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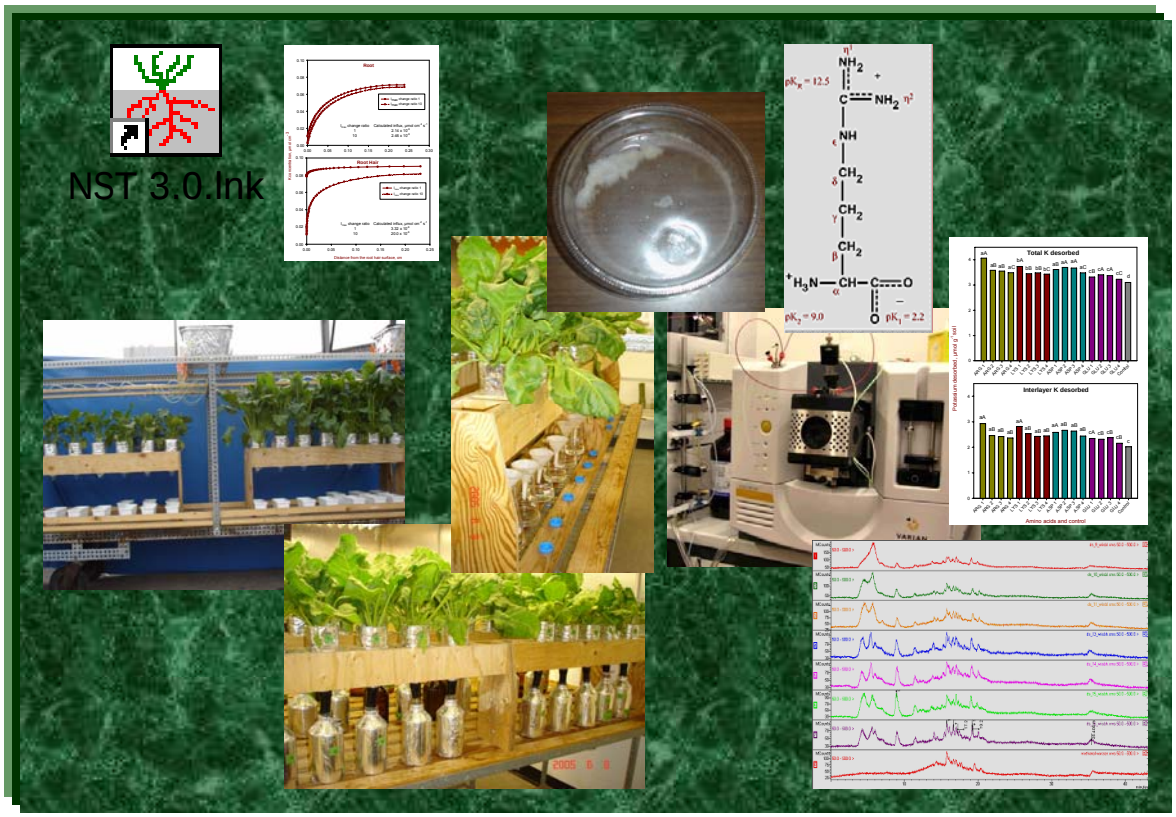
Potassium Uptake Efficiency Mechanisms and Root Exudates of Different Crop Species



Potassium Uptake Efficiency Mechanisms and Root Exudates of Different Crop Species

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Dedicated to my parents

Abstract

Potassium uptake efficiency is the ability of plants to take up sufficient K under low soil K availability. Plant species differ in their K uptake efficiency. This study was done with the objective to investigate the possible mechanisms responsible for the differences in K uptake efficiency of crop species. Potassium uptake efficiency and K dynamics in the rhizosphere of maize, wheat and sugar beet were evaluated by a pot experiment which was conducted on K deficient soil with and without K fertilization. Sugar beet and wheat can take sufficient K under low soil K supply and therefore are uptake efficient for K. High K uptake efficiency in wheat was mainly due to its large root system. Sugar beet has few roots, but it could acquire more K per unit shoot dry weight, because of higher K influx. The nutrient uptake model (NST 3.0) could satisfactorily predict K influx in all the crops under high K supply, however under low K supply; the model prediction was 0.64, 0.68 and 0.31 times the measured K influx for maize, wheat and sugar beet, respectively. The severe under prediction in case of sugar beet indicated that processes not considered in the model were important for the high K uptake efficiency. Results of sensitivity analysis showed that initial soil solution K concentration (C_{Li}) is the most important parameter responsible for the differences in the measured and calculated K influx of wheat, maize and sugar beet. However, the mechanisms responsible for increasing C_{Li} in rhizosphere of different plant species is not clear yet, whether it is due to the capacity of plant root to release some organic compounds, which can solubilize K from the non-exchangeable fraction of soil or it is due to the indirect effect of higher I_{max} (maximum K uptake capacity) of the root and/or root hairs.

To study the root exudation pattern, wheat and sugar beet plants were grown in quartz sand supplied with modified Hoagland nutrient solution of low and high K levels at two growing conditions, one in screen house under natural environmental conditions and another in growth chamber under control conditions. Root exudates were collected by percolation method. Root exudation rate was many-fold higher under low K compared to high K supply in both the crops and was higher in young

plants and at natural sun light, perhaps due to higher light intensity in the screen house. HPLC analysis of the root exudates showed that exudation rate of organic acids, amino acids and sugars was higher under low K supply in both the crops and it was higher in wheat compared to sugar beet. Arginine was the amino acid detected only in root exudates of sugar beet. The results of mobilization of K in a K fixing soil by amino acids, as found in root exudates showed that total K desorbed by Arginine was the highest. Arginine might work like long chain n-alkyl ammonium compound, which could widen the interlayer of clay mineral resulting in a higher soil solution K concentration. Though amino acids can desorb K in K fixing soil, but degree of desorption does not seem to be sufficient to explain the differences in soil solution K concentration in the rhizosphere of wheat and sugar beet grown on low K soil.

Non-targeted metabolite profiling was done by separating the root exudates collected from plants grown in the growth chamber by HPLC coupled with ESI-MS. Several signals and change in intensity of certain signals specific for root exudates from K deficient plants were found. Signal corresponding to m/z value 475 was relatively stronger under low K supplied sugar beet. From KEGG data base, one of the possible structures for m/z 475 was Amastatin ($C_{21}H_{38}N_4O_8$), which resembles to n-alkyl ammonium compound in chemical structure. Further investigation is needed to identify the compounds corresponding to the signals and to study their effect in desorbing K in low K soil.

Zusammenfassung

Pflanzenarten unterscheiden sich in ihrer Kaliumaufnahmeeffizienz, d.h. der Fähigkeit auch bei geringer K-Verfügbarkeit im Boden ausreichend K aufzunehmen. In dieser Arbeit wurden mögliche Ursachen für die Unterschiede in der Effizienz untersucht. In einem Topfexperiment mit einem gedüngten und ungedüngten K-Mangelboden wurde die K-Aufnahme von Mais, Weizen und Zuckerrübe sowie die K-Dynamik im Boden bestimmt. Zuckerrübe und Weizen zeigten sich aufnahmeeffizient, da sie auch ohne Düngung genügend K aufnehmen konnten. Die hohe Aufnahmeeffizienz von Weizen war in seinem großen Wurzelsystem begründet. Zuckerrübe hatte vergleichsweise wenig Wurzeln, konnte aber dennoch höhere K-Sprossgehalte als Weizen erzielen, weil sein K-Influx hoch war. Der gemessene K-Influx wurde mit Ergebnissen eines Nährstoffaufnahmemodells (NST 3.0) verglichen, das die Sorption, den Bodentransport und die Aufnahmephysiologie beschreibt. Bei hohem K-Angebot im Boden stimmten Mess- und Simulationsergebnisse gut überein, jedoch unter K-Mangel errechnete das Modell für Mais, Weizen und Zuckerrübe nur 64%, 68% bzw. 31% der gemessenen Aufnahme. Die deutliche Unterschätzung bei Zuckerrübe deutet darauf hin, dass weitere Prozesse als die im Modell berücksichtigten für die K-Aufnahmeeffizienz verantwortlich waren. Eine Sensitivitätsanalyse zeigte, dass die K-Konzentration der Bodenlösung ein wichtiger Parameter ist, so dass die Erhöhung dieser Konzentration eine mögliche Ursache für die Aufnahmeeffizienz darstellt. Allerdings ist bislang nicht bekannt, in welcher Weise Pflanzen die K-Konzentration in der Bodenlösung beeinflussen können. Eine Möglichkeit wäre die Exsudation organischer Stoffe, die nichtaustauschbares K in Lösung bringen könnten. Ein weiterer Effizienzmechanismus könnte die Erhöhung der Aufnahmekapazität (ein erhöhtes I_{max}) der Wurzel und/oder Wurzelhaare sein.

Zur Untersuchung der Wurzelexsudation wurden Zuckerrübe und Weizen bei niedriger und hoher Kaliumversorgung in Quarzsand angezogen. Die Pflanzen standen sowohl im Freiland (Drahthaus) als auch in der Klimakammer. Die

Wurzelexsudate wurden durch Perkolation gewonnen. Die Exsudationsraten beider Arten waren bei K-Mangel um ein mehrfaches erhöht im Vergleich zu gut versorgten Pflanzen. Zudem waren sie höher bei jüngeren Pflanzen und im Freiland, vermutlich wegen der höheren Einstrahlung. Die Analyse der Exsudate mittels HPLC zeigte, dass unter K-Mangel die Ausschüttung an organischen Säuren, Aminosäuren und Zucker erhöht war. Dies galt für beide Pflanzenarten, allerdings war die Exsudation bei Weizen stärker erhöht als bei Zuckerrübe. Arginin wurde ausschließlich in den Ausscheidungen der Zuckerrübe gefunden. Inkubationsversuche, in denen die Fähigkeit der Aminosäuren untersucht wurde, K im Boden zu mobilisieren, zeigten, dass Arginin die höchste Mobilisierungskapazität hat. Die Wirkung des Arginin könnte ähnlich der von langkettigen n-alkyl Ammoniumverbindungen sein, die die Zwischenschichten der Tonminerale aufweiten und so die Desorption nichtaustauschbaren Kaliums erhöhen. Obwohl Aminosäuren die Kaliumdesorption in stark fixierenden Böden anregen, ist die resultierende Erhöhung der K-Bodenlösungskonzentration nicht in der Größenordnung wie sie gemäß der Modell-Sensitivitätsanalyse sein müsste, um die Aufnahmeeffizienz von Weizen und Zuckerrübe zu erklären.

Die Wurzelexsudate der Klimakammerpflanzen wurden mittels HPLC gekoppelter ESI-MS getrennt, um weitere Bestandteile zu charakterisieren. Es wurden einige Signale gefunden, die bei K-Mangel auftraten, bspw. das Signal mit dem m/z-Wert 475 war bei Zuckerrübe deutlich erhöht. Laut der KEGG-Datenbank könnte dies Amastatin ($C_{21}H_{38}N_4O_8$) sein, das ähnliche Strukturen wie n-Alkyl Ammonium aufweist. Weitere Untersuchungen sind nötig, diese Substanz genau zu bestimmen und ihre K-Mobilisierungskapazität im Boden zu messen.

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General introduction

1 General introduction

Potassium (K^+) is an essential macronutrient and the most abundant cation in higher plants. Potassium plays an essential role for enzyme activation, protein synthesis and photosynthesis. It also mediates osmoregulation during cell expansion and stomatal movements. Furthermore, K^+ is necessary for phloem-solute transport and for the maintenance of cation: anion balance in the cytosol as well as in the vacuole (Mäser et al., 2002). With the progressive intensification of agriculture and introduction of high yielding varieties, the soils are getting depleted in reserve K at a faster rate. As a consequence, K deficiency is becoming one of the major constraints to crop production. A key question is whether present K management recommendations are adequate to meet future needs. Recent research suggests that (i) commonly used soil tests may not always reflect the actual crop response to K, (ii) crop K requirements per unit yield are not constant, but vary with the absolute yield levels and crop management factors, (iii) spatial variability of soil K affects K management strategies, (iv) genotypic differences exist in response to soil and fertilizer K and (v) non-yield traits such as stalk strength or product quality must be taken into account in K management decisions. Therefore future K management recommendations should be more robust and accommodate different crops, cropping systems, crop management technologies, soil conditions, and climate-driven yield potential (Dobermann, 2001). Screening K efficient cultivars and growing those under low K supply conditions could be one of the major components contributing to more specific K management recommendations.

1.1 Potassium availability and nutrient dynamics in the rhizosphere

Potassium is the fourth most abundant nutrient, constituting about 2.5% of the lithosphere. However, actual soil concentrations of this nutrient vary widely, ranging from 0.04 – 3% (Sparks and Huang, 1985). The availability of potassium to the plant is highly variable, due to complex soil dynamics, which are strongly

influenced by root–soil interactions. In accordance with its availability to plants, soil K is ascribed to four different pools: (i) soil solution, (ii) exchangeable K, (iii) non-exchangeable K and (iv) lattice K (Syers, 1998). As plants can only acquire K^+ from solution, its availability is dependent upon the K dynamics as well as on total K content. The exchange of K between different pools in soil is strongly dependent upon the concentration of other macronutrients in the soil solution, for example, nitrate (Yanai *et al*., 1996). The release of exchangeable K is often slower than the rate of K^+ acquisition by plants (Sparks and Huang, 1985) and consequently, soil solution K^+ concentration in some soil is very low (Johnston, 2005). Plant K status may further deteriorate in the presence of high levels of other monovalent cations such as Na^+ and NH_4^+ that interfere with K uptake (Qi and Spalding, 2004). Apart from long-term deprivation, plant roots can experience transient shortages of K because of spatial heterogeneity and temporal variations in the availability of this nutrient. The main source of soil heterogeneity is often the plant roots themselves, the K^+ transport activity of which creates zones with elevated or reduced nutrient concentration. Contact between a root and nutrient may occur because of (i) root growth into the area where a nutrient is located (root interception), and (ii) transport of a nutrient to the root surface through the soil (Jungk and Claassen, 1997). Root interception constitutes less than 1-2% of total K^+ uptake because of rapid removal of K^+ at the root surface (Barber, 1985; Rosolem et al., 2003). The second process, K^+ translocation through the soil to the root surface, is facilitated by diffusion and mass flow (Barber, 1962). Diffusion is the most dominant mechanism of K^+ delivery to the root surface (Seiffert et al., 1995) and constitutes up to 96% of total soil K^+ transport (Oliveira et al., 2004). Therefore, K^+ depletion around the root is the most frequently observed phenomenon associated with plant-evoked soil K perturbations. If K^+ delivery by diffusion is always associated with the reduction of K^+ content in the areas adjacent to the root surface, mass flow may conversely result in K^+ accumulation around the root if transpiration and K concentration in soil solution is high (Ca-lactate extractable K of the soil was 366 µmol K kg^{-1} soil) (Vetterlein and Jahn, 2004). Experimentally, development of a depletion profile around individual maize root segments has been demonstrated

using ^{86}Rb as a potassium tracer (Jungk and Claassen, 1997). These data are consistent with results obtained by Yamaguchi and Tanaka (1990), who demonstrated that roots compete for K if half distance between them is less than 4 mm. Similar results were obtained with flat mats of maize (*Zea mays* L.), rape (*Brassica napus* L.), and rice (*Oryza sativa* L.) roots (Jungk and Claassen, 1997; Hylander et al., 1999; Vetterlein and Jahn, 2004).

Variations in soil density may also affect potassium availability. Soil compaction is associated with higher volumetric water content and therefore tends to facilitate K^+ transport to the root surface (Kuchenbuch et al., 1986). However, the dense soil may also cause a reduction in the root length and so the higher bulk density does not necessarily result in increased K^+ accumulation (Seiffert et al., 1995). The spatial heterogeneities in K^+ distribution encountered by a root are often superimposed with temporal variations in K^+ availability, caused by continuously changing soil moisture content. In dry soils, bulk K^+ content is normally higher, but mass flow and diffusion are restricted (Seiffert et al., 1995; Vetterlein and Jahn, 2004; Kuchenbuch et al., 1986). The negative effects of drought on K^+ transport in soil are likely to be more significant than increases in K^+ concentration and therefore these environmental conditions lead to reduced availability of the nutrient (Seiffert et al., 1995; Liebersbach et al., 2004).

Potassium starvation is known to activate K^+ uptake in plants (Fernando et al., 1990; Shin and Schachtman, 2004). This activation has been conventionally associated with induction of expression of high affinity transporters, and was considered as a major mechanism of adaptation to K^+ starvation. Growing roots continuously experience variations in potassium availability, to which they have to adjust their physiology and growth pattern. In order to optimize their performance as nutrient uptake organs and to compete for K^+ uptake in the dynamic and heterogeneous environment, plant roots developed mechanisms of acclimation to the current K^+ status in the rhizosphere. All these acclimation strategies enable plants to survive and compete for K in a dynamic environment with a variable availability of K (Ashley et al., 2006).

1.2 Potassium efficiency mechanisms

It is known from long term experiments that plants differ in their K efficiency, i.e. some plant species obtain higher yield despite a low soil K supply whereas other species fail (Meyer, 1993; Trehan and Claassen, 1998; Sadana and Claassen 1999; Zhang et al., 1999; Steingrobe and Claassen, 2000). This efficiency can be due to different mechanisms i.e. use and uptake efficiency. Use efficient plants can obtain relative high yield with a low K concentration in their dry matter, whereas uptake efficient plant can take up sufficient K despite a low soil K supply level. The size of the root system, the physiology of uptake and the ability of plants to increase K solubility in the rhizosphere are considered as mechanisms of uptake efficiency. Sugar beet and wheat both are uptake efficient for K (Dessougi et al., 2002). However, both species use different mechanisms.

Potassium uptake and supply level of the soil can be described by a mechanistic model. The model calculates the diffusive and convective transport of nutrients towards the root under consideration of sorption and desorption processes. The uptake rate is calculated by Michaelis-Menten kinetics (Claassen et al., 1986; Claassen and Steingrobe, 1999). Applying nutrient uptake model calculations (Claassen and Steingrobe, 1999) on wheat, it can be shown that the high K uptake efficiency of wheat is mainly due to its large root system, where calculated transport and uptake agreed well with measured data. However, sugar beet could realize much higher uptake rate than calculated by the model despite of having few roots. This indicates besides diffusion, convection and desorption, other processes may be important for K supply, which are not described by the model. Sugar beet seems to increase the chemical availability of K in the soil. Usually, only K in solution and K sorbed at clay minerals, which is in equilibrium with solution K, counts as plant available. Only this exchangeable K is considered in the model calculations. However, it has been shown that non-exchangeable K can also be used by plants when the available fraction is too low for sufficient supply. Until now, it is not known in which way plants increase the availability of non-exchangeable K and why some plant species perform better than others. Plant

species with increased capacity to render sparingly soluble nutrient forms into plant available ones or with a higher capacity to transport nutrients across the plasma membranes are considered to possess high nutrient uptake efficiency (Rengel, 1999). However, if the rate of nutrient replenishment at the root surface is much lower than the capacity of the root cells to take up nutrients, uptake will be governed by the nutrient supply rather than by the nutrient uptake capacity of the root cells (Rengel, 1993). Hence greater uptake capacity of the root cells such as high affinity nutrient uptake systems would have an insignificant contribution to higher uptake efficiency for transport limited nutrients, for example, P, K, Zn, Mn and Cu (Rengel, 1999).

Chemical mobilisation of nutrients in the rhizosphere is reported to be caused by (i) changes in pH through H^+ release which is related to increase in cation: anion uptake ratio, (ii) root exudates and (iii) the presence of micro-organisms and their interactions with plant roots and/or exudates (Marschner, 1995). The released protons take part in the exchange processes at the edges of the inter-layers of clay minerals, widen them and increase the exchangeability of the interlayer K. The occurrence of root-induced release of K from K bearing minerals has been frequently associated with the lowering of K concentration in the solution resulting from root uptake as a result of dynamic equilibrium reaction between the phases of soil K (Hinsinger and Jaillard, 1993). The decrease of K-concentration in the vicinity of rye grass roots shifts the exchange equilibrium between internal- and external-K at the mica-solution interface. When K-concentration in the solution fell below a threshold value of about 80 µmol L^{-1}, the release of interlayer-K became significant. The release of interlayer K increases when the concentration of soil-solution K and/or exchangeable K decreases due to K uptake by plants and leaching (Hinsinger and Jaillard, 1993; McLean and Watson, 1985). In rape (*Brassica napus* cv Drakkar) after 8 days of cropping, the contribution of non-exchangeable K to K uptake ranged from 50% in the fine clay to 80-100% in the coarser fractions. The silt fractions provided a major part of the supply of K by these soils due to their high supplying power and their relative abundance (Niebes et al., 1993). Meyer and Jungk (1993) reported that 64 and 79% of the K taken up

by wheat and sugar beet plants, respectively, grown on luvisol in pot experiment was derived from the rapidly released 'exchangeable' and 21-36% from the less mobile 'non-exchangeable' soil K fraction. Wang et al. (2000) reported that the net release of K from the mineral K pool was significantly enhanced when the crops grew in feldspar and the enhanced mobilization of mineral K might be attributed to the release of organic acids from the plant roots. When gneiss of various particle sizes was exposed to malic and tartaric acids, both acids had a direct positive influence on the release of mineral K from gneiss.

1.3 Role of K^+ transporters and K^+ channel in K uptake

Potassium is the most abundant cation in plants and is required for plant growth. To ensure an adequate supply of K^+, plants have developed a number of highly specific mechanisms to take up K^+ from the soil; these include the expression of K^+ transporters and K^+ channels in root cells. Potassium channels play an important role in K^+ uptake as well as the control of membrane potential (Brüggemann et al., 1999), growth and turgor driven movements (Moran et al., 1988; Schroeder et al., 1984). Potassium channels can be divided into outward rectifiers (K_{out}) which excrete potassium from the cell, inward rectifiers (K_{in}) which transport potassium ions into the cell and largely voltage-independent channels ($K_{in/out}$) which are able to catalyze both processes. Despite the fact that root epidermal and hair cells are in direct contact with the soil, the role of these tissues in K^+ uptake is not well understood. Downey et al. (2000) reported the molecular cloning and functional characterization of a novel potassium channel KDC1, which forms part of a new subfamily of plant K_{in} channels. KDC1 was isolated from carrot root RNA and *in situ* hybridization experiments show KDC1 to be highly expressed in root hair cells. A combination of *in situ* hybridization experiments and comparative electrophysiological studies of the gene product expressed in Chinese Hamster Ovary (CHO) cells and K^+ channels in root hair cells, identified KDC1 as the major inwardly rectifying K^+ channel of carrot root hair cell plasma membranes. Root hairs and the endodermis with the casparian strip are exposed places for

potassium uptake (Tester and Leigh, 2001). In Arabidopsis root hairs, AtKC1 and AKT1 are part of a functional K^+-influx channel. As AtKC1 influences the apparent K^+ conductance of whole-cell inward currents and has a maximum expression in root hairs and endodermis with the casparian strip, AtKC1 is likely to be a K^+-uptake modulator subunit needed to adjust the characteristics of plant potassium uptake channels such as AKT1 (Reintanz et al., 2002). A family of 13 genes, named AtKT/KUP is involved in K^+ transport and translocation. In Arabidopsis, ten AtKT/KUPs were expressed in root hairs, but only five were expressed in root tip cells which suggested an important role for root hairs in K^+ uptake (Ju et al., 2004). Even though not much research has been done on effect of root hairs on K uptake of different crop species, but there are some evidences for phosphorus. Several researchers reported the contribution of root hair to total P uptake in different plant species. Root hairs increased P uptake over that due to the plant root alone in six different plant species that varied widely in root hair length, density and radius and sensitivity analysis showed a significant contribution of root hairs to P uptake (Itoh and Barber, 1982). The basis for large proportion of P uptake by root hairs was explained by several researchers as (i) root hairs increase the absorbing surface area- in case of spinach, it was 1.9-fold higher than that of the root cylinder (Föhse et al., 1991), (ii) root hairs have a very small radius (approximately 0.005 mm; Barber, 1995), so that P concentration at the root hair surface remains higher than that at the root cylinder, which leads to a higher influx per unit surface area, (iii) root hairs grow into soil perpendicular to the root surface and thereby increase the radius of the P absorbing body (root cylinder plus root hairs). This causes greater transport of P to the root (Föhse et al., 1991; Claassen, 1990; Kovar and Claassen, 2005).

1.4 Root exudates- an overview

The hidden half of a plant system thrives in a diverse, ever changing environment with bacteria, fungi, and other microorganisms feeding on an array of organic material (Ryan and Delhaize, 2001). Thus, the area of soil surrounding a plant root

represents a unique physical, biochemical, and ecological interface between the roots and the external environment. This so-called rhizosphere is in part regulated by the root system itself through chemicals exuded/ secreted into the surrounding soil. The release of all forms of carbon from roots has been termed as rhizodeposition (Marschner, 1995). Rhizodeposition products, which are available for microbial metabolism in the rhizosphere (zone adjacent to the root) and on the rhizoplane (root surface), can be categorized as exudates, lysates, secretions and gases. The difference between exudates and secretions is that, exudates are passively released and secretions are actively released. Secretions include polymeric carbohydrates and enzymes (Whipps, 1990). The products of extensive cell degeneration have been termed "root lysate" for example: sloughed-off root hairs or root cap, epidermal, and cortical cells (Liljeroth et al., 1990). The most common definition of the term "root exudates" is the substances which are released into the surrounding medium by healthy and intact plant roots (Rovira, 1969) and is the definition used in our study.

Root exudates include high and low molecular weight compounds. High molecular weight compounds in root exudates include the mucilage, gelatinous material covering root surfaces, and ectoenzymes. Phosphatase is an ectoenzyme that mobilizes organic P in the soil for plant use. Low molecular weight root exudates are released in larger quantities and include organic acids, sugars, phenolics, amino acids, phytosiderophores, flavonoids (Marschner, 1995), and vitamins (Whipps, 1990). Phytosiderophores are natural chelating agents known to be important for plant iron nutrition. The term "root exudates" is used in the literature to describe all organic compounds released from roots. An inclusive list of root exudates component found in the literature (Uren, 2001), which includes over 100 different compounds, is also representative of a list of potential cell chemical constituents. The major source of the addition of cell contents to the rhizosphere is root border cells, formerly known as sloughed off root cells. These cells are living when released from the root and act as an interface between the soil and root through protection of the root as it grows though the soil and interacts with soil microbes (Hawes et al., 1998). Although quantitative comparisons of exudates

vary widely, average estimates have been reported in the literature. Using axenic wheat, Prikryl and Vancura (1980) expressed root exudates as 50% of the root dry weight or 12% of the whole plant dry weight over a growing season. Based on a compilation from the literature, Lynch and Whipps (1990) described rhizodeposition as 4-70% of carbon allocated to the roots, which is 30-60% of net photosynthetic carbon. Soil-chemical changes related to the presence of these compounds and products of their microbial turnover are important factors affecting microbial populations, availability of nutrients, solubility of toxic elements in the rhizosphere and thereby, enabling the plants to cope with adverse soil-chemical conditions.

Organic acids are low-molecular weight compounds which are found in all organisms and which are characterized by the possession of one or more carboxyl groups. Depending on the dissociation properties and number of these carboxylic groups, organic acids can carry varying negative charge, thereby allowing the complexation of metal cations in solution and the displacement of anions from the soil matrix. For this reason, they have been implicated in many soil processes including the mobilisation and uptake of nutrients by plants and microorganisms (e.g., P and Fe), the detoxification of metals by plants (e.g., Al), microbial proliferation in the rhizosphere, and the dissolution of soil minerals leading to pedogenesis (e.g., podzolisation) (Marschner, 1995). A full assessment of their role in these processes, however, cannot be determined unless the exact mechanisms of plant organic acid release and the fate of these compounds in the soil are more fully understood (Jones, 1998). Typically the total concentration of organic acids in roots is around 10-20 mM (1–4% of total dry weight) which can be compared, at least for maize, with the other main organic solutes present in root cells, namely amino acids (10–20 mM) and sugars (90 mM) (Jones and Darrah, 1994, 1996).

Root exudation is affected by multiple factors such as light intensity, temperature, nutritional status of the plants, various stress factors, mechanical impedance, sorption characteristics of the growth medium and microbial activity in the rhizosphere. When plants are nutrient deficient, the amount of exudates released

by the root often increases (Kraffczyk et al. 1984). Differences in root exudation have been reported for different crop species (Neumann et al., 1999; Subbarao et al., 1997). Amino acid content of root exudates of maize genotypes was higher than those reported for legumes (Singh, 2000). Only limited information is available on effects of K supply on root exudation. Increased exudation of sugars, organic acids and amino acids has been detected in maize as a response to K limitation (Kraffczyk et al., 1984). Root exudation of organic acids, amino acids and sugars generally occurs passively via diffusion and may be enhanced by stress factors affecting membrane integrity such as nutrient deficiency (e.g. K, P, Zn), temperature extremes or oxidative stress (Rovira, 1969; Cakmak and Marschner, 1988; Bertin et al., 2003). This may be related to preferential accumulation of low molecular weight N and C compounds at the expense of macromolecules (Marschner, 1995). Soil extraction experiments with carboxylates, amino acids and sugars revealed that only citrate applied in extraordinary high concentrations (6 mmol g^{-1} soil) was effective in K desorption (Gerke, 1995; Steffens and Zarhoul, 1997). The composition of root-derived substances is of great importance for the understanding of processes in the rhizosphere. Therefore, methods allowing a comprehensive collection and chemical analysis of the organic root exudates are necessary.

1.5 Methods used in root exudates research

1.5.1 Collection of root exudates

1.5.1.1 Root washing method

The most common way to collect water soluble root exudates is by immersing the root systems into aerated trap solutions for a defined time period and afterwards collecting the root washings. The technique is easy to perform and allows kinetic studies by repeated measurements using the same plants (Neumann and Römheld, 2000). It is possible to get a first impression about qualitative exudation patterns and even quantitative changes in response to different pre-culture

conditions, the technique also includes several restrictions which should be taken into account for the interpretation of experimental data. Application should be restricted to plants grown in nutrient solution, since removal of root systems from solid media (soil, sand) is almost certainly associated with mechanical damage of root cells, resulting in overestimation of exudation rates. On the other hand, it has been frequently demonstrated that the mechanical impedance of solid growth media leads to alterations in root morphology and stimulates root exudation (Boeuf-Tremblay et al., 1995; Groleau-Renaud et al., 1998). In liquid culture media, simulation of the mechanical forces imposed on roots of soil-grown plants may be achieved by addition of small glass beads (Groleau-Renaud et al., 1998; Barber and Gunn, 1974).

1.5.1.2 Percolation method

Collection of root exudates from plants grown in sand culture may be performed by percolating the culture media with de-ionized water for a defined time period, after removal of rhizosphere products accumulated during the preceding culture period by repeated washings (Johnson et al., 1996). For this approach, however, recovery experiments and comparison with results obtained from experiments in liquid culture are essential, since sorption of certain exudate compounds to the matrix of solid culture media cannot be excluded. Root exudates recovery is only about half of that in the dipping method in maize, but the analyzed group (sugars, amino acids and carboxylic acids) was the same in both the methods (Gransee and Wittenmayer, 2000). As a modification of the percolation technique, cartridges filled with selective adsorption media (e.g. XAD resin for hydrophobic compounds, anion exchange resins for carboxylates), which are installed in the tube below the plant culture vessel, can be employed for the enrichment of distinct exudate constituents (Petersen and Böttger, 1992). After adsorption to a resin, exudate compounds are also protected against microbial degradation.

Trap solutions employed for collection of water soluble root exudates are nutrient solutions of the same composition as the culture media (Johnson et al., 1996),

solutions of 0.5-2.5 mM $CaSO_4$ or $CaCl_2$ to provide Ca^{2+} for membrane stabilization (Ohwaki and H. Hirata, 1992) or simply distilled water (Lipton et al., 1987; Neumann et al., 1999). Since the osmotic strength of nutrient solutions is generally low, short term treatments (1-2 h) even with distilled water are not likely to affect membrane permeability by osmotic stress. Accordingly, comparing exudation of amino acids from roots of *Brassica napus* L. into nutrient solution, 20 mM KCl, or distilled water respectively, revealed no differences during collection periods between 0.5 and 6 hours (Shepherd and Davies, 1994). In contrast, Cakmak and Marschner (1988) reported increased exudation of sugars and amino acids from roots of wheat and cotton during a collection period of 6 h when distilled water instead of 1 mM $CaSO_4$ was applied as trap solution. Thus, for longer collection periods or for repeated measurements, only complete or at least diluted nutrient solutions should be employed as trap solutions in order to avoid depletion of nutrients and excessive leaching of Ca^{2+}. Long-term exposure of plant roots to external solutions of very low ionic strength is also likely to increase exudation rates due to an increased transmembrane concentration gradient of solutes (Jones and Darrah, 1993).

