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Table of content

List of tables

List of figures

List of Appedixes

List of Abbreviations

APX	Ascorbate peroxidase
BARI	Bangladesh agricultural research institute
CAT	Catalase
CIP	International potato center
Cl	Chloride ion
CWL	Cumulative water loss
DAT	Date after transplanting
DMRT	Duncan's multiple range test
DW	Dry weight
GR	Glutathione reductase
IRGA	Infrared gas analyser
K	Potassium ion
kPa	Kilopascal
LA	Leaf area
mM	Millimole
Na	Sodium ion
NaCl	Sodium chloride
POX	Peroxidase
rH	Relative air humidity
ROS	Reactive oxygen species
RZS	Root zone salinity
SE	Standard error
SOD	Superoxide dismutase
SP	Sweet potato
Ug^{-1}	Unit per gram
VPD	Vapor presure deficit

Summary

Soil salinity is a global problem that threatens sweet potato production worldwide. To mitigate the future food crisis, salt-tolerant sweet potato genotypes are important because they have high calorie and diverse nutritional value. For the development of salt-tolerant sweet potato varieties, either through breeding or biotechnology, an appropriate salinity screening tool is necessary for the identification of tolerant or sensitive genotype. To serve this purpose, understanding the genotypic response and physiological mechanism of salt tolerance sweet potato exposed to salinity could be an important approach. Most studies on salt tolerance mechanisms focus on a limited number of genotypes or a short period of salt stress that cannot provide information on physiological traits or yield. Additionally, there is very little literature to date on the mechanism of salt tolerance of sweet potato in terms of genotypic salt threshold, salt threshold factor, leaf-level ion uptake/distribution through transpiration, and leaf-level activities of reactive oxygen scavenging antioxidant enzymes, which are unavoidable for the development of a suitable, rapid, and reliable screening tool. Our overall objectives for this study were to develop a suitable, reliable and rapid salinity screening tool in view of salt tolerance mechanism in sweet potato under salinity. To better understand the tolerance mechanisms; leaf level ion uptake and distribution patterns by transpirational water loss and leaf level ROS scavenging antioxidant enzyme activities were evaluated under salinity. Additionally, different ion extraction methods were tested which will contribute to the development of reliable salinity screening tool in sweet potato genotypes. All the experiments were conducted in the greenhouse and VPD (vapor pressure deficit) chambers of the Hans-Rutenberg Institute of Tropical Agricultural Sciences, University of Hohenheim, Germany, in a hydroponic system. Twelve genotypes of sweet potato were collected from Bangladesh Agricultural Research Institute (BARI) and used to evaluate salt thresholds with salt tolerance mechanisms for a wide range of salinity levels (0, 50, 100, and 150 mM NaCl).

First, genotypic thresholds were determined for 12 sweet potato genotypes exposed to salinity, whereupon it was found that 75 mM root zone salinity (NaCl) was the threshold for sweet potato. The genotypic threshold was estimated from the dry matter accumulation that began to decrease under the influence of salinity. It was found that genotypic thresholds were negatively linearly correlated with the difference between tissue K content at 75 mM NaCl and tissue K content at controlled salinity in the root zone. This information is very important for identifying the salt tolerant and sensitive genotype of sweet potato. From this point of view, K content is identified as a key salt tolerance mechanism in sweet potato with respect to the classification of these 12 sweet potato genotypes.

Second, the uptake and distribution of Na, K, and Cl ions by transpiration, across different-aged leaves, were studied to better understand the mechanisms of salt tolerance in sweet potato. Two different sweet potato genotypes were subjected to salt stress of 0 and 50 mM NaCl in artificially dry (VPD 2.27 kPa) and humid (VPD 0.76 kPa) chambers. We found that cumulative water loss per unit leaf area was twice as high at a VPD of 2.27 kPa, but Na uptake remained the same. No relationship was observed between water loss from individual leaves and Na or Cl uptake. About 30% more Na was distributed in the petioles of salt tolerant genotype compared to leaf blades, while the opposite was observed in salt sensitive sweet potato genotype and VPD had no effect on Na distribution. Similarly to Na; K was preferentially accumulated in the petioles and under saline condition was up to twice as much Na by tolerant genotype. Moreover, young petioles of the tolerant genotype showed higher K content than the older ones under salinity. The Na and K distribution patterns in the leaf blades and petioles at different ages of the leaves of the tolerant and sensitive genotypes of sweet potato indicate that the leaf-age-dependent K distribution represents physiological characteristics of salt-tolerant sweet potato.

Third, the activities of ROS scavenging antioxidant enzymes were evaluated with respect to different leaf age, in two different genotypes of sweet potato under 100 mM salinity. In general, antioxidant enzymes in sweet potato do not respond to salt stress but are altered by the effects of leaf position, leaf age, duration of stress, and genotype. No effect of Na on antioxidant enzyme activities was found under salt stress in sweet potato leaves. Therefore, the responses of antioxidant defense mechanisms in sweet potato cannot be generalized for all abiotic stresses, but should be further investigated for the particular stress situation. However, the significant positive correlation between K concentration and the level of SOD (super oxide dismutase) in older leaves suggests that SOD contributes to the maintenance of a high K concentration to protect photosynthetic activity. At the same time, the activity of CAT (catalase) in younger leaves of tolerant genotypes increased at low K concentration, indicating a strategy to protect younger leaves (against membrane damage) with higher CAT activities. Tolerant genotypes also showed a rapid and early increase in GR (glutathione reductase) activities under salinity, which could be another potential salt-tolerant trait of sweet potato. However, before using the results to develop appropriate screening tools for antioxidant enzymes in sweet potato, contradictory factors such as genotypic variations in the affinity of ROS feeding enzymes for their respective substrates and metabolic pathways should be investigated in detail.

In summary, this study shows that sweet potato responds differently to salinity depending on the genotype, and that the threshold beyond which yield decreases is 75 mM NaCl. Genotypic threshold strongly linked to high tissue K content under increasing salinity that suggests a salt tolerance mechanisms in sweet potato. Salt-tolerant sweet potatoes distribute significant amounts of Na and K in their petioles. Young leaves of the tolerant genotype contain more K under salt stress. GR and positive relationship between K concentration and SOD in salt tolerant genotypes indicate some tolerance mechanisms. Therefore, a screening tool is proposed for sweet potato based on the genotypic ability to maintain high tissue K levels under increasing salinity level. Since, K plays an important role in maintaining dry matter under salt stress in sweet potato, K-containing fertilizers may increase salt tolerance to some extent, resulting in higher yields in salt-prone areas. More research on K level in the nutrient solution in a hydroponic system, or multilocation field trials on salinity prone soils addressing K fertilizers should be conducted for getting more information on screening tools. Actual ion transport processes in leaf petioles should be further studied, looking at ion uptake and distribution. To learn more about the antioxidant enzymes in salt-stressed sweet potato many impenetrable factors such as individual genotypic variations, ROS scavenging enzymes to their respective substrates, metabolic pathways, proline content, hormonal activities, etc. need to be studied.

Zusammenfassung

Bodenversalzung ist ein globales Problem, das auch die Süßkartoffelproduktion weltweit bedroht. Um die künftige Nahrungsmittelkrise zu kompensieren, können salztolerante Süßkartoffelgenotypen einen wichtigen Beitrag leisten, da sie einen hohen Kaloriengehalt und einen vielfältigen Nährwert haben. Für die Entwicklung salztoleranter Süßkartoffelsorten, sei es durch Züchtung oder Biotechnologie, ist ein geeignetes Screening-Tool erforderlich, um tolerante oder empfindliche Genotypen zu identifizieren. Zu diesem Zweck könnte das Verständnis der genotypischen Reaktion und des physiologischen Mechanismus der toleranten, dem Salzgehalt ausgesetzten Süßkartoffel ein wichtiger Ansatz sein. Die meisten Studien über die Mechanismen der Salztoleranz konzentrieren sich auf eine begrenzte Anzahl Genotypen oder eine kurze Salzstressdauer, die keine Informationen über physiologische Merkmale oder Erträge liefern können. Darüber hinaus gibt es bisher nur sehr wenig Literatur über Mechanismen der Salztoleranz von Süßkartoffeln in Bezug auf den genotypischen Salzschwellenwert, den Salzschwellenfaktor, die Ionenaufnahme/-verteilung durch Transpiration und die Aktivitäten von reaktiven Sauerstoff bindenden antioxidativen Enzymen auf Blattebene, die für die Entwicklung eines Screening-Tools unverzichtbar sind. Die übergeordneten Ziele für diese Studie waren die Entwicklung eines geeigneten, zuverlässigen und schnellen Screening-Instruments für die Salztoleranz von Süßkartoffeln unter Salinität.
Um die Toleranzmechanismen besser zu verstehen, wurden die Ionenaufnahme und die Verteilungsmuster auf Blattebene durch den transpiratorischen Wasserverlust sowie die Aktivitäten der antioxidativen ROS-Fängerenzyme auf Blattebene unter Salzbelastung bewertet. Außerdem wurden verschiedene Methoden zur Ionenextraktion getestet, die zur Entwicklung eines zuverlässigen Screening-Tools für den Salzgehalt von Süßkartoffel-Genotypen beitragen werden. Alle Versuche wurden im Gewächshaus und in VPD-Kammern (Vapor Pressure Deficit) des Hans-Rutenberg-Instituts für Tropische Agrarwissenschaften der Universität Hohenheim in einem Hydrokultursystem durchgeführt. Zwölf Genotypen von Süßkartoffeln wurden vom Bangladesh Agricultural Research Institute (BARI) gesammelt und zur Bewertung von Salzschwellenwerten mit Salztoleranzmechanismen für eine breite Palette von Salzgehalten (0, 50, 100 und 150 mM NaCl) verwendet.

Zunächst wurden die genotypischen Schwellenwerte für 12 Süßkartoffelgenotypen bestimmt, die dem Salzgehalt ausgesetzt waren. Dabei stellte sich heraus, dass 75 mM Wurzelzonensalzgehalt (NaCl) der Schwellenwert für Süßkartoffeln ist. Der genotypische Schwellenwert wurde anhand der Trockenmasseakkumulation geschätzt, die unter dem Einfluss der Versalzung zu sinken begann. Es wurde festgestellt, dass die genotypischen Schwellenwerte negativ linear mit der Differenz zwischen dem Gewebe-K-Gehalt bei 75 mM NaCl und dem Gewebe-K-Gehalt bei kontrolliertem Salzgehalt in der Wurzelzone korreliert waren. Diese Information ist sehr wichtig für die Identifizierung des salztoleranten und -empfindlichen Genotyps der Süßkartoffel. Unter diesem Gesichtspunkt wird der K-Gehalt als Schlüsselmechanismus für die Salztoleranz bei Süßkartoffeln im Hinblick auf die Klassifizierung dieser 12 Süßkartoffel-Genotypen identifiziert.

Zweitens wurde die Aufnahme und Verteilung von Na-, K- und Cl-Ionen durch Transpiration über die Blätter unterschiedlichen Alters untersucht, um die Mechanismen der Salztoleranz bei Süßkartoffeln besser zu verstehen. Zwei verschiedene Süßkartoffel-Genotypen wurden in künstlich trockenen (VPD 2,27 kPa) und feuchten (VPD 0,76 kPa) Kammern einer Salzbelastung von 0 und 50 mM NaCl ausgesetzt. Wir stellten fest, dass der kumulative Wasserverlust pro Blattflächeneinheit bei einem VPD von 2,27 kPa doppelt so hoch war, die Na-Aufnahme jedoch gleich blieb. Es wurde kein Zusammenhang zwischen dem Wasserverlust einzelner Blätter und der Na- oder Cl-Aufnahme festgestellt. Etwa 30 % mehr Na wurde in den Blattstielen des salztoleranten Genotyps im Vergleich zu den Blattspreiten verteilt, während beim salzempfindlichen Süßkartoffel-Genotyp das Gegenteil beobachtet wurde

und der VPD keinen Einfluss auf die Na-Verteilung hatte. Ähnlich wie Na wurde auch K bevorzugt in den Blattstielen akkumuliert, und unter salzhaltigen Bedingungen war der Na-Gehalt beim toleranten Genotyp bis zu doppelt so hoch. Darüber hinaus wiesen die jungen Blattstiele des toleranten Genotyps unter Salinitätsbedingungen einen höheren K-Gehalt auf als die älteren. Die Na- und K-Verteilungsmuster in den Blattspreiten und Blattstielen in verschiedenen Altersstufen der Blätter des toleranten und des empfindlichen Genotyps der Süßkartoffel deuten darauf hin, dass die vom Alter der Blätter abhängige K-Verteilung physiologische Merkmale der salztoleranten Süßkartoffel darstellt.

Drittens wurden die Aktivitäten der ROS-bindenden antioxidativen Enzyme bei zwei verschiedenen Genotypen von Süßkartoffeln unter 100 mM Salzgehalt in Abhängigkeit vom Blattalter bewertet. Im Allgemeinen reagieren die antioxidativen Enzyme der Süßkartoffel nicht auf Salzstress, sondern werden durch die Auswirkungen der Blattposition, des Blattalters, der Dauer des Stresses und des Genotyps verändert. Bei Süßkartoffelblättern wurde unter Salzstress keine Auswirkung von Na auf die Aktivitäten antioxidativer Enzyme festgestellt. Daher können die Reaktionen der antioxidativen Abwehrmechanismen in Süßkartoffeln nicht für alle abiotischen Stressfaktoren verallgemeinert werden, sondern sollten für die jeweilige Stresssituation weiter untersucht werden. Die signifikante positive Korrelation zwischen der K-Konzentration und dem Gehalt an SOD (Superoxiddismutase) in älteren Blättern deutet jedoch darauf hin, dass SOD zur Aufrechterhaltung einer hohen K-Konzentration zum Schutz der photosynthetischen Aktivität beiträgt. Gleichzeitig stieg die Aktivität von CAT (Katalase) in jüngeren Blättern toleranter Genotypen bei niedriger K-Konzentration an, was auf eine Strategie zum Schutz jüngerer Blätter (vor Membranschäden) durch höhere CAT-Aktivitäten hinweist. Tolerante Genotypen zeigten auch einen raschen und frühen Anstieg der GR-Aktivitäten (Glutathionreduktase) unter Salzgehalt, was eine weitere potenzielle salztolerante Eigenschaft der Süßkartoffel sein könnte. Bevor jedoch die Ergebnisse zur Entwicklung geeigneter Screening-Instrumente für antioxidative Enzyme in Süßkartoffeln verwendet werden, sollten widersprüchliche Faktoren wie genotypische Variationen in der Affinität von ROS-zuführenden Enzymen für ihre jeweiligen Substrate und Stoffwechselwege im Detail untersucht werden.

Zusammenfassend zeigt diese Studie, dass Süßkartoffeln je nach Genotyp unterschiedlich auf den Salzgehalt reagieren und dass die Schwelle, ab der der Ertrag sinkt, bei 75 mM NaCl liegt. Der genotypische Schwellenwert steht in engem Zusammenhang mit einem hohen K-Gehalt im Gewebe bei steigendem Salzgehalt, was auf einen Salztoleranzmechanismus bei Süßkartoffeln schließen lässt. Salztolerante Süßkartoffeln akkumulieren erhebliche Mengen an Na und K in ihren Blattstielen. Junge Blätter des toleranten Genotyps enthalten unter Salzstress mehr K. GR und die positive Beziehung zwischen K-Konzentration und SOD in salztoleranten Genotypen deuten auf Toleranzmechanismen hin. Daher wird ein Screening-Instrument für Süßkartoffeln vorgeschlagen, das auf der genotypischen Fähigkeit basiert, hohe K-Gehalte im Gewebe bei steigendem Salzgehalt aufrechtzuerhalten. Da K bei Süßkartoffeln eine wichtige Rolle bei der Aufrechterhaltung der Trockensubstanz unter Salzstress spielt, könnten K-haltige Düngemittel die Salztoleranz bis zu einem gewissen Grad erhöhen, was zu höheren Erträgen in salzgefährdeten Gebieten führt. Weitere Untersuchungen zum K-Gehalt in der Nährlösung in einem hydroponischen System oder Feldversuche an mehreren Standorten auf salzanfälligen Böden mit K-Düngern sollten durchgeführt werden, um mehr Informationen über Screening-Tools zu erhalten. Die tatsächlichen Ionentransportprozesse in den Blattstielen sollten weiter untersucht werden, um die Ionenaufnahme und -verteilung zu untersuchen. Um mehr über die antioxidativen Enzyme in salzgestressten Süßkartoffeln zu erfahren, müssen viele verschiedene Faktoren wie individuelle genotypische Variationen, ROS-fangende Enzyme und ihre jeweiligen Substrate, Stoffwechselwege, Prolingehalt, hormonelle Aktivitäten usw. untersucht werden.

Chapter 1

General introduction

1.1 Sweet potato (*Ipomoea Batatas* L.)

Sweet potato is a member of Convolvulaceae family with the genus *Ipomoea* and the species *Batatas*. The total number of chromosomes of sweet potato is 2n=6x=90. Moreover, it is naturally very heterogeneous and self-incompatible, which makes conventional breeding of sweet potato difficult (Cervantes-Flores et al., 2011). The life history of sweet potato was elucidated in 2017 at the Max Plank Institute, Germany, and it revealed that sweet potato followed a similar evolutionary pattern about 5000 years ago (Yang et al., 2017).

Sweet potato usually develops three types of tuberous roots (swell tuberous roots, pencil roots and fibrous roots) with a horizontal stem and five leaf types: round, reniform, cordate, triangular and lobed (Ravi & Indira, 2010). It has an intermediate growth habit where new leaves are constantly formed during its life cycle. The number of leaves, leaf area, and specific leaf weights vary greatly among the genotypes. The number of leaves in sweet potato can vary from 60 to 300 depending on the genotype (Rajeshkumar, 1993), with specific leaf weights usually ranging from 2 to 4.4 mg cm^{-2} (Nair and Nair, 1995). Sweet potato stem length and the leaf size are determined by genotypes and influenced by the growing environment. Normally, sweet potato has three types of branching (primary, secondary, and tertiary) that vary with genotype, with stem length averaging 0.5 to 2.2 m (Rao et al., 1992; Rajeshkumar et al., 1993). Yield of sweet potato generally ranges from 10 to 25 tonnes per hectare and depends on the genotypes planted, the cropping period (~16 to ~20 weeks), and the suitability of the environment (Golder et al., 2007; Ravi & Indira, 2010). The yield and total dry matter production are highly dependent on many factors such as irrigation (Goswami et al., 1995), K and N fertilisers (Satapathy et al., 2005), photoperiod (Patil et al., 1990), photosynthetic rate (Zhong, 1991), photosynthetically active radiation (Cen and Sage, 2005), air temperature (Bhagsari & Asley, 1990), salinity (Begum et al., 2015), relative air humidity (Mortley et al.,

1994), transpiration (Kelm et al., 2000), growth regulators (Singh et al., 2019) and enzymes (Zhang et al., 2005). Generally, sweet potato is propagated by the stem cuttings and roots.

Considering annual production, sweet potato is ranked as the 5th most important food crop in the tropics and the 7th in the world food production after wheat (*Triticum aestivum*), rice (*Oryza sativa*), maize (*Zea mays*), potato (*Solanum tuberosum*), barley (*Hordeum vulgare*) and cassava (*Manihot esculenta*), (FAO, 2016). It is also considered as a unique vegetable (Tennant et al., 2014) that reduces the risk of cancer, heart attack, diabetes, and inflammatory problems in the human body due to its huge antioxidant properties (Mohanraj & Sivasankar, 2014). The leaves of sweet potatoes are also a good source of vitamins and are commonly used as a vegetable in many Asian countries (Ghasemzadeh et al., 2016).

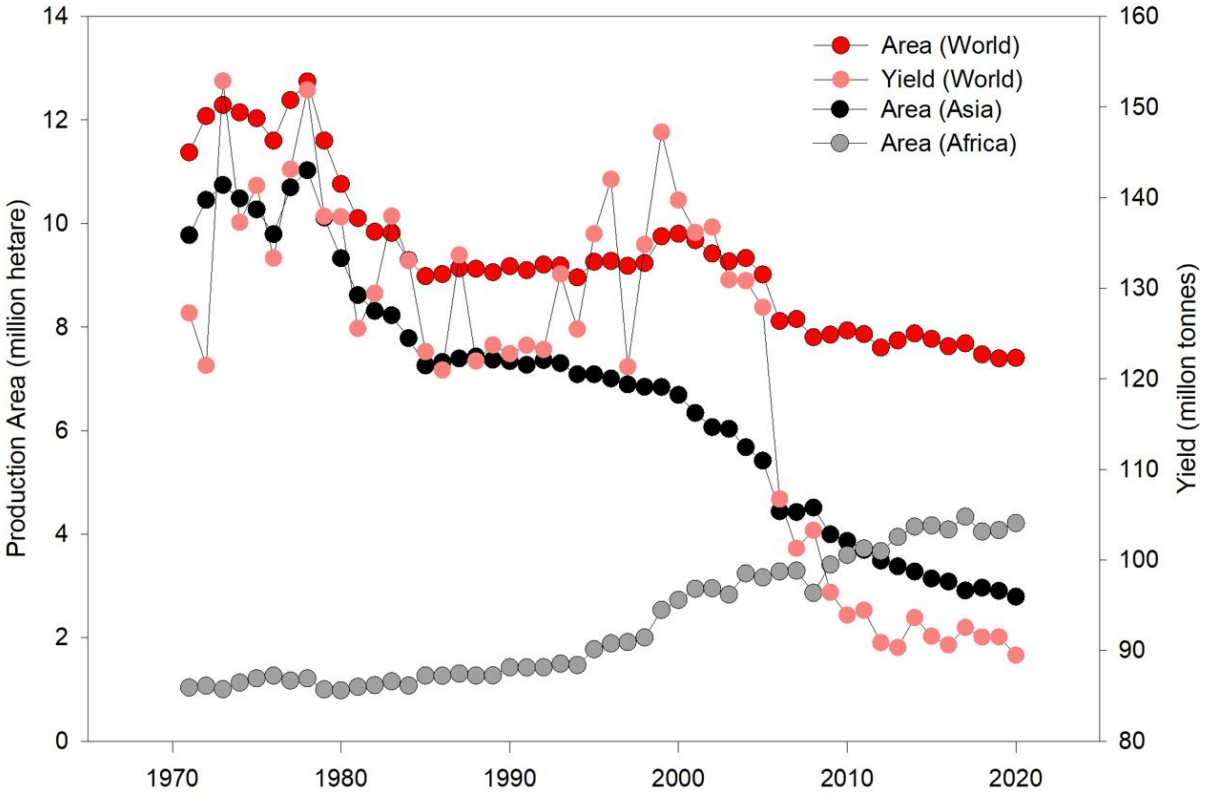

Figure 1.1 Temporal trends in global sweet potato production area and yield together with the area of Asia and Africa from 1970 to 2020. Data source (FAOSTAT, 2022).

Global sweet potato production is declining due to rapidly increasing climate change, with salinity, drought, and temperature as the main stressors. Guo et al. (2006) and Zhai et al. (2016) reported that sweet potato productivity is greatly affected by many biotic and abiotic stresses such as drought and salinity. In general, Asian countries production of sweet potatoes is higher than elsewhere in the world, accounting for about 80% of the total world production, China alone produces nearly 68% of the total world production (FAO, 2014). However, between 2000 and 2020, sweet potato production area and yield have gradually declined worldwide (Figure 1.1) (FAOSTAT, 2022). Figure 1.1 likewise shows the average production area and yield of sweet potato in the world, with both production area and yield drastically decreased from ~12 to ~8 million hectares, and from 140 to 90 million tonnes, respectively, over the last 50 years (FAOSTAT, 2022). Interestingly, the trends of production area in Africa has been gradually increasing over time, the Asian land are under cultivation has been sharply decreasing (Figure 1.1). This implies that a specific problem may be causing the dramatic regional decline in Asian production. Quadir et al. (2014) reported that salinity in Asia is increasing excessively, day by day, threatening overall crop production in this region. On the other hand, cultivated land in Africa is more threatened by drought rather than salinity (Van Oort, 2018). So, it is thought that reduced trends in Asian sweet potato production area might be due to excessive soil salinity. If excessive soil salinity is one of the main drivers for Asian sweet potato reduction, salt tolerance could offer a way forward for production in the region.

1.2 Soil salinity: A global problem and a threat to sweet potato production

Soil salinity is a massive problem for sustainable crop production all over the world. Globally, nearly 800 million hectares of arable land are threatened by soil salinization (Munns and Tester, 2008).The causes of increased soil salinity are diverse and include climate change-related events (sea level rise, cyclones, tsunamis and floods), shrimp farming, excess irrigation with poor drainage, land clearing, etc. (Munns & Gilliham, 2015; Zaman et al., 2018; Zörb et al., 2019; Schneider & Asch, 2020). It is estimated that about 50% of earth arable land

will be affected by salinization by 2050 if proper mitigation strategies are not implemented (Blumwald and Grover, 2006; Butcher et al., 2016).

In rice subsistence countries, sweet potato is mostly grown on river islands or marginal land in coastal areas (BARI Annual Report, 2014; Rahaman et al., 2015), where salinity is the main stressor for crop production (Chen & Mueller, 2018). For example, salinity has increased sharply over the past century in coastal Bangladesh, where more than 1 million ha of cropland have been seriously affected (SRDI, 2010) including much of the land used to grow sweet potato (BARI Annual Report, 2014; Rahaman et al., 2015). Not only in Bangladesh but sweet potato productivity and expansion are also greatly hampered everywhere where salinity is the problem (Dasgupta et al., 2008). A report on sweet potato in relation to soil salinity found that where sweet potato is generally water-loving plant that is resistant to water stress because of its deep roots, it is nonetheless very sensitive to salinity (Mukherjee et al., 2012). Although the salinity threshold is controversial, yield reduction in sweet potato may significantly reduce with salinity (Mukharjee et al., 2009) and salt tolerance sweet potato genotypes could be the best option to solve the problem (Dasgupta et al., 2008).

1.3 Salinity stress and tolerance mechanisms in plants

Soil salinity is considered to exist where there is high amount of sodium, chlorine, calcium, potassium, and/or magnesium ions dissolved in water in the soil (USDA, 2008). It is commonly thought that about 40 mM NaCl or 4 dSm^{-1} can produce an osmotic force of nearly 0.2 MPa, resulting in significant impairment of growth and development in most of the crops which is considered as salt stress (Munns and Tester, 2008). In general, 4 to 8 dSm^{-1} salt stress reduces yield by 50 to 90% in most glycophytic crops (Panta et al., 2014). Salinity stress in the plant body may be affected by environmental factors such as vapor pressure deficit (VPD), solar radiation, temperature. In addition to the level of soil salinity (Chinnusamy et al., 2005). The effects of salinity are also highly dependent on plant species (Estes, 2002) and their developmental stage. For example, some plant species are very sensitive at the seedling

stage, while others are more sensitive at the reproductive stage (Howat, 2000; Houle et al., 2001).

Plants have evolved several mechanisms that allow them to tolerate or recover from different types of salt stress. To date, six main salt tolerance mechanisms have been identified: i) avoidance or minimization of initial Na input; ii) maximization of Na efflux; iii) minimization of Na loading to the xylem; iv) maximization of Na recycling to the phloem; v) intracellular compartmentalization or Na redistribution in specific organs such as old leaves; and vi) secretion of salt onto a leaf surface (Greenway & Munns,1980; Tester and Davenport, 2003; Munns & Tester, 2008). In addition, some halophytic plants may deposit their Na in specific types of glands. Generally, for halophytic and glycophytic plants; Na, K, and Cl are the most important cations and anion to understand the tolerance mechanism to salinity. For example, in most crops, potassium is important for maintaining ion homeostasis and plays a functional role in mitigating the effects of Na (Munns & Tester, 2008; Kumari et al., 2021). In addition, ion (Na, K and Cl) uptake and distribution patterns in plants may provide another clue to the mechanisms of salt tolerance, which are poorly understood in most crop plants. Besides the ion homeostasis of Na, K and Cl, salinity may stimulate the production of reactive oxygen species, and the role of antioxidant enzymes in reducing their deleterious effects could be another important aspect of plants' salt tolerance mechanisms (Bowler et al., 1992; Kumar et al., 2021). Therefore, a comprehensive understanding of various phenotypic and physiological aspects is requird for screening in any crop plants under specific stress conditions.

The primary goal of this thesis is to creat a fast and reliable screening tool for sweet potato concerning salt tolerance mechanisms in different phenotypic responses and physiological processes. To that end, genotypic thresholds (dry matter reduction) in terms of anion-cation relationship; ion uptake (transpiration) and partitioning in different organs and antioxidant enzymes activities have been examined to understand salt tolerance mechanisms in sweet potato under salinity. Before exploring the three approaches to salt tolerance mechanisms

listed above, different ion (Na, K and Cl) analysis methods related to salt stress in sweet potato and rice were implemented to improve the comparability of the results of different studies.

1.3.1 Salinity stress in sweet potato and some clues for tolerance mechanisms

Information on the development of salt-tolerant sweet potato is limited to the development of different cultivar responses (Ekanayake and Dodds, 1993; Mukherjee, 2001). There is limited information on the tolerance mechanisms related to different physiological aspects and their relative thresholds. Different salt threshold can be observed in sweet potato depending on the culture medium used for screening (hydroponics, soil, or in vitro media) and/or the stage of development. Salgado-Garciglia et al. (1985) reported that sweet potato growth in cell cultures was strongly inhibited by 170 mM NaCl concentrations used as selection pressure. Another experiment on the "Effect of NaCl on auxiliary bud production" showed that explants of sweet potato decreased slightly by 1 to 2 grams per litre of NaCl and the response depended on the genotype (Mukharjee, 2001). Paulino & Marutani (2016) found that newly emerged root and shoot growth in *in-vitro* culture was completely arrested at NaCl concentrations of 170 mM and 256 mM, with 85 mM NaCl able to produce more roots and shoots than the control. Anwar et al. (2010) established an in vitro salinity screening system using 0, 25, 50, 75, 100, 125, 150, 175, and 200 mM NaCl for phenotypic analysis of sweet potato and showed that a gradual negative effect started at 75 mM NaCl. Most studies addressed short-term (days to weeks) salinity tolerance in sweet potato with less information on yield and physiological traits (Anwar et al., 2010; Yang et al., 2020). Begum et al. (2015) found that young sweet potato plantlets responded strongly to 200 mM NaCl and this was mainly dependent on genotype potentiality. They found that salt tolerant sweet potato could establish their root up to 200 mM salt stress and that K always decreased with increasing Na. Evoi et al. (2017) found that sweet potato growth and development in soil were greatly reduced by 200 mM NaCl stress, with Na antagonistic to K and Ca.

However, majority of the literatures disagree on the specific threshold and level of salinity that is severely detrimental in sweet potato under salt stress.

1.3.2 Ion uptake and distribution patterns in sweet potato

The uptake of ions (Na, K, and Cl) by water and their distribution among the root, shoot, stem, and leaves (different ages) are essential in salt tolerance mechanisms in plants. For example, Na uptake in rice plants is proportional to transpirational water loss (Yeo et al., 1985; Asch et al., 1997; Wimmer & Asch, 2005), and Na and K are largely distributed in the leaf sheath. In contrast, Naito et al. (1994) found that transpiration or loss of water in rice did not result in ion uptake. Besides Na, K and Cl uptake, ion distribution by different organs may play an important role in the tolerance mechanisms in many plants. For example, Munns et al. (1988) showed that salt tolerant barley plants reduce the concentration of Na concentration in their apical reproductive tissues, compared to the sensitive genotype. Similar findings were found by Yasar et al. (2006) in the case of green beans exposed to salt stress. The large difference in Na, K and Cl concentrations may occur at different ages of leaves or other organs in the same plants exposed to salt stress. Salt tolerant genotypes tend to deposit their Na and Cl mostly in expanded or older leaves (Greenway & Munns, 1980).

Sweet potato is a water-loving plant, and water deficit also affects the growth and yield of sweet potato (Yooyongwech et al., 2014; Yooyongwech et al., 2017). Since water has a close relationship with Na uptake in most cases, it is important to study water loss and the mechanisms that control water uptake in sweet potatoes, because this may play in the development mitigation of salt stress, which may lead to understanding salt tolerant mechanisms in sweet potato. For sweet potato, we have no idea on how Na, K and Cl uptake changes among leaves of different ages under salinity. The leaf level Na, K and Cl uptake distribution patterns could be another set of understanding of salt tolerance mechanisms in sweet potato.

1.3.3 Antioxidant activities in sweet potato under salt stress

Under salt stress, cell damage by reactive oxygen species (ROS) is one of the limiting factors for plant growth (Munns & Tester, 2008). Most ROS are generated by the disruption of electron transport chains in the thylakoid membrane in the chloroplast and are directly involved in electron loss (Asada et al., 1999; Khorobrykh et al., 2020) and lipid peroxidation. Five types of antioxidant enzymes such as peroxidase (POX), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), and superoxide dismutase (SOD) are essential as the first line of defesce (Rajput et al., 2021). Highly electronegative molecules such as O2•-, OH•-, and H_2O_2 must be converted to non reactive molecules. However, very little is known about the antioxidant activities of sweet potato under salt stress. Dasgupta et al. (2008) found that only SOD, GPX, and CAT increased in leaves of tolerant sweet potato genotypes when exposed to 1% NaCl in an in vitro culture. Another report showed that CuZnSOD and APX played a significant role in the salt tolerance of sweet potato plants when exposed to 100 mM NaCl stress (Yan et al., 2016). Similarly, Kim et al. (2015) found that CuZnSOD and APX reduced SO_2-induced oxidative stress in sweet potato. For understanding salt tolerance mechanisms in plants, antioxidant activities under salt stress during physiological growth and development is essential (Fan et al., 2014). Besides these, the linkage between Na, K and Cl with antioxidant enzymes exposed to salinity specifies a salt tolerant mechanism in many glycophytic plants. For example, K is reported to be involved in the augmentation of antioxidant enzymes under water and salt stress (Ahanger et al., 2017) whereas Na (Kumar et al., 2021) and Cl (Shelke et al., 2019) do the opposite. From this perspective, leaf level antioxidant enzyme activities linked to Na, K and Cl are integral to understanding the salt tolerant mechanisms or physiological processes of sweet potato, especially when evaluating metabolic traits for salt tolerance attributes.

1.3.4 Determination of Na, K and Cl concentration by different methods

Na, K, and Cl ions are the most important elements related to salinity and salt tolerance mechanisms of crop plants. Accurate measurement of Na, K and Cl is a prerequisite for obtaining reliable results, especially in the field of salt tolerance mechanisms. Mainly, Na and K content in plant tissues is analysed by flame photometry (Harve, 1961; Puffeles & Nessim, 1957), atomic absorption spectrometry (AAS) (Dionisio-Sese & Tobita, 2000) or inductively coupled plasma atomic emission spectrometer (ICP-AES) (Garcia-Morales et al., 2012), whereas Cl concentrations are studied by titration methods. Attempts have been made to optimize methods for Na, K, and Cl determination, but no comparative analysis of different extraction methods has yet been published. We compared six different extraction methods for Na and K and three for Cl-free using sweet potato and rice tissues to determine the differences between extraction methods.

1.4 Research justification

To ensure food security for the world's growing population, vast saline areas should be considered for crop production, and sweet potato could be used on the frontier due to its higher yield and nutritional value. Currently, traditional management practices like soil mulching, fresh water application, and raised bed techniques are practiced to reduce soil salinity for sweet potato cultivation in many developing countries like Bangladesh (Islam et al., 2013; BARI Annual Report, 2015). In addition to soil management, varietal development of sweet potato is needed to minimize the impact of salinity and increase the total production area available. For varietal improvement, information on the physiological response to salinity which affect phenotypic traits could play a vital role in developing a reliable salt tolerant screening tool for the sweet potato which is urgently needed to solve the problem.

In general salt tolerance mechanisms of sweet potato regarding phenotypic responses and physiological processes are sparsely investigated. Generally, most of the literatures regarding

salt stress and tolerance mechanisms in sweet potato were associated with short periods of salt exposure time, fewer variations of salinity levels, young plantlet, and smaller numbers of genotypes (Begum et al., 2015; Cusma-Ticlla et al., 2016; Ekanayake and Dodds, 1993; Evoi et al., 2017). These do not support the development of reliable screening tools in sweet potato with respect to salt stress. Most literatures can not give complete information on the genotypic thresholds linked to Na, K or Cl that could be reliable screening tools in sweet potato salinity tolerance/vulnerability. This is important not only for the identification of salt tolerance/sensitive genotype of sweet potato, but also for direct information on yield potentiality against salinity to the farmers. Then, it is thought that transpiration, Na, K and Cl uptake and distribution could play a vital role in salt tolerance mechanisms in plants (Greenway and Munns, 1980; Munns and Tester, 2008) but there is no information to date on the effects of transpiration on salt ion uptake and distribution in sweet potato. In addition, leaf level antioxidants linked to Na, K and Cl ions may give further information on ROS scavenging potentiality in sweet potato, which is also indicative of salt tolerance mechanisms in sweet potato. No studies have been published to date on leaf level antioxidant activities as related to ion uptake and distribution, subject to salinity.

So, there are three big research gaps in the way of developing a suitable and reliable screening tool, as well as knowledge on salt tolerance mechanisms in sweet potato. Ultimately, a reliable and quick screening, regarding phenotypic behaviors and physiological processes, is not only helpful for identifying tolerant genes but also gives direct yield information to the farmers. It is noted that comparative study of different ion analysis methods by different plant's tissue samples are imperative to prove a reliable screening tools in a specific plant.

1.5 Specific Objectives

Sweet potatoes are extremely important to global food and energy security. However, research on sweet potato physiology related to salinity is limited, hindering progress in genetic improvement. An in-depth study of salt tolerance mechanisms concerning various aspects of physiological processes could lead to the development of a quick, suitable and reliable screening tool for the identification of salt tolerant/sensitive genotypes of sweet potato. Therefore, salt tolerance traits could play an important role in mitigating the global food crisis by increasing agricultural production on marginally and highly saline soils. Given the foregoing, we established four primary objectives, which are listed below:

i) to compare different Na, K and Cl extraction methods to optimize screening protocols for determining ion concentrations in plant tissues.

ii) to understand the mechanisms of salt tolerance and to develop a rapid and reliable screening tool in sweet potato subjected to salinity.

iii) to determine to what extent transpiration can control Na, K and Cl uptake and distribution in sweet potato genotypes under two different VPD conditions.

iv) to evaluate the activities of ROS detoxifying antioxidant enzymes in leaves of sweet potato plants of different ages exposed to salt stress.

