UNIVERSITY HOHENHEIM

FACULTY AGRICULTURAL SCIENCE

INSTITUTE OF AGRICULTURAL SCIENCES IN THE TROPICS (HANS-RUTHENBERG-INSTITUTE)

CROP WATERSTRESS MANAGEMENT IN THE TROPICS AND SUBTROPICS

UNIVERSITÄT HOHENHEIM

Bachelor Thesis

Influence of salt stress and vapour pressure deficit on the transpiration of Sweet potato varieties

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Kaltenleutgeben, 02 September 2020

I. Summary

Sweet potato (*Ipomea batatas* L.) is an important crop all over the world especially in tropics and subtropics in order to its nutritional value. But salinity is a big problem for the expansion area as it is increasing for the harsh climatic condition. Now it is important to investigate the effect of salt stress on Sweet potato plants and how it behaves with different vapor pressure deficit (VPD) level or control transpiration to understand salt tolerance mechanism. The ultimate objective of the experiment is to understand how Sweet potato plants carry on their growth and development under salt stress and how it's linked with transpiration or water loss. In a hydroponic experiment four varieties of Sweet potatoes CIP 189151.8 (variety 1), CIP 188002.1 (variety 2), and CIP 106082.1 (variety 3), CIP 420001 were tested individually with the same duration (6 days) of 100 mM NaCl stress in the different growth stage of plants. After seventeen days of growth, salt stress was imposed on each alternate day until 22 days. The first harvesting had completed in 23 days and continued till 28 days. So, six days, two treatments (0, 100) and four varieties were maintained day-wise (23 day = 8 plants) for data collection after testing in the artificial VPD chamber. The transpiration was recorded from different VPD levels in the chamber and from the greenhouse (cumulative). The Na uptake related to transpiration (cumulative) were intensively observed and found that variety CIP 188002.1 and CIP 420001 transpired less water and up took less Na whereas variety CIP 189151.8 and CIP 106082.1 performed a higher amount on both sides. In the different level of VPD, all varieties transpired more according to their increased VPD level. Among the four varieties, CIP 188002.1 and CIP 420001 moderately regulated transpiration in young to older leaves even in high VPD level whereas CIP 106082.1 transpire more especially from older leaves under stress condition. Interestingly older leaves in CIP 189151.8 transpire less water even at a high VPD level. From the regression analysis, there was no significant relationship under salt stress in SPAD and Leaf area ($p<0.05$), however, CIP 188002.1 and CIP 420001 performed better over control in leaf area compared with the other two varieties. To get more information, further, root Na concentration was estimated and found that CIP 188002.1 and CIP 420001 regulate root Na gradually with the plant development whereas CIP 106082.1 showed more concentration even in aging plants. It might be due to the higher transpiration of water by CIP 106082.1 that already observed in cumulative transpiration as well as different VPD levels. From the experimental observation, it is clear that CIP 420001 and CIP 188002.1 showed the controlled mechanism of Na in order to different VPD and transpiration whereas CIP 106082.1 and CIP 189151.8 showed their sensitive behaviour. Further investigation could be helpful designing with replication to understand clearly the Na regulation among these four varieties of Sweet potato. The reason for low/high Na concentration in different varieties should also be investigated to know exclusion or other mechanisms that are quite important in the tolerance mechanism in Sweet potato.

II. List of tables

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IV. List of abbreviations

Etc.	and so on
e.g.	For example
RH	Relative humidity
VPD	Vapour pressure deficit
cm	Centimetre
l	Liter
g	Gram
mmol	Millimolar
mM	Millimolar
min	Minute
W	Watt
kPa	Kilo Pascal
K	Potassium
Na	Sodium
Cl	Chlorine
H_20	Water
°C	Degree Celsius
SPAD	Single Photon Avalanche Diode
DW	Dry weight
LA	Leaf area

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1 Introduction

1.1 Background

Farmers have to deal more and more with disruptions, so having fitting genotypes and varieties helps balance or compensate the complexity of climate and regional changes (Ledesma et al., 2016, p. 589). These difficulties are seen with the sweet potato.

The sweet potato (*Ipomea batatas*) is part of the *Convolvulaceae* family and is a tropical plant with close similarities to the potato (*Solanum tuberosum*). Both plants have their origin in South America. Peculiar to sweet potato is that the varieties have the most diverse forms and there are many wild species (Huaman, 1992, p. 5,6). Under normal growing conditions it is a perennial plant, but mostly used annually by vegetative reproduction from stem cuttings or storage roots. The plant has rapidly expanding vine systems and its root system consists of fibrous and storage roots. The first is for nutrient uptake and stability, and the second for photosynthetic product storage and, from an agricultural point of view, the main crop (Huaman, 1992, p. 8). The plant, and in particular the roots, have a high nutritional value in mineral nutrients and starch with evident potential for economic benefit in farming systems (Yooyongwech et al., 2014, p. 361). Sweet potato is more and more used as a source for bio energy and fuel production, often on farmland on the outskirts (Liu et al., 2014, p. 2).

More and more soil types are obstructed by salt and different accumulations of ions. When plants have a higher transpiration, salt tolerance can be increased, also the humidity level influences the impact of NaCl accumulation (P. An et al., 2001, p. 405). In salt stress, plants uptake salt ions through different systems and distribute them via the transpiration stream to all vegetative plant parts (Backhausen et al., 2005, p. 229). The nutrient uptake is vital for a healthy plant, but high amounts of Na^{+} disturb the efficient stomatal regulation for K^{+} and Ca^{2+}, the result will have a negative impact on growth and low photosynthesis (Tavakkoli et al., 2010).

Sweet potato is described as sensitive to salt but the variety and the conditions describe the range of tolerance and varietal behaviour (Backhausen et al., 2005, p. 230). Similarly to other crops, it is also seen that the tolerance level determines the hydraulic conductivity and the transpiration rate (Mahlooji et al., 2018, p. 550).

1.1.1 Salt stress

One of the most stressful abiotic influences in farming is salt and more importantly, the tolerance level of a crop on its development (Liang et al., 2018). The initial stress for a plant is the osmotic stress with instant effect on growth, followed by ion toxicity because of a lack of homeostasis. In particular, salt stress results in decreasing photosynthesis, lower leaf area or dry weight, quality and yield of the crop (Liang et al., 2018). Plants are weaker under stress, so a state outside their optimal range for development. Na^{+} and Cl^{-} toxicity is stress for the plant and the most damaging and limiting factor for growth (Backhausen et al., 2005, p. 235). Following to these accumulation, K+ and Ca2+ is reduced and leads to an ion imbalance, ensued by inhibition of cell growth and division (Rasool et al., 2013, p. 16). Ion accumulation causes increased amounts of reactive oxygen species (ROS), which result in the degradation of DNA, proteins and cell death. The majority of ROS formation under salt stress in form of the disturbance in the electron transport chains in the chloroplasts. Another form is due to the reduced stomatal conductivity with reduction in the internal CO2 concentration (Rasool et al., 2013). The internal damage is the basis for what is seen on the outsight in form of chlorosis and necrosis on leaf and after long exposure on stem and root (Ledesma et al., 2016).

To adapt to salt stress, a plant accumulates osmolytes to lower the osmotic potential and with glycerol and sucrose the biological macromolecules can be protected, these will be found in the bulk water of the cell for direct interaction (Rasool et al., 2013, p. 9). In other experiments on salt stress with different species, it was seen that different substances in plants can be important to prevent damage and illness e.g. soluble sugars and free amino acids increase salt-tolerance in rocket or soluble sugar and carbohydrates in pea (Hniličková et al., 2017; Yooyongwech et al., 2014). Better transpiration rate or so called lower transpiration under changing environmental conditions are seen in more adapted plants or specifically tolerant varieties (Yooyongwech et al., 2014).

Genotypic differences can be a main reason of salt tolerance (Munns & James, 2003, p. 201). There are alternatives for more tolerant varieties, such as transgenic plants with higher salt tolerance (Begum et al., 2015; Liang et al., 2018; Liu et al., 2014). Tolerance of salt is necessary, because salt is left behind in the soil from the pre-crop, when the water table is lowered, or through the better use of poor irrigation water in drought-suffering regions (Munns & James, 2003, p. 201). A general classification of salinity is listed in the figure below.

Table 1. Salinity classification and concentration for water (*Water Salinity and Plant Irrigation*, 2019)

EC (mS/m)	Approximate total dissolved solids (ppm or mg/L)	Status
0-80	0-456	Low salinity
80-250	456-1425	Moderately salty
250-500	1425-2850	Salty
>500	>2850	Very salty

Stress or injuries of the plant can be from water stress, ion accumulation of Na or Cl in the leaf or deficiency of other ions, but also osmotic potential can cause senescence or leaf death and is directly seen in the field (Munns & James, 2003, p. 202).

Figure 1. First signs of salt stress; spots, wilt (leaf edge) and conductors increasingly visible (SCHOPFHAUSER, 2019)

Through membranes and diffusion, water and solutes enter the plant (Muenscher, 2019, p. 311). Haberlandt already showed with his work in 1892 that salt movement plays a major role in diffusion, next to transpiration. High humidity with high temperatures reduce the transpiration rate (Muenscher, 2019, p. 313). For a plant it is necessary to have a larger amount of a diluted solution rather than a concentrated one for the best possible growth and nutrient supply (Muenscher, 2019, p. 314).