Exudates collection in trap solutions usually requires subsequent concentration steps (vacuum evaporation, lyophilization) due to the low concentration of exudate compounds. Depending on the composition of the trap solution, the reduction of sample volume can lead to high salt concentrations, which may interfere with subsequent analysis or may even cause irreversible precipitation of certain exudate compounds (e.g. Ca-citrate, Ca-oxalate, proteins). Therefore, if possible, removal of interfering salts by use of ion exchange resins prior to sample concentration is recommended. Alternatively, solid phase extraction techniques may be employed for enrichment of exudate compounds from the diluted trap solution (Johnson et al., 1996). High molecular weight compounds may be concentrated by precipitation with organic solvents (methanol, ethanol, acetone 80% (v/v).

1.5.1.3 Localized sampling techniques

In several cases, exudation is not uniformly distributed along the plant roots and considerable longitudinal gradients or hot spots of exudation can exist in different root zones. Thus, collection techniques based on root washings or percolation with trap solutions, integrating root exudation over the whole root system, can only give limited information for rhizosphere processes, which frequently depend on the local concentrations of root exudates in the rhizosphere of distinct root zones (e.g. apical root zones, root hairs, cluster roots). Therefore, localized sampling techniques can be done by applying various sorption media onto the root surface (Grierson, 2000; Neumann and Römheld, 1999) or by collecting rhizosphere soil solution by micro-suction cups (Wang et al., 2004; Göttlein et al., 1996).

The root washing method is mainly confined to plants grown in hydroponics. Percolation with trap solutions can be applied for plants cultivated in solid substances, such as sand. All these techniques mentioned so far are applicable for laboratory studies if no spatial resolution is required, e.g.: for demonstration of basic physiological reactions related to changes in root exudation; for collection of exudate compounds on a preparative scale or for quantification of total carbon flow from roots by use of isotopic labeling techniques (Neumann and Römheld, 2000).

A major problem of all techniques used for collection of root exudates is the risk of microbial degradation during the collection period and the difficulties to differentiate between root exudates and microbial metabolites in the rhizosphere. In hydroponic systems, axenic culture can be employed to avoid microbial degradation of root exudates. Root washing and percolation methods are important for basic model studies, but the localized collection techniques with soil grown plants provides opportunity for rhizosphere studies under more realistic conditions.

1.5.2 Analysis of root exudate samples

Determination of low-molecular weight compounds in root exudates is usually based on standard analytical methods used in biochemistry. Analytical techniques

for determination of different classes of compounds, such as sugars, amino acids, phenolics are mainly based on derivatization reactions with subsequent spectrophotometric detection and calibration with representative standards for the respective group of compounds. These methods give a first overview concerning quantitative relations of low molecular weight organic compounds present in the root exudates. For more detailed information, individual compounds are analyzed after chromatographic separation. High performance liquid chromatography (HPLC) systems with stationary phases, based on reversed-phase silica or ion-exchange resins and subsequent spectrophotometric, fluorescence or conductivity detection with or without derivatization are most frequently employed in rhizosphere research (Weiß, 2004; Harborne, 1998). Gas chromatography (GC) (Tang and Young, 1982) and most recently capillary electrophoresis (CE) (Weinberger, 2000) and with alternative detection modes, such as mass spectrometry (MS) have been introduced (Walker et al., 2003; Roepenack-Lahaye et al., 2004).

1.6 Outline of the thesis and objective of the study

Crop species differ widely in their K efficiency. Wheat and sugar beet can take up sufficient K under low K supply, therefore are uptake efficient for K. Applying nutrient uptake model (Claassen, 1994) on wheat, it can be shown that the high K uptake efficiency of wheat is mainly due its large root system, where calculated transport and uptake agreed well with measured data. Sugar beet had fewer roots, but it could realize much higher uptake rate than calculated by the model (Dessougi et al., 2002). The mechanisms enabling sugar beet to obtain a high K influx need to be studied. In sugar beet, the K concentration in soil solution was approximately six times (94.2 µmol K L^{-1}) the concentration found in un-planted soil under low K supply [Initial soil solution concentration (C_{Li}), 15 µmol K L^{-1}] (Dessougi, 2001). Whether the increase in soil solution K concentration was due to chemical mobilisation of K by sugar beet root exudates or it was due to the problems in measuring K in soil solution i.e. K was not actually in solution, instead

in fine soil particles dispersed in the soil solution and was measured by flame emission? Further question is whether chemical mobilisation plays a role or it is due to the efficient uptake kinetics of root or both the mechanisms contribute to high K uptake efficiency.

In the present investigation three experiments were conducted in order to study the K efficiency mechanisms in different crop species. General introduction is given in the first chapter.

In the second chapter, K uptake efficiency and dynamics in the rhizosphere of maize, wheat and sugar beet were studied and evaluated by a mechanistic model. Potassium uptake was simulated by a nutrient uptake model NST 3.0 including root hairs (Claassen, 1994). Sensitivity analysis was done to study the importance of uptake kinetics responsible for the differential K uptake efficiency of different crop species.

In the third chapter, experimental set up was designed to grow K deficient and sufficient plants in sand culture to collect root exudates under different growth conditions. First experiment was conducted in screen house under natural environmental conditions in which wheat and sugar beet plants were grown in coarse quartz sand with continuous supply of nutrient solution of low and high K concentration. Cold and warm water soluble root exudates were collected from plants by percolation method at different growth stages. Organic acid, amino acid and sugar composition of root exudates were analyzed quantitatively by High Performance Liquid Chromatography (HPLC). Second experiment was conducted in growth chamber under controlled environmental conditions, where wheat and sugar beet plants were grown in medium coarse quartz sand with nutrient solution of low and high K supply and root exudates were collected in similar manner. Non-targeted metabolite profiling was done by separating the root exudates by HPLC coupled with Electrospray Ionisation-Mass Spectrometry (ESI-MS).

In the fourth chapter, mobilization of K by amino acids component of root exudates was studied in a K fixing soil. The study is summarized in the fifth chapter.

Chapter II

Potassium uptake efficiency and dynamics in the rhizosphere of maize, wheat and sugar beet evaluated by a mechanistic model

2 Potassium uptake efficiency and dynamics in the rhizosphere of maize, wheat and sugar beet evaluated by a mechanistic model

2.1 Introduction

Potassium uptake efficiency is the ability of plants to take up more K under low soil K availability. Plant species differ in their K uptake efficiency. It has been reported that K uptake efficiency of potato is less as compared to that of wheat and sugar beet (Trehan and Claassen, 1998). Dessougi et al. (2002) studied the K efficiency of spring wheat, spring barley and sugar beet under controlled conditions on a K fixing sandy clay loam soil and reported that at low K concentration (5-20 µmol L^{-1}) in soil solution, sugar beet had a 7-20 times higher K influx (K uptake per cm of root per second) than wheat and barley, indicating that sugar beet was more efficient in removing low available soil K. To understand the differences in K uptake efficiency of different crop species one has to look for the underlying mechanisms. The size of the root system, the physiology of uptake and the ability of plants to increase K solubility in the rhizosphere are considered as mechanisms of uptake efficiency (Steingrobe and Claassen, 2000).

From models for simulating of nutrient flux from soil to plant roots (Claassen, 1990), which consider soil solution concentration as a main input parameter, the factors influencing soil solution concentration with decreasing distance from the root can be derived. Apart from buffer capacity for a specific ion, which is related to binding sites for an ion in soil, solubility of related salts and chemical equilibrium in soil solution, soil moisture, transport distance and nutrient uptake capacity of the root are important. Transport distance depends on root length density and distribution. Nutrient uptake capacity of a certain unit of root length depends on root diameter (surface area), root/shoot ratio and affinity of the transporters for the ion (Engels and Marschner, 1993; Rodriguez-Navarro, 2000). To ensure an adequate supply of K^+, plants have developed a number of highly specific mechanisms to take up K^+ from the soil; these include the expression of K^+ transporters and K^+ channels in root cells especially in root hair cells (Brüggemann

et al., 1999; Ju et al., 2004; Reintanz et al., 2002). Even though not much research has been done on effect of root hairs on K uptake of different crop species, but there are some evidences for phosphorus. Root hairs increased P uptake over that due to the plant root alone in six different plant species that varied widely in root hair length, density and radius. A sensitivity analysis showed a significant contribution of root hairs to P uptake (Itoh and Barber, 1982). Diffusion conditions around a root are cylindrical. Therefore, the soil volume that can be depleted is influenced by root radius and is much greater for root hairs than for the same surface area of root cylinder. Assuming the same uptake rate for roots and root hairs, the depletion zone around root hairs is less extended due to the greater soil volume for nutrient supply (Δx is smaller). Hence, the concentration gradient ($\Delta C/\Delta x$), necessary for any rate of uptake can be established with a lower ΔC. Therefore, the concentration at the surface remains higher for a root hair or a thin root compared to thicker one. A higher concentration at the root surface enables a greater decrease of this concentration by an increasing I_{max}, resulting in a greater gradient, a higher influx, and a higher uptake rate. Root hairs achieve a higher proportion of I_{max} and are therefore more efficient in nutrient uptake from soil at low solution concentration (Claassen and Steingrobe, 1999).

Potassium uptake and supply level of the soil can be described by a mechanistic model. The model calculates the diffusive and convective transport of nutrients towards the root under consideration of sorption and desorption processes and the uptake rate is calculated by Michaelis-Menten kinetics (Claassen et al., 1986; Claassen and Steingrobe, 1999). Applying nutrient uptake model (Claassen, 1994) on wheat, it can be shown that the high K uptake efficiency of wheat is mainly due to its large root system, where calculated transport and uptake agreed well with measured data. Sugar beet has fewer roots, but it could realize much higher uptake rate than calculated by the model (Dessougi et al., 2002). The mechanisms enabling sugar beet to obtain a high K influx need to be studied. In sugar beet, the K concentration in soil solution was approximately six times (94.2 µmol K L^{-1}) the concentration found in un-planted soil under low K supply [Initial soil solution concentration (C_{Li}), 15 µmol K L^{-1}] (Dessougi, 2001). Whether the increase in soil

solution K concentration was due to chemical mobilisation of K by sugar beet root exudates or it was due to the problems in measuring K in soil solution i.e. K was not actually in solution, instead on fine soil particles dispersed in the soil solution and was therefore measured by flame emission could not be cleared definitely.
The objective of this study was to better understand the K uptake mechanisms of maize, wheat and sugar beet under low K supply by the help of nutrient uptake model calculations which also take into account the contribution of root hairs for nutrient uptake (NST 3.0). Soil and plant parameters were determined from three different plant species grown on a silty clay loam soil of low K status at two K levels. Where simulated K influx differed from measured K influx, a sensitivity analysis was done by changing different soil and plant parameters influencing K uptake, alone or by combination. The purpose was to have some clue regarding what actually occur in the rhizosphere of sugar beet, whether uptake kinetics alone could explain the differences in measured and calculated influx under low K supply or we need to study the chemical mobilisation in the rhizosphere.

2.2 Materials and Methods

A pot culture experiment was conducted to study K uptake efficiency and dynamics in the rhizosphere of maize (*Zea mays* L. cv. 8481IT), wheat (*Triticum aestivum* L. cv. Thasos), and sugar beet (*Beta vulgaris* L. cv. Monza) and to determine the soil and plant parameters for nutrient uptake model calculations. The experiment was conducted in a growth chamber located in the U.S. Department of Agriculture - Agricultural Research Service (USDA-ARS) National Soil Tilth Laboratory in Ames, Iowa. A light/dark regime of 16/8 hours at 25/18^{o}C, relative humidity of 60/75 % and PAR (photosynthetic active radiation during the day time) of 41 W m^{-2} were utilized for the study. Taintor-silty clay loam soil (Fine, smectitic, mesic, Vertic, Agriaquolls) of low K status [soil solution K concentration, 58 µmol L^{-1}; pH (0.01 M $CaCl_2$), 7.8] was collected from the upper 15 cm at the edge of a production farm near Washington, Iowa. Field-moist samples were sieved to 2-mm particle size. Soil was fertilized with 0 and 250 mg K kg^{-1} soil as

KCl. A basal dose of 340 mg N kg^{-1} soil as NH_4NO_3 and water up to field capacity (-33 kPa) moisture content (27% by weight) was applied to all the pots and the pots were incubated for one week. The experiment was designed for two harvests. The plants were grown in pots filled with soil equivalent to 1.6 kg at oven-dryness for the first harvest and 3.0 kg for the second harvest. Before transplanting, seeds were pre-germinated in folded tissue paper placed vertically in a glass beaker containing aerated tap water in the growth chamber. Number of plants per pot was 6 and 3 for the first and second harvest, respectively. Each treatment was replicated three times. Three pots per treatment were left un-planted as control for measurement of soil parameters and to determine the moisture loss through evaporation. Maize plants were harvested at 15 and 21 days of germination for first and second harvest, respectively. Wheat and sugar beet plants were harvested at 19 and 26 days of germination for first and second harvest, respectively. At harvest, shoots were cut at the soil surface and roots were carefully separated from the soil by gently shaking and sieving. Collected roots were washed repeatedly with distilled water by flooding over a sieve. The roots were stored in glass jar for root scanning. After removal of roots, total soil weight was recorded. A sub sample of 500 g soil was taken for washing by a hydro pneumatic elutriation method as described by Smucker (1982) to make sure that no fine roots were left on the soil. A second soil sub sample was taken for determination of soil solution K, exchangeable K and pH.

2.2.1 Soil chemical analysis

2.2.1.1 Soil solution K concentration

A column displacement method was used to determine soil solution K. This method permits accurate determination of the unaltered composition of soil solution (Adams, 1974). A sample of moist soil equivalent to 500 g at oven-dryness was packed into a Plexiglass column with a hole at the bottom to a density of approximately 1.3 Mg m^{-3}. Filter paper was placed on the top of each soil

column to avoid the evaporation loss of moisture during the collection. De-ionized water was added to each column at a rate of 4 mL h^{-1} until the soils reached "field capacity" water content. The samples were allowed to equilibrate for 24 h and then 40 mL of de-ionized water were added at a rate of 4 mL h^{-1}. The displaced solution was collected and filtered through a 0.20 µm filter. The solutions then were analyzed for K by atomic emission spectroscopy in the Iowa State University Soil Testing Laboratory.

2.2.1.2 Exchangeable K and pH

Two grams of field moist soil were weighed into an extraction flask. Appropriate number of blanks and reference samples was taken. 20 mL of extracting solution (1 molar NH_4OAc solution, pH 7) were added to the extraction flask and shaken for 5 minutes on a reciprocating shaker at 200 epm (excursions per minute). The suspension was filtered through Whatman No. 2 filter paper. The K concentration in the extracts was determined by Inductively Coupled Plasma (ICP) Spectroscopy in MDS Harris Labs in Lincoln, Nebraska (Carson, 1980). The soil exchangeable K content was calculated on dry weight basis.

To determine pH, five g of soil were weighed into a paper cup and 5 mL of 0.01 M $CaCl_2$ were added to the sample. The slurry was stirred vigorously for 5 seconds and then allowed to stand for 30 minutes with occasional stirring. pH meter was calibrated over the appropriate range using the manufacturer's instructions. Electrodes were placed in the slurry, swirled carefully and the pH reading was taken.

2.2.1.3 Soil parameter for model calculation

Initial soil solution concentration (C_{Li}): For the first harvest, plants were grown in soil equivalent to 1.6 kg at oven-dryness, but for the second harvest it was 3.0 kg. Model calculates the K influx for the duration between first and second harvest. It means model will calculate the influx for the plants grown in larger pot because at

second harvest plants were growing in larger pot. Soil solution concentration (C_{Li}) was measured for planted and unplanted pot at the time of each harvest. Therefore the measured C_{Li} at the time of first harvest from the smaller pot (planted pot) was lower than the actual C_{Li}. For which C_{Li} was calculated from the calibrated curve plotted between measured soil solution K concentration and the corresponding shoot K uptake for low and high K supplied plants and low and high K supplied control pot (no plant) at both the harvest. Two curves were plotted, one curve for low K supplied treatment for both the harvest and from the equation; C_{Li} for low K treatment was calculated. Second curve was plotted for high K supplied treatment for both the harvest for calculating C_{Li} for high K treatment (Fig 2.1).

D_L: Diffusion coefficient of K in water at 25°C, $cm^2 s^{-1}$ (Parsons, 1959).

θ: Volumetric water content of the soil in $cm^3 cm^{-3}$.

f: Impedance factor, calculated from formula: $f = 0.97\theta - 0.17$ (Kaselowsky,1990)

b: Buffer power for high and low K supply conditions was calculated as the ratio of soil exchangeable K and the soil solution K concentration of soil with (+K) and without (-K) K fertilization, respectively.

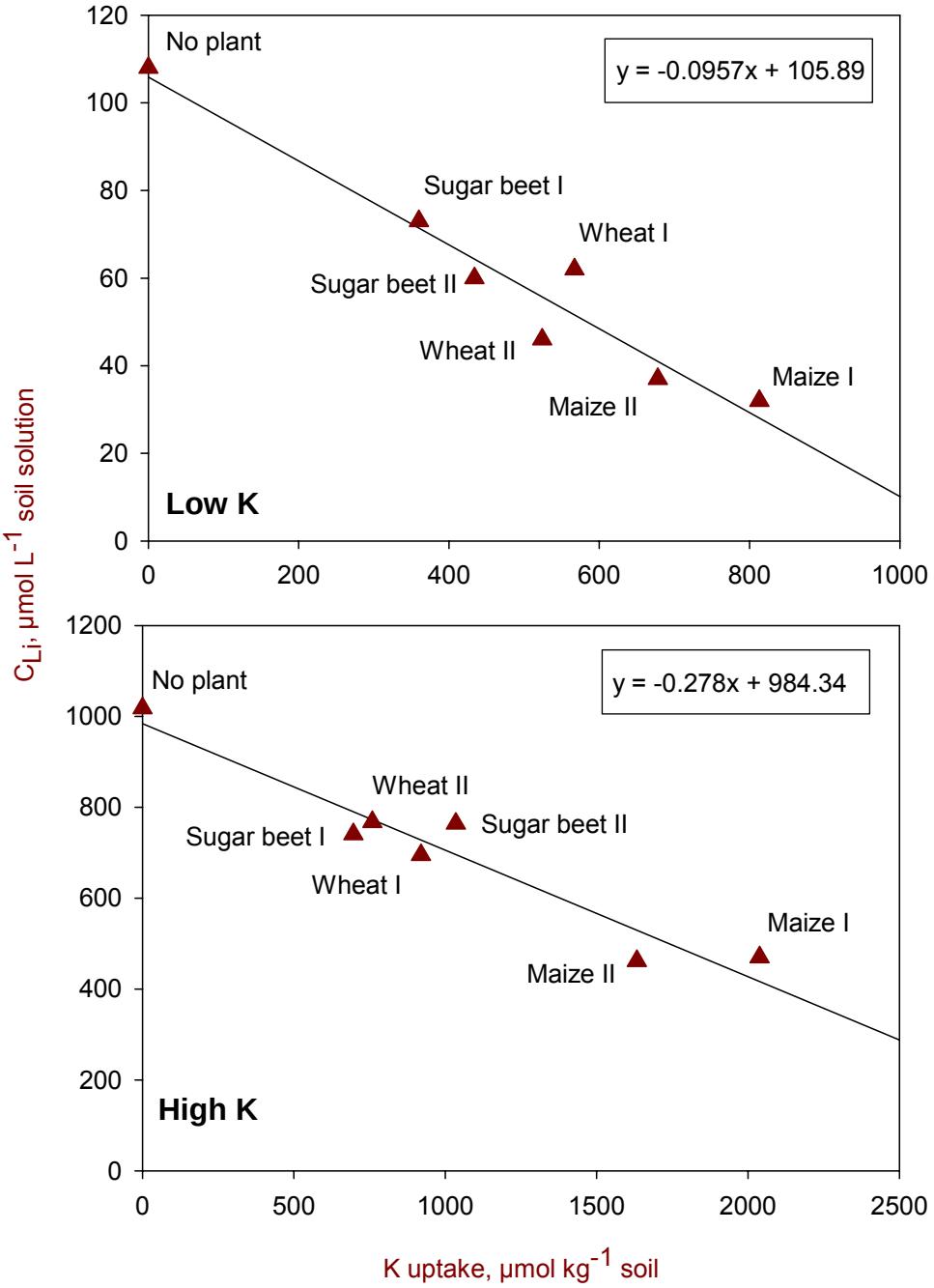


Figure 2.1: Soil solution K concentration (C_{Li}) and corresponding K uptake of no plant, maize, wheat and sugar beet grown on soil of low and high K supply at first and second harvest.

Pot size for first harvest (I) was smaller than that of second harvest (II). K uptake given in the figure is calculated per kg soil. Therefore for wheat and maize the K uptake per kg soil at second harvest was smaller than that of first harvest.

2.2.2 Plant chemical analysis

At harvest, after taking the fresh weight, shoot samples were dried at 60°C for 24 hours and then dried at 105°C till a constant weight. Sub samples of ground shoot material were wet digested under pressure using concentrated H_2SO_4 and 30% H_2O_2 and K concentration was measured by Inductively Coupled Plasma (ICP) Spectroscopy in the Analytical Lab of the USDA-ARS National Soil Tilth Laboratory.

2.2.2.1 Root length and root surface area

Collected roots were washed repeatedly with distilled water by flooding over a sieve. Separation of fine roots from 500 g of soil sub sample was done using water, air and 530 µm mesh screens in a hydro pneumatic elutriation system. Debris was removed manually and roots were stored at 5°C in 20% (v/v) ethanol in glass jar. The roots were removed from the storage solution and rinsed with water to remove most of the ethanol. The roots were then stained dark blue by placing them for 5 min in a heated (50°C) crystal violet solution made at a ratio of 1 g of crystal violet stain to 100 mL of water. After staining, the roots were returned to storage. Immediately before scanning, the stained roots were rinsed thoroughly with water. To determine the root length and root surface area, binary image of the stained root was acquired by a desktop scanner and then image analysis was done by using ROOTEDGE software (Kaspar and Ewing, 1997). Compared with most digitizing video cameras, desktop scanners have the advantage of greater resolution over a large area. ROOTEDGE is a computer program written for DOS machines that uses the edge chord algorithm (Ewing and Kaspar, 1995) to measure areas, perimeters, lengths and widths of objects in binary images. Ratios of ROOTEDGE length measurements to manual line-intersection length measurements (Newman, 1966) ranged from 0.98 to 0.88 for four corn root samples of different sizes (Kaspar and Ewing, 1997).

2.2.2.2 Average half distance between neighboring roots

Average half distance between neighboring roots (r_1) was calculated from the formula:

$$r_1 = \sqrt{\frac{\text{soil volume}\,(\text{cm}^3)}{\pi \times \text{root length}(\text{cm})}}$$

2.2.2.3 Water influx

Water influx (v_0) was calculated from the formula:

$$v_0 = \frac{T_2 - T_1}{RA_2 - RA_1} \frac{\ln(RA_2 / RA_1)}{t_2 - t_1}$$

Where T_2-T_1 is the amount of water transpired between t_1 and t_2, cm^3 and RA is root surface area, cm^2. Total evapo-transpiration loss of water was determined from the water loss from the planted pot and total evaporation loss of water was determined from the water loss from the unplanted pot. Transpiration loss of water was calculated by deducting the evaporation loss of water from the evapo-transpiration loss of water.

2.2.2.4 Mean root radius

Mean root radius (r_0) was calculated from fresh root weight (FRW) and root length (RL) assuming specific gravity of root 1 g cm^{-3}.

$$r_0 = \sqrt{\frac{FRW}{\pi . RL}}$$

2.2.2.5 Relative shoot growth rate

Relative shoot growth rate (RGR) was calculated from the formula:

$$RGR = \frac{\ln(SDW_2 / SDW_1)}{t_2 - t_1}$$

Where SDW is shoot dry weight in g and is the average of three replications, t is time of harvest in seconds.

2.2.2.6 Plant parameters related to K uptake kinetics

The K uptake kinetics describe the relationship between the net K influx (I_n) and its concentration at the root surface (C_{L0}). This relation can be described by a modified Michaelis-Menten function (Nielsen, 1972):

$$I_n = \frac{I_{\max}(C_{L0} - C_{L\min})}{K_m + C_{L0} - C_{L\min}}$$

Where I_{max}, K_m and C_{Lmin} are described below:

2.2.2.6.1 Maximum net influx

Maximum net influx (I_{max}) was obtained from the influx measured from the treatment with the highest K level for each crop. As the influx was calculated per cm of root, it was recalculated per cm^2 of total root surface area including the surface area of root hairs per cm root. Root and root hair surface area were calculated from the formula:

Root surface area = $2\pi r_0 RL$

Where r_0 is the root radius and RL is root length. In this case total surface area was calculated for 1 cm of root, therefore RL was 1 cm.

Root hair surface area = $2\pi r h_0 RHL$

Where rh_0 is the root hair radius (0.0005 cm as reported by Drew and Nye, 1969 and Barber 1984) and RHL is the total root hair length per cm of root. The RHL value was taken from Hofbauer (1990) and given in Appendix 1-3, where similar experiment was conducted in a comparable growth condition.
Since I_{max} is extrapolated for infinite concentration, the measured value was increased by 10%.

2.2.2.6.2 Minimum solution concentration

Minimum solution concentration (C_{Lmin}) is the concentration at which net influx equals zero. The value was taken from Meyer (1993).

2.2.2.6.3 Michaelis-Menten constant

Michaelis-Menten constant (K_m) is the difference between concentration at which influx is half of I_{max} and C_{Lmin}. The values were taken from Meyer (1993).

2.2.2.6.4 Net K influx

The influx is the net amount of K taken up per unit root length (or root surface area) per unit time. Assuming that young plants have exponential root growth, the net K influx (I_n) was calculated from formula of Williams (1948):

$$I_n = \frac{U_2 - U_1}{RL_2 - RL_1} \frac{\ln(RL_2 / RL_1)}{t_2 - t_1}$$

Where U is K content in µmol plant^{-1}, RL is root length per plant in cm; t is time of harvest in seconds; subscripts 1 and 2 refer to first and second harvest, respectively.

2.2.3 Nutrient uptake model calculation- basis of the model

Nutrient uptake model is useful to improve the understanding of the processes

governing soil supply and plant uptake of mineral nutrients. To simulate K uptake by different plant species, the model (NST 3.0) of Claassen (1994), which encompasses nutrient uptake by root hairs as well, was used in this study. The model is based on three basic processes: (i) release of nutrients from the solid phase into the solution which is governed by sorption and desorption processes, (ii) transport of nutrients to roots in the soil liquid phase by mass flow and diffusion (Barber, 1962), (iii) nutrient uptake into the root which is dependent on the nutrient concentration in the soil solution at the root surface and can be described by a modified Michaelis-Menten equation derived from enzyme kinetics and applied by Epstein and Hagen (1952) and modified later by Nielsen (1972).

2.2.4 Data analysis

Statistical analysis were performed by using two way analysis of variance (ANOVA), where significant difference were found, mean values were compared by using Tukey's procedure.

2.3 Results

2.3.1 Root-shoot relations in acquiring K from soil

2.3.1.1 Root length and shoot K concentration

Under low K supply, K deficiency symptoms were observed in maize leaves at 9 days after transplanting, where as in wheat and sugar beet till the second harvest no K deficiency symptoms were detected. The results pertaining to root length and shoot K concentration of maize, wheat and sugar beet at second harvest are given in Figure 2.2. At second harvest, under low K supply, shoot K concentration was 2% in maize, but that of wheat and sugar beet was 3.5 and 4.0%, respectively.

Under high K supply, shoot K concentration was increased significantly in all the crops. The crop species varied widely in their root length both under low and high K supply. Under low K supply, absolute root length of maize was 2 and 6 times higher compared to wheat and sugar beet, respectively. Potassium supply resulted in an increased root length in all the crops. The root length of maize was 72% of its maximum, but that of wheat and sugar beet was 98 and 87 % of maximum. Root length of sugar beet was only 18% of that of maize in no K treatment, but the shoot K concentration was two times higher than that of maize.

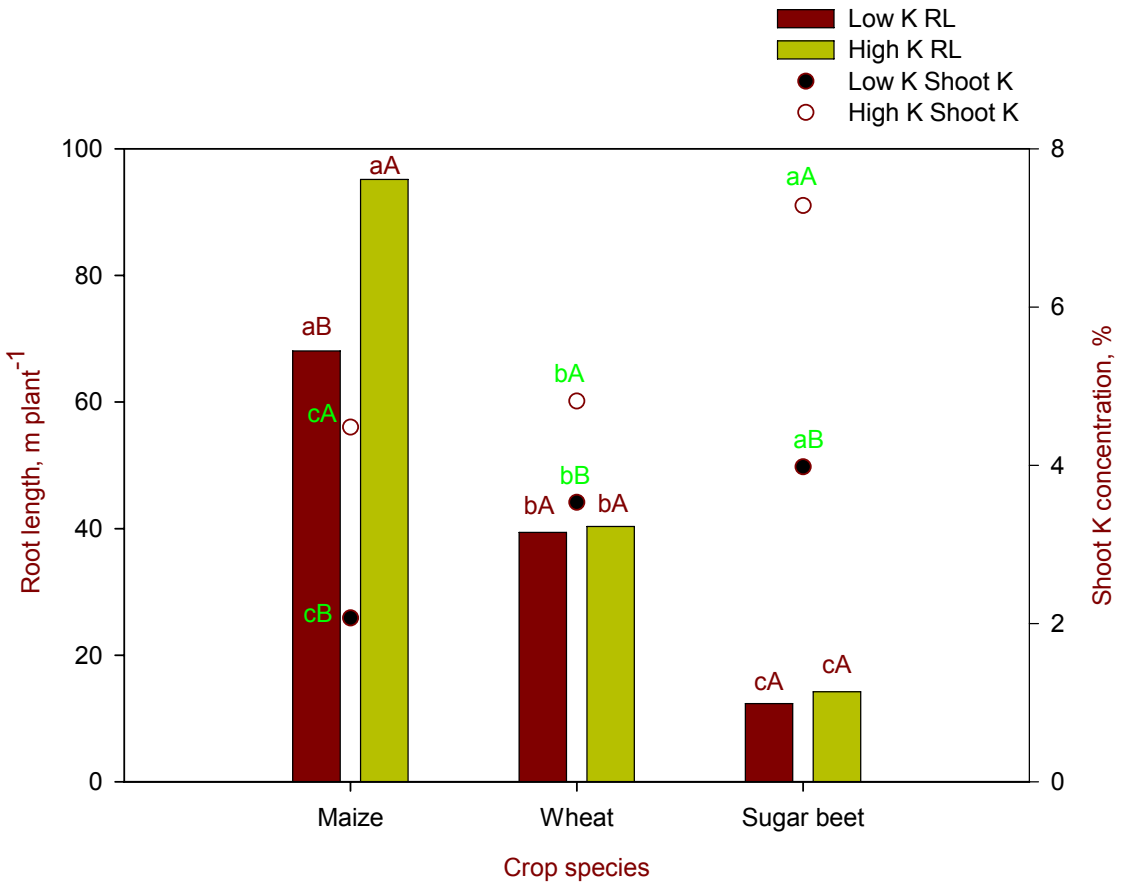


Figure 2.2: Root length and shoot K concentration of maize, wheat and sugar beet grown on low and high K supply at second harvest.

Data are mean of 3 replicates. Lower case letters indicate significant difference of root length and shoot K concentration among main effect of different crops at the same K level ($P \leq 0.001$, Tukey-test). Upper case letters indicate significant difference of root length and shoot K concentration between different K levels for the same crop species ($P \leq 0.001$, Tukey-test).