1.6 Thesis structure and outline

The thesis consist of four chapters for the publication as depicted in Figure 1.2. The structure of the publications is consistent with the journal where they were published or submitted for the peer review.

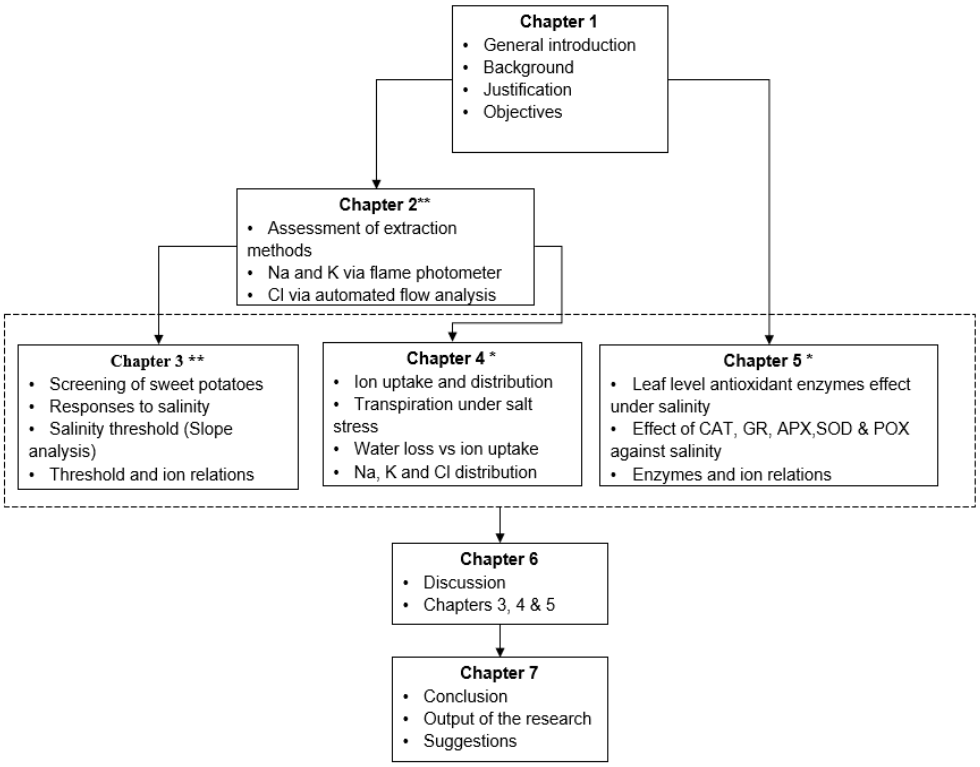

Figure 1.2 Schematic illustration of the structure and outline of this thesis. Double asterisk (**) denotes published and single asterisk (*) denotes submitted articles for the peer review.

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Chapter 2

Comprehensive assessment of extraction methods for plant tissue samples for determining sodium and potassium via flame photometer and chloride via automated flow analysis

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Abstract

Determination of sodium (Na), potassium (K), and chloride (Cl) content in plant tissue is required for research related to salinity resistance in plants. Standard methods are available to extract these elements from dried plant material, but these methods are often costly, relatively dangerous, or time consuming. Many authors modify extraction methods substantially without proof of comparability across methods. Here, dried tissue of two varieties of rice and three varieties of sweet potato subjected to salt stress were extracted for Na and K using six different extraction methods (1-6) and for Cl using three Cl-free extraction methods (2, 4, 5) for Cl: (1) the VDLUFA standard method, consisting of ashing, and heat extraction in hydrochloric acid (HCl), (2) hot water pressure extraction via autoclave, (3) extraction with 1 M HCl overnight, (4) hot water extraction at 90 °C for 1 h, (5) acetic acid extraction in hot 1 M acetic acid for 2 h, and (6) extraction with a microwave using nitric acid. Na and K were determined via flame photometer and Cl via automated flow analysis. Na and K concentrations varied little among different extraction methods as compared to the VDLUFA standard method, and for Cl, all extractions resulted in similar tissue Cl concentrations. Ultimately, the choice of extraction method depends on the instrumentation and lab equipment necessary, available budget, the available amount of sample, and time constraints which should be decided according to the experiment. For reasons of comparability among publications, methods applied should be clearly described since results vary depending on the method chosen.

Keywords: autoanalyzer, *Ipomoea batatas*, *Oryza sativa*, salt stress

2.1 Introduction

Determination of sodium (Na), potassium (K), and chloride (Cl) concentrations and content in plant tissue is at the heart of any research related to salinity resistance in plants. Na and K contents in plant tissues are often determined via flame photometry (Havre 1961; Puffeles and Nessim, 1957), atom absorption spectrometry (AAS) (Dionisio-Sese and Tobita, 2000) or inductively coupled plasma-atomic emission spectrometer (ICP-AES) (García-Morales et al., 2012).

Samples are either liquid, such as that of a hydroponic solution, rain, or irrigation water, and can be analyzed directly or after dilution, or dry plant material that needs to be extracted prior to analysis. There are some standard methods available to extract these elements from dried plant material, but these methods are often tedious, costly, relatively dangerous, or time-consuming. Many authors modify the extraction methods substantially without proof of comparability across methods.

In Germany, the official standard method has been defined by VDLUFA, the Association of German Agricultural Analytic and Research Institutes e. V., an organization that sets standards for many laboratory tests. In the standard method for determining Na and K (VDLUFA, 2012), 2 g of sample are ashed, extracted using hydrochloric acid (25%), heated in a water bath, filtered, and then analyzed. This method is time, energy, and material intensive and, due to the use of strong acid, more hazardous than other methods. The VDLUFA method has not been recognized as a global standard, and 2 g of dried plant material required for this method are often not available. Therefore, generally applicable methods for smaller sample sizes are needed, such as published earlier by Leyton (1951), Puffeles and Nessim (1957), or the similarly hazardous method of Hald (1947) who applied 4 N sulfuric acid before ashing the sample and digesting it in concentrated hydrochloric acid.

Many different extraction methods are available from literature; however, few have been directly compared using the same samples. In this study, we focus on rice and sweet potato,

as examples for important monocotyledonous and dicotyledonous C3 staples that are often threatened by salinity, for a first-time direct comparison of various extraction methods.

Although the German standard method works from ash and thus requires large amounts of dry sample, in other methods dry plant samples are extracted directly in acid or hot water. For acid digestion, different acids and concentrations can be used. For rice tissues, for example, Campbell et al. (2017) digested the samples in 0.1 molar nitric acid for 8 h at 70 °C, whereas Kibria et al. (2017) used a mixture of nitric acid and perchloric acid and heated the mixture until dense white fume was observed. Another approach extracts the samples in 1 M hydrochloric acid on a shaker overnight before filtering to remove all organic particles (Asch et al., 1999; Yoshida et al., 1976). Gerona et al. (2019) extracted the samples in 0.1 M acetic acid for two hours at 85 °C and left them overnight at room temperature before filtration similarly to Yeo (1992) and Tatar et al. (2010) who extracted at 90 °C. For sweet potato, Evoi et al. (2017) used nitric and perchloric acid mixture after pretreatment with concentrated nitric acid to digest 1.0 g of sample for determining the Na and K concentrations.

Hot water extraction is not so widely reported in literature but has some advantages. It is not hazardous, and the extract allows determination of ions otherwise masked by the extraction agent, such as chloride (Cl) for example, when tissue samples are extracted with HCl. For rice samples, Matsushita and Matoh (1991) boiled 0.05 g of dried sample for 60 min to extract Na whereas Dionisio-Sese and Tobita (2000) used an autoclave to extract the samples at 121 °C for 20 min following a boiling water treatment of 1 h. For sweet potato, the only hot water extraction for Na and K was reported by Begum et al. (2015), following the protocol of Karmoker and Van Steveninck (1978).

Chloride concentrations from plant extracts are determined via titration, for example, using mercury nitrate after extraction in acidic sodium nitrate solution (VDLUFA, 2012). Silva et al. (1998) compared different titration methods for determining Cl concentrations in coffee leaf extracts, including a potentiometric titration where silver wire was used as indicator electrode to determine the endpoint of titration. They stated that the mercurimetric method, using

mercury nitrate and diphenycarbazone, was most convenient. Alamgir et al. (2007) extracted Cl from rice samples in 0.1 M acetic acid at 90 ºC for 2 h whereas Islam et al. (1983) used an ion-selective electrode to avoid dry ashing or wet oxidation procedures. Gaines et al. (1984) extracted dried and milled tissue of soybean using 0.1 M sodium nitrate solution, 5 minutes of shaking, and measured the filtered solution in an Auto-Analyzer II with mercuric thiocyanate for colorimetric determination. Finally, Karmoker and Van Steveninck (1978) extracted labelled ^{36}Cl with hot water and related the radioactive counts to the concentration in the tissue.

The aim of this study is to test different extraction methods for comparability of determining Na, K, and Cl concentrations from plant tissue samples. Extracts of dried tissue of two varieties of rice (*Oryza sativa*) and three varieties of sweet potato (*Ipomoea batatas*) subjected to salt stress were compared using six different extraction methods for Na and K (1-6) as well as three Cl-free extraction methods (2, 4, 5) for Cl: (1) the VDLUFA standard method, consisting of ashing, and heat extraction in hydrochloric acid (HCl), (2) hot water pressure extraction via autoclave, (3) extraction with 1 M HCl overnight, (4) hot water extraction at 90 °C for 1 h, (5) acetic acid extraction in hot 1 M acetic acid for 2 h and (6) extraction with a microwave using nitric acid.

2.2 Materials and methods

All solutions and standards described here were prepared with pro-analysis grade chemicals (obtained from Sigma-Aldrich, Germany) in deionized water.

2.2.1 Plant material and treatment

Seeds of two rice varieties (IR 64, and IR 31785-58-1-2-3-3 (further IR31785)) were pre-germinated on filter paper at room temperature for 2 days, followed by 7 days growth in sand. Seedlings were transplanted in 1-L pots containing half strength Yoshida nutrient solution at pH 5.8 (Yoshida et al.,1976). Nutrient solution was changed to full strength after 1 week, and

renewed weekly. The salt treatment, nutrient solution containing 60 mM NaCl, was started 4 weeks after transplantation for half of the plants. For each variety and treatment there were five replicates.

Three sweet potato varieties (CIP 189151.8, CIP 106082.1, CIP 420001) were propagated via stem cuttings transferred to aerated 50% Yoshida nutrient solution (Yoshida et al.,1976). After 1 week, nutrient solution was changed to 100% and renewed weekly. Three weeks after transplanting, a salt treatment of 100 mM NaCl was applied. Five weeks (sweet potato) or six weeks (rice) after transplanting, the plants were harvested, rinsed with deionized water, divided in roots, stems, and leaves. The plant material was oven dried until constant weight for 2 days at 70 °C in a drying oven (ULM500; Memmert, Germany). The dried samples were milled in 20-mL scintillation vials (Nerbe, Germany), using six small and three large steel milling balls (3 and 5 mm diameter, VWR, Germany). The powdered samples were used for all extraction methods (rice) or for VDLUFA, Autoclave and HCl extraction (sweet potato).

2.2.2 Extraction methods

Not that 20-mL plastic vials (Scintillation vials, Germany) and qualitative filter paper 413 (VWR, Germany) were used for all extraction methods.

2.2.2.1 VDLUFA method 10.1.1 / 10.2.1

The original method was used with small modifications in the used amounts, 1/5 of all amounts was used (Puffeles and Nessim, 1957). Approximately 2 g of each sample were ashed in a muffle furnace at 450 °C for 3 h. The ash was transferred to 100-mL measuring flasks, using 50 mL deionized water, and 10 mL hydrochloric acid (25%). The solutions were heated at 90 °C for 2 h in a water bath, cooled down, transferred to 100-mL volumetric flasks, and filled up to the mark using deionized water. Samples that were not clear were filtered prior to measurements.

2.2.2.2 Autoclave

For hot water pressure extraction, the method of Dionisio-Sese and Tobita (2000) was followed with modifications. Note that 0.1– 0.15 g dried, milled material was weighed in 15-mL centrifuge tubes, 10 mL of deionized water was added and the lids closed loosely. Samples were autoclaved at 121 °C for 1 h, subsequently cooled, filtrated, transferred to 100 mL volumetric flasks, and filled up to the mark using deionized water.

2.2.2.3 Hydrochloric acid (HCl)

We followed the method described by Yoshida et al. (1976) with minor modifications. Note that 0.1 - 0.15 g of dried, ground sample were weighed into 20-mL plastic vials (Scintillation vials, Nerbe, Germany), 10 mL 1 N hydrochloric acid was added while strong shaking was avoided to make sure to keep the sample totally submerged. The samples were shaken overnight (16 h) at room temperature (20 °C). Samples were filtrated into 100 mL volumetric flasks and made up to 100 mL using deionized water.

2.2.2.4 Hot water

The extraction with hot water was described by Matsushita and Matoh (1991). Note that 0.1 – 0.15 g of dried, milled sample were extracted in 100 mL deionized water in a 90 °C water bath for 60 minutes. After cooling the samples, the extracts were filtrated to remove particles.

2.2.2.5 Acetic acid

Following Yeo (1992), we used 0.1 - 0.15 g dried and ground material weighed in 15-mL centrifuge tubes (Roth, Germany), added 10 mL 1 M acetic acid and heated the samples at 90 °C for 2 h. Samples were cooled, filtrated into 100 mL volumetric flasks, and volume was made up using deionized water.

2.2.2.6 Microwave

For extracting plant tissue samples with a microwave, we modified the protocol for microwave digestion for determination of multi-elements by inductively coupled plasma mass spectrometry from Wu et al. (1997). Note that 0.15-0.2 g dried and ground material was weighed into microwave extraction tubes (mws Mikrowellen-Laborsysteme, Germany), 2 mL deionized water, 4.5 mL concentrated nitric acid, and 1.5 mL hydrogen peroxide were added. Tubes were closed and samples were immediately extracted in a ETHOS.lab (mws Mikrowellen-Laborsysteme) microwave (15 min to heat to 170 °C, then 20 min at 200 °C), transferred into 100 mL volumetric flasks and volume was made up using deionized water.

2.2.3 Standards and flame photometer measurements

Standards were purchased from Jenway (UK) as 1000 ppm sodium or potassium standard and diluted to 100 ppm, 50 ppm, 25 ppm, 12.5 ppm using deionized water.

Sodium and potassium concentration was determined via flame photometer (PFP7; Jenway, UK) using an exponential calibration curve. Samples were diluted with deionized water to fit the concentration range of the calibration.

2.2.4 Determination of chloride

Chloride concentrations were determined via an automated flow system connected to a chart recorder (ABB Goerz SE120, Germany). The main driving solution was 0.5 M potassium nitrate, containing 3 mL 1 M nitric acid, 3 mL 0.003 M sodium chloride solution, and 0.5 mL Brij 30 per 1000 mL. Samples were mixed with the potassium nitrate solution and pumped through a silver tube coated with 0.19 M iron (III) chloride and 20 mL 1 M hydrochloric acid in 100 mL. The connection scheme and the flow rates according to the diameter of tubes used are given in Figure 2.1. A chart recorder records the electrical difference to a second coated silver tube containing potassium nitrate solution as reference. Every 5000 samples, the tubes

were cleaned using 0.2 M nitric acid and coated again using the iron (III) chloride solution. Standard stock solution was 100 mM sodium chloride solution, the standards for calibration were diluted to obtain 30, 20, 10, and 2.5 ppm. Chloride concentrations of 0.5–30 ppm can be determined, extracts with higher concentrations have to be diluted. Forty samples were measured per hour.

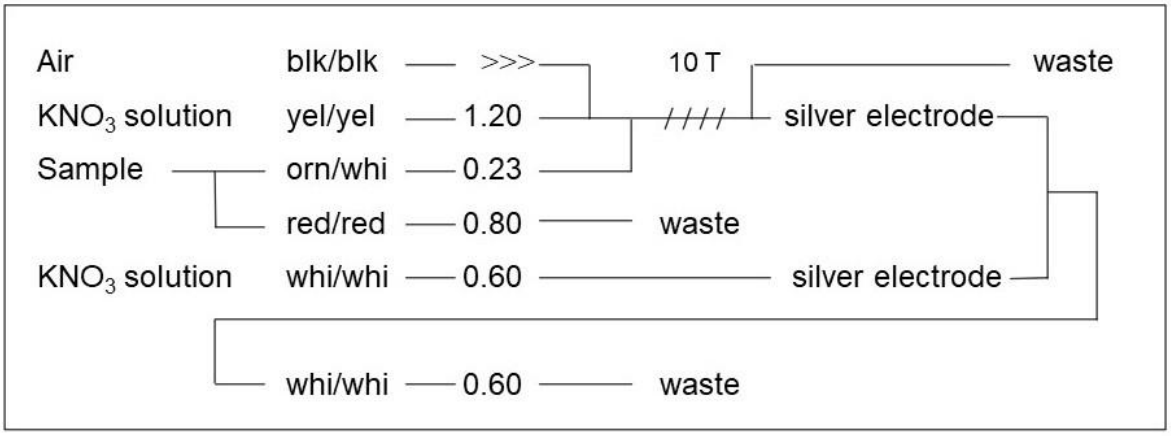

Figure 2.1 Flow diagram for automated chloride analysis. Numbers given are pump flow rates in mL min^{-1}. Color coded pumping tubes (Alliance Instruments, Austria). Abbreviations: blk, black; orn, orange; red, red; yel, yellow; whi, white. >>> = no flow rate. /// =mixing coil. T = number of turns of mixing coils.

2.3 Results

2.3.1 Comparison of extraction methods for sodium and potassium

Na and K concentrations for all extracts obtained via the different extraction methods were determined via flame photometer. Figure 2.2 compares sodium concentrations determined from rice samples extracted with the VDLUFA extraction methods to the sodium concentrations determined for the same samples extracted with the other extraction methods. In general, sodium concentrations from 0 to 25 mg Na per g dry weight were quite similar among the different extraction methods and followed a linear trend close to the 1:1 line. Extraction with HCl resulted in a slight overestimation of sodium concentrations, whereas extraction with hot water, acetic acid, with an autoclave extraction, or microwave resulted in

small underestimations of Na concentrations. Treatment or variety did not affect the extraction efficiency for Na.

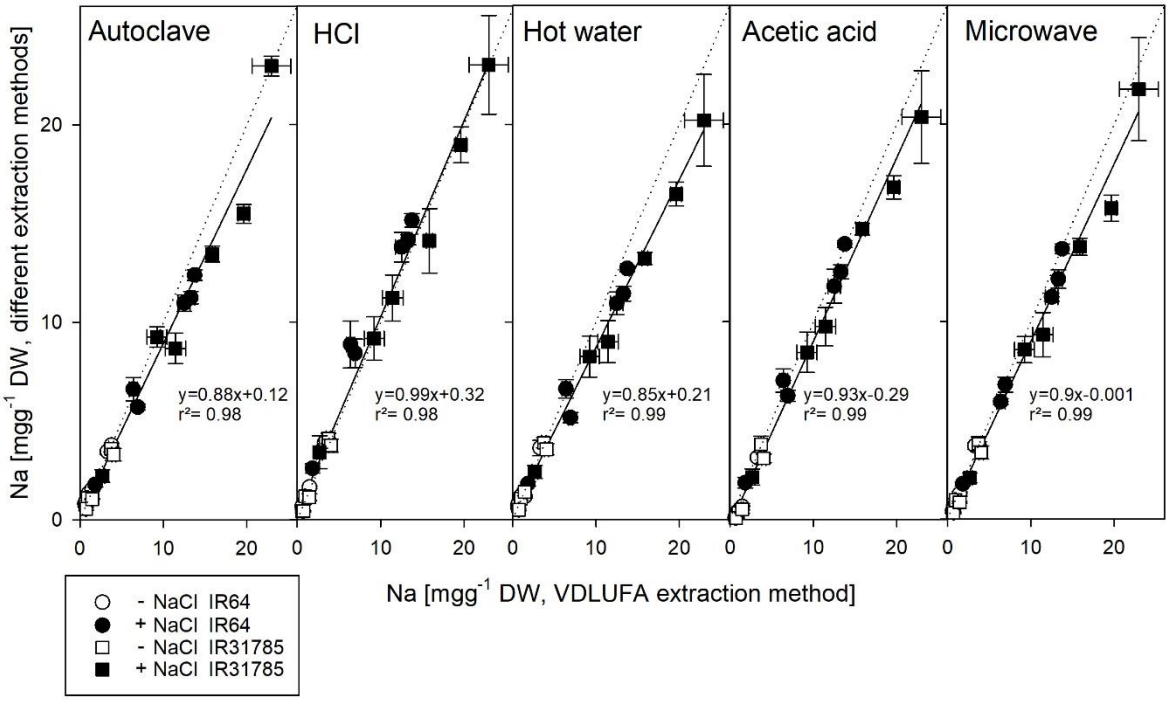

Figure 2.2 Sodium concentration of rice samples determined via different extraction methods compared to the standard method (VDLUFA). White = nutrient solution (no NaCl added); black = 60 mM NaCl added to the nutrient solution; circles depict samples of IR 64, squares from IR 31785–58–1–2–3–3. The dotted lines in the subgraphs show the 1:1 relation. The solid lines show linear regressions across all measured values. Error bars = standard error of means (n = 5).

K concentrations (Figure 2.3) determined from the same extracts showed the same linear trend for each extraction method compared to the VDLUFA extraction, following the 1:1 line. Relative to the VDLUFA method, extracting with HCl leads to slightly higher K concentrations compared to the standard method, whereas extracting via autoclave resulted in minor underestimations of the K concentrations. The closest linear agreement was found between K concentrations in hot water extracts, extraction via microwave, and those extracted according to the VDLUFA method. The saline treatment did not change this relation, and no difference related to extraction method was found between the two varieties.

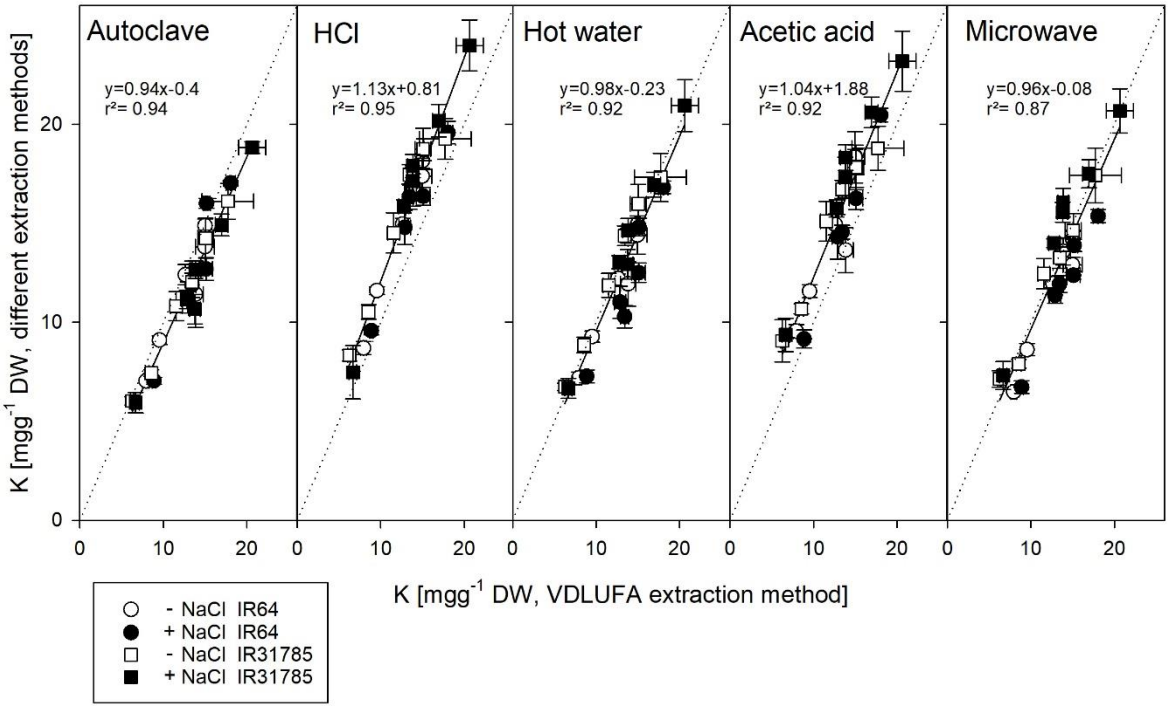

Figure 2.3 Potassium concentration of rice samples determined via different extraction methods compared to the standard method (VDLUFA). White = nutrient solution (no NaCl added); black = 60 mM NaCl added to the nutrient solution; circles depict samples of IR 64, squares from IR 31785–58–1–2–3–3. The dotted line in the subgraphs shows the 1:1 relation. The solid lines show linear regressions across all measured values. Error bars = standard error of means (n = 5).

Figure 2.4 compares the two extraction methods commonly used in the laboratory of the authors, namely extraction with HCl and via autoclave. Extraction with HCl results in Na and K concentrations 13% and 30% higher, respectively, compared to water extraction via autoclave. Neither treatment nor variety affected this relationship.

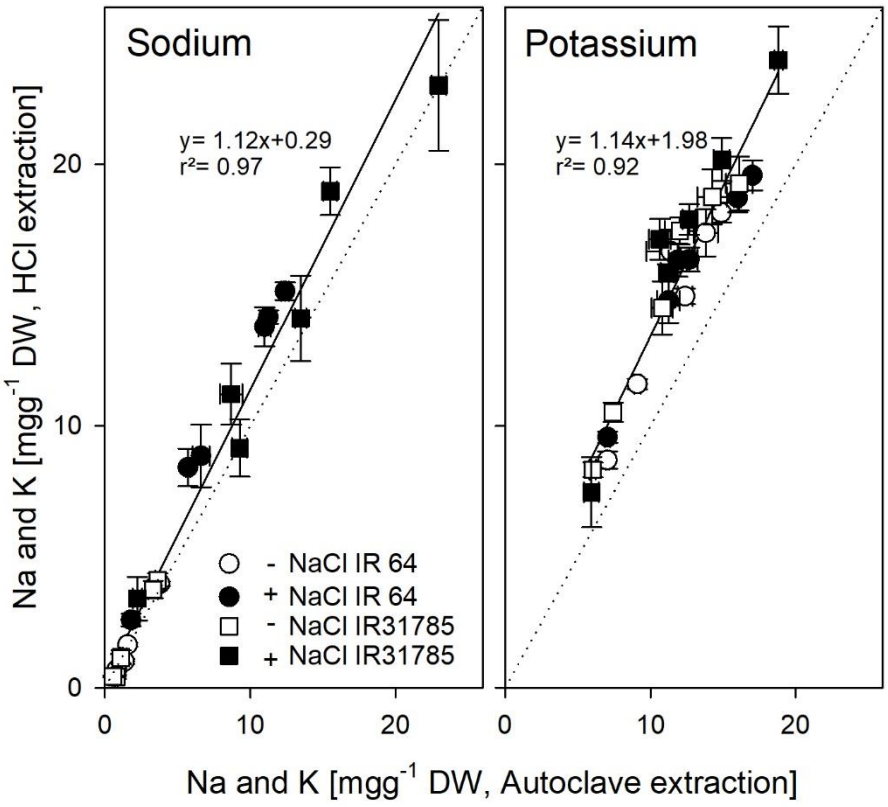

Figure 2.4 Sodium and potassium concentrations of HCl extracted rice samples compared to the corresponding concentrations obtained via extraction by autoclave. White = nutrient solution (no NaCl added); black = 60 mM NaCl added to the nutrient solution; circles depict samples of IR 64, squares from IR 31785–58–1–2–3–3. The dotted line in the subgraphs shows the 1:1 relation. The solid lines show linear regressions across all measured values. Error bars = standard error of means (n = 5).

2.3.2 Determination of chloride concentration in extracted samples

Cl concentration was measured in extracts obtained via hot water, acetic acid, and autoclave. Figure 2.5 compares the Cl concentrations found from hot water and acetic acid extraction to the values found after extraction via autoclave. Extraction with acetic acid leads to slightly underestimated Cl concentrations compared to extraction via autoclave, whereas extraction with hot water shows almost the same results as extraction via autoclave.

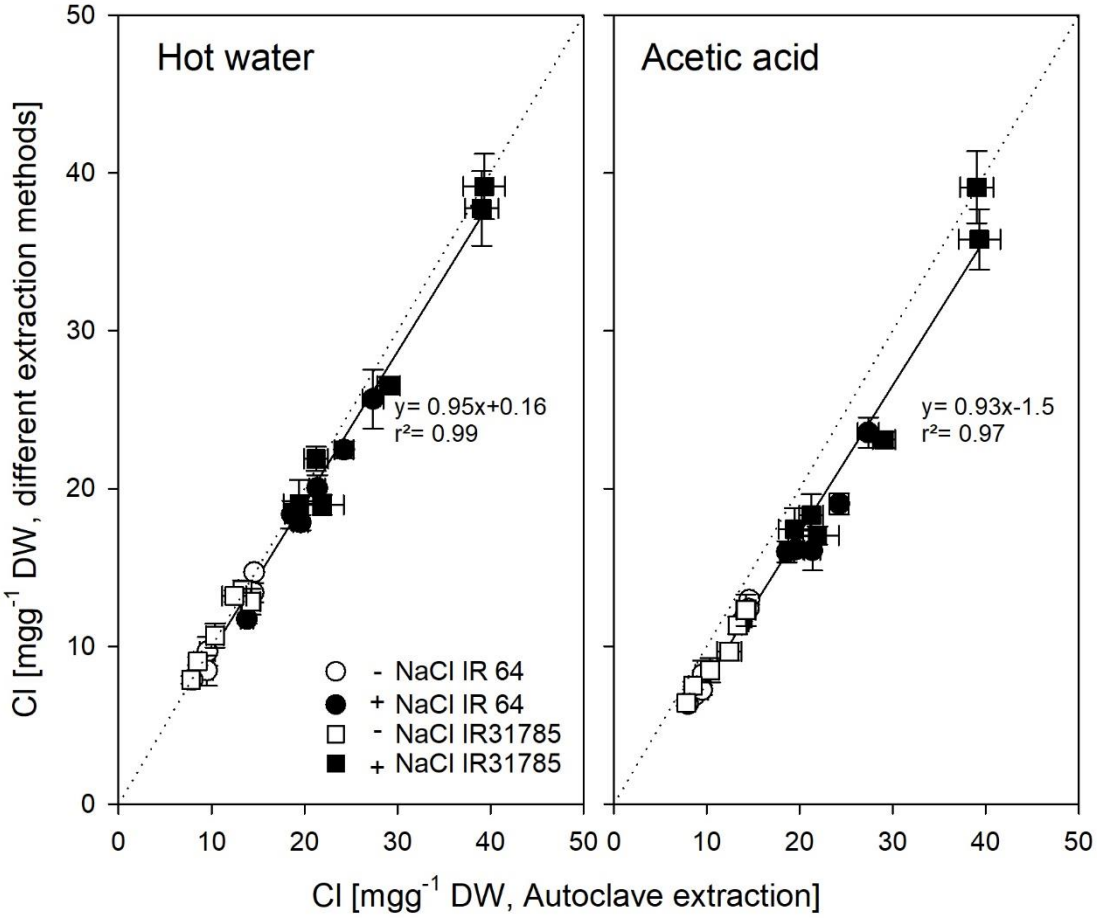

Figure 2.5 Chloride concentration of hot water and acetic acid extracted rice samples compared to the corresponding concentrations obtained via autoclave extraction. White = nutrient solution (no NaCl added); black = 60 mM NaCl added to the nutrient solution; circles depict samples of IR 64, squares from IR 31785–58–1–2–3–3. The dotted line in the subgraphs shows the 1:1 relation. The solid lines show linear regressions across all measured values. Error bars = standard error of means (n = 4).

2.3.3 Test of extraction methods using a dicotyl plant

A small subset of sweet potato samples was used to determine the applicability of the findings to other crops. VDLUFA extraction was compared to autoclave and HCl extraction (Figure 2.6). Na concentration of samples extracted via the VDLUFA method was linearly correlated to Na concentrations found in samples extracted via autoclave (R^2 = 0.98) and HCl (R^2 = 0.97). K concentrations also correlated between the extraction methods, but the correlation factor was lower (R^2 = 0.88 and 0.82, respectively). Extraction with HCl detected higher K concentrations than the VDLUFA method.

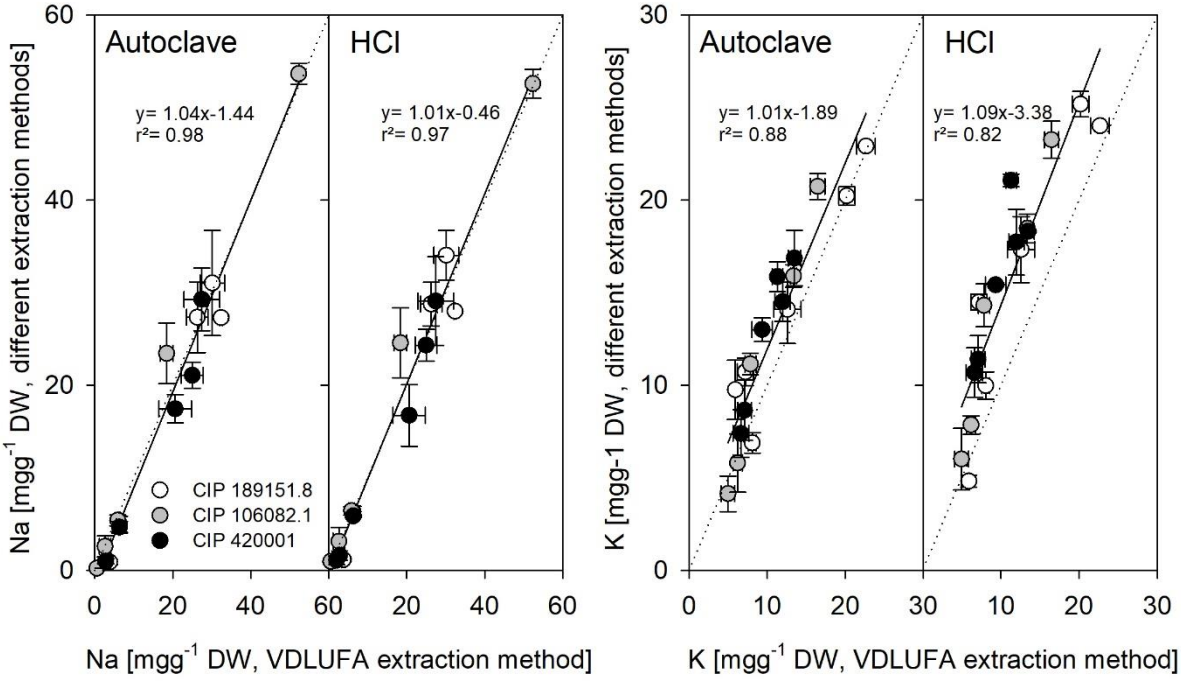

Figure 2.6 Sodium (left) and potassium (right) concentrations of tissues of three sweet potato varieties (CIP 189151.8, CIP 106082.1, CIP 420001) extracted via autoclave and HCl extraction method compared to the standard method (VDLUFA). The dotted line in the subgraphs shows the 1:1 relation. The solid lines show linear regressions across all measured values. Error bars = standard error of means (n = 3).

2.4 Discussion

Out of many different extraction methods for the determination of Na, K, and Cl from plant samples, we tested six against each other for Na and K concentrations from the same tissue samples. The Na and K concentrations found in rice tissue with the different methods showed reasonable agreement (Figures 2.2 and 2.3). This confirms reports from Hodson et al. (1985), who showed that extraction with nitric acid compared to extraction with hot water for a wild grass (*Agrostis stolonifera*) produced similar results. For dicots, Matejovic and Durackova (1994) compared several dry and wet digestions methods for Alfalfa and found no differences in Na and K concentrations when using different extraction methods, which was confirmed our study with sweet potato (Figure 2.6). Digestion and extraction of ions with HCl generally

produced higher values for K than all other methods (Figures 2.3, 2.4, and 2.6); thus, it is important to know which extraction method was used when directly comparing K results across studies (Gholizadeh & Navabpour, 2011).

Chloride determination is not possible from all tested extracts. A pH below 2, as in extracts obtained with the VDLUFA method and from microwave digestion, might damage the silver electrodes in the autoanalyzer. High concentration of Cl in the extraction solution, such as in the VDLUFA method and when extracting with HCl, results in high background Cl values that do not allow differentiating sample Cl concentrations from the extraction solution. Nevertheless, Cl determination from water-based extractions, particularly with hot water or using an autoclave, produced plausible and reproducible results for both Cl in rice (Figure 2.5) and sweet potato tissue (data not shown).

Since we could show that all extraction methods potentially produce comparable results, the final choice of method may depend on other factors such as required instrumentation, costs per sample, consumables required, or time to complete the analysis.

Table 2.1 Requirements for the extraction of sodium, potassium, and chloride. Costs for machinery are rough estimates, costs per sample are calculated according to consumables and chemicals required for the method.

	VDLUFA method	**Autoclave**	**HCl**	**Hot water**	**Acetic acid**	**Microwave**
Amount of sample	2 g	0.1-0.15 g	0.1-0.15 g	0.1-0.15 g	0.1-0.15 g	0.15-0.2 g
Hazardous substances	25% HCl	-	1 M HCl	-	1 M CH_3COOH	Conc. HNO_3
Required machinery	Muffle furnace, waterbath	Autoclave	Shaker	Waterbath	Waterbath	Microwave digestion system, fume hood
Estimated minimum cost for machinery	3500 €	1500 €	800 €	500 €	500 €	10,000€
Cost per sample	0.65 €	0.24 €	0.22 €	0.24 €	0.24 €	0.23 €

For the VDLUFA standard method, a muffle furnace, a large number of crucibles, and a water bath are required for the extraction (Table 2.1). Ashing samples is usually done overnight, due to the muffle furnace' relatively long heating and cooling requirements, leading to a relatively long minimum time requirement per batch (Table 2.2). The number of samples that can be processed in one batch is limited by the size of both water bath and furnace. The standard method has some drawbacks: (1) the use of highly concentrated acid (25% HCl), (2) high costs per sample (Table 2.1) (mainly due to laboratory chemicals and consumables), and (3) a relatively large amount of dried sample, which is hard to obtain in salt stress experiments when single plants or individual plant organs will be analyzed.