In other experiments with different crops but similar conditions of a transpiration chamber with differing humidity levels, it was pointed out that under dry conditions a bigger ash content was found, but is dependent on the species and the variety (Muenscher, 2019, p. 312). The rate of which salts enter the cell is independent from the rate of water uptake (Muenscher, 2019, p. 311).

1.1.2 Vapour Pressure Deficit

Basically the VPD is the difference between the amount of moisture in the air and how much moisture the air could hold when it is fully saturated, in other words, how close the air is to saturation. When the VPD is 0 the air is 100 % saturated with water vapour. The relative humidity of the atmosphere as well as the temperature plays an important role for the VPD. Many factors such as the difference in water vapour concentration between the intracellular spaces of the leaves, the atmosphere around the stomata and the resistance of diffusion are decisive for the transpiration rate. The transpiration rate increases with increased temperatures and decreasing humidity (Larcher, 2001). So higher humidity would lead to decreased water movement in the plant, and under salt stress a reduction of transpiration and of water flow in the root takes place.

Assimilation products are essential for the sinks in the plants. Difference in leaf to air water vapour pressure is a limiting factor for assimilation and the carbon dioxide assimilation rate. To increase the leaf water VPD at a constant temperature leads to a decrease in stomatal conductance to air water VPD. High VPD reduces assimilation of internal carbon dioxide (Bunce, 2003, pp. 37, 38). In field experiments Bunce showed that high VPD reduces assimilation rates on leaves when carbon dioxide is limited. Direct response to stomatal conductance and VPD cannot always be seen, but sensitivity of stomatal conductance to VPD is increased through water deficits and differences in species (Bunce, 2003, p. 39).

Higher air humidity leads to a decrease in the water movement in the plant and resolves in lower accumulation of salt in leaves and shoots and overall in a reduction of transpiration (Backhausen et al., 2005). For tomato (*Lycopersicon esculentum* Mill.) the influence of salt and VPD has a major impact on the growth rate, which determines the possible yield. Salt exposure and higher relative humidity (70 %) lead to reduced growth and higher NaCl amount (Ping An et al., 2005).

1.1.3 Transpiration

The diffusion of water vapour from an evaporating surface to the atmosphere is the process by transpiration through the mesophyll cells and further to the stomata. In transpiration there are resistances from stomata, boundary layers and air, when neglecting the cuticular transpiration (Lippert, 1987, p. 178) .

Temperature is one of the determining factors in transpiration. The change from liquid to vapour (evaporation) costs the plant energy in the form of heat. In this way the plant forms a reply to the radiation of the sun (Lippert, 1987, p. 179). The sweet potato is an isohydric plant, so the plant is able to close its stomata in hot situations under stress, for example significant water loss and decreasing transpiration (Lippert, 1987, p. 182).

The sweet potato is plant that flourishes under warm and humid conditions. Climatic change result immediately in the transpiration of the individual plant. In the chapter 1.2.2. the example of different humidity e.g. in tomato show the importance of the relationship between transpiration and VPD (relative humidity). Linked to this relationship is the experiment with 4 Sweet potato varieties. Sometimes counted as a minor crop, the importance considering VPD or transpiration is not yet discovered. A suitable screening tool could be identified for measuring tolerance level of the sweet potato plant.

1.2 Objective

Closer investigation will be on the effect of salt stress on Sweet potato plants and how it behaves with different vapor pressure deficit (VPD) level or control transpiration to understand salt tolerance mechanism. The ultimate objective of the experiment is to understand how Sweet potato plants carry on their growth and development under salt stress and how it's linked with transpiration or water loss. So what are the plants doing over time with the effect of salt exposure? Important will be the difference in the varieties and their influence in their transpiration by showing the varietal behaviour.

Varietal and salt effects are expected in relation to transpiration and VPD. Raised leaf area will lead to increased transpiration in the control plants and reduced transpiration in the salt-treated plants. More transpiration enhances more sodium uptake in sweet potato, dependent on VPD. With higher pressure more transpiration will take place and more sodium should be recorded and could cause lethal damage for plant cells.

1.3 Current knowledge

China has, with more than 50 % of the sweet potato production, an output of 70.963.630 metric tons every year Following with significantly less annually production in Africa and South America like Nigeria, Tanzania, Ethiopia or Indonesia (Wee, 2017). Climatic changes have made a new way for sweet potato in non-tropical areas e.g. Germany (Stoewer, 2019).

Climate change, and often as a result weather changes, make farming more difficult. There are many affected areas of salinity, for example agricultural regions in Bangladesh, and a lack of possible varietal alternatives (Begum et al., 2015, p. 249). In most cases human influence increases salinity. The agricultural consequences have a major impact on the actions of farmers, and in the end on consumers (Backhausen et al., 2005, p. 229). The way of applied irrigation can be one part, but the change of climate (drought, temperature, extreme weather events, etc.) and the missing adaptations are another part of a complex issue (Ledesma et al., 2016, p. 585; Motsa et al., 2015, p. 2). More severe and more frequent droughts and weather conditions will force suitable adaptations in agriculture and crops which will secure food and nutrient supply (Motsa et al., 2015; Rahaman et al., 2015, p. 74).

2 Material and Methods

2.1 Plant cultivation

For the experiment four varieties were selected, which showed different responses to salt. The varieties originated from the Bangladesh Agricultural Research Institute (BARI). In the course of the experiment these varieties were taken according to their different morphological attributes (e.g. leaf size).

48 plants were taken for this experiment with 12 each per variety. Of these, 6 were the control plants and the other 6 were treated with salt (100 mmol NaCl) in a Yoshida solution with iron (EDTA) (Bado et al., 2016).

The stock solutions were weighed according to the value and placed in a 1-litre volumetric flask, filled with deionized water. The chemical was dissolvent in the water using a magnetic stirrer (Monotherm IKA, VELP Scintifica, Italy). For the first 10 days a 50 percent diluted Yoshida solution with distilled water was used. The next step was to take a 100 percent Yoshida solution for 6 days. Each pot was refilled regularly for optimum pH conditions of 5,7 to 5,9. If the pH value was too high, it was adjusted using 1 M HCl acid. The amount of water transpired was checked using a balance. Approximately every 2 days the pH and electric conductance were checked.

Table 2. Compound of stock solution "Yoshida Culture Solution" with IronEDTA (SCHOPFHAUSER, 2020)

Label	Element	Chemical	Stock [g/L]	Stock / final [ml/L]	Solubility [g/L]
A	N	NH_4NO_3	114.29	1	2089
B	P	$NaH_2PO_4 * 2H_2O$	50.37	1	850
C	K	K_2SO_4	89.14	1	111
D	Ca	$CaCl_2 * 2H_2O$	146.73	1	986
E	Mg	$MgSO_4 + 7H_2O$	405.64	1	710
F	Fe	FeNa – EDTA	15.080	1	N.N.
G	Mn	$MnCl_2 * 4H_2O$	1.875	1	700
	Zn	$ZnSO_4 * 5H_2O$	0.0440		965
	Cu	$CuSO_4 * 4H_2O$	0.0393		203
	Mo	$(NH_4)_6Mo_7O_{24} * 4H_2O$	0.0920		430
	B	H_3BO_3	11.675		50

2.2 Transpiration measurement

2.2.1 Experiment set-up and cultivation

4 varieties with different characteristics were taken by vegetative propagation. The cuttings came from the offspring of the mother plant´s vines and were separated with a razor blade. To prevent pests, the razor blade and the cuttings were immersed in a biocide (Neudorff Spruzit). The plants were transferred to standardised pots around 1 to 1,2 l and filled with Yoshida culture solution (iron EDTA).

For growing the sweet potato plants, a hydroponic system was taken. The system was connected with a pump via rubber pipes to aerate the plants and roots in the nutrient solution for 45 minutes with 15 minutes of pause till the next run. The overall growth and development of the sweet potato plants in the glass house was at a temperature between 25 to 28 °C with a relative humidity of 36 to 47%.