2.3.1.2 Shoot dry weight, root length to shoot dry weight ratio and K uptake

Results pertaining to shoot dry weight (SDW), root length to shoot dry weight ratio (RL/SDW) and shoot K uptake of maize, wheat and sugar beet at second harvest are given in table 2.1. Absolute SDW was different for different crops. Maize attained highest dry matter yield i.e. 2 and 3 times higher than that of wheat and sugar beet. Applying K there was not significant increase in SDW. Shoot dry weight was 91, 94 and 78 % of their maximum in maize, wheat and sugar beet respectively.

Table 2.1: Shoot dry weight (SDW), root length to shoot dry weight ratio (RL/SDW) and K uptake of maize, wheat and sugar beet grown on K deficient soil with (+K) and without (-K) K fertilization at second harvest.

Crop species	K levels	SDW	RL/SDW	K uptake
		g plant^{-1}	m g^{-1}	µmol Plant^{-1}
Maize	-K	1.29 a A	53 b A	678 a B
	+K	1.42 a A	67 b A	1633 a A
Wheat	-K	0.58 b A	68 a A	524 b B
	+K	0.62 b A	66 a A	759 b A
Sugar beet	-K	0.43 b A	29 c A	434 b B
	+K	0.55 b A	26 c A	1035 b A

Data are mean of 3 replicates. Lower case letters indicate significant difference of SDW, RL/SDW and K uptake among main effect of different crops at the same K level ($P \leq 0.001$, Tukey-test). Upper case letters indicate significant difference of SDW, RL/SDW and K uptake between different K levels for the same crop species ($P \leq 0.001$, Tukey-test).

Root length to shoot dry weight ratio was significantly higher in wheat as compared to maize and sugar beet under low K supply. In case of sugar beet, RL/SDW was only 29 m g^{-1}, but in wheat and maize it was 68 and 53 m g^{-1}, respectively. With high K supply there was no significant increase in RL/SDW.

Potassium uptake i.e. the shoot K content was significantly higher in maize as compared to wheat and sugar beet. There was no significant difference in potassium uptake in wheat and sugar beet. Under high K supply, K uptake was increased significantly in all the crops, but the increase was more in maize and sugar beet compared to wheat.

2.3.1.3 Relative shoot growth rate and shoot demand on root

The data pertaining to relative shoot growth rate and shoot demand on root (SD) of maize, wheat and sugar beet are given in table 2.2. During the growth period between first and second harvest, relative shoot growth rate of sugar beet was 23% higher than that of wheat and maize. Shoot demand on root is the K acquisition loads imposed by shoot growth on each cm of root and is calculated by dividing the shoot growth rate by the average root length assuming that the roots of young plant shows exponential growth.

$$SD = \frac{SDW_2 - SDW_1}{RL_2 - RL_1} \; \frac{\ln(RL_2) - \ln(RL_1)}{t_2 - t_1}$$

Under low K supply, shoot demand of sugar beet was 3 and 2 times higher as compared to wheat and maize and this increase was even more under high K supply.

Table 2.2: Relative shoot growth rate and shoot demand on root of wheat, maize and sugar beet grown on K deficient soil with (+K) and without (-K) K fertilization.

Crops	K levels	Relative shoot growth rate 10^{-6} s^{-1}	Shoot demand on root 10^{-10} g s^{-1} cm^{-1}
Maize	-K	2.25 b A	3.84 b A
	+K	2.22 b A	3.46 b A
Wheat	-K	2.24 b A	3.00 b A
	+K	1.89 b A	2.64 b A
Sugar beet	-K	2.76 a A	8.66 a A
	+K	2.86 a A	10.2 a A

Data are means of 3 replicates. Lower case letters indicate significant difference of shoot growth rate and shoot demand among main effect of different crops at the same K level ($P \leq 0.05$, Tukey-test). Upper case letters indicate significant difference of Shoot growth rate and shoot demand between different K levels for the same crop species ($P \leq 0.05$, Tukey-test).

2.3.2 Soil parameters

The soil properties of interest that are required for K uptake model calculations are: potassium concentration in the soil solution (C_{Li}), it's buffering by the solid phase (b) i.e. desorption and sorption of K and diffusion coefficient in water (D_L). The data pertaining to soil parameters are presented in Table 2.3.

Table 2.3: Plant and soil parameters used for nutrient uptake model calculations.

Parameters	K applied					
	0	250	0	250	0	250
	mg kg^{-1} soil					
	Maize		Wheat		Sugar beet	
Plant parameters						
I_{max}, 10^{-6} µmol cm^{-2} s^{-1}	5.02	4.00	3.73	3.55	21.6	22.5
K_m, 10^{-2} µmol cm^{-3}	3.2	3.2	1.0	1.0	1.2	1.2
C_{Lmin}, 10^{-3} µmol cm^{-3}	2.0	2.0	2.0	2.0	2.0	2.0
r_0, 10^{-2} cm	1.01	1.13	1.05	1.08	1.05	0.96
v_0, 10^{-7} cm^3 cm^{-2} s^{-1}	4.33	6.30	38.05	34.40	95.38	120.04
r_1, 10^{-2} cm	18.8	18.7	27.2	24.4	56.6	55.9
k, d^{-1}	0.1517	0.2086	0.1584	0.1321	0.2008	0.2169
RL_0, cm	2703	2719	1298	1601	299	306
Soil parameters						
C_{Li}, µmol cm^{-3}	0.085	0.833	0.092	0.916	0.097	0.933
θ, cm^3 cm^{-3}	0.30	0.30	0.30	0.30	0.30	0.30
f	0.12	0.12	0.12	0.12	0.12	0.12
D_L, 10^{-5} cm^2 s^{-1}	1.98	1.98	1.98	1.98	1.98	1.98
b	16.1	6.2	16.1	6.2	16.1	6.2

2.3.2.1 Soil solution K concentration

Results of soil solution K concentration of the mixture of rhizosphere and bulk soil of maize, wheat and sugar beet and unplanted pot at time of each harvest are given in table 2.4.

Table 2.4: Soil solution K concentration of the soil (mixture of rhizosphere and bulk soil) of maize, wheat and sugar beet grown on K deficient soil with (+K) and without (-K) K fertilization at time of harvest. Control - Soils collected from unplanted pot.

Harvest	Crops	Soil solution K	
		- K	+ K
		µmol L^{-1}	
First	Maize	32 c A	470 c B
	Wheat	62 b A	695 b B
	Sugar beet	74 b A	741 b B
	Control	109 a A	1030 a B
Second	Maize	37 c A	462 c B
	Wheat	46 b A	768 b B
	Sugar beet	60 b A	764 b B
	Control	105 a A	994 a B

Data are means of 3 replicates. Lower case letters indicate significant difference of soil solution K among main effect of different crops at the same K level ($P \leq 0.001$, Tukey-test). Upper case letters indicate significant difference of soil solution K between different K levels for the same crop species ($P \leq 0.001$, Tukey-test).

Soil solution K concentration was lower in the soil of all the crops as compared to that of unplanted pot in both the harvest. However it varied among the crops. Soil solution K concentration in case of sugar beet was 3 and 1.5 times higher compared to that of maize and wheat in no-K treatment. The difference might be

due to lower uptake of K by sugar beet as compared to maize and wheat. The difference in soil solution concentration was in accordance to K uptake difference between the crops i.e. uptake of K per pot in sugar beet was 2.3 and 1.6 times lower than that of maize and wheat. Applying K to the soil, there was a significant increase in soil solution K concentration in both planted and unplanted pot.

2.3.2.2 Soil exchangeable K concentration

Exchangeable K concentration of the soil was measured from the mixture of rhizosphere and bulk soil collected from all the three crops and also from unplanted pot at the time of first and second harvest for calculating buffer power (Table 2.5). At second harvest, there was no significant difference between the soil exchangeable K of wheat, sugar beet and unplanted soil, but in maize the difference from unplanted soil was significant. To know whether the change in exchangeable K after plant growth was in accordance to the plant K uptake, both the parameters were compared by a scattered plot. Surprisingly all the dots corresponding to both the parameters for low K soil after first and second harvest were above the 1:1 symmetry line (Figure 2.3). This result indicated that about 50% of the K taken by plants was from non-exchangeable source.

Table 2.5: Exchangeable K of the soil (mixture of rhizosphere and bulk soil) of maize, wheat and sugar beet grown on K deficient soil with (+K) and without (-K) K fertilization at time of harvest. Control - Soils collected from unplanted pot.

Harvest	Crops	Exchangeable K	
		- K	+ K
		μmol kg^{-1} soil	
First	Maize	949 c A	3043 c B
	Wheat	1068 b A	3889 b B
	Sugar beet	1111 b A	3889 b B
	Control	1256 a A	4538 a B
Second	Maize	863 b A	3299 b B
	Wheat	1026 a A	4111 a B
	Sugar beet	1103 a A	3940 a B
	Control	1231 a A	4154 a B

Data are means of 3 replicates. Lower case letters indicate significant difference of soil exchangeable K among main effect of different crops at the same K level ($P \leq 0.001$, Tukey-test). Upper case letters indicate significant difference of soil exchangeable K between different K levels for the same crop species ($P \leq 0.001$, Tukey-test).

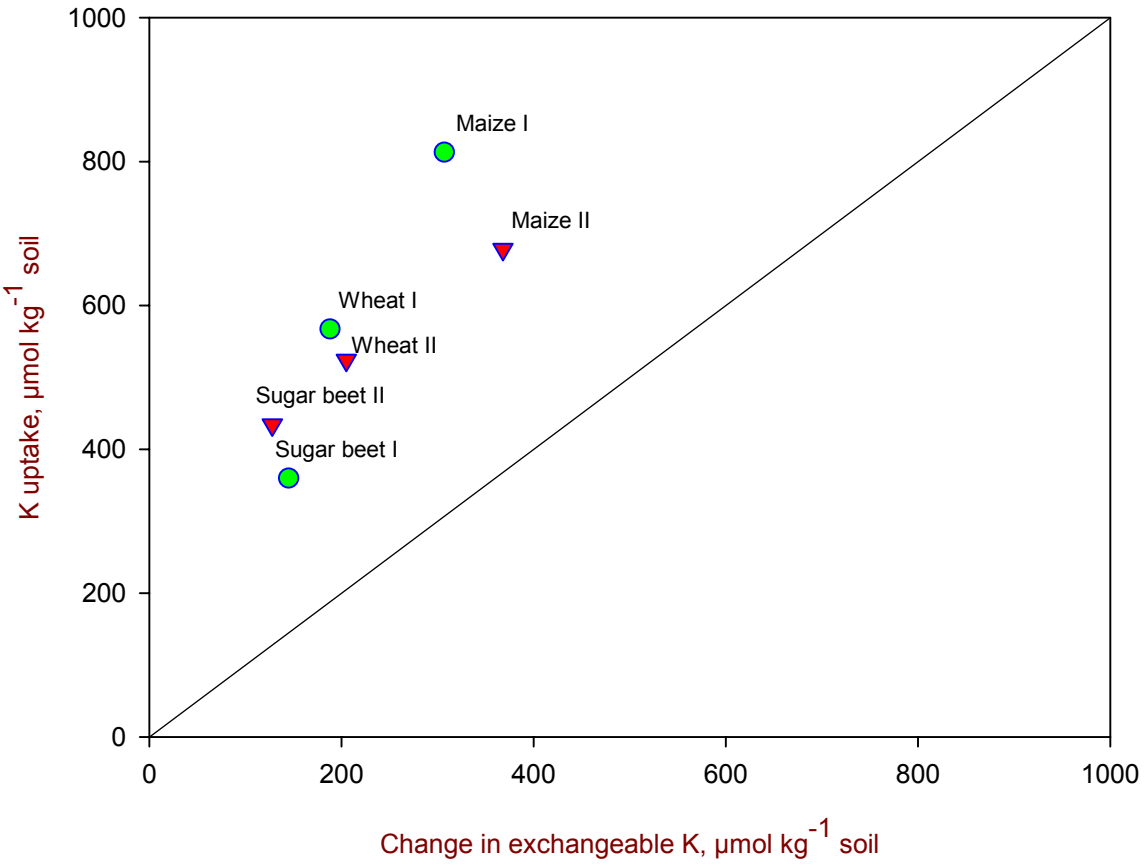


Figure 2.3: Potassium uptake and corresponding change in exchangeable K in soil (average bulk and rhizosphere) of maize, wheat and sugar beet grown on K deficient soil (-K) at first and second harvest.

2.3.3 Simulation of K uptake by a computer model

The measured soil and plant parameter (Table 2.3) were used to simulate K influx by maize, wheat and sugar beet using nutrient uptake model (NST 3.0) which includes the contribution of root hairs for K uptake.

2.3.3.1 Measured and calculated K influx

Potassium influx is a measure of the physiological activity of the roots. Results pertaining to measured and calculated K influx by maize, wheat and sugar beet are summarized in table 2.6. In no-K treatment, measured K influx was 8.45 µmol

cm^{-1} s^{-1} in sugar beet, which was 4 and 3 times higher than that of maize and wheat, respectively. With increasing K supply, measured K influx was increased by approximately two times in maize and sugar beet and 1.2 times in wheat. Under high K supply, K influx in sugar beet was 5 and 6 times higher than that of maize and wheat, respectively. The results show that under both low and high K supply, sugar beet had a higher K influx than the other crops.

Table 2.6: Measured and calculated K influx in the roots of maize, wheat and sugar beet grown on K deficient soil with (+K) and without (-K) K fertilization.

Crops	K levels	K influx		
		Calculated	Measured	Calculated/ Measured
		10^{-7} µmol cm^{-1} s^{-1}		
Maize	-K	1.27	1.99	0.638
	+K	4.33	3.87	1.119
Wheat	-K	1.77	2.59	0.683
	+K	3.90	3.22	1.211
Sugar beet	-K	2.64	8.45	0.312
	+K	15.10	19.00	0.795

In order to find an explanation for the different K influx of the crops, model calculations that included root hairs were done. Results in figure 2.4 show the calculated depletion of K in the rhizosphere of maize, wheat and sugar beet after different days of uptake at low and high K supply. Sugar beet could decrease the K concentration at the root surface to a lower value of $3x10^{-3}$ µmol cm^{-3} as compared to wheat ($7.6x10^{-3}$ µmol cm^{-3}) and maize ($9.9x10^{-3}$ µmol cm^{-3}) after 7 days of uptake at low K supply (for maize it was 6 days of uptake). Under high K supply, at C_{Li} of $933x10^{-3}$ µmol cm^{-3}, sugar beet could decrease K concentration at the root surface to $110x10^{-3}$ µmol cm^{-3}, whereas wheat decreased it to $608x10^{-3}$ µmol cm^{-3} and maize to $320x10^{-3}$ µmol cm^{-3}.

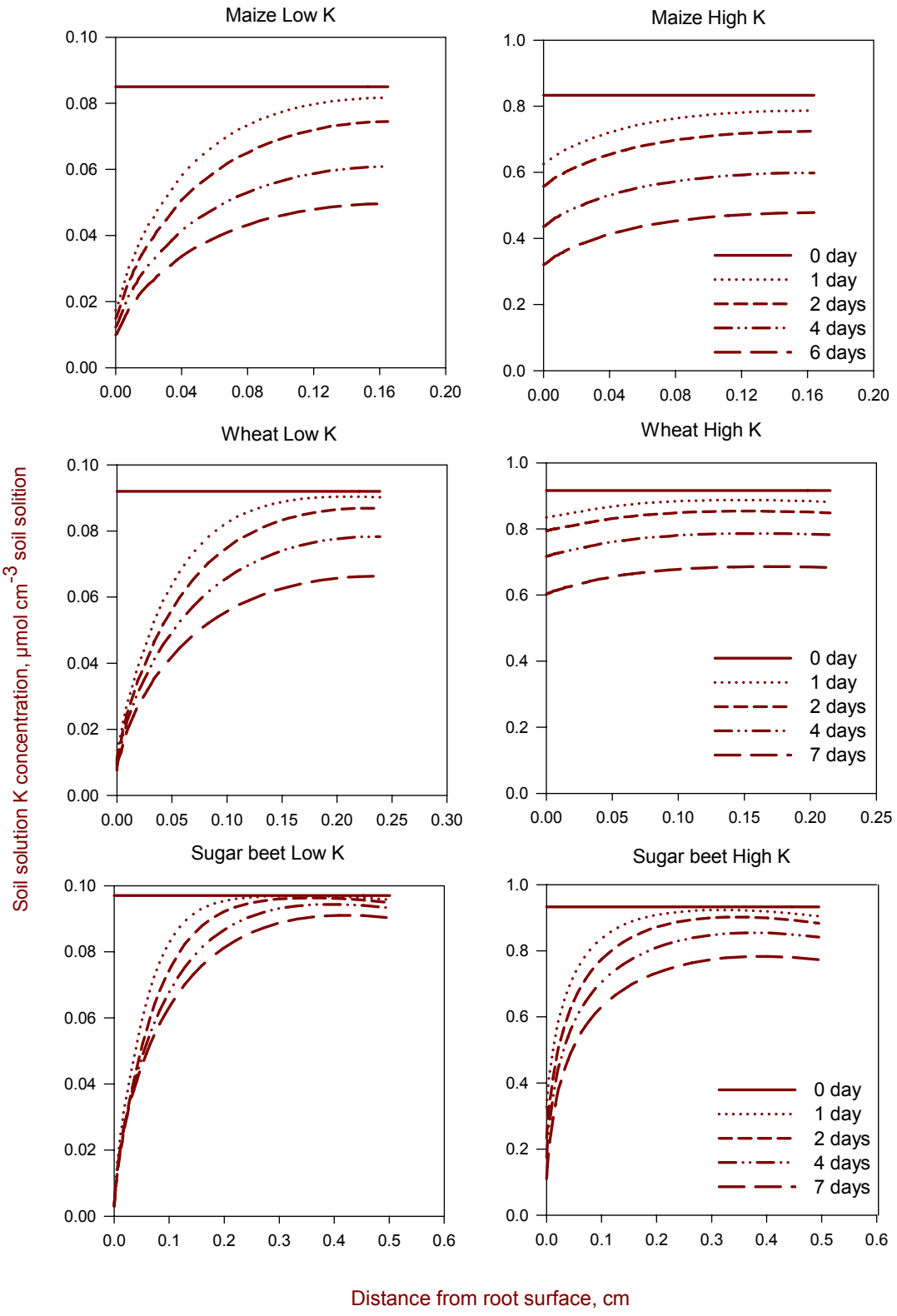


Figure 2.4: Calculated K depletion in the rhizosphere of maize, wheat and sugar beet grown on K deficient soil with (+K) and without (-K) K fertilization.

These results suggest that higher influx of sugar beet was due to its capability to decrease the K concentration at the root surface to a relatively much lower value thereby increasing the concentration gradient and so the transport to the root surface. Furthermore, the average half distance between the roots (r_1) in sugar beet was more than two times as compared to wheat and maize, irrespective of K supply. Therefore the inter root competition was much lower in sugar beet than that of wheat and maize.

As per the model calculation, there was a close prediction for K influx under high K supply for wheat and maize, but a slight under prediction was there for sugar beet. Under low K supply there was under prediction for K influx in all the crops. However the prediction was very low for sugar beet and that was only 31% of the measured influx and that of wheat and maize was 68 and 64% (Table 2.6). Which emphasized either incorrect measurement of the input parameters or there are some other processes which played a role and that had not been considered in the model. To find the reason for this under prediction, a sensitivity analysis was done by changing C_{Li}, I_{max} and buffer power alone and in combination of I_{max} and buffer power.

2.3.3.2 Sensitivity analysis

Evaluation of soil and plant properties can be done by using the model in a sensitivity analysis. In such an analysis, several calculations are performed by changing one input parameter while keeping the other input data constant. This enables evaluation of the influence of an input parameter on the calculated influx. Sensitivity analysis for soil solution K concentration (C_{Li}) shows that, increasing (C_{Li}) by a factor of 1.6 we could get 100% prediction for measured K influx in wheat and maize, but in sugar beet the same was achieved by increasing C_{Li} by a factor of 3.5 (Figure 2.5).

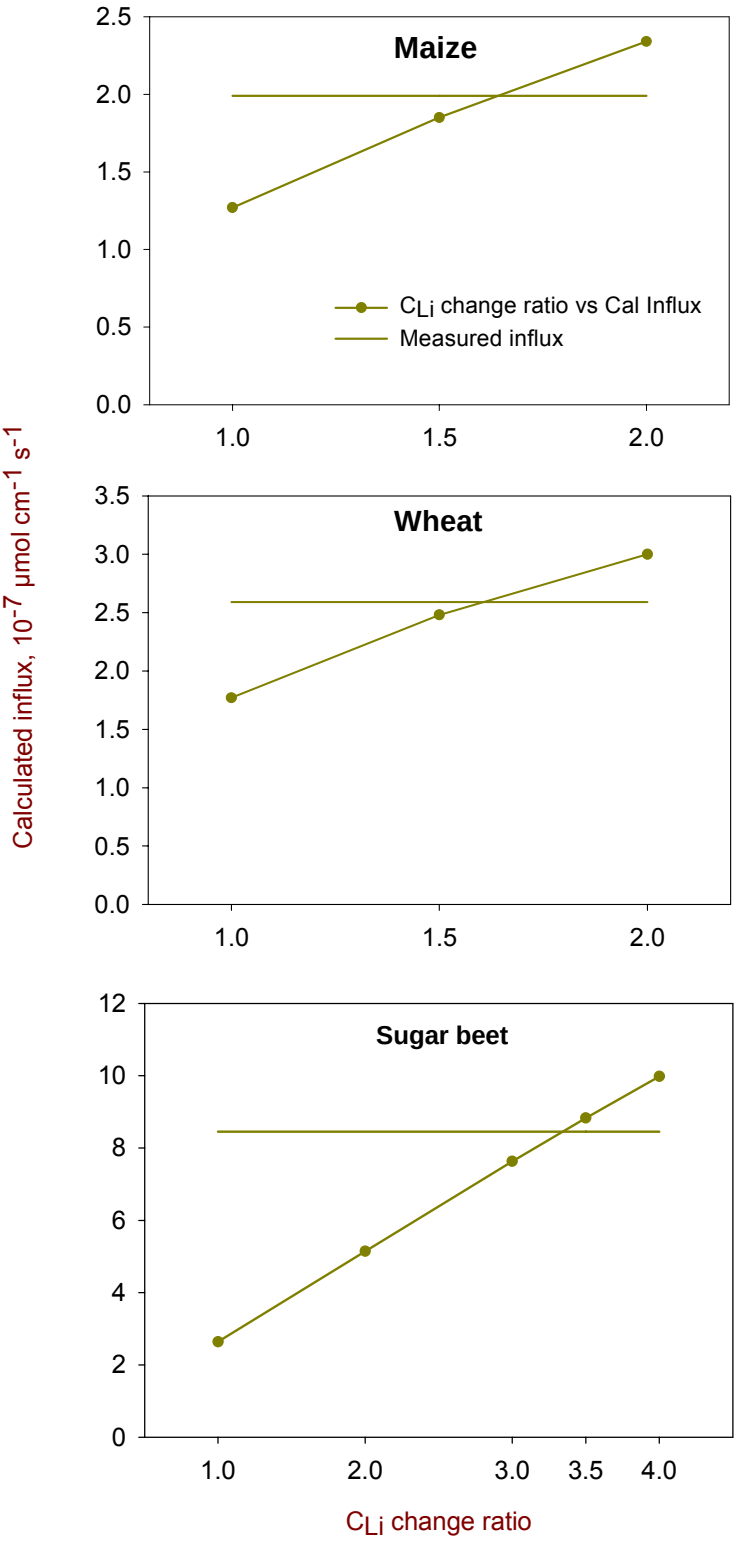


Figure 2.5: Effect of initial soil solution concentration (C_{Li}) on calculated influx. Measured influx of respective crop species is represented as a straight line.

As C_{Li} is a parameter which can be measured very precisely, it is a very reliable model input parameter. The parameters like I_{max} and buffer power cannot be measured very accurately. There are several ways to calculate I_{max} and buffer power of the soil. With plant growth, soil buffer power may also change because of the plant influence e.g. through root exudation, which mobilize K from solid phase, uptake of K from non-exchangeable fractions by cation exchange or simply by chemical desorption of K from the solid phase due to the steeper concentration gradient created by plant root uptake. Maximum uptake capacity (I_{max}) is sometimes taken from solution experiments, where the growing conditions are totally different from soil experiments. Therefore a sensitivity analysis for these parameters is justified. Increasing the buffer power by a factor of 10, there was 100 % prediction by the model for wheat and maize, however in sugar beet the same was achieved by increasing the buffer power 50 times (Figure 2.6).

Figure 2.7 shows the effect of increasing I_{max} on calculated influx by total root, root cylinder and root hairs of maize, wheat and sugar beet under low K supply. Sensitivity analysis for I_{max} showed that increasing I_{max} increased the influx for all the three crops. However, even by increasing I_{max} by 25 times, the model prediction for K influx was 0.33, 0.53 and 0.83 times the measured K influx for sugar beet, wheat and maize, respectively. It is interesting to note that the effect of I_{max} in increasing calculated K influx was only due to the root hairs and increasing I_{max} did not affect much K influx of the root cylinder. In case of sugar beet the root hair K influx was higher than that of root cylinder for all the I_{max} change ratio, but in wheat and maize, root hair K influx was lower than that of root cylinder, but increasing I_{max} gradually increased the root hair K influx.

In order to know why increasing I_{max} increased root hair influx but not that of root cylinder, we run the model for wheat without including root hairs, where only root radius parameter was changed. In order to know the effect of I_{max} on root cylinder K influx, the actual root radius was taken and to know the effect of I_{max} on root hair K influx, instead of root radius, root hair radius (0.0005 cm) was taken. The purpose was to look at the concentration profile around the root and root hair surface (Figure 2.8).

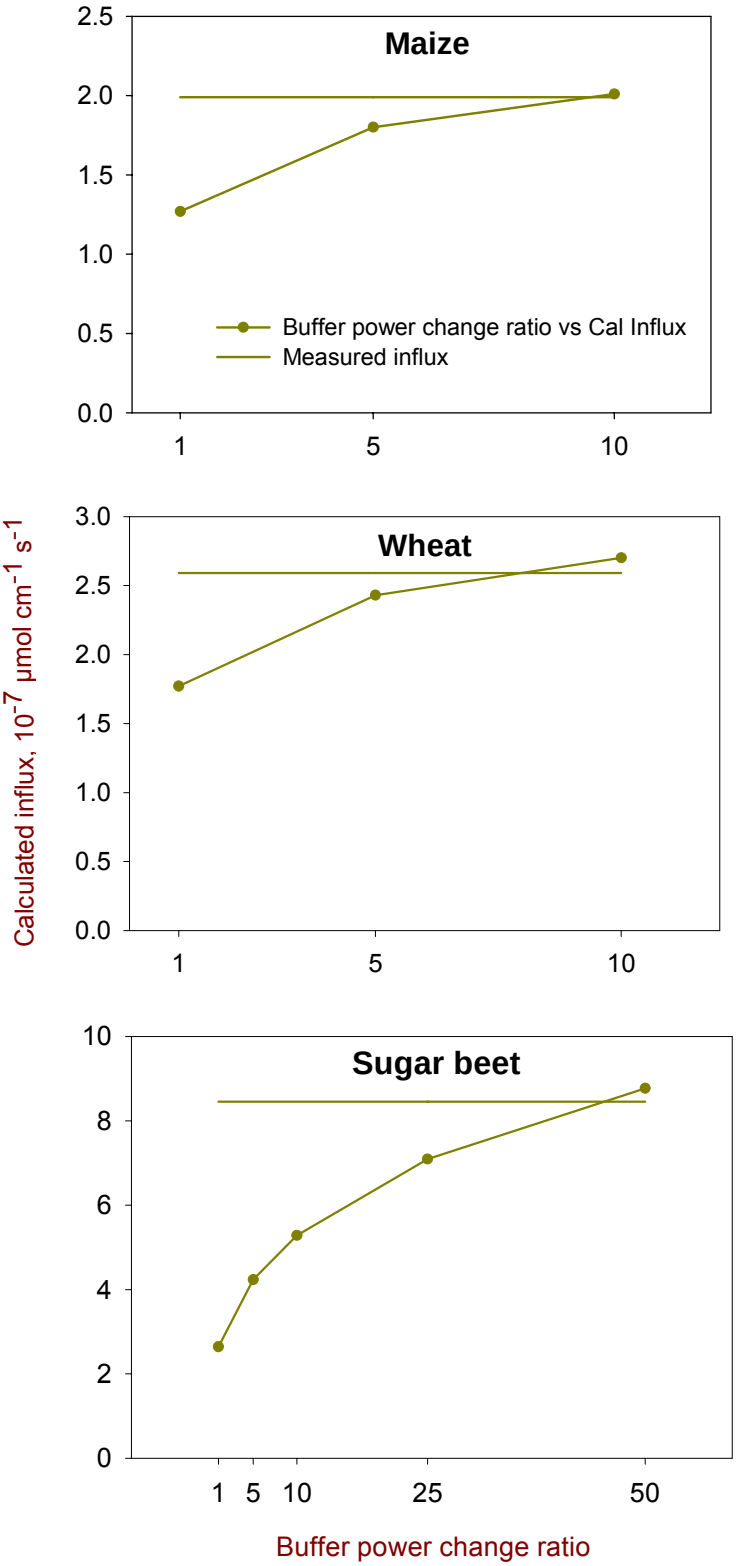


Figure 2.6: Effect of change in buffer power (b) on calculated influx. Measured influx of respective crop species is represented as a straight line.

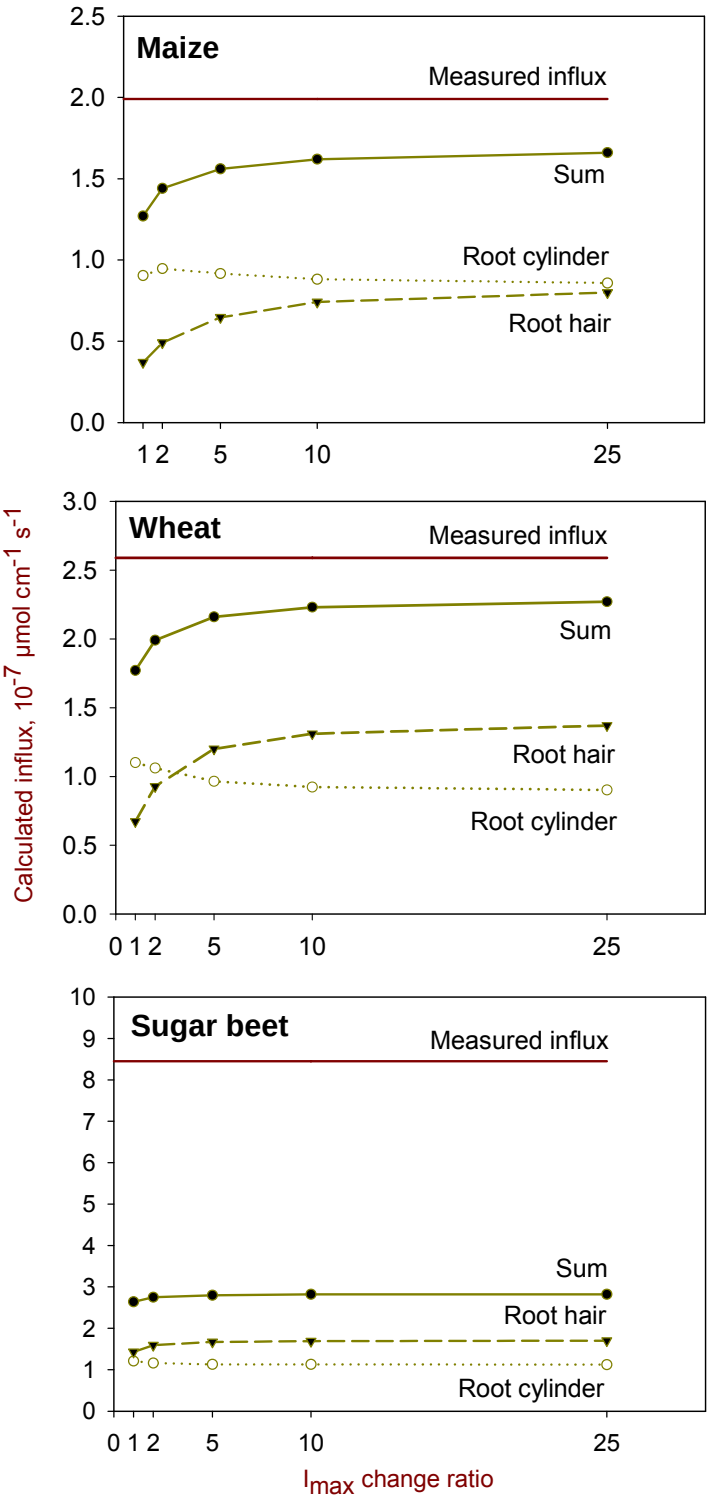


Figure 2.7: Effect of I_{max} on calculated influx per cm of root through the root cylinder, the root hairs and the sum of both of maize, wheat and sugar beet grown on low K supply. Measured influx of respective crop species is represented as a straight line.

