Chapter 1: **Prologue**

The world's climate is currently undergoing an era of rapid change, characterized by increased average temperatures and shifting precipitation patterns. Such incidences has led to increased drought occurrences and durations in many regions worldwide (IPCC, 2014: Climate Change 2014: Synthesis Report, Shepherd, 2014). Since agriculture is the biggest consumer of water on this planet, continuous water shortages will inevitably endanger the food security of the growing numbers of its inhabitants.

From the estimation of world population, by 2030 (compared to 1998) 50% more food is required (FAO 2008). These estimations together with the limitations driven by water shortages, have raised concerns in the area of agricultural production. This indicates that the current agricultural practices are obliged to promptly improve in order to provide the demanded capacity in the next two decades.

The latest improvements in agricultural science and technology has led to the development of high yielding cultivars with increased resistance to pathogens and drought stresses. However, these high yielding materials require also high nutrient input, otherwise the soil nutrient resources will be rapidly diminished. Therefore, the soils subjected to modern agriculture, have to be replenished with nutrients more often than the ones used for conventional agriculture. In this regard, a new area in plant nutrition has emerged which aims at optimization of nutrient supply to the plants aiding them to reach their potential growth and simultaneously improve the efficiency of nutrient use. Modern plant nutrition studies also try to tackle the problems related to environmental changes and water shortages by optimizing the concentrations of necessary nutrients in soil and plants.

One of the necessary nutrients, which significantly contributes to osmoregulation in plants being important under drought stress conditions, is potassium. Studies show that supplying adequate levels of potassium (K) to the soil, increases soil water holding capacity (Holthusen et al., 2012) but more importantly improves plant water use efficiency (WUE) (Zörb et al., 2014; Jákli et al., 2016). Such properties of potassium makes it an asset to both plant and soil under drought stress conditions.

This study has focused on the integrated effects of potassium supply and osmotic stress conditions in barley (*Hordeum vulgare* L.). The aspects that have been covered in this research comprises plant water relations, Reactive Oxygen Species (ROS) and antioxidant dynamics. Furthermore, whole genome response of barley under selected potassium levels and osmotic stress conditions have been studied and compared to control conditions. The

preliminary introduction to the respective scientific gaps and the aspects under focus is provided stepwise in the following.

1.1 Plant preliminary growth and water relations affected by K supply

Potassium is one of the most abundant solutes in the cells taking the highest share after nitrogen in plant biomass up to 50 g.kg^{-1}. Potassium (K) together with its associated anions considerably affects the osmotic potential of plant cells emerging in cell extension and stomatal movements (Marschner, 2012). It is known that the rate of cell elongation and expansion is the most important element in plant growth. A study by Mengel and Arneke, (1982) on *Phaseolus vulgaris* has shown that potassium deficiency negatively affects cell size and leaf area. Similar findings have been obtained in recent studies on maize (Jordan-Meille and Pellerin, 2008) and wheat (Jákli et al., 2016) indicating the importance of potassium in leaf area expansion and growth of field crops.

Plants alter the amount of leaf transpiration via stomata regulation in accordance with soil water availability. In this process, the concentration of abscisic acid (ABA) in the guard cells plays a crucial role as a positive regulator of stomatal closure (Huang et al., 2008). In drought stress conditions, ABA signaling starts from the roots (Jiang and Hartung, 2008) and in leaf vascular parenchyma cells (Endo et al., 2008) which is then followed by transport to the apoplast and finally to the guard cells through specific transporters (Kuromori et al., 2011).

Upregulation of genes involved in ABA biosynthesis in barley and rice has also proven the importance of ABA signaling under drought conditions in cereals (Seiler et al., 2011; Ye et al., 2011). In this process, the accumulation of ABA causes the efflux of K$^+$ through the GORK channel (Jeanguenin et al., 2008) and thereby reduces the turgor pressure and contributes to stomatal closure. Contrastingly, under sufficient water supply ABA catabolism is activated which contributes to the influx of potassium into the guard cells (Pilot et al., 2001) leading to initiation of turgor pressure required for stomatal aperture.