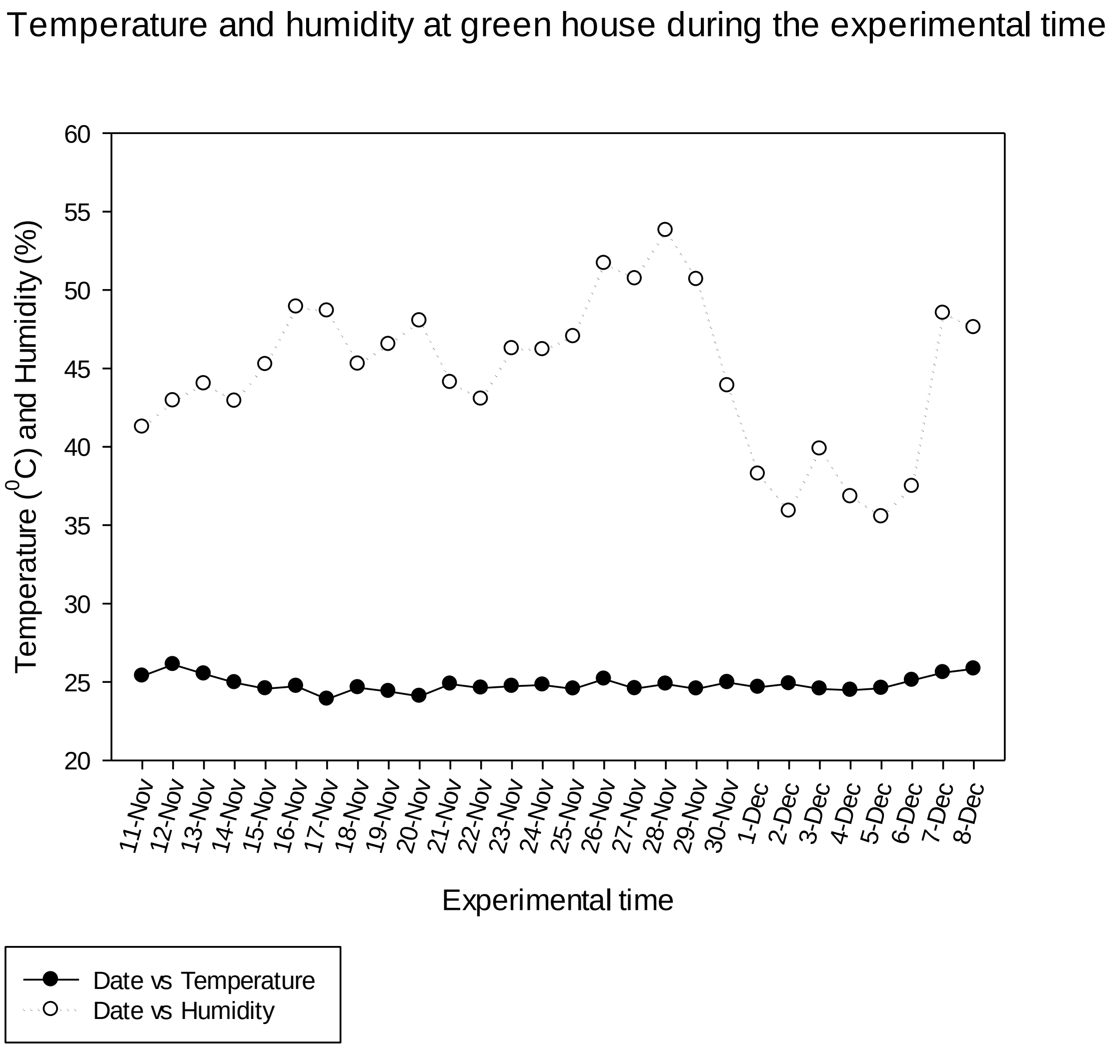

Figure 2. Temperature and humidity in the greenhouse during the experiment

The timeframe of the experiment was from early November to early December with 15 days of growth of the cuttings in the greenhouse, followed by the staggered salt application. Important was the different plant development, because 8 plants were treated each day (4 control/ 4 with salt). So there were 6 days of development over time, but the same length of salt uptake in the nutrient solution over a period of 7 days.

Chamber 1. day - growing 15 days – salt day 16 – test day 23

Chamber 2. day - growing 16 days – salt day 17 – test day 24

Chamber 3. day - growing 17 days – salt day 18 – test day 25

Chamber 4. day - growing 18 days – salt day 19 – test day 26

Chamber 5. day - growing 19 days – salt day 20 – test day 27

Chamber 6. day - growing 20 days – salt day 21 – test day 28

The typical characteristic of sweet potatoes is to grow vines and to grow under optimum conditions very fast. A supporting aid made out of plastic pots, guaranteed a controlled growth and optimal light conditions for all plant parts. Two plastic scaffolds of 15 to 20 cm each were glued together and fixed with adhesive tape on the lid of the pot (see figure 2). Due to the short experimental set up, the duration was just 3 weeks, so a simple construction was possible for the plant exposition.

After the salt treatment, the sweet potato plants were transferred into a perspiration chamber to be tested at different VPD levels and to test the influence of salt stress in staggered repetitions.

The development period was about 22 days, and so a location change for the plants from the greenhouse to the perspiration/ transpiration chamber was necessary. The distance between the greenhouse and the chamber location was 300 m. The transport has been carried out with a plastic waste bag over a plastic box, filled with 4 plants and airtight sealed to keep temperature constant and the influence of the environment minimal. The outside temperatures varied around 5 to 8 °C and at the end of November 2019 the temperature was 1 to 3 °C.

Figure 3. Design of the hydroponic system after planting (SCHOPFHAUSER 2019)

Figure 4. Plastic aid for stabilisation for controlled vine growth (SCHOPFHAUSER, 2020)

2.2.2 Transpiration chamber and VPD

The chamber was built by the Institute of Agricultural Ecology in the Tropics and Subtropics in the cellar of the University of Hohenheim. The description of the chamber was taken and modified with small adaptations from Asch and Häfele, 2018. With the chamber it is possible to measure the transpiration rate, work with different levels of VPD and temperature and other possible options. The chamber is made of 2 sections: the mixing chamber for air and fog, and the main measurement “transpiration chamber”.

Both chambers have the same volume of 100 cm x 68 cm x 80 cm (Asch & Häfele, 2018, p. 9). The materials used were wood for the structure and transparent acrylic glass for all the sides. At the front there was a moveable plastic plate/ door to enable working inside the chamber.

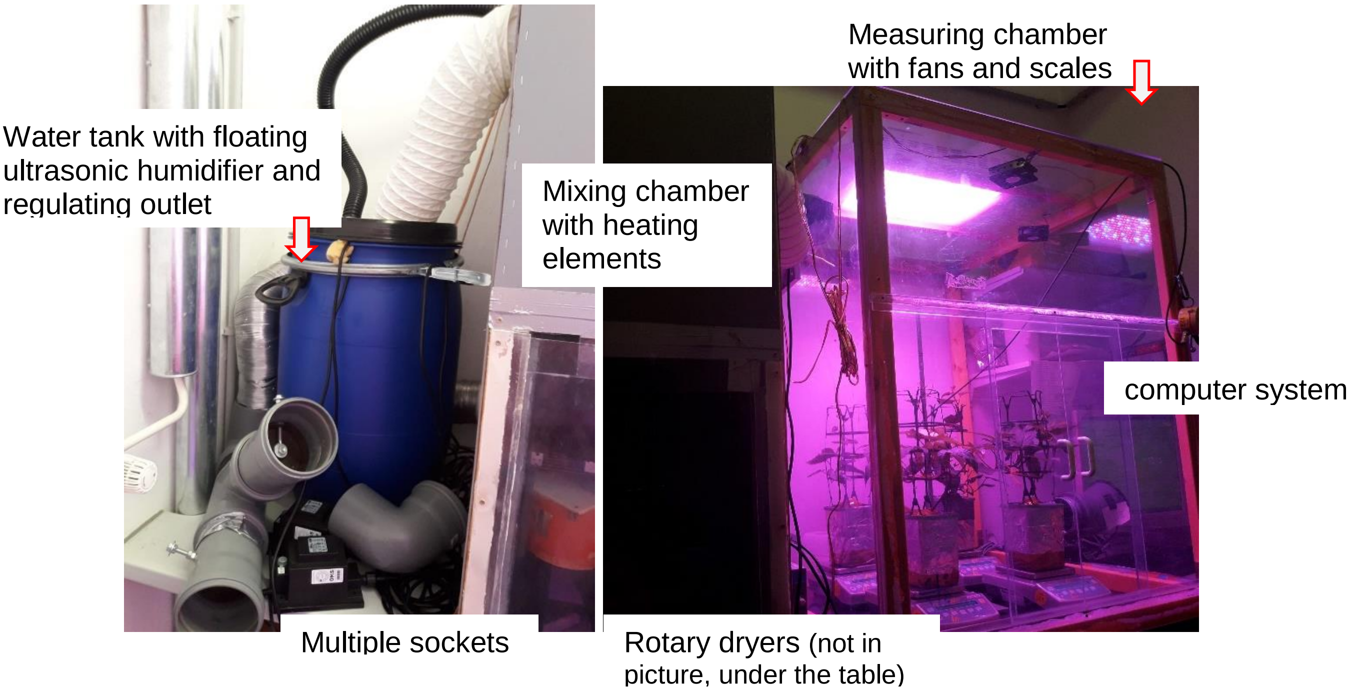

Figure 5. Transpiration measuring chamber Hohenheim

To be able to change air humidity an absorption dryer (DST Seibu Girken, Modell Consorb DC-10) was used. The air flow was regulated by a 60 l plastic tank filled with water for steam production with connected pipes for different outlets to manage and control the chamber. On the ground there was an ultrasonic humidifier (Seliger GmbH, Modell Fogstar 300) for more precise regulations and fine atomisation of the water. For both chambers, microcontrollers (Atmel Corporation, Modell ATMEGA 132) were used for checking the fan, the heating and the humidifier. The air conditions had to be suitable, as there was a risk that if there was too much or too little air movement, the stomata would have got used to it, which would have affected the result.

From the plastic tank a pipe went over to the mixing chamber and further to the main chamber. An 80 mm fan blew the preheated and regulated air into the main chamber. Self-built ceramic 12 V heating elements were used to regulate and heat the temperature in the mixing chamber. In the inflow and outflow of the main transpiration chamber a sensor carrier plate was fitted and was operated by the microcontroller. There were 3 fans in the main chamber which could be regulated manually or using a computer-controlled power supply. The regulation of the air flow and air humidity was important for minimizing air differences. 4 scales (Kern & Sohn GmbH Modell 2400-2N d=0,01g) were placed in the main chamber. The Graslog-software is used to measure the weight intervals and changes from 1 minute or longer. Controlling software such as Hterm adjusted the preferred atmosphere in the mixing and the transpiration chamber. A prepared stencil for every minute helped measure the relative air humidity in percentage and the temperature in °C for the vapour pressure deficit (VPD) (Asch & Häfele, 2018, p. 10).