Sensitivity analysis for I_{max} of root cylinder showed that increasing I_{max} 10 times did not affect much the calculated K influx. But in case of root hair, increasing I_{max} by a factor of 10 resulted in 6 times increase in calculated K influx. To explain the reason, the K depletion curve for wheat was drawn for root and root hairs from the model output. At the root surface the soil solution K concentration was already very low with the original I_{max} (control) i.e. $10x10^{-3}$ µmol cm^{-3}, so by increasing I_{max} to a higher value, which means increasing the K uptake capacity of the root cylinder, there was not much possibility for the root cylinder to further decrease the K concentration at the root surface i.e. soil transport was limiting K uptake. In contrast at the root hair surface, the K concentration of the control was much higher i.e. $80x10^{-3}$ µmol cm^{-3}, therefore root hairs had a large possibility to decrease the concentration further thereby creating the concentration gradient for higher transport of K towards the root and resulting in higher K influx.

A sensitivity analysis of single parameter could be insufficient, because sometime they are interrelated (Claassen and Steingrobe, 1999). Sensitivity analysis was done by increasing I_{max} and b at the same time and by doing so there was a closer prediction of K influx. By increasing both the parameters by 2.5 times there was 100% agreement for measured K influx in wheat and maize (Figure 2.9). But in sugar beet 100% agreement was achieved by increasing both the parameters by 25 times. Increasing I_{max} alone by a factor of 25 times, prediction was only 33% in sugar beet.

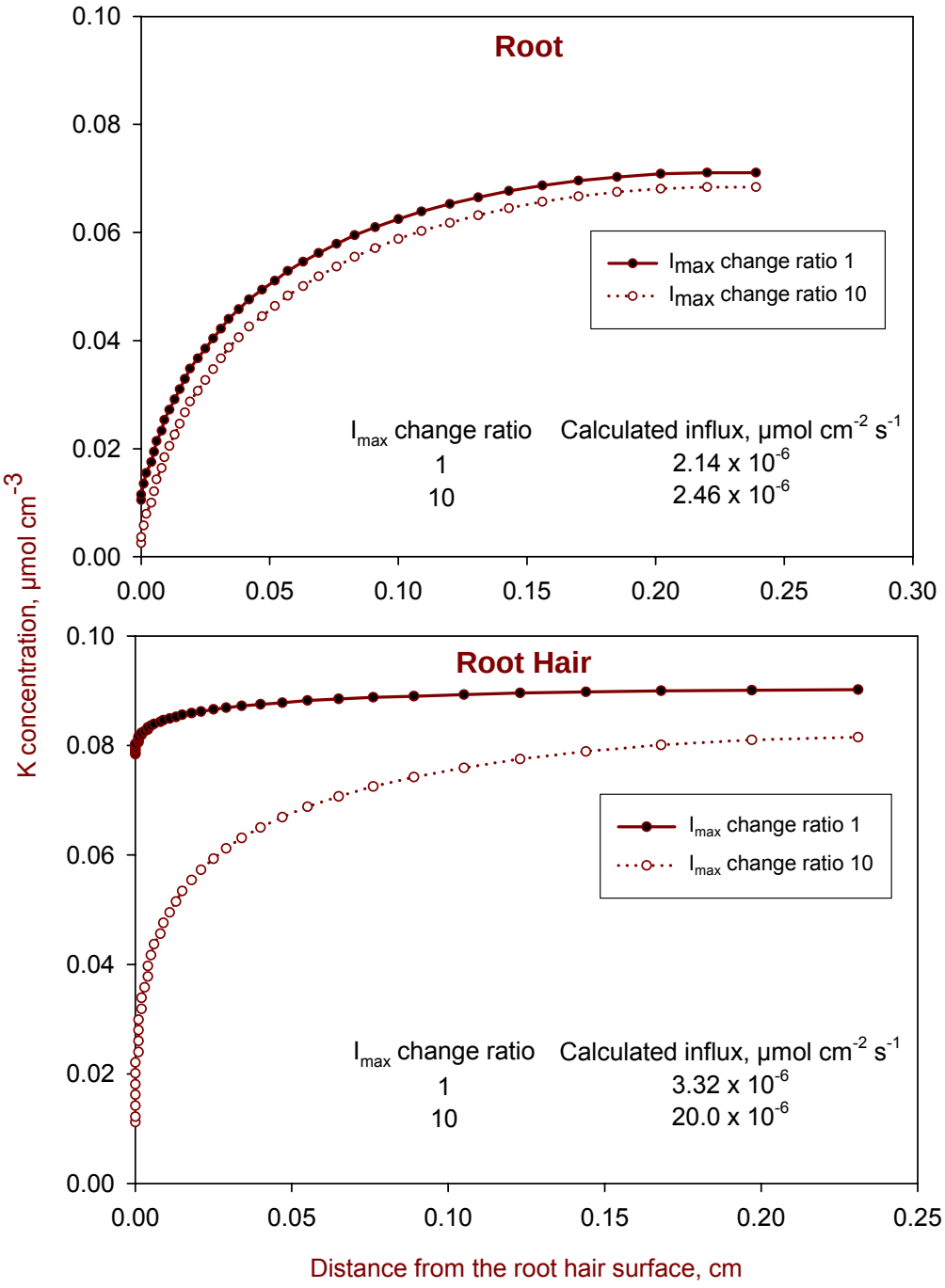


Figure 2.8: Effect of I_{max} on soil solution concentration at different distances from the root and root hair surfaces of wheat.

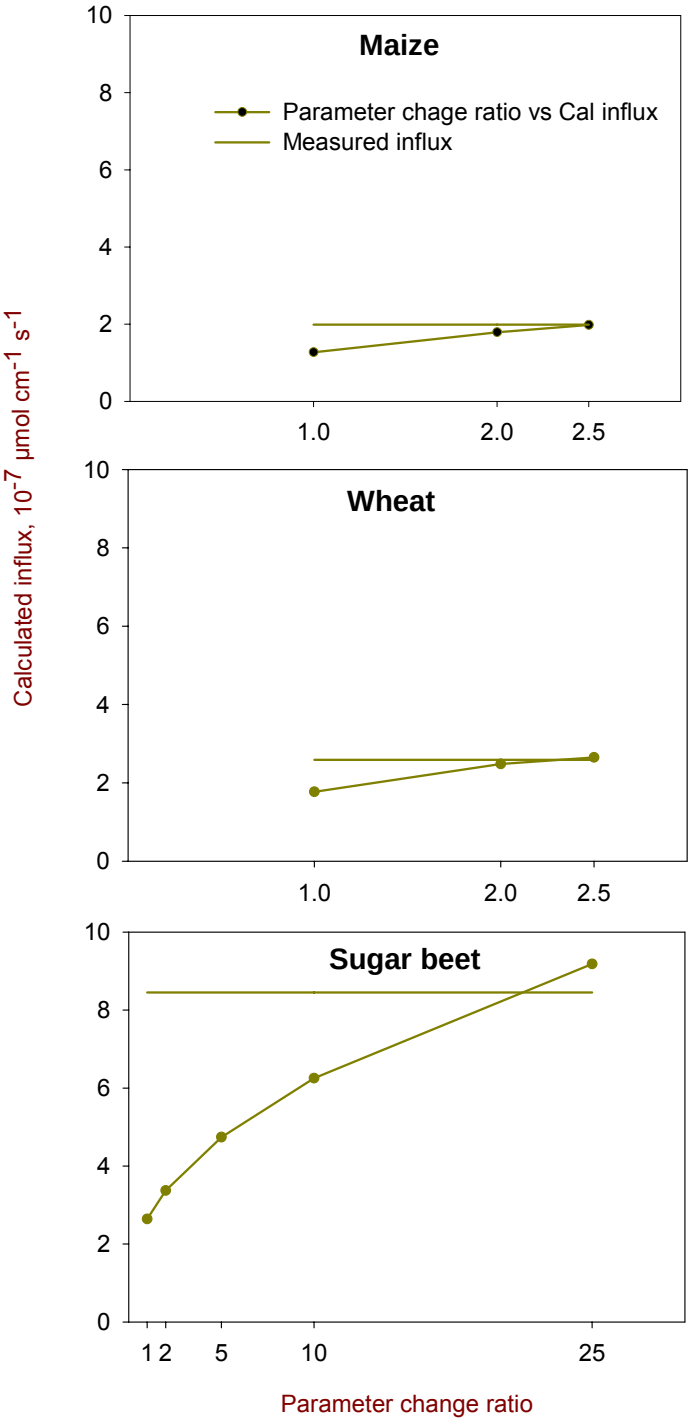


Figure 2.9: Effect of simultaneous change of I_{max} and buffer power (b) (for example I_{max} and b, both are increased by 5 times) on calculated influx. Measured influx of respective crop species is represented as a straight line.

2.4 Discussion

This study focuses on K uptake efficiency mechanisms of maize, wheat and sugar beet by the help of a nutrient uptake model calculations and a sensitivity analysis. Sugar beet and wheat were found to have a higher K uptake efficiency as compared to maize.

The shoot K concentration was higher in sugar beet and wheat than that of maize (Figure 2.2). Wheat could take higher K from low K supplied soil because of having higher root length to shoot dry weight ratio (Table 2.1). Steingrobe and Claassen (2000) reported that in both soil and solution experiment wheat and sugar beet were more K efficient than potato because wheat had a large root system and both species had an efficient uptake physiology. Root length of sugar beet was only 18% of that of maize in no K treatment, but the shoot K concentration was two times higher than that of maize. Sugar beet could acquire more K per unit shoot dry weight because of having 4 and 3 times higher K influx (K uptake per cm of root per second) as compared to maize and wheat, respectively and thereby able to compensate the higher shoot demand on unit root length (Table 2.6). The higher K uptake efficiency of wheat was due to higher root length to shoot dry weight ratio and lower shoot demand as compared to sugar beet and maize (Table 2.2). Dessougi and coworkers (2002) studied the K efficiency of spring wheat, spring barley and sugar beet under controlled conditions on a K fixing sandy clay loam soil and reported a higher shoot K concentration with only 30-50% of the root length and 15-30% of the relative shoot growth rate in sugar beet as compared to wheat and barley. Sugar beet had a 7-20 times higher K influx than wheat and barley, indicating that sugar beet was more efficient in removing low available soil K. Sadana and Claassen (1999) from one pot culture experiment with K fixing sandy clay loam soil reported lowest root/shoot ratio and highest shoot growth rate in sugar beet resulting in 3.2 and 2 times higher shoot demand on root than wheat and maize, respectively.

From the calculated concentration profile around the root surface of maize, wheat and sugar beet it was deduced that the higher K influx in sugar beet was due to the

capacity of the sugar beet root to reduce the concentration at the root surface to a lower value as compared to wheat and maize thereby increasing the concentration gradient and so the transport of K to the root surface. Furthermore, the average half distance between the roots (r_1) in sugar beet was more than two times as compared to wheat and maize, irrespective of K supply, which means that the volume of soil that supply K to root of sugar beet was more than that of wheat and maize, resulting in greater concentration gradient for K influx (Figure 2.4).

The nutrient uptake model could satisfactorily predict K influx in maize and wheat under high K supply conditions and even in sugar beet, prediction was 80% (Table 2.6). The close agreement between model calculated and measured K influx under high K supply indicated that the concept of diffusion, mass flow and K uptake physiology as the most important processes for K transport and uptake are appropriate and the model input parameters were accurately measured. However under low K supply, the model prediction was 0.64, 0.68 and 0.31 times the measured K influx for maize, wheat and sugar beet, respectively.

Initial soil solution concentration (C_{Li}) is a parameter which can be measured very precisely; it is a reliable model input parameter. But C_{Li} is an average value for the whole soil, but in rhizosphere may be it is different, therefore sensitivity analysis for C_{Li} was done. By increasing C_{Li} by factor of 1.6 times for wheat and maize and 3.5 times for sugar beet, there was 100 % prediction for K influx (Figure 2.5). Which indicate the possibilities of chemical mobilization of K by plant root. Maximum net influx (I_{max}), K_m and b are the parameters which cannot be measured directly in soil so there are chances of error in determining these values. 100% model prediction for K influx was achieved by increasing buffer power by a factor of 10 in maize and wheat and by a factor of 50 in sugar beet (Figure 2.6). In maize and wheat the K concentration at the root surface was 2.5 and 3 times higher than that of sugar beet, therefore increasing buffer power by a factor 10, the concentration gradient needed for driving flux could be established. Because by increasing b, we are increasing the soil replenishing power for K i.e. more K is coming to the exchangeable fraction from non-exchangeable fraction for example from the interlayer K. In our results we also found on an average about 50% of the total K

uptake by plant was from non-exchangeable fractions, but close to the root it was probably much more (Kuchenbuch and Jungk, 1984) and this would cause a much higher b in the rhizosphere (Figure 2.3). But in sugar beet, K concentration at the root surface was already very low i.e. $3x10^{-3}$ µmol cm^{-3}, for which 100% prediction achieved at a very high buffer power. Increasing buffer power, though we were increasing the soil supplying capacity for K, plant could not reduce the K concentration at the root surface further unless I_{max} of the plant increased.

In case of wheat and maize at the original b, inter root competition was already there, which resulted in a lower concentration gradient. At a higher b, more K was coming to soil solution and there was no inter root competition which resulted in a steeper concentration gradient for K influx. Therefore increasing b by only a factor of 10 in maize and wheat, model could simulate K influx 100%. But in case of sugar beet, at a low b, there was no inter root competition hence the influence of soil K transport to the root surface or the buffer power was less. For which similar K influx could be achieved at a higher value of b in sugar beet. Steingrobe et al. (2000) reported that in situations of root competition, the difference between the K depletion at a low and high value of buffer power became important and the influence of buffer power on K uptake increased.

Similar was the case if we increase the I_{max} alone keeping buffer power constant. By increasing I_{max}, we are increasing the K uptake capacity of root and root hairs which results in a very low soil solution K concentration at the root surface. The equilibrium between K in solution and K in exchangeable and non- exchangeable fractions is disturbed when root absorbs more K. To re-establish the equilibrium, K from non-exchangeable fraction is released into the solution and solution concentration is thus buffered. For which even increasing I_{max} by a factor of 25, we could not achieve 100% prediction in all the crops in low K supplied plants (Figure 2.7). By doing so only we were increasing the maximum uptake capacity of plant without increasing the soil supplying capacity. But to maintain this high I_{max}, buffer power of the soil has to be increased. In soil-plant system (rhizosphere) probably these are interrelated parameters. Plants develop mechanisms to survive under low nutrient supply conditions for example by increasing the maximum uptake

capacity i.e. may be through expression of genes of high affinity K transporters, K channels in root or root hair surface. As a consequence it can deplete K at the root surface to a very low value thereby creating a concentration gradient for diffusive flux of K from the non-exchangeable sources.

Several researchers reported that plants have developed a number of highly specific mechanisms to take up K^+ from the soil; these include the expression of K^+ transporters and K^+ channels in root cells especially in root hair cells to ensure an adequate supply of K^+ under low K supply (Brüggemann et al., 1999; Ju et al., 2004; Reintanz et al., 2002). Even though not much research has been done on effect of root hairs on K uptake of different crop species, but there are some evidences for phosphorus. Root hairs increase P uptake over that due to the plant root alone in six different plant species that varied widely in root hair length, density and radius and sensitivity analysis showed a significant contribution of root hairs to P uptake (Itoh and Barber, 1982). The basis for large proportion of P uptake by root hairs was explained by several researchers as (i) root hairs increase the absorbing surface area- in case of spinach, it was 1.9-fold higher than that of the root cylinder (Föhse et al., 1991), (ii) root hairs have a very small radius (approximately 0.005 mm) (Barber, 1995), so that P concentration at the root hair surface remains higher than that at the root cylinder, which leads to a higher influx per unit surface area, (iii) root hairs grow into soil perpendicular to the root surface and thereby increase the radius of the P absorbing body (root cylinder plus root hairs). This causes greater transport of P to the root (Föhse et al., 1991; Claassen, 1990; Kovar and Claassen, 2005). In our study, we observed from the sensitivity analysis that the increase in model prediction for K influx by increasing I_{max} was due to the root hairs only. It was due to the fact that the K concentration at the root hair surface was considerably higher as compared to that of root surface. Diffusion conditions around a root are cylindrical (Claassen and Steingrobe, 1999). Therefore, the soil volume that can be depleted is influenced by root radius and is much greater for root hairs than for surface area of root cylinder. Assuming the same uptake for roots and root hairs, the depletion zone around root hairs is less extended due to the greater soil volume for nutrient supply. Hence, the

concentration gradient necessary for any rate of uptake, can be established with a lower difference in concentration between root surface and bulk soil (Figure 2.8). Therefore concentration at the root surface remains higher for a root hair. A higher concentration at the root hair surface enables a greater decrease of this concentration by an increase of I_{max}. For which in maize and wheat better prediction was there by increasing I_{max} as compared to sugar beet.

Sensitivity analysis was done by increasing I_{max} and b at the same time. Surprisingly, only by increasing I_{max} and b by a factor of 2.5 times, model could predict measured K influx 100% in maize and wheat under low K supply conditions (Figure 2.9). However the same was achieved for sugar beet by increasing both the factor by 25 times. To maintain this high I_{max}, soil solution concentration had to be increased. But now the question is whether because of the steeper concentration gradient created by the roots and/or root hairs, more K is desorbing to soil solution from non-exchangeable fraction i.e. b is higher or it is due to some organic compounds secreted by sugar beet roots which can solubilize K from soil minerals and increase K concentration in soil solution close to the root surface. Springob and Richter (1998) reported that an exudation of organic acids and/or protons seems to be not necessary to make non-exchangeable K available. Already the decrease of the K concentration in soil solution below 3.5 µmol L^{-1} initiated a release of interlayer K of a Luvisol. The minimum concentration of most of the plant species is well below 3.5 µmol L^{-1}. Hinsinger and Jaillard (1993) demonstrated that release of interlayer K in phlogopite occurred in the rhizosphere of ryegrass (*Lolium perenne* L.) when the K concentration in the rhizosphere solution decreased below a threshold of about 80 *µ*mol L^{-1} and the release involves exchange of interlayer K by cations of high hydration energy and the consequent expansion of the inter layer space. The source and the releasing processes of non-exchangeable K from the maize rhizosphere were evaluated by Moritsuka et al. (2004) and they reported that interlayer K in 2:1 type phyllosilicate was the main source of non exchangeable K for maize and K was releasing through cation (Ca^{2+}, Mg^{2+} and Na^{+} accumulated in the rhizosphere) exchange of the K rather than mineral dissolution by protons. The occurrence of root-induced

release of K from K bearing minerals has been frequently associated with the lowering of K concentration in the solution resulting from root uptake as a result of dynamic equilibrium reaction between the phases of soil K (Hinsinger and Jaillard, 1993).

As per the earlier model calculations, for the under prediction of K influx under low K supply, attention always goes towards the chemical mobilisation of K in the rhizosphere based on the fact that soil solution concentration increases in the rhizosphere, but actually we never measure directly the soil solution concentration in the rhizosphere. On the other hand, root hair uptake kinetics may be different from that of roots. Therefore in the future study, attempts should be taken to measure directly the K depletion around the root and root hair surface and to measure the uptake kinetics (I_{max}, K_m and C_{Lmin}) for root hairs.

Chapter III

Root exudates composition and release rate of wheat and sugar beet at low and high K supply

3 Root exudates composition and release rate of wheat and sugar beet at low and high K supply

3.1 Introduction

The release of all forms of carbon from roots has been termed as rhizodeposition (Marschner, 1995). Rhizodeposition products, which are available for microbial metabolism in the rhizosphere (zone adjacent to the root) and on the rhizoplane (root surface), can be categorized as exudates, lysates, secretions and gases. The difference between exudates and secretions is that, exudates are passively released and secretions are actively released compounds. However, the most common definition of the term “root exudates” is all the substances which are released into the surrounding medium by healthy and intact plant roots (Rovira, 1969) and is the definition used in this chapter. Root exudates include high and low molecular weight compounds. High molecular weight compounds in root exudates include the mucilage, gelatinous material covering root surfaces, and ectoenzymes. Low molecular weight root exudates are released in larger quantities and include organic acids, sugars, phenolics, amino acids, phytosiderophores, flavonoids and vitamins (Marschner, 1995; Whipps, 1990).

Root exudation is affected by multiple factors such as light intensity, temperature, nutritional status of the plants, various stress factors, mechanical impedance, sorption characteristics of the growth medium and microbial activity in the rhizosphere. Root exudation of organic acids, amino acids and sugars generally occurs passively via diffusion and may be enhanced by stress factors affecting membrane integrity such as nutrient deficiency (e.g. K, P, Zn), temperature extremes or oxidative stress (Rovira, 1969; Cakmak and Marschner, 1988). This may be related to preferential accumulation of low molecular weight N and C compounds at the expense of macromolecules (Marschner, 1995). When plants are nutrient deficient, the amount of exudates released by the root often increases (Kraffczyk et al., 1984; Neumann et al., 1999; Subbarao et al., 1997). Root

exudates composition and exudation rate varies among plant species. Singh and Pandey (2003) reported that green gram, a legume crop, had greater root exudation compared to maize. However, the amino acid content of the total root exudates in maize was two-fold as compared to green gram. Root exudates play a role in chemical mobilization of nutrients in the rhizosphere (Marschner, 1995; Wang et al., 2000). Soil extraction experiments with carboxylates, amino acids and sugars revealed that only citrate applied in extraordinary high concentrations (6 mmol g^{-1} soil) was effective in K desorption (Gerke, 1995; Steffens and Zarhoul, 1997). The composition of root-derived substances is of great importance for the understanding of bio-chemical processes in the rhizosphere.

Sugar beet and wheat both are uptake efficient for K. However, both species use different mechanisms. Applying nutrient uptake model on wheat, it could be shown that the high K uptake efficiency of wheat was mainly due its large root system, where calculated transport and uptake agreed well with measured data. However, sugar beet could realize much higher K uptake rate than calculated by the model. In the previous chapter, results of sensitivity analysis showed that by increasing soil solution K concentration (C_{Li}) or buffer power, model prediction for K influx was 100% under low K supply. Which indicates that sugar beet probably increase the soil solution K concentration in the rhizosphere by exuding some organic compounds under K deficiency. Root exudates may mobilize K from non-exchangeable source and an enhanced mobilization of K increases soil solution K concentration, which in turn increases transport of K towards the root. Wang et al. (2000) reported that the net release of K from the mineral K pool was significantly enhanced when the crops were grown in feldspar. The enhanced mobilization of mineral K might be attributed to the release of organic acids from the plant roots. When gneiss of various particle sizes was exposed to malic and tartaric acids, both acids had a direct positive influence on the release of mineral K from gneiss.

This chapter will focus on the release of water soluble organic root exudates in response of low and high K nutrition and characterization of their composition. The purpose was to check if sugar beet is releasing some specific compound under low K supply, which might be responsible for solubilizing K from non-exchangeable

sources. For this an experimental set up was designed to grow K deficient and sufficient plants in sand culture and to collect root exudates under different growth conditions. The first experiment was conducted in a screen house in which wheat and sugar beet plants were grown in coarse quartz sand with continuous supply of nutrient solution of low and high K concentration. Cold and warm water soluble root exudates were collected from plants by a percolation method at two different growth stages. Organic acid, amino acid and sugar composition of root exudates were analyzed quantitatively by High Performance Liquid Chromatography (HPLC) coupled with different detectors. For quantitative analysis of organic acids, HPLC was coupled with photodiode array detector; for amino acids with Fluorescence detector and for sugars with differential refractometer. The second experiment was conducted in a growth chamber under controlled environmental conditions, where wheat and sugar beet plants were grown in medium coarse quartz sand with nutrient solution of low and high K supply and root exudates were collected in similar manner. Non-targeted metabolite profiling was done by separating the root exudates by HPLC coupled with electro-spray mass spectrometry (ESI-MS).

3.1.1 Screen house experiment

3.1.1.1 Materials and methods

3.1.1.1.1 Experimental set up

Wheat (*Triticum aestivum* L. cv. Thasos) and sugar beet (*Beta vulgaris* L. cv. Semper) plants were grown in inverted open mouth bottles containing 1400 g of quartz sand in a screen house. Three seeds of wheat or sugar beet were placed over 1200 g of coarse sand (1-2 mm diameter) and covered by 200 g of medium coarse sand (<0.7 mm diameter) to reduce the evaporation loss of moisture. The bottles were completely covered with aluminium foil in order to avoid transmission of light through the bottle. The plants were supplied with modified Hoagland solution with two K levels (K was supplied as KCl) of the following composition [in mmol L^{-1}]: $NH_4(H_2PO_4)$ [1], $Ca(NO_3)_2$ [7], $MgSO_4$ [2]; [in µmol L^{-1}]: Fe(III)-EDTA

[100], H_3BO_3 [46], $MnCl_2 . 4H_2O$ [9], $ZnSO_4 . 7H_2O$ [0.8], $CuSO_4 . 5H_2O$ [0.3], $(NH4)_6Mo_7O_{24} . 4H_2O$ [0.014]. Plants were supplied with nutrient solution drop wise continuously through plastic tubes graduated from a plastic pot containing 14 L of nutrient solution. Loss in nutrient solution was replenished by fresh nutrient solution in every alternate day. For deficient K level wheat and sugar beet plants were supplied with all the nutrients except K for one week after germination. Afterwards plants were supplied with 50 µmol K L^{-1} for deficient K level and 1000 µmol K L^{-1} for sufficient K level. Potassium concentration of the leachate collected in every alternate day from the bottom of each pot and K concentration was measured to determine the K depletion in the nutrient solution. Accordingly low and high level of K applied to wheat and sugar beet was increased from 50 to 100, 150, 200 and 300 µmol K L^{-1} for low K level and 1000 to 1500 and 2000 µmol K L^{-1} for high K level. As the rate of exudation of each plant is very low, in order to collect sufficient amount of root exudates for further chemical analysis, each treatment was replicated 21 times. Root exudates of wheat and sugar beet were collected two times at 21 and 42 days of growth. Three pots from each treatment were harvested at 21 days of growth after first collection of root exudates for determining root length, shoot dry weight (SDW) and shoot K concentration of wheat and sugar beet and the same parameters were determined for rest of the replications (18) at 42 days of growth after second collection of root exudates.

Picture 3.1: Experimental set up to grow K deficient and sufficient sugar beet plants in quartz sand with continuous supply of modified Hoagland nutrient solution of low and high K levels.

3.1.1.1.2 Collection of root exudates by percolation method

Cold water soluble root exudates (CRE) were collected after 21 days of germination. Supply of nutrient solution was stopped before collection of root exudates. The growing media was washed with distilled water in order to make the sand free from nutrient ions especially K^+ and NO_3^-. As NO_3^- disables the accurate determination of organic acid anions by HPLC (High Pressure Liquid Chromatography) and the root exudates were collected to study their effect in mobilizing K in soil. Potassium and nitrate concentration of the leachate was measured and washing was continued till no K^+ and NO_3^- was detected. Mouth of each bottle was closed from the bottom for one hour. 200 mL of double distilled

water was added to the growing medium to allow the roots to exude. In order to avoid O_2 stress in the roots the cap of the bottles was opened after one hour and leached root exudates were collected from bottom of each bottle and immediately the collected root exudates were refilled to the growing medium and mouth of each bottle was again closed from the bottom for another one hour to allow the roots to exude. Just before 5 minutes of collection, 50 mL of double distilled water was added in order to displace the root exudates from top portion of the growing medium. After two hours, the cap of the bottles was opened and root exudates were collected from bottom of each bottle. Root exudates were filtered through Schleicher and Schuell folded filter paper of ∅ 150 mm to make them free from any foreign particles. The weight of the cold water soluble root exudates (Root exudates with 250 mL water) was recorded and it was frozen at -32°C. The frozen root exudates were transferred to the freeze dryer (Epsilon 2-40 – Christ and LPC-16 was the software used to run the freeze dryer). Weight of freeze dried exudates was recorded. At 42 days of germination both cold and warm water soluble exudates (WRE) were collected. For WRE, the double distilled water was heated to 60°C and 200 mL of warm water (60°C) was added to each pot and the leachate was collected from the bottom and again heated to 60°C and added to the bottle and it was repeated three times and finally WRE was collected. Warm water soluble exudates are assumed to be high molecular weight mucilage, which are not soluble in cold water.

3.1.1.1.3 HPLC analysis of organic acids, amino acids and sugars

The organic acids, amino acids and sugars present in the root exudates sample were analyzed with HPLC for CRE collected at 21 days of germination, but only organic acids and sugars were analyzed for CRE and WRE collected at 42 days of germination.

3.1.1.1.4 Analysis of organic acids

For analyzing organic acids, 10 mg of freeze dried root exudates were weighed in

an eppendorf cup and dissolved in 1 mL of 18 mmol L^{-1} KH_2PO_4 (adjusted to pH 2.5 with H_3PO_4) solution. It was mixed by an ultrasonic mixer, centrifuged and filtered through Teflon membrane filter (0.2 µm) for HPLC injection. The organic acid anions in root exudates samples were analyzed by reversed phase HPLC in the ion suppression mode. Separation was conducted on a reversed phase column, Li ChroCART 250 x 3 mm, Purospher STAR RP-8, 5 µm particle size, equipped with a Li ChroCART 4 x 4 mm, Purospher STAR RP-8, 5 µm particle size, guard column (Merck, Darmstadt, Germany). Sample solutions of 20 µL were injected into the column, and 18 mmol L^{-1} KH_2PO_4 pH adjusted to 2.5 with H_3PO_4 was used for isocratic elution, with a flow rate of 0.15 mL min^{-1} at 30° C and detection was done by photodiode array detector 996 (Waters, Milford, MA, USA). Identification of organic acids was done by comparing retention times and absorption spectra with those of known standards.

3.1.1.1.5 Analysis of amino acids and sugars

For analyzing amino acids, 10-20 mg of freeze dried root exudates were weighed in an eppendorf cup and dissolved in 500 µL of HPLC water. It was mixed by an ultrasonic mixer, centrifuged and filtered through Teflon membrane filter (0.2 µm) for HPLC injection. The amino acids in root exudate samples were analyzed by reversed phase HPLC in the ion suppression mode. Separation was conducted on a reversed phase column (Li ChroCART 250 x 3 mm, Purospher STAR RP-8, 5 µm particle size) equipped with a Li ChroCART 4 x 4, Purospher STAR RP-8, 5 µm particle size, guard column (Merck, Darmstadt, Germany). Sample solutions of 10 µL were injected into the column and eluent gradient of 50 mmol L^{-1} CH_3COONa (pH 7.0) and methanol (71/29 - 20/80, v/v) was used for elution, with a flow rate of 0.6 mL min^{-1} at 45° C and detection was done by fluorescence detector 474 (Waters, Milford, MA, USA) for amino acids. For sugar analysis, separation was done by reversed phase column, LiChroCART 250 x 4 mm, LiChrospher 100 NH_2, 5µm particle size, equipped with a LiChroCART 4 x 4 mm, LiChrospher 100 NH_2, 5 µm particle size, guard column (Merck, Darmstadt, Germany). Sample

solutions of 20 µL were injected into the column, and Acetonitrile and water (80/20, v/v) was used for elution, with a flow rate of 1 mL min^{-1} at 30° C and detection was done by differential refractometer 198.00 (Knauer, Berlin, Germany). Identification of amino acids and sugar was done by comparing retention times and absorption spectra with those of known standards.