Table 2.2 Time requirements of different steps in the procedure of extracting Na, K, and Cl with various methods. Minimum time per batch is calculated by adding time requirements of all steps. Working time per sample comprises weighing in, transferring, filling up of samples and cleaning and preparation time per batch divided by the number of samples per batch. Times given are empirical values for one technically versed staff from the lab of the authors.

	VDLUFA method	**Autoclave**	**HCl**	**Hot water**	**Acetic acid**	**Microwave**
Ash samples [min]	900	-	-	-	-	-
Extraction time [min]	120	60	900	60	120	100
Filter [min]	-	60	60	60	60	-
Working time per sample [min:s]	5:30	1:30	1:30	2:00	2:00	4:00
Samples per batch	25	120	120	50	50	14
Minimum time per batch [min]	1240	300	1260	220	280	156

Hydrochloric acid extraction only requires a laboratory shaker of any kind (Table 2.1) and some materials such as scintillation vials and filter paper. Theoretically, even the shaking could be done manually. Samples are extracted in weakly concentrated acid and the costs per sample are quite low (Table 2.1). However, filtration is required to remove any particles prior to flame photometry. Overnight extraction on the shaker leads to similarly long minimum batch times as in the VDLUFA method, (Table 2.2); however, depending on the shaker, a large number of samples can be processed per batch and the entire procedure is not labor-intensive.

Hot water extraction does not need expensive instrumentation and has low costs for consumables (Table 2.1). It also needs a short extraction time of only 60 min (Table 2.2), and the number of samples per batch depends on the size of the water bath. Filtrating the samples is necessary to avoid blockage in the flame photometer caused by particles in the extract.

Acetic acid extraction only needs a water bath that is adjustable to 90 °C (Table 2.1), the extraction takes 2 h per batch and the size of one batch depends on the size of the water bath.

The costs per sample are low due to low requirements for consumables and chemicals. The extraction medium is weakly concentrated acetic acid.

Autoclave extraction requires an autoclave, which is standard equipment in many labs. Due to the relatively large size of autoclaves and the use of 15-mL centrifuge tubes, the potential number of samples per batch is high (Table 2.2). The extraction depends on pressure and high temperatures and, as before, subsequent filtration of the extracts is required. The costs per sample are low (Table 2.1). Only centrifuge tubes, filter papers, and vials for measurement and storage are needed. Few examples for extraction by autoclave exist in literature. Dionisio-Sese and Tobita (2000) autoclaved their samples for 20 min and then boiled them for 1 h in a water bath. We found that extending the extraction time in the autoclave can replace the subsequent boiling. This renders the method faster and less tedious since the hot samples do not need to be handled again, and the results are still comparable to the other methods (Figures 2.2–2.4 and 2.6).

The nitric acid microwave extraction needs more sophisticated instrumentation. The ETHOS lab microwave is often used to extract samples for determining nutrients such as phosphate in plant tissue. The results presented here show that K and Na could be determined reliably from the same extract (Figure 2.3). Since the extraction using the microwave is time-consuming (Table 2.2), allows only for a small number of samples per batch, requires expensive instruments and extraction tubes, and uses hazardous chemicals for the extraction (Table 2.1), we do not recommend this method for determining Na and K from plant samples.

2.5 Conclusions

Since no comprehensive comparison of extraction methods for Na, K and Cl is available in literature, but substantial research focusses on salinity and its effects on plants, we tested here six extraction methods for determining Na and K via flame photometry and three methods for determining Cl with an autoanalyzer. Tissue samples from rice and sweet potato obtained

from plants grown hydroponically in the absence and presence of salt were subjected to all extraction methods. Neither plant species and variety and nor salinity influenced the reliability of the different extraction methods. All methods gave reliable and reproducible results. Only overnight extraction with HCl found tissue K concentrations that were several percent higher than other methods, including the German standard method, VDLUFA. We included a comparison of costs, applicability, time efficiency, and sample throughput to allow researchers finding the method most adapted to their conditions. With this, we hope to provide a comprehensive reference to extraction methods for future publication to increase comparability of results across studies.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Chapter 3

Potassium content is the main driver for salinity tolerance in sweet potato before tuber formation

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Abstract

Sweet potato (*Ipomoea batatas* L.) is mostly grown in Asia, which accounts for 86% of global production. However, its production is under threat by salinity. Little is known about genotypic responses to salinity in sweet potato. Phenotypic responses or physiological processes linked to salt tolerance that could be developed into a reliable screening tool to assist breeding have not yet been developed for sweet potato. In a hydroponic cultivation system, 12 contrasting sweet potato genotypes were subjected to 0, 50, 100, and 150 mM root zone salinity (RZS). Genotypic thresholds for dry matter accumulation and the genotypic slopes for additional dry matter reduction when the RZS increased beyond the genotypic threshold were determined. Sodium, chlorine, and potassium (K) were determined from above ground biomass and correlated with the genotypic thresholds found. Genotypic threshold levels were linearly negatively correlated with the difference in tissue K content at 75 mM RZS and the tissue K content at control levels. Based on the genotypic ability to retain high tissue potassium levels under increasing RZS, we propose a screening tool based on these experimental data that can distinguish between salt tolerant and salt sensitive genotypes and indicate the potential yield level of the sweet potato genotypes.

Keywords: chloride uptake, dry matter, sodium uptake, slope, salinity threshold

Key points

- Sweet potato threshold for salinity damage is at 75 mM (RZS)
- Genotypic threshold levels correlated with the difference in tissue K content at 75 mM RZS and the tissue K content at control levels
- Shoot potassium content is the main driver for salinity tolerance in sweet potato
- We propose a screening tool that distinguishes salt tolerant and salt sensitive genotypes and indicates their potential yield level

3.1 Introduction

Sweet potato (*Ipomoea batatas* L.) is mostly grown in Asia, which accounts for 86% of global production. Sweet potato is often grown on less fertile soils, and in coastal areas where it is threatened by salinity (Shahid et al., 2018). Climate change aggravates this threat through extreme weather events such as storms and floods as well as sea level rise leading to salt intrusion into production systems based in the major delta regions of Asian rivers (Schneider and Asch, 2020). For these reasons, both area and production have been greatly reduced over the last 3 to 4 years. However, the high nutritional as well as the high market value makes sweet potato an important commodity in Bangladesh in addition to rice (Alam et al., 2016; Sorwar et al., 2015). Traditional agronomic management of salinity, such as mulching, raised beds, or rainwater application will only have an effect if accompanied by at least moderately salt-tolerant and high-yielding varieties (BARI Annual Report, 2015; Islam et al., 2013).

Very little is known about genotypic responses to salinity in sweet potato. Phenotypic responses or physiological processes linked to salt tolerance that could be developed into a reliable screening tool to assist breeding have not yet been developed for sweet potato.

Potential mechanisms have been described and shown for other crops, such as exclusion of sodium and/or chlorine at the root level (for wheat - Wu et al., 2018; for mustard – Chakraborty et al., 2016), excretion of salt from roots or leaves (for halophytes - Flowers et al., 2015; for *Aeluropus*, a coastal grass, - Barhoumi et al., 2007), partitioning of unwanted ions in non-transpiring or non-photosynthetically active tissues (for rice -Asch et al., 1997; for *Lotus tenuis* – Teakle et al., 2007), control of transpiration to reduce sodium uptake (in rice – Wimmer and Asch 2005), rapid senescence and renewal of leaves (in cereals - Munns and Tester, 2008) or protection of young parts through relocation of sodium into older parts (in green bean - Yasar et al., 2006), or relocation of potassium into younger parts (in barley - Wolf et al., 1991). For sweet potato, however, few of these mechanisms have been investigated and if, only for a limited number of genotypes (Evoi et al., 2017), or a short period of salt stress in young plantlets (Begum et al., 2015; Cusma-Ticlla et al., 2016). If the genetic basis of salinity

tolerance was studied it was performed *in vitro* where plants were grown only for a short time and no information was provided on yield or physiological traits (Anwar et al., 2010; Yang et al., 2020) and if the yield response to salinity was studied, no physiological or genetic traits were included (Rahaman et al., 2015).

Literature does not agree on at what level salinity becomes detrimental to sweet potato and little is known on the genotypic range of resistance (Begum et al., 2015; Evoi et al., 2017; Rahaman et al., 2015).

To develop a quick and reliable screening tool for salinity resistance in sweet potato, we subjected 12 sweet potato varieties, contrasting in salinity tolerance, to a wide range of salinity levels in a hydroponic cultivation system under greenhouse conditions.

3.2 Materials and methods

An experiment was set up in the greenhouse of the Hans-Ruthenberg-Institute for Tropical Agricultural Sciences, University of Hohenheim, Germany. Twelve sweet potato genotypes, namely CIP 106082.1, CIP 400039, CIP 420001, CIP 194281.2, CIP 188002.1, BARI SP 8, CIP 199062.1, BARI SP 4, CIP 440181, CIP 440004, CIP 189151.8, and BARI SP 12 were obtained from the Bangladesh Agricultural Research Institute and cultivated as mother plants in the green house in 10L pots containing sandy soil. The plants were kept at 20°C and 28°C during night and day, respectively and received 12 hours artificial light with an intensity of about 400 µmol $m^{-2}s^{-1}$ photosynthetic active radiation at canopy level (27-30°C). From the mother plants, the required number of cuttings were cut as 13 cm of the apical section of each vine. The cuttings were transferred to a hydroponic nutrient solution (Yoshida et al., 1976) in 1.2 L polyethylene pots (Gies GmbH & Co., Niederaula, Germany) and grown for 7 days in half-strength nutrient solution followed by full-strength nutrient solution which was renewed every 5 days for the rest of the experiment. Seventeen days after transplanting plants were subjected to 0, 50, 100, and 150 mM root zone salinity (RZS) by adding the appropriate

amount of NaCl to the nutrient solution for 3 weeks. The pH of the nutrient solution was kept between 5.5 and 6.5.

3.2.1 Data collection and harvesting procedure

All plants were harvested 38 days after planting and 21 days after the onset of treatments. The lengths of the main vine and of all side branches were determined. Leaf area (Li-Cor Inc., LI3000C, USA) and SPAD values (SPAD-502 Plus, Konica Minolta, Japan) were measured for all leaves on the vine. Leaves, stems, side branches, and dead leaves were sampled individually. Roots were rinsed with distilled water before packing. All samples were kept in paper bags and dried in an oven at 60°C for 72 hours. The dry weight of the samples was determined with a fine balance (XB 220 A, Precisa). All dry samples were transferred to scintillation vials (Nerbe plus GmbH & Co. KG), six 5 mm, three 3 mm stainless steel balls were added, and samples were ground for 10 min in a ball mill (Retsch).

3.2.2 Determination of sodium, potassium, and chlorine concentrations

Following the extraction method described by Asch et al. (2022), 10 mL of de-ionized water were added to approximately 0.1 g of ground dry material in 15 mL centrifuge vials (Roth GmbH & Co. KG) and shaken until thoroughly homogenized. Samples were heat extracted with an autoclave (SanoClav MMCS) at 120°C for 60 minutes and centrifuged (Allegra X-15®, Beckman Coulter GmbH) for 5 min. From the supernatant 9 mL were collected and made up to 100 mL with de-ionized water. Sodium and potassium were determined by flame photometer (Jenway, PFP 7). Chloride was determined from the same sample with an auto-analyser (Autoanalyser II, Technicon, America) according to Asch et al. (2022) and standard solutions.

3.2.3 Statistical analyses

Means were compared using Duncan's Multiple Range Test (DMRT) at P≤0.05 and ≤0.01, following a 2-factorial ANOVA with salinity and variety as the main factors using R statistical software. Thresholds and slopes were determined by regression analyses with SigmaPlot 14 (Systat Inc.). The threshold was defined as the theoretical salinity level at which the slope reaches the initial dry weight of plants not subjected to salinity. In order to determine genotypic sodium, potassium, and chlorine contents and concentrations at any given salinity level, linear (potassium) and quadratic (sodium, chlorine) regressions were performed with SigmaPlot 14 used (Appendix 3.2). Graphs were plotted with SigmaPlot 14.0.

3.3 Results

3.3.1 Sweet potato responses to increasing levels of salinity

In general, increasing salinity decreased total dry weight, leaf dry weight, leaf number, leaf area, SPAD values, length of the main vine, and length of the side branches branching and increased the number of dead leaves as a function of RZS across all genotypes (Table 3.1). For most traits, the reduction under 50 mM RZS was numerical but not statistically significant ($p < 0.05$) except for total leaf number and leaf senescence, which significantly increased as indicated by the increased number of dead leaves. Salinity levels beyond 50 mM significantly reduced the values for all observed traits. Total dry weight under non-saline conditions was about 7 g per plant and was reduced by 33% (4.7 g) and 60% (2.8 g) under 100 mM and 150 mM RZS, respectively. Leaf dry weight was affected similarly with reductions of 47% and 77% relative to non-saline conditions under 100 mM and 150 mM RZS, respectively. SPAD values, indicating the greenness of the leaves and thus senescence when the values decline, were significantly and severely reduced by 35% under 100 mM and 70% under 150 mM RZS. Salinity effects on main vine length, in contrast, were not as severe but still significant with 24% under 100 mM and 42% under 150 mM RZS (Table 3.1).

Table 3.1 Mean effects of different levels of salinity treatments across 12 sweet potato genotypes on selected morphological and physiological traits.

	Salt treatment (mM)								
Trait	0		50		100		150		LSD
Total dry weight, mg	7058±243	a	6712±250	a	4735±296	b	2784±192	c	637
Leaf dry weight, mg	1934±145	a	1630±193	a	1016±157	b	442±140	c	377
Leaf number	21±1	a	18±2	b	14±2	c	6±2	d	2.4
Leaf area, cm^2	689±30	a	627±68	a	424±65	b	176±56	c	128
SPAD	38±2	a	32±2	a	25±3	b	12±4	c	6
Main vine length, cm	73±6	a	68±7	a	56±6	b	43±4	c	9
Side branch length, cm	11±5	a	10±3	a	7±2	ab	3±1	b	6
Number of dead leaves	1±0.5	a	4±2	b	6±2	cd	8±2	d	3

Note. Values are means ± standard error, n = 12.

LSD, least Significant Difference ($p<0.05$). Data for individual genotypes are provided in Appendix 3.1.

3.3.2 Genotypic salinity thresholds and slopes for total dry weight

Total dry weight was affected differently among the genotypes. Figure 3.1 shows the dry weight dynamics for the 12 genotypes subjected to four levels of salinity.

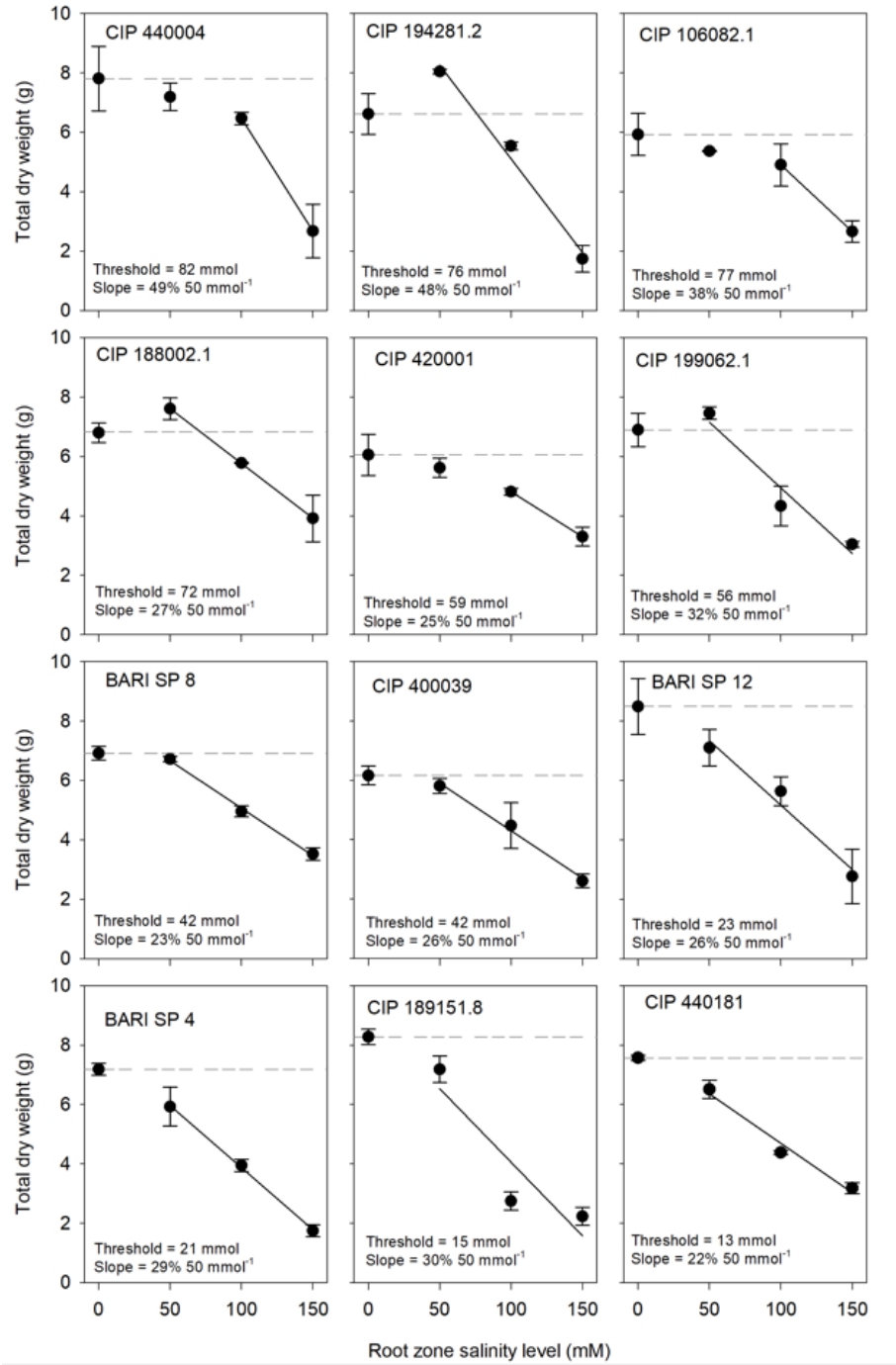

Figure 3.1 Total dry weight of 12 sweet potato varieties subjected to 4 levels of RZS for 21 days. 0 mM = control treatment. Dashed line = control level dry weight. Linear regressions were used to determine the threshold and the slope for salinity-induced dry matter reduction. Error bars = standard error of means, n = 3.

Total dry weight under non-stressed conditions (0 mM) varied among the genotypes between 8.2 and 6.0 g per plant and under the severest stress level (150 mM) between 3.2 and 1.8 g per plant. Regression analyses were used to determine the genotypic salt level at which dry matter accumulation started to decrease as a function of RZS (threshold) and the magnitude of the salinity effect on dry matter accumulation per 50 mM increase in RZS (slope). Thresholds for salinity effects on dry matter accumulation varied strongly between genotypes with CIP 189151.8 and CIP 440181 (15 mM and 13 mM respectively) being most sensitive and CIP 194281.2 and CIP 440004 (76 mM and 82 mM, respectively) being most tolerant. Slopes indicating the percentage dry matter reduction caused by additional 50mM of salinity varied between 22% (CIP 440181) and 49% (CIP 440004).

In order to evaluate whether threshold levels determine slope values, we plotted them against each other (Figure 3.2). Up to about 75 mM thresholds, almost all slopes range roughly between 20 and 30%. With thresholds exceeding 75 mM, slopes increased roughly by factor two.

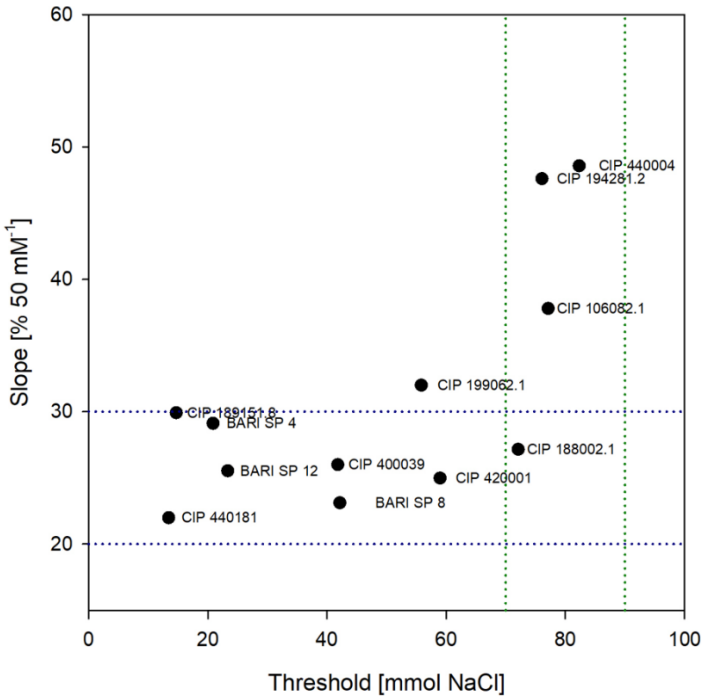

Figure 3.2 Relationship between threshold for salinity induced dry matter reduction and the percent reduction in dry matter per 50 mM increase in salinity beyond the threshold. Dotted horizontal lines include varieties with contrasting thresholds but similar slopes. Dotted vertical lines include varieties with a high threshold (beyond 75 mM) and contrasting slopes.

Thus, the threshold for salinity effects on dry matter slopes was identified for this set of genotypes at about 75 mM RZS.

3.3.3 Potassium, Sodium and Chlorine uptake

For all 12 genotypes and treatments potassium (K), sodium (Na) and chlorine (Cl) contents of the above ground dry matter were determined (Figure 3.3).

K content under non-stressed conditions varied among the varieties roughly between 250 mg and 350 mg per plant and decreased in all varieties linearly with increasing RZS to levels between 80 mg (CIP 194281.2) and 180 mg (CIP 180002.1). Na and Cl showed very similar uptake patterns and their accumulation with increasing RZS followed a quadratic function for all varieties (all regression equations are listed in Appendix 3.2). Tissue content of Cl (mg) was always higher than or equal to tissue Na content (mg). Maximum tissue contents for Cl and Na were always found at the same RZS levels. For example, the highest Cl and Na contents were observed in CIP 194281.2 with 350 mg Cl and 200 mg Na and in BARI SP 12 with 390 mg Cl and 280 mg Na, respectively, at RZS levels of 100 mM, whereas in CIP 189151.8 maximum values were observed at 50 mM RZS with 340 mg Cl and 240 mg Na. There was no significant relationship between Cl or Na content and the observed threshold levels. On a molar basis the ratio between Na and Cl was 1 to 1.2 for most genotypes (data not shown). Due to the dry matter kinetics under increasing RZS (Figure 3.1) and the uptake kinetics for K, Na and Cl under increasing RZS (Figure 3.3), K concentrations in the plants stayed constant although total uptake was reduced and Cl and Na concentrations increased, while uptake was constant or reduced with increasing RZS. Genotype specific tissue concentrations are given in Appendix 3.3.

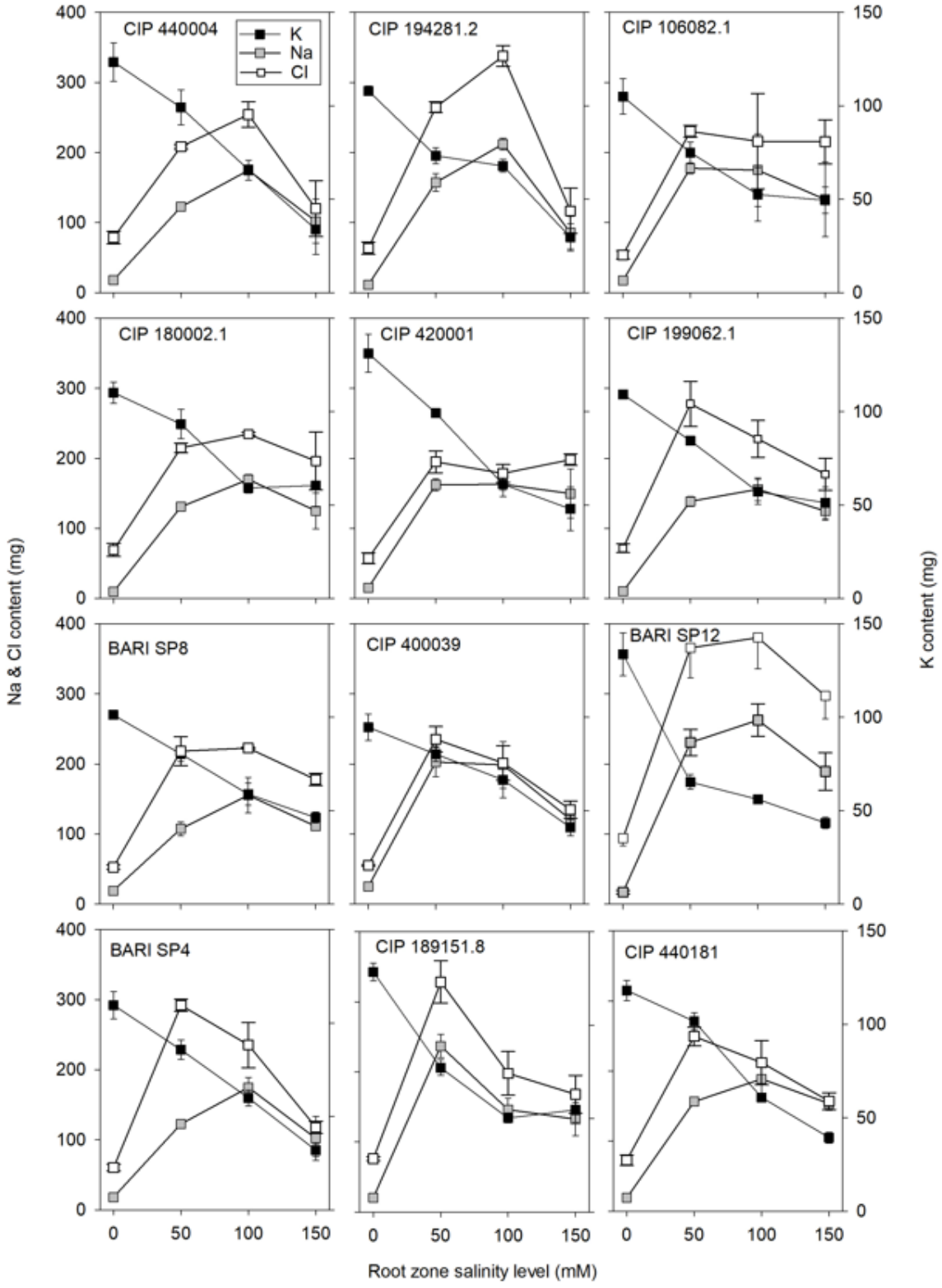

Figure 3.3 Total sodium (Na), potassium (K), and chlorine (Cl) content in the dry matter of 12 sweet potato varieties subjected to 4 different levels of RZS. Error bars = standard error of means, n = 3.

3.3.4 Threshold and ion relations

From first order regressions of the K content data in Figure 3.3 and second order regressions of the Na and Cl content data in Figure 3.3 (see Appendix 3.2 for details), molar concentrations of the ions were calculated for RZS levels and K/Na ratios at the genotypic thresholds were derived. Figure 3.4 shows a negatively correlated relationship between the genotypic tissue molar K/Na ratio at the threshold and the genotypic threshold. This indicates a functional relationship between salinity tolerance and tissue K and Na contents which is revealed when the threshold is known.

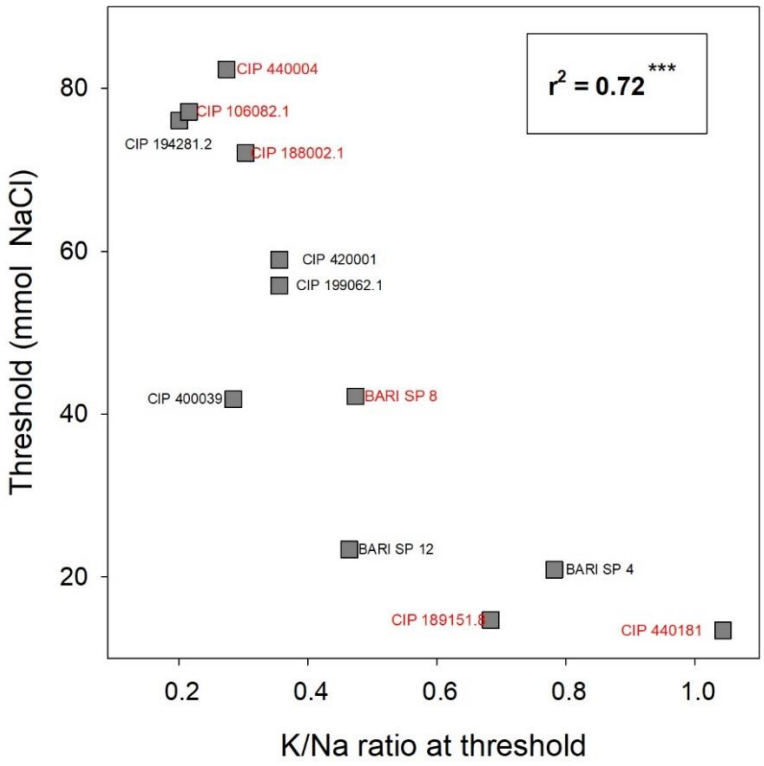

Figure 3.4 Relationship between the threshold for dry matter reduction and the K/Na ratio of the above ground tissue at threshold salinity levels. K/Na ratios were calculated from regressions of the data shown in Figure 3.3 after conversion into mM. Regressions are given in Appendix 3.2.

When from the same regressions the K/Na ratio at a given RZS was calculated (we chose here the apparent limit for flat slopes at 75 mM (Figure 3.2)) no significant correlation between the K/Na ratio and the threshold was found (Figure 3.5a). From the first order regressions for the K content at increasing RZS, the K content at 75 mM RZS was calculated and was

subtracted from the K content measured for non-salt stressed plants. The difference was plotted against the genotypic threshold in Figure 3.5b and revealed a highly significant ($p < 0.001$) linear relationship between the genotypic threshold and the difference in potassium content which is the same as the slope from the linear regression of the potassium content shown in Appendix 3.2.

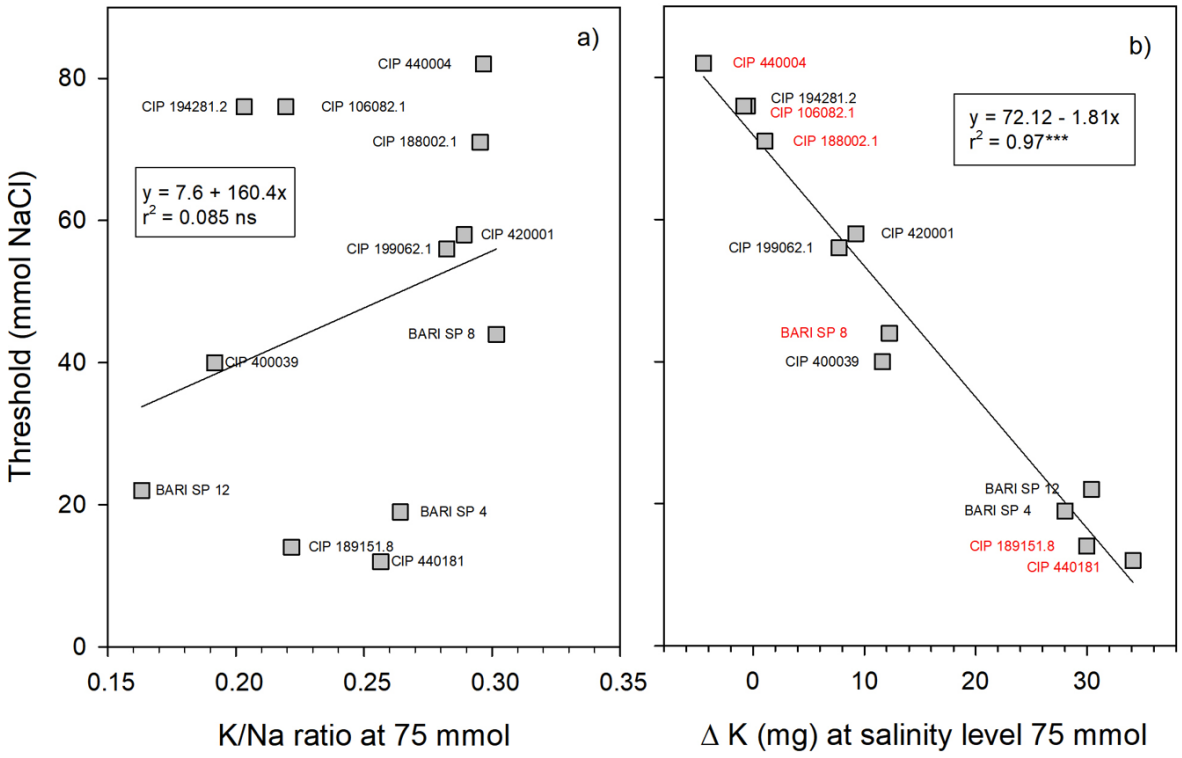

Figure 3.5 (a) Threshold for dry matter reduction as correlated to the tissue K/Na ratio at 75 mM RZS. K/Na ratios were calculated from regressions given in Appendix 3.2 after conversion to molar concentrations. (b) Threshold for dry matter reduction as correlated to the difference (Δ) in tissue potassium content between control and 75 mM RZS. Δ were calculated from regressions given in Appendix 3.2.

3.4 Discussion

3.4.1 Salinity effects on sweet potato

At present, literature on salinity tolerance traits in or salinity effects on sweet potato genotypes is extremely limited. The main focus in literature at the moment is on genomic, transcriptomic, and proteomic studies in a limited number of sweet potato (gene-modified) genotypes (e.g. Jin et al., 2017; Zhang et al., 2022; Zhu et al., 2021). Martin and Carmer (1985) compared a large number of sweet potato genotypes in their response to a multitude of abiotic and biotic stresses and found salinity being the severest stressor and the most unique one with hardly any cross tolerances to other stresses. Across the genotypes in this study, increasing levels of RZS affected all traits negatively i.e. total dry weight, leaf dry weight, leaf number, leaf area, SPAD values, length of the main vine, and length of the side branches branching and increased the number of dead leaves (Table 3.1). These results confirm findings from O'Sullivian et al. (1997) who reported severe growth reductions at RZS levels of 2.9, 5.6, 10.7, or 20.4 dSm^{-1} (which corresponds roughly to 30, 60, 100 and 200 mM NaCl in the root zone solution) resulting in root necrosis, shedding of leaves, and, eventually, death of the plant. In the present study, plants exposed to 150 mM NaCl in the root zone for 3 weeks, suffered strongly but did not die.

3.4.2 Genotypic salinity thresholds and slopes

Commonly, salinity tolerance in a crop is expressed via the crops threshold to salinity induced yield loss and the decrease in yield per unit salt level increase thereafter (Tanji and Kielen, 2002). A high threshold in combination with a flat slope indicates a high salinity tolerance. In sweet potato, to date, little information has been published on this relationship. Summary information, regardless of genotypes, classifies sweet potato as medium sensitive crop with a threshold of about 15 mM NaCl in the root zone ($\approx$ 1.5 dSm^{-1}) and a slope of 11% per unit EC

or 50% yield loss at 60 mM RZS (≈ 6 dSm^{-1}) (Jan et al., 2000; Tanji and Kielen, 2002). Begum et al. (2015) tested 10 genotypes for rooting ability under four levels of nutrient solution salinity. Although they found strong genotypic variation in the effect of salinity on root growth under saline conditions, they derived a general threshold of 80 mM of RZS for sweet potato based on the total plant K/Na ratio after 2 weeks of growth under saline conditions. In contrast, Evoi et al. (2017) derived 200 mM RZS as the critical concentration to distinguish genotypic effects of salinity on vine length and dry matter accumulation. The major difference between the two studies was the rooting medium. Whereas Begum et al. (2015) used Hoagland solution, a highly concentrated nutrient solution with an initial EC of 1.8 dSm^{-1}, Evoi et al. (2017) used a soil mixed with NaCl to reach soil solution concentrations of 200 mM and 600 mM. At a moisture content of 24% most of the rooting medium is not saline and, therefore, the actual stress level at the root surface is much lower.

In the present study, we used a Yoshida nutrient solution (Yoshida et al., 1976), with an initial EC of 0.8 dSm^{-1}, added NaCl to reach the desired salinity levels. In this system, the entire root system is exposed to the same level of salinity. We found strong genotypic variation in thresholds (13 mM in CIP 440181 to 82 mM in CIP 440004 – Figure 3.1) based on RZS effects on dry matter accumulation and a much less pronounced variation in genotypic slopes which ranged between 22% and 32% per 50 mM increase in RZS for thresholds lower than 75 mM. Beyond a 75 mM threshold, slopes increased rapidly to 50% (Figure 3.2).

3.4.3 Chlorine, Sodium, and Potassium - Threshold and ion relations

High tissue concentrations of potassium are generally accepted as beneficial under salt stress, whereas restricting the uptake of sodium and chlorine has been shown to increase resistance to salinity in many crop plants (Munns and Tester, 2008). Teakle and Tyerman (2010) argue in their review that genotypic salt tolerance may be related to the ability to regulate Cl and Na uptake independently to avoid potential toxicity of either element, the severeness of which depends on their respective concentrations in the cytoplasm. Under 100 mM and 150 mM

RZS, Cl tissue concentrations of 48 mgg^{-1} to 80 mgg^{-1} were found (Appendix 3.3). Xu et al. (2000) defined the toxicity threshold of Cl for plant tissue at concentrations between 15 mgg^{-1} and 50 mgg^{-1} for Cl tolerant species and placed the tolerance level of sweet potato to Cl at the lower limit of this range. However, as shown in Figure 3.3, the actual uptake of Cl to the shoots of the different genotypes decreased with increasing RZS while the biomass accumulation also decreased leading to increased tissue concentrations. Therefore, reaching toxic Cl concentrations in the tissue, may not have been the cause for the decrease in shoot dry weight, but rather its consequence.