For illumination a 360 W LED-panel on the top of the chamber was used. The intensity of the light was measured (Methen Irradiance Meter) with 169 to 250 micro mol per square meter per second, in the middle part 430 to 450, upper middle part with 600 to 700 and the upper part with 1952 micro mol per square meter per second.

In the transpiration chamber the influence on the sweet potato plant by the transpiration through salt stress over one week and the influence of different VPD levels was tested. The RH (relative humidity) in the chamber was shown to reduce gradually from 85%> 70%> 50%> 30% at a constant temperature of 28 to 29°C. The RH was afterwards converted into the chosen VPD levels 0,57kPa > 1,13kPa > 1,89kPa > 2,65kPa.

The chamber is equipped with 4 scales and the varieties and pots were chosen in such a way that 2 control plants and 2 salt-treated plants, 4 in total, were given one passageway. To prevent evapotranspiration from the pots, they were wrapped in aluminium foil.

2.3 Data collection

2.3.1 Leaf area, Dry weight and Ion concentration analysis

After testing in the transpiration chamber, the leaf area (green weight), root length and dry weight were determined. Dry matter determination after 72 hours (at 60°C) was made (Memmert GmbH drying cabinet), for examination of ion concentrations.

The grinding of the plant material with the measurement of around 0.1 gram for nutrient analysis in the autoclave for Na, K and Cl analysis. For this stainless steel balls for grinding were used (3 and 5 mm size) and deionized water was added and mixed for perfect homogenisation and autoclaved for around 60 min. at 120°C and afterwards centrifuged. For the ion concentration the determination with a flame photometer and AutoAnalyzer was used.

2.3.2 SPAD

Before each test in the perspiration chamber, the chlorophyll content was determined in the greenhouse at 10 am, with SPAD measurement for the chlorophyll content (SPAD-502Plus, Konica Minolta, Japan). Three leafs of the bottom, the middle and the upper part of the Sweet potato plant were tested.

2.3.3 Transpiration rate and VPD

During the growth in the greenhouse the daily transpired amount and refilled amount of water was been noted. Furthermore in the transpiration chamber the 4 balances registered the water loss, which was taken into account in the data collection.

The different VPD levels were tested in the transpiration chamber with gradual reduction (85%> 70%> 50%> 30%) at a constant temperature of 28 to 29°C and correlated with the transpiration for the different varieties.

2.4 Statistical evaluation

All data was plotted with Excel, SigmaPlot 2010 and 2013, ANOVA and R Software using Scatter plots to illustrate the different varietal behaviour under different parameters, Bar charts with Linear Regression and correlation to show possible relations.

2.5 Literature research

A literature review with keywords like "sweet potato", "salt stress", "transpiration in sweet potatoes" and "VPD and transpiration" was conducted. Relevant or similar sources were selected with reference to the research question and the results.

3 Results

The difference of development stage (seen in section 2.2) has been strongly involved in the processes and is visible in the results presented. In order to describe the salt effects on the different parameters, the evaluation refers to the influencing factors time of leaf development and salt application. Different reactions must be expected due to the development of leaves and varietal behaviour under their physiological reactions.

3.1 Morphology

During growth in the greenhouse, stronger root growth was observed in varieties 2 and 4 compared to 1 and 3. Variety 4 was characterized by habitus with significantly stronger side shoot growth and increased leaf growth.

Figure 6. Varietal leaf difference under 100mmol salt treatment with slight signs of wilt and different growth

3.1.1 Leaf dry weight

In the following figure the dry weight of CIP 189151.8 under control shows a significantly higher over the measured period, significantly lower under salt stress but still increasing. For CIP 188002.1 and CIP 42001 the leaf dry weight increased under both treatments, only very small changes under salt. The R-squared (R2) is a statistical measure that represents the proportion of the variance for a dependent variable that is explained by an independent variable and is very low in the total DW. One reason could be the data availability. The time increase, leads to a DW increase. Variety 2 (CIP 188002.1) is significant and, together with variety 3 (CIP 106082.1), are showing a positive relationship and response in DW over time, especially in day 28.

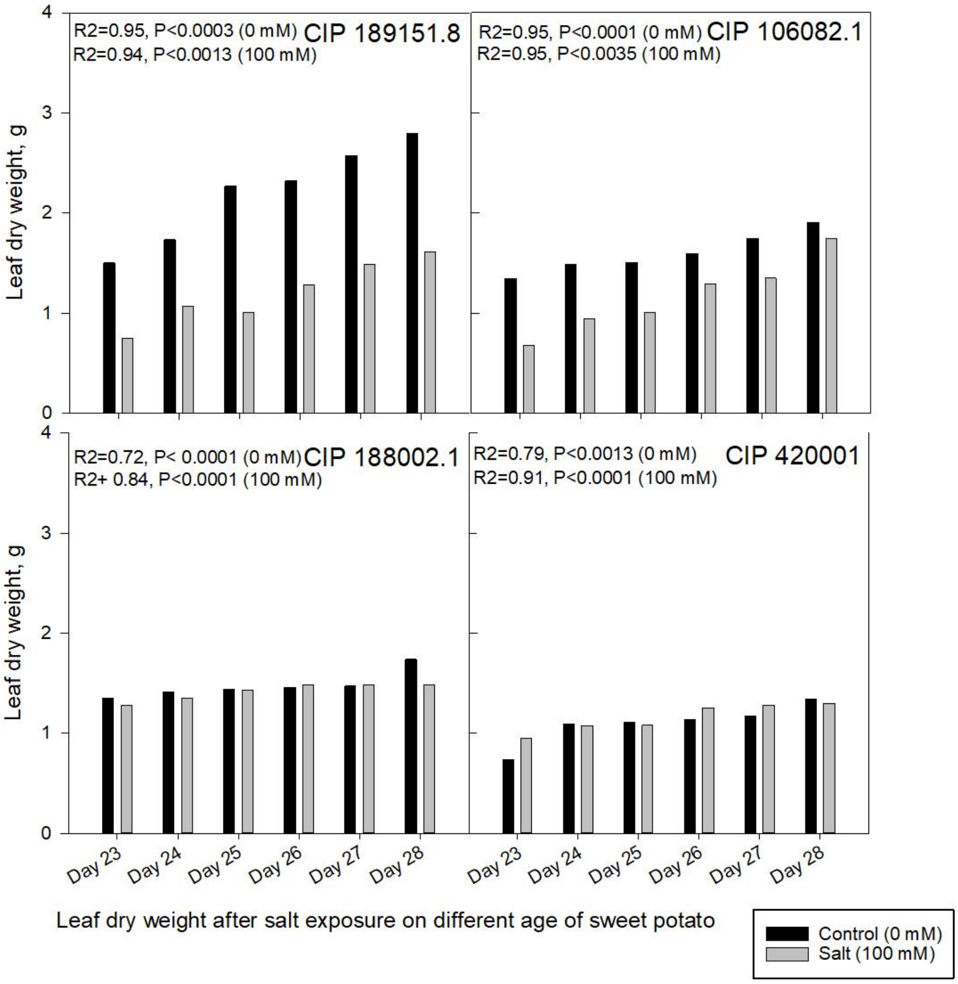

Figure 7. Leaf dry weight (g) over time under salt treatment and under control of Sweet potato of the varieties CIP 189151.8, CIP 106082.1, CIP 188002.1 and CIP 42001

3.1.2 Leaf area and concentration

In the results, the LA was very significant, interestingly highest at CIP 189151.8 under control conditions of about 880 cm^2, which is not surprising, since no external stress factors were present, but is much more pronounced than in the other varieties. Under salt influence it is also very high up to 650 cm^2.

The varieties CIP 189151.8 and CIP 106082.1 have similar leaf area and development rates. Relation is visible in CIP 106082.1 with a high LA for the control treatment. Under the influence of salt, their leaf area growth is reduced by about 1/3 and has a clear varietal effect. In CIP 188002.1 the control is going down, which was not expected. However, the salt treatment had a higher DW performance and a high LA in CIP 188002.1, with R^2 of 0.95.

