Chapter 1: Prologue

The world's population reached 7.3 billion in 2015 and according to the United Nations, the world's population will continue to grow by 1.18 % per year, projected to reach 8.5 billion in 2030 and 9.7 billion in 2050 (United Nations, 2015). Consequently, global demand for food is steadily rising and exerts a pressure on the need for more efficient crop production. However, efficient crop production is hampered by biotic and abiotic stress factors, such as drought. Drought poses the most important limitation to crop productivity in the world (Lauer et al. 2012) and increases in the frequency and intensity of drought events are predicted (Pachauri and Meyer 2014). This raises the concern about availability of water resources in cropping systems and concomitantly the topic of understanding how water-use efficiency (WUE) can be improved. In this context, one aspect is the role of plant management practices like plant nutrition by fertilizer application. Plant nutrient management has an indirect effect on WUE by affecting plants physiology and its modification can increase WUE by 15 to 25 % (Hatfield et al. 2001). The plant macronutrients magnesium (Mg), nitrogen (N) and potassium (K) are inevitable for maximal plant performance and deficiency in either of them will cause disturbances in proper functioning of the plants' physiology.

1.1 Water-use efficiency and its distinction between leaf-WUE and biomass-WUE

Stomata play an essential role in regulating both water losses by transpiration and CO_2 uptake for photosynthesis and thus, plant growth (Damour et al. 2010). As plants usually grow in continually fluctuating conditions, the capacity for rapid adaptation, i.e., optimizing CO_2 uptake and water loss, is crucial for plant viability. In this context, already in 1927 Scarth stated: "When stomata regulate one process they must regulate the other also but the question remains as to which of these actions represents the real role of the stomata in the economy of the plant […] When they do function, i.e. open, is it primarily to allow of transpiration or the assimilation of CO_2?" (Scarth 1927). Plants aim at maximizing CO_2 assimilation for a fixed amount of water loss which is described in the stomata optimization theory (Cowan 1978). The ratio of assimilated carbon to transpired water by the plant is described by the water-use efficiency (WUE) which can be considered a measure for the efficiency in optimizing carbon assimilation while minimizing water use (Bramley et al. 2013). The term water-use efficiency is quite flexible and depends on the

scale which is considered. It can either be considered on short term on the leaf level (leaf-WUE) or on long term on the whole plant level (biomass-WUE). Leaf-WUE is defined as

$$\text{Leaf-WUE} = \frac{A}{g_s} \quad (1)$$

where A is the net CO_2 assimilation rate and g_s is the stomatal conductance to CO_2 describing the ratio of transpiration to air-to-leaf vapour pressure deficit. From Fick's law of diffusion, A can be expressed as

$$A = g_s(C_a - C_i) \quad (2)$$

where C_a denotes the CO_2 concentration in ambient air and C_i is the CO_2 concentration in sub-stomatal cavities. Under steady state conditions, A can also be expressed as the product of mesophyll conductance (g_m) and the difference of CO_2 concentrations at the sub-stomatal cavities (C_i) and carboxylation site in the chloroplasts (C_c):

$$A = g_m(C_i - C_c) \quad (3)$$

Combining Eq. 2 and Eq. 3, A can be expressed as (Niinemets et al. 2009)

$$A = \frac{1}{\frac{1}{g_s}+\frac{1}{g_m}}(C_a - C_c) \quad (4)$$

The latter equation indicates that assimilation depends on g_s and g_m. A change in g_s affects the water use of a plant, whereas g_m only affects photosynthesis and thus enhancing the g_m/g_s ratio can improve both WUE and yield (Flexas et al. 2013). The assimilation rate reflects changes in both stomatal conductance and mesophyll capacity for photosynthesis. The latter depends on the capacity for electron transport to regenerate ribulose bisphosphate and on the activity of Rubisco (von Caemmerer and Farquhar 1981) as this contributes to the CO_2 drawdown from internal air space to chloroplasts. However, environmental cues such as nutrient deficiency can alter the photosynthetic capacity, thus affecting C_c, and stomatal aperture has to be synchronized with the CO_2 requirement at the site of carboxylation in the mesophyll (Raschke 1975). Under these conditions, the ratio of C_i/C_a was reported to remain constant (Wong et al. 1979) and Raschke (1975) proposed that stomata sense the demand for CO_2 by sensing the CO_2 concentration influenced by photosynthesis or CO_2 fixation in the dark of the intercellular spaces. Hence stomatal opening is a response to CO_2 concentrations in the leaf and stomata constitute a component of feedback loops. This was shown in studies with DCMU, an inhibitor of photosynthesis, which was applied to plants through the transpiration flow (Cummins et al. 1971; Wong et al. 1979). By inhibiting photosynthesis in the mesophyll, intercellular CO_2 concentrations increased and thereby stomatal closure was indirectly evoked.