Regulation of the stomata is mainly done by the plants to optimize the use of water based on the external water availability. The most common definition of water use efficiency (Tallec et al., 2013) is the total biomass production per the amount of water used in a spatial scale (Eq1).

$$Eq1. \quad WUE = \frac{Biomass\ production}{water\ used}$$

Whereas on the leaf level, the terms instantaneous and intrinsic water use efficiency are defined (Tambussi et al., 2007). The term instantaneous refers to the rate of net CO_2 assimilation (A_n) to the amount of water transpired (E) within equal time scale, however the intrinsic water use efficiency refers to the amount of assimilation (A) to stomatal conductance (g_s) where vapor pressure deficit (VPD) is not determinative (Eq2).

$$\text{Eq2.} \quad \text{Leaf-WUE} = \frac{A_n}{E} \, VPD^{-1} = \frac{A}{g_s}$$

A temporal integration model of leaf-WUE can be reflected in the ratio of stable carbon isotopes ^{13}C to ^{12}C (Δ) in the dry matter. These models in C3 plants (Farquhar et al., 1989) have been developed with similar structure considering the differential diffusivities of $^{12}CO_2$ and $^{13}CO_2$ through the stomata which is followed by RUBISCO's Discrimination (Eq3.).

$$\text{Eq3.} \quad \Delta = a + (b - a) \, \frac{p_i}{p_a}$$

Where (a) is the discrimination against $^{13}CO_2$ occurring during diffusion through the stomata, (b) is the fractioning by RuBisCO during carboxylation and (pi/pa) is the ratio of leaf-internal to atmospheric CO_2 partial pressure.

Numerous studies imply the significance of potassium supply in improving the biomass water use efficiency of crops (Kanai et al., 2011; Jákli et al., 2016) specifically under osmotic stress conditions. In spite of the general agreements that properties such as improvements in plant osmotic potential, regulation of stomata, improvement in photosynthesis (Peoples and Koch, 1979) and reduced dark respiration (Bottrill et al., 1970) fairly explain an improvement in biomass-WUE, the detailed causation of positive effects K on biomass and leaf water use efficiency is still not yet clear.

In fact, there are contrasting reports on how K deficiency affects plant water relations. Some studies (Benlloch-Gonzalez et al., 2008; Benlloch-González et al., 2010) reported higher transpiration occurring under K deficiency when plants are suffering from drought induced stress conditions. These findings are later associated with the reduction of water use efficiency in the respective studies. Gonzalez has backed up the results with the hypothesis that under multiple stress conditions (low potassium and drought stress) the closure of stomata by ABA signaling is impaired by ethylene, leading to an imperfect closure of stomata causing an inefficient water loss.

In contrast to these reports, there are studies done by Tomemori et al. (2002), Jin et al., (2011) which generally indicate stomatal closure under potassium deficiency conditions.

Moreover, Egilla et al. (2005) reported lower stomatal conductance under combination of drought and potassium deficiency in *Hibiscus* plants. Therefore, to fill the respective scientific gaps further research is required.

Potassium and drought have individually been the focus in many plant abiotic stress studies, however few (Wei et al., 2013; Jákli et al., 2016) have interactively studied the impact of potassium deprivation under drought conditions on plant water relations.

In this regard, plant physiological responses including plant water relation and its relation to potassium nutrition and PEG induced osmotic stress was studied in chapter 2 where water use efficiency on the leaf and plant scale has been mainly focused. Moreover, the other aim of chapter 2 is to address the contradiction between the studies related to stomatal conductance and potassium supply under osmotic stress conditions.

1.2 Effects of K nutrition on mitigation of oxidative stress in plants

Plant initial response to environmental stress with in the first minutes and hours after stress occurrence is alterations in the levels of phytohormones such as ABA as well as changes in the levels of toxic compounds known as reactive oxygen species (ROS). Both ABA and ROS act as signalers to environmental stress conditions activating downstream cascades contributing to plant survival in the stress period.

The most important ROS species in plants are hydrogen peroxide (H_2O_2), super oxide (O_2^-), hydroxyl radical (OH°) and singlet oxygen ($^1O_2$) (Apel and Hirt, 2004; Asada, 2006). There are several sources of ROS production from which the majority belong to the chloroplasts instantly responding to environmental stresses such as drought and/or high light intensity. Impairing photosystem II (PSII) manganese (Mn) clusters which catalyzes the extraction of electrons from water is one of the initial ROS productions sites. Under high light, the proton transfer by quinone molecules to lumen could reach more than the H^+-ATPase proton pump resulting in acidification of lumen. The latter eventuates in inhibition of the protection that extrinsic proteins provide to Mn cluster and thus water hydrolysis will be impaired leading to H_2O_2 production. The other known source of ROS production is ferredoxin (Fd) which is placed in the last step of $NADP^+$ reduction in photosystem I (PSI). As electrons are excited and passed through PSII and PSI, they reach Fd. Fd reduces $NADP^+$ to NADPH which is then used by ribulose bisphosphate carboxylase (RuBisCO), the enzyme in dark reactions of photosynthesis fixing carbons from CO_2. Ferredoxin has also the capacity to provide electrons to oxygen generating O_2^- in the chloroplast. Other important

ROS sources in plant cells are free transition metal ions (Shcolnick and Keren, 2006) and NADPH oxidases (Kadota et al., 2015).