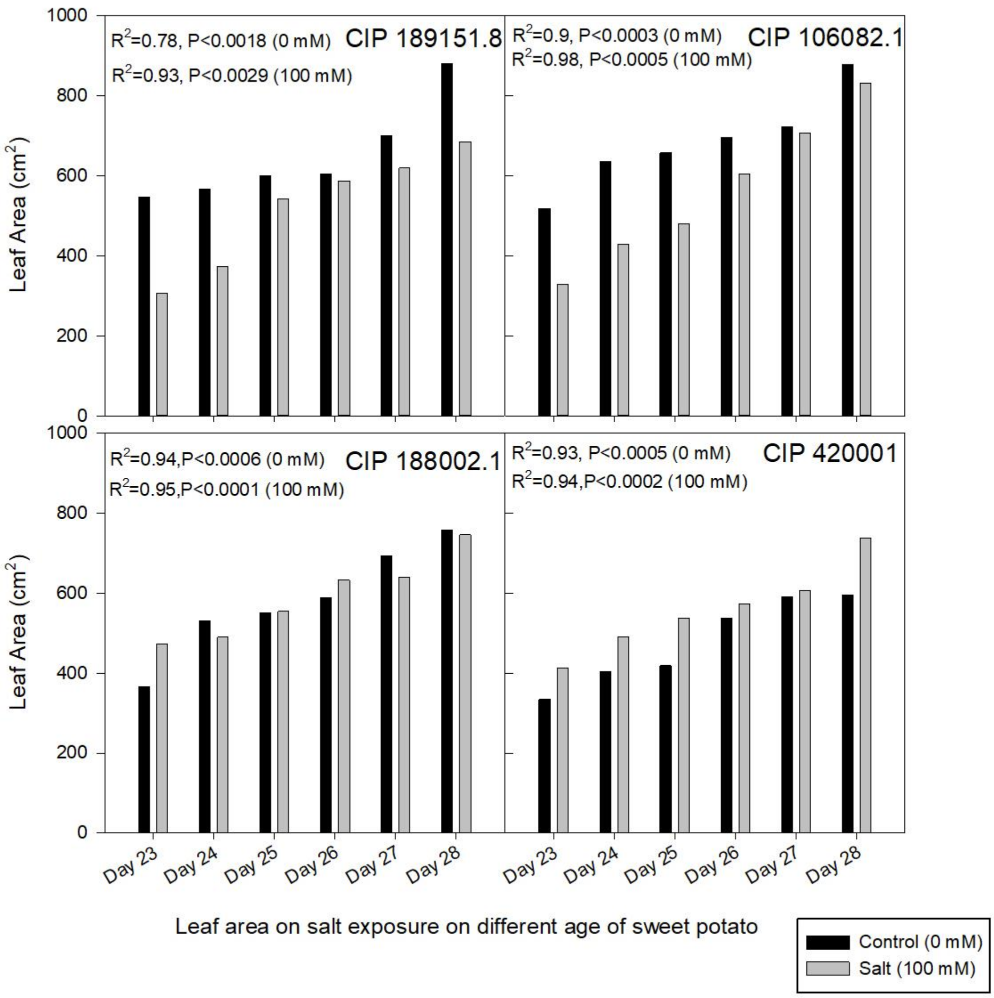

Figure 8. Leaf area (cm^2) over time under salt treatment and control for Sweet potato

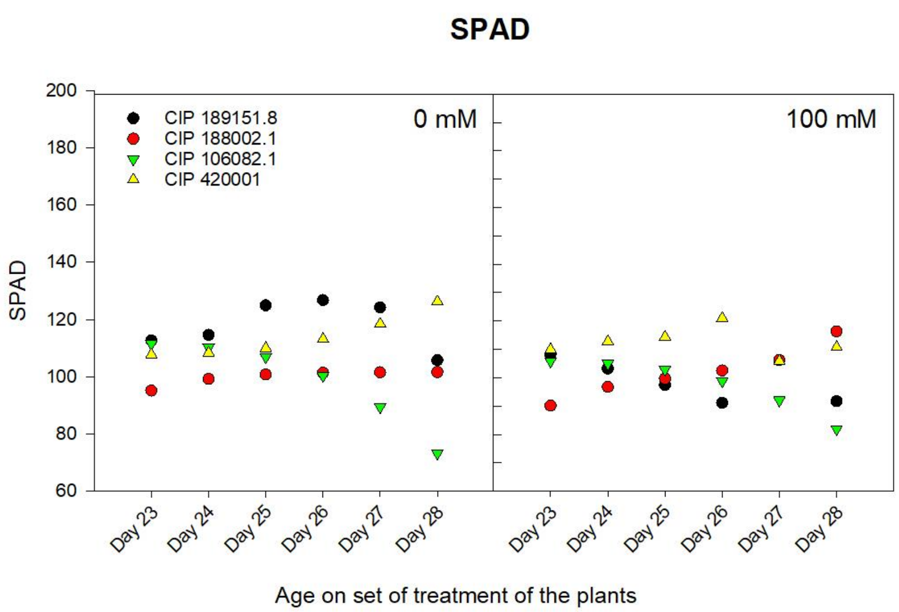

Figure 9. SPAD measurement over time under control and salt treatment

The leaf chlorophyll concentration is an indication of the health of the plant and is important for energy absorption from light (Larcher, 2001). Treatment with salt resulted in a lower chlorophyll content in the leaf. The exception was V1 (CIP 188002.1) which showed an increase in growth under salt influence.

The variety CIP 106082.1 shows a clear downward bending or a negative trend in both treatments. Similar for CIP 189151.8 with significant negative curve, due to lower SPAD and possible higher Na^+ content in the leaf. Under salt CIP 188002.1 and CIP 420001 show an increase, which can be explained by further growth, despite higher Na^+ uptake and possible exclusion mechanisms of the plant. The SPAD measurements are significantly correlated with their chlorophyll content, which means that more stress reduces the chlorophyll amount over time as seen under 100 mM.

From mg the Na^+ content was transformed to micromoles. For the variety CIP 189151.8 a higher Na^+ content measured, which has an impact on the SPAD. CIP 106082.1 contains a lower proportion of Na^+ and more SPAD, so higher chlorophyll content seen on the lower mean value line. Lowest Na^+ was seen in CIP 420001 with maintained SPAD value.

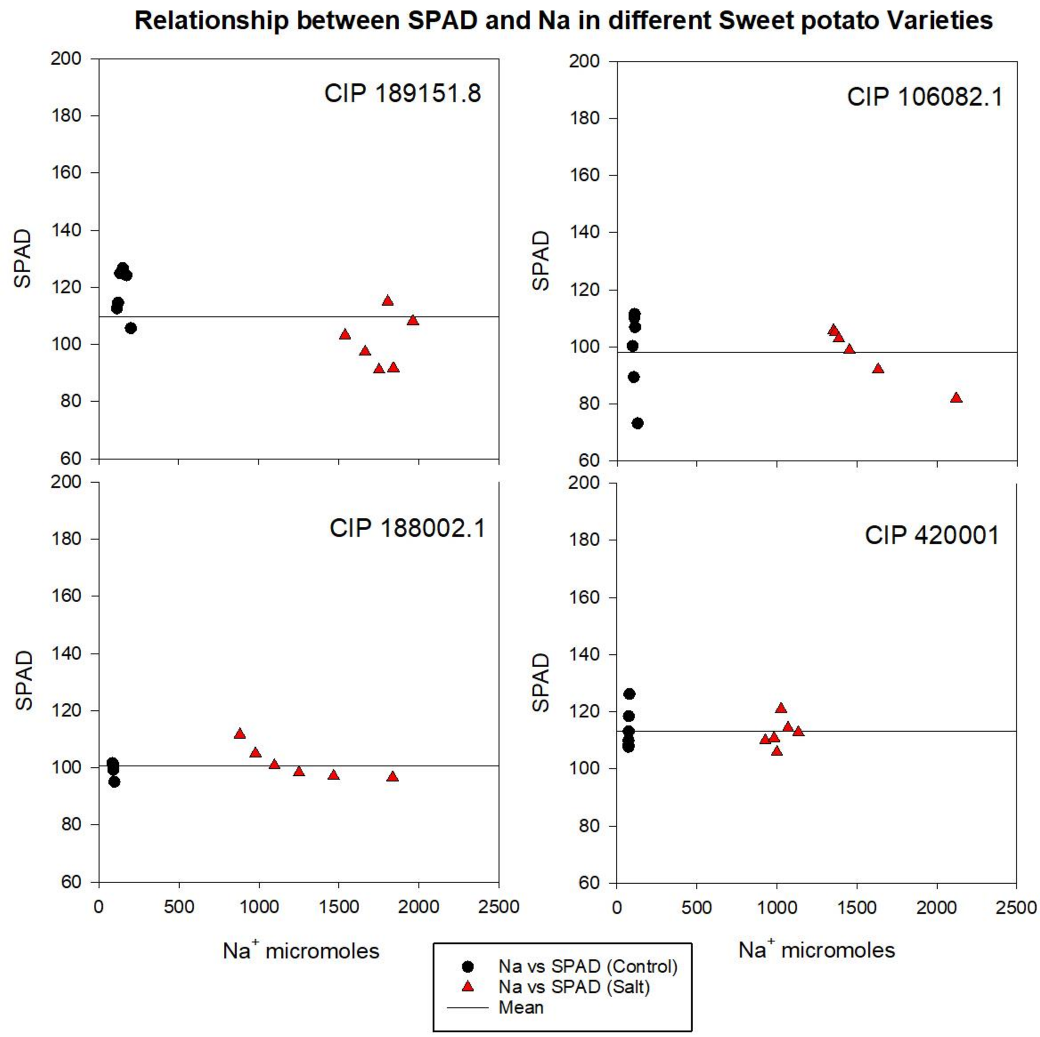

Figure 10. SPAD measurement on the Na^+ (micromoles) content for different Sweet potato varieties

3.2 Distribution of sodium

Under salt exposure there is much difference in uptake in comparison to the control treatment. CIP 189151.8 (black) is showing very high Na^+ concentration of about 44 $^{mg-1}$ in the leaf on day 23, but steadily reducing $y=44.19+(-1.8)*(ln*x)^2$. CIP 188002.1 is much lower, starting with 29 $^{mg-1}$ of Na^+ down to 12 $^{mg-1}$ on day 28. Only CIP 106082.1 has a positive trend in the leaf concentration. The varieties can contain salt over their growth/ age seen here for the sweet potato, because young plants have higher concentrations and more metabolic energy resolving in a higher uptake, which is for older ones much lower. The bend in CIP 420001 is not clear, so the equation is fitted with $y=21.5+47.23*ln*x/x^2$.