By stomata regulation, the leaf is provided with a simple mean to change the partial pressure of CO_2 at the site of carboxylation and thus, assimilation (Farquhar and Sharkey 1982), and transpiration. In reverse, stomatal opening and closing can be a response to CO_2 concentrations ($[CO_2]$) in the leaf. However, it is not certain which feedback loop determines the stomatal apertures as CO_2 exchange and water-vapour loss are always simultaneously affected and stomata control system is rarely in a steady state making stomata responses difficult to interpret. However, clearly regulation of stomata aperture at the leaf level can be considered a strategy for optimal economizing carbon-water balances of a whole plant (Katul et al. 2010).

Biomass-WUE takes into account the whole plant biomass and can be defined as plant dry matter production per unit of water loss via transpiration during the vegetation period (Tallec et al. 2013). Several parameters affect biomass-WUE related to biomass formation such as photosynthesis, respiratory carbon loss, and whole plant transpiration such as transpiration and unproductive water-loss by e.g. nocturnal transpiration (Claussen 2002; Wang et al. 2013) and thereby making this parameter more complex compared to leaf-WUE. Biomass-WUE can be written as (Farquhar and Richards 1984)*:*

$$\text{Biomass-WUE} = \frac{A(1-Q_c)}{E(1+Q_W)} \quad (5)$$

where E is transpiration, Q_c is the fraction of assimilated carbon loss (e.g., respiration, root exudation) and Q_w is the fraction of total water loss that is 'unproductive' (e.g., from non-photosynthetic parts of the plant or through open stomata at night). Under field conditions, only aboveground biomass (shoot) is considered for calculation of WUE as determination of belowground biomass (roots) is fairly challenging, whereas in greenhouse experiments, where roots can easily be harvested, total biomass can be used for calculation of WUE (Bramley et al. 2013). Environmental variables such as nutrient supply, water scarcity and elevated CO_2 concentrations were shown to impact biomass-WUE (Cernusak et al. 2009; Lewis et al. 2011).

With 200 to 1000 g of transpired water per 1g of assimilated carbon, the amount of water which is transpired by the plant is relatively high compared to the amount of carbon that is fixed (Bramley et al. 2013). Manipulating this ratio towards less transpiration and relatively more assimilation might contribute to the stabilization of yields under present and future conditions of diminishing freshwater supply and increasing food demand. As the regulation of both stomata and CO_2 fixation plays a central role in adaptation to stress conditions, such as nutrient deficiency, it is of paramount importance to try to improve our understanding of the way WUE changes in response to these environmental constrains.

1.2 The plant nutrient magnesium

The discovery of magnesium (Mg) dates back to 1729 when Hoffmann, a German chemist, was able to distinguish magnesia from lime. Research on this element continued throughout the centuries and in the early 20th century, Richard Willstätter elaborated the structure of chlorophyll and that Mg is present as the central metal within that molecule. Up to today, research on this element is conducted and still not all of the functions and influences on diverse physiological roles in plants are well understood (Grzebisz 2015). For plants, Mg is an essential element which is present in concentrations of 15-20 mM in the plant cell. In the metabolic pool, Mg concentrations in the cytosol are 0.2-0.4 mM, in the chloroplast 10-15 mM and in the mitochondria 0.2-0.5 mM, and metabolically inactive Mg is stored in the vacuole with concentrations of 5-80 mM (Hermans et al. 2013). Magnesium is taken up by plant roots from the soil solution where the Mg concentration ranges from 0.125 to 8.5 mM. However, a concentration of 0.3 to 0.4 mM in the soil solution is enough to achieve the optimum plant tissue concentration of 1-3 g Mg kg^{-1} dry matter. Magnesium in soils originates from silicates and carbonates and varies between 0.05 % and 0.5 % depending on the Mg content in the source rock (Gransee and Führs 2013). Magnesium deficiency can occur due to low Mg contents in source rocks (Papenfuß and Schlichting 1979), to Mg losses by leaching (Grzebisz 2011) and/or Mg losses due to crop removal with concurrent unbalanced crop fertilization (van der Pol and Traore 1993). Imbalances in crop fertilization practices can also lead to cation competition which can cause low Mg availability. The uptake of Mg is influenced by the availability of other cations (Gransee and Führs 2013). Soils with a low pH and sandy soils, especially where high rates of potassium or ammonium fertilisers are applied (Senbayram et al. 2015), have a high risk of Mg deficiency.