3.1.1.1.6 Data analysis

Statistical analysis were performed by using two way analysis of variance (ANOVA), where significant difference were found, mean values were compared by using Tukey's procedure.

3.1.1.2 Results

3.1.1.2.1 Shoot dry weight, root length and shoot K concentration

Wheat and sugar beet showed K deficiency symptoms under low K supply at both first and second harvest. At first harvest, under low K supply, shoot dry weight (SDW) and root length (RL) was 9.7 and 10% of the maximum, respectively with shoot K concentration of 1.22% in wheat (Table 3.1). Shoot dry weight and RL was 21% and 19% of the maximum, respectively with shoot K concentration of 0.97% in sugar beet. Considering relative shoot dry weight as a measure of K efficiency, sugar beet was found to be more K efficient as compared to wheat. Under high K supply conditions, shoot K concentration was increased by 3.7 and 4.7 times in wheat and sugar beet, respectively. At second harvest, relative shoot yield was similar as reported at first harvest in both wheat and sugar beet, where as relative root length was greater in sugar beet than in wheat i.e. it was 9.3 and 35% of the maximum in wheat and sugar beet respectively under low K supply. At second harvest, the shoot K concentration was increased by 1.5 and 2.3 times as

compared to first harvest in wheat and sugar beet, respectively.

Table 3.1: Shoot dry weight (SDW), root length (RL) and shoot K concentration of wheat and sugar beet at low and high K levels after 21 and 42 days of growth.

Harvest	Crops	K levels	SDW	RL	Shoot K concentration
			g pot^{-1}	cm $plant^{-1}$	%
First	Wheat	Low	0.086 a B	141 a B	1.22 a B
		High	0.885 a A	1409 a A	4.48 a A
	Sugar beet	Low	0.096 b B	150 b B	0.97 a B
		High	0.451 b A	798 b A	4.53 a A
Second	Wheat	Low	0.44 b B	399 b B	1.89 b B
		High	4.87 b A	4280 b A	4.78 b A
	Sugar beet	Low	3.48 a B	4062 a B	2.27 a B
		High	15.49 a A	11506 a A	5.72 a A

Data are means of 3 replicates for first harvest and 5 replicates for second harvest. Lower case letters indicate significant difference of SDW, RL and shoot K concentration between main effect of wheat and sugar beet at the same K level ($P \leq 0.001$, Tukey-test). Upper case letters indicate significant difference of SDW, RL and shoot K concentration between different K levels for the same crop species ($P \leq 0.001$, Tukey-test).

3.1.1.2.2 Root exudation rate of CRE and WRE

Results indicate the differences in root exudation between wheat and sugar beet at low and high K supply (Figure 3.1). At first harvest, under low K supply, the rate of exudation of cold water soluble root exudates (CRE) was 19 times higher compared to high K supply in both wheat and sugar beet. At second harvest, rate of root exudation was decreased in wheat and sugar beet as compared to first harvest both under low and high K supply. The rate of exudation was also significantly greater under low K supply compared to high K supply at second harvest. However, reduction in exudation rate due to high K supply was greater in

wheat compared to sugar beet. At second harvest after collection of cold water soluble root exudates, warm water soluble exudates (WRE) were collected. Under low K supply, the rate of exudation of WRE was 9.2 and 2.5 times higher than under high K supply in wheat and sugar beet, respectively. The rate of exudation of both CRE and WRE was higher in wheat than in sugar beet both at first and second harvest. However differences were remarkable at second harvest.

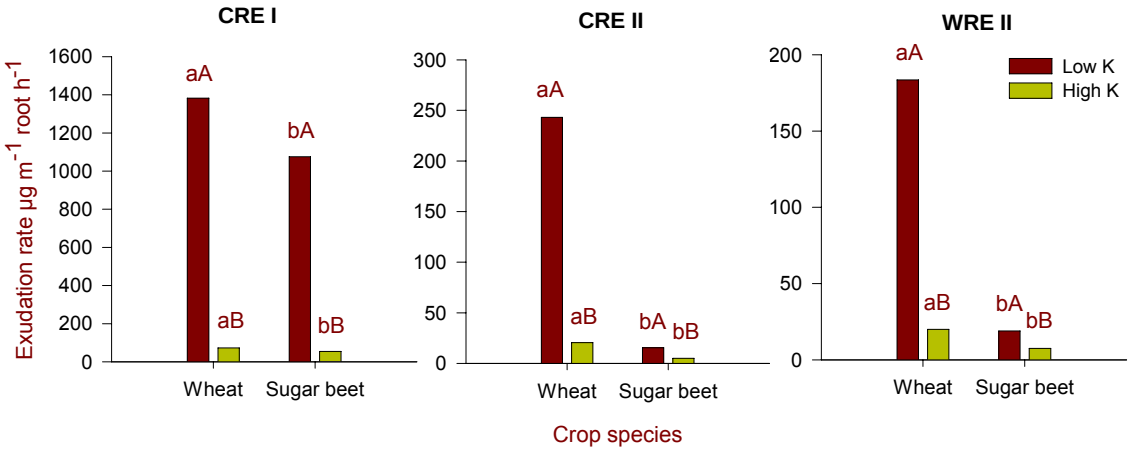


Figure 3.1: Exudation rate of cold water soluble root exudates at first harvest (CRE I) and cold and warm water soluble root exudates at second harvest (CRE II and WRE II) of wheat and sugar beet under low and high K supply grown in the screen house under natural sunlight.

Data are mean of 3 replicates for first harvest and 5 replicates for second harvest. Lower case letters indicate significant difference of exudation rate between main effect of different crops at the same K level ($P \leq 0.001$, Tukey-test). Upper case letters indicate significant difference of exudation rate between different K levels for the same crop species ($P \leq 0.001$, Tukey-test).

3.1.1.2.3 HPLC analysis of organic acids, amino acids and sugars

Organic acids and sugars detected in root exudates of wheat and sugar beet and their exudation rate are given in table 3.2 and 3.3. Under high K supply, the rate of exudation of organic acids and sugars was decreased by many folds as compared to that of low K supply and this decrease was higher in wheat than in sugar beet. Lactic acid exudation rate was highest followed by acetic, malic, citric and fumaric acid. Citric acid exudation rate was higher in WRE than in CRE in both wheat and sugar beet at second harvest and acetic acid exudation rate was higher in WRE

than in CRE only in wheat. Sucrose and t-aconitic acid were detected only in WRE in both wheat and sugar beet.

Aspartic acid (ASP), glutamic acid (GLU), serine (SER), arginine (ARG), glycine (GLY), threonine (THR), alanine (ALA), valine (VAL), phenylalanine (PHE), isoleucine (ILE), leucine (LEU) and lysine (LYS) were the twelve amino acids detected in root exudates of wheat and sugar beet collected at first harvest (Figure 3.2). Arginine was detected only in root exudates of sugar beet both under low and high K supply. Amino acids exudation rate was greater in wheat as compared to sugar beet. Under low K supply amino acids exudation rate was greater than under high K supply in both wheat and sugar beet.

The proportion of the reported organic acids, sugars and amino acids was only 2, 2 and 0.2% of the collected root exudates, respectively.

Table 3.2: Organic acids exudation rate of wheat and sugar beet at low and high K supply.

Organic compounds	Crops	K levels	Exudation rate		
			I CRE	II CRE	II WRE
			nmol m^{-1} root h^{-1}		
Malic acid	Wheat	Low	5.67 a A	1.61 a A	1.06 a A
		High	0.34 a B	0.05 a B	0.04 a B
	Sugar beet	Low	3.92 a A	0.06 b A	0.02 b A
		High	0.37 a B	0.04 b B	0.01 b B
Citric acid	Wheat	Low	4.73 a A	0.92 a A	4.05 a A
		High	0.24 a B	0.21 a B	0.23 a B
	Sugar beet	Low	0.66 b A	0.07 b A	2.32 a A
		High	0.06 b B	0.03 b B	0.18 a B
Lactic acid	Wheat	Low	149 b A	49.6 a A	54.9 a A
		High	17.0 b B	2.92 a B	3.96 a B
	Sugar beet	Low	298 a A	1.66 b A	5.60 b A
		High	19.5 a B	0.72 b B	0.97 b B
Acetic acid	Wheat	Low	49.4 a A	1.68 a A	3.91 a A
		High	0.41 a B	0.17 a A	0.04 a B
	Sugar beet	Low	3.77 b A	0.16 a A	0.09 b A
		High	0.30 b B	0.22 a A	0.38 b B
Fumaric acid	Wheat	Low	0.2562 a A	0.0047 a A	0.0042 a A
		High	0.0017 a B	0.0005 a B	0.0037 a B
	Sugar beet	Low	0.0280 b A	0.00007 b A	0.0007 b A
		High	0.0004 b B	0.0001 b B	0.00007 b B
t-Aconitic acid	Wheat	Low	*ND	ND	0.011 a A
		High	ND	ND	0.005 a A
	Sugar beet	Low	ND	ND	0.014 a A
		High	ND	ND	0.001 a A

*ND- not detected.

Data are means of 3 replicates for first harvest and 5 replicates for second harvest. Lower case letters indicate significant difference of organic acids exudation rate between main effect of wheat and sugar beet at the same K level ($P \leq 0.001$, Tukey-test). Upper case letters indicate significant difference of organic acids exudation rate between different K levels for the same crop species ($P \leq 0.001$, Tukey-test).

Table 3.3: Sugars exudation rate of wheat and sugar beet at low and high K supply.

Sugar	Crops	K levels	Exudation rate		
			I CRE	II CRE	II WRE
			nmol m^{-1} root h^{-1}		
Glucose	Wheat	Low	134 a A	35 a A	42.5 a A
		High	11 a B	6.2 a B	6.0 a B
	Sugar beet	Low	113 a A	2.0 b A	8.7 b A
		High	24 a B	1.5 b B	2.5 b B
Sucrose	Wheat	Low	ND	ND	2.32 b A
		High	ND	ND	0.36 b B
	Sugar beet	Low	ND	ND	2.93 a A
		High	ND	ND	1.30 a B

*ND- not detected

Data are means of 3 replicates for first harvest and 5 replicates for second harvest. Lower case letters indicate significant difference of sugar exudation rate between main effect of wheat and sugar beet at the same K level ($P \leq 0.001$, Tukey-test). Upper case letters indicate significant difference of organic acids exudation rate between different K levels for the same crop species ($P \leq 0.001$, Tukey-test).

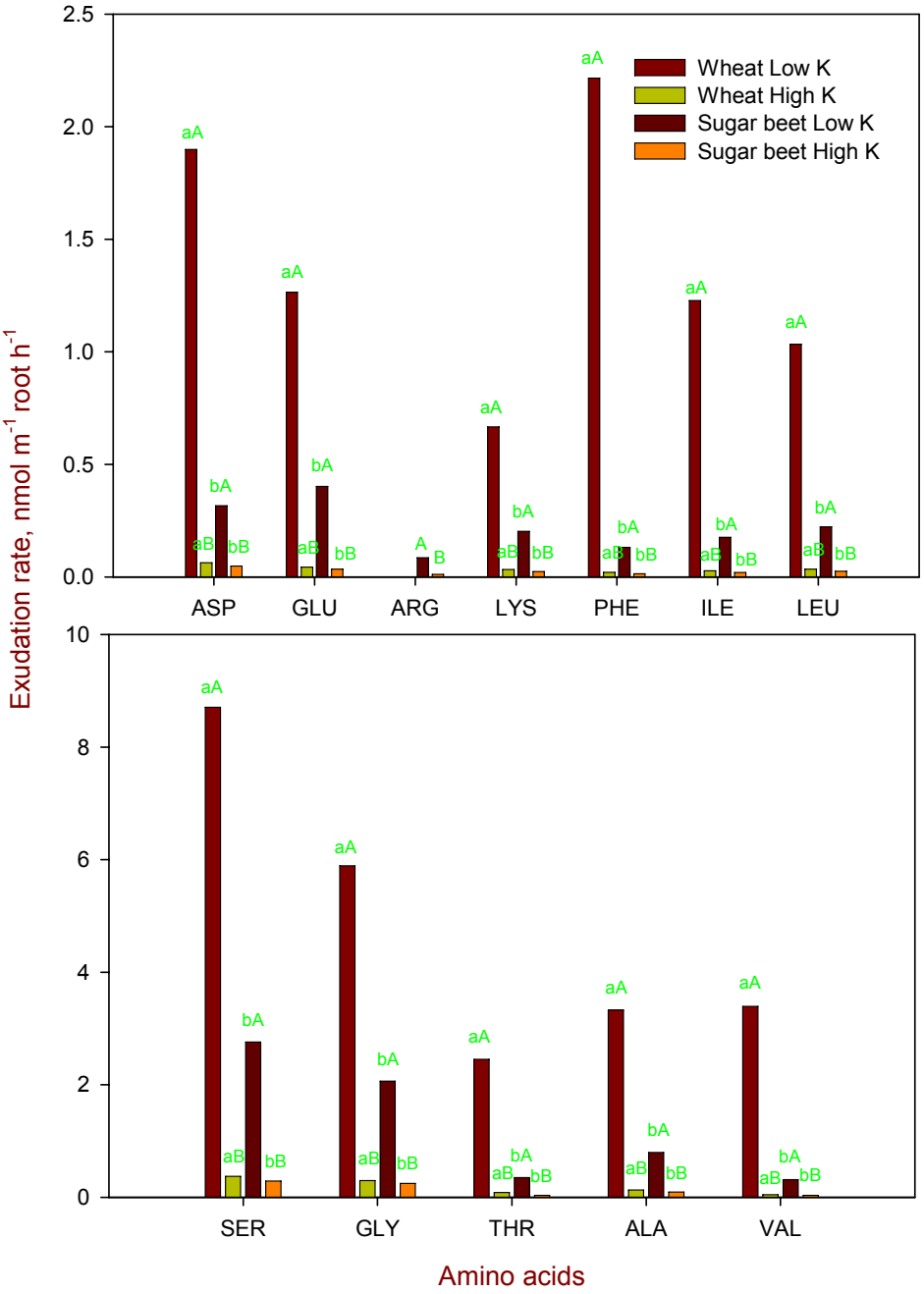


Figure 3.2: Amino acid exudation rate of wheat and sugar beet under low and high K supply.

Data are mean of 3 replicates. Lower case letters indicate significant difference of amino acid exudation rate between main effect of different crops at the same K level ($P \leq 0.001$, Tukey-test). Upper case letters indicate significant difference of amino acid exudation rate between different K levels for the same crop species ($P \leq 0.001$, Tukey-test).

3.1.1.3 Discussion

At first harvest, shoot K concentration was approximately 1% in wheat and sugar beet, which is lower than the critical shoot K concentration of 2% which shows that the plants were severely K deficient (Table 3.1). Shoot K concentration at second harvest was around 2% in wheat and sugar beet which shows K deficiency was more severe at first harvest as compared to second harvest. Under high K supply shoot K concentration was more than 4%, which was far above the critical concentration for K deficiency. Results of relative shoot dry weight and root length shows that, K deficiency was more severe in wheat as compared to sugar beet through out the growth period.

Under low K supply, the rate of exudation of cold water soluble root exudates (CRE) was several-fold higher as compared to high K supply in wheat and sugar beet at both the harvest (Figure 3.1). When plants are nutrient deficient, the amount of exudates released by the root often increases (Kraffczyk et al., 1984). Differences in root exudation have also been reported for different crop species (Neumann et al., 1999; Ohwaki and Hirata, 1992; Subbarao et al., 1997). With age of plant, the rate of exudation was decreased in wheat and sugar beet both under low and high K supply. However, at second harvest, reduction in exudation rate due to high K supply was greater in wheat as compared to sugar beet. Kamh et al. (2001) observed a decrease of the organic anions exudation rate with time under P deficiency. Rovira (1956) demonstrated differences in the exudates of different plants and differences were due to the age of the plant.

The rate of root exudation in wheat was significantly higher as compared to sugar beet at both the harvests; however this difference was remarkable at second harvest. As the K deficiency was more severe in wheat, it released more organic compounds as compared to sugar beet. Singh and Pandey (2003) reported both inter- and intra-species differences among maize and green gram in terms of root exudation, P uptake, and shoot and root P content and in general, green gram, a legume crop had greater root exudation compared to maize. There was no noticeable difference between the rate of release of cold and warm water soluble

root exudates. The rate of root exudation of WRE was higher under low K supply as compared to high K supply and it was higher for wheat than sugar beet as reported in CRE.

Exudation rate of all reported organic acids except lactic and t-aconitic acid was higher in wheat compared to sugar beet (Table 3.2). Glucose was the only sugar detected in CRE; where as in WRE, glucose and sucrose were detected. Sucrose and t-aconitic acid was only detected in WRE (Table 3.3). Plants are capable of releasing large amounts of organic acids into the rhizosphere in response to P deficiency (Hoffland et al., 1992; Gerke, 1994). Enhanced release of organic acids has been reported under P deficiency in dicots in general and legumes in particular (Lipton et al., 1987). Malate and citrate appear to be the primary components released by roots under P deficiency (Jones, 1998). Iron deficiency induces a substantial accumulation of organic acids in root tissues and also induces 5-10 fold increase in organic acid excretion (Ohwaki and Sugahara, 1997). Neumann and Römheld (1999) reported that P-deficiency induced exudation of carboxylic acids in chickpea and white lupin and was associated with a larger increase of carboxylic acid concentrations in the roots and lower accumulation of carboxylates in the shoot tissue and this depends on the ability to accumulate carboxylic acids in the root tissue, which in turn is determined by biosynthesis, degradation and partitioning of carboxylic acids or related precursors between roots and shoot. In some plants such as white lupin, there are indications for a specific transport mechanisms (anion channel) involved in root exudation of extraordinary high amounts of citric acid. Exudation rate of amino acids was significantly higher in wheat compared to sugar beet both under low and high K supply (Figure 3.2). Singh and Pandey (2003) reported that amino acid content of the total root exudates in maize was two-fold as compared to green gram. Amino acids exudation rate was significantly higher under low K supply as compared to high K supply. Arginine was detected only in root exudates of sugar beet under both low and high supply. Rovira (1956) reported that Peas excreted considerable amounts of amino material during 21 days growth with 22 different amino compounds, while oats excreted less consisting of 14 amino compounds. The proportions of the

various amino acids in the exudates differed between peas and oats. The proportion of the reported organic acids, sugars and amino acids was only 2, 2 and 0.2% of the collected root exudates, respectively. The results show that only 4.2% of the total root exudates were analyzed by HPLC. The very low proportion of organic acids, sugars and amino acids indicate the possibility of microbial degradation of the root exudates during the time of collection and filtration.

Root exudation rate was many-fold higher under low K supply as compared to high K supply conditions in both wheat and sugar beet. However, rate of root exudation was higher in wheat as compared to sugar beet. Rate of exudation decreased with plant age. Exudation rate of organic acids (acetic acid, malic acid, citric acid and fumaric acid) except lactic acid and t-aconitic acid was higher in wheat compared to sugar beet. Exudation rate of amino acids was higher in wheat compared to sugar beet. Glucose was the only sugar detected in CRE; where as in WRE, glucose and sucrose were detected.

3.1.2 Growth chamber experiment

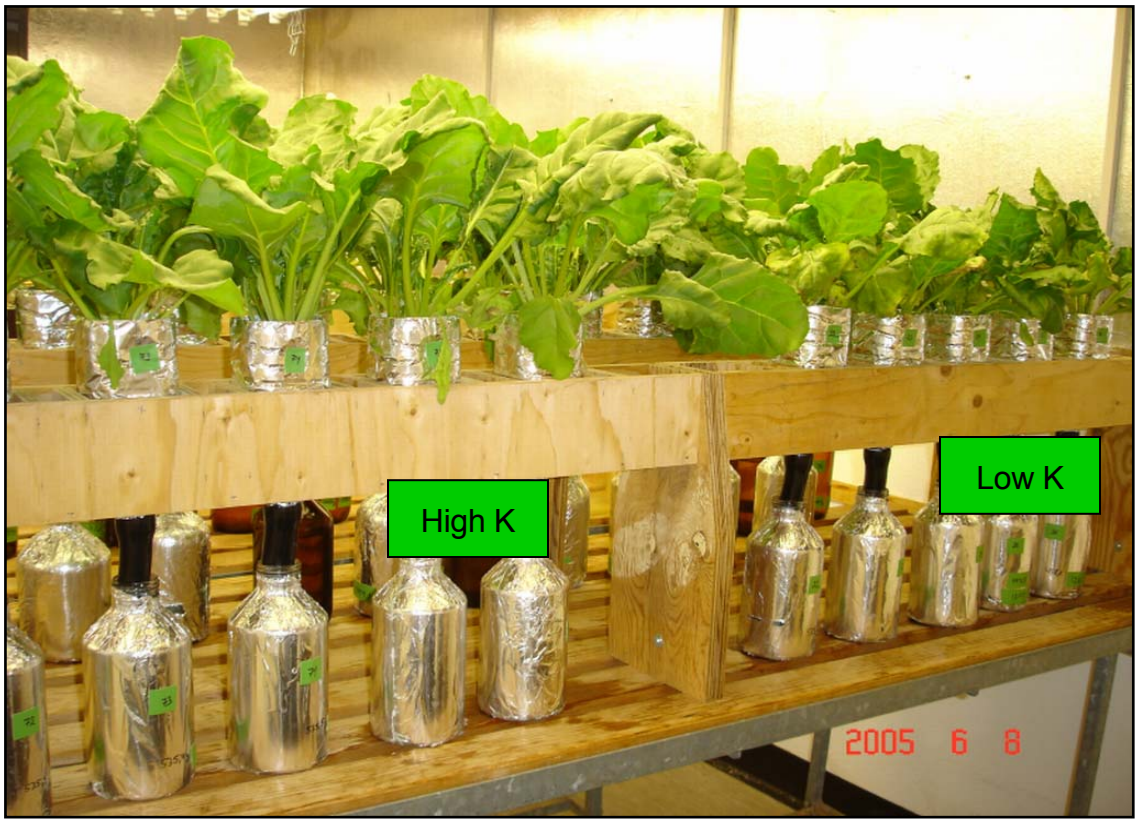
3.1.2.1 Materials and methods

3.1.2.1.1 Experimental set up

Wheat (*Triticum aestivum* L. cv. Thasos) and sugar beet (*Beta vulgaris* L. cv. Semper) plants were grown in inverted open mouth bottles containing 1400 g of medium course quartz sand (0-7 mm diameter) in a growth chamber with day/night regime of 16/8 hours, temperature of 25/18 $^{\circ}$C and relative humidity of 70 %. The photosynthetic active radiation during the day time was 250 µE m^{-2} s^{-1}. The bottles were completely covered with aluminium foil in order to avoid transmission of light through the bottle. Number of plants per pot was 3 and 2 for wheat and sugar beet, respectively. The plants were supplied with modified Hoagland solution with two K levels with similar composition as described in the earlier screen house experiment. From the beginning all the plants were supplied with 500 µmol K L^{-1} from one liter bottle placed immediately below the bottom of inverted bottle in

which plants were grown. The mouth of the inverted bottle and the bottle containing 1 L of solution which serve as reservoir for nutrient solution were connected by black color plastic tubes. Everyday nutrient solution was recycled from the 1 L bottle placed at the bottom and after recycling the weight loss from the growing medium was replenished by distilled water. Every alternate day, K concentration from the reservoir bottle was measured. Plants were allowed to grow under sufficient K for some days and afterwards K concentration in nutrient solution was gradually reduced to 100 µmol K L^{-1} (low K supplied plants) for half of the pots and for other half it was gradually increased to 4000 µmol K L^{-1} (high K supplied plants).

As the rate of exudation of each plant is very less, in order to collect sufficient amount of root exudates for further chemical analysis, each treatment was replicated 20 times. Root exudates of wheat and sugar beet were collected two times at 21 and 42 days of growth. Three pots from each treatment were harvested at 21 days of growth after first collection of root exudates for determining root length, shoot dry weight (SDW) and shoot K concentration of wheat and sugar beet and the same parameters were determined for rest of the replications (17) at 42 days of growth after second collection of root exudates.



Picture 3.2: Experimental set up to grow K deficient and sufficient sugar beet plants in quartz sand supplied with modified Hoagland nutrient solution of low and high K levels.

3.1.2.1.2 Collection of root exudates by percolation method

Root exudates were collected from wheat and sugar beet at 21 and 42 days of growth, respectively by percolation method as described earlier. The weight of the cold and warm water soluble root exudates (Root exudates with 250 mL water) was recorded and it was frozen at -32°C. The frozen root exudates were transferred to the freeze dryer (Epsilon 2-40 – Christ and LPC-16 was the software used to run the freeze dryer). Weight of freeze dried exudates was recorded. Differential metabolite profiling for comparative analysis of organic components in root exudates was done by HPLC-MS (High Pressure Liquid Chromatography – Mass Spectrometry) in department of Crop Sciences- Molecular Plant Pathology and Mycotoxins research group, Georg-August-Universität, Göttingen. The purpose was to identify the metabolic signals which occur or change with potassium deficiency. The analytical strategy was to separate and detect as many

metabolites as possible in a single analysis, but only the signals which were different for different treatments were considered for characterization. Therefore metabolic profiles were recorded using the separation of dissolved root exudates samples by HPLC on reversed phase material in combination with mass spectrometry and photometric detectors. Profiling schemes for Arabidopsis and other plants have been developed in recent years (Roessner et al., 2000, 2001; Fiehn et al., 2000; Wagner et al., 2003). The main focus of these mostly gas chromatography (GC)-mass spectrometry (MS)-based approaches have been metabolites of the primary metabolism such as sugars, amino acids and organic acids. Several hundred compounds can be robustly and reliably detected. However, these first pioneering reports already emphasize the need for complementary liquid chromatography (LC)-MS based approaches to allow a more comprehensive profiling of metabolites (Roessner et al., 2000). The coupling of electrospray ionization (ESI) MS with capillary (Cap) electrophoresis (Soga et al., 2002) and hydrophilic interaction chromatography (Tolstikov and Fiehn, 2002) has been successfully applied to metabolomics problems. Every analytical procedure is necessarily limited as to what type of compounds can be separated and detected. GC-MS is predominantly applied to very polar or unpolar substances and the main application range of LC-MS is more related to compounds of medium polarity. Furthermore, LC coupled to an MS technique providing soft ionization and high mass accuracy has the potential to generate information useful for the identification of unknown compounds because molecular ions and characteristic fragments can be detected (De Hoffmann, 1996; Niessen, 1999). Roepenack-Lahaye and coworkers (2004) described a profiling approach in Arabidopsis that combines separation by capillary liquid chromatography with the high resolution, high sensitivity, and high mass accuracy of quadrupole time-of-flight mass spectrometry. About 2,000 different mass signals could be detected in extracts of Arabidopsis roots and leaves. Many of these originate from Arabidopsis secondary metabolites. Detection based on retention times and exact masses was robust and reproducible. The dynamic range was sufficient for the quantification of metabolites. Assessment of the reproducibility of the analysis showed that

biological variability exceeds technical variability.

3.1.2.1.3 Advantages of linking High Performance Liquid Chromatography with Mass Spectrometry

Mass Spectrometry (MS) coupling with the separation power of High Pressure Liquid Chromatography (HPLC) has become a widely used analytical technique for qualitative and quantitative analysis of semi-volatile, thermo labile and polar substances. The mass spectrometer acquires mass to charge ratio (m/z) of ions. In many analyses, the compounds of interest are found as part of a complex mixture and the role of the chromatographic technique is to provide separation of the components of that mixture to allow their identification or quantitative determination and identification is based on retention time of an unknown with those of reference materials. Even if the retention characteristics of an unknown and a reference material are identical, the analyst cannot say with absolute certainty that the two compounds are the same. It is not always possible to effect complete separation of all the components of a mixture and which prevent precise and accurate quantitative determination of the analyte of interest. The mass spectrometry lies in the fact that the mass spectra of many compounds are sufficiently specific to allow their identification with a high degree of confidence. The combination of the separation capability of chromatography to allow pure compounds to be introduced into the mass spectrometer with the identification capability of the mass spectrometer is therefore advantageous, particularly as many compounds with similar or identical retention characteristics have quite different mass spectra and therefore be differentiated (Snyder and Kirkland, 1974).

3.1.2.1.4 Sample preparation for HPLC-MS analysis

About 0.5 mg of freeze-dried root exudates was weighed in a 1.5 mL capacity HPLC Vial. 100 µL Methanol was added to let dissolve the less polar compound, after few minutes, 900 µL of double distilled water was added. The vial was

shaken with a vortexer. The dissolved root exudates solution was filtered through a Teflon membrane filter (0.2 µm). Vials were covered by screw cap with a Teflon septum. Samples were analyzed within 6 hours of its preparation. Autosampler was cooled to 8°C.

3.1.2.1.5 Instrumental Analysis

High Performance Liquid Chromatography – Photometric detector - Electrospray-Ionization/Mass Spectrometry (HPLC-DAD-ESI/MS)
Injection:

- injection volume: 10 µL

Separation:

- stationary phase: reversed phase column
- mobile phase solvents: water with 5 % acetonitrile (A), methanol (B)
- gradient elution: increase of methanol starting at 10% to 98%, 0.2 mL min^{-1}
- column oven: 40°C

Detection:
Diode array detector (DAD): UV absorption 200-800 nm
The eluate runs through a cell where the UV-Absorption is measured without changing or destroying the analytes, so that afterwards the eluate can be submitted to electrospray ionization for mass spectrometric detection.

Electrospray-Ionisation interface hyphenated to Triple Quadrupole Mass spectrometer:

- Electrospray-Ionisation
  ESI negative: Needle Voltage – 4400V, Shield Voltage -600 V
  ESI positive: Needle Voltage +5000V, Shield Voltage +250 V
  Drying Gas: N_2, pressure18 psi, at 250°C

Nebulizing Gas: Air, pressure 50 psi

- Scan-Mode: Full Scan from m/z 50 to m/z 500, Scan time 1 sec, Centroid.

Data analysis:

- Analysis Software for chromatograms with mass spectrometric detection: MS Data Review
- Analysis of DAD chromatograms and UV-Spectra: Polyview

Solvents and materials

Syringe filter with Teflon membrane 0.2 µm (WICOM, Heppenheim, Germany).
HPLC column: Polaris C18-A, 150 x 2 mm, Particle size 5 µm (Varian, Darmstadt, Germany).
Solvents: Methanol, HPLC Gradient Grade (VWR, Darmstadt, Germany) and double distilled water.

Instruments and software

Autosampler: ProStar 430 Autosampler (Varian, Darmsatdt, Dtl.)
HPLC-Pumps: ProStar 210 Solvent Delivery Module (Varian, Darmstadt, Dtl.)
Column oven: Mistral, ProStar 510 column oven (Varian, Darmstadt, Dtl.)
Diodearray-Detector (DAD): ProStar 330 Photodiode Array Detector (Varian, Darmstadt, Dtl.)
Mass Spectrometer: 1200 LC/MS Triple Quadrupole- Mass spectrometer coupled with Electrospray Ionisation-Interface (Varian, Darmstadt)
Software for acquisition: MS Workstation Version 6.41 (Varian, Darmstadt)
Software for data analysis: Polyview, MS Data Review, MS Workstation Version 6.41

3.1.2.1.6 Purification of freeze dried root exudates

5 mg of cold water soluble root exudates, collected from low K supplied sugar beet at second harvest was loaded onto the column containing Sephadex LH-20. Analytes were eluted with 10% methanol: 90% water. Several fractions of 0.5 mL each were collected and analyzed by direct infusion into the ESI-MS.

3.1.2.2 Results

In the earlier experiment the low K supplied plants were severely K deficient, because for deficient K level, wheat and sugar beet plants were supplied with all the nutrients except K for one week after germination. Afterwards plants were supplied with nutrient solution of very low K concentration i.e. 50 µmol K L^{-1}. As K is the major essential nutrient for plant growth, the plant stopped growing after 15 days of supplying solution of 50 µmol K L^{-1}. Afterwards increasing K concentration had no effect in mitigating the severe K deficiency. To avoid that, the present experiment was designed to grow the plants with sufficient K for some days and afterwards K concentration in nutrient solution was gradually reduced (low K supplied plants) for half of the pots and for other half it was gradually increased (high K supplied plants).