In turn, metabolic toxicity of Na results from its competition with K in protein synthesis which requires high concentrations of K. Disrupting protein synthesis could therefore be the main driver behind Na toxicity in plant tissues (Tester and Davenport, 2003). Na and Cl uptake (Figure 3.3) and tissue concentrations for the two elements (Appendix 3.3) were highly positively correlated across all genotypes tested. Thus, it can be assumed that both elements contributed equally to the damage inflicted by salinity and that neither the regulation of Na influx nor of Cl uptake was related to the genotypic variation in salinity resistance.

Whereas tolerance has been defined via high tissue tolerance to sodium combined with a stay green trait in tetraploid wheat (Munns and James, 2003), Asch et al. (2000) showed that the discrimination against sodium and the maintenance of a high K/Na ratio reduced salinity induced yield loss in rice. Sweet potato is a K-favoring crop which is reflected in its nutrient demand ratio of N:P:K of about 2:1:4 (Wang et al., 2015). It has been shown that potassium management strongly affects tuber yield in sweet potato and 4% to 6% K in leaf tissues to be the critical level for high yields (Lv and Lu, 2021) in a hydroponic system with regular changes of nutrient solution, plants have a luxury supply of nutrients available and a direct K deficiency cannot occur. However, in competition with Na (and maybe Cl) in the root zone, relative K availability may have been reduced, and thus, the total uptake of K to the shoot decreased with increasing RZS (Figure 3.3). Tang et al. (2015) showed that K deficiency disrupted leaf chlorophyll biosynthesis and photosynthate accumulation, and also disturbed protective

enzymes involved in the antioxidative defense system. Since K uptake under increasing RZS was strongly reduced, it is possible that an indirect leaf level potassium deficiency was responsible for a large share of the damage inflicted by RZS. Increasing RZS resulted in decreased K uptake while at least up to 100 mM RZS Na uptake remained high. This indicates that a high tissue ratio of K (the beneficial element) over Na (the unwanted element) indicates genotypic resistance to RZS as proposed by Begum et al. (2015). In contrast, when relating the genotypic threshold to dry matter accumulation under RZS to the tissue K/Na ratio (Figure 3.4), we found a negative correlation and thus the opposite of what would have been expected according to Asch et al. (2000) and Begum et al. (2015). Similarly, when we calculated the K/Na ratio at the threshold level for slope steepness (75 mM, Figure 3.2), we found no correlation with the genotypic threshold (Figure 3.5a). We also calculated the K/Cl ratio and the K/Na+Cl ratio and found similar results (data not shown). This indicates that the genotypic tolerance levels to RZS is not determined by the relationship between K and any other potentially toxic element. Using the regressions given in Appendix 3.2, we calculated tissue K content for plant not subjected to RZS and for a plant theoretically subjected to 75 mM RZS and plotted this difference against the genotypic threshold for dry matter accumulation under RZS. We found a highly significant ($p<0.01$) negative linear correlation between the two parameters, showing that the genotypic threshold levels depend on the ability of the plant to maintain high potassium levels in the above-ground shoot tissue.

3.4.4 Screening for salinity tolerance in sweet potato

The main challenge of any breeding program is a reliable screening technique. We tried to develop a method to reliably select potentially salt tolerant sweet potato genotypes with a potential high yield. Chowdhury et al. (2002) reported that dry mater accumulation and final fresh tuber yield are strongly correlated. Mbah and Eke-Okoro (2015) showed across 7 contrasting genotypes highly significant correlations ($p<0.01$) between final tuber yield and dry matter accumulation, which were strongest at 4 weeks after planting. Since, in the present

study, salinity linearly decreased dry matter accumulation from a genotype specific threshold according to a genotype specific slope (Figure 3.1), it can be assumed that tuber yield would have been reduced in a similar manner. Equally, a strong correlation between tissue K content and sweet potato yield was established in several reports (e.g. Lv and Lu, 2021 and Wang et al., 2015). Chen et al. (2005) developed a screening tool for salt tolerance in wheat based the ability of genotypes to retain K under saline conditions, making the beneficial element K the focal point for salinity tolerance and not the toxic elements Na or Cl. We followed a similar way by determining tissue K content at critical RZS levels and comparing it to the tissue K content at non-saline levels. Two aspects, namely the absolute K content under non-saline conditions and the relative reduction of K content at the critical RZS, allow distinguishing high yielding from low yielding genotypes and at the same time salt tolerant from salt sensitive varieties. A high tissue K content under non-saline levels indicates high yield and a small difference in K content at the critical RZS, here defined as 75 mM NaCl, indicates salt tolerance. Therefore, the system we propose for quickly screening large numbers of genotypes consists of a hydroponic system using Yoshida nutrient solution (Yoshida et al.,1976), vine cuttings of three nodes (one in the solution, two above the solution), non-saline growth for two weeks and exposure of half of the plants to 75 mM NaCl in the root zone for three weeks, sampling of the above ground biomass for all plants and analyze the tissue K content according to Asch et al. (2022).

3.5 Conclusion

In this study, we have shown that a large variation in genotypic salt tolerance exists in sweet potato. By subjecting 12 contrasting genotypes to increasing levels of RZS in a hydroponic system, we were able to determine genotypic thresholds for dry matter accumulation and the genotypic slopes for additional dry matter reduction when the RZS increased beyond the genotypic threshold.

We showed that the genotypic threshold is strongly related to the ability to retain high tissue potassium levels under increasing salinity. We propose a screening tool based on these experimental data allowing to distinguish between salt tolerant and salt sensitive genotypes and indicating the potential yield level of the sweet potato genotypes.

More research is needed on the potassium level in the nutrient solution to see if an increase in root zone potassium would produce even more pronounced results even faster. In addition, validating the results of our screening tool in multi-location field trials on salinity prone soil under different potassium management needs to be the next step.

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Data Avaiability Statement

Aditional data will made available by the author upon reasonable request.

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Appendix 3.1 Mean effects of different levels of root zone salinity on selected morphological and physiological traits of 12 sweet potato clones. Values are means ± standard error, n = 3.

VAR	RZS (mM)	TDW (mg)	LDW (mg)	LNo	LA cm^2	SPAD	MVL (cm)	SBL (cm)	DLNo
1	0	6897±563 b	2474±363a	22±1 a	726±50 ab	47±1 a	58±3 a	8±3 a	0±0 b
1	50	7459±210 a	2703±208a	22±1 a	825±66 a	44±1 ab	75±7 a	9±3 a	0±0 b
1	100	4336±667 c	1577±189b	21±2 a	627±48 b	35±6 bc	70±15 a	2±2 a	0±0 b
1	150	3047±98 d	864±106b	11±1 b	302±59 c	30±3 c	53±7 a	3±1 a	3±1 a
	LSD	*183*	*769*	*4*	*183*	*12*	*30*	*8*	*2*
2	0	6620±689 b	1962±533a	18±2 a	631±74 a	45±2 a	103±16 a	16±10 a	1±0.3b
2	50	8046±76 a	2144±126a	19±1 a	696±52 a	43±2 ab	104±7 a	3±1 a	0±0 b
2	100	5546±131 b	1043±49 b	13±1 b	451±47 b	31±6 b	71±4 ab	17±2 a	4±1 a
2	150	1744±448 c	272±89 b	4±1 c	98±31 c	16±3 c	45±13 b	4±2 a	5±1 a
	LSD	*1362*	*909*	*4*	*173*	*12*	*35*	*17*	*3*
3	0	8276±263 a	3193±144a	25±3 a	824±71 a	45±1 a	96±2 a	3±1 ab	1±0 c
3	50	7180±446 a	1558±412b	14±4 b	767±139a	31±1 b	74±10 a	15±8 a	8±3 b
3	100	2748±306 b	0±0 c	0±0 c	0±0 b	0±0 c	48±7 b	6±4 ab	17±2 a
3	150	2231±304 b	0±0 c	0±0 c	0±0 b	0±0 c	37±6 b	1±1 b	15±1 a
	LSD	*1099*	*712*	*8*	*253*	*3*	*22*	*14*	*6*
4	0	6800±326 ab	2028±95 b	24±3 ab	710±41 b	39±0 a	72±14 ab	7±7 a	1±1 b
4	50	7610±367 a	2403±111a	27±1 a	829±23 a	39±1 a	83±2 a	1±1 a	0±0 b
4	100	5782±21 b	1867±41 b	25±1 a	731±21 b	33±2 a	71±1 ab	2±1 a	0±0 b
4	150	3917±785 c	1392±51 c	13 6 b	580±32 c	22±11 a	50±8 b	1±1 a	3±0 a
	LSD	*1511*	*262*	*12*	*98*	*19*	*27*	*12*	*1*
5	0	5930±708 a	1640±270a	19±2 a	772±125a	33±5 a	86±6 a	1±1 b	2±1 b
5	50	5370±22 a	1373±135ab	15±1 ab	676±86 ab	28±1 a	87±6 a	2±2 b	3±1 b
5	100	3739±1237ab	795±400bc	9±5 b	352±179bc	18±9 a	59±22 a	3±2 ab	6±2 ab
5	150	2664±363 b	0±0 c	0±0 c	0±0 c	0±0 b	57±9 a	7±0 a	13±4 a
	LSD	*2399*	*817*	*8*	*382*	*17*	*41*	*5*	*8*
6	0	7812±1084a	1685±454a	25±4 a	804±109a	30±3 a	85±9 a	37±16 a	1±1 a
6	50	7195±464 a	1957±50 ab	25±2 a	899±47 a	34±1 a	92±13 a	7±3 ab	0±0 a
6	100	6472±210 a	1687±160ab	23±2 ab	759±59 a	37±2 ab	72±10 ab	15±8 b	0±0 a
6	150	2677±905 b	805±335b	13±5 b	280±128b	21±7 b	40±8 b	6±1 b	1±1 a
	LSD	*2448*	*960*	*11*	*300*	*13*	*33*	*30*	*3*
7	0	6056±692 a	1233±317a	18±4 a	522±78 a	33±5 a	84±7 a	4±4 b	5±2 a
7	50	5620±321 a	1022±277a	15±4 a	456±97 ab	34±5 a	65±4 b	26±10 a	5±2 a
7	100	4814±119 a	953±285a	16±5 a	422±62 ab	29±5 a	65±4 b	18±3 ab	7±3 a
7	150	3302±320 b	539±271a	9±3 a	251±39 b	19±11 a	60±5 b	9±2 ab	9±4 a
	LSD	*1362*	*939*	*14*	*235*	*22*	*17*	*18*	*9*
8	0	8491±948 a	1631±227a	22±0 a	833±34 a	41±1 a	61±4 a	15±2 a	0±0 b
8	50	7103±613 ab	1789±111a	19±1 a	814±43 a	29±2 b	44±2 ab	11±3 ab	1±1 b
8	100	5632±489 b	1158±22 b	11±1 b	399±41 b	28±2 b	49±7 ab	6±3 bc	8±1 a
8	150	2770±918 c	125±125c	3±3 c	0±0 c	3±3 c	36±12 b	2±2 c	11±3 a
	LSD	*2503*	*461*	*5*	*111*	*7*	*24*	*8*	*5*
9	0	7180±203 a	1737±342a	21±4 a	553±154a	35±4 a	57±17 a	33±17 a	0±0 c
9	50	5925±653 b	1824±233a	17±8 a	535±152a	31±9 a	50±18 a	6±3 ab	3±1 b
9	100	3947±208 c	855±183b	14±3 ab	339±77 ab	31±5 a	34±7 a	7±4 ab	4±1 b
9	150	1744±200 d	0±0 c	0±0 b	0±0 b	0±0 b	25±2 a	1±1 b	13±1 a
	LSD	*1211*	*738*	*15*	*375*	*18*	*42*	*29*	*3*
10	0	6913±230 a	1746±142a	21±1 a	637±85 a	38±1 a	73±8 a	1±1 b	0±0 a
10	50	6718±87 a	1654±58 a	18±5 a	613±104a	30±5 a	57±17 a	14±4 a	1±1 a
10	100	4952±184 b	1277±65 b	21±1 a	582±20 a	35±1 a	72±1 a	0±0 b	3±1 a
10	150	3523±212 c	1074±60 b	15±4 a	420±30 a	31±6 a	50±3 a	0±0 b	3±2 a
	LSD	*609*	*288*	*10*	*228*	*13*	*31*	*6*	*4*
11	0	7569±88 a	2147±46 a	19±1 a	608±87 a	44±3 a	56±4 a	5±2 bc	0±0 b
11	50	6507±309 b	467±96 b	7±1 b	226±60 b	24±7 b	43±8 ab	22±4 a	16±2 a
11	100	4381±65 c	234±294b	9±5 b	197±20 b	12±6 b	35±0 b	12±3 b	10±4 a
11	150	3180±191 d	511±117b	5±3 b	181±90 b	8±5 b	33±1 b	3±2 c	12±1 a
	LSD	*618*	*545*	*9*	*230*	*18*	*15*	*9*	*7*
12	0	6156±318 a	1733±265a	16±1 a	650±28 a	26±4 a	43±3 a	4±1 a	0±0 b
12	50	5805±250 ab	668±122b	11±1 ab	185±55 b	13±2 a	44±2 a	2±1 ab	13±1 a
12	100	4471±768 b	463±232bc	7±3 b	225±16 b	13±7 a	33±2 b	1±1 ab	11±1 a
12	150	2604±230 c	0±0 c	0±0 c	0±0 c	0±0 b	26±1 c	0±0 b	11±1 a
	LSD	*1465*	*608*	*6*	*104*	*13*	*7*	*3*	*3*

Note: Values are mean± standard error, n = 3.

VAR = Variety: 1=CIP 199062.1, 2= CIP 194282.1, 3= CIP 189151.8, 4=CIP 188002.1, 5=106082.1, 6= CIP 440004, 7= CIP 420001, 8= BARI SP 12, 9= BARI SP 4, 10= BARI SP 8, 11= CIP 440181, 12=CIP 400039. TDW = Total dry weight; LDW = Leaf dry weight; LNo = Number of leaves on the main vine; LA = leaf area; SPAD = greenness of the leaf; MVL = length of the main vine; SBL = length of the side branches; DLNo = Number of dead leaves. LSD = least significant difference at $p<0.05$.

Appendix 3.2 First order (K) and second order (Na, Cl) regressions for ion accumulation in above ground tissue under increasing RZS.

Variety	Ion	a	b	c	r^2
CIP 199062.1	K	105.69	-0.403		0.848
	Na	13.44	3.114	-0.016	0.916
	Cl	84.49	4.380	-0.026	0.718
CIP 194281.2	K	105.80	-0.481		0.886
	Na	6.92	4.642	-0.027	0.920
	Cl	55.34	6.795	-0.042	0.919
CIP 189151.8	K	114.86	-0.497		0.764
	Na	39.52	3.943	-0.023	0.630
	Cl	100.18	4.520	-0.028	0.554
CIP 188002.1	K	108.23	-0.366		0.709
	Na	9.62	3.263	-0.017	0.910
	Cl	72.65	3.563	-0.018	0.808
CIP 106082.1	K	98.86	-0.378		0.654
	Na	23.53	3.720	-0.020	0.575
	Cl	64.20	3.594	-0.018	0.582
CIP 440004	K	125.92	-0.605		0.857
	Na	14.62	3.261	-0.018	0.824
	Cl	73.96	4.297	-0.026	0.807
CIP 420001	K	128.12	-0.576		0.853
	Na	22.09	3.209	-0.016	0.798
	Cl	66.91	2.581	-0.012	0.803
BARI SP 12	K	117.59	-0.588		0.746
	Na	20.82	5.419	-0.029	0.933
	Cl	102.12	6.606	-0.036	0.838
BARI SP 4	K	110.57	-0.517		0.937
	Na	14.62	3.261	-0.018	0.824
	Cl	71.66	5.472	-0.035	0.861
BARI SP 8	K	99.66	-0.373		0.921
	Na	16.50	2.639	-0.013	0.854
	Cl	58.22	3.919	-0.021	0.917
CIP 440181	K	121.56	-0.554		0.946
	Na	20.86	3.462	-0.017	0.934
	Cl	82.73	3.898	-0.023	0.770
CIP 400039	K	96.84	-0.350		0.792
	Na	30.43	4.400	-0.026	0.835
	Cl	73.96	4.297	-0.026	0.819

Note: Regressions are based on the data presented in Figure 3.3.

Function used to regress tissue K content against RZS: $y = a + bx$. Function used to regress root Na and Cl tissue content against RZS: $y = a + bx + cx^2$.

Appendix 3.3 Organ specific and total plant ion concentrations (mgg^{-1}) for 12 sweet potato clones subjected to 3 levels of RZS.

V	T	Root			Stem			Leaf			Total plant		
		Na	K	Cl	Na	K	Cl	Na	K	Cl	Na	K	Cl
1	0	4±0 c	17±1 b	20±1 b	1±0 c	12±0 a	11±0 c	1±0 c	14±1 a	6±1 d	1± 0 b	16±1 a	10±0 c
1	50	22±1 b	17±1 b	55±20 a	20±1 b	7±1 b	31±3 b	10±1 c	10±1 b	25±1 c	19± 0 a	11±0 b	37±3 a
1	100	36±1 a	23±2 ab	52±2 ab	35±5 a	9±1 b	49±5 a	32±4 b	12±1 ab	56±5 b	37± 4 a	13±1 c	54±4 ab
1	150	37±3 a	34±8 a	50±2 ab	34±2 a	8±1 b	41±5 ab	45±4 a	14±1 a	73±1 a	41± 3 a	17±2 c	58±6 b
	LSD	*5*	*14*	*33*	*9*	*2*	*13*	*9*	*3*	*8*	*8*	*4*	*13*
2	0	7±1 d	19±4 a	20±1 c	1±0 d	13±0 a	11±1 d	1±0 d	17±2 ab	6±1 d	2± 0 a	17±2 a	10±0 c
2	50	28±1 c	13±1 a	41±2 b	13±1 c	6±0 c	23±2 c	19±2 c	7±1 c	35±3 c	20± 1 b	9±1 b	33±1 b
2	100	43±3 b	16±0 a	57±4 a	27±2 b	9±1 b	38±3 b	44±3 b	14±2 bc	80±5 b	38± 2 c	12±0 b	61±4 a
2	150	52 2 a	22 5 a	51 2 a	36 1 a	9 1 b	50 3 a	63 3 a	21 2 a	105 1 a	49 1 d	18 2 c	66 2 c
	LSD	*6*	*11*	*8*	*4*	*2*	*8*	*7*	*6*	*10*	*4*	*4*	*7*
3	0	7±1 c	21±2 a	12±1 b	1±0 d	13±0 b	10±1 b	2±0 b	12±0 a	7±0 b	2± 0 c	16±1 a	9±1 c
3	50	28±1 b	15±2 b	38±5 a	16±1 c	9±1 b	23±2 b	42±5 a	6±1 b	64±6 a	33± 2 a	11±1 b	46±4 a
3	100	45±1 a	17±2 ab	44±2 a	47±2 b	12±1 b	58±4 a	0±0	0±0	0±0	53± 1 b	19±2 c	71±4 b
3	150	49±2 a	18±1 ab	47±3 a	55±3 a	23±4 a	71±7 a	0±0	0±0	0±0	59± 4 b	25±1 c	75±6 b
	LSD	*5*	*5*	*10*	*6*	*7*	*14*	*14*	*3*	*18*	*9*	*4*	*13*
4	0	7±0 c	14±1 a	22±0 b	0.3±0 d	12±0 a	13±0 c	1±0 c	18±1 a	6±1 c	1± 0 c	16±1 a	10±1 b
4	50	22±1 b	19±10 a	40±3 ab	12±1 c	8±0 a	26±2 bc	16±1 b	9±0 b	22±3 b	17± 1 ab	12±1 a	28±1 a
4	100	31±1 a	10±1 a	48±1 a	20±0 b	7±0 a	35±1 b	27±3 a	8±1 b	38±3 a	29± 1 a	10±0 b	41±0 a
4	150	28±3 ab	19±3 a	55±11 a	26±2 a	12±3 a	60±8 a	32±3 a	10±1 b	42±5 a	32± 1 b	16±0 b	50±2 a
	LSD	6	17	19	4	5	14	7	3	11	3	2	4
5	0	11±1 c	22±0 ab	13±1 c	0.3±0 c	12±0 a	10±0 c	3±0 c	24±1 a	6±0 c	3± 0 b	18±1 a	9±0 b
5	50	31±2 b	21±2 b	39±3 b	16±4 b	12±2 a	24±5 bc	49±5 b	9±0 b	62±4 b	33± 1 a	14±1 ab	43±1 a
5	100	36±1 b	27±3 ab	40±4 b	27±6 b	15±4 a	30±9 b	63±3 a	9±0 b	83±5 c	50± 6 a	15±1 b	61±6 a
5	150	46±4 a	28±3 a	53±1 a	43±5 a	19±1 a	52±2 a	0±0	0±0	0±0	47±15 ab	19±2 b	81±2 a
	LSD	*8*	*7*	*9*	*15*	*8*	*17*	*12*	*2*	*13*	*27*	*4*	*10*
6	0	9±0 d	14±3 a	19±0 d	1±0 c	10±1 a	12±0 c	1±0 c	23±1 a	6±0 b	2± 0 c	16±1 a	10±1 b
6	50	26±1 c	12±3 a	39±3 c	17±2 b	6±0 b	24±2 bc	11±0 bc	20±1 ab	29±2 ab	17± 1 ab	14±1 a	29±2 a
6	100	33±0 b	8±0 a	47±16 b	28±0 ab	5±0 b	35±1 b	24±2 b	15±1 c	45±3 a	27± 1 a	10±0 b	38±1 a
6	150	48±2 a	20±6 a	54±3 a	38±7 a	6±0 b	51±8 a	51±10 a	16±2 bc	42±16 a	40± 3 b	12±2 c	48±7 b
	LSD	*3*	*12*	*7*	*12*	*2*	*14*	*17*	*5*	*27*	*5*	*4*	*12*
7	0	8±2 d	22±2 a	12±2 c	1±0 d	16±1 a	8±0 c	3±0 d	23±1 a	7±0 c	3± 0 b	22±1 a	9±0 b
7	50	29±2 c	20±1 a	37±1 b	16±0 c	9±1 bc	18±0 b	35±1 c	8±1 b	44±2 b	29± 0 a	18±1 b	35±1 a
7	100	35±0 b	18±1 a	43±1 b	23±1 b	8±0 c	27±4 b	43±1 b	11±1 b	42±4 b	34± 1 a	13±1 c	37±3 a
7	150	51±1 a	17±4 a	56±5 a	41±3 a	12±2 b	44±4 a	59±0 a	11±4 b	73±3 a	44± 8 a	14±3 c	61±4 a
	LSD	*5*	*8*	*8*	*6*	*6*	*10*	*4*	*4*	*8*	*15*	*5*	*8*
8	0	7±0 d	20±4 ab	24±3 d	0.35±0 d	9±1 ab	11±0 c	2±0 c	18±2 a	7±0 b	2± 0 c	16±1 a	11±0 b
8	50	26±0 c	15±1 b	45±1 c	14±1 c	6±1 b	32±2 b	55±2 b	5±1 b	76±5 a	33± 1 ab	9±1 b	51±2 a
8	100	37±1 b	22±1 ab	58±1 b	26±1 b	6±0 b	50±2 a	78±4 a	6±1 b	77±5 a	47± 0 a	10±1 bc	67±2 a
8	150	40±1 a	25±1 a	68±5 a	40±6 a	12±3 a	66±10 a	0±0	0±0	0±0	51± 3 b	14±3 c	77±5 ab
	LSD	*2*	*7*	*10*	*10*		*17*	*9*	*4*	*14*	*5*	*5*	*9*
9	0	11±0 c	17±0 a	17±1 a	0.48±0 b	10±0 a	5±1 c	2±0 c	20±1 a	6±0 c	3± 0 c	15±1 a	8±0 c
9	50	42±1 b	15±2 a	15±2 a	15±5 b	13±5 a	21±1 b	24±3 b	14±0 a	64±3 b	24± 1 a	15±2 b	50±4 a
9	100	49±3 a	14±1 a	14±2 a	16±1 b	10±1 a	30±1 b	35±3 a	17±4 a	77±2 a	35± 2 a	15±0 c	59±5 a
9	150	55±2 a	8±2 b	8±4 b	53±9 a	12±1 a	72±10 a	0±0	0±0	0±0	51± 6 b	19±2 d	69±9 b
	LSD	*6*	*5*	*5*	*17*	*9*	*16*	*9*	*8*	*7*	*10*	*5*	*18*
10	0	12±2 d	6±0 c	21±5 b	1±0 d	8±0 a	6±0 c	2±0 c	28±0 a	6±0 c	3± 0 c	15±1 a	8±0 c
10	50	29±0 c	10±1 b	32±4 b	10±1 c	6±0 b	14±1 b	16±2 b	17±1 b	51±3 b	16± 1 b	12±0 b	32±3 a
10	100	42±2 b	13±0 a	51±3 a	18±1 b	6±0 b	26±1 a	36±2 a	18±1 b	68±1 a	31± 4 a	12±1 c	45±1 a
10	150	49±2 a	9±1 b	57±1 a	21±1 a	7±0 b	29±2 a	35±1 a	19±1 b	67±0 a	32± 1 ab	13±0 c	51±2 b
	LSD	*5*	*2*	*11*	*3*	*1*	*4*	*4*	*3*	*5*	*7*	*2*	*6*
11	0	10±2 c	32±3 a	19±0 b	1±0 b	10±1 a	4±0 c	1±0 c	16±1 ab	13±3 c	3± 0 b	16±1 a	10±1 c
11	50	31±2 b	34±2 a	40±7 ab	9±0 b	7±0 b	16±0 bc	45±4 b	10±3 b	61±11 a	24± 2 a	16±1 b	38±2 a
11	100	43±3 a	25±0 b	63±4 a	20±6 a	8±1 ab	31±9 ab	62±2 a	12±5 b	33±17 ab	43± 5 a	14±0 c	48±7 ab
11	150	49±2 a	14±2 c	49±17 ab	24±1 a	8±0 b	37±2 a	58±6 a	21±7 a	22±12 b	48± 1 a	12±1 d	50±4 b
	LSD	*7*	*7*	*31*	*10*	*2*	*16*	*12*	*6*	*19*	*9*	*3*	*14*
12	0	13±1 b	19±1 ab	20±2 b	1±0 d	12±1 ab	3.5±0 c	2±0 b	17±1 a	7.4±0 b	4± 0 c	15±0 a	9±1 c
12	50	40±4 a	24±2 a	44±2 a	13±1 c	10±0 b	27±2 b	42±5 a	9±1 b	68±6 a	35± 2 a	14±1 ab	40±1 a
12	100	41±1 a	25±5 a	46±5 a	25±6 b	14±3 ab	43±9 ab	51±0 a	8±0 b	80±3 a	45± 2 a	15±1 b	46±3 a
12	150	42±2 a	11±2 b	47±1 a	38±3 a	17±2 a	59±6 a	0±0	0±0	0±0	46± 2 b	16±1 c	52±3 b
	LSD	*7*	*9*	*9*	*11*	*5*	*18*	*10*	*3*	*12*	*6*	*2*	*7*

Note: Values are means ± standard error, n = 3

LSD = least significance difference ($p<0.05$). V = Variety: 1=CIP 199062.1, 2=CIP 194282.1, 3=CIP 189151.8, 4=CIP 188002.1, 5=106082.1, 6=CIP 440004, 7=CIP 420001, 8=BARI SP 12, 9=BARI SP 4, 10=BARI SP 8, 11=CIP 440181, 12=CIP 400039. T = Treatment.

Chapter 4

Ion uptake and distribution in sweet potato genotypes subjected to salt stress is not driven by transpiration

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Abstract

Whereas potassium is taken-up actively to the plant, sodium uptake and distribution often is driven by the transpirational volume flow in the shoots of plants grown under salinity. Thus, reducing transpiration rate is regarded as adaptation mechanism to reduce tissue salt load. In combination with a high K uptake, plants may be able to maintain growth and are, thus, seen as salt tolerant. Little is known about these mechanisms in sweet potato (*Ipomoea batatas* L.). Therefore, cuttings of two sweet potato genotypes contrasting in salinity tolerance (CIP 188002.1, tolerant; CIP 189151.8, sensitive) were subjected to 0 and 50 mM NaCl root zone salinity in a hydroponic system and grown under low (40%) and high (80%) relative air humidity (rH) to create difference in transpiration. After 18 days of initial hydroponic growth, NaCl was added for another 33 days. Cumulative plant water loss and total ion uptake were determined in terms of air humidity and genotypes. The amount of water loss per unit leaf area is the double in low air humidity than high air humidity but the Na accumulation remained almost at the same amount. Cumulative water loss per unit leaf area subjected to 50 mM salt stress significantly increased ($P<0.003$) in tolerant and decreased ($P<0.01$) in sensitive genotype under low air humidity whereas it had no difference under high air humidity. In addition, water loss and ion uptake were calculated in leaves (age) from the days after salt application. There was no relationship (very low r^2) between the water loss from individual leaves and Na or Cl uptake in both of genotypes. However, young leaves of tolerant genotype that were mostly developed under salt stress maintained more than the double amount of K than the sensitive genotype under high air humidity where higher amount of water was lost. We also figured out the distributing pattern of Na, K and Cl by the different organs of sweet potato and found that CIP 188002.1 hold 50% more Na, K and Cl by their petioles whereas it was almost a similar ratio in case of CIP 189151.8. The rH, water uptake and leaf age had no significant relationship for Na, K and Cl uptake and distribution exposed to salt stress. We conclude that transpirational volume flow is not a main driving force for Na and Cl uptake and distribution within the plant. However at least at high air humidity, high levels of K in young leaves may

maintain a larger accumulation of dry matter. Mostly, Na and Cl are distributed to the petioles by CIP 188002.1 whereas it was opposite in CIP 189151.8 (distributed by leaf blade). Na distributing pattern (Na mostly deposited in leaves and petioles) by active ion transport linked with ATP trade-off could be suggested for further study.

Keywords: vapor pressure deficit (VPD), water uptake, ion uptake and salinity stress.

Key points

- Water lost by transpiration proportionally increased with the increased levels of Vapor Pressure Deficit (VPD) in sweet potato plants
- Water loss does not lead to Na, K and Cl ion uptake
- Tolerant genotypes of sweet potato shared significantly higher percentage of Na and Cl by their petioles
- Sensitive genotypes of sweet potato shared Na and Cl more or less at a similar ratio with leaf blades and petioles
- Young leaves of tolerant genotype share higher amount of K that leads to dry matter maintenance in sweet potato

4.1 Introduction

Uptake of sodium and chlorine in glycophytes grown under root zone salinity is thought to be passive with the transpiration stream via apoplastic breaks in the endodermis (Greenway & Munns, 1980; Yeo et al., 1987). In support of this it has been shown that the effects of salinity are aggravated in dry seasons relative to rainy seasons due to differences in relative air humidity or vapor pressure deficit (VPD) (Lauter & Munns, 1987; Asch et al., 1999). This may be due to increased sodium accumulation due to higher transpiration und high VPD conditions as sodium uptake is positively related to transpiration rate (Hirai et al., 1985; Asch et al., 1997a; Yeo et al., 1985; Wimmer & Asch, 2005). However, the relationship between transpiration and salt accumulation has been controversially discussed. According to Flowers et al. (1988) the net transport of NaCl to the shoot of rice is positively correlated with the transpiration rate. In contrast, Naito et al. (1994) found that increased transpiration did not result in increased sodium accumulation in the shoot and Katerij et al. (2009) reported similar results for wheat and barley. Yeo et al. (1985) showed that although overall transpiration may be linked to overall NaCl accumulation, on an individual leaf level it is not which may not explain later results showing an unequal distribution of sodium within plants (Yamanouchi et al., 1987, Asch et al., 1997b). Yeo et al. (1985) proved a preferential flux of sodium towards older leaves which transpired significantly less than the younger leaves and Sharma (1996) reported that older wheat leaves contained six to eight times more Na and Cl than the flag leaves and that transpiration, stomatal conductance, and assimilation were higher in young and active flag leaves. Yasar et al. (2006) reported similar results for green bean where under salt stress conditions, Na accumulated mainly in older leaves and was not distributed to the young leaves. Potentially, reducing the uptake and accumulation of NaCl through reduced transpiration, sequestration of sodium in older, less active leaf tissues, and maintaining high potassium levels (as proposed by Mondal et al., 2022) could constitute traits for salinity tolerance in sweet potato. However, to date, no data are available on the relationship between transpiration and ion accumulation in sweet potato grown under salinity neither in general nor

on leaf level. To elucidate this relationship, we subjected two sweet potato genotypes contrasting in salinity tolerance to root zone salinity of 50 mM and 0 mM NaCl and investigated the overall and leaf-individual transpirational water loss and the accumulation of Na, Cl, and K in 11 sequential leaves on the main vine. To create differences in transpiration, plants were grown under two contrasting VPD levels.

4.2 Materials and methods

Two genotypes of sweet potato namely- CIP 188002.1 (salt tolerant) and CIP 189151.8 (salt sensitive) were obtained from Bangladesh Agricultural Research Institute (BARI), Bangladesh and propagated in a greenhouse at the University of Hohenheim, Germany at about 27-30 ^{0}C. The experiment was conducted in two climate chambers (Percival Scientific Inc., USA) of the Institute of Tropical Agricultural Sciences (Hans-Ruthenberg Institute) at the University of Hohenheim, Germany. The chambers were set to 12h light period with 550 µmolm^{-2}s^{-1} at mid canopy height and day-night temperature of 28 ^{0}C and 22 ^{0}C, respectively. The chambers differed in VPD (1) = VPD ~0.76 kPa and (2) VPD ~2.27 kPa. Chamber temperatures and relative air humidities were monitored using tinytag data loggers (Gemini Data Loggers, UK). Tender vines of approximately five to seven cm length with two nodes were grown in Yoshida nutrient solutions (Yoshida, 1976) in 1 L plastic pots with a continuous 15-minute air supply at 45-minute intervals. During the first week of plant growth, the Yoshida nutrient solution was provided as half strength and at full strength for the rest of the time until harvest after 15 days of salt exposure. The pH of the solution was kept in the range of 5.5 to 6.5. The nutrient solution was renewed twice a week to maintain the solutions pH between 5.5 and 6.5.16 plants of each variety were positioned in each chamber with half the plants subjected to 50 mM NaCl at 18 days after planting whereas the other half served as control treatment. According to Mondal et al. (2022), 50 mM NaCl root zone salinity creates a sufficiently stressful environment to study leaf level salt tolerance mechanisms of sweet potato.

4.2.1 Whole plant transpiration

For the duration of the experiment, transpirational water loss was determined daily at about 11 am for each pot by difference weighing using an electric balance (Kern, KB 2400-2N, Germany) with 4 replicates per variety, treatment, and VPD level. To correct for evaporation additional non-planted pots were placed and measured in each chamber. After weighing, pots were refilled to original weight with deionized water and the pH of the nutrient solution was checked again.

4.2.2 Measurement of gas exchange

The porometer LCi-SD (ADC BioScientific Ltd, UK) was used to measure stomatal conductance, assimilation rate, and transpiration rate based on an infrared gas analyzer (IRGA) system. All fully developed leaves on each vine were measured in sequence from base to top with 4 replicates. All gas exchange measurements were conducted during the light period from 9:00 am to 6:00 pm.

4.2.3 Measurement of leaf area

The EASY LEAF AREA software (Easlon et al., 2014) was used to measure the leaf area of individual leaves non-destructively. The start date was day 18 for of the first fully developed leaf. Measurements of new leaves were repeated until for the individual leaf area had not changed for two consecutive days. Final leaf area was measured destructively 15 days after onset of treatments using a leaf area meter (Li-Cor Inc., LI 3000C).

4.2.4 Estimation of actual water loss by individual leaves

In order to link salt uptake and distribution to the transpirational history of individual leaves, it is important to know the amount of water lost from individual leaf surfaces. However, in

chambers differing so strongly in VPD, gas exchange measurement with a porometer give biased results due to the fact that stomata in low VPD environments are wide open, but do not transpire much water since the atmospheric conditions are quite saturated. On the other hand, in high VPD environments stomata may be somewhat closed but atmospheric water demand and thus transpiration rates may still be high. When measuring transpiration rates of individual leaves with an IRGA in low VPD conditions, the atmosphere in the porometer chamber is much drier due to the vapor being transported to the IRGA which results in apparently higher transpiration rates than under high VPD conditions, which of course is not true. In addition, we found that fully grown leaves reduce stomatal conductance with age, leading to changes in transpiration rates from the same surface over time. Using the transpiration rates determined for individual leaves with the IRGA and multiplying them with the individual leaf surface calculates the water loss from that leaf surface on that day. We measured the same leaf 48, 96, 192, and 288 hours after onset of treatments and calculated the respective water loss from the leaf surface based on a linear decrease. The respective transpiration rate kinetics are shown exemplarily for the leaves 1-4 for variety CIP188002.1 in Figure 4.1.

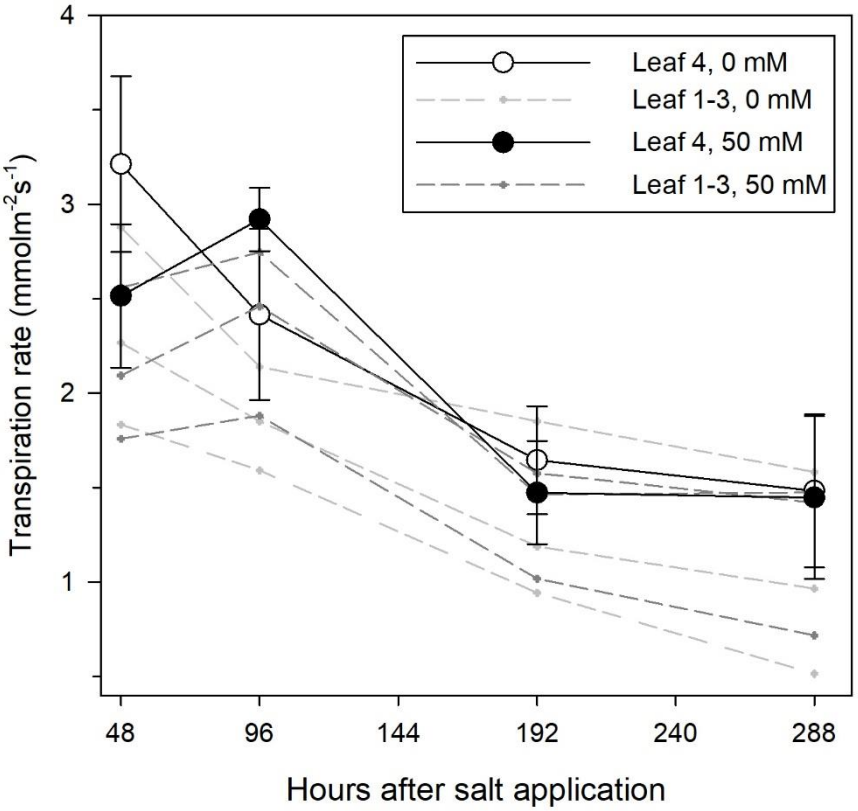

Figure 4.1 Transpiration rate kinetics for aging leaves of sweet potato. Exemplarily shown for sweet potato variety CIP188002.1 grown under a VPD of 2.27 kPa. Dark grey and grey dotted lines depict the kinetics of the transpiration rate of oldest leaves (1-3) for 50 mM and 0 mM root zone salinity. Circles show the kinetics for the leaf that was the youngest fully developed leaf at the beginning of the salt treatment. Values are means of 4 replications, error bars = standard error of means.

