281 |
| **PEP** | 42.45 ± 13.37 | 16.46 ± 2.96 | 2.43 ± 0.55 | 3.62 ± 1.01 | 3.2 ± 1.92 | 0.39 ± 0.23 |
| **Hexose-P** | 75.28 ± 7.55 | 47.66 ± 10.09 | 58.4 ± 16.89 | 62.21 ± 28.37 | 41.62 ± 13.79 | 52.8 ± 8.86 |

**Continue, table 4.** Primary metabolites profiling, using three different barley lines under **high K supply** and terminal drought stress.

| | Medium K- Control | | | Medium K- Stress | | |
|---|---|---|---|---|---|---|
| | **LP104** | **LP110** | **LP106** | **LP104** | **LP110** | **LP106** |
| **Trehalose-6-P** | 0.81 ± 0.14 | 0.59 ± 0.14 | 1.24 ± 0.22 | 1.19 ± 0.14 | 0.78 ± 0.16 | 1.58 ± 0.24 |
| **Sucrose-6-P** | 1.12 ± 0.11 | 1.32 ± 0.31 | 1.31 ± 0.23 | 1.65 ± 0.28 | 2.11 ± 0.65 | 1.98 ± 0.62 |
| **Succinate** | 2.3 ± 0.95 | 2.59 ± 0.61 | 2.41 ± 0.37 | 2.38 ± 0.6 | 2.2 ± 0.32 | 2.57 ± 0.72 |
| **Oxoglutarate** | 301± 44 | 192± 51 | 365.97 ± 94.56 | 255± 32 | 129± 51 | 205± 85 |
| **ADP-Glucose** | 0.16 ± 0.03 | 0.06 ± 0.04 | 0.04 ± 0.03 | 0.02 ± 0.01 | 0.07 ± 0.04 | 0.05 ± 0.01 |
| **NADH** | 0.27 ± 0.16 | 0.39 ± 0.13 | 0.2 ± 0.05 | 0.5 ± 0.13 | 1.38 ± 0.33 | 2 ± 0.077 |
| **3PGA** | 515 ± 66 | 301 ± 65 | 417± 67 | 325 ± 84 | 96.21 ± 57 | 46.79 ± 17.1 |
| **Isocitrate** | 100.18 ± 16 | 92.92 ± 26 | 88.32 ± 20.24 | 93.16 ± 20 | 124.96 ± 44 | 95.81 ± 30 |
| **UDP-Glucose** | 39.03 ± 8.09 | 38.38 ± 8.14 | 40.94 ± 11.69 | 32.1 ± 6.37 | 24.23 ± 3.73 | 23.21 ± 4.74 |
| **ADP** | 15.01 ± 3.43 | 11.05 ± 1.8 | 10.13 ± 3.23 | 9.34 ± 1.44 | 4.54 ± 2.04 | 1.76 ± 0.49 |
| **Fru1,6bisP** | 2.95 ± 0.55 | 2.55 ± 0.47 | 3.22 ± 0.53 | 13.08 ± 2.5 | 4.81 ± 2.15 | 5.48 ± 0.97 |
| **NADPH** | 0.89 ± 0.45 | 1.91 ± 0.76 | 1.18 ± 1.19 | 1.92 ± 1.23 | 1.35 ± 0.41 | 0.42 ± 0.15 |
| **UDP** | 5.5 ± 0.67 | 3.07 ± 0.78 | 3.92 ± 0.26 | 4.86 ± 0.88 | 1.96 ± 0.57 | 1.46 ± 0.41 |
| **ATP** | 19.15 ± 6.74 | 20.64 ± 5.38 | 17.89 ± 7.74 | 14.85 ± 3.19 | 4.27 ± 3.49 | 3.41 ± 3.18 |
| **Malate** | 12979± 1959 | 7965± 970 | 9743± 758 | 6420± 671 | 7320± 466 | 6204± 921 |
| **Fumarate** | 4070± 507 | 2920± 330 | 3189± 330 | 3279± 330 | 2630± 217 | 2592± 150 |
| **Citrate** | 13720± 3806 | 6523.83 ± 6.45 | 10108± 853 | 6687± 915 | 4688± 482 | 5388± 196 |
| **Isocitrate** | 10872± 2060 | 7886± 1397 | 13132± 1134 | 10685± 1678 | 6084± 1569 | 9561± 345 |
| **Pyruvate** | 603± 100 | 535± 94.78 | 1088. ± 204 | 600± 110 | 649.38 ± 181 | 1029± 352 |
| **PEP** | 19.23 ± 8.66 | 9.11 ± 3.09 | 27.43 ± 4.42 | 5.76 ± 1.71 | 0.66 ± 0.49 | 0.17 ± 0.04 |
| **Hexose-P** | 79.31 ± 15.4 | 53.73 ± 16.86 | 55.33 ± 13.73 | 86.41 ± 21.82 | 60.04 ± 9.13 | 79.39 ± 16.21 |