3.1.2.2.1 Shoot dry weight, root length and shoot K concentration

Wheat and sugar beet plants showed K deficiency symptoms under low K supply at both first and second harvest. At first harvest, under low K supply shoot dry weight (SDW) and root length (RL) was 77% and 55% of the maximum, respectively with shoot K concentration of 1.9% in wheat (Table 3.4). Shoot dry weight and RL was 46% and 39% of the maximum, respectively with shoot K concentration of 1.4% in sugar beet. The shoot K concentration was lower than the critical concentration of 2% which shows that the plants were K deficient. Under high K supply, shoot K concentration was increased by 2.3 and 1.9 times in wheat and sugar beet, respectively. At second harvest, relative shoot yield was

reduced in wheat and it was 58% of the maximum, but in sugar beet it was similar as reported at first harvest i.e. 46.5% of the maximum. Relative root length was greater in sugar beet than in wheat i.e. it was 45 and 50% of the maximum in wheat and sugar beet, respectively under low K supply. At second harvest shoot K concentration was 2.0 and 1.8% in wheat and sugar beet, respectively under low K supply. Under high K supply, shoot K concentration was increased by 2.0 times in both wheat and sugar beet.

Table 3.4: Shoot dry weight (SDW), root length (RL) and shoot K concentration of wheat and sugar beet at low and high K levels after 21 and 42 days of growth.

Harvest	Crops	K levels	SDW	RL	Shoot K concentration
			g pot^{-1}	cm $plant^{-1}$	%
First	Wheat	Low	1.2 b B	1833 b B	1.9 a B
		High	1.5 b A	3351 b A	4.3 a A
	Sugar beet	Low	1.1 a B	1726 a B	1.4 b B
		High	2.4 a A	4468 a A	2.6 b A
Second	Wheat	Low	9.2 a B	5708 b B	2.0 a B
		High	15.7 a A	12624 b A	4.3 a A
	Sugar beet	Low	6.1 b B	7893 a B	1.8 b B
		High	12.9 b A	15749 a A	3.8 b A

Data are means of 3 replicates for first harvest and 5 replicates for second harvest. Lower case letters indicate significant difference of SDW, RL and shoot K concentration between main effect of wheat and sugar beet at the same K level ($P \leq 0.001$, Tukey-test). Upper case letters indicate significant difference of SDW, RL and shoot K concentration between different K levels for the same crop species ($P \leq 0.001$, Tukey-test).

3.1.2.2.2 Root exudation rate of CRE and WRE

Results indicate the differences in root exudation between wheat and sugar beet at low and high K supply (Figure 3.3). At first harvest, under low K supply, the rate of exudation of cold water soluble root exudates (CRE) was 1.6 times higher compared to high K supply in wheat, but in sugar beet it was 7.0 times higher. The rate of exudation decreased with time. At second harvest the exudation rate of CRE was 1.2 and 2.7 times higher in low supply plants compared to high K supply in wheat and sugar beet, respectively. The exudation rate of WRE was 3.6 and 7.0 times higher in low K supply plants compared to high K supply in wheat and sugar beet, respectively. The exudation rate was higher in sugar beet, because K deficiency was more severe in sugar beet compare to wheat.

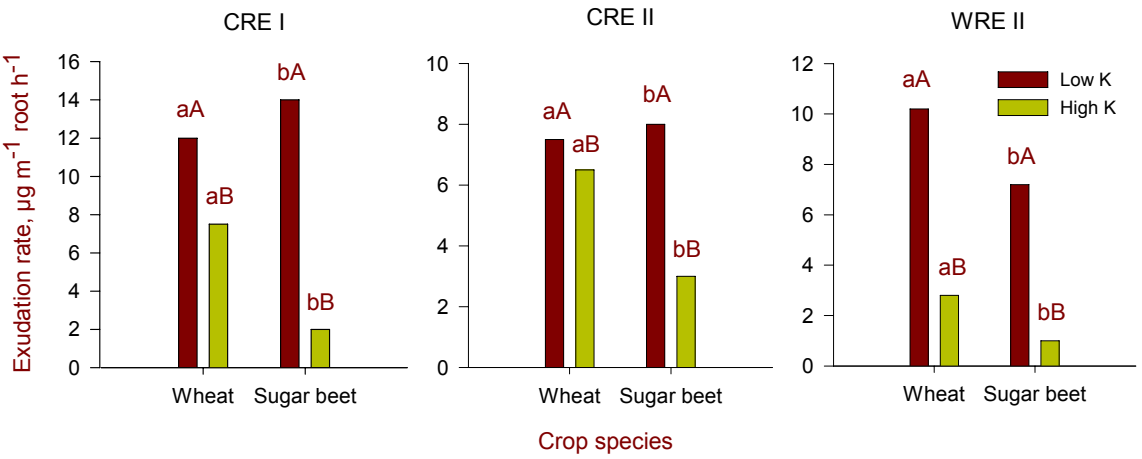


Figure 3.3: Exudation rate of cold water soluble root exudates at first harvest (CRE I) and cold and warm water soluble root exudates at second harvest (CRE II and WRE II) of wheat and sugar beet under low and high K supply grown in growth chamber under controlled condition.

Data are mean of 3 replicates for first harvest and 5 replicates for second harvest. Lower case letters indicate significant difference of exudation rate between main effects of different crops at the same K level ($P \leq 0.001$, Tukey-test). Upper case letters indicate significant difference of exudation rate between different K levels for the same crop species ($P \leq 0.001$, Tukey-test).

In growth chamber experiment, root exudation rate of cold water soluble root exudates of wheat and sugar beet was reduced by approximately 100 times as compared to screen house experiment under low K supply and reduced by approximately 20 times under high K supply at first harvest. But this reduction in root exudation rate was lower at second harvest i.e. the rate of exudation of wheat and sugar beet was reduced by 15 and 2 times in the growth chamber experiment as compared to screen house experiment under low and high K supply, respectively.

3.1.2.2.3 HPLC-MS analysis

During sample preparation it was observed that part of the weighed root exudates samples was not dissolved in the solvent. They were removed by filtration. May be those were unpolar waxes from the roots or silica polymer of the quartz sand. Samples were measured within 6 hours of preparation to avoid degradation and

transformation of some signals.

Comparison of HPLC-MS profiles of root exudates showed some signals specific to root exudates collected from low and high K supplied wheat and sugar beet. Total ion current for the cold and warm water soluble root exudates were recorded at ESI (Electro spray ionization) positive and negative mode. But the major differences in signals between low and high K supply and between wheat and sugar beet at the same K level were observed in the ESI negative mode.

ESI negative mode

The total ion current (TIC) recorded in ESI negative mode for mass range (m/z values) 50 to 500 D for the cold water soluble root exudates (CRE) collected at first harvest and for both cold and warm water soluble root exudates (CRE and WRE) collected at second harvest from wheat and sugar beet under low and high K supply are given in figure 3.4 and 3.5. Several signals were detected in root exudates samples, but not in the HPLC mobile phase (10% methanol). There was difference between TIC of CRE collected at first and second harvest and also between TIC of cold and warm water soluble root exudates. There were several signals detected specific to CRE collected at first and second harvest and also some signals were specific to WRE. The intensity of the signal also varied from first harvest to second harvest and from CRE to WRE. In this chapter the m/z values for cold water soluble root exudates collected at second harvest are described in detail.

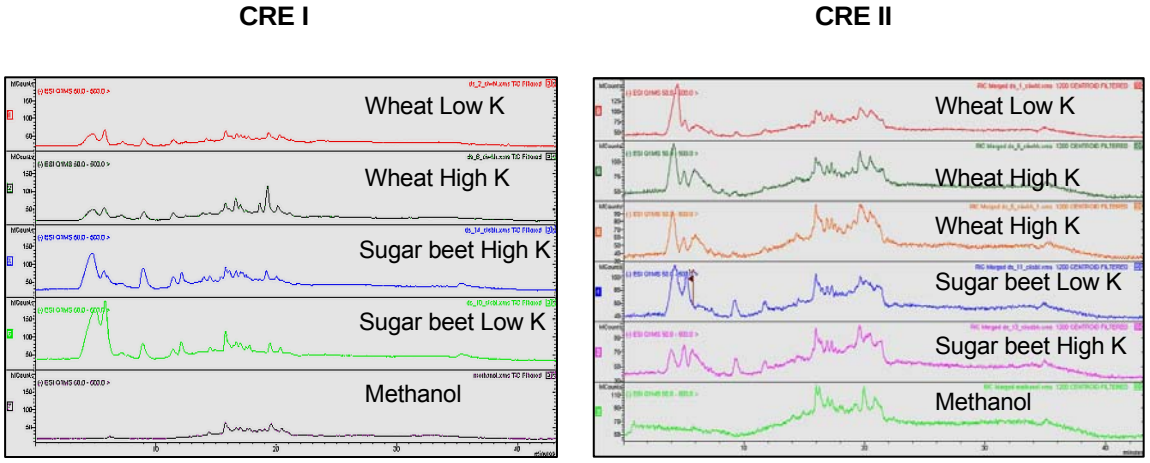


Figure 3.4: Total ion chromatogram (Full scan m/z 50-500 D) at ESI negative mode of CRE collected from wheat and sugar beet under low and high K supply at first (I) and second (II) harvest.

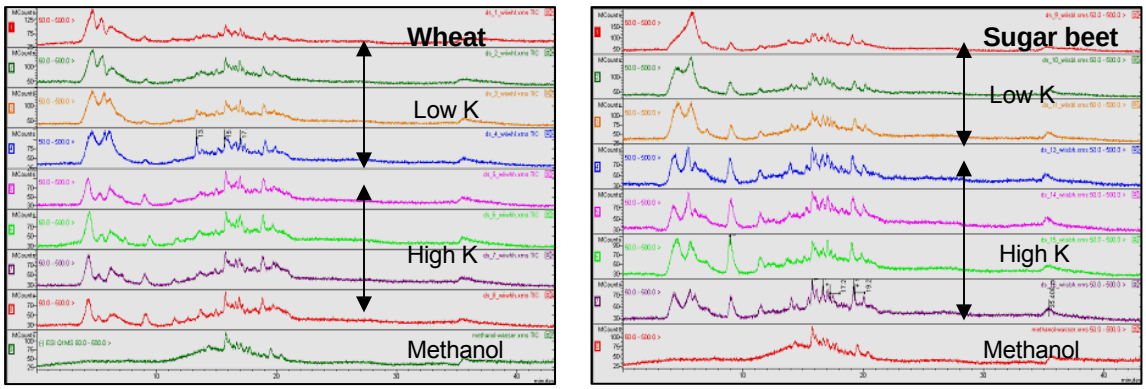


Figure 3.5: Total ion chromatogram (Full scan m/z 50-500 D) at ESI negative mode of WRE collected from wheat (Four replicates each for low and high K supply) and sugar beet (3 replicates for low K and four replicates for high K supply) under low and high K supply at second harvest.

Mass spectrometric signals of CRE at second harvest

Out of the several signals, detected in CRE collected at second harvest, only 18 were different in their intensities in root exudates of wheat to sugar beet at same K level and also in low and high K supply for the same crop. The m/z values with their retention time (t_R) and the corresponding intensities (which is represented as areas per mg of freeze dried root exudates) of those 18 signals are given in figure 3.6 and 3.7. The intensity of the signals corresponding to m/z values- 62, 225, 89,

210, and 119 were relatively stronger among all the signals detected in CRE. The intensity of the signals corresponding to m/z values- 210, 119, 164, 179, 189, 147 (t_R = 5.22 minutes), 173, 116, 145 (t_R = 7.19 minutes), 191 and 254 were relatively higher in CRE collected from low K supplied wheat and sugar beet as compared to high K supply. For CRE collected from high K supplied wheat and sugar beet, the intensity of the signals corresponding to m/z values- 59 and 89 were relatively higher as compared to that of low K supply and that of 225 was higher under high K supply as compared to low K supply only in sugar beet. The intensity of the signals corresponding to m/z values- 225, 179, 210, 159, 59, 145 (t_R = 6.00 minutes), 62 and 254 were relatively higher in CRE of sugar beet as compared to that of wheat and the intensity of the signal of m/z value 119, 116, 173, 103 (only under low K), 145 (t_R = 7.19 minutes), 191 were relatively higher in wheat as compared to sugar beet under both low and high K supply.

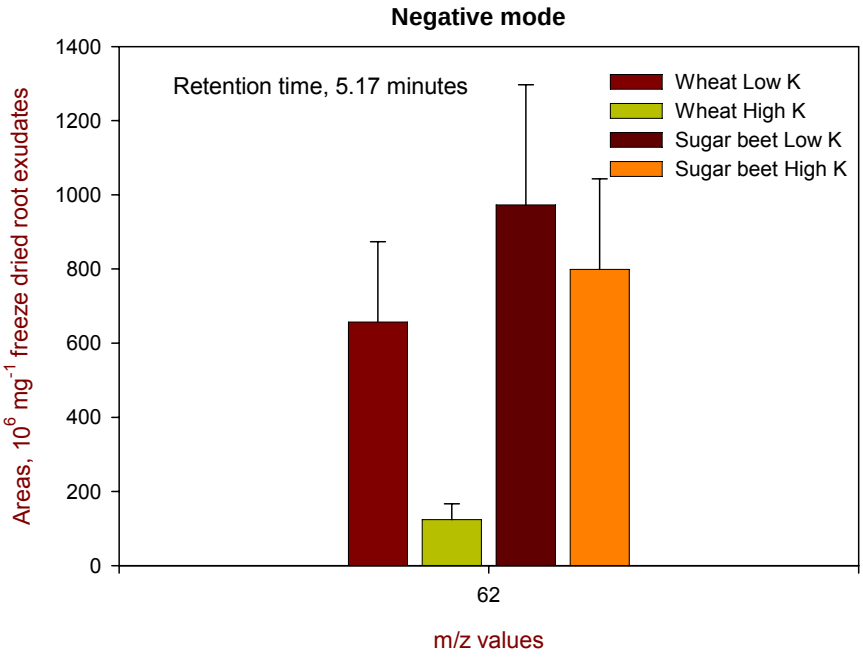


Figure 3.6: Areas of signal corresponding to m/z value 62 detected in cold water soluble root exudates collected from wheat and sugar beet under low and high K supply at second harvest.

Data are mean of 4 replicates for wheat and 3 replicates for sugar beet. Standard error is given in the error bar.

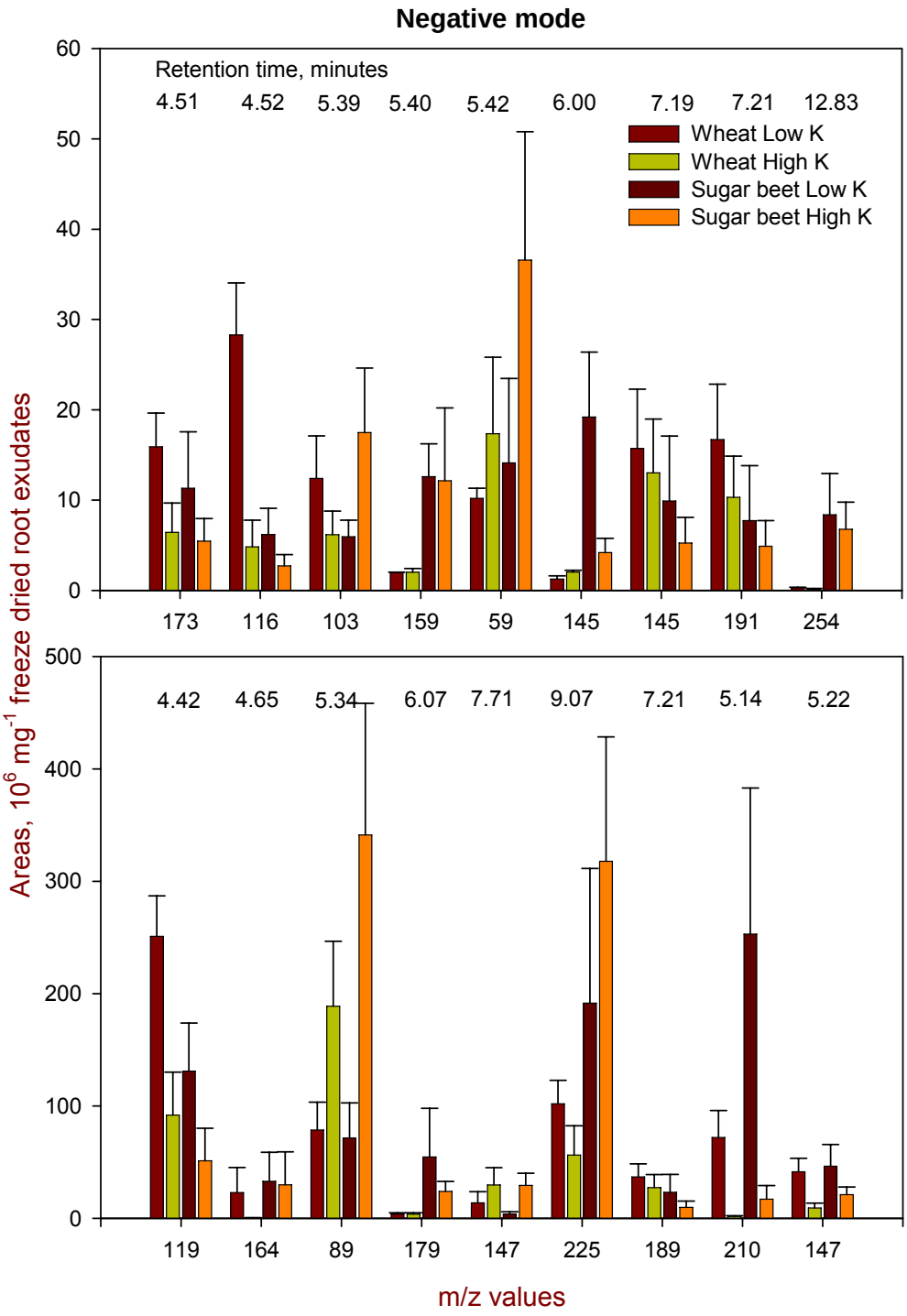


Figure 3.7: Different m/z values with corresponding areas detected in cold water soluble root exudates collected from wheat and sugar beet under low and high K supply at second harvest.

Data are mean of 4 replicates for wheat and 3 replicates for sugar beet. Standard error is given in the error bar.

Mass spectrometric signals of CRE at first harvest

Twenty five signals were detected in cold water soluble root exudates collected at first harvest which varied in their intensities in wheat and sugar beet at the same K level and under low and high K supply for the same crop species. The m/z values with their retention time (t_R) and the corresponding intensities (which is represented as areas per mg of freeze dried root exudates) of those 25 signals are given in table 3.5. Several peaks corresponding to m/z values 176, 97, 261, 193, 199, 242, 175, 199, 277, 356, 385, 376 were detected in cold water soluble root exudates at first harvest which were not detected in CRE collected at second harvest. Surprisingly, intensities of all the peaks detected except m/z values 189 and 191 were higher in sugar beet as compared to wheat under low K supply. Under high K supply, the intensities of the signals corresponding to m/z values 356, 189 and 191 were relatively higher in wheat as compared to sugar beet in CRE. As compared to CRE collected at second harvest, the intensity of the dominant signals [m/z 97 (t_R - 4.8), 119, 116, 210, 62, 147 and 226] were relatively stronger in CRE collected at first harvest. The intensities of almost all detected signals were higher under low K supply as compared to high K supply in both wheat and sugar beet. The m/z values 145, 147, 189, 191 eluted at the same retention time. All these masses may belong to one compound. The m/z values 62, 147, 210 eluted at the same retention time, possibly belong to one compound. Similar for m/z values 179 and 242 which eluted at the same retention time, may belong to one compound.

Table 3.5: Areas of mass spectrometric signals corresponding to m/z values detected in cold water soluble root exudates (CRE) at first harvest under low and high K supply.

m/z values	Retention time	Areas			
		Wheat		Sugar beet	
		Low K	High K	Low K	High K
	minutes	10^6 mg^{-1} CRE			
97	4.8	1208 (105)	646 (41)*	9430 (546)	6131 (2146)
119	4.9	234 (12)	138 (18)	1162 (106)	609 (211)
116	4.8	10 (3)	6 (1)	204 (28)	139 (57)
210	5.7	809 (75)	325 (105)	1318 (201)	512 (187)
62	5.7	239 (17)	101 (16)	4605 (1342)	993 (340)
147	5.7	71 (24)	13 (4)	103 (35)	70 (26)
226	5.7	133 (18)	14 (9)	228 (76)	35 (17)
176	4.7	4.61 (0.77)	5.88 (1.36)	6.96 (1.85)	4.37 (1.52)
261	4.9	22.67 (8.19)	13.51 (1.33)	39.49 (4.73)	20.82 (7.06)
356	5.2	5.93 (2.58)	29.31(13.13)	3.03 (1.26)	0.71 (0.23)
97	5.4	13.68 (4.32)	8.06 (1.43)	0 (0)	7.37 (1.66)
193	5.6	1.98 (0.62)	1.48 (0.13)	8.93 (1.95)	6.25 (2.65)
199	5.7	7.6 (2.55)	3.75 (1.04)	18.42 (3.95)	10.75 (3.84)
242	6.0	0.61 (0.15)	1.13 (0.47)	1.23 (0.16)	4.8 (2.69)
179	6.0	1.13 (0.44)	2.43 (0.17)	4.15 (1)	10.09 (5.17)
175	7.0	0.83 (0.29)	7.83 (2.18)	1.45 (0.28)	4.4 (1.6)
145	7.2	26.45 (5.52)	23.08 2.96)	82.15 (13.8)	50.43 (16.03)
189	7.2	43.92 (8)	51.55 (5.72)	35.66 (5.83)	31.97 (7.6)
147	7.2	15.15 (2.9)	11.69 (3.38)	37.13 (3.58)	37.62 (4.64)
191	7.3	21.13 (5.12)	21.99 (1.31)	11.27 (3.84)	11.28 (4.81)
199	7.8	2.03 (0.44)	2.58 (0.42)	33.04 (18.26)	2.07 (0.44)
225	9.8	6.2 (1.99)	5.55 (2.76)	9.01 (2.81)	28.2 (5.17)
385	11.5	0.43 (0.11)	0.45 (0.12)	1.48 (0.82)	4.45 (2.05)
376	11.5	4.75 (2.07)	6.84 (3.6)	17.34 (7.15)	7.94 (3.95)
277	13.9	3.43 (0.48)	8.95 (3.24)	13.42 (2.25)	10.05 (1.95)

*Standard error is given in the bracket

Mass spectrometric signals of WRE at second harvest

Twenty four signals were detected in warm water soluble root exudates collected at second harvest which varied in their intensities in wheat and sugar beet at the same K level and under low and high K supply for the same crop species. The m/z values with their retention time (t_R) and the corresponding intensities of those signals are given in table 3.6. Several peaks corresponding to m/z values 344, 120, 104, 367, 129, 130 and 134 were detected in warm water soluble root exudates at second harvest which were not detected in CRE. The intensities of signals corresponding to m/z values 367, 129, 130 and 134 were relatively higher in WRE collected from wheat as compared to sugar beet at both low and high K supply. Intensities of these four signals were relatively higher under low K supply as compared to high K supply. This result indicates that WRE might contain different compound than CRE. As the warm water soluble root exudates contain some high molecular weight compounds like mucilage, the composition of WRE could be different from CRE. Rests of the signals were common to both CRE and WRE. The intensity of the signal corresponding to m/z value 164 was remarkably higher in wheat as compared to sugar beet both under low and high K supply. For all the dominant signals, the trend for change in intensities was similar as reported in CRE i.e. the intensities of those signals were higher in WRE collected from sugar beet as compared to wheat both under low and high K supply and were higher under low K than that of high K supply for both wheat and sugar beet.

Table 3.6: Areas of mass spectrometric signals corresponding to m/z values detected in warm water soluble root exudates (WRE) at second harvest under low and high K supply.

m/z values	Retention time	Areas			
		Wheat		Sugar beet	
		Low K	High K	Low K	High K
	minutes	10^6 mg^{-1} WRE			
97	4.4	2136 (1122)	789 (386)*	2295 (1458)	2222 (359)
119	4.4	279 (152)	57 (28)	227 (156)	170 (28)
116	4.4	78 (40)	4.5 (2.3)	44 (27)	18 (8.9)
344	4.6	15 (6.9)	12 (4.1)	7.7 (3.8)	12 (4.7)
210	5.3	152 (29)	1.1 (0.4)	246 (157)	240 (103)
62	5.4	2167 (1131)	50.4 (27)	3018 (1980)	1594 (108)
120	5.4	5.4 (2.1)	0.2 (0.1)	5.8 (3)	8.5 (2.5)
147	5.5	37 (6.9)	1.8 (1.1)	77 (50)	41 (5.7)
89	5.7	59 (7.9)	17 (11)	121 (56)	293 (96)
242	6.0	6.9 (3.2)	0.6 (0.3)	6.8 (4.5)	2.1 (0.3)
145	6.0	36 (12)	0.8 (0.4)	28 (12)	43 (25.8)
104	6.1	13 (2.3)	2.4 (2.3)	3 (1.2)	7.7 (3.1)
179	6.1	40 (7.1)	5.8 (5.1)	27 (15)	35 (7.1)
367	6.2	30 (4.4)	13 (8.5)	8.8 (4.1)	19 (5.9)
116	6.3	8.9 (2.7)	2.2 (1.3)	5.9 (3.5)	12 (1)
129	6.3	42 (17.1)	4.5 (2.4)	2.3 (0.9)	1.8 (0.3)
130	6.9	42 (6.4)	8.4 (4.7)	11.9 (8.1)	11 (2)
134	7.0	14 (3.4)	0.3 (0.1)	6.8 (4.1)	8.9 (3.3)
147	7.2	43 (9.5)	20 (13)	15 (11)	30 (4.8)
225	9.0	227 (100)	412 (133)	762 (242)	1524 (166)
261	9.0	13 (3.1)	15 (3.3)	30 (10)	53 (5)
164	13	250 (74)	44 (13)	0.2 (0.1)	0.3 (0.2)
197	17	16 (2.6)	9.8 (2.3)	15 (5.6)	14 (1.3)

*Standard error is given in the bracket

ESI positive mode

Signals corresponding to m/z values 475, 197, 363 and 429 were different for CRE collected from wheat and sugar beet under low and high K supply at second harvest in ESI positive mode (Figure 3.8). For CRE collected from low K supplied wheat and sugar beet, the intensity of the signal corresponding to m/z value 475 was relatively higher as compared to high K supply and that of 363 was higher under high K supply as compared to low K supply. For m/z value 197, the intensity was higher under low K supply as compared to high K supply only in wheat and there was no change in intensity from low to high K supply in sugar beet. In wheat, the intensity of the signal corresponding to m/z value 429 was higher under high K supply than that of low K supply.

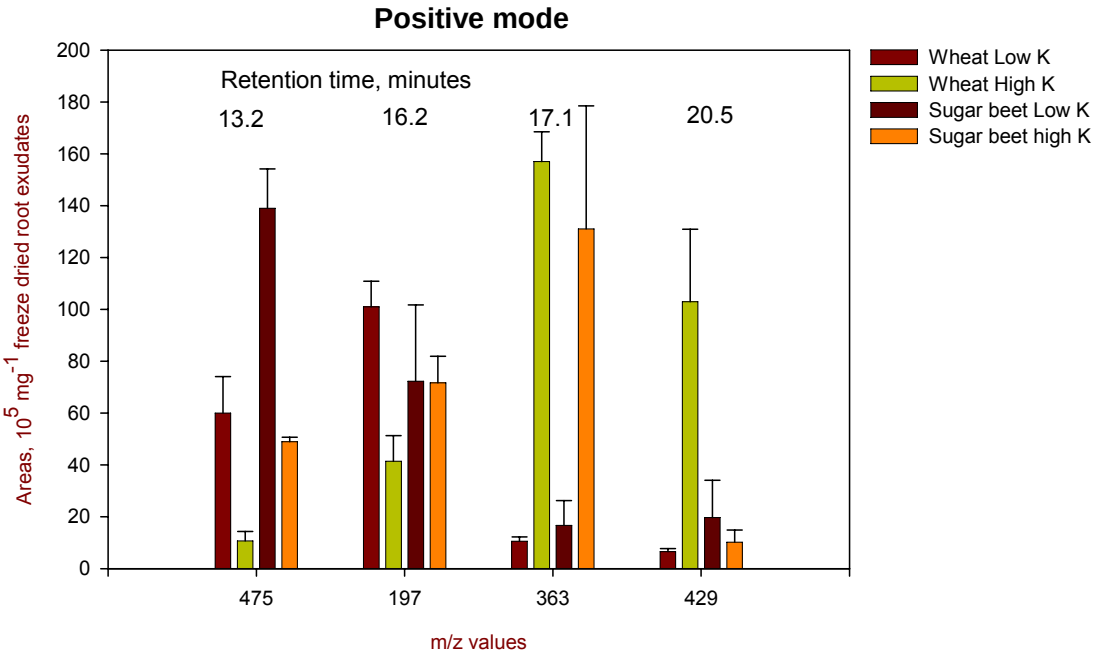


Figure 3.8: Different m/z values with corresponding areas detected in cold water soluble root exudates collected from wheat and sugar beet under low and high K supply at second harvest.

Data are mean of 4 replicates for wheat and 3 replicates for sugar beet. Standard error is given in the error bar.

3.1.2.2.4 Purification and preliminary identification of root exudates components

Root exudates samples were purified to collect the pure fractions of different components for further identification. Dominant peaks were found in the early fractions from purification column (Sephadex LH-20). In fractions 5-7, signals corresponding to m/z values 119, 116, 191 and 171 and in fractions 8-12, signals corresponding to m/z 62, 147, 210 and 226 were detected. Highly polar compounds used to come out of the purification column early which indicates the compounds were very polar. Figure 3.9 shows the relation between m/z values 210, 62, 147 and 226. The maximum intensity of all these four m/z values were detected at the same retention time, which means all these m/z values belong to one substance. The signal corresponding to m/z value 210 was also detected in agar which contains KNO_3. To confirm whether m/z 210 belongs to KNO_3 or not, the mass spectrum of KNO_3 and CRE collected from wheat at second harvest were compared both in positive and negative ionization mode. Figure 3.10 and 3.11 shows the mass spectrum of m/z 210 of CRE collected from wheat at the second harvest and KNO_3. It was found that in both positive and negative ionization mode there was a peak at 5.9 minutes in negative mode m/z 210 and in positive mode m/z 102 (Figure 3.14). Mass spectrums of m/z 102 of CRE collected from wheat at second harvest and KNO_3 are given in figure 3.12 and 3.13. From these results it was confirmed that the dominant peak which was detected in relatively higher amount in low K supplied sugar beet was KNO_3. As the objective of our study was to find some organic compounds which may play a role in mobilizing K under low K supply, it was of minor importance for our study. But at least it shows the importance of mass spectrometry like how the mass spectrum of certain m/z value could be helpful for further identification of the compound.

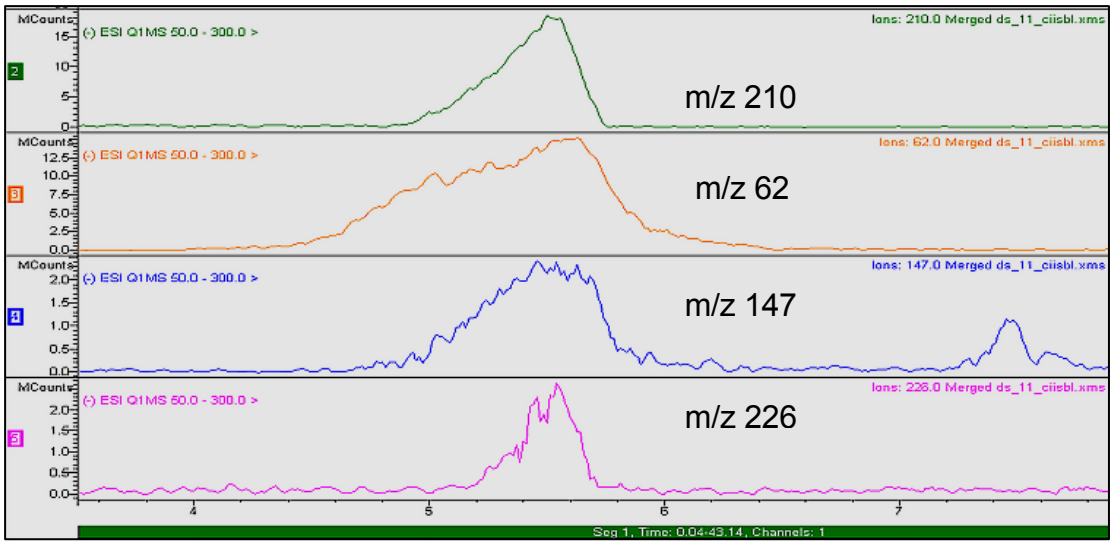


Figure 3.9: Relation between m/z values 210, 62, 147 and 226.