The individual regressions were used to calculate daily water losses for each existing leaf surface, allowing to calculate the daily total water loss based on the IRGA measurements.

As described in section 4.2.1 we measured the actual total water loss from each pot by difference weighing. By using the ratio between the actual water loss and the calculated water loss from the IRGA measurements as described above, we obtained the necessary correction factors to calculate the actual transpirational water loss from each existing leaf surface for the duration of the experiment.

4.2.5 Measurement of dry matter

Dry weights of petioles and individual fully developed leaves were determined after 72 hours of drying the samples at 60 ^{0}C with a digital balance (XB 220 A, Precisa, Switzerland) after 15 minutes of dehumidification (Exicator, Duran, Germany). All samples were analyzed separately for Na, K, and Cl.

4.2.6 Analysis of Na^+, K^+ and Cl^- concentrations

Samples were analyzed for Na, K and Cl content following the procedure for autoclave extraction as detailed by Asch et al. (2022). Six 5-mm stainless steel balls and three 3-mm balls were used for grinding to obtain a fine powder. After grinding, about 0.1 g of dry sample was used as a subsample for the final analysis. Samples were extracted with 10 mL distilled water in 15 mL centrifuge tubes (Roth GmbH & Co. KG) by heat digestion in an autoclave (SanoClav MMCS) at 120°C for 60 minutes and then centrifuged for 5 minutes (Allegra X-15®, Beckman Coulter GmbH). Finally, 9 mL of the supernatant were collected and made up to 100 mL with distilled water. The sodium and potassium concentrations of the sample solutions were determined using a flame photometer (Jeanway, PFP 7). Chloride content was determined with an auto analyzer (Autoanalyser II, Technicon, America) as described by Asch et al. (2022).

4.2.7 Statistical analysis

The experiment was designed as a factorial experiment to investigate the individual and additive effects/distributions of Na^+, Cl^-, and K^+ ions under different rH values in two sweet potato genotypes differing in salt tolerance. Results are presented as means ± standard errors. Significance tests were performed with a value of $P \leq 0.05$ and ≤ 0.01 using R statistical software (RStudio Team, 2020). Both paired and unpaired t tests were performed using Microsoft Excel and Sigma Plot 14.0 software, as appropriate. Graphs were generated with

Sigma plot 14.0 (Systat Inc). Linear regressions were used to determine individual leaf water loss as described by (Gomez and Gomez, 1984).

4.3 Results

4.3.1 Salinity and VPD effects on dry weight, Na, K, and Cl concentration in leaf blades and petioles

Two sweet potato varieties (CIP 188002.1, salt tolerant and CIP 189151.8, salt sensitive) were subjected to two root zone salinity treatments (0 mM and 50 mM NaCl) and grown in contrasting atmospheric moisture environments (VPD 0.76 kPa equivalent to about 80% relative air humidity (rH) and VPD 2.27 kPa equivalent to about 40% rH). After 15 days of stress treatments, dry weights of all fully developed leaf blades on the vine and their petioles and concentrations of Na, K, and Cl therein were determined. Table 4.1 shows the results for the 50 mM root zone salinity treatment for the two varieties and atmospheric moisture conditions. Results for non-stressed conditions are shown in Appendix 4.1.

Salinity reduced dry matter in both varieties and under both atmospheric moisture conditions (data not shown, see also Appendix 4.1). Generally, the dry weight of leaf petioles was smaller than that of the leaf blades. On average petiole dry weight was 230 mg and 260 mg for salt sensitive CIP 189151.8 and 510 mg and 830 mg for salt tolerant CIP 188002.1 under high and low VPD, respectively. This corresponded to an average of 15% and 18% of total leaf weight for CIP 189151.8 and 24% and 32% for CIP 188002.1 which leaves in general were heavier. Quite in contrast to the dry weight distribution, ion concentrations (Na, K, and Cl) in the petioles were generally significantly higher than in the leaf blades. In tolerant CIP 188002.1 leaf blade concentrations were on average across all leaf positions 26 mgg^{-1} (Na), 5 mgg^{-1} (K), and 23 mgg^{-1} (Cl) under high VPD conditions with no significant effect of VPD (low VPD: 20 mgg^{-1} (Na), 5 mgg^{-1} (K), and 20 mgg^{-1} (Cl)). In sensitive CIP 189151.8 average leaf blade concentrations of Na and Cl were significantly higher than in CIP 188002.1 (35 mgg^{-1} (Na), 34

mgg^{-1} (Cl) under high VPD and 48 mgg^{-1} (Na) and 31 mgg^{-1} (Cl) under low VPD conditions) whereas no significant difference was observed for K concentrations (4.5 mgg^{-1} (high VPD) 7.5 mgg^{-1} (low VPD). Leaf petiole Na concentrations were with 116 mgg^{-1} (high VPD) and 90 mgg^{-1} (low VPD) on average across all leaf positions about factor 4.5 times higher than leaf blade concentrations in CIP 188002.1. For CIP 189151.8 with 119 mgg^{-1} (high VPD) and 92 mgg^{-1} (low VPD) the factors were 3.4 and 1.9, respectively. This indicates a less efficient protection of leaf blades against sodium accumulation in CIP 189151.8. In CIP 188002.1 average K concentrations in petioles across all leaf positions were about 10 times higher than in leaf blades under both VPD regimes (53 mgg^{-1} and 46 mgg^{-1}, respectively) whereas in CIP 189151.8 K concentrations in petioles were about 6.2 times higher than leaf blade concentration in high VPD (29 mgg^{-1}) and 2.8 times higher in low VPD (21 mgg^{-1}). At the same time, the average K concentration in petioles across all leaf positions of CIP 188002.1 was about twice as high as in petioles of CIP 189151.8 under both VPD regimes.

Leaf ion concentrations differed strongly depending on leaf position, atmospheric moisture conditions, and variety. In tolerant CIP 188002.1 under high VPD conditions mean Na, K, and Cl concentrations of leaf blades of the oldest leaves (1-6) were about twice as high as the mean concentrations of the youngest leaves (7-11), this trend was not observed in the petioles. With average concentrations across all leaf positions similar to high VPD conditions, ion concentrations did not differ among leaf positions under low VPD conditions indicating a more equal distribution of ions among leaf positions. In sensitive CIP 189151.8 under high VPD conditions ion concentrations of leaf blades were generally higher than in CIP 188002.1 in all leaves, however older leaf blades also accumulated ion concentrations 1.5 to 2.3 times higher than young leaf blades which was not reflected in the petioles. Low VPD did not affect ion concentrations across leaf positions in CIP 189151.8.

Table 4.1 Dry weight (mg), Na, K, and Cl concentrations (mgg^{-1}) in leaf blades and petioles of two contrasting sweet potato genotypes subjected to 50 mM root zone salinity and two contrasting atmospheric moisture conditions. Values are means ± standard error (SE), n=4.

		VPD 2.27 kPa (40% rH)								VPD 0.76 kPa (80% rH)							
VAR 1	L/P 1	DW L 1	DW P 1	Na L 1	Na P 1	K L 1	K P	Cl L 1	Cl P	DW L 1	DW P 1	Na L 1	Na P 1	K L 1	K P 1	Cl L 1	Cl P
1	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	64±11	68±2	25±4	107±54$^{\#}$	5 ± 1	58±29$^{\#}$	27±2	112±9$^{\#}$
1	2	92±1	30±0*	25±2	77±38	10±1	75±38	20±4	125±40	86±1	65±3	16±0	89±45	6 ± 1	78±39	21±1	91±23$^{\#}$
1	3	89±22	40±3*	37±3*	107±54	9±1	39±20	32±4	100±50	103±12	75±10	21±4	73±36	7 ± 1	46±23	23±4	77±12
1	4	123±16	51±5$^{\#}$	54±6*	75±37	7±2	42±21*	29±2*	107±53	127±10	60±7$^{\#}$	21±2	122±61$^{\#}$	5 ± 1	81±41$^{\#}$	21±1	156±6
1	5	181±23	57±4	34±2*	107±2	5±0	37±5	26±6	167±5*	181±29	80±11$^{\#}$	26±2	83±20	4 ± 1$^{\#}$	30±7	23±2	104±22
1	6	163±22	50±11*	31±3	161±80*	5±1	59±29$^{\#}$	32±3	25±12$^{\#}$	200±10$^{\#}$	90±2$^{\#}$	23±3$^{\#}$	81±40	5 ± 0	32±16	24±6	107±14
1	7	226±12$^{\#}$	70±2$^{\#}$	20±3	92±46	3±1	33±17	22±4	58±29*$^{\#}$	210±20$^{\#}$	80±8$^{\#}$	23±2$^{\#}$	89±45$^{\#}$	4 ± 0$^{\#}$	31±15	20±1$^{\#}$	132±22
1	8	212±19	70±8*$^{\#}$	16±5$^{\#}$	119±60*	3±1	48±24*$^{\#}$	19±5	54±26$^{\#}$	250±20$^{\#}$	100±5$^{\#}$	18±2$^{\#}$	66±33	4 ± 0	19±9	16±2$^{\#}$	68±27
1	9	180±23$^{\#}$	60±10$^{\#}$	20±6$^{\#}$	105±52	4±2	58±29$^{\#}$	16±5$^{\#}$	22±11*$^{\#}$	200±30$^{\#}$	70±7$^{\#}$	18±1$^{\#}$	107±54	6 ± 1	51±25$^{\#}$	16±4$^{\#}$	173±8$^{\#}$
1	10	160±7$^{\#}$	30±7*	17±6$^{\#}$	239±119*	3±1	110±55$^{\#}$	11±1$^{\#}$	49±24*$^{\#}$	196±19$^{\#}$	100±8$^{\#}$	19±2$^{\#}$	79±40$^{\#}$	6 ± 2	42±21$^{\#}$	24±4	141±13
1	11	169±15$^{\#}$	60±3$^{\#}$	21±5	128±64	5±1	60±30	27±5	59±29*$^{\#}$	135±53	38±7	10±4	140±70	5 ± 2	93±46	12±4	221±38
Mean		***167±6***	***54±2$^{\#}$***	***28±2***	***116±7.2***	***5±1***	***53± 11$^{\#}$***	***23±1***	***88±8***	***178± 15***	***79±5***	***20±1***	***91±8***	***5 ± 1***	***47±4***	***21±2***	***128±12***
2	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	45±21	0±4	52±16	36±18	13 ± 2	6±3	69±29	32±0
2	2	26±0	10±0	53*±0	121±61	14±0	18±9	72±0	206±0	85±19	27±6	20±3	82±41	6 ± 1	12±6	26±5	98±11
2	3	36±0	13±0	35±0	71±35	13±0	19±10	50±0	149±0	87±17	21±5	26±6	95±47	4 ± 0	10±5	25±6	103±11
2	4	125±15	35±8	51*±9	119±59	6±2	52±26	31±4	251±99	147±43	31±10	26±5	93±46	6 ± 1	17±8	25±8	104±32
2	5	108±0	19±0	77*±0	92±0	4±0	26±0	39±0	146±0	161±23	46±5	34±11	95±5	7 ± 1	19±2	25±6	87±5
2	6	127±26	25±4	30±8	142*±71	5±2	18±9	38±8	194±65	140±22	32±6	37±4	88±44	8 ± 1	21±11	32±3	107±53
2	7	190*±21	33±3	19*±7	106±53	2*±1	23±11	28±11	130±44	129±10	28±8	36±5	131±65	6 ± 0	32±16	32±5	84±42
2	8	187±8	30±4	16*±9	130±65	2±1	25±12	11*±2	106±59	137±37	25±8	50±19	97±49	13 ± 8	30±15	25±3	82±41
2	9	170*±32	21±2	41±6	106±53	7±4	26±13	45±7	129±48	84±23	19±4	40±4	94±47	8 ± 2	30±15	47±10	128±64
2	10	170*±15	20±1	30±4	124±62	3*±0	27±14	34±4	185±40	72±10	16±4	40±4	136±68	7 ± 1	37±19	42±7	166±16
2	11	114±13	20±1	34±6	191±96	5±0	45±23	43±6	277±53	90±0	19±0	28±0	n.d.	5 ± 0	n.d.	28±0	76±0
Mean		***143±4***	***26±2***	***34±2***	***129±19***	***5±1***	***29±4***	***36±2***	***213±25***	***113±14***	***30±6***	***36±4***	***96± 2***	***8 ± 1***	***22±5***	***33±3***	***118±20***

Note. values are mean± standard error, n=4

VAR = Variety: 1= CIP 188002.1 (salt tolerant), 2= CIP 189151.8 (salt sensitive). L/P: number of leaves in the order of appearance. Na = Sodium, K = Potassium, Cl = Chlorine, L = Leaf, P = Petiole, DW = Dry weight. n.d. = no data. * = significant difference ($p<0.5$) between the VPD levels; $^{\#}$ = significant difference ($p<0.5$) between varieties in the same VPD level.

4.3.2 Cumulative water loss and total uptake of Na, K, and Cl

Cumulative water loss (CWL) over the experimental period and the respective leaf area (LA) were determined in two contrasting genotypes of sweet potato to elucidate the relationship between CWL and Na, K, and Cl uptake and distribution. Table 4.2 shows cumulative sodium, potassium, and chlorine uptake into the leaves of salt tolerant CIP 188002.1 and salt sensitive CIP 189151.8 and the CWL as well as the CWL/LA under two root zone salinity levels (0 mM and 50 mM) when exposed to high VPD and low VPD environments. The cumulative uptake of Na and Cl was significantly ($p<0.05$) positively and the cumulative K uptake significantly negatively affected by 50 mM root zone salinity, independent of VPD and variety. Higher atmospheric water demand in the high VPD environment, increased the CWL/LA by factor 1.7 and factor 2.2 in salt tolerant CIP 188002.1 and by factor 1.5 and factor 3.7 in salt sensitive CIP 189151.8 under 0 mM and 50 mM root zone salinity, respectively. Uptake of Na in to leaf tissues was not as strongly affected by high VPD and varieties differed in their response to VPD. For salt tolerant CIP 188002.1, high VPD increased Na uptake under 0 mM root zone salinity by factor 1.2, however, the same conditions decreased Na accumulation by factor 0.93 under 50 mM root zone salinity. For salt sensitive CIP 189151.8, high VPD increased Na uptake under 0 mM root zone salinity by factor 1.3 and the same VPD conditions under 50 mM root zone salinity increased Na accumulation by factor 1.5. In CIP 188002.1, high VPD decreased K accumulation (0.7 and 0.8 under 0 mM and 50 mM root zone salinity, respectively) whereas in CIP 189151.8 increased (1.15 under 0 mM) or remained unchanged (1.0 under 50 mM root zone salinity). High VPD decreased the accumulation of Cl into the leaves of CIP 188002.1 by about 10% and 20% under 0 mM and 50 mM root zone salinity, respectively, whereas the same conditions increased the accumulation of Cl in the leaves of CIP 189151.8 by factor 1.2 and 1.7, respectively. This indicates a certain potential link between water lost through transpiration and the accumulation of Na and Cl at least for CIP 189151.8. However, calculating the amount of Na or Cl transported into the leaves per unit of water lost from the leaves under 50 mM root zone salinity shows for Na in 45.7 $\mu molmmol^{-1}m^{-}$

^{2} (CIP 188002.1) and 46.3 µmolmmol^{-1}m^{-2} (CIP 189151.8) under high VPD conditions and 80.7 µmolmmol^{-1}m^{-2} (CIP 188002.1) and 160.5 µmolmmol^{-1}m^{-2} (CIP 189151.8) which is an increase of factor 2-4 in Na accumulation per unit water lost under low VPD. The results for Cl accumulation per unit water lost from leaf surfaces are similar as for Na, but in general about 10% to 20% lower.

Table 4.2 Estimation of cumulative water loss (per unit leaf area) and Na, K, and Cl ion uptake into the leaves of a salt tolerant and a salt sensitive variety of sweet potato subjected to 2 levels of root zones salinity (0 and 50 mM). Values are means ± standard error (SE), n=4.

VAR	TR, mM	VPD, kPa	Na, µmol	K, µmol	Cl, µmol	CWL, M	CWL/LA
CIP 188002.1	0	2.27	632 ± 50	2172 ± 98	2564 ± 104	118	21.7
CIP 188002.1	50	2.27	5986 ± 281	1494 ± 99	4435 ± 265	131	28.6
CIP 189151.8	0	2.27	551 ± 64	2633 ± 82	2462 ± 221	123	37.8
CIP 189151.8	50	2.27	5001 ± 454	1093 ± 249	4626 ± 384	108	30.4
CIP 188002.1	0	0.76	538 ± 50	2994 ± 247	2798 ± 329	81	13.2
CIP 188002.1	50	0.76	6453 ± 552	1882 ± 190	5720 ± 429	80	12.6
CIP 189151.8	0	0.76	428 ± 42	2280 ± 135	2064 ± 202	87	26.0
CIP 189151.8	50	0.76	3371 ± 357	1053 ± 121	2757 ± 112	21	8.3

Note. values are mean± standard error, n=4.

VAR = Variety: CIP 188002.1 =tolerant and CIP 189151.8 =sensitive. rH=Humidity: 40% and 80%: Air humidity at the artificial VPD chambers. TR= Treatment. LA=Leaf Area in cm^{2}; CWL= Cumulative water loss over the experimental period in Mol.

4.3.3 Leaf area, transpiration rate, and water loss from individual leaves

Leaf area development and the kinetics of transpiration rates were recorded for 18 days after salt application (Figure 4.2). Leaf area increased linearly in the beginning but levelled out in all treatments at about 25 days after transplanting (DAT) in CIP 188002.1 and at about 29 DAT in CIP 189151.8, probably due to light constraints in the climate chambers (Figure 4.2a). Under 0 mM root zone salinity LA was generally largest, however, leaf area of CIP 189151.8 as compared to CIP 188002.1 was about 27% and 37% reduced in low and high VPD environments, respectively. 50 mM root zone salinity induced a statistically non-significant reduction in LA of about 10% in CIP 188002.1, under high VPD conditions, whereas in CIP189151.8 under the same condition no effect on leaf area was observed. In contrast, 50 mM root zone salinity under low VPD reduced LA in CIP 189151.8 significantly ($p<0.05$) by about 30%, whereas no salinity effect was observed in CIP 188002.1 under the same VPD

conditions. Whole plant transpiration rates showed a quasi-linear decrease over time for both varieties, all treatments, and atmospheric conditions, but differed among genotypes, depending on root zone salinity and atmospheric moisture conditions (Figure 4.2b). In CIP 188002.1, transpiration rate increased by about 50% as compared to low VPD conditions when plants were grown at high VPD. However, there were no significant differences on whole plant transpiration rates between 0 mM and 50 mM root zone salinity. In CIP 189151.8, whole plant transpiration rates were higher than CIP 188002.1 under both VPD conditions, but 50 mM root zone salinity combined with high VPD conditions resulted in a significantly reduced transpiration rates whereas, combined with low VPD transpiration rates significantly increased ($p < 0.05$). However, total cumulative water loss was lower in CIP 189151.8 (Table 4.2) than in the CIP 188002.1 genotype, probably due to the larger leaf area of CIP 188002.1 (Figure 4.2a).

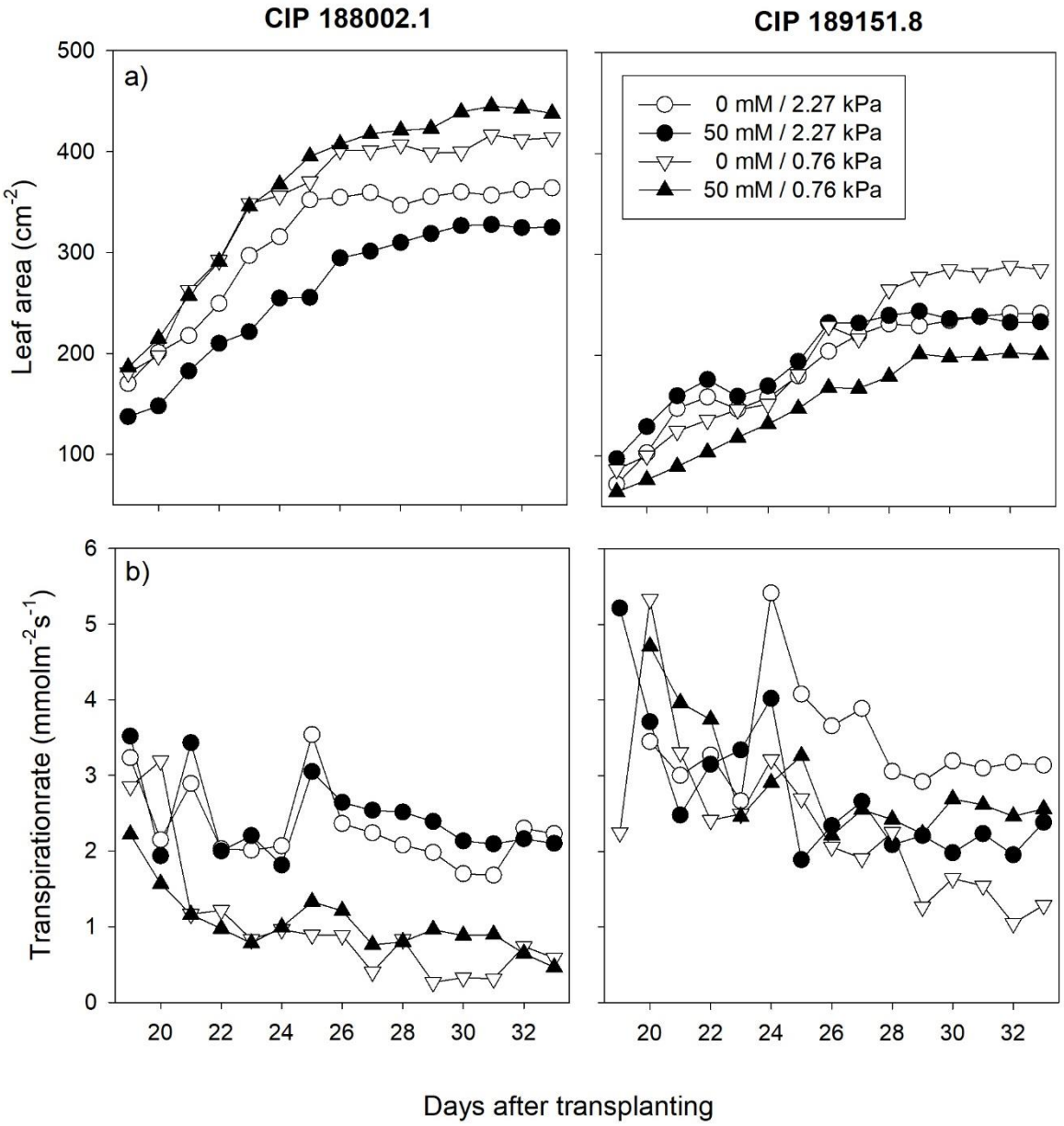

Figure 4.2 Total leaf area development and kinetics for total plant transpiration rate for sweet potato varieties CIP 188002.1 and CIP 189151.8 subjected for 15 days to 2 levels of root zone salinity (0 mM and 50 mM) and grown in 2 contrasting atmospheric moisture regimes (VPD = 2.27 kPa and VPD = 0.76 kPa). Error bars have been omitted for better readability.

Based on the leaf area of individual leaves and applying the procedure describe above, we calculated the cumulative transpirational water loss from individual leaves for the two varieties, the salinity treatments and the different atmospheric conditions (Figure 4.3). As with total plant transpiration, water loss from individual leaves as compared with low VPD conditions increased significantly under high VPD conditions in both varieties, but here at the same level of magnitude (Figure 4.3 a, c). Under high VPD conditions in general, younger leaves showed higher individual water losses than older leaves, a trend that was not observed under low VPD conditions and was not affected by root zone salinity. Although transpirational water loss at individual leaf level was not statistically significantly different between the two root zone salinity treatments, water loss from individual leaves at high VPD conditions under 50 mM root zone salinity was lower in both varieties than under 0 mM root zone salinity (Figure 4.3 a, c), whereas no systematic difference was observed under low VPD conditions (Figure 4.3 b, d).

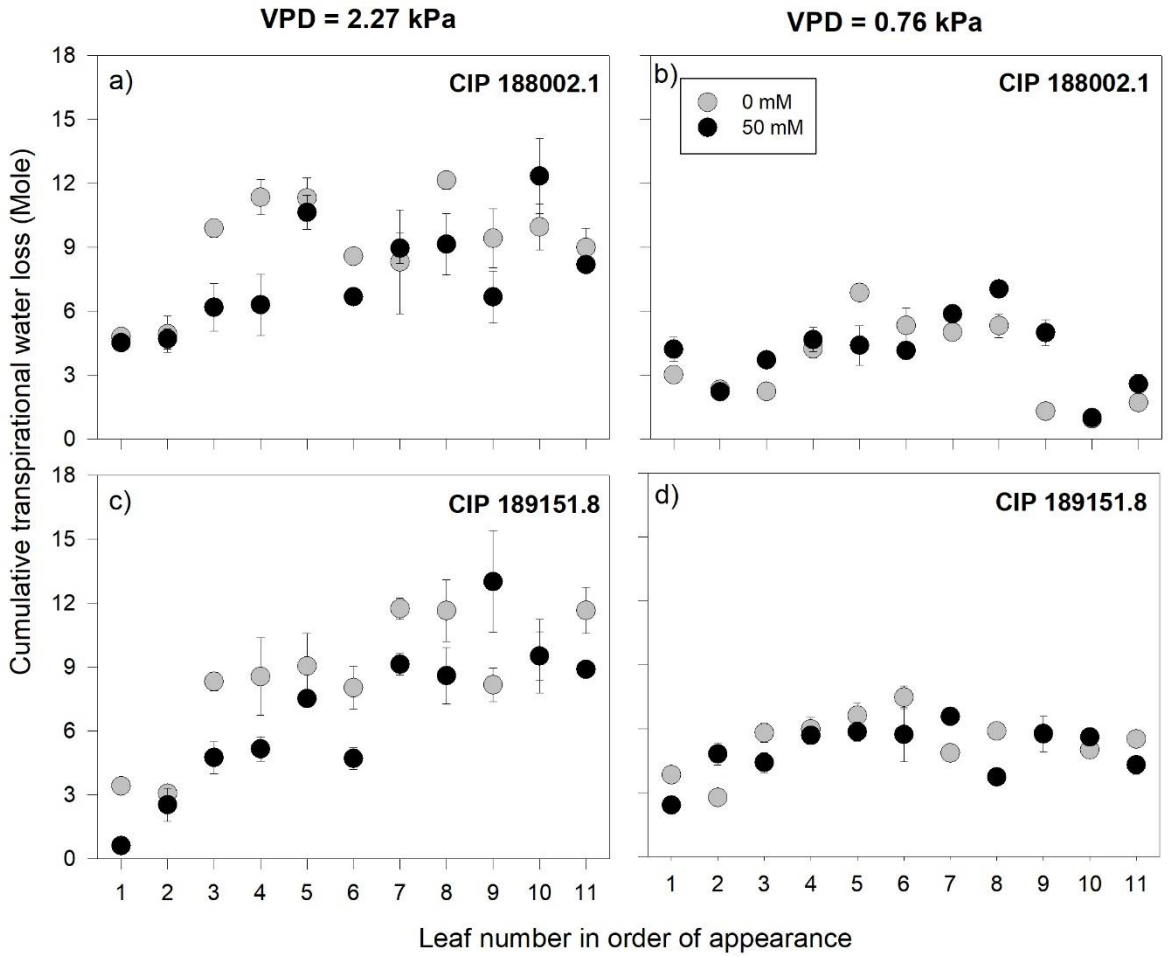

Figure 4.3 Cumulative transpirational water loss from individual leaves for sweet potato varieties CIP 188002.1 and CIP 189151.8 subjected for 15 days to 2 levels of root zone salinity (0 mM and 50 mM) and grown in 2 contrasting atmospheric moisture regimes (VPD = 2.27 kPa and VPD = 0.76 kPa). Leaves are shown in the order of appearance with leaf 1 being the oldest leaf. Data shown as means of 4 replications. Error bars = standard error of means. Missing error bars are due to the data symbol being larger than the error.

4.3.4 Water loss from individual leaves and ion accumulation

Assuming that at least sodium and chlorine are taken up into the plant via the transpirational volume flow, the amount of water passing through a plant should be reflected in the amount of sodium and/or chlorine taken up to the plant. Ion distribution via the transpiration stream should result in higher accumulation of ions in tissues that lose more water. The relative share of ions in a leaf should, thus, be indicative of its relative share of water loss. From individual leaf concentrations we calculated the individual and, by summing those up, the total amount

of Na, K and Cl found in the leaf biomass. Dividing the individual amount of ions in the leaf by the total sum, we calculated the share of ions in that leaf. Similarly, summing up the cumulative water loss from individual leaves and dividing the individual water loss by this sum, we determined the individual share of water loss from that leaf relative to the total water lost from all leaves. Figure 4.4 shows the results of this exercise for sodium, potassium, and chlorine distribution among individual leaves as related to their share of water lost from their surfaces under 50 mM root zone salinity for the two genotypes and the two contrasting atmospheric moisture regimes.

Despite contrasting total amounts of water lost under the contrasting VPD conditions (Table 4.2), the relative share of water lost from individual leaves differed more strongly among the leaf positions then among the varieties or between the respective VPD levels. Medium aged leaves (4-8) lost on average roughly 10% of the total water transpired, thus, accounting for about 40% of total water loss. Of the total sodium taken up into the leaves, these medium aged leaves of CIP 188002.1 stored on average of 13% (high VPD), and 12.5 % (low VPD) accounting for 65% and 62.5% of the total amount of sodium taken up into the leaves. In CIP 189151.8 the same leaf positions accumulated on average about 15% under both VPD conditions and thus accounting for about 75% of the total sodium. Differences between the VPD regimes became apparent in the young leaf positions. Under high VPD conditions, young leaves (9-10) in both varieties lost similar or larger shares of water (15%, leaf 10, CIP 188002.1; 17.5%, leaf 9, CIP 189151.8) than the medium aged leaves while accumulating on average about the same share of sodium as the medium aged leaves. In contrast, under low VPD conditions, the same leaf positions of both varieties accumulated on average 7.5% of the total sodium while losing on average only slightly less water than the medium aged leaves. Old leaves (1-3) lost the least water and accumulated the smallest share of sodium independent of variety or VPD conditions.

As expected, no linear relationship was found between the relative accumulation of K into the leaf tissue and the water lost from the same tissue. Nonetheless, VPD conditions and variety

did affect the distribution of K among the leaf positions. In CIP 188002.1 independent of leaf position and VPD, the share of K in the leaves was between 7% and 15%. In CIP 189151.8, VPD conditions had a strong influence on the K distribution among leaf positions. Whereas under high VPD conditions the youngest leaves (8-11) had the largest share of K (12%-17%), under low VPD conditions this share was reduced to 4% - 10%. In all cases, the share of K in any leaf was strongly related to the share of Na in the same leaf.

Also for Cl no linear relationship was found between its relative accumulation into the leaf tissue and the water lost from the same tissue. Leaf position did not influence Cl accumulation, although older leaves (1-4) on average accumulated slightly less Cl than younger leaves, but the difference was not statistically different. Except for some inexplicable extremes in leaf 5 and leaf 10 in CIP 188002.1 under high VPD conditions (Figure 4.4i), the share of Cl among the leaves was between 5% and 15%. Only in CIP 189151.8, high VPD conditions resulted in a large difference of Cl accumulation among leaf positions with leaf 6 and 7 accumulating almost 40% of the total Cl (Figure 4.4k).

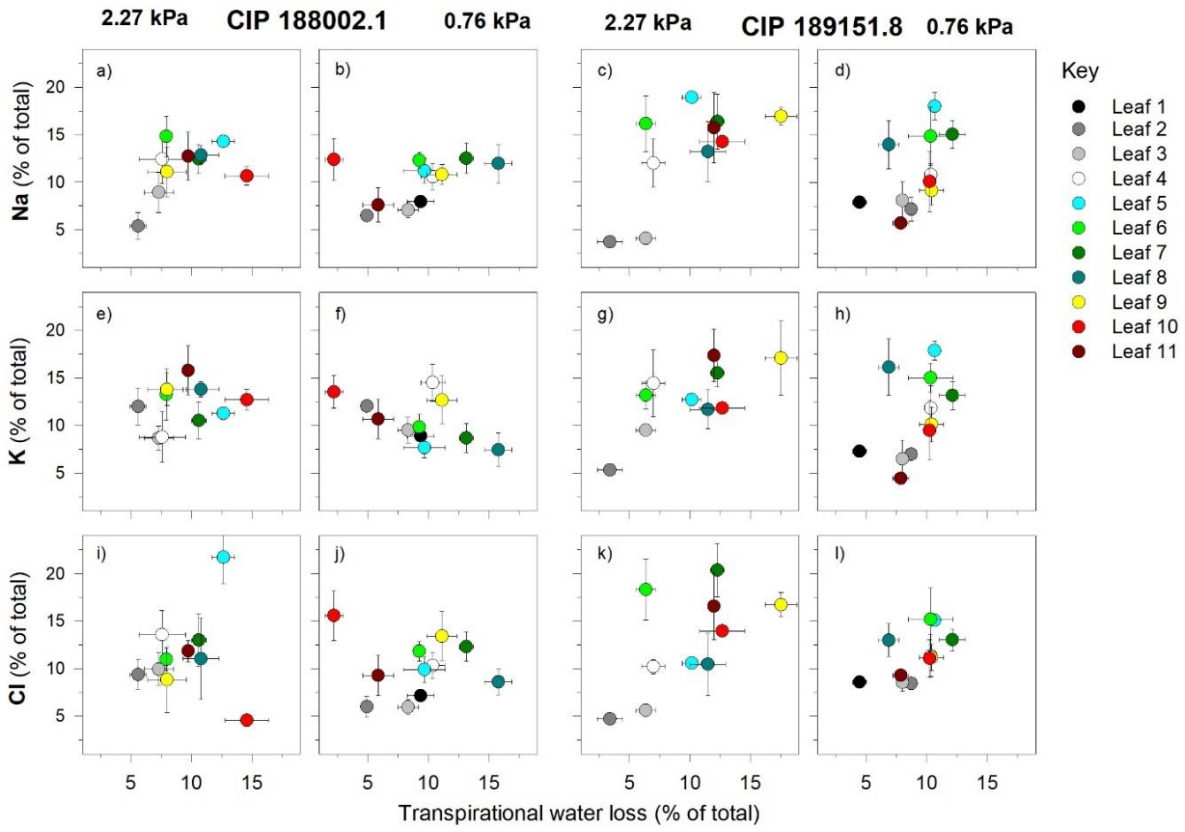

Figure 4.4 Relative shares of individual leaf positions for Na, K, and Cl of the respective total accumulation for all leaves plotted against the relative share of transpirational water loss in total transpirational water loss for the same individual leaf positions. All shares are expressed in %. Data are shown for leaf positions in order of appearance (1=oldest fully expanded leaf; 11 = youngest fully expanded leaf) for sweet potato varieties CIP 188002.1 and CIP 189151.8 subjected for 15 days to 2 levels of root zone salinity (0 mM and 50 mM) and grown in 2 contrasting atmospheric moisture regimes (VPD = 2.27 kPa and VPD = 0.76 kPa). Data are presented as means ± SE, n=4. Missing error bars are due to the data symbol being larger than the error.

4.3.5 Distribution of dry matter, sodium, potassium, and chlorine accumulation among leaf organs

Figures 4.5 to 4.8 shows the relative accumulation of dry matter, sodium, potassium, and chlorine of leaf petioles and leaf blades for all leaf positions, root zone salinity treatments and atmospheric moisture conditions. For dry weight, the relative share of petioles for all leaves was on average 3.5% and 1.8% under 0 mM root zone salinity and 3% and 2% under 50 mM root zone salinity in CIP188002.1 and CIP 189151.8, respectively, with no effect of atmospheric moisture conditions on that share. The relative share of dry weight for the leaf blades under 0 mM root zone salinity was on average 7.4% and 7.7% in CIP188002.1 and CIP 189151.8, respectively, with no effect of atmospheric moisture conditions on that share. Thus, the relative share in dry weight was about factor 2 larger for the leaf blades than for the leaf petioles. Under 50 mM root zone salinity, this relative share increased on average for both varieties under high VPD conditions and was strongly affected by leaf position. In CIP 188002.1, leaf blades from leaves 7-11 had a larger relative share of total dry weight (11% on average), whereas in leaves 1-6, this share was strongly reduced (6% on average). In CIP189151.8, a similar pattern but with larger differences was found. Here, leaf blades of leaves 6-10 accounted for about 75% of the dry weight and the share of leaf blades 1-5 as well as 11 were greatly reduced. Results in low VPD under 50 mM root zone salinity were different. Here, a strong linear increase in the relative share of dry weight in the leaf blades from leaves 1-8 was found followed by a decrease in this share for leaves 9-11. On average across all leaves positions, the relative share of dry weight in the leaf blades was with 6.8% about 0.6% smaller than under non-saline conditions. For CIP189151.8, again a similar pattern with larger differences was found. Here, the linear increase in the relative share of dry weight in the leaf blades was strong from 2.5% in leaf 1 to 12% in leaf 4 with leaf blades form leaf 4–

8 accounting for about 60% of the total dry weight, followed by a strong reduction in this share for the leaf blades of leaves 9-11 (Figure 4.5).

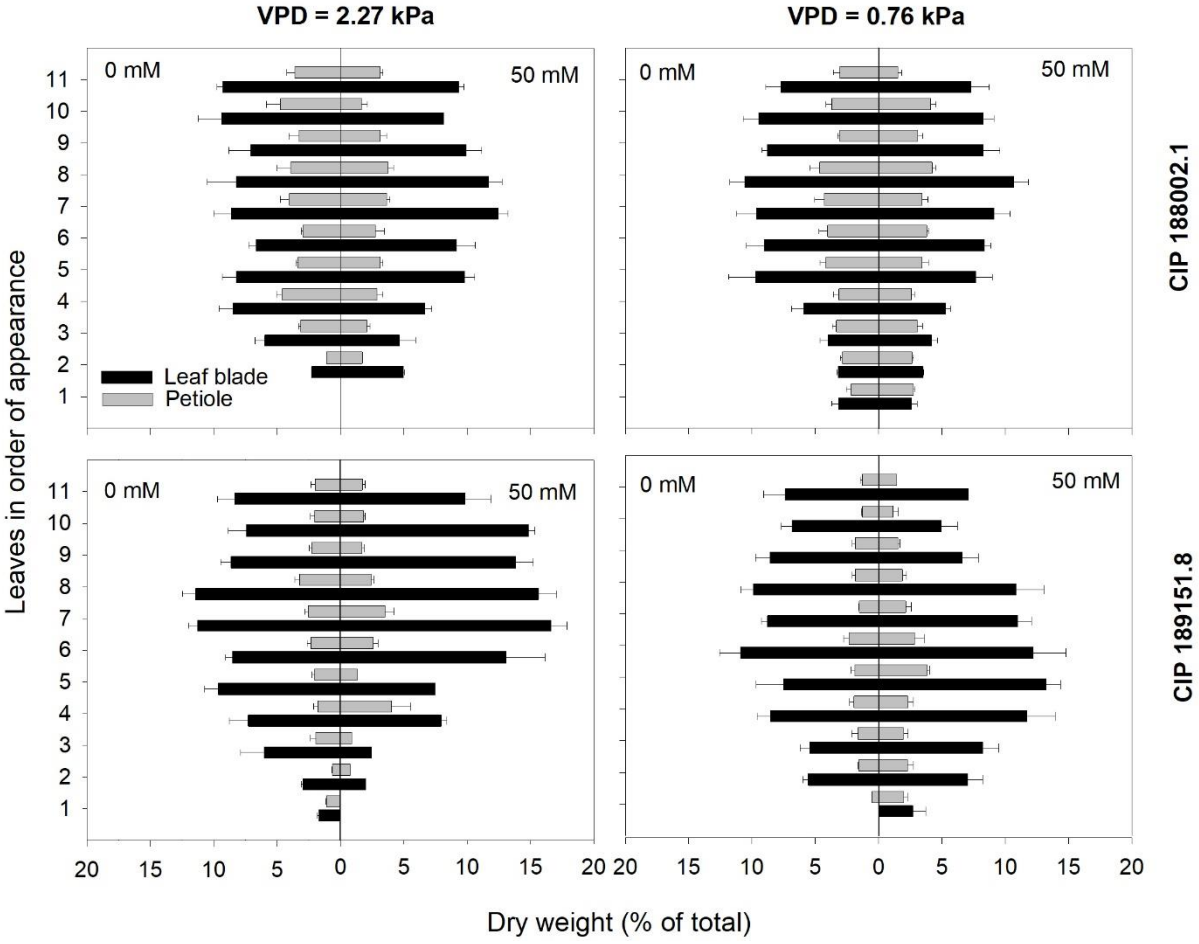

Figure 4.5 Percent share of total dry weight was plotted for different ages of leaf (leaf 1 to leaf 11) where leaf 1 was fully developed and 11 was the youngest one. Two genotypes of sweet potato, CIP 188002.1 and CIP 19151.8 were considered under two levels of rH subjected to 50 mM salt stress. Means ± SE, n=4.

In strong contrast to the dry weight distribution, leaf petioles play a major role in the distribution and accumulation of sodium, potassium, and chlorine with strong varietal and treatment effects for sodium accumulation and distribution among leaf positions and leaf organs (Figure 4.6), strong varietal effects for potassium accumulation and distribution among leaf positions and leaf organs (Figure 4.7) and strong treatment, varietal, and VPD effects for chlorine accumulation and distribution among leaf positions and leaf organs (Figure 4.8).

Since there is basically no sodium in the 0 mM root zone salinity treatment, the sodium found in the leaf tissue is weakly concentrated and originates from some sodium containing compounds of the nutrient solution. Nonetheless, under high VPD conditions leaf petioles accumulated even under non-stress conditions significantly larger shares of sodium in most of the petioles. This effect was more pronounced in CIP 188002.1 than in CIP189151.8. Under low VPD conditions under 0 mM root zone salinity, either equal shares between petiole and blade (CIP188002.1) or larger shares in the leaf blades (CIP189151.8) were observed. Under 50 mM root zone salinity, varietal differences become more apparent. Whereas in CIP188002.1, a strong linear decrease in the relative share of sodium in the leaf blades was found (from 7.5% leaf 4 to 2.5% leaf 10) and no such gradient was seen in CIP189151.8. In CIP188002.1, the relative share of sodium in the petioles of leaf 4 to leaf 11 was with on average 7.5% always larger than the relative share of the corresponding leaf blade. In contrast, in CIP189151.8, no clear pattern was observed, however the relative share of sodium in the leaf blades was in most cases larger than or equal to the relative share of sodium in the corresponding petioles. Under low VPD conditions, the relative share of sodium in all petioles under 50 mM root zone salinity was on average 7.5% and always larger than in the corresponding leaf blades. Particularly in the older leaves (1-4), the relative share of sodium in the leaf blades was minor. In CIP189151.8, under low VPD conditions under 50 mM root zone salinity, the relative share of sodium in the petioles was only in the 3 oldest leaves larger than in the leaf blades. In all other leaves, the blades accumulated a larger share than the corresponding leaf petioles.

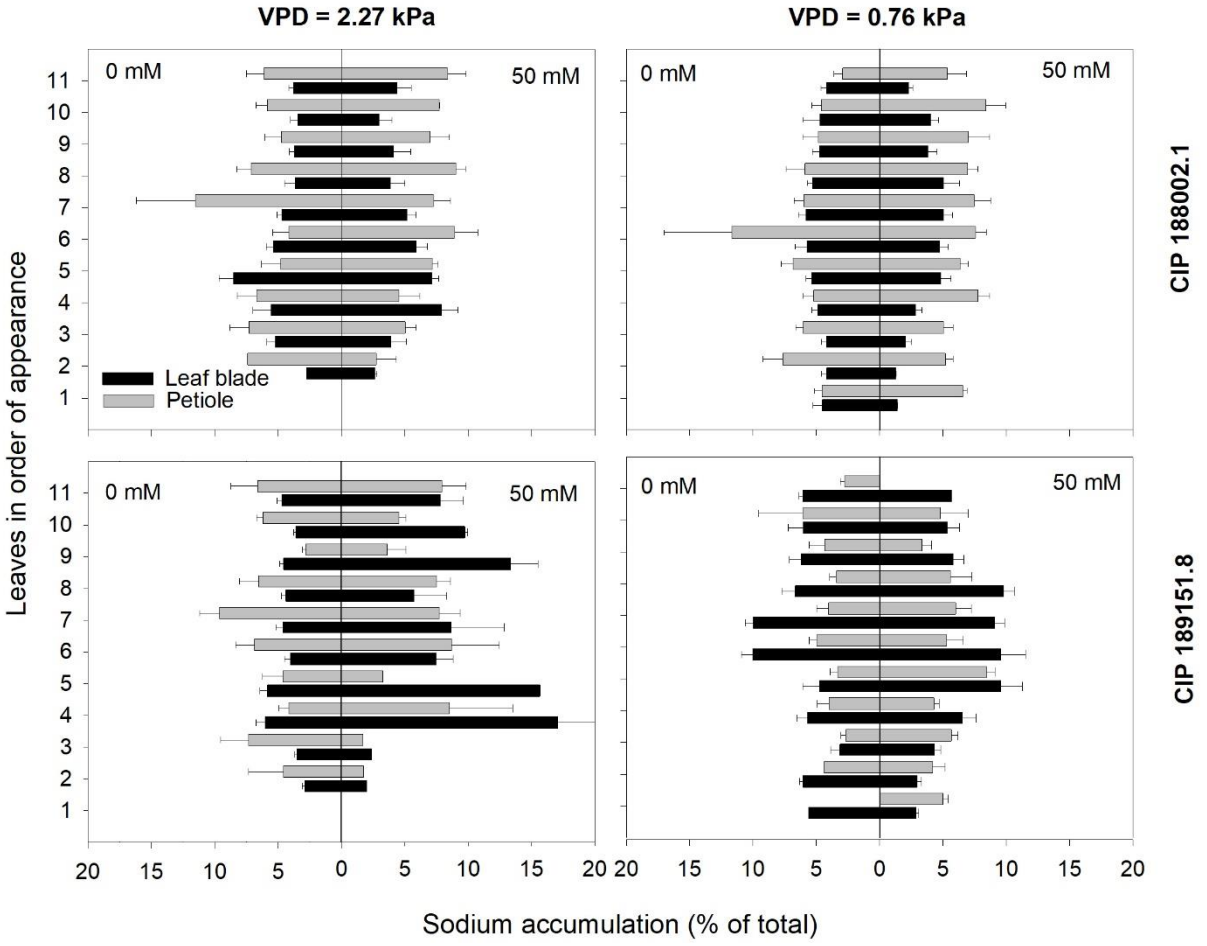

Figure 4.6 Percent share of sodium (Na) in leaf blades and petioles (% of total) was plotted for different ages of leaf (leaf 1 to leaf 11) where leaf 1 was fully developed and 11 was the youngest one. Two genotypes of sweet potato, CIP 188002.1 and CIP 19151.8 were considered under two levels of rH subjected to 50 mM salt stress. Means ± SE, n=4.

Potassium in the nutrient solution was provided in equal concentrations in the two root zone salinity treatments. For potassium accumulation and distribution, strong varietal differences were observed. In CIP 188002.1, the relative share of potassium in the petioles was always larger than in the leaf blades with only small effects related to leaf positions. This difference was even more pronounced under 50 mM root zone salinity than under 0 mM root zone salinity. Under high VPD conditions, the relative accumulation of potassium in the leaf petioles was on average about factor 2 larger than in the corresponding blades under 0 mM root zone salinity and factor 3.4 under 50 mM root zone salinity. Under low VPD conditions, these factors

were 3.0 and 4.3, respectively (Figure 4.7). This constitutes in accumulation relationships the opposite to dry matter accumulation between petioles and blades as shown in Figure 4.5. In CIP 189151.8, in contrast, relative shares of sodium accumulation between leaf blades and petioles were generally more equal and no clear root zone salinity effect was found. Under low VPD conditions and 0 mM root zone salinity, petioles tended to accumulate larger shares of potassium then the corresponding leaf blades, however, under 50 mM root zone salinity this trend was reversed particularly in the medium age leaves 4-8.

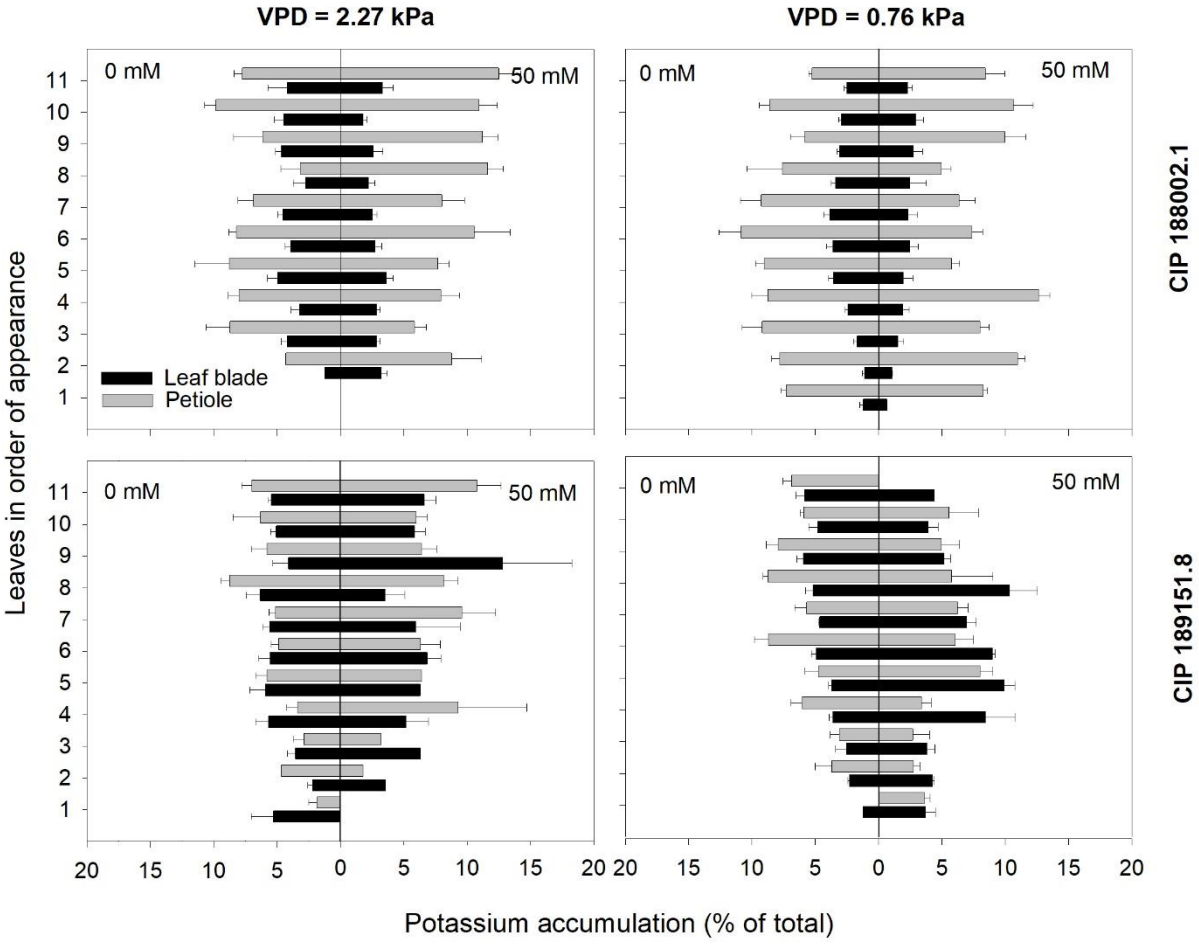

Figure 4.7 Percent share of potassium (K) in leaf blades and petioles (% of total) was plotted for different ages of leaf (leaf 1 to leaf 11) where leaf 1 was fully developed and 11 was the youngest one. Two genotypes of sweet potato, CIP 188002.1 and CIP 19151.8 were considered under two levels of rH subjected to 50 mM salt stress. Means ± SE, n=4.

Similar to sodium, not much chlorine was added to the 0 mM root zone salinity solution. Nonetheless, we found similar distribution and relative accumulation patterns as for sodium under 0 mM root zone salinity in both varieties. In contrast to sodium (Figure 4.6), relative chlorine accumulation was less affected by leaf position but more strongly distributed among the leaf organs in CIP 188002.1 (much larger share in the petioles than in the blades) as compared to CIP 189151.8 (Figure 4.8). The similar pattern was also observed under low VPD conditions. Varieties differed not strongly in the distribution of relative shares of chlorine between leaf blade and petiole under high VPD condition under 50 mM root zone salinity. In older leaves (2-5) of CIP 188002.1, a larger share of chlorine was accumulated in the petioles, and this was not found in CIP189151.8. Whereas a larger relative share of chlorine was accumulated in the blades of younger leaves which was more pronounced in CIP189151.8. Under low VPD conditions and 50 mM root zone salinity, however, a strong and significant contrast between the varieties was found in the accumulation of relative shares of chlorine between the leaf blades and petioles. In CIP188002.1, the relative share of chlorine in petioles was on averages 7.3% and thus about factor 2.7 times larger than in the blades whereas, in CIP189151, on average leaf petioles accumulated 5.3% of the total chlorine and leaf blades 6.1%, which is about 2.3 times more than in blades of CIP188002.1.

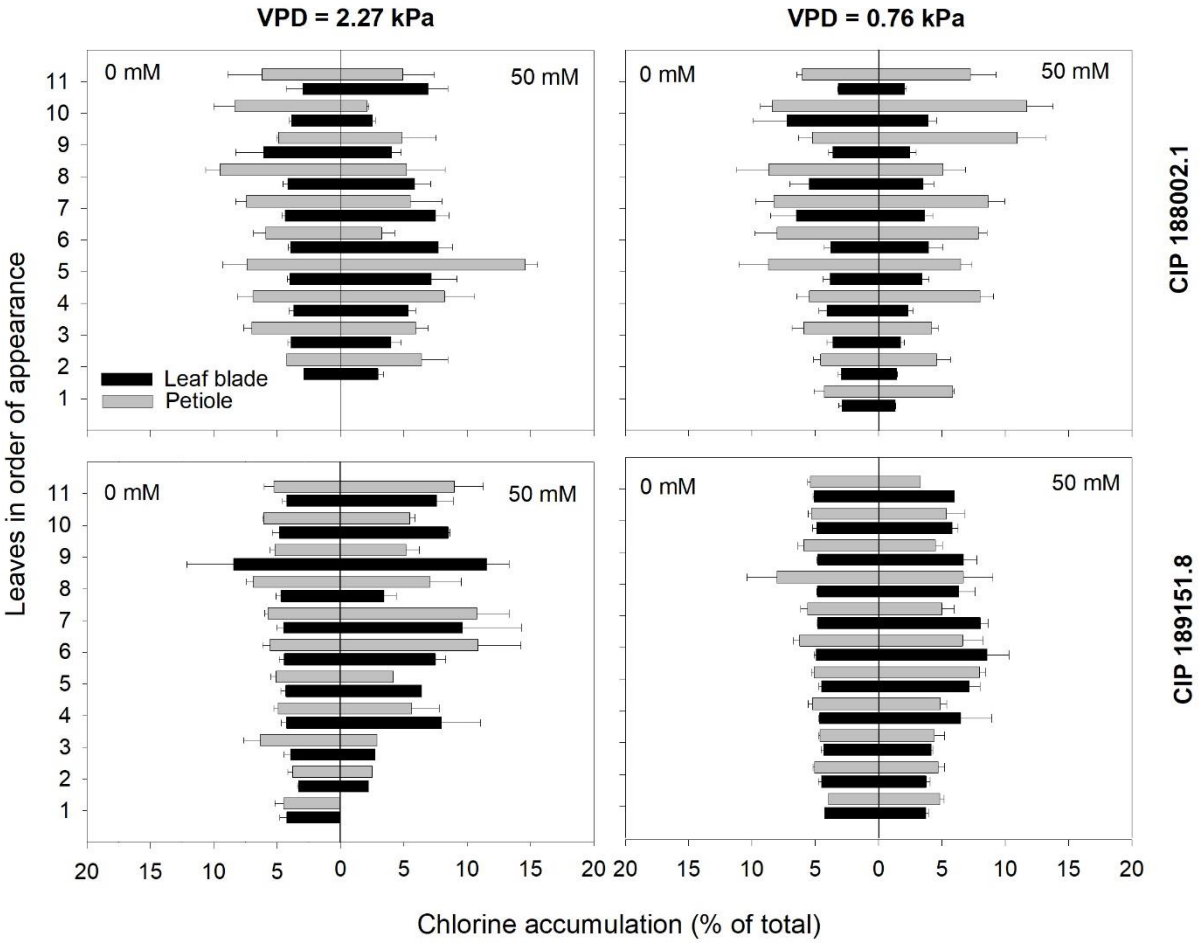

Figure 4.8 Percent share of chlorine (Cl) in leaf blades and petioles (% of total) was plotted for different ages of leaf (leaf 1 to leaf 11) where leaf 1 was fully developed and 11 was the youngest one. Two genotypes of sweet potato, CIP 188002.1 and CIP 19151.8 were considered under two levels of rH subjected to 50 mM salt stress. Means ± SE, n=4.

4.4 Discussion

Root zone salinity results in the accumulation of sodium and chlorine in plant tissues. Earlier work in rice shows that sodium is transported and taken up with the transpirational volume flow (Yeo et al., 1985; Asch et al., 1997b). Several barriers and pathways modifying the concentration of sodium and chlorine in the transpirational volume flow have been described and discussed, e.g. an apoplastic pathway for Na in rice (Yeo et. al., 1987) and an epidermal barrier for Cl combined with a cortex barrier for Na in durum wheat (Läuchli et al., 2008). In sweet potato, nothing is known to date about root traits mitigating sodium or chlorine uptake under root zone salinity conditions. Therefore, we have to rely on information obtained from other species to identify potential physiological traits related to salinity tolerance in sweet potato. It has been shown in rice (Asch et al., 1997a) and in soybean (An et al., 2001) that overall sodium accumulation is linked to water uptake, with weakly transpiring plants taking up less sodium than strongly transpiring plants. In sweet potato, we found large differences in transpirational water loss between the two VPD conditions but only small differences in sodium accumulation (Table 4.2), indicating no direct link between transpiration and sodium uptake. Which is in line with reports from other species e.g. Naito et al. (1994) found that higher transpiration reduced the amount of sodium carried by the transpiration stream of rice, Munns (1985) showed in barley plants that transpiration plays no role in Na uptake, and Katerij et al. (2009) found no significant relationship between sodium uptake and transpiration in wheat and barley under salinity.

In general, water loss from transpiring surfaces modified by some barriers and filters may drive overall sodium and chlorine uptake into the plant, however, individual leaf age and position may be determining the final amount of water lost from a given leaf surface and how much sodium or chlorine will be accumulated in that leaf (Tester & Davenport, 2003). We found that with increasing age, leaf stomatal conductance decreases (Figure 4.1), which results in smaller amounts of water passing through an aging leaf as compared to a newly developed one. Consequently, older leaves also transpired less than medium aged and young leaves but

only under high VPD conditions with no strong effect of root zone salinity on individual leaf transpiration, and this pattern was not found under low VPD conditions, where the youngest leaves showed the lowest transpiration rates (Figure 4.3). Yeo et al. (1985) described a strong effect of leaf position on sodium accumulation in rice that was inversely linked to transpirational water loss. They found under saline conditions the highest transpiration rate but the lowest sodium accumulation in the youngest leaf and, conversely, the lowest transpiration rates combined with greater accumulation of sodium in the older leaves. Older wheat leaves can contain 6 to 8 times more Na and Cl than the flag leaves, while transpiration, stomatal conductance, and assimilation were higher in young and active flag leaves (Sharma, 1996). Yasar et al. (2006) showed for green bean a preferential and higher accumulation of sodium in older leaves as compared to younger ones, but they did not investigate leaf transpiration. In the present study, we determined transpirational water loss from individual leaves of two sweet potato clones under 50 mM root zone salinity, and showed that the accumulation of ions (Na, K, Cl) in the respective leaves was not linked to the corresponding water loss (Figure 4.4). The relative shares of the different ions were similar across a wide range of leaf positions, whereas the medium age leaves (5-9) lost the largest share of water. The share of potassium and chlorine in individual leaves seemed to be strongly influenced by the sodium accumulation in the respective leaf.

Leaves are structured into a support part that links the leaf to the rest of the plant (petiole in dicot plants and leaf sheath in monocot grasses) and a leaf blade that transpires water and takes up carbon dioxide via the stomata. Under root zone salinity conditions, leaf sheaths of rice have been shown to accumulate more sodium than the leaf blades, thus protecting the blade from excess sodium loads (Asch et al., 1997c). The difference in leaf sheath sodium concentration and leaf blade sodium concentration decreased with increasing leaf age and varieties differed strongly in this respect. Wimmer and Asch (2005) investigated the relationship between individual transpirational histories of different leaf positions and the sodium uptake into these leaves. Using boron (B) as a marker, transpiration was found to

happen only in the leaf blades and not in the sheaths. Nonetheless, the sheaths contained more than 75% of all the sodium taken up into the individual leaves, clearly indicating the independence of sodium distribution from water transport, at least in rice. In the present study, the leaf petioles of the two varieties on average accounted for 25% (CIP188002.1) and 15% (CIP189151.8) of the respective individual leaf dry weight (Table 4.1). Since leaves differ strongly in size and weight depending on position and age, the individual share of a petiole in the overall leaf biomass is even smaller (Figure 4.5). Similar to the results from rice (Asch et al., 1997b, c), under 50 mM root zone salinity, sodium accumulated together with potassium to a large extend (in some cases more than 10% of total in one petiole) in the petioles of the leaves of the salinity tolerant variety, CIP 188002.1, thus, protecting the respective leaf blades from excess amounts of sodium (Figure 4.6 and 4.7). Across VPD conditions a total of about 60% to 70% of the sodium taken up by CIP 188002.1 was found in the petioles as compared to about 30% to 40% in the leaf blades. In contrast, CIP 189151.8 accumulated about 40% of the sodium in the petioles, as compared to about 60% accumulated in leaf blades. Varieties differed even more strongly in leaf potassium accumulation. Whereas under all treatments and atmospheric moisture conditions, tolerant CIP188002.1 accumulated the largest share of potassium in the petioles, sensitive CIP 189151.8 in general accumulated a smaller share of potassium in the leaf petioles and, under salinity and independent of VPD, accumulated up to four times as much potassium in the leaf blade as tolerant CIP 188002.1 (Figure 4.7). This is in line with our earlier results, showing that tolerant varieties can sustain much lower K/Na ratios in their tissues at high salinity levels than sensitive ones (Mondal et al., 2022). We calculated the K/Na ratio for different sections of the shoot and leaves (Appendix 4.2) and found in contrast to sensitive CIP189151.8, high K/Na ratios in the distal part of the shoot as well as in the younger leaves of tolerant variety CIP 188002.1. High K/Na ratios have been shown to indicate a high level of salt tolerance, e.g. higher leaf K/Na ratios reduce yield loss in lowland rice (Asch et al., 2000), wheat (Munns & James, 2003), and constitute a tolerance trait in barley (Chen et al., 2007). In sweet potato, not the ratio between potassium and sodium seems to be important but the amount of potassium stored in the tissue per se (Begum et al.,

2015; Mondal et al., 2022). Literature in general concentrates on sodium or chlorine as the detrimental elements when salt stress in plants is investigated (e.g. Greenway & Munns, 1980; Munns & James, 2003; Munns & Tester; 2008, Teakle & Tyerman, 2010), and although high tissue concentrations of potassium are generally seen as beneficial under salinity, potassium has not been much in the focus. Consequently, Tester and Davenport (2003) argued that the restriction of sodium transport to the leaf is the key determinant of salt tolerance in glycophytic plants, which was supported by Asch et al. (2000) for rice while arguing in favor of a high K/Na ratio. Munns, R. (1985) showed that in contrast to salt sensitive varieties, salt-tolerant barley varieties protect their younger leaves by reducing sodium uptake but found no effect of potassium. Munns, R. (1988) investigated sodium, chlorine, and potassium uptake and distribution in *Lupinus alba* to the level of ion concentrations in xylem sap passing through different tissues including the petiole and the mid rib of individual leaves. She found a linear increase of sodium and chlorine concentrations in petiole and midrib under increasing salinity, but low xylem concentrations for potassium in petioles and midribs. High transpiration rates seem to have deposited large quantities of sodium and chlorine into the leaf blade, whereas the fate of potassium remained unclear. Since Munns, R. (1988) did not investigate the accumulation of potassium in the petioles, we can only speculate if the constant concentration of potassium in the xylem flux was the result of active storage of potassium in the surrounding tissue. For sweet potato, we show here a strong varietal difference in potassium distribution between petioles and leaf blades and we argue that high accumulation of potassium in the petioles of CIP 188002.1 probably constitutes a salinity tolerance trait.

Chlorine has been shown to be the detrimental element in salt stress inhibiting photosynthetic activity or dry matter accumulation in crops such as citrus (Arbona et al., 2008), beans (Tavakkoli et al., 2010), barley (Tavakkoli et al., 2011), and strawberry (Barroso et al., 1997). Except for Mondal et al. (2022) there are no reports available for chlorine effects on sweet potato under salt stress. When comparing the distribution of the relative shares of sodium, chlorine, and potassium among the individual leaf petioles and blades (Figure 4.6, 4.7, and

4.8), no preferential accumulation of neither of these ions was found in sensitive CIP189151.8, neither for the petioles nor for the blades, no strong leaf position effect, and only a weak effect of root zone salinity. The picture is quite different for tolerant CIP188002.1, where we found a highly preferential accumulation of potassium in the leaf petioles for both treatments and the two atmospheric moisture conditions; A less but still pronounced preferential accumulation of sodium in petioles under salinity for both atmospheric moisture conditions and under non-saline conditions only at high VPD. Leaf position effects were rather visible in the share of sodium in the leaf blades than in the petioles. In contrast, for chlorine, we found a preferential accumulation in petioles, particularly in the younger leaves, under low VPD conditions for both salinity treatments, whereas under high VPD and 50 mM root zone salinity, chlorine preferentially accumulated only in the petioles of older leaves. These results indicate that potassium seems to play a major role in sequestering sodium in leaf petioles to protect leaf blades from excess sodium accumulation and that the anion chlorine may be helpful in maintaining balanced charges in the tissue but is not the key element for sequestering sodium in the petioles. Varietal differences manifest themselves mainly in maintaining potassium uptake and distribution under root zone salinity but not in transpirational effects on sodium uptake and distribution.

4.5 Conclusion

We investigated the relationship between transpirational water loss of individual leaves and their respective uptake of sodium, chlorine and potassium for two sweet potato varieties contrasting in salinity tolerance. Transpirational water loss was manipulated via atmospheric moisture conditions by establishing a high and a low VPD environment. VPD had a strong influence on total water uptake and transpiration in both genotypes, but sodium and chlorine differed little between the VPD environments. This clearly shows that transpiration is not the driving force for sodium and chlorine uptake under root zone salinity conditions. Linking the transpirational history of individual leaves to the relative share of sodium and chlorine

accumulated in them showed no direct link between leaf position, water lost from it and ions accumulated. Medium aged leaves lost most of the water, but did not differ from other leaves in sodium or chlorine accumulation. A detailed analyses of the distribution of the different elements according to leaf position and within them between petiole and leaf blade showed strong varietal differences in potassium distribution between petiole and leaf blade became evident. Tolerant variety CIP 188002.1 stored up to 5 times more potassium in the petioles than in the leaf blades under all conditions and up to twice as much sodium under saline conditions. Since this phenomenon was absent in sensitive CIP189151.8, we conclude that the leaf-internal management (between petiole and blade) as well as the leaf age dependent distribution of potassium (younger leaves accumulate more potassium and less sodium) constitute physiological traits of salt tolerance in sweet potato aiming at sequestering sodium. The actual transport processes needed to achieve this preferential distribution requires further research into the physiology of the leaf petioles.

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Data Avaiability Statement

Aditional data will made available by the author upon reasonable request.

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Appendix 4.1 Mean effect of dry weight (g), Na, K and Cl concentrations (mgg^{-1} dw) in control condition (50 mM root zone salinity) on leaves and petioles of two contrasting sweet potato clones. Values are ±Standard error, n=4.

		40% Air Humidity				80% Air Humidity			
VAR	Leaf	DW	Na	K	Cl	DW	Na	K	Cl
1	L1	0±0	0±0	0±0.00	0.00±0	0.08±0.02	3.31±0.47	12.96±2.52	20.64±3.47
1	P1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.05±0.01	5.03±0.95	118.55±22.47	41.93±5.16
1	L2	0.05±0.00	5.00±0.00	22.34±0.00	31.40±0.00	0.08±0.00	2.93±0.19	11.46±1.48	19.26±0.97
1	P2	0.02±0.00	26.92±0.00	100.98±0.00	93.60±0.00	0.07±0.00	6.22±1.70	89.20±8.67	33.39±4.13
1	L3	0.13±0.02	3.30±0.50	18.22±2.17	15.40±2.47	0.09±0.01	2.54±0.31	13.19±0.43	17.58±1.54
1	P3	0.07±0.01	8.19±1.22	69.36±6.63	50.67±7.48	0.07±0.00	4.21±0.40	84.92±8.06	32.89±2.50
1	L4	0.19±0.03	2.57±0.80	8.77±2.50	10.16±1.90	0.13±0.01	1.97±0.18	13.87±2.13	14.49±2.30
1	P4	0.10±0.01	5.25±1.01	39.53±9.83	33.55±5.46	0.07±0.01	4.16±1.10	89.59±7.36	34.90±3.12
1	L5	0.18±0.02	3.87±0.49	16.08±1.81	11.10±1.08	0.22±0.03	1.35±0.13	12.84±2.33	8.30±0.80
1	P5	0.07±0.00	5.64±1.99	56.48±16.71	50.66±13.62	0.09±0.00	3.79±0.30	70.97±7.94	39.66±4.77
1	L6	0.15±0.02	3.07±0.48	18.81±4.21	13.47±1.48	0.20±0.02	1.45±0.06	12.96±0.98	8.57±0.82
1	P6	0.07±0.00	5.73±2.40	59.02±6.91	45.18±9.10	0.09±0.01	6.00±1.83	87.56±10.29	38.69±2.49
1	L7	0.19±0.03	2.11±0.31	16.22±1.31	11.96±1.53	0.22±0.03	1.46±0.17	13.27±1.34	18.33±10.43
1	P7	0.09±0.02	15.35±10.30	47.08±3.32	41.94±3.70	0.10±0.01	3.38±0.45	70.40±7.41	39.33±5.23
1	L8	0.18±0.05	1.89±0.27	8.97±4.06	17.05±6.67	0.24±0.01	1.19±0.13	10.41±0.67	10.03±1.92
1	P8	0.09±0.02	7.95±1.48	55.12±33.43	69.26±18.57	0.10±0.01	2.72±0.56	45.71±15.65	34.30±5.57
1	L9	0.16±0.04	2.39±0.66	16.65±2.34	20.30±5.26	0.22±0.00	1.21±0.13	11.57±0.43	8.51±0.08
1	P9	0.07±0.02	6.90±3.38	46.09±19.59	42.21±12.08	0.08±0.00	3.75±1.08	63.24±14.36	36.16±9.26
1	L10	0.21±0.04	1.48±0.34	13.49±3.76	10.68±2.83	0.24±0.03	1.12±0.16	10.69±0.79	16.24±5.09
1	P10	0.10±0.02	4.79±0.61	45.98±6.26	39.44±1.46	0.10±0.01	2.91±0.06	79.62±3.24	50.34±2.09
1	L11	0.21±0.02	1.38±0.11	14.98±0.49	10.01±0.36	0.20±0.03	1.31±0.10	11.22±0.87	9.41±1.05
1	P11	0.08±0.02	7.45±3.43	63.85±12.12	60.03±14.69	0.08±0.01	2.19±0.11	60.22±7.62	44.07±1.72
2	L1	0.03±0.00	5.81±1.13	61.08±22.00	49.61±8.93	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	P1	0.02±0.00	6.25±0.52	31.384±11.14	79.57±13.23	0.03±0.00	0.00±0.000	0.06±0.00	43.81±0.00
2	L2	0.06±0.00	2.91±0.29	15.33±3.28	23.10±0.82	0.10±0.01	2.86±0.50	9.40±0.47	15.58±0.34
2	P2	0.01±0.00	19.24±11.14	137.36±0.00	122.20±6.21	0.03±0.00	6.98±0.373	56.24±23.97	61.10±2.55
2	L3	0.10±0.02	2.38±0.69	14.32±2.38	17.18±4.79	0.11±0.03	1.53±0.45	10.46±5.22	16.55±3.77
2	P3	0.03±0.00	11.26±1.93	31.533±6.31	71.21±15.43	0.02±0.00	5.15±1.589	52.52±3.63	70.00±10.16
2	L4	0.14±0.03	2.82±0.59	17.48±3.87	14.36±4.54	0.16±0.03	1.44±0.13	9.31±1.56	10.52±1.77
2	P4	0.03±0.01	8.56±3.11	41.345±11.09	64.57±14.45	0.04±0.01	4.55±0.749	70.78±14.57	57.39±17.01
2	L5	0.18±0.01	1.89±0.21	12.62±2.27	9.15±0.48	0.14±0.04	1.54±0.47	13.58±4.79	14.76±5.12
2	P5	0.04±0.01	6.75±2.32	55.295±9.84	52.36±3.68	0.04±0.01	4.07±0.816	52.29±5.93	53.96±10.48
2	L6	0.16±0.02	1.46±0.21	13.82±3.38	10.78±1.49	0.21±0.03	2.10±0.36	10.66±2.89	8.98±1.84
2	P6	0.04±0.01	9.27±2.64	42.355±2.28	48.52±2.04	0.05±0.01	4.77±0.683	80.92±11.94	51.33±8.47
2	L7	0.21±0.01	1.26±0.13	10.06±0.77	8.07±0.33	0.17±0.03	2.51±0.33	11.70±2.09	10.26±1.37
2	P7	0.05±0.00	12.32±2.56	41.215±4.06	46.94±3.50	0.03±0.01	5.89±1.752	89.77±30.46	69.56±11.72
2	L8	0.21±0.01	1.18±0.04	11.51±1.95	8.36±0.11	0.19±0.02	1.46±0.24	11.41±2.34	9.12±0.75
2	P8	0.06±0.01	6.46±1.56	56.479±2.20	45.81±6.25	0.04±0.01	3.93±0.095	108.09±24.89	98.22±48.35
2	L9	0.17±0.03	1.70±0.28	9.15±2.65	18.72±6.55	0.17±0.04	1.56±0.22	15.24±2.13	10.81±1.51
2	P9	0.04±0.00	3.88±0.39	53.653±11.60	47.68±4.80	0.04±0.01	4.78±0.788	98.82±19.31	62.68±9.56
2	L10	0.14±0.04	1.62±0.23	15.77±3.61	14.31±2.39	0.13±0.02	1.91±0.31	14.64±1.55	13.29±1.35
2	P10	0.04±0.01	10.00±1.50	67.425±29.43	65.43±9.21	0.03±0.01	12.97±8.991	107.31±21.97	80.41±12.45
2	L11	0.16±0.03	1.92±0.45	14.74±3.14	12.20±3.81	0.13±0.02	1.99±0.40	18.19±3.02	13.57±1.65
2	P11	0.04±0.01	9.62±1.65	75.339±10.07	60.20±16.57	0.03±0.01	5.00±1.294	122.61±26.42	79.50±9.88

Note: values are mean± standard error, n=4.