# 10 Acknowledgements

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**Seyed Abdollah Hosseini**

# 11 Publications and proceedings related to the submitted thesis

- Seyed Abdollah Hosseini, Nese Sreenivasulu, Mohammad Reza Hajirezaei and Nicolaus von Wirén. Effect of terminal drought stress on metabolites and hormone regulation of two contrasting senescence barley lines. 7th European Workshop on Plant Senescence 10-14 November 2014, Aarhus University, Sondbjerg state, Denmark.

- Seyed Abdollah Hosseini, Nese Sreenivasulu, Mohammad Reza Hajirezaei and Nicolaus von Wirén. Influence of potassium deficiency on metabolite and hormonal regulation in different barley lines during terminal drought stress. International conference of the German Society of Plant Nutrition, 10-12 September 2014, Halle, Germany.

- Seyed Abdollah Hosseini, Nese Sreenivasulu, Mohammad Reza Hajirezaei and Nicolaus von Wirén. Influence of potassium deficiency on metabolites in different barley lines during drought stress, oral presentation. 17th international plant nutrition conference, 19– 22 August 2013, Istanbul, Turkey.

- Seyed Abdollah Hosseini, Nese Sreenivasulu, Mohammad Reza Hajirezaei and Nicolaus von Wirén. Influence of potassium deficiency on metabolites in different barley lines during drought stress, oral presentation. 9th plant Science Student Conference 28th May – 31th May 2013, Leibniz-Institute of Plant Biochemistry, Halle, Germany.

- Seyed Abdollah Hosseini, T. Harshavardhan, Bernhard Bauer, Nese Sreenivasulu, Mohammad Reza Hajirezaei and Nicolaus von Wirén. Effect of potassium on terminal drought stress in barley during anthesis and post-anthesis. Poster presentation, 8th plant Science Student Conference 4th July – 7th July 2012, IPK, Gatersleben, Germany.

- Seyed Abdollah Hosseini, Nese Sreenivasulu, Mohammad Reza Hajirezaei, Bernhard Bauer, Vokkaliga T. Harshavardhan and Nicolaus von Wirén. Dissecting the physiological mechanisms of potassium starvation in terminal drought stress in barley lines during anthesis and post anthesis. Oral presentation, nutrient and

water supply of crops under the conditions of global warming, 18-19 October 2012, Anhalt University of Applied Sciences, Bernburg, Germany.

# 12 Curriculum Vitae

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**Nationality**: Iranian

**Date of Birth**: 29/12/1978

**Place of Birth**: Karaj / Iran

**Gender**: Male

**Marital status**: Married

## EDUCATION

**2011-2014** **Ph.D.;** Biology, Martin Luther University, Halle, Germany. Thesis prepared at Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

**Thesis title:** Influence of potassium deficiency on metabolites in different barley lines during drought stress

**Supervisor:** Prof Nicolaus von Wirén

**2007-2009** **M.Sc.;** Agronomy, Azad Islamic University of Takestan, Iran. Thesis prepared at Agricultural Biotechnology Research Institute of Iran, Karaj (ABRII).