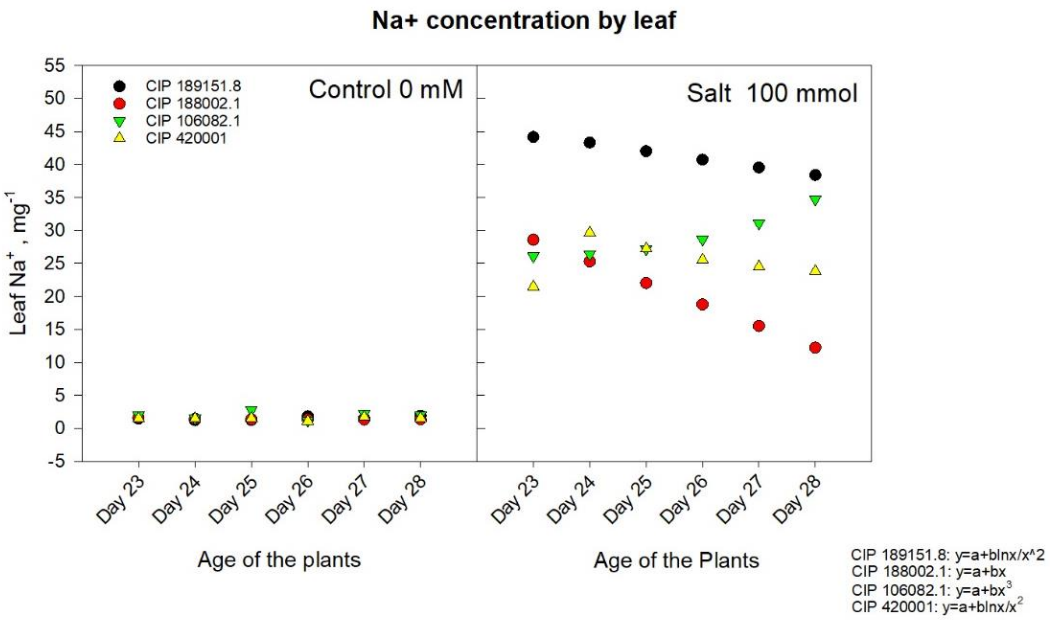

Figure 11. Na^+ Concentration by leaf (mg) over time

Shortly after the salt application, physiological drought was observed. The salt captures the power and no water is taken up, so the plant wilts. Black (CIP 189151.8) is going up under salt, but is able to maintain the Na^+ $y=45+(-56.3)*ln*x/x^2$. CIP 420001 is similar in its reaction between control and salt exposure. For CIP 188002.1 the equation is $y=42.17+(-12)*ln*x$ with a negative trend and significant lower Na^+ uptake. The growth has increased, but the sodium intake has decreased, which could be understood that sodium is not being accepted or liked by the plant. Over time it could avoid and exclude sodium with mechanisms e.g. in the root. The growth is different in the varietal behaviour and can be brought back with the salt intake. The expected scenario would be more growth with higher sodium uptake. For green (CIP 106082.1) the growing increase is nicely described with the equation of $y=30.99+0.04*e^x$.

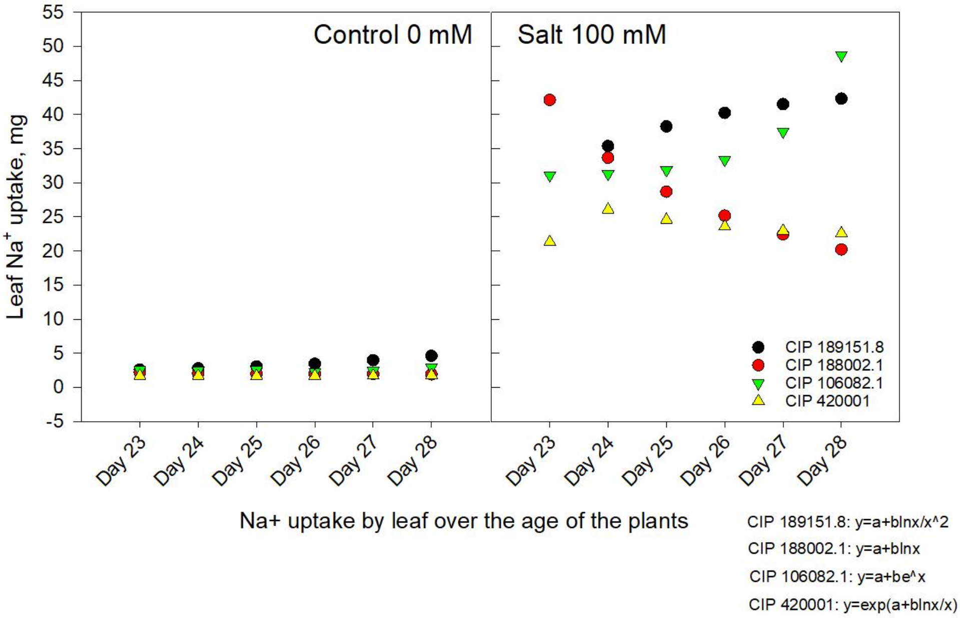

Figure 12. Na^+ Uptake by leaf (mg) over time

After harvesting the plants, there was not big difference in root development between the varieties, except that under control conditions the roots were longer. For CIP 1880002.1 and CIP 420001 a downward bending is evident, so a lower Na^+ concentration in the root over time. The same trend can be seen for the leaf concentration for this two varieties. In day 23 for all plants a higher Na^+ concentration can be seen, what relates to a higher uptake of the young sweet potato roots. CIP 106082.1 has exceptional high Na^+ concentration in the roots, possible due to the different leaf form and plant habitus.

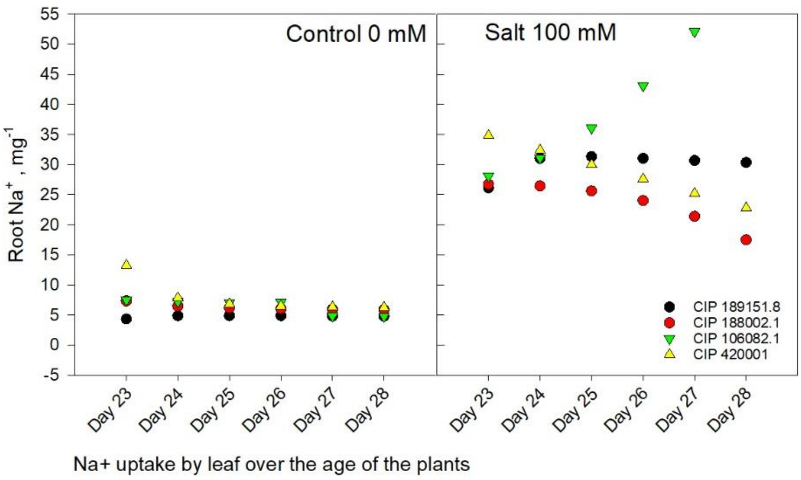

Figure 13. Na^+ Concentration by root (mg) over time

The following table is listing the leaf concentrations of Na^+. V2 (CIP 188002.1) has a significantly lower concentration of sodium. V1 (189151.8) shows a higher sodium uptake in all parts. V4 also shows a lower sodium concentration

Table 3. Sodium concentration in the leaf (mg g); 100mmol salt treatment, 0 control; V1 = CIP 189151.8; V2 = CIP 188002.1; V3 = CIP 106082.1; V4 = CIP 420001; [x no available data]

PLANT PART	DAYS OF STRESS	V1 0	V1 100	V2 0	V2 100	V3 0	V3 100	V4 0	V4 100
UPPER	1	1.41	28.64	1.65	23.42	1.45	2.77	28.05	25.07
UPPER	2	1.45	52.87	4.89	18.18	0.91	X	1.99	30.50
UPPER	3	0.92	31.15	0.91	20.31	1.46	21.24	1.44	23.69
UPPER	4	0.91	41.29	0.94	13.47	0.94	24.07	0.95	18.61
UPPER	5	0.91	37.32	0.93	18.93	3.66	51.30	2.22	21.62
UPPER	6	1.45	40.09	X	11.05	1.42	46.38	0.94	22.39
MIDDLE	1	1.48	49.22	2.02	34.30	1.95	41.77	2.54	20.01
MIDDLE	2	0.93	49.06	0.95	17.63	1.44	1.63	1.46	37.50
MIDDLE	3	1.42	40.32	1.46	18.38	2.50	23.41	2.02	30.33
MIDDLE	4	1.94	30.30	1.47	20.94	0.95	32.65	0.92	29.42
MIDDLE	5	0.93	25.66	1.44	7.79	1.46	18.10	1.42	29.28
MIDDLE	6	1.97	50.95	1.46	12.29	1.43	38.97	2.54	18.34
BOTTOM	1	1.48	38.02	0.90	X	2.57	27.25	1.93	26.88
BOTTOM	2	1.46	46.78	1.47	27.44	1.92	44.41	0.94	28,03
BOTTOM	3	1.42	X	1.48	30.41	3.69	28.39	0.95	18,20
BOTTOM	4	1.93	38.97	1.46	16.23	1.42	23.76	1.47	22,98
BOTTOM	5	2.01	18.82	1.46	19.40	2.47	31.74	2.00	29,15
BOTTOM	6	2.00	x	1.44	14.92	3.06	30.82	0.94	23,46

3.3 VPD and transpiration

For the cumulative transpiration from the pots and from the plant/ leaves are shown. In CIP 188002.1, transpiration is relatively low with the equation of $y=152.96+(-7.7)*x+(-204.7)/x+134.1/x^2$ and in CIP 42001 $y=659.2+(-188.6)*x+62.2*x*\ln*x+(-765.6)/x+e/x^2$, both show a significantly lower cumulative water absorption under salt than under control treatment. In control CIP 106082.1, closely followed by CIP 189151.8 show a strong transpiration. Also under salt conditions, they transpire more and therefore have a higher Na^+ concentration, possible signs of lower tolerance, combined with more LA, so bigger surface.