Plant uptake of Mg ions (Mg^{2+}) from the soil solution can be either via an apoplasmic pathway where Mg^{2+} is passively transported into the apoplast of the root cortex, or via a symplasmic pathway where Mg^{2+} enter the cortex cells through carriers and diffuse from cell to cell by plasmodesmata (Hermans et al. 2013). Magnesium transport inside the plant is achieved by transporters belonging to the *MRS2* family (*Mitochondrial RNA Splicing 2*) and *Magnesium/Proton Exchanger* (*MHX*). However, molecular details of Mg^{2+} transport are not yet well understood and precise information on the physiological functions of the respective genes is limited. Magnesium is a phloem-mobile element and is translocated to fruits, seeds and tubers and to growing leaves under Mg-deficient conditions, hence deficiency symptoms appear on older leaves usually as interveinal chlorosis.

The functions of Mg in plants are diverse and in the following only some are described in more detail. The probably best known function is as the inner metal of chlorophyll molecules. Here, Mg cannot be replaced by other ions. Between 6 and 25 % of total Mg is bound to chlorophyll, with in general a higher proportion being associated with chlorophyll under Mg deficiency (Marschner 2012). Biosynthesis of chlorophyll includes the insertion of Mg^{2+} into the protoporphyrin IX which is catalysed by Mg chelatase. The activity of this enzyme is in turn affected by Mg^{2+} concentrations in the stroma and requires ATP, thus additional Mg. Another involvement of Mg in photosynthesis is its influence on grana stacking in chloroplasts (Hall et al. 1972; Ceppi et al. 2012). The degree of stacking increases with increasing Mg concentrations (Stys 1995). Low Mg supply leads to disruption of grana stacks which may adversely affect photosynthetic performance of plants. Magnesium ions affect the ribosomal subunit association and its activity and control the structural flexibility of numerous ribosomal proteins (Yamamoto et al. 2010). It was shown by high resolution X-ray structures that more than hundred Mg ions are associated with the large ribosomal subunit (Petrov et al. 2012). Hence, Mg plays a crucial role in protein synthesis. Many enzymes require Mg, such as glutathione synthase, phosphoenolpyruvate carboxylase, fructose-1,6-bisphosphate and Rubisco (Marschner 2012). The latter enzyme binds Mg and thereby the affinity for CO_2 and the turnover rate are increased. As this enzyme catalyses the first step of carbon fixation, it is not surprising that a reduction of net assimilation rates was observed in a wide variety of plant species supplied with low levels of Mg (Fischer and Bremer 1993; Yang et al. 2012; Tränkner et al. 2016). The enzyme ATPase uses a Mg-ATP complex as substrate. Plasma membrane ATPases are involved in phloem loading of sucrose, thus contributing to carbon partitioning. During phloem loading, sucrose has to be transported from the apoplasm into the phloem against a concentration gradient (Ainsworth and Bush 2011). This is achieved by using the pH gradient as metabolic energy which is generated by H^+-ATPases. Under low Mg supply, cytosolic Mg concentrations are insufficient which leads to dissociation of the MgATP complex. As a consequence, sucrose and other carbohydrates accumulate in leaves and carbohydrate supply to the roots and other young growing organs is impaired. The accumulation of sugars in leaves is the first of metabolic events responding to Mg deficiency, followed by a disturbance of dry matter partitioning, chlorosis, enhanced production of reactive oxygen species (ROS) and necrosis (Marschner 2012).