**Thesis title:** Proteins analysis of rice under salinity stress.

**Supervisor:** Dr. Ghasem Hosseini Salekdeh

**1996-2000** **B.Sc.** Agronomy, Department of Agriculture, Faculty of Agronomy, Azad Islamic University of Karaj, Iran.

**Job Experiences**

- Working on Ph.D. thesis as an employee at the Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany (2011- 2014).
- 
- Technical Assistant at Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran (2003-2011).

**Research**

- Influence of potassium deficiency on metabolite and hormone regulation in different barley lines during drought stress, IPK, Germany.

- Detection and Characterization of Phytoplasma Infecting Ornamental and Weed Plants in Iran. Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran.

- Proteome analysis of rice nucleus in response to salt stress, Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran.

- Proteome analysis of leaf under salt stress, Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran.

- Proteome analysis of root under salt stress, Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran.

- Proteome analysis of wheat under cold stress, Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran.

**Workshops**

- First Barlomic Summer School workshop, Plant Genetics and Crop Plant Research (IPK), Gatersleben, 9-12th September Germany,

- Basic statistic techniques, Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, 13-19th April 2012.

- Self-presentation, Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, 23-24th May 2012.

- Basic, Structure & Documentation of General Requirement for the Competence of Testing and Calibration Laboratories with EN ISO/IEC 17025:2000. 4-5 March 2007.

- Bioinformatics Applications in Biological Sciences, Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran, 2010.

- Good Laboratory Practice (GLP), Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran, 2010.

- International Workshop, Crop improvement under drought: an integrated approach, Agricultural Biotechnology

Research Institute of Iran (ABRII), Karaj, Iran, January 31-February 2 2009.

- Instructor, Good Laboratory Practice (GLP), Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran, 2007.

**Conferences**

**International:**

- 7th European Workshop on Plant Senescence 10-14 November 2014, Aarhus University, Sondbjerg state, Denmark. **Seyed Abdollah Hosseini**, Nese Sreenivasulu, Mohammad Reza Hajirezaei, and Nicolaus von Wirén. Effect of Terminal drought stress of metabolites and hormone regulation of two contrasting senescence barley lines.

- International conference of the German Society of Plant Nutrition 10-12 September 2014, Halle, Germany. **Seyed Abdollah Hosseini**, Nese Sreenivasulu, Mohammad Reza Hajirezaei, and Nicolaus von Wirén. Influence of potassium deficiency on metabolite and hormonal regulation in different barley lines during terminal drought stress.
- 
- 17th international plant nutrition Conference19– 22 August 2013, Istanbul, Turkey. **Seyed Abdollah Hosseini**, Nese Sreenivasulu, Mohammad Reza Hajirezaei, and Nicolaus von Wirén. Influence of potassium deficiency on metabolites in different barley lines during drought stress, **oral presentation**.

**National:**

- 9th plant Science Student Conference 28th May – 31th May 2013, Leibniz-Institute of Plant Biochemistry, Halle,

Germany. **Seyed Abdollah Hosseini**, Nese Sreenivasulu, Mohammad Reza Hajirezaei, and Nicolaus von Wirén. Influence of potassium deficiency on metabolites in different barley lines during drought stress, oral presentation.

- 8th plant Science Student Conference 4th July – 7th July 2012, IPK, Gatersleben, Germany. **Seyed Abdollah Hosseini**, T. Harshavardhan, Bernhard Bauer, Nese Sreenivasulu, Mohammad Reza Hajirezaei, and Nicolaus von Wirén. Effect of potassium on terminal drought stress in barley during anthesis and post-anthesis, poster presentation.

- Nutrient and water supply of crops under the conditions of global warming 18-19 October 2012, Anhalt University of Applied Sciences, Bernburg, Germany. **Seyed Abdollah Hosseini**, Nese Sreenivasulu, Mohammad Reza Hajirezaei, Bernhard Bauer, Vokkaliga T. Harshavardhan and Nicolaus von Wirén. Dissecting the physiological mechanisms of potassium starvation in terminal drought stress in barley lines during anthesis and post anthesis, oral presentation.