Over time it can be seen that a higher transpiration is linked to more Na^+ uptake and the individual reaction of the variety. The different genetics of the sweet potato varieties have different reactions for salt. CIP 188002.1 and CIP 420001 are showing a lower trend, so less transpiration and less Na^+ uptake. While CIP 189151.8 and CIP 106082.1 are increasing significantly.

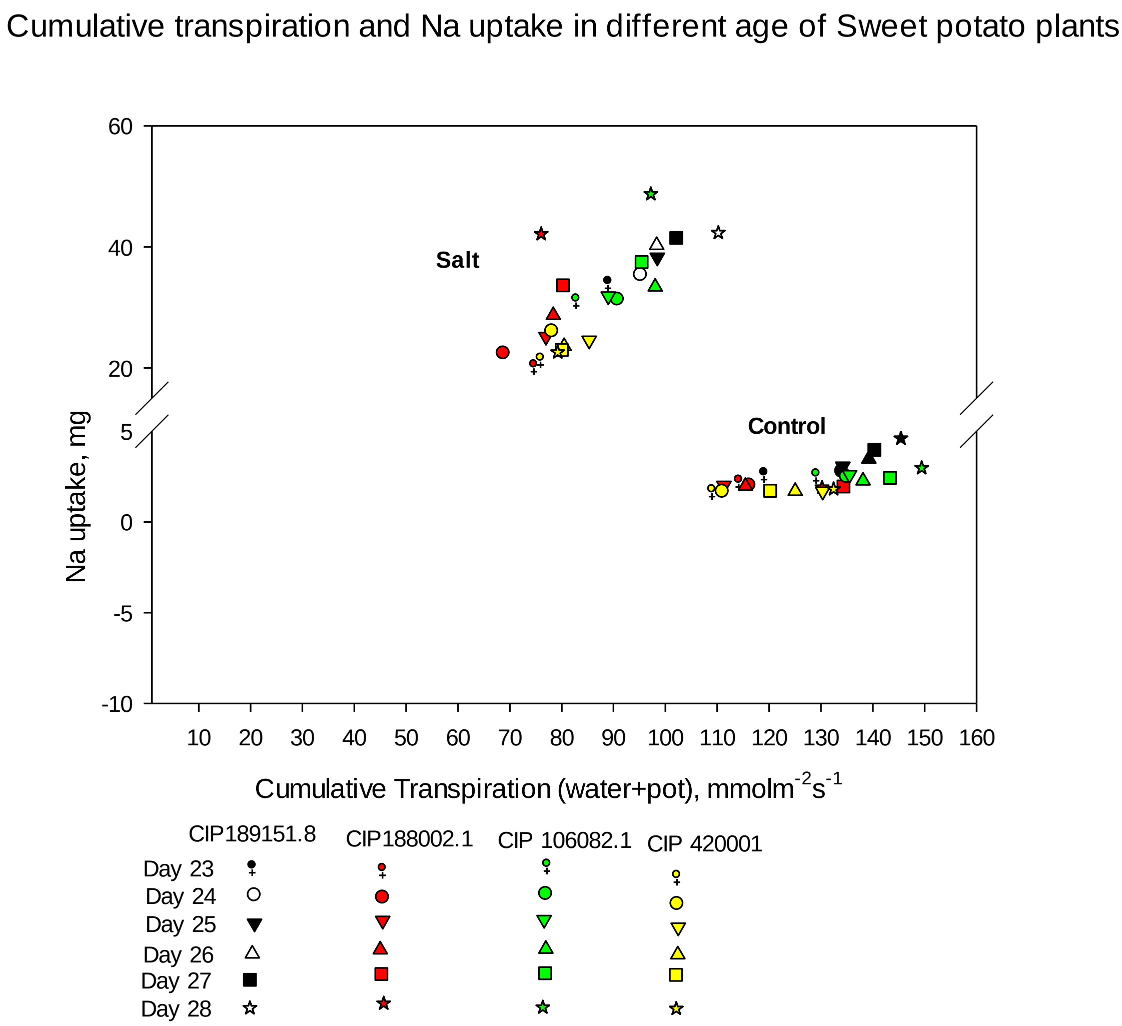

Figure 14. Cumulative transpiration over time for salt and control treatment

The results of the tests in the transpiration chamber display a great response for the variety CIP 189151.8 and CIP 106082.1 in their transpiration under 100 mM salt. Under control conditions there is not much response in the transpiration from CIP 1880002.1. The big error bars are due to more variability of the data, the reason where some technical issues. Variety CIP 189151.8 is responding with the highest transpiration over all varieties, in particular on day 23 and 24 with salt and high VPD, but transpiring similar under control conditions. CIP 1880002.1 and CIP 420001 are transpiring less under salt exposure, but even under control is the transpiration significantly higher. CIP 106082.1 shows a particularly high perspiration, especially on day 28. The VPD was fitted to the chosen levels with a temperature of 28 - 29°C. The relationship between higher transpiration and higher pressure is nicely displayed.

Salt treatment, VPD and Transpiration

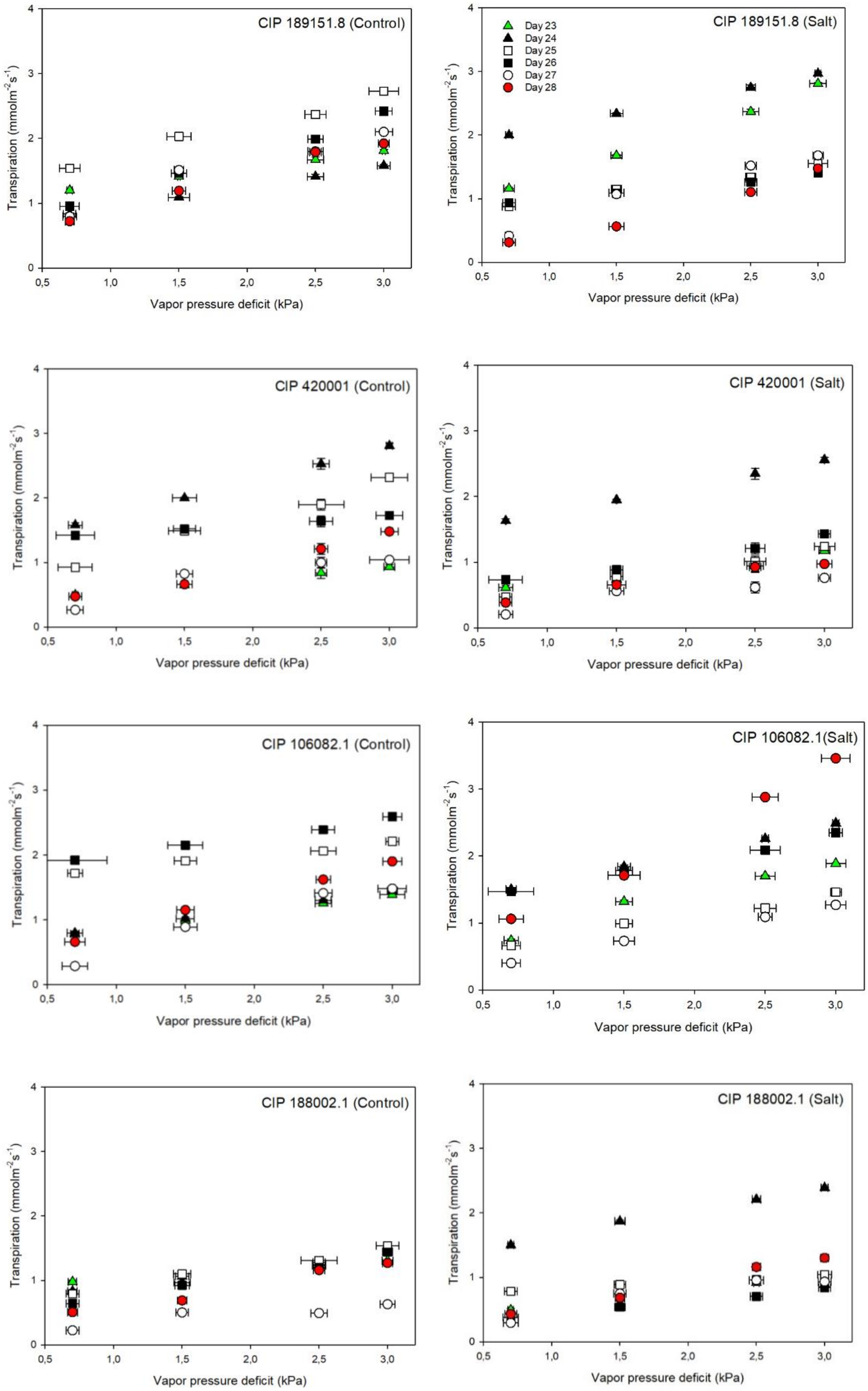

Figure 15. Transpiration and different VPD levels of Sweet potato under control and salt treatment

The correlation was used to outline relations between the different parameters. There is a negative relation (+/- 0.5) between sodium concentration and total dry weight, meanwhile between concentration and uptake show a significant correlation in Sweet potato. The negative correlation for the cumulative water transpiration for sodium uptake and concentration, furthermore with root sodium concentration can be traced back to the reduction in transpiration under salt influence. Concentration of sodium at the root have a significant relation with the uptake and concentration of sodium in the leaf and total plant. If the root sodium concentration increases, the sodium concentration in the plant will increase. For leaf weight and leaf number there is a correlation, causally linked to development and varietal character. Close related is the relation between root weight and leaf area, so more growth could be related to that.