The plants however, have evolved stress avoidance and detoxification mechanisms. There stress avoidance or safety valves include chlorophyll fluorescence and heat dissipation in a process known as non-photochemical quenching (Baker, 2008; Ruban, 2016). Both of these safety valves return the excited states of electrons to ground state by absorbing their energy and therefore, avoiding ROS concentration from reaching damaging levels. As soon as ROS concentration reach crucial levels, the antioxidant machinery comes into action and detoxifies ROS into non-toxic compounds (Asada, 2000, 2006).

The most important antioxidant enzymes in plants are superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) (Asada, 2006). When super oxide is produced by donation of electron from Fd to oxygen molecules, SOD which is sitting on thylakoid membrane immediately gets involved and convert it to H_2O_2. H_2O_2 subsequently will change back to water by the function of ascorbate peroxidase, which in return oxidizes ascorbic acid (AsA) into mono-dehydroascorbate (MDA). MDA could either be reduced back directly to AsA by receiving the electron of reduced Fd or by receiving the electron from NADPH in a reaction catalyzed by MDA reductase. The other fate of MDA is to spontaneously disproportionate into AsA and dehydroascorbate (DHA). DHA will then be converted to AsA via reduced glutathione (GSH) in a reaction catalyzed by dehydroascorbate reductase (Asada, 2000,). The function of GR is to keep GSH levels sufficient enough for detoxification activities. Since these series of reactions start with electrons driven by water and finish with turning back ROS into water, they are called the water-water cycle (Asada, 2000).

It is known that the water-water cycle is an alternative to the extra electron excitation under environmental stresses, however when the stress reach crucial levels, plants will suffer from photo-oxidative damage and chlorosis and necrosis symptoms will occur indicating chloroplast degradation.

Studies show that K deficiency inhibits translocation of assimilates negatively affecting the assimilation in plants (Cakmak et al., 1994; Cakmak, 2005). As a result of reduced assimilation, the dark reactions of photosynthesis (Calvin cycle) will not be able to receive the energy from excited electrons with the same rate as the light reactions are providing. Such occurrences will eventuate in increased ROS concentration in the plants. Similar scenario will happen to the plants when drought is occurring. Under drought, the stomata

will close or narrow resulting in reduced CO_2 concentration in the mesophyll cells. This means that RuBisCO will have reduced substrate (CO_2) and simultaneously plenty of energy is available (in the form of NADPH). Under these conditions, the reduced Fd pool will alternatively reduce O_2 molecules and super oxide is generated.

There are several studies reporting osmotic stress conditions as positive stimulator of plant ROS concentration up to several folds (Kubiś, 2008; Luna, 2004; Selote and Khanna-Chopra, 2010). The increased ROS surveyed in these studies was followed by increased antioxidant activity. Moreover, studies reported the impact of K deficiency on ROS generation and antioxidant defense (Shin and Schachtman, 2004; Tewari et al., 2007; Hafsi et al., 2011). Most of these studies have measured ROS and antioxidants at one time point after the stress was initiated while masking out the dynamical changes in ROS production and antioxidant response throughout the experiment. In fact, there are very few studies which have done such measurements with a particular focus on few time points after the onset of treatments (Türkan et al., 2005; Pompelli et al., 2010). Nevertheless, no study so far has focused on the kinetics of ROS and antioxidant response under combined effects of low potassium supply and osmotic stress conditions.

Accordingly, in chapter 3, the oxidative stress and antioxidant responses of the plants are evaluated under potassium and osmotic stress conditions to see how the dynamics between ROS and antioxidants is affected and how much of it could be explained by the antioxidants gene expression at the peak of each stress.

1.3 Whole genome responses to K starvation and osmotic stress

Plants are notified of the abiotic stress by sensing the increased levels of signalers such as H_2O_2, O_2^- (Kreslavski et al., 2012; Suzuki et al., 2012) and ABA (Raghavendra et al., 2010). Similar to the function of ABA in stomata closure, H_2O_2 can activate calcium channels in the guard cells which eventuates in release of K^+ from the guard cell and thereby closes the stomata (Köhler et al., 2003). Studies show that low potassium supply causes reduction in plant transpiration via stomatal closure (Tomemori et al., 2002; Jin et al., 2011). However, as already explained in section 1.1 of the prologue, there are a number of studies (Benlloch-Gonzalez et al., 2008; Benlloch-González et al., 2010) in contrast to this report that low K conditions when specially combined with osmotic stress, inhibits ABA signaling and leaves the stomata open negatively affecting WUE. Therefore, it is still under question