- 1th National Biosafety Congress, February 16- 17, 2005, Agriculture Biotechnology Research Institute of Iran, Karaj, Iran.

- The second Iranian proteomics congress. 23- 24 April 2009, Royan Institute, Tehran, Iran Elham Sarhadi, Siroos Mahfoozi, , **Seyed Abdolah Hosseini**, Ghasem Hosseini Salekdeh. Low- temperature tolerance and proteome analysis of vernlization requiring wheat (Triticum aestivum L. em.Tell).

- The second Iranian proteomics congress, 23- 24 April 2009, Royan Institute, Tehran, Iran. Manzar Heidari, **Seyed Abdollah Hosseini**, Saeed Vazan, Jahanfar Daneshian, Ghasem Hosseini Salekdeh. Total and nuclear proteome analysis of rice leaf under salt stress.

**Publication**

- **Seyed Abdollah Hosseini,** Javad Gharechahi, Manzar Heidari, Parisa Koobaz, Shapour Heidari, Mehdi Mirzaei, Babak Nakhoda, Ghasem Hosseini Salekdeh. Comparative proteomic and physiological characterization of two closely related rice genotypes with contrasting responses to salt stress. Functional Plant Biology, 2015.

- Amanda Rasmussen, **Sayed Abdollah Hosseini**, Mohammad-Reza Hajirezaei, Uwe Druege, and Danny Geelen. Adventitious rooting declines with vegetative to reproductive switch and involves a changed auxin homeostasis. Journal of Experimental Botany, 2014.

- Elham Sarhadi, Siroos Mahfozi, **Seyed Abdollah Hosseini**, Ghasem Hosseini. Cold Acclimation Proteome Analysis Reveals Close Link between the UP – Regulation of Low- Temperature Associated Proteins and Vernalization Fulfillment. Journal of Proteome Research. 2010

- Babaie, G., Khatabi, B., Bayat, H., Rastgou, M., **Hosseini., A**., Salekdeh, GH. 2007. Detection and Characterization of Phytoplasmas Infecting Ornamental and Weed Plants in Iran. J. Phytopathology 155: 368–372.

**Honors and Awards**

- Poster award in 7th European Workshop on Plant Senescence 10-14 November 2014, Aarhus University, Sondbjerg state, Denmark. **Seyed Abdollah Hosseini**, Nese Sreenivasulu, Mohammad Reza Hajirezaei, and Nicolaus von Wirén. Effect of Terminal drought stress of metabolites and hormone regulation of two contrasting senescence barley lines.

- Khwarizmi International Award for fundamental research (2010), Iran, Tehran.

- The first rank among 2009 M.Sc .graduates of Agronomy with the best thesis (the mark of 20/20), Department of Plant breeding, Faculty of Agriculture, Azad Islamic University University, Takestan, Iran.

- The first Agronomy and plant breeding award for the Poster presentation entitled Study of antioxidant enzymes activity in leaf of rice under salinity stress, 2009, Beheshti University, Tehran, Iran

# 13 Affirmation

I hereby declare that the submitted work has been completed by me, the undersigned, and that I have not used any other than permitted reference sources or materials or engaged any plagiarism. All the references and the other sources used in the presented work have been appropriately acknowledged in the work. I further declare that the work has not been previously submitted for the purpose of academic examination, either in its original or similar form, anywhere else.

Hiermit erkläre ich, dass ich diese Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Die den benutzten Hilfsmitteln wörtlich oder inhaltlich entnommenen Stellen habe ich unter Quellenangaben kenntlich gemacht. Die vorliegende Arbeit wurde in gleicher oder ähnlicher Form noch keiner anderen Institution oder Prüfungsbehörde vorgelegt.

**Seyed Abdollah Hosseini**

**Gatersleben,**