Table 4. Correlations among SPAD, total dry weight of whole plant, Na uptake, Na concentration, cumulative water transpiration, root Na concentration, total leaf number, leaf area, leaf weight and root weight in four Sweet potato varieties contrasting in salt tolerance

	SPAD	TDW	NaUp	NaC	CWT	RNAC	LNr	LA	LW	RW
SPAD	1									
TDW	0.07664	1								
NaUp	-0.24319	-0.34195	1							
NaC	-0.24969	**-0.52695**	**0.94766**	1						
CWT	0.05237	0.36678	**-0.71639**	**-0.72611**	1					
RNAC	-0.17141	-0.48005	**0.89225**	**0.92491**	**-0.81041**	1				
LNr	0.14532	0.50936	0.00593	-0.14447	-0.11214	-0.10573	1			
LA	-0.27807	0.51977	-0.12338	-0.1649	0.27721	-0.19877	0.08826	1		
LW	0.03414	0.50932	-0.37587	-0.45742	0.58156	-0.47897	0.12794	**0.57442**	1	
RW	0.00576	0.59547	0.17197	0.06352	0.06162	0.08467	0.22861	**0.56453**	0.30549	1

4 Discussion

The aim of this work was to assess the influence of salt and VPD on the transpiration of the sweet potato. Closer, with look on the transpiration effect and sodium uptake. The sweet potatoes were grown at different levels of VPD and salt treatment in a hydroponic system. The effect of transpiration was recorded in the chamber for control and salt treated plants. Variation was made between the four varieties and the salt treatment. The biggest effect is expected between the varieties with different reactions on salt.

4.1 Differences in the morphology and variety

The different habitus can be a possible reason for different responses in transpiration, uptake and stress (Lippert, 1987). CIP 106082.1 had a particular high transpiration in the chamber, exceptional high Na^+ and from the habitus bigger leafs than the other varieties, which resulted in a higher leaf area.

The LA was not as large, especially for the salt-intolerant varieties, which can be attributed to the reduced growth. Bhagsari et al. (1986) have made in their research assumptions and following experiments about the possible influence of leaf characteristics on different crops and in particular on the sweet potato. The field of leaf form and influence is still not fully explored, but the negative yield or reduced LA is an important factor which needs to be taken into account, probably from importance for the sweet potato crop. Varieties with reduced growth or sensitivity to ion accumulation or too high transpiration will affect the result achieved on the field.

Variety CIP 1880002.1 was ovate shaped in leaf form and CIP 420001 serrated or lobbed. The leaf could have had an influence on the screening method and the leaf form and size. Proofed is, that the VPD and the ion concentration in the leaf is closely related to the LA in response to humidity, growth and photosynthetic activity (Ohsumi et al., 2008). Lower chlorophyll content became more visible and from salt influence on leafs were slight wilted features at the edges and slightly developed spots on the leaves. Tavakkoli et al. (2010) recorded similar reactions of lower growth under salt for faba bean, but this reactions to very high ion accumulation and resolving stress and inoperable plants are known and proven for many plant species.

4.2 Salt exposure

Begum et al. (2015) and Tavakkoli et al. (2010) their investigations of the negative influence of salt on growth rates for different species were also visible for sweet potato, but there were clear differences between the varieties. For example CIP 1880002.1 showed steady growth even with salt and a significantly increased tolerance limit to Na^+. There was no significant relationship under salt stress between SPAD and LA, but the short length of salt exposure (one week) inhibited just minor the leaf development, a bigger decrease could have been visible under a longer salt exposure (Plaut et al., 2000).

CIP 1880002.1 and CIP 420001 reveal in nearly all examinations and data a lower Na^+ concentration in the root and for the leaf concentration compared to the other two varieties. Except the higher Na^+ concentration and uptake in the first day for all plants, what relates to a higher uptake of the young sweet potato roots. The gradual root Na regulation with the plant development is also the case with soybean (P. An et al., 2001). The same phenomenon was detected with specific difference for the variety Tachiyutaka (soybean), in this case similar with CIP 106082.1 (sweet potato), that more transpiration, lower root growth and higher root Na+ content is seen, which means a lower tolerance level of certain varieties. The same result for sweet potoato as from Vysotskaya et al. (2010) for barley was observed for the two varieties CIP 1880002.1 and CIP 420001, that a smaller restriction in leaf area and root mass was characteristic. Not that significantly seen for the sweet potato was the lower SPAD or chlorophyll content.

On the other side the varieties CIP 189151.8 and CIP 106082.1 have similar reactions as An et al. for soy (2001) and for tomato (2005), showing that the sensitive varieties show lower growth under salt stress, additionally the effect is enhanced by a higher relative humidity. These results were clearly visible in the varieties that did not tolerate that well salt. In the chamber the turgor loss was visible due to the salt application.

4.3 Transpiration and VPD

The different mechanisms from high to low concentration for uptake and from low to high from the osmotic transport are important for the distribution and furthermore the different developments stages from the plant. This will not be discussed in detail here, but in order to include the possible effects they will be mentioned, even so reactions with K and Cl are not taken into account.

All varieties transpired more according to an increased VPD level. For CIP 1880002.1 and CIP 420001 a same result was seen with low Na^+ concentrations, as well as lower transpiration. This indicates effective mechanisms to exclude salt and even growth under suboptimal conditions. A similar relationship between Na+ concentration and transpiration can be seen for soybean (P. An et al., 2001). For soybean there was no significant relationship for VPD on root Na^+ content, this aspect could eventually reveal further information for the varietal character and reaction.

The higher the VPD the more the sweet potato plant transpired. Backhausen et al. (2005) received similar results for potato plants under high light intensity and air humidity. The Na^+ accumulation was lower at high air humidity. Although this was seen also for sweet potato, it just occurred to CIP 1880002.1 and to CIP 420001. More transpiration from older leaves under stress conditions for CIP 106082.1 and the opposite for CIP 189151.8 at high VPD show different regulation for their transpiration. The different reactions can be due to different outer factors, but these were reduced to a minimum, so e.g. the decrease of the transpiration can be caused by lower stomatal conductance for H_20 (Hniličková et al., 2017). Furthermore due to higher NaCl concentration and chlorophyll content transpiration can be influenced.

Muenscher´s (2019) experiments on transpiration demonstrate the reduction of transpiration with increasing humidity. Following that the ash content is not related to the amount that is transpired. The more “sensitive” sweet potato varieties CIP 189151.8 and CIP 106082.1 had a higher Na^+ uptake in combination with a higher VPD and significantly higher transpiration. Plaut et al. (2000) discovered the same for sugarcane, so transpiration is not directly in reaction with salinity and leaf development, but salt increases the stress and in combination with higher vapour pressure on the plant a higher transpiration occurs. From the observation, CIP 420001 and CIP 188002.1 showed clear mechanism for Na with different VPD stages and transpiration.

4.4 Conclusion

The toxic influence of salt is presenting a research field for further future projects. Needed adaptations in genetics and plant breeding, will be a key factor for success under changing and new climatic conditions (Ledesma et al., 2016).

A 100 mM salt treatment and different development stages, present already on the small scale of the experiment could results on different varietal behaviour. It became clear that the different varieties reacted differently to the influence of salt and consequently their transpiration rate. The cumulative transpiration related to Na uptake was closely observed, with high in both for CIP 189151.8 and CIP 106082.1. For the different VPD levels, all varieties transpired more according to higher level. CIP 188002.1 and CIP 420001 regulated transpiration in young to older leaves.

In particular CIP 188002.1 and CIP 420001 showed a better tolerance with high Na^+ concentration, additionally this did not hinder growth, while transpiration could be reduced and controlled despite salt stress. The varieties CIP 189151.8 and CIP 106082.1 have a high leaf area for the control treatment, but reduced chlorophyll content under salt exposure. Both varieties transpired under high VPD significantly more ($p < 0.05$). LA and root Na^+ concentration is in close relation to their transpiration recording to the increasing VPD level. The first day showed the highest concentration in Na^+, only CIP 188002.1 showed a reduction closely followed by CIP 420001. In root Na concentration, CIP 188002.1 and CIP 420001 regulated root Na gradually with their development.

Further investigation could be helpful with more replications to understand clearly the Na regulation among these four varieties of Sweet potato. The reason for low/high Na concentration in different varieties should also be investigated to know exclusion or other mechanisms that are quite important in the tolerance mechanism in Sweet potato.

4.5 Limitations

Due to time limits in the experiment, a longer period could have brought a closer look at the impact and the duration of salt application. The transpiration chamber had some technical problems in the middle of the experiment, which made the regulation of humidity and temperature much more difficult. This can be seen with irregularities e.g. in day 3. So an improved technical equipment and standardised handling with the transpiration chamber could improve future results.

Water was lost from the plant-pots due to the pump in the greenhouse, which resulted in missing data in the weight measurement. The space was limited for growing, which led to some slight damage to the plant leafs. This could have been prevented with more space for growth or extra plants to take the strongest for the experiment.

The cold climate in November and December could possibly have had an influence on the plants in the transfer from greenhouse to transpiration chamber. A more detailed view on the ion accumulation can provide better information about the distribution and effect on the plant, even so the not included data from Cl and other ions, other gathered plant parts and their results. More specific research for stomatal conductance and mechanisms for uptake could give an insight of the reactions in the plant to find more fitting sweet potato varieties for different soil and saline conditions.

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