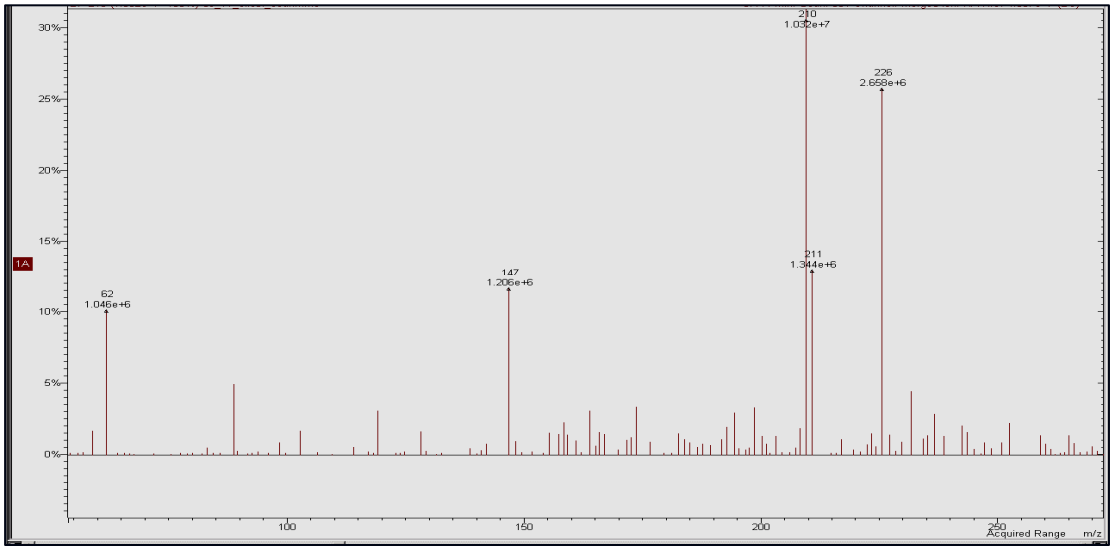


Figure 3.10: Mass spectrum of m/z value 210 in CRE collected from wheat at the second harvest in ESI negative mode.

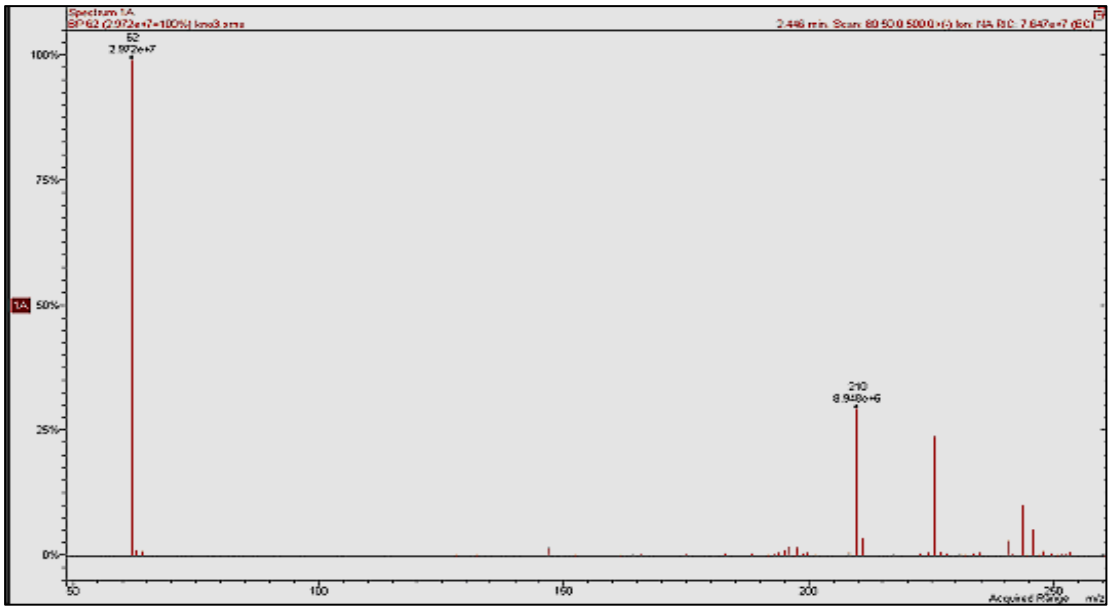


Figure 3.11: Mass spectrum of m/z 210 KNO_3 in ESI negative mode.

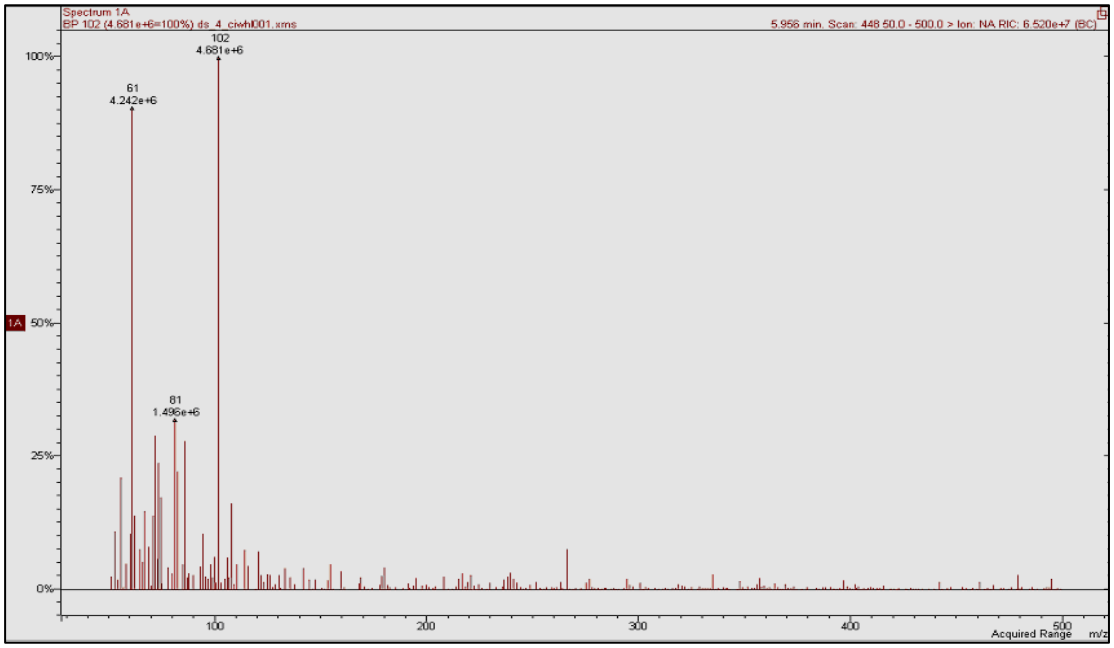


Figure 3.12: Mass spectrum of m/z 102 of CRE collected from wheat in ESI positive mode.

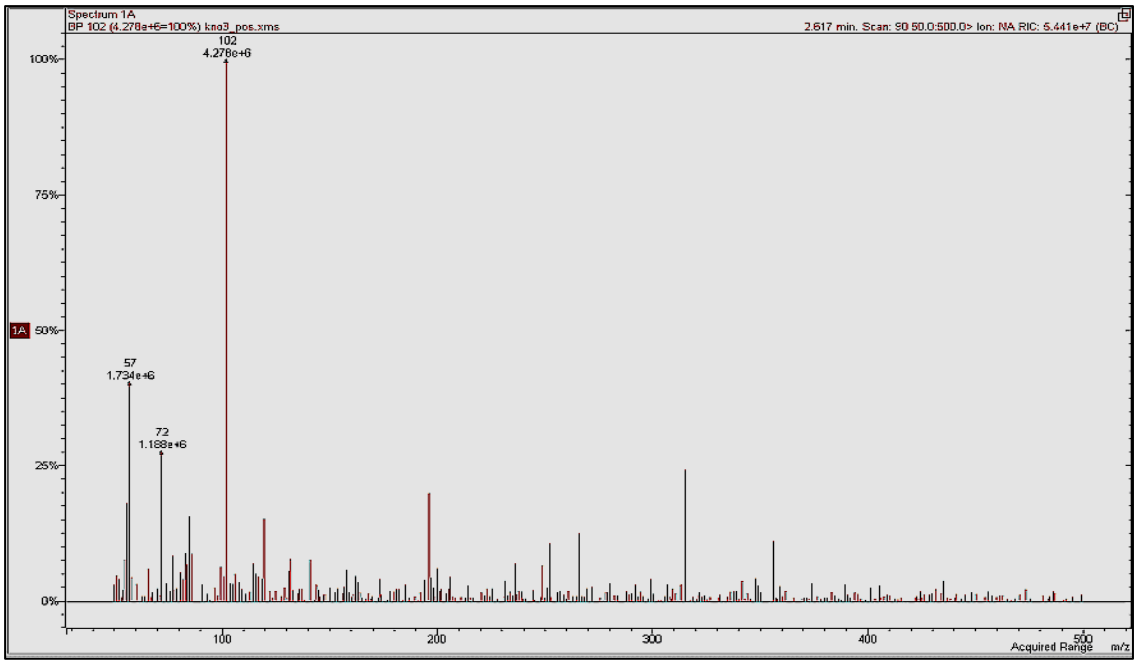


Figure 3.13: Mass spectrum of m/z 102 KNO_3 in ESI positive mode.

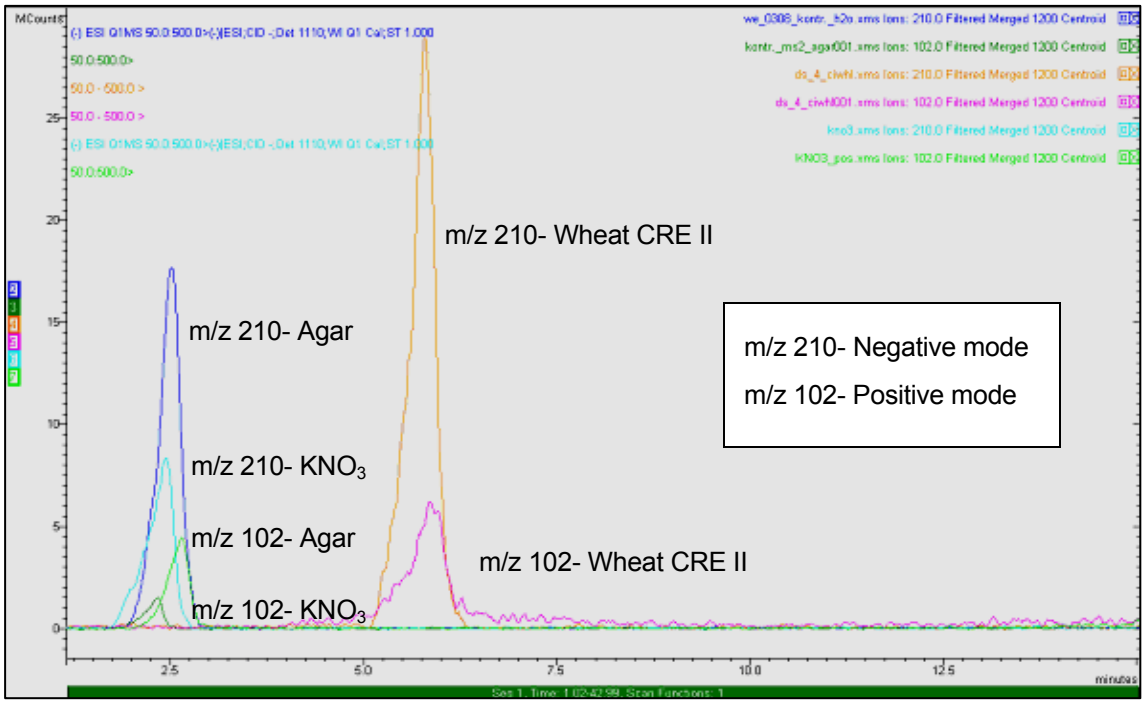
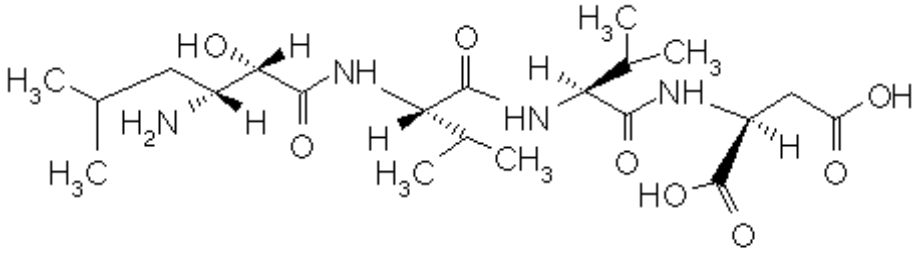


Figure 3.14: Full scan of m/z 210 (ESI negative) and m/z 102 (ESI positive) overlaid.

Possible structure for different m/z values from KEGG data base

KEGG (Kyoto Encyclopedia of Genes and Genomes) is a "biological systems" database integrating both molecular building block information and higher-level systemic information. Molecular building blocks are distinguished between genetic building blocks (KEGG GENES) and chemical building blocks (KEGG LIGAND), while the systemic information is represented as molecular wiring diagrams (KEGG PATHWAY) and hierarchies and relationships among biological objects (KEGG BRITE). KEGG LIGAND contains the knowledge on the universe of chemical substances and reactions that are relevant to life. It is a composite database consisting of compounds, drugs, glycans, reactions and enzymes. From this data base one of the possible compounds for m/z value 475 (Exact mass 474.24) was found to be Amastatin ($C_{21}H_{38}N_4O_8$): 3-amino-2-hydroxy-5-methylhexanoyl-L-valyl-L-valyl-L-aspartic acid with molecular weight 474.26. As m/z 475.24 was detected in positive mode the actual molecular weight of the possible compound is 474.24.



Amastatin (3-amino-2-hydroxy-5-methylhexanoyl-L-valyl-L-valyl-L-aspartic acid)

Other possible structures for m/z values 89, 164 and 173 from the KEGG data base are Lactic acid, Phenylalanine and t-Aconitic acid, respectively.

3.1.2.3 Discussion

Wheat and sugar beet plants were K deficient under low K supply condition; however K deficiency was relatively more severe in case of sugar beet as

compared to wheat at both first and second harvest. Results of relative shoot dry weight and root length shows that, K deficiency was more severe in sugar beet than in wheat throughout the growth period.

It was observed that the rate of root exudation was several folds higher when wheat and sugar beet were grown under natural environmental conditions in screen house as compared to controlled conditions in the growth chamber under both low and high K supply; however this difference in exudation rate was 5 times higher under low K supply as compared to high K supply. The difference in exudation rate between natural and controlled condition could be because of difference in light intensity. Probably under screen house condition, the light intensity was higher as compared to the growth chamber. Since a large proportion of the organic carbon released into the rhizosphere is derived from photosynthesis, changes in light intensity are likely to modify the intensity of root exudation (Johnson et. al., 1996). Rovira (1959) demonstrated changes in quantity and quality of amino acids in exudates of tomato and clover with decreasing light intensity. Rovira (1956) also reported that the number of micro-organisms which developed on the surfaces of tomato roots was less with plants grown in artificial light than in day light suggested that light could also influence exudation. In P-deficient white lupin, citrate release from proteoid roots followed a diurnal rhythm with exudation peaks during the light period (Watt and Evans, 1999). This behavior might reflect the diurnal variations in carbohydrate (sucrose) supply by the shoot as precursors for citrate biosynthesis (Richter, 1996).

The rate of root exudation of CRE was significantly higher in sugar beet as compared to wheat at both the harvest as the K deficiency was more severe in sugar beet than in wheat. Potassium deficiency increased the root exudates release rate several folds both in wheat and sugar beet; however the increase in exudation rate was higher in sugar beet as compared to wheat. Similar to CRE, the rate of exudation of WRE was higher under low K supply as compared to high K supply in both the crops; however the rate of exudation was significantly higher in wheat as compared to sugar beet both under low and high K supply.

Comparative analysis of the signals recorded by mass spectrometer was applied

to identify the subtle differences between metabolites found in root exudates of wheat and sugar beet grown with low and high K level. Several signals and changes in intensity of certain signals specific for root exudates from K deficient plants were found. At both first and second harvest, several signals detected which were relatively stronger under low K supply as compared to high K supply both in wheat and sugar beet and was also different from wheat to sugar beet at the same K level. At first harvest almost all signal detected were stronger in CRE collected from sugar beet as compared to wheat both under low and high K supply. As compared to first harvest, the signals were relatively less strong at second harvest. There were several signals detected specific to first harvest and some specific to second harvest. Most dominant signals (m/z 210, 62, 147) were detected in negative mode and those belong to one compound. Other important signals which were different between samples were m/z 119 and 116. Besides these dominant signals around 20 other mass signals were detected in ESI negative mode, which were different in their intensities under low and high K levels and between sugar beet and wheat samples at the same K level. One interesting peak was found in ESI positive mode was m/z value 475 and the intensity of the signal was relatively stronger in sugar beet as compared to wheat both under low and high K supply.

MS spectra were used for preliminary characterization of these metabolites. Lists of possible compounds were given for some mass signals by search in database (KEGG). Selective metabolites specific for K deficiency were purified for characterization by passing through Sephadex LH-20. Dominant peaks were also found in the early fractions from purification column which indicated those are very polar compounds. It was confirmed that m/z 62, 147, 210, 226 belong to the mass spectrum of KNO_3. The amount of root exudates left was not enough for structural elucidation by nuclear magnetic resonance spectroscopy (NMR-spectroscopy) to identify the different m/z values detected in root exudates. For which further analysis could not be done.

Possible structure for some of the m/z values were found from KEGG data base. From the data base the possible compound for m/z value 475 was Amastatin (3-amino-2-hydroxy-5-methylhexanoyl-L-valyl-L-valyl-L-aspartic acid). The chemical

structure of Amastatin resembles to long carbon chain n-alkyl ammonium compounds. It has been reported that long chain n-alkyl ammonium compounds can penetrate through the inter layer of clay mineral and widen the interlayer space and making the inter layer K accessible to the plants. With increasing chain length of n-alkyl ammonium compounds, the ability to expand the interlayer increases (Stanjek et al., 1992). Further investigation is needed to confirm whether m/z 475 belong to Amastatin.

Non-targeted metabolite profiling of the root exudates collected from wheat and sugar beet by HPLC coupled with ESI-MS showed some strong signals which were specific to root exudates collected from low K supplied wheat and sugar beet; however further identification of those signals could not be done because the amount of collected root exudates was not sufficient for the structural elucidation by nuclear magnetic resonance spectroscopy (NMR-spectroscopy). Further investigation is needed to identify the compounds corresponding to those signals and to run K desorption experiment in soils of low available K with those compounds which may explain the differences in measured and calculated K uptake of wheat and sugar beet.

Chapter IV

Mobilization of potassium in K fixing soil by amino acids component of root exudates

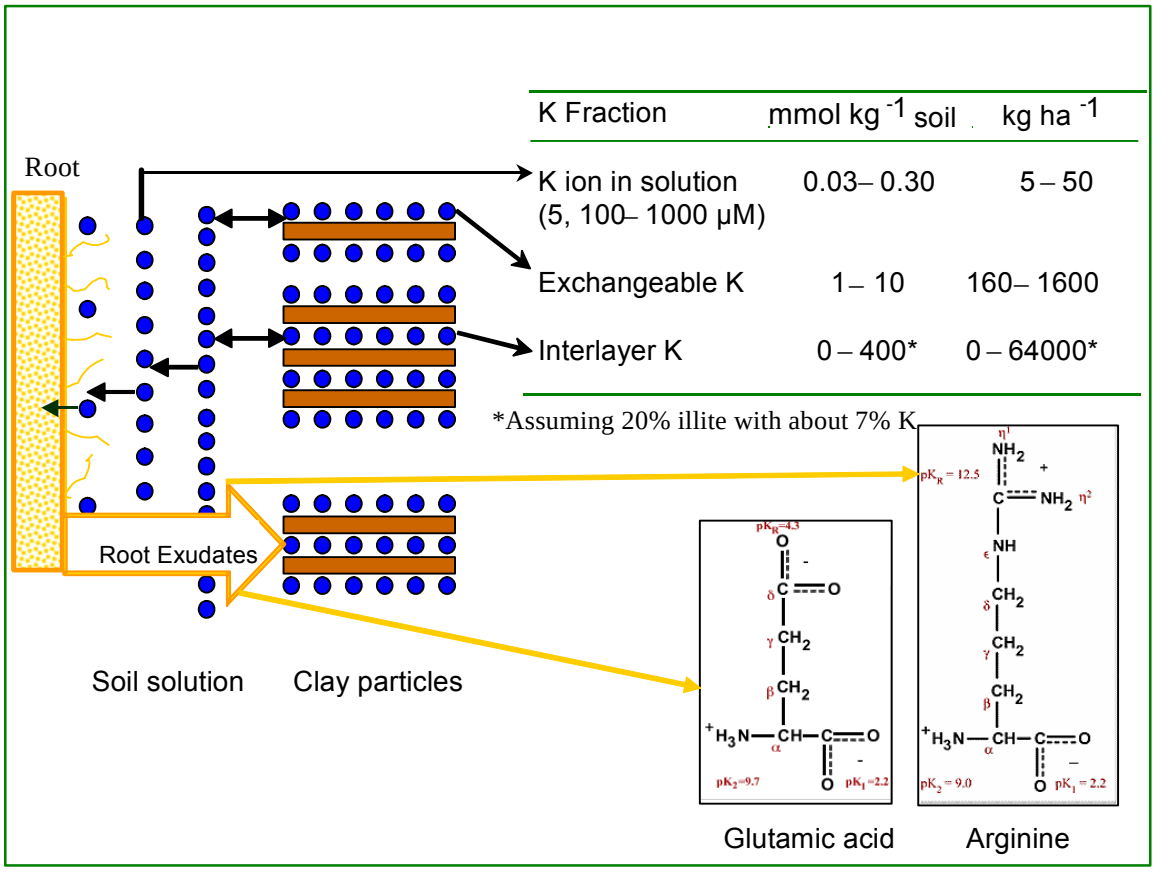
4 Mobilization of potassium in K fixing soil by amino acids component of root exudates

4.1 Introduction

The availability of potassium to the plant is highly variable, due to complex soil dynamics, which are strongly influenced by root–soil interactions. In accordance with its availability to plants, soil K is ascribed to four different pools: (i) soil solution, (ii) exchangeable K, (iii) fixed K, and (iv) lattice K (Syers, 1998). As plants can only acquire K^+ from solution, its availability is dependent upon the K dynamics as well as on total K content. The release of exchangeable K is often slower than the rate of K^+ acquisition by plants (Sparks and Huang, 1985) and consequently, soil solution K^+ concentration in some soil is very low (Johnston, 2005). In order to optimize their performance as nutrient uptake organs and to compete for K^+ uptake under low K supply conditions, plant roots developed mechanisms of acclimation to the current K^+ status in the rhizosphere. The size of the root system, the physiology of uptake and the ability of plants to increase K solubility in the rhizosphere are considered as mechanisms of K uptake efficiency. Earlier research on K efficiency of different crop species indicated that sugar beet seems to increase the chemical availability of K in the soil (Dessougi et. al., 2002; Sadana and Claassen, 1999) and in the previous chapter; results of sensitivity analysis also indicated the same. Usually, only K in solution and K sorbed at clay minerals, which is in equilibrium with solution K, counts as plant available. However, it has been reported that non-exchangeable K can also be used by plants when the available fraction is too low for sufficient supply (Hinsinger and Jaillard, 1993; Moritsuka et al., 2004). Until now, it is not clear in which way plants increase the availability of non-exchangeable K and why some plant species perform better than others. Chemical mobilization of K in the rhizosphere by the plant through root exudation could be the possible mechanism of K efficiency.

It has been reported that long chain n-alkyl ammonium compounds can penetrate

the inter layer of clay mineral and widen the interlayer space and making the inter layer K accessible to the plants. With increasing chain length of n-alkyl ammonium compounds, the ability to expand the interlayer increases (Stanjek et al. 1992). Arginine was the only amino acid detected in root exudates of sugar beet, but not in that of wheat in the earlier experiment. Arginine, Lysine, Glutamic acid and Aspartic acid are polar (charged) long chain amino acids and in the previous experiment it was reported that these amino acids are component of root exudates collected from wheat and/or sugar beet at low and high K supply. These four amino acids were selected for the K mobilization study as they resemble to the n-alkyl ammonium compounds in their chemical structure. To investigate the possibility of K mobilization by amino acids component of root exudates, high K fixing Anglberg soil was desorbed by these four amino acids. The hypothesis concerning why the following 4 amino acids were chosen for K desorption study



The soil-root system for K- Schematic representation of the hypothesis

and how it might work in soil-root system for K is represented by a schematic diagram.

The proportion of soil solution K concentration is too low (0.03-0.30 mmol kg^{-1} soil) as compared to exchangeable K (1-10 mmol kg^{-1} soil) and interlayer K (0-400 mmol kg^{-1} soil). Plants can only acquire K^+ from soil solution; therefore K^+ availability is dependent upon the K dynamics in soil. Plant root may influence the K dynamics by chemical mobilization of K through root exudation. The chemical structure of Arginine and Glutamic acid are given in the schematic diagram, which resemble to n-alkyl ammonium compound in their chemical structure. The objective of this experiment was to study whether these four amino acids (Arginine, Lysine, Aspartic acid and Glutamic acid) could chemically mobilize K in the similar way as n-alkyl ammonium compound does.

4.2 Materials and methods

4.2.1 Calculation of the ratio between root exudates and rhizosphere soil that they can affect during two hours

- The concentration gradient of carboxylic acids can range 0.2 to 1 mm from the rhizoplane into the soil (Jones, 1998). Accordingly, it was assumed that the root exudates can affect a cylinder with radius of 0.5 mm around the roots.
- For a root radius of 0.12 mm, calculated soil volume was 0.012 cm^3 or 0.015 g soil (Bulk Density = 1.3 g cm^{-3}) around 1 cm of root.
- During two hours of exudation the plants exuded at the rate of 0.1 - 28 μg cm^{-1} root.
- Root exudates to soil ratio 0.006 - 1.87 mg g^{-1} soil.
- According to these assumptions and calculations and by taking into account that the concentration of root exudates is higher near the root tips, four treatments were decided i.e. 0.5, 2.0, 8.0 and 16.0 mg root exudates g^{-1} soil.

4.2.2 Preparation of amino acids solution

Amount of amino acids present in 0.5, 2.0, 8.0 and 16.0 mg root exudates were calculated as per the amino acids concentration detected in root exudates collected from wheat and sugar beet plants in the screen house experiment as mentioned in the previous chapter (Figure 3.2). Accordingly four different amino acid solutions were prepared designated as amino acids 1 to 4 with increasing concentration for example Arginine 1 to Arginine 4 (ARG 1- ARG 4) (Table 4).

Table 4.1: Concentration of amino acids solution used for mobilization of K in K fixing Anglberg soil.

Amino acids	Concentration of amino acids			
	1	2	3	4
	nmol L^{-1}			
Arginine (ARG)	4	16	64	128
Lysine (LYS)	13	52	208	416
Aspartic acid (ASP)	28	112	448	896
Glutamic acid (GLU)	21	84	336	672

4.2.3 Desorption of K by Amino acids in K fixing Anglberg soil

Two gram of soil was weighed in 50 mL centrifuge tubes and 30 mL of amino acid solution was added to it and was shaken for 6 hours and centrifuged for 10 minutes at the rate of 8000 revolution per minute. 20 mL of clear supernatant was pipette out and K concentration was measured by flame photometry. Again 20 mL of fresh amino acid solution was added to the residual soil solution and kept in the cool room for further desorption of K. Control was run where two gram of soil was treated with double distilled water. Every day, the tube was shaken for 30 minutes and the above mentioned procedure was done for 14 days till desorption rate of K

became constant. At 14 days, 20 mL of 1.5 molar Ammonium acetate was added to the residual soil solution and after shaking and centrifugation the K concentration of the clear supernatant was measured. Two gram of soil was treated with 30 mL of 1 molar Ammonium acetate and K concentration was measured to determine the total exchangeable K present in the soil.

4.2.4 Data analysis

Statistical analysis were performed by using two way analysis of variance (ANOVA), where significant difference were found, mean values were compared by using Tukey's procedure.

4.3 Results

The results of soil solution K concentration of the filtrate after different days of K desorption by amino acids of increasing concentration and the control without amino acids are shown in figure 4.1 and 4.2. After 6 hours of desorption, the soil solution concentration in the filtrate was 35.3 μmol L^{-1} in control and 44.6, 41.1, 36.4, 34.1 μmol L^{-1} in case of desorption by lowest concentration of Arginine, Lysine, Aspartic acid and Glutamic acid, respectively. Soil solution K concentration was highest after one day of desorption for all the four amino acids of four increasing concentrations and also for the control. The soil solution K concentration in the filtrate after desorption with amino acids and control without amino acids were decreased with time and became constant after 10 days of desorption. Through out the study, the soil solution K concentrations in the filtrate after desorption with Arginine and Lysine was higher than that of control and for Aspartic and Glutamic acid, the increase in soil solution K concentration as compared to control was reported after 3 days of desorption. The soil solution K concentration in the filtrate was highest by desorbing soil with the solution of lowest amino acid concentration for Arginine and Lysine (ARG 1 and LYS 1), but for Aspartic and Glutamic acid, the highest soil solution K concentration was

reported for the second lowest concentration i.e. (ASP 2 and GLU 2) through out the study.

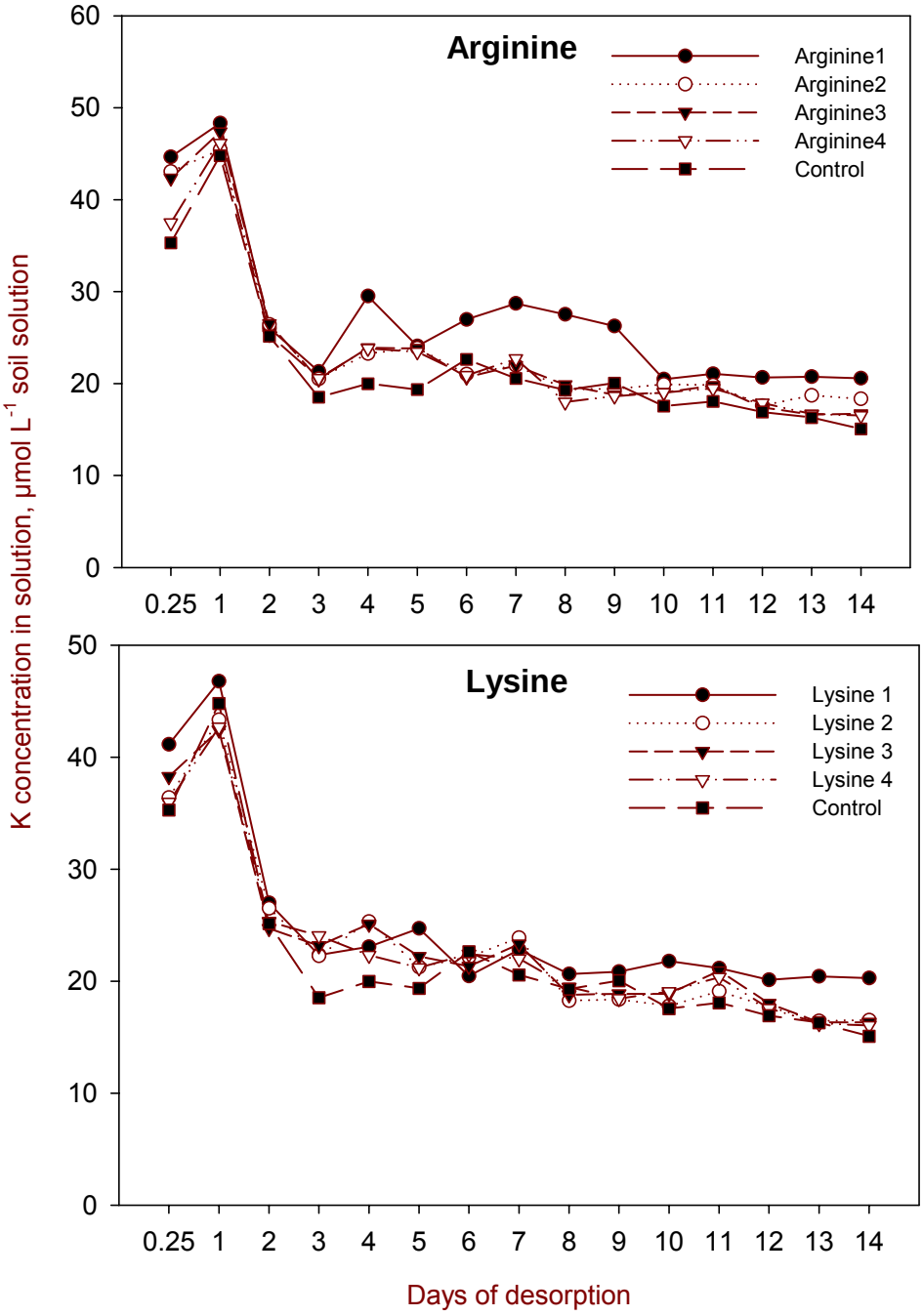


Figure 4.1: Effect of Arginine, Lysine and control (double distilled water without amino acid) on amount of soil solution K concentration after different days of desorption.