VAR = Variety: 1= CIP 188002.1 (tolerant), 2= CIP 189151.8 (sensitive). Chr.= Characters; L1= Leaf 1(Oldest), P1= Petiole 1(Oldest). DW = Dry weight (total).

Appendix 4.2 Mean K/Na ratio in upper, middle and bottom sections are determined in terms of molar basis in control and salt stress condition (50 mM root zone salinity) of two contrasting sweet potato clones linked to two different level of rH. Values are ± standard error, n=4.

		40% rH		80% rH	
VAR	Sections	K/Na-0	K/Na-50	K/Na-0	K/Na-50
CIP 188002.1	Upper	7.01	0.76	8.88	0.96
CIP 188002.1	Middle	3.14	0.39	1.87	0.42
CIP 188002.1	Bottom	3.60	0.36	3.20	0.41
CIP 188002.1	Upper	9.07	0.28	4.71	0.27
CIP 188002.1	Middle	5.69	0.35	8.45	0.30
CIP 188002.1	Bottom	4.42	0.37	3.26	0.36

Note: values are mean± standard error, n=4.

VAR = Variety: 1= CIP 188002.1 (tolerant), 2= CIP 189151.8 (sensitive); K/Na= Potassium Sodium ratio, Na-0=Control; Na-50= Salt stress; rH= Relative humidity.

Chapter 5

Salinity effects on the activities of ROS scavenging enzymes in leaves of two sweet potato clones

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Abstract

Sweet potato production, particularly in coastal areas is often prone to salinity. Salt-tolerant clones will be needed to maintain production, but to date little is known about salt tolerance traits in sweet potato. Salt stress may result in excessive uptake of unwanted ions into plant tissues leading to the formation of reactive oxygen species (ROS), which in turn may destroy membranes and reduce photosynthesis and growth. Antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), glutathione reductase (GR), and ascorbate peroxidase (APX) scavenge ROS and early changes in the activities of such enzymes could be used to identify salinity tolerant genotypes. Cuttings of two contrasting cultivars of sweet potato, BARI SP 8 (tolerant) and BARI SP 4 (sensitive) were greenhouse-cultivated in nutrient solution for 21 days and then exposed to 100 mM NaCl for seven days. Three, five, and seven days after salt application the youngest leaves were sampled individually and enzyme activities, potassium (K) and sodium (Na) concentrations, and SPAD (as a proxy for chlorophyll content) were determined. In both varieties leaf growth was not affected by salinity and young leaves grown under salinity had higher SPAD values than older leaves. Na concentration increased over time, particularly in earlier and in older leaves, whereas K was reduced in younger leaves. In general, enzyme activities were strongly affected by leaf age and leaf position. SOD and APX showed varietal but no salinity effects, CAT increased under salinity in both varieties, whereas POX was strongly reduced and GR was strongly increased under salinity in BARI SP 8 with no effect in BARI SP 4. Enzyme activities were not correlated to leaf Na, neither in relation to leaf age, nor leaf number or duration of salt stress in both varieties. However, varietal differences were observed regarding leaf K. Activities of SOD were highly positively and of CAT highly negatively correlated with leaf K under salinity in BARI SP 8 but not in BARI SP 4, whereas activities of GR and POX were strongly positively correlated with leaf K in BARI SP 4 under salinity but not in BARI SP 8. We conclude that potassium may have a strong regulating role on leaf stress levels and therefore on the activities of antioxidant enzymes. Varieties may differ in their tolerance strategy and we have shown that salinity does not generally increase levels of ROS-

scavenging enzymes in sweet potato leaves under salt stress. Confounding factors such as leaf age and leaf position as well as maintaining high leaf level K concentrations need to be considered when evaluating metabolic traits for salinity tolerance traits.

Keywords: antioxidant enzymes, leaf age, leaf potassium concentration, leaf sodium concentration, tolerance strategies

Key points

- Generally salinity does not increase level of ROS-scavenging enzymes in sweet potato
- The responses of antioxidant enzymes are overlaid by leaf position, leaf age, duration of salt stress and genotypes
- GR strongly increase in salt tolerant sweet potato genotype under salinity
- Leaf K concentration and SOD are strongly correlated in salt tolerant genotype under salt stress
- CAT can be increased in low K concentration in tolerant genotype under salt stress
- We are now not proposing a salinity screening tools regarding leaf level ROS scavenging antioxidant enzyme activities in sweet potato under salinity before investigating genotypic variation in the affinity of ROS scavenging enzymes to their respective substrates and the possibility of several strategic metabolic pathways

5.1 Introduction

Salt stress is known to induce reactive oxygen species (ROS) in plant cells (e.g. for tobacco, Banu et al., 2009; for Arabidopsis, Sofo et al., 2015; for rice, Kaur et al., 2016). Excess concentrations of ROS in plant cells commonly lead to metabolic disorders, cellular damage, premature senescence, or necrosis in leaf tissues (Møller et al., 2007; Jaleel et al., 2009; Miller et al., 2010 & Habib et al., 2016). Four types of reactive oxygen species are most harmful to plant tissues, namely, superoxide ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydroxide anion ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2).

Among other traits, enzymatic detoxification of salinity induced ROS is seen as a tolerance mechanism to salt stress. Five antioxidant enzymes, peroxidase (POX), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX), and superoxide dismutase (SOD) are essential for the first line of defence where $O_2^{\bullet -}$ is converted first to hydrogen peroxide (H_2O_2) by SOD in the chloroplast, cytoplasm, mitochondria, peroxisome, and apoplast (Bowler et al., 1992) and subsequently POX, CAT, and APX, detoxify H_2O_2 to H_2O (Mittler, 2002). Whereas CAT scavenges at high levels of H_2O_2, APX is more active at low levels of H_2O_2 (Mittler, 2002; Noctor & Foyer, 1998; Willekens et al., 1997). In addition, GR plays a central role in the cell against ROS by maintaining cellular reduction of oxidized glutathione (GSSG) to glutathione (GSH) involving the oxidation of NADPH (Contour-Ansel et al., 2006). The ascorbate glutathione cycle is mainly controlled by GR as it regenerates GSH, GSSG, MDHAR (Monodehydroascorbate) and DAR (Dehydroascorbate) using NADPH as electron donor.

In sweet potato, little is known about antioxidant enzyme activities under salt stress. Dasgupta et al. (2008) found that in an in-vitro culture subjected to 1% NaCl, SOD, GPX (Glutathione Peroxidase) and CAT increased in the leaves of tolerant sweet potato genotypes as compared to a non-stressed control. In cuttings of transgenic sweet potato clones Yan et al. (2016) showed that 100 mM NaCl stress induced the expression of CuZnSOD and APX leading to higher levels of antioxidant enzyme activity as compared to the wild type. Similar results were

shown by Kim et al. (2015) for SO_2 stress induced antioxidant activity in transgenic sweet potato plants overexpressing CuZnSOD and APX in chloroplast.

It has also been shown that potassium (K) not only significantly effects the yield of sweet potatoes (Tang et al., 2015; Wang et al., 2015) but also plays a vital role in activating most of the essential enzymes (Anjaneyulu et al., 2014; Erel et al., 2015) that reduce ROS in plants subjected to salt or water stress (Cakmak, 2005). Begum et al. (2015) found that K has an antagonistic effect with sodium in sweet potato genotypes subjected to salt stress, and recently, Mondal et al. (2022) found that K content is negatively correlated with the threshold for dry matter reduction for 12 sweet potato genotypes subjected to 0, 50, 100, and 150 mM NaCl stress.

The role of ROS detoxifying enzymes in salinity tolerance expression has been controversially argued in literature (Chen et al., 2005; Moradi & Ismail, 2007; Abogadallah, 2010; Parida & Jha, 2010). In sweet potato however, leaf level antioxidant activities as related to ion uptake and distribution in plants subjected to salinity have not yet been reported upon. We hypothesized that leaf-level antioxidant activities may be linked to leaf K concentration and act in concert in early detoxification of ROS under salt stress in salt-tolerant sweet potato genotypes.

5.2 Materials and methods

An experiment was set up in the greenhouse of the Hans-Ruthenberg Institute for Tropical Agricultural Sciences, University of Hohenheim, Germany. Two contrasting varieties, BARI SP 4 and BARI SP 8, were obtained from Bangladesh Agricultural Research Institute and grown in a greenhouse (27-30° C) to obtain vines for cuttings. Cuttings were cultivated in modified Yoshida nutrient solution (Yoshida et al., 1976), where iron was added as Fe-EDTA. Air was percolated through the nutrient solutions for 15 min per hour. Salt stress (0 and 100 mM NaCl) was initiated at 17 days after transplanting, which was also the day of the first sampling. Two

sets of plants were grown, set A was used to measure enzyme activities whereas set B was used to collect data on SPAD, fresh weight, dry matter, and tissue concentrations of Na^+ and K^+. At the day of salt application, the four youngest fully developed leaves were marked on all plants during the first sampling.

5.2.1 Sampling

The youngest fully developed leaf was defined as the second leaf from the vine tip and labelled as “leaf 4” at all plants, the next 3 older leaves were labelled “leaf 3-1”. The samplings took place at day 0, 3, 5, and 7 after salt application. Only fully developed leaves were sampled, so at each sampling date more leaves per plant were harvested. SPAD values (SPAD-502 Plus, Konika Minolta) were averaged for three positions on each leaf immediately before the harvest. The sampling scheme is illustrated in Figure 5.1.

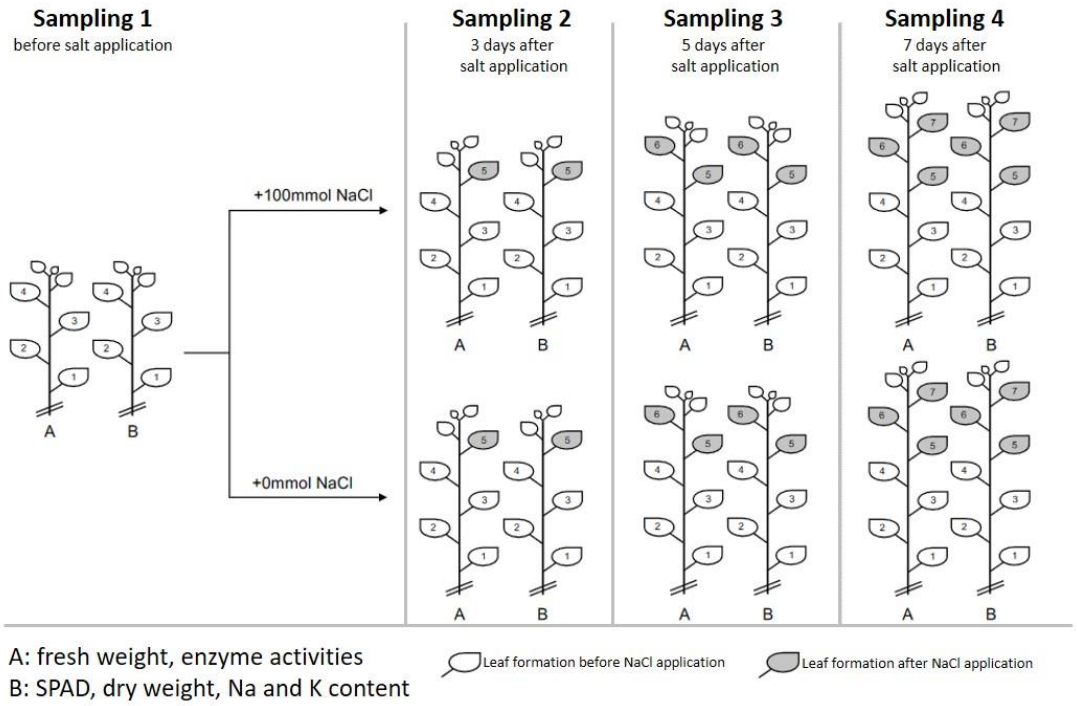

Figure 5.1 Sampling scheme. Data were collected on four different sampling days (0, 3, 5 and 7). The four youngest fully developed leaves were marked on all plants during the first sampling. The youngest fully developed leaf was identified as a second leaf in front of the shoot tip and the leaves were numbered in an ascending order towards the shoot tip. The four leaves were sampled at the first sampling time. Leaves of two plants per repetition (n=3) were sampled, one for the determination of enzyme activities, the other one for the determination of sodium and potassium content.

At day 0 only enzyme activities were measured, leaf concentrations of Na^+ and K^+ were not determined. A randomized complete block (RCB) design with three replications was used where each block shared the same air pump.

5.2.2 Enzyme activity analysis

All solutions and standards described here were prepared with pro-analysis grade chemicals (obtained from Sigma-Aldrich, Germany). Millipore ultrapure water was used in every step of sample preparation. Enzyme activity was determined spectrophotometrically (Infinite M200, Tecan, Männedorf, Switzerland) following established and widely used methods. All methods for the determination of the enzyme activities were adjusted from the originally published methods to facilitate the use of 96 well plates and a plate reader. For the extraction, approximately 0.02 g fresh material from the leaf central part (free of vascular bundles) was weighed and analyzed the same day to avoid any storage effect on enzyme activity (Hartmann and Asch, 2019). For extraction and sample dilution various concentrations of a Potassium Phosphate Buffer (PPB) consisting of a mixture of KH_2PO_4 and K_2HPO_4 adjusted with H_3PO_4 to pH 7 were used. CAT, SOD, POX, and GR were measured from the same extract, using 1 ml extraction buffer (50 mM PPB) + 1% PVP40 + 0.2 mM EDTA), whereas for APX, 5 mM ascorbate was added additionally. Samples were kept on ice until measurement. Prior to sample homogenization (Fast Prep-24, MP Biomedicals, Fisher Scientific GmbH, Schwerte for 60 s at 6 ms^{-1}) 0.2g ceramic beads (Preqlab, VWR, Germany) of diameters 2.8 mm and 1.4 mm were added. The homogenates were centrifuged for 5 min at 13000 $Umin^{-1}$ at 4 °C and the supernatant was used to determine enzyme activities.

APX activities were determined according to Nakano & Asada (1981). For this, 10 µL extract were added to 190µL reaction mixture (50 µL 200 mM PPB + 50 µL 0.8 mM ascorbate + 40 µL H_2O + 50 µL 3.6 mM H_2O_2). CAT and POX activities were determined according to Chance & Maehly (1955). For CAT, 20 µL extract were added to 180 µL reaction mixture (100 µL 200 mM PPB + 30 µL H_2O + 50 µL 40 mM H_2O_2). For POX, 20 µL extract were added to 180 µL

reaction mixture (50 µL 100 mM PPB + 50 µL 40 mM Guaiacol + 30 µL H_2O + 50 µL 200 mM H_2O_2). GR activities were determined according to Foyer & Halliwell (1976). For this, 20 µL extract were added to 170 µL reaction mixture (120 µL 50 mM PPB + 30 µL 1% BSA (Bovine Serum Albumin) solution + 10 µL 49 mM GSSG + 10 µL 3.6 mM NADPH). SOD activities were determined according to Giannopolitis & Ries (1977). For this, 20 µL extract were added to 180 µL reaction mixture (20 µL 0.5 M Na_2CO_3 + 20 µL 130 mM Methionine + 20 µL 1.3 µM Riboflavin + 20 µL 21 µM NBT (nitro blue tetrazolium) + 100 µL H_2O). For determination of SOD activity, the 96 well plate placed under a strong light source (LED growth lamp, spLED, Flensburg, Germany). After 60 s, initial absorbance was measured at 560 nm. The plate was exposed to the light treatment another 5 min before absorbance was measured again. Since the presence of SOD inhibits the reduction of NBT, the amount of inhibition can be used to quantify the enzyme activity as compared to a standard curve made with a commercially available standard (SOD standard S9697, Sigma Aldrich, Taufkirchen, Germany) (Hartmann and Asch, 2019). For determining the activities of APX, CAT, GR and POX plates were measured for 180s at 290, 240, 340, and 470 nm, respectively. The respective absorbance was plotted and enzyme activities calculated based on the slope.

5.2.3 Determination of Na^+ and K^+ concentrations

Samples were oven dried at 60°C for 72 hours, dry weight was recorded, and samples were milled to a fine powder (5 mm and 3 mm stainless steel balls). About 0.1 g of the sample material was weighed in 15 mL centrifuge vials (Roth GmbH& Co.KG, Karlsruhe, Germany) and 10 ml of deionized H_2O was added for the water extraction in an autoclave as described by Asch et al. (2022). Samples were autoclaved for 60 min at 120 °C and then centrifuged for 5 min at 4000 $Umin^{-1}$. The supernatant was made up to 100 mL in a volumetric flask with de-ionized water for further analysis. Sodium and potassium concentrations of the sample solutions were determined by flame photometer (Jenway, PFP 7, UK) whereas chloride

concentrations were measured with an Auto-Analyzer (Alliance Instruments, Freilassing, Germany).

5.2.4 Statistical analysis

Experiments were set-up as randomized complete blocks with three replications. Results are presented as means ± standard error. Both paired and unpaired t-tests were done using Microsoft Excel and regression analyses and graphs were done with Sigma Plot 14.0 (Systat Inc.).

5.3 Results

5.3.1 Plant growth and Sodium and Potassium concentration in leaves

Plants of 2 sweet potato varieties, namely BARI SP 4 and BARI SP 8, were subjected to 100 mM root zone salinity for one week. Each plant had four fully developed leaves at the beginning of the stress and 7 fully developed leaves at the end of the stress phase. Leaf growth was not affected by salinity (data not shown) and SPAD values were slightly but significantly ($p<0.01$) increased under salinity. In both varieties young leaves grown under 100 mM root zone salinity had higher SPAD values than older leaves and SPAD values of individual leaves increased with increasing leaf age (Figure 5.2).

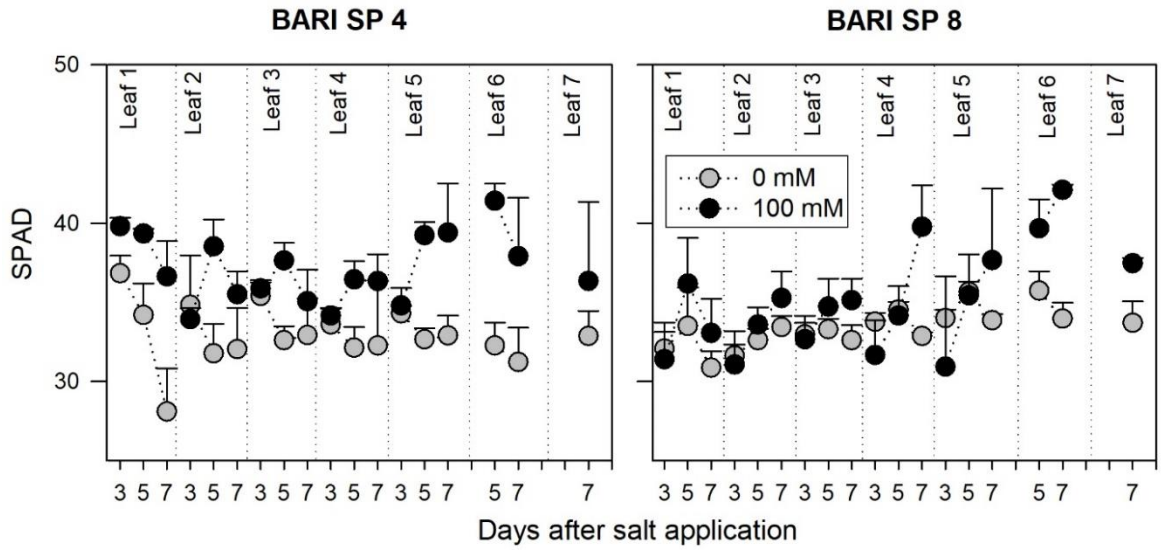

Figure 5.2 SPAD values of leaves of two hydroponically grown sweet potato varieties subjected to two levels of root zone salinity for 7 days. Leaves were sampled according to the sampling scheme presented in Figure 5.1. Error bars = ±SE, n=3.

Figure 5.3 shows the sodium and potassium concentrations in the leaves of both varieties for the two treatments over time. In both varieties salt stress increased the leaf concentrations of sodium as compared to the control. In BARI SP 8, sodium concentrations in the leaves increased little during the first 5 days of stress but then more than tripled over the course of the next 2 days in the oldest 4 leaves whereas sodium concentrations in the youngest leaves were only about half of the concentrations in oldest leaves (3 mgg^{-1} in leaf 6 as compared to about 6 mgg^{-1} on average in leaves 1 and 2). In BARI SP 4, a similar trend was observed, however, sodium concentrations increased sharply over the first 5 days of stress in leaves 1-4 but with increasing leaf age (except for leaf 3) balanced out at about 3 mgg^{-1} across all leaves. Potassium concentrations in leaves of BARI SP 4 stayed at control levels of about 55 mgg^{-1} in the leaves 1-4, but increasingly decreased in leaves newly formed under salt stress (5-7). In BARI SP 8, potassium concentrations in leaves 1-4 under salt stress were about 20% higher than under control conditions but decreased to levels below control values in youngest leaves. Under saline conditions, a clear gradient in potassium concentrations was observed from the oldest leaf (leaf 1, day 7, 65 mgg^{-1}) to the youngest leaf (leaf 7, day 7, 35 mgg^{-1}). With a similar gradient seen in final sodium concentrations in BARI SP 8 K/Na ratios stayed nearly

constant across all leaves, whereas in BARI SP 4 K/Na ratios decreased in the youngest leaves due to the almost constant sodium concentrations across all leaves.

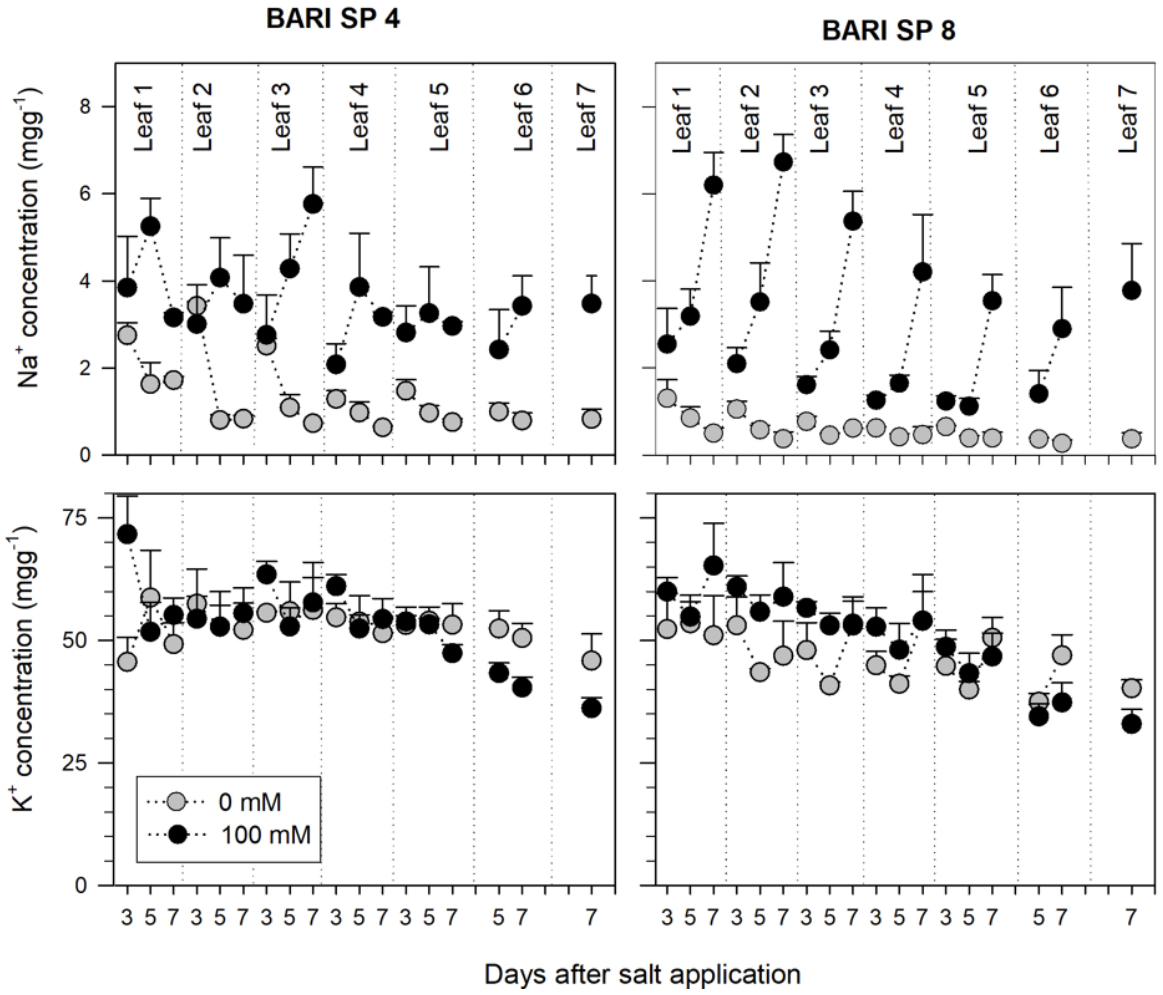

Figure 5.3 Sodium and potassium concentrations of leaves of two hydroponically grown sweet potato varieties subjected to two levels of root zone salinity for 7 days. Leaves were sampled according to the sampling scheme presented in Figure 5.1. Error bars = ±SE, n=3.

5.3.2 Antioxidant enzyme activities

Activities of five antioxidant enzymes i.e., POX, CAT, GR, APX, and SOD were determined at three, five, and seven days after plant exposure to 100 mM root zone salinity and compared to activities under non-stressed conditions. SOD activities varied between 0.075 and 0.15 Ug^{-1}_{FW} in both varieties under both conditions and for all leaves (data not shown). Figure 5.4 shows the activities of POX, CAT, GR and APX for all leaves under non-stressed control conditions and under salt stressed conditions for both varieties.

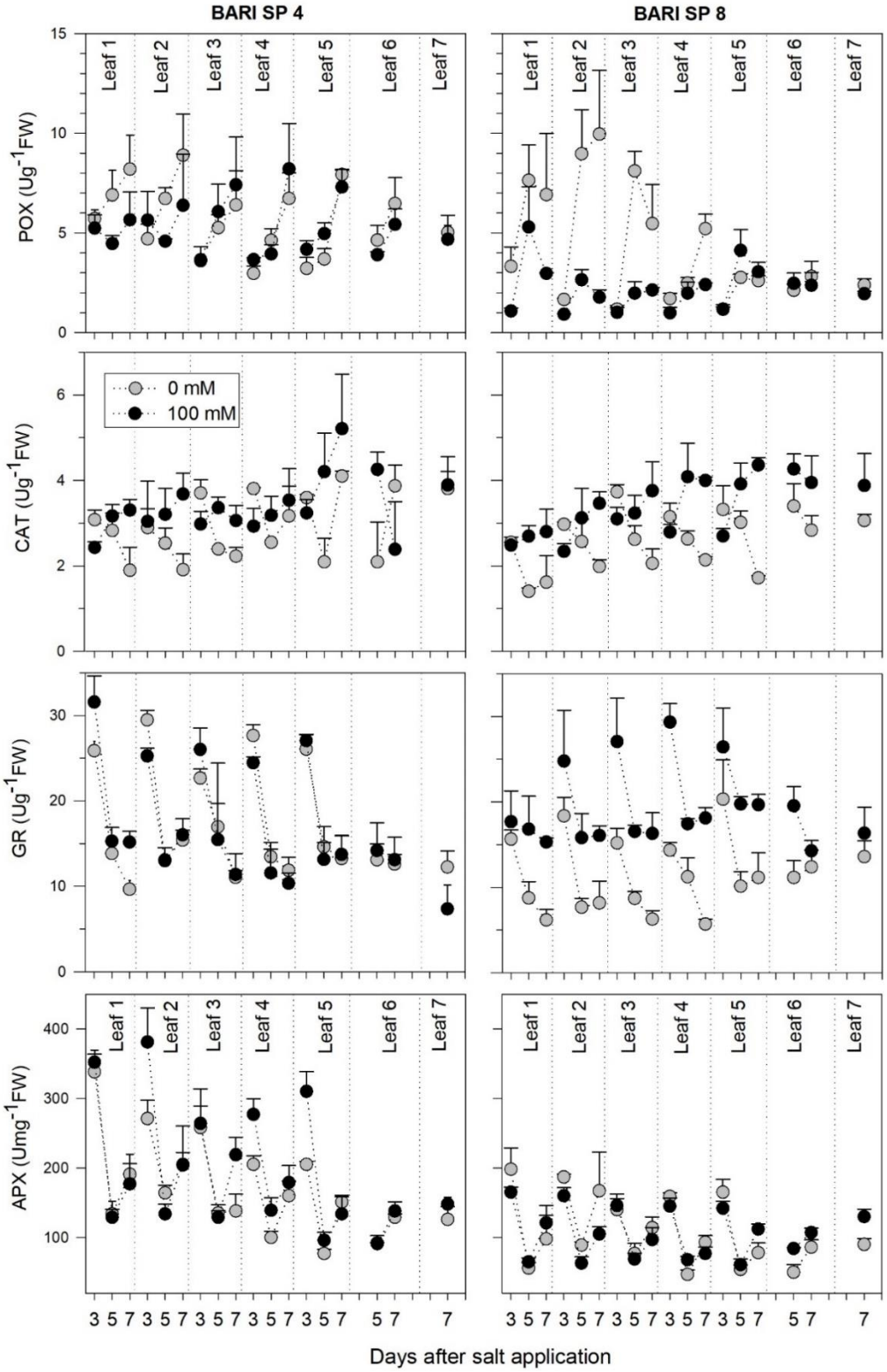

Figure 5.4 Activities of peroxidase (POX), catalase (CAT), glutathione reductase (GR), and ascorbate peroxidase (APX) of leaves of two hydroponically grown sweet potato varieties subjected to two levels of root zone salinity for 7 days. Leaves were sampled according to the sampling scheme presented in Figure 5.1. Error bars = ±SE, n=3.

In contrast to SOD, activities of the antioxidant enzymes POX, CAT, GR and APX varied between varieties and treatments and often showed an influence of leaf age on activity. POX increased with leaf age in both varieties under control conditions. About 4 Ug^{-1} in BARI SP 4 and about 5.4 Ug^{-1} on average in BARI SP 8 in leaves 1-4. Under saline conditions POX activities in leaves of BARI SP 4 were at control levels whereas in BARI SP 8 POX activity in leaves 1-4 was strongly reduced as compared to the control but was at control levels for leaves 5-7. CAT activities varied roughly between 1 Ug^{-1} and 4 Ug^{-1} for all leaves treatments and varieties. However, in both varieties with increasing age of individual leaves CAT activities decreased under control conditions but increased under saline conditions for all leaves but for 6 and 7. GR activities generally decreased with increasing age of individual leaves in both varieties and under both treatments. However, under salinity GR activities were significantly ($p<0.05$) increased by more than 70% compared to the control in BARI SP 8 in all leaves except for 6 and 7, whereas no salinity effect on GR activities was observed in BARI SP 4. APX activities differed strongly between the varieties in magnitude but followed a similar pattern of initially higher values followed by a strong decrease and then again, a sharp increase over time in the individual leaves. The amplitude of this pattern decreased with increasing leaf number but no salinity effect was observed in either variety. APX activities were about 1.5 to 2 times higher in BARI SP 4 than in BARI SP 8.

5.3.3 Leaf ion concentrations and antioxidant enzyme activity

Leaf sodium concentrations and antioxidant enzyme activities were neither correlated in relation to leaf age, nor leaf number or duration of salt stress (correlations not shown). The only exception of this was a significant ($p<0.05$) negative correlation between the activities of GR and the sodium concentration from the youngest to the oldest leaves in which GR activity was reduced by up to 50% whereas sodium concentration increased by factor 6 (data not shown). Figure 5.5 shows the correlations between leaf potassium concentrations and leaf

antioxidant enzyme activities for five enzymes and two varieties under saline and non-saline conditions.

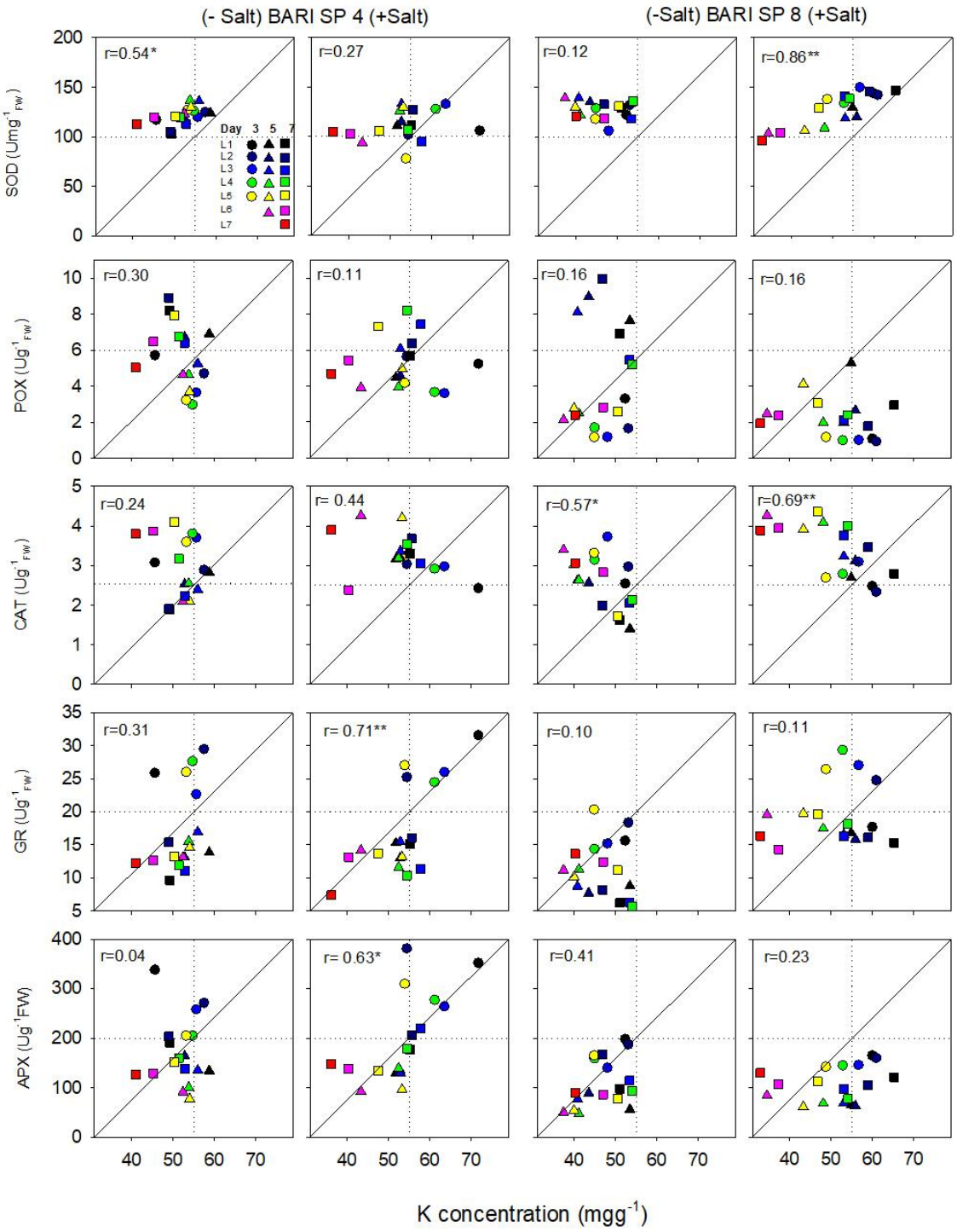

Figure 5.5 Potassium concentration of leaves of two hydroponically grown sweet potato varieties subjected to two levels of root zone salinity for 7 days regressed against the respective activities of superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), glutathione reductase (GR), and ascorbate peroxidase (APX) under saline and non-saline conditions. Circle, triangle and square symbols indicate three, five, and seven days of salt exposure for individual leaves differing in age. For better readability error bars were omitted. N = 3.

Root zone salinity treatments resulted in genotypically different responses of SOD activities to leaf potassium concentrations. Whereas there was a significant positive correlation ($r=0.54$, $p<0.05$) under non-saline conditions and no significant correlation between leaf K concentrations and SOD activities under saline conditions in BARI SP 4, in BARI SP 8, in contrast, the opposite was observed with a highly significant positive correlation ($r=0.86$, $p<0.01$) between leaf K concentration and SOD activities under saline conditions. For POX none of the enzymatic activities under any treatment were correlated with leaf potassium concentrations in neither variety. Genotypic differences were found for the activities of CAT. Whereas no correlation with leaf K concentration was observed for neither treatment in BARI SP 4, in BARI SP 8 significant ($r = 0.57$, $p<0.05$) and highly significant ($r = 0.69$, $p<0.01$) negative correlations between CAT and leaf K concentrations were found under non-saline and saline conditions, respectively. Leaf K concentrations were highly significantly correlated with GR and APX activities under root zone salinity of 100 mM in BARI SP 4 ($r = 0.71$, $p<0.01$ and $r = 0.63$, $p<0.05$). Highest GR, APX, and K values were found in early measurements of leaves 1-5 and lowest values in the final measurements on day seven of salinity treatment in leaves 5-7. On the other hand, no significant correlation was found for non-saline conditions, nor for either treatment and both enzymes in BARI SP 8.

5.4 Discussion

5.4.1 Salt stress effects on plant health

The salt stress plants were subjected to this study, was intentionally short (only 7 days) and with 100 mM root zone salinity just above the sweet potato's threshold of 75 mM (Mondal et al., 2022). Since ROS de-toxification is known as the first line of defence against abiotic stress, we expected to see strongest enzymatic activities during the early phase of the stress when all other inhibitions and injuries caused by excessive sodium accumulation (Hossain & Delowar, 2014; Begum et al., 2015; Evoi et al., 2017) did not yet occur. During the duration of

the stress, leaf sodium accumulation with maximum values of 6-7 mgg^{-1} dry matter was moderate but significantly higher than in leaves grown under non-stress conditions in both genotypes (Figure 5.3). E.g. root zone salinity of 100 mM for three weeks resulted in leaf sodium concentrations between 24 mgg^{-1} and 78 mgg^{-1} in a spectrum of 12 genotypes (Mondal et al., 2022). Similarly, salinity did not affect leaf dry matter in either genotype but moderately increased SPAD values, particularly in the youngest leaves (Figure 5.2), indicating no limitations in N uptake or photosynthesis (Asch et al., 2000). Except for leaves 6 and 7 in BARI SP 4 where potassium concentrations decreased by 10-20%, potassium concentrations in leaves of plants subjected to 100 mM root zone salinity in general remained at control (0 mM root zone salinity) levels (Figure 5.3) at about 45 to 55 mgg^{-1} indicating an adequate supply of K to the leaves (Lv & Lu, 2021).

5.4.2 Activities of antioxidant enzymes

Antioxidant enzyme activities have been studied widely in relation to plant abiotic stress responses, including salinity, in many crop genera (e.g. Parida & Jha, 2010 (*Salicornia*); Fan et al., 2014 (*Hordeum*); Habib et al., 2016 (*Abelmoschus*); Kaur et al., 2016 (*Oryza*)).

For sweet potato, information on antioxidant enzymes, their activities, their triggers, and their effects on tolerance to abiotic stress is scarce. Adamski et al. (2012) determined activities of SOD, CAT, and APX in leaves of a non-described sweet potato clone subjected to different levels of iron toxicity for two stress durations in a hydroponic system. Activities were reported as product obtained per time and total leaf protein. Since total leaf protein was not reported, a direct comparison to enzyme activity levels reported here, is not possible. Nonetheless, in their study activities of APX doubled under tripled iron levels independent of stress duration, the same was found for SOD under long stress duration and no change in activity was found for CAT. In contrast, we found a different pattern in leaf level CAT activities under salt stress and also pronounced differences between cultivars. In BARI SP 4, CAT activities after 7 days of salt stress increased by about 50% in the oldest 3 leaves but no clear salinity effect was

present in the younger leaves. In contrast, in BARI SP 8, CAT activities almost doubled in the oldest 5 leaves and were elevated by at least 25% in the youngest leaves. SOD activities remained at constant levels all through our experiment and APX decreased with time and leaf age independent of salt stress. Wang et al. (2019) reported on antioxidant enzyme activities in roots of two sweet potato clones stored for various lengths of time under low and normal temperatures. Compared to our results in leaves, activities reported for APX and CAT were lower by a factor 5, SOD by a factor 2 and GR by a factor 10, respectively, indicating strong differences in antioxidant enzyme activities among plant organs. Under chilling, APX activities rapidly and permanently increased by about 70% in both varieties, whereas CAT and SOD activities initially increased sharply but regained control levels after about two weeks. These results are in strong contrast to our results in leaves under salt stress. The only two rapid responses to salt stress were found in BARI SP 8 with a strong early reduction in the activities of POX and a strong early response in GR activities in the medium aged leaves that lasted only for about 3 days. Liu et al. (2017) reported for two sweet potato clones genotypic differences in elevated activities of POX, CAT, and SOD under potassium deficiency. However, values were given in U $mg_{protein}^{-1}$ without reporting total leaf protein content which renders a direct comparison of enzyme activities impossible. Finely, Lin & Pu (2010) reported on APX, SOD, CAT, and GR activities in leaves of three soil-grown sweet potato clones subjected for either 24 or 48h to 4 levels of root zone salinity. SOD may be a critical component of salinity tolerance since it has been shown to protect chloroplast functions under high levels of salinity (Singh, 2022). Similar to the current study, Lin & Pu (2010) found no effect of genotype or salt treatment on SOD activities, indicating that H_2O_2 levels in the tissue were not the result of SOD activity. These results are in line with data from salt-stressed cucumber reported by Lechno et al. (1997). Under, for sweet potato (Mondal et al., 2022), extremely high salinity levels (450 mM), Lin & Pu (2010) found elevated levels of APX activities which were linked to secondary water stress levels due to the negative osmotic potential at the root surface. In contrast, GR activity levels did not increase which was to be expected if water stress was the cause for the APX response (Gamble & Burke, 1984). Root zone osmotic

potentials in the current study did not lead to secondary water stress in sweet potato, which could explain why there was no link between the APX activities and salt stress. In general, CAT activities increase with level and duration of salt stress (Lechno et al., 1997 for cucumber; Lin & Pu, 2010 for sweet potato), but the slope of the increase may be genotype dependent (Lin & Pu, 2010), which is in line with what we found in the current study.

The effects of salinity on the oxidative defense mechanism in plants have been reviewed recently by Singh (2022). Although a large number of studies have investigated the link between salinity tolerance and the activities of SOD, APX, CAT, POX and others, as well as the expression of genes coding for those, no clear link between the level of activity or expression and the level of salt tolerance has been established in crops. Particularly for sweet potato, the number of studies available is too small to come to any valid conclusions yet. For wild halophytes on the other hand, Ghanem et al. (2021) recently proposed two different strategies to cope with the saline environment: one based on the activation of antioxidant enzymes and biosynthesis of proline and the other one based on the biosynthesis of antioxidant compounds such as carotenoids, phenolics, and flavonoids which may need less energy than needed activating antioxidant enzymes. This implies that a wider array of metabolic compounds needs to be taken in consideration when trying to identify physiological traits of salt tolerance in crops.

In the present study, the activity of antioxidant enzymes in contrasting clones subjected to 100 mM salt for 7 days was not linked to salinity tolerance levels established earlier (Mondal et al., 2022) for BARI SP 4 (sensitive) and BARI SP 8 (tolerant). Only in BARI SP 8 under salt stress conditions activities of GR and CAT exceeded activities under non-stressed conditions in most of the leaves. The swift and early increase of GR activity in leaves indicates a potential salt tolerance trait as an early defense mechanism since in BARI SP 4, as the salt sensitive genotype, GR activities did not change under salinity. Similar to the current study, Panda & Khan (2009) investigated responses of antioxidant enzymes to short term salinity in *Vigna radiata* and found GR activity significantly increased but not the activities of SOD, CAT, and

APX. GR is a crucial enzyme in the GR-APX cycle at the thylakoid membrane in Photosystem I (Asada, 2006) protecting leaf tissue against oxidative damage (Foster & Hess, 1982; Gamble & Burke, 1984) by supplying electrons through the reduction of oxidized glutathione to reduced glutathione, thus, scavenging H_2O_2 in the cycle of GR-APX via oxidation to glutathione regeneration of GR using NADPH (Noctor & Foyer, 1998 & Hasanuzzaman et al., 2012). GR and APX have been discussed as fine regulators at relatively low levels of H_2O_2 in the cell, whereas CAT, with its lower affinity to H_2O_2, increases activities at higher stress levels (Asada, 1992; Willekens et al., 1997; Mittler, 2002). In view of this, one should expect during early stress phases, when the stress related H_2O_2 concentration in the cells should still be low, increased activities of GR and APX and no or only little change in CAT activities. In contrast, in the current study, CAT activities were slightly increased in the leaves of the sensitive genotype BARI SP 4 whereas GR and APX activities remained at non-stressed levels. In the tolerant genotype, however, CAT activities where significantly increased after 7 days of salt stress, particularly in the younger leaves, GR activities were significantly increased after 3 days of salt stress particularly in the older leaves, and no response in APX activities was observed. These results imply that affinity levels of enzymes to their respective substrates may be genotype specific and additionally influenced by third factors, such as individual leaf age or leaf position.

The role of POX activities in salt stressed plants remains unclear. We determined POX as guaiacol peroxidase. The heme-containing guaiacol peroxidase reduces surplus H_2O_2 during the normal metabolism as well as in stressed (Das & Roychoudhury, 2014). Whereas, in the current study, POX activities in the sensitive genotype were similar under stressed and non-stressed conditions and tended to increase with leaf age, in the tolerant genotype POX activities in the older leaves were strongly suppressed but increased with leaf age under non-stressed conditions (Figure 5.3). This indicates leaf-level POX activities may not be linked to salt stress but rather to leaf age, confirming an earlier finding in salt stressed barley by Fan et

al. (2014) who showed no salinity effect on POX activity but a strong correlation with leaf age across all treatments.

5.4.3 Leaf ion concentrations and antioxidant enzyme activities

Root zone salinity results in uptake of sodium into plant tissues, often driven by the transpirational volume flow (Wimmer & Asch, 2005). Therefore, strongly transpiring tissues such as actives leaves are often more threatened by high sodium concentrations. In maize and rice, leaf K concentrations decreased as Na concentrations increased under root zone salinity of 100 mM and 40 mM respectively (Asch et al., 1997; Abdelgawad et al., 2016). Whereas no competitive relationship between K and Na was found in sweet potato leaves, the capacity of maintaining high tissue concentrations of K determined the genotypic tolerance levels independent of the sodium uptake to the leaves (Mondal et al., 2022). Both K nutrition and K tissue concentrations have been shown to greatly affect growth and yield in sweet potato (Wang et al., 2015; Lv & Lu, 2021). AbdElgawad et al. (2016) have shown for maize that higher leaf Na concentrations were correlated with increased antioxidant enzyme activities and Che et al. (2002) have shown in tobacco that K can improve plant salt tolerance by alleviating oxidative damage induced by high sodium concentrations in the leaves and regulating hormone signal transduction to influence transpiration and photosynthesis. In the current study we did not find any correlation between sodium concentration or sodium content in leaf tissues of any age and any of the antioxidant enzyme activities in neither genotype (data not shown). The role of potassium as an important osmolyte has often been discussed as important in the plants' antioxidant defense system (Ahanger et al., 2017). However, K concentrations in leaves were differently affected by salinity among the genotypes as well as their effects on antioxidant enzyme activities. K concentration were generally high in older leaves as well as in leaves that had been exposed to salt stress for only a short time, independent of leaf age. Younger leaves tended to have lower K concentrations than older leaves (Figure 5.2). When correlating these concentrations with the different antioxidant

enzyme activities, in tolerant BARI SP 8, a strong positive linear correlation ($p<0.01$) between leaf K concentration and SOD activities was found under salt stress conditions with young leaves having lowest K concentrations and lowest SOD activities (Figure 5.5). These results are in line with observations of Lechno et al. (1997) who showed that salinity generally decreased SOD activities relative to non-stressed controls in cucumber. They also showed a strong increase in CAT activities that was mitigated by high external K concentrations. In the present study, CAT activities were in all cases negatively correlated with leaf K concentrations. Consequently, in BARI SP 8 highest CAT activities were found under saline conditions in the youngest leaves with the lowest K concentrations. It is not clear, however, if the CAT activities were higher because of the lower K concentrations in the youngest leaves or due to salt stress in the tissue, which could explain the negative correlations of CAT activities with leaf K concentrations in salinity tolerant BARI SP 8 under both root zone salinities. A strong effect of leaf potassium concentrations on the activities of GR ($p<0.01$) and APX ($p<0.05$) were found in BARI SP 4 under salinity (Figure 5.5). Particularly younger leaves during the early stress stages showed elevated GR and APX activities which subsided with decreasing K concentrations and stress duration. None of these effects were observed in BARI SP 8. Similar results were shown by Ahanger & Agarwal (2017) in wheat under drought stress, who reported a strong effect of K on antioxidant activities in general and in particularly a strong increase in the activities of GR and APX in one of two varieties. In the other variety APX and GR activities were significantly lower or not elevated at all as compared to the fully watered control, however, in this variety free amino acids and free sugars were strongly increased and this increase was strongly correlated with K nutrition. In view of the recent suggestions by Ghanem et al. (2021), one can speculate about genotype specific stress tolerance mechanisms of which one involves a stronger defense via detoxification of ROS, whereas others may involve completely different metabolic compounds. For sweet potato to date, reports on metabolic responses to abiotic stresses are generally scarce and studies involving several genotypes at varying stress levels with multiple observations on the same organs over the stress duration basically do not exist.

5.5 Conclusions

We reported here on the responses of antioxidant enzymes in successive leaves of two sweet potato genotypes contrasting in salt tolerance subjected to short term moderately severe root zone salinity. Responses of antioxidant enzymes in general were not related to salt stress alone, but modified or overlaid by effects of leaf position, leaf age, duration of the stress, and genotype. The results clearly show that the leaf sodium load of plants grown under salinity was not the trigger or the cause for any of the changes observed in the activities of the antioxidant enzymes. Comparison of the current results with other studies on sweet potato is rendered difficult due to the limited number of studies available and an incompatibility of data due to incomplete data sets. However, when the current results were seen in light of data obtained under abiotic stresses other than salinity (i.e. drought, iron toxicity or K deficiency), it became evident that the same antioxidant enzymes respond differently when plants were subjected to different stresses. This may be partly confounded by differences in experimental conditions and genotypes, however, we believe responses of antioxidant defense mechanisms in sweet potato cannot be generalized across abiotic stresses but should be further investigated for the individual stress situations. In terms of potential traits for salt tolerance, our results suggest the swift and early increase in GR activities observed in BARI SP 8 as a candidate. In concert with elevated leaf K concentrations, an increase in SOD activities could be triggered in older leaves that maintain high K concentrations to protect their photosynthetic activity. Along the same line, the observed increase in CAT activities in younger leaves of BARI SP 8 low in K could indicate a strategy to protect younger, still growing leaves from damage to membranes and other cell constituents. However, before these results could be developed into an effective screening tool, confounding factors such as genotypic variations in the affinity of ROS scavenging enzymes to their respective substrates and the possibility of several strategic metabolic pathways need to be cleared. We further believe to fully understand how salinity tolerance manifests itself on a metabolic level in sweet potato a larger set of substances including proline, phenols, or free amino acids as well as some

hormones such as ABA and auxins should be included in the analyses across a wider range of genotypes to identify different tolerance strategies.

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Data Avaiability Statement

Aditional data will made available by the author upon reasonable request.

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Chapter 6

General discussion

For better understanding of the physiological mechanisms of sweet potato that may contribute to the plants' salt tolerance, we investigated the threshold with specific ion effects; ion uptake and distribution, and antioxidant enzyme activities subjected to salt stress. Our ultimate goal was to develop a quick, reliable, and suitable screening tool to evaluate salt tolerance mechanisms in sweet potato, taking into account phenotypic attributes and physiological processes. To achieve the goals, a total of twelve sweet potato genotypes, watered with different mM concentrations of NaCl, were considered for screening by determinging genotypic threshold (dry matter accumulation) and genotypic slope (slope for dry matter reduction) when the root zone salinity (RZS) increased beyond the genotypic threshold. We also investigated how the threshold could be related to different ions. Subsequently, two genotypes (tolerant and sensitive) were chosen and used to develop a better understanding of Na, K and Cl uptake and distribution. This may lead to more ideas on salt tolerance mechanisms in sweet potato. We also investigated general antioxidant effects in relation to varied ion concentrations in two contrasting genotypes (tolerant and sensitive) of sweet potato, pursuant of further understating of ROS effect in the photosystem that may obstruct the photosynthesis activities in thylakoid membrane.

6.1 Salinity screening tools in sweet potato

So far, most of the researchers mainly focus on the salinity stress responses of cereal crops such as rice, corn, wheat and barley, since they are used as food crops worldwide. But sweet potato also an important crop, and developing genotypes with high yied potential is an important step in boosting its productivity in situations of salt stress. For the development of reliable screening tools on this issue, salt tolerance mechanisms is obviously important to identify the salt tolerant and salt sensitive genotypes. To achieve this aim, identification of salt

tolerance mechanisms is key to identify the salt tolerant and salt sensitive genotypes. To this end, we analyzed the genotypic threshold for the 12 sweet potato genotypes subejected to 0, 50, 100, and 150 mM salt stress (three weeks) in a hydroponic system.

6.1.1 The threshold for salinity damage is at 75 mM RZS in sweet potato

Threshold salinity in sweet potato is a variable phenomenon and can vary from 15 mM to 100 mM NaCl root zone salinity. Many authors found that the threshold value for sweet potato root zone salinity can be 15 mM (Jan et al., 2000), 30 mM (O'Sullivan et al., 1997), 35 mM (Rodríguez- Rodríguez-Delfín et al., 2012), 80 mM (Begum et al., 2015), 90~120 mM (Rahaman et al., 2015), 100~120 mM (Abdullah-Al-Mahmud & Akhter, 2018). The authors found that the range of 80~120 mM salinity threshold was responsive only for tolerant sweet potato genotypes. However, the different genotypic thresholds of sweet potato depend on the culture medium, environmental conditions, salt stress duration, salt amount, and genotypic potential, etc. Evoi et al. (2017) found different thresholds for the different salinity levels in soil medium treatments and Begum et al. (2015) in soilless (hydrophonic) crops. It is not only the case in sweet potato; Tavakkoli et al. (2010) showed that barley plants respond differently to salinity in hydroponics and in soil.

In this study, we have estimated the genotypic threshold for 12 sweet potato genotypes subjected to 0, 50, 100, and 150 mM NaCl stress (21 days) is 75 mM NaCl (Figure 3.2) in a hydroponic system (Mondal et al., 2022). The genotypic threshold was calculated from dry matter accumulation started to decrease by the effect salinity and the dry matter accumulation per 50 mM NaCl increase (slope) in root zone salinity after that slope values were plotted against each other (Figure 3.2, Appendix 3.2). Anwar et al. (2010) found similar threshold concentration in an in vitro salinity screening system using 0, 25, 50, 75, 100, 125, 150, 175 and 200 mM NaCl for phenotypic analysis of sweet potato. He found that at 75 mM NaCl, a gradual negative effect on sweet potato phenotype began. But, he did not show the reason behind 75 mM threshold in sweet potato. However, Jan et al. (2000) estimated that sweet

potato may experience 50% yield loss at 60 mM (6 dSm^{-1}) stress, with a threshold of 15 mM NaCl. Except for Jan et al. (2000), most researchers found that the threshold for salinity in sweet potato was between 30 and 100 mM NaCl. This information is very important because very few research articles have been published regarding the salinity threshold on the reduction of total biomass of sweet potato. Munns and James (2003) argued that biomass yield is directly related to economic yield in most crops under saline conditions. Thus, our results (75 mM threshold) provide farmers with information on where to grow sweet potato to avoid risks of economic yield loss. The comprehensive salinity threshold is also useful for developing appropriate salinity screening tools.

6.1.2 K content in sweet potato: A suitable screening trait for tolerance to salinity

K plays a major role ameliorating most abiotic stresses such as drought (Nieves-Cordones et al., 2017), salinity (Assaha et al., 2017), cold (Cakmak, 2005), waterlogging (Xiong et al., 2013), heavy metal toxicity (Dhiman et al., 2022), and so on. In addition, K appears to be a major element for most enzyme activations, protein synthesis, charge balancing, osmoregulation, stomatal regulation, cell tension, leaf development, and maintenance of dry mass in the plant body (Marschner, 2011; Shabala, 2017; Munns & Tester, 2008; Lindhauer, 1985; Xu et al., 2020).

Generally, higher K/Na ratio in plants appears to be an important salt tolerance mechanism under salinity (Almedia et al., 2017; Katschnig et al., 2015). For example, Asch et al. (2000) showed the K/Na ratio was associated with yield reductions in rice. A similar result was also found in sweet potato, where Begum et al. (2015) found that the genotypic threshold was based on the K/Na ratio. However, in our study, we found an opposite result when we compared the genotypic threshold for dry matter accumulation under root zone salinity to tissue K/Na ratio. A negative correlation (r^2=0.72***) was found between them (Figure 3.4). We also calculated the K/Na ratio at the threshold levels for slope-steepness (75mM, Figure 3.2) and plotted against threshold for dry matter reduction, but no correlation was found

(Figure 3.5a). A similar results were found in case of K/Cl and K/Na+Cl (data not shown). These results indicate genotypic threshold in sweet potato under salininy is not determined the relationship between K and Na (or Cl).

To investigate the reason that threshold for dry matter decrease under salinity was as high as 75 mM, we estimated the difference between K content in non-saline and 75 mM saline solution (threshold), and plotted against the genotypic threshold for dry matter accumulation. We have followed regression analysis to determine the genotypic salt level (Appendix 3.2). A significant negative correlation (r^2=0.97***) (Figure 3.5 (b)) was found between plotted potassium content, which was the difference (∆) between control and 75 mM root zone salinity, against dry matter accumulation (Mondal et al., 2022). Thus, our results indicate that the presence of K in non-saline soils leads to higher yields and the small difference (∆) in K content up to 75 mM NaCl is the tolerance mechanism in sweet potato.

In some studies, sweet potato was found to be K-loving crop and yield reduction was strongly correlated with K content in leaf tissue (Wang et al., 2015; Lv and Lu, 2021). Not only yield, but also chlorophyll biosynthesis, photosynthesis, and protective enzymes are severely affected by K deficiency in sweet potato (Tang et al., 2015). In our study, we showed that the genotypic threshold level of salinity at which dry matter decreased (up to 75 mM NaCl) depends on the ability of the sweet potato plant to muster higher K content in the aboveground tissues.

Chen et al. (2005) developed a screening tool based on gentypic K retention under salinity for wheat where Na and Cl were not the main factors. In our case, Na and Cl also are not relevant to the development of screening tools for salinity in sweet potato, which is similar to that of Chen et al. (2005). Sun et al. (2015) found that natural variation in salinity tolerance of *Arabidopsis* accession is achieved by a variably strong capacity of K retention. They found, neither any of the seven salt tolerant accessions nor the wild type wild Col-0 had any difference in tissue Na content.

Therefore, absolute and relative K content under non-saline and saline conditions allow for discrimination between salt-tolerant and salt-sensitive genotypes of sweet potato. This could confirm the development of a rapid and reliable screening tool in a hydroponic system under salt stress. The K containing screening tool is not only important for identifying salt-tolerant and salt-sensitive sweet potato genotypes, but also shows that potassium fertilization management in salinity prone soil could be another option to improve the salinity problem or yield increase.

6.1.3 Sweet potato is not sensitive to Cl

It is generally believed that Na is more toxic to the plant than Cl. However, for many plants, Cl may play a greater role in ion toxicity than Na. For example, citrus (Arbona et al., 2008), beans (Tavakkoli et al., 2010), barley (Tavakkoli et al., 2011), legumes (Lauchili, 1984), and strawberries (Barroso et al., 1997) are all very sensitive to Cl. It is therefore, important to know the effect of Cl on sweet potato under conditions of salt stress. We have no or very little information in the literature to date. In our study, we closely examined the effect of Na and Cl ions on plants in 0, 50, 100, and 150 mM NaCl saline solutions in a hydroponic system. In our study, we found that the Cl tissue concentrations in sweet potato grown in 100 and 150 mM NaCl solution (root zone salinity) ranged from 48 to 80 mgg^{-1} (Appendix 3.3). Xu et al. (2000) argued that the toxicity level of Cl in plant tissue generally ranges from 15 to 50 for Cl tolerant species, which placed the tolerance level of sweet potato to Cl at the lower limit of this range. Our study showed that while different genotypes of sweet potato decreased their Cl uptake in shoots with increasing salinity, biomass accumulation also decreased (Figure 3.3), resulting in net increases in tissue concentrations (Mondal et al., 2022). In most plant species, Na^+ first has toxic effects before Cl^- does (Munns & Tester, 2008). In sweet potatoes, Na^+ likely plays the same role compared to Cl^-. Thus, a reduction in shoot dry weight may not be the reason for an increase in Cl concentration in sweet potatoes with respect to salt stress.

6.2 Leaf level ion uptake and distribution in sweet potato via the transpiration in saline conditions

To learn more about the mechanisms of salt tolerance in sweet potato, it is necessary to know how Na, K, and Cl are taken up and distributed. Transpiration control mechanisms (Harris et al., 2010; Barbieri et al., 2012; Hasanuzzaman et al., 2017) and the distribution of Na (Munns, R., 1988; Tester and Davenport, 2003) could be other salt tolerance mechanisms in glycophytic plants. In our study, we investigated the status of transpirational water loss from leaves of different ages, Na, K, and Cl uptake, and distribution patterns of these ions in two contrasting genotypes of sweet potato exposed to 50 mM salt stress at two VPD levels (Low VPD: 0.76 kPa; High VPD: 2.27 kPa).

6.2.1 Transpirational water plays no role in Na, K and Cl uptake in sweet potatoes

In general, the initial osmotic phase of the plant always responds to salinity (Munns and Tester, 2008), and ion uptake by plants is usually associated with transpirational water loss. Some studies have reported that the relationship between Na and water uptake is positively correlated with traspirational water loss (for rice: Wimmer and Asch, 2005; Yeo et al., 1987; for soybean: An et al., 2001). However, in sweet potato, no report has been found on the relationship between ion uptake and water under salt stress conditions. In our study, we found that water uptake per unit leaf area was almost four times higher under dry conditions (VPD: 2.27kPa) than under wet conditions (VPD: 0.76 kPa), while Na, K, and Cl uptake were almost the same both conditions (Table 4.2).

Leaf-level studies of water loss and ion uptake yielded similar results to water loss per unit leaf area. Middle-aged leaves transpired more water than old and young leaves (Figure 4.3), but Na, K, and Cl showed no relationship with the age of leaves of plants exposed to salt stress (Figure 4.4). Some studies also reported that transpiration volume flow does not lead to Na uptake in many plants such as rice, wheat, and barley (Munns, 1985; Naito et al., 1994;

Katerji et al., 2009). In view of the above, ion uptake in sweet potato probably occurs through an active transport system, using ATP (Adenosine triphosphate) as energy. Fan et al. (2014) found that genes responsible for Na^+/H^+ antiporters in Arabidopsis play an important role in Na compartmentalization in transgenic sweet potato. Thus, the tolerant sweet potato genotypes taken up more water under salt stress than the sensitive genotypes, which is necessary for maintaining cell tension and overall physiological activities.

6.2.2 The tolerant genotype stores more Na, K, and Cl in its petioles than in the leaf blades

We analyzed the distribution patterns of Na, K, and Cl in two different genotypes of sweet potato across their roots, stems, leaf blades, and petioles grown in high and low VPD under 2 weeks salt stress. We found that there was no significant difference between the VPD for Na and K distribution, but ion distribution between petioles and leaf blades was varied highly significantly with genotype. For example, the salt tolerant genotype distributed about 30% more Na to its petioles compared to leaf blades, while the opposite was observed in salt sensitive sweet potato genotype and VPD had no effect on Na distribution (Figure 4.6). Similarly to Na, in the tolerant genotype K was preferentially accumulated in the petioles and under saline condition was up to twice as much Na. In this context, the leaf blade of the tolerant genotype always had lower Na content. It was also found that young petioles of the tolerant genotype always showed higher K content than the older ones. A similar type of results were reported for rice plants where leaf blades and panicles had lower Na concentration in salt tolerant genotype (Asch et al.,1997b & 1997c). However, in this study, the sensitive genotype showed no control over Na, Cl, and K through its leaf blades and petioles. Thus, K distribution in the leaf interior of different aged leaf might be another salt-tolerant mechanism in sweet potato. The higher concentrations of K relative to Na in petioles of the salt-tolerant genotype of sweet potato also suggests sequestration of Na or discharge of Na in the xylem. Probably, the re-sequestering of Na or the high loading capacity in petioles, together with the control of

Na and Cl ion loading in leaf blades of the tolerant genotypes could be important for salt tolerance mechanisms in sweet potato. Long-distance transport of Na and the ability to retrieve Na in the xylem through the leaves and the movement of Na from the leaf to the phloem have been repeatedly reported to be important salt tolerance traits in many plant species (Munns and Tester, 2008; Shabala et al., 2013; Davenport, 2007; Berthomieu, 2003).

6.3 Leaf level ROS scavenging antioxidant effect in sweet potato under salt stress

It is generally believed that increased level of antioxidant activities under salt stress could be a good indicator of salt tolerance in plant body (Parida & Jha, 2010; Sabra et al., 2012; Naji Alhasnawi et al., 2014 and Das & Roychoudhury, 2014). We investigated the activities of five major antioxidant enzymes in two different sweet potato genotypes considering different leaf developmental stages grown with 100 mM NaCl. We investigated the activities of the five major antioxidant enzymes such as glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and peroxidase (POX), might be increased in sweet potato with high tissue tolerance to NaCl. We hyphothesized that, activities of antioxidant will in sweet potato with high tissue tolerance to NaCl.

6.3.1 Salinity does not increase ROS in sweet potato leaves under salt stress

We found that antioxidant enzymes were generally not related to salt stress alone but were altered or overridden by the effects of leaf position, leaf age or duration of stress, and genotypes. CAT activities increased more in the oldest leaves of both genotypes of sweet potato, whereas the youngest leaves in tolerant genotypes yielded only 25% higher activity under salt stress. The increase of CAT activity in both old and young leaves of the tolerant genotype of sweet potato suggests that the activities of ROS are probably reduced because CAT is functional during the leaf development period. CAT always seems to be the major H_2O_2 scavenger in many plants, such as maize (de Azevedo Neto, 2006); cotton (Meloni et al.,

2003); mulberry (Sudhakar et al., 2001); and barley (Liang et al., 2003). In this study, GR increased in almost all leaves of the tolerant genotype exposed to salinity, while it was unchanged in the sensitive genotype (Figure 5.4). Several authors suggested that the activities of GR in plants under salt stress could be an important indicator of salt tolerance mechanisms in many plants (Hernandez et al., 2000; Sudhakar et al., 2001; Meloni et al., 2003). Besides CAT and GR, the activity of APX differed greatly among both genotype of sweet potato, but no significant salt effect was found at any stage of leaf age and timing of salt exposure. Our study also showed that APX was reduced in salt tolerant genotypes under salt stress compared to the control. GR and APX are very important for the thylakoid membrane, which can detoxify ROS in photosystem I (Asada, 2006). Both GR and APX play important roles in the detoxification of ROS. Although GR has been proposed as a vital enzyme (Foster & Hess, 1982 and Gamble & Burke, 1984; Hasanuzzaman et al., 2012) for the GR-APX cycle (Foyer & Halliwell,1976), it does not alone lead to detoxification of ROS without APX in salt-tolerant sweet potato.

There are very few studies looking at ROS scavenging antioxidant enzyme levels under salt stress in sweet potato. Dasgupta et al. (2008) showed that the activities of CAT under salt stress are higher than control by up to 70.20%. However, our study suggests that CAT alone or GR alone do not provide conclusive evidence for salt tolerance mechanisms in sweet potato under salt stress. It should also be noted that our salt-sensitive genotypes also exhibited unchanged levels of CAT under salt stress (Figure 5.4).

6.3.2 Na and Cl concentration had no relationship in leaf level antioxidant activities under salinity but K was different

The leaf level activities of antioxidant enzymes and the tissue ionic effect are important for understanding the salt tolerance mechanisms, because young and active leaves showed higher transpiration rates and took up more Na or Cl. In our previous chapter 4, we found no relationship between water loss and ion uptake in sweet potato considering different age of

leaves. In chapter 5, we examined leaf-level activities of antioxidant enzymes in relation to ions and found no correlation between sodium concentration as well as Cl in leaf tissues of any age and antioxidant enzyme activities in either genotype (data not shown). To date, very little or no literature has been found on this relationship in sweet potato. Dasgupta et al. (2008) found that superoxide dismutase, peroxidase, and catalase increased in general in sweet potatoes exposed to 1% NaCl in an in vitro culture but she did not show any relationship between antioxidant enzymes and ions. However, Rios-Gonzalez et al. (2002) and AbdElgawad et al. (2016) showed that Na concentrations in sunflower and maize leaves were correlated with increased antioxidant enzyme activities. In addition to Na, higher leaf Cl content can also increase antioxidant enzyme activity in *Cassia angustifolia* (Agarwal & Pandey, 2004). However, in sweet potato, we did not find any correlation between leaf Na or Cl concentration and antioxidant enzyme activity (data not shown).

In Chapter 3, we discussed in detail about the importance of K content for salt tolerant mechanisms in sweet potato. From this point of view, we keenly investigated leaf-level K concentration and antioxidant activities. We found that K concentration and SOD activities were strongly positively correlated under salt stress conditions ($p<0.01$). The young leaves of the salt tolerant genotype always had the lowest K concentrations and the lowest SOD activities (Figure 5.5). Increased level of SOD activities in older leaves (Figure 5.5) with high K concentration indicate a mechanism to protect their photosynthetic potential. A similar type of observation was made by Lechno et al. (1997) in the case of cucumber which showed that SOD activities decreased under salinity stress, relative to control. They also showed that K played a significant role for CAT increase in their study. But, in our study, salt-tolerant sweet potato showed higher CAT activities at low K concentrations, which contrasts with Lechno's study. A study on the effect of K-containing fertilizer and antioxidant enzyme activities showed that the activity of CAT decreased after 30 days in eggplants when KCl and K_2SO_4 were used as K containing fertilizer treatments (Marques et al., 2014). In this point of view, plant age (time) long time K application may be involved for the CAT fluctuation in plants. Gondim et al.

(2012) reported that CAT is the most responsive enzyme that plays a key role for the salt induced H_2O_2. Though K and CAT was not positively related in salt tolerant sweet potato in our study but substantial difference of CAT activity under salt stress condition in very young leaves (especially leaf 6&7) indicates a tolerance mechanisms in sweet potato which was not found in salt sensitive genotype (Figure 5.4). However, it is not clear how low K concentrations lead to higher CAT in our study. Besides CAT, no correlation was found between K concentration and the activities of GR or APX considering leaf age and duration of salt exposure in the tolerant genotype (Figure 5.5). However, a strong correlation was found in the salt-sensitive genotype, where younger leaves had increased levels of GR and APX in the early phase (Figure 5.5). Ahanger & Agarwal (2017) found the similar elevated level of APX and GR activities in one wheat variety under drought stress whereas other variety showed a lower level. But, the K led to increase the free amino acid and free sugar in that wheat variety who produced lower APX and GR. Very recent, Ghanem et al. (2021) suggested that salt tolerance mechanisms involve both activation of antioxidant enzyme activities and biosynthesis of metabolic compounds. Therefore, before proposing a screening tool for salt tolerance sweet potato in relation to antioxidant enzyme activities, other metabolic compound activities like proline, phenols, free amino acids, and hormones (ABA, Auxin) should be well studies under salinity in a wider genotypes. However, elevated level of GR, CAT as well as the nature of K concentration in relation to SOD (See Figure 5.4; Figure 5.5) in salt tolerant genotype under salinity in this study should be concern for salt tolerance mechanisms in sweet potato.

In the overall discussion, we found that the genotypic thresholds of 12 sweetpotato genotypes (75 mM root zone salinity) were strongly related to tissue potassium content (Chapter 3), but not to the K/Na ratio with increasing salinity. Higher K content in young leaves (Chapter 4) and a positive correlation between K concentration and SOD activity (Chapter 5) in salt-tolerant genotypes suggest that K plays a critical role in salt tolerance mechanisms in sweetpotato.

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Chapter 7

Conclusion

Overall, our study aimed at developing a screening tool for salinity by understanding the mechanisms of salt tolerance in sweet potato exposed to salt stress. To achieve this goal, we determined the salt threshold with an appropriate factor, ion uptake and distribution patterns via transpiration at the leaf level, and the role of antioxidant activities at the leaf level under salt stress in sweet potato.

First, we were able to determine the genotypic threshold of 12 contrasting sweet potatoe varieties at 75 mM NaCl. It was determined from dry matter accumulation and genotypic slopes for further dry matter reduction when root zone salinity increased above the genotypic threshold. A strong negative relationship between the difference in tissue K content at 75 mM NaCl and tissue K content at controlled root zone salinity indicates that tissue K content above soil is the salt tolerance mechanism of sweet potato.

Second, we found that cumulative transpiration water loss from two different sweet potato leaves was twice as high at a vapor pressure deficit of 2.27 kPa as at 0.76 kPa, but sodium uptake was almost the same. No correlation was found between water loss in the transpiration history of individual leaves and Na and Cl accumulation independent of VPD with 50 mM salt stress. Outstandingly, the tolerant genotype of sweet potato accumulated five times more K in its petioles than in its leaf blades under all conditions and up to twice as much Na under salt stress. Additionally, K content in young leaves was very high in the tolerant genotypes under salt stress. Thus, internal leaf ion management and the distribution of Na and K account for salt tolerance in sweet potato.

Third, no clear response to salt stress was observed in antioxidant enzymes in two different genotypes. However, leaf position, leaf age, duration of 100 mM salt stress, and genotypes showed a modifying effect. Leaf Na and Cl content were not related to antioxidant activities under any conditions. However, a strong positive relationship between high leaf K

concentration and SOD activities was observed in the tolerant genotype under salt stress, where older leaves showed higher SOD activities. On the other hand, CAT activities in younger leaves showed a negative relationship with low K concentration in salt tolerant genotype. This result suggests that K concentration in tolerant genotype leads to the maintenance of photosynthetic activity in older leaves, in terms of SOD activity and protects younger leaves from membrane damage with an increased level of CAT activity. It is noted that GR significantly increased in salt-tolerant genotypes in almost all leaves of the tolerant genotypes whereas no correlation was found in case of K concentration. Therefore, before proposing a screening tool for salinity in relation to antioxidant enzyme activities in sweet potato leaves, other important activities of metabolic compound such as proline, phenol, free amino acids, hormones (ABA and auxins) should be studied.

In conclusion, salt tolerance of sweet potato genotypes is highly related to tissue K content. However, our study suggests that genotypic salt tolerance strategies in sweetpotato in terms of different K contents in nutrient solutions, and K management in field trials at different locations on salt-prone soils; active ion (Na) transport processes through petioles, and Na distribution patterns through ATP balance; antioxidant activities with their metabolic values across a wider range of genotypes need to be further investigated.

Affidavit

Declaration in lieu of an oath on independent work

according to Sec. 18(3) sentence 5 of the University of Hohenheim's Doctoral Regulations for the Faculties of Agricultural Sciences, Natural Sciences, and Business, Economics and Social Sciences

1. The dissertation submitted on the topic

Physiological mechanisms and growth responses of sweet potato subjected to salinity

is work done independently by me.

2. I only used the sources and aids listed and did not make use of any impermissible assistance from third parties. In particular, I marked all content taken word-for-word or paraphrased from other works.

3. I did not use the assistance of a commercial doctoral placement or advising agency.

4. I am aware of the importance of the declaration in lieu of oath and the criminal consequences of false or incomplete declarations in lieu of oath.

I confirm that the declaration above is correct. I declare in lieu of oath that I have declared only the truth to the best of my knowledge and have not omitted anything.

Stuttgart, April 26, 2023

Place, Date

Signature

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EDUCATION

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EMPLOYMENT

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