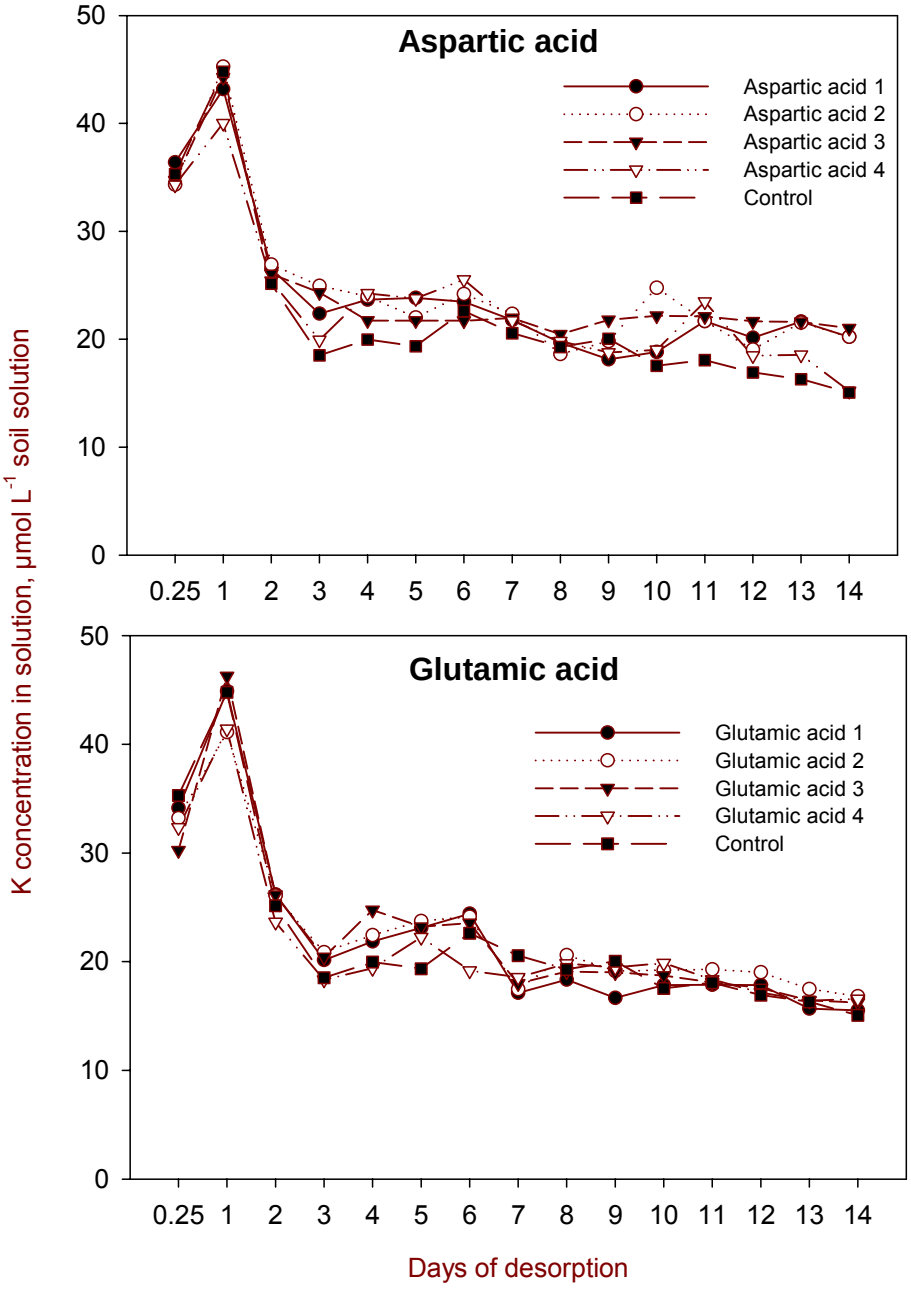


Figure 4.2: Effect of Aspartic acid, Glutamic acid and control (double distilled water without amino acid) on soil solution K concentration after different days of desorption.

The results of desorption of K by amino acids of increasing concentration at different time interval are shown in figure 4.3 and 4.4. From the data of soil solution K concentration in the filtrate recorded after different days of desorption,

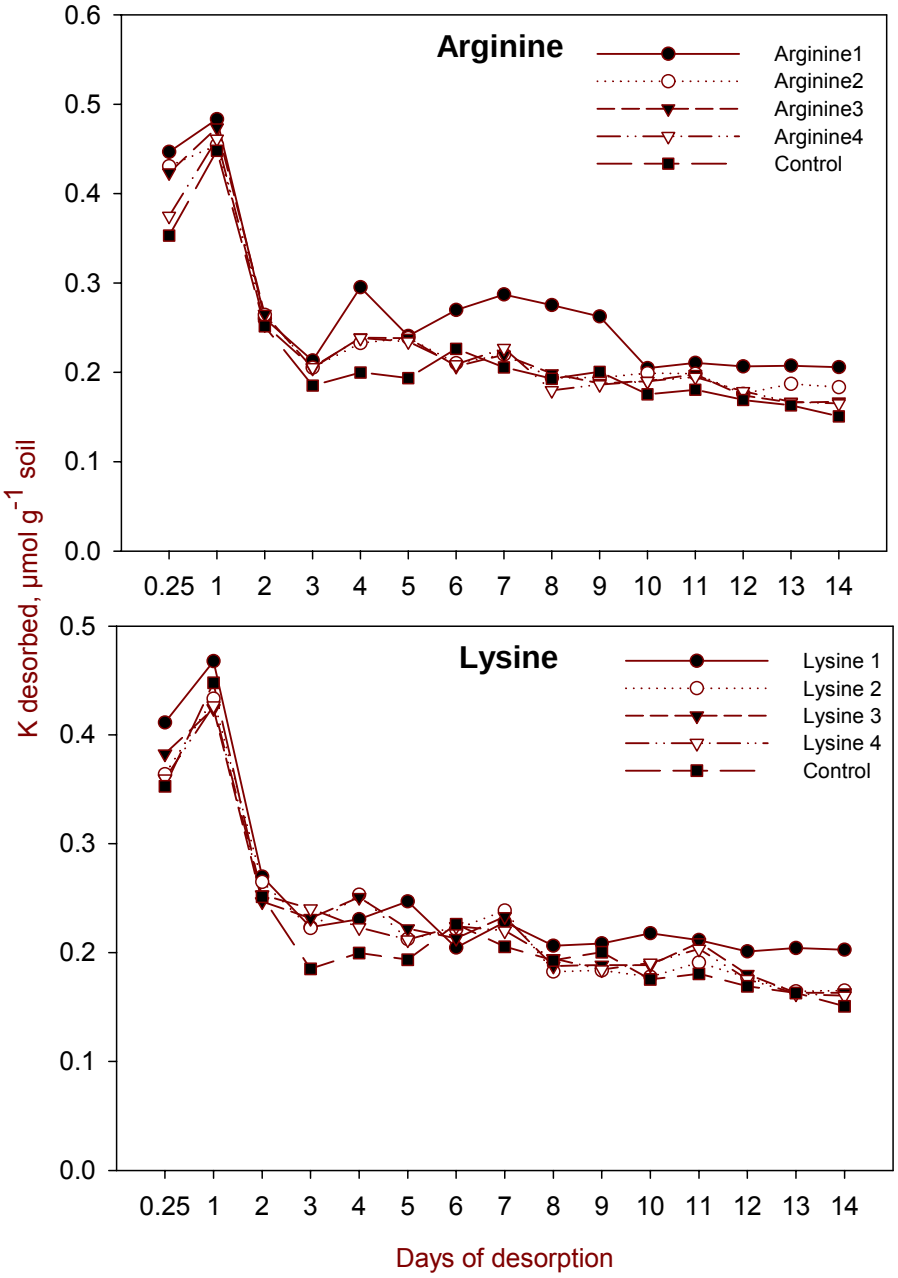


Figure 4.3: Effect of Arginine, Lysine and control (double distilled water without amino acid) on amount of K desorbed after different days of desorption.

corresponding amount of K desorbed was calculated. Desorption of K was highest after one day of desorption for all the four amino acids of four increasing concentrations and also for the control without amino acids. The rate of K desorption was decreased with time and became constant after 10 days of desorption.

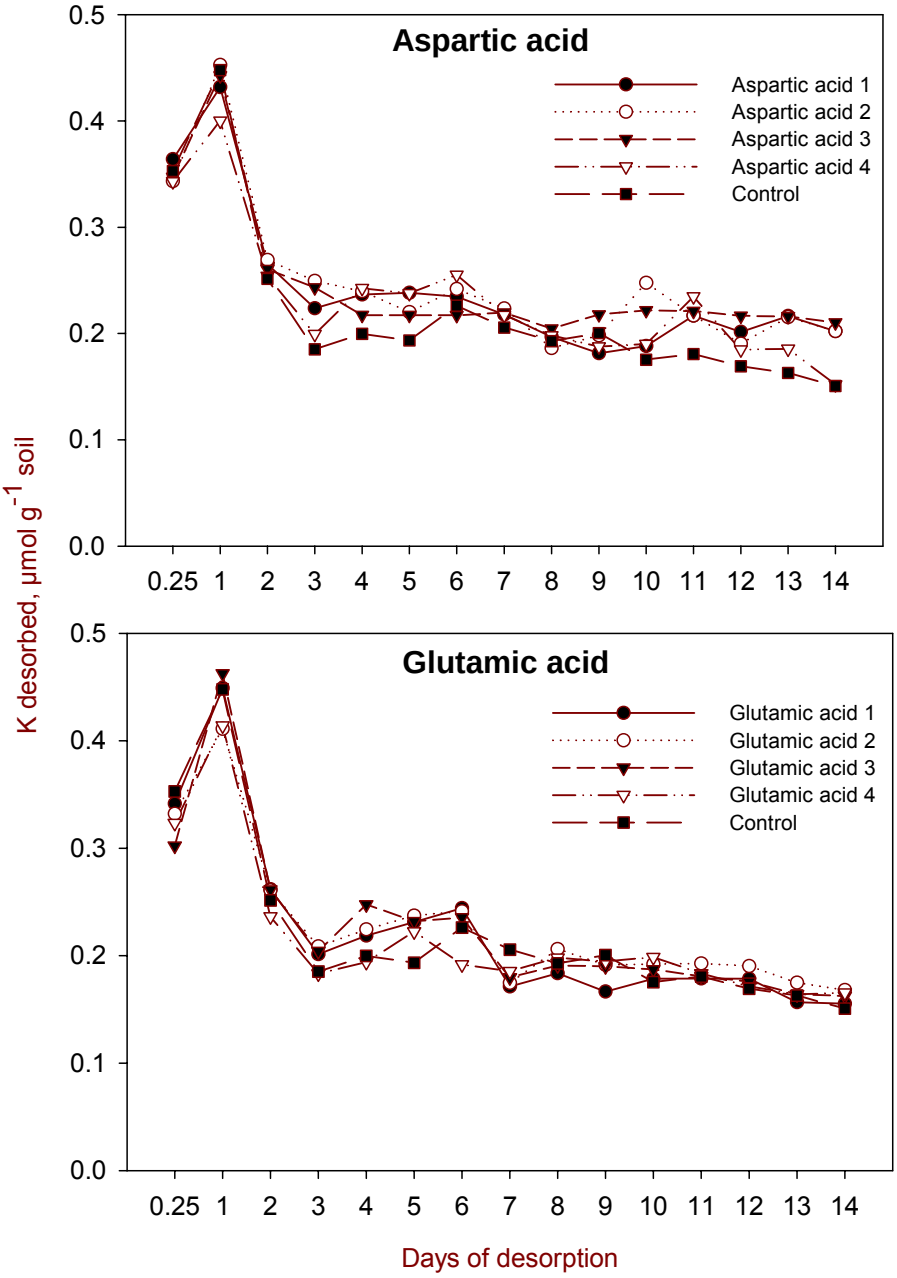


Figure 4.4: Effect of Aspartic acid, Glutamic acid and control (double distilled water without amino acid) on amount of K desorbed after different days of desorption.

The total desorbed K by the lowest concentration of Arginine and Lysine (ARG 1 and LYS 1) were increased by 31 and 20% as compared to control (Figure 4.5). This increase in K desorption from inter layer K was even higher i.e. it increased by 45 and 39% due to desorption by ARG 1 and LYS 1 as compared to the control.

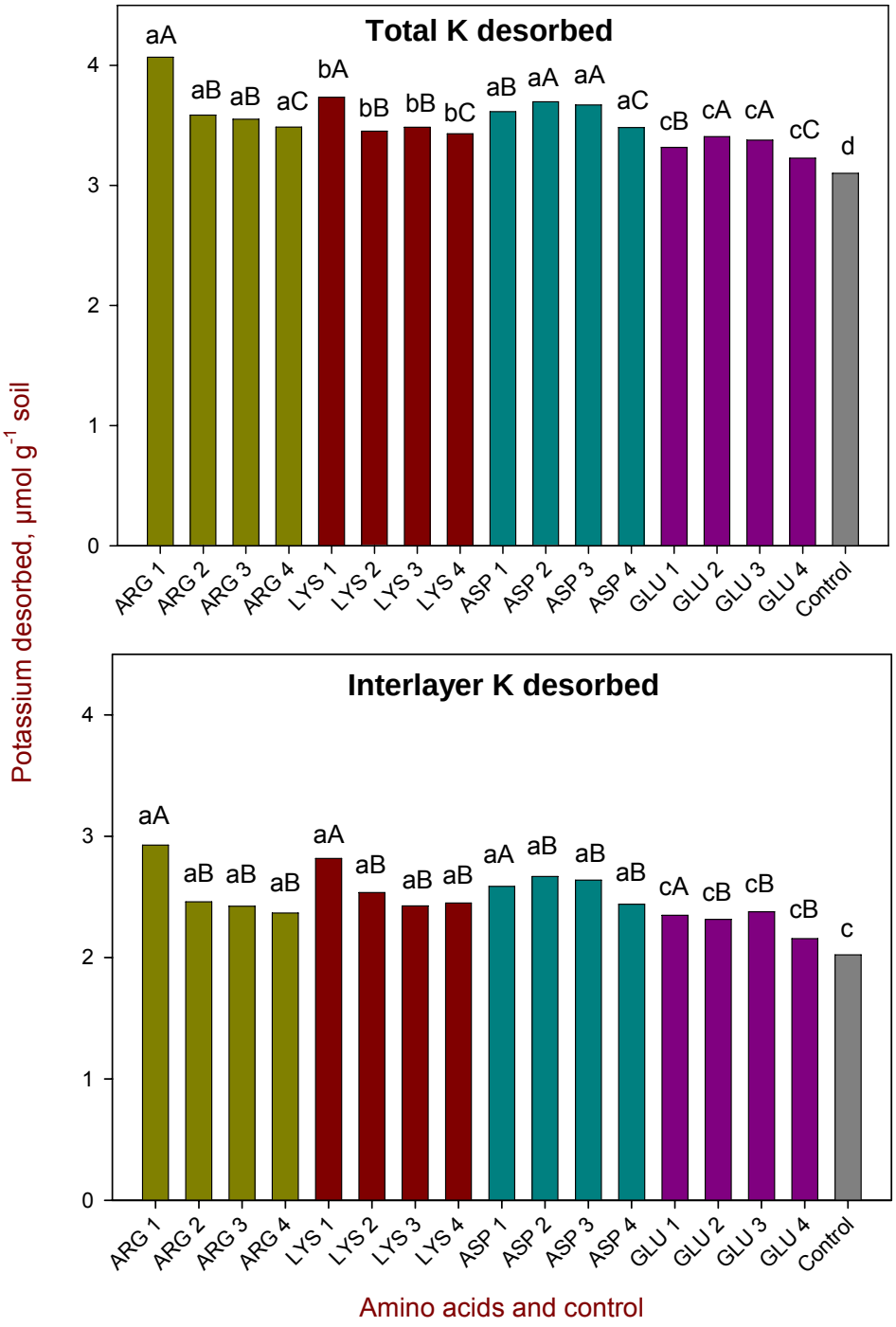


Figure 4.5: Total and interlayer K desorbed by different amino acids and control with only double distilled water without amino acid.

Data are mean of 2 replicates. Lower case letters indicate significant difference of total and interlayer K desorbed among main effect of different amino acids treatments at the same amino acid level ($P \leq 0.001$, Tukey-test). Upper case letters indicate significant difference of total and interlayer K desorbed among different levels of the same amino acid ($P \leq 0.001$, Tukey-test).

However in case of Aspartic and Glutamic acid, maximum K desorption (Total and inter layer K) occurred by the second lowest amino acid concentration (ASP 2 and GLU 2). The total K desorption were increased by 19 and 10% by ASP 2 and GLU 2 as compared to control and that of inter layer K was increased by 32 and 14%, respectively (Figure 4.5). With increasing concentration of Arginine and lysine, the rate of total and inter layer K desorption decreased. But for Aspartic acid and Glutamic acid the K desorption was highest for ASP 2 and GLU 2, respectively and further increasing concentration has negative effect in desorbing K from soil. Among the amino acids studied, total K desorbed by Arginine was the highest followed by Aspartic acid, Lysine and Glutamic acid and the difference in K desorption by different amino acids was significant.

4.4 Discussion

Through out the study, the soil solution K concentrations in the filtrate after desorption with Arginine and Lysine was higher than that of control and for Aspartic and Glutamic acid, the increase in soil solution K concentration as compared to control was reported after 3 days of desorption. Soil solution K concentration was highest after one day of desorption for all the four amino acids of four increasing concentrations and also for the control. This could be due to the fact that the equilibrium between the soil solution and soil solid was not established after 6 hours of desorption.

Desorption of K was highest after one day of desorption for all the four amino acids of four increasing concentrations and also for the control without amino acids. The rate of K desorption decreased with time and became constant after 10 days of desorption. The total and inter layer K desorbed was significantly higher as compared to the control, however the difference was greater in Arginine and Lysine than in Aspartic acid and Glutamic acid. The results indicate that Arginine and Lysine behaves differently than Aspatic and Glutamic acid in desorbing K. This may be due to the differences in their chemical structure. Aspartic acid and Glutamic acid has only one amino group and short carbon chain as compared to 4

and 2 amino group with relatively longer carbon chain in Arginine and Lysine, respectively.

The increase in K desorption by amino acids as compared to control was more pronounced in inter layer K than in total K. Which show that amino acids could mobilize more K from the inter layer. Earlier research on K desorption study showed no significant solubilization of K by different amino acids. The role of proteinaceous amino acids in rhizosphere nutrient mobilization was assessed both experimentally and theoretically. The degree of adsorption onto the soil solid phase was dependent on both the amino acid species and on soil properties. On addition of amino acids to soil, no detectable mobilization of nutrients (K, Na, Ca, Mg, Cu, Mn, Zn, Fe, S, P, Si and Al) was observed, indicating a very low complexation ability of the acidic, neutral and basic amino acids (Jones et al., 1994). Soil extraction experiments with carboxylates, amino acids and sugars revealed that only citrate applied in extraordinary high concentrations (6 mmol g^{-1} soil) was effective in K desorption (Gerke, 1995; Steffens and Zarhoul, 1997). It has been reported that long chain n-alkyl ammonium compounds can penetrate into the inter layer of clay mineral and widen the interlayer space and making the inter layer K accessible to the plants. With increasing chain length of n-alkyl ammonium compounds, the ability to expand the interlayer increases (Stanjek et al., 1992). Like long chain n-alkyl ammonium compounds, these amino acids might widen the interlayer space and therefore more interlayer K could come to the soil solution. The maximum amount of K was already desorbed at lower amino acid concentration and further increasing the concentration had negative effect in desorbing K from soil. This could be due to the blocking of the interlayer space by amino group of the amino acids after certain concentration and also with time; therefore further K could not come out of interlayer. Stanjek et al. (1992) reported that once a single layer is occupied with alkyl ammonium chains, K in adjacent layers may be bound more strongly due to the polarizing effect of K depleted or alkyl ammonium-occupied layers on to the adjacent layers.

The results also showed that Arginine could desorb significantly higher amount of K than other amino acids and the increase in K desorption from that of control was

more pronounced at the lower concentration. This might be due to its chemical structure. It might work like long alkyl ammonium compound which could widen the interlayer resulting in a higher soil solution K concentration. The results of root exudates composition in the previous experiment showed that Arginine was the amino acid, which was detected only in root exudates of sugar beet not in wheat; however the rate of exudation was low as compared to other amino acids (Figure 3.2). But in the present study, the influence of Arginine was pronounced under lowest concentration. This result shows that even though the rate of exudation of Arginine is low in sugar beet, it may increase the soil solution concentration in its rhizosphere. The results of sensitivity analysis in the previous chapter showed that increasing C_{Li} by a factor of 3.5, 100 % prediction could be achieved in case of sugar beet. In the present study, we observe that amino acid at the lowest concentration could increase the soil solution K concentration by 27% as compared to control (Figure 2.5) which shows Arginine may play a considerable role in increasing soil solution concentration in low K soil.

Amino acid can desorb K in the K fixing soil but the degree of desorption does not seems to be sufficient to explain the differences in measured and calculated K influx by sugar beet in soil of very low K supply. It is also not clear why the maximum K desorption attained at lower amino acid concentration. Further investigation is needed to identify the component present in root exudates of sugar beet which may explain the differences between the soil solution K concentration in the rhizosphere of wheat and sugar beet.

Chapter V

Summary

5 Summary

Potassium uptake efficiency is the ability of plants to take up high K under low soil K availability. Plant species differ in their K uptake efficiency. Growing roots continuously experience variations in K availability, for which they have to adjust their physiology and growth pattern. In order to optimize their performance as nutrient uptake organs and to compete for K uptake in the dynamic and heterogeneous environment, plant roots develop mechanisms of acclimation to the current K status in the rhizosphere. This study was done with the objective to investigate the possible mechanisms responsible for the differences in K uptake efficiency of crop species.

Potassium uptake efficiency and K dynamics in the rhizosphere of maize, wheat and sugar beet were evaluated by a pot experiment which was conducted on K deficient soil with and without K fertilization. Sugar beet and wheat could acquire more K per unit shoot dry weight as compared to maize. The higher K uptake efficiency of wheat was due to higher root length to shoot dry weight ratio and lower shoot demand on root as compared to sugar beet and maize. Root length of sugar beet was only 18% of that of maize under low K supply, but the shoot K concentration was two times higher than that of maize. Sugar beet could acquire more K per unit shoot dry weight because of having 4 times higher K influx (K uptake per cm of root per second) as compared to maize.

Potassium uptake by different crop species was simulated by the nutrient uptake model NST 3.0, which calculate nutrient transport towards the root by mass flow and diffusion and nutrient uptake by the root following a Michaelis-Menten kinetic taking into account nutrient uptake by root including root hairs. From the calculated concentration profile around the root of maize, wheat and sugar beet it was deduced that the higher K influx in sugar beet was partly due to the capacity of the sugar beet root to reduce the concentration at the root surface to a lower value as compared to wheat and maize thereby increasing the concentration gradient and so the transport of K to the root surface. The nutrient uptake model could satisfactorily predict K influx in all the crops under high K supply conditions.

However under low K supply, the model prediction was 0.64, 0.68 and 0.31 times the measured K influx for maize, wheat and sugar beet, respectively. The severe under prediction in case of sugar beet indicated that processes not considered in the model were important for the high K uptake efficiency.

A sensitivity analysis was done by changing different soil and plant parameters influencing K uptake, alone or in combination. Increasing soil solution concentration (C_{Li}) by a factor of 1.6 for wheat and maize and 3.5 for sugar beet, resulted in a 100 % prediction for K influx. This indicates the possibilities of chemical mobilization of K by plant roots. Increasing I_{max} by a factor of 25 had only limited effect on calculated K influx, but 100% model prediction was achieved by increasing buffer power by a factor of 10 to 50. However, it is unlikely that plant can change soil buffer power to this extent. Surprisingly, only by increasing I_{max} and b both by a factor of 2.5 times, model could predict measured K influx 100 % in maize and wheat under low K supply conditions and the same was achieved in case of sugar beet by increasing I_{max} and b by 25 times. An increased b at a constant C_{Li} would denote a higher available K concentration in the soil. This might be due to a higher exploitation of non-exchangeable K, which was confirmed by the fact that on average of about 50% of the total K uptake by the plants was from the non-exchangeable fractions, but close to the root it was probably much more and this would cause a much higher b in the rhizosphere. The increase in model prediction for K influx by increasing I_{max} was due to an increased K uptake of root hairs only. It was due to the fact that the K depletion at the root hair surface was much lower than at the root cylinder surface.

To study the root exudation pattern of wheat and sugar beet under deficient and sufficient K supply, an experimental set up was designed to grow low and high K supplied wheat and sugar beet plants in quartz sand at two different growing conditions, one in a screen house under natural environmental conditions and another in a growth chamber under controlled conditions. Cold and warm water soluble root exudates (CRE and WRE) were collected at two different growth stages. Root exudation rate was many-fold higher under low K supply compared to high K supply conditions in both wheat and sugar beet. However, rate of exudation

was higher in wheat compared to sugar beet. Rate of exudation was higher in young plants and at natural light conditions, perhaps due to the higher light intensity under natural sun light conditions in the screen house than that of growth chamber. Results of HPLC analysis of the root exudates collected from wheat and sugar beet grown in the screen house showed that exudation rate of organic acids (acetic acid, malic acid, citric acid and fumaric acid) except lactic acid and t-aconitic acid was higher in wheat compared to sugar beet. Glucose was the only sugar detected in CRE; where as in WRE, glucose and sucrose were detected. Also exudation rate of amino acids was higher in wheat as compared to sugar beet. Arginine was the amino acid detected only in root exudates collected from low and high K supplied sugar beet. Mobilization of K by amino acids component of root exudates was studied in a K fixing soil and the results showed that though amino acids can desorb K in K fixing soil, but the degree of desorption does not seem to be sufficient to explain the differences in soil solution K concentration in the rhizosphere of wheat and sugar beet grown in soil of very low available K.

Non-targeted metabolite profiling was done by separating the root exudates collected from wheat and sugar beet grown in the growth chamber by HPLC coupled with Electrospray Ionisation-Mass Spectrometry (ESI-MS). Several signals and change in intensity of certain signals specific for root exudates from K deficient plants were found. Mostly differences in mass signals were detected in ESI negative mode. Mass signals with m/z values 210 and 62 were most dominant, but belonged to the mass spectrum of KNO_3, which cannot explain the differences in K efficiency of different crop species. In addition, there were around 24 mass signals in ESI negative mode, which showed differences under low and high K supply and between sugar beet and wheat root exudates under similar K supply.

In positive ESI mode, only few signals were detected which were different in their intensities in root exudates collected from wheat and sugar beet under low and high K supply. Among these, signal corresponding to m/z value 475 was found to be interesting and was relatively stronger under low K supply sugar beet. From the KEGG (Kyoto Encyclopedia of Genes and Genomes) data base, one of the possible structure for m/z 475 was Amastatin ($C_{21}H_{38}N_4O_8$) which resembles to n-

alkyl ammonium compound in chemical structure. Further investigation is needed to confirm whether m/z 475 belong to Amastatin.

Non-targeted metabolite profiling of the root exudates collected from wheat and sugar beet by HPLC coupled with ESI-MS showed some strong signals which were specific to root exudates collected from low K supplied wheat and sugar beet; however further identification of those signals could not be done because the amount of collected root exudates was not sufficient for the structural elucidation by nuclear magnetic resonance spectroscopy (NMR-spectroscopy). Further investigation is needed to identify the compounds corresponding to those signals and to run K desorption experiment in soils of low available K with those compounds which may explain the differences in measured and calculated K uptake of wheat and sugar beet.

C h a p t e r V I

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Chapter VII

Appendices

7 Appendices

Appendix 1: Parameters of maize root hairs used for calculating K uptake at different K levels between first and second harvest.

Distance from root, cm	K applied			
	0		250	
	mg kg^{-1} soil			
	$N^{(1)}$	$r_1h^{(2)}$, 10^{-3} cm	$N^{(1)}$	$r_1h^{(2)}$, 10^{-3} cm
0.00-0.02	304	11.7	316	10.3
0.02-0.03	44	46.6	134	26.0
0.04-0.06	5	159.0	23	82.4
0.06-0.08	1	385.5	3	253.8
0.08-0.10	1	963.3	-	-
Total length, cm cm^{-1} root		7.8		9.4
Average length, cm		0.022		0.020

$N^{(1)}$ = number of root hairs per cm root.

$r_1h^{(2)}$ = half distance between neighboring root hairs.

$N^{(1)}$ and $r_1h^{(2)}$ are from Hofbauer (1990).

Appendix 2: Parameters of wheat root hairs used for calculating K uptake at different K levels between first and second harvest.

Distance from root, cm	K applied			
	0		250	
	mg kg^{-1} soil			
	$N^{(1)}$	$r_1h^{(2)}$, 10^{-3} cm	$N^{(1)}$	$r_1h^{(2)}$, 10^{-3} cm
0.00-0.02	196	11.3	226	11.3
0.02-0.03	112	26.3	81	26.8
0.04-0.06	25	73.4	28	54.1
0.06-0.08	4	241.4	10	104.5
0.08-0.10	1	924.6	6	206.9
0.10-0.12	-	-	1	985.5
Total length, cm cm^{-1} root		8.9		9.5
Average length, cm		0.026		0.027

$N^{(1)}$ = number of root hairs per cm root.

$r_1h^{(2)}$ = half distance between neighboring root hairs.

$N^{(1)}$ and $r_1h^{(2)}$ are from Hofbauer (1990).

Appendix 3: Parameters of sugar beet root hairs used for calculating K uptake at different K levels between first and second harvest.

Distance from root, cm	K applied			
	0		250	
	mg kg^{-1} soil			
	$N^{(1)}$	$r_1h^{(2)}$, 10^{-3} cm	$N^{(1)}$	$r_1h^{(2)}$, 10^{-3} cm
0.00-0.02	328	9.9	513	9.2
0.02-0.03	91	30.2	79	35.8
0.04-0.06	13	84.6	5	145.3
0.06-0.08	3	140.3	2	422.5
0.08-0.10	2	202.0	-	-
0.10-0.12	2	336.1	-	-
0.12-0.14	1	663.4	-	-
Total length, cm cm^{-1} root		8.0		9.0
Average length, cm		0.017		0.015

$N^{(1)}$ = number of root hairs per cm root.

$r_1h^{(2)}$ = half distance between neighboring root hairs.

$N^{(1)}$ and $r_1h^{(2)}$ are from Hofbauer (